



4TH INTERNATIONAL CONGRESS OF VETERINARY BIOCHEMISTRY AND CLINICAL BIOCHEMISTRY

4-7 September 2025

SİVAS CUMHURİYET UNIVERSITY TÜRKİYE



EDITOR

Prof. Dr. Nazlı ERCAN

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adapted by Mariam Rasulan

CONGRESS ID

CONGRESS TITLE

**4TH INTERNATIONAL CONGRESS OF VETERINARY BIOCHEMISTRY
AND CLINICAL BIOCHEMISTRY**

DATE and PLACE

September 4-7, 2025 / Sivas, Türkiye

ORGANIZATION

**Sivas Cumhuriyet University
Balıkesir University
Düzce University**

**TUBITAK | The Scientific and Technological Research Council of Türkiye
IKSAD-Institute of Economic Development and Social Research, Türkiye**

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NUMBER of ACCEPTED PAPERS-118 (*Türkiye- 51, Other Countries- 67*)

NUMBER of REJECTED PAPERS-29

PARTICIPANTS COUNTRY

**Türkiye, India, Uzbekistan, Pakistan, Morocco, Nigeria, Poland, Bangladesh,
Ukraine, Romania, Iran, Tunus, Italy, Japan**

PRESENTATION

Oral & Poster presentation

EVALUATION PROCESS

All applications have undergone a double-blind peer review process

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Sivas Cumhuriyet University
**4th INTERNATIONAL CONGRESS OF VETERINARY
BIOCHEMISTRY AND CLINICAL BIOCHEMISTRY**

September 04-07, 2025 / Sivas, Türkiye



-CONGRESS PROGRAM-

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

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- Moderator is responsible for the presentation and scientific discussion (question-answer) section of the session.

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Opening Ceremony

Sivas Cumhuriyet University, 100th Year Cultural Center

04.09.2025 09:30-10:15	PRESIDENT OF THE CONGRESS Prof. Dr. Nazlı ERCAN Sivas Cumhuriyet University Faculty of Veterinary Department of Biochemistry Dr. Yeter ÇİLESİZ Member of the Scientific Board of the IKSAD Institute HONORARY PRESIDENT OF THE CONGRESS Prof. Dr. Ahmet ŞENGÖNÜL Sivas Cumhuriyet University Rector
04.09.2025 10:15-10:30	<i>COFFEE BREAK</i>

1st In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Hamdi UYSAL & Prof. Dr. Zafer BULUT	
04.09.2025 10:30-11:00	INVITED SPEAKER Prof. Dr. Khalid Rahman- Liverpool John Moores University <i>“Aged Garlic Extract and Inhibition of Platelet Aggregation, the Inside Story!”</i>
04.09.2025 11:00-11:30	INVITED SPEAKER Prof. Dr. Omar M. Atrooz- Mutah University & Al-Ahliyya Amman University <i>“Calixarene as a New Approach for Global Health”</i>
04.09.2025 11:30-12:00	INVITED SPEAKER Prof. Dr. Ralf JACOB- Philipps University of Marburg <i>“Cell Biology of Galectin-3”</i>
04.09.2025 12:30-13:30	<i>LUNCH</i>

2nd In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Berrin SALMANOĞLU & Prof. Dr. Atila ATEŞ

04.09.2025
13:30-14:30

EPIGENETICS AND THE EPIGENETIC EFFECTS OF VITAMIN D
Fatmagül YUR, Güzide Zeynep TAN

THE RELATIONSHIP BETWEEN TROPONIN AND ADAMTS IN
PATIENTS WITH ACUTE CORONARY SYNDROME
*Kübra MADEN, Hasan Basri ÇETİNKAYA, Adnan Adil
HİŞMİOĞULLARI*

INTRACELLULAR METABOLISM OF NaF
Semiha DEDE, Ayşe USTA, Veysel YÜKSEK

EFFECTS OF NON-THERMAL PLASMA APPLICATION ON AIR
QUALITY AND PERFORMANCE PARAMETERS IN BROILER
PRODUCTION
Melisa TAŞCI

3rd In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Semiha DEDE & Prof. Dr. Cevat NİSBET

04.09.2025
14:30-15:30

BEYOND ANTIOXIDANTS: APOPTOTIC PATHWAY MODULATION
BY ONOPORDUM ACANTHIUM IN CANCER CELL LINES AND
COMPUTATIONAL MODELS
Dilek Nur BESTİL, Hamdi UYSAL, Osman TUGAY

INVESTIGATION OF THE POSSIBLE PROTECTIVE EFFICACY OF
RUTIN AND TAXIFOLIN AGAINST ISCHEMIA/REPERFUSION
INJURY IN THE RAT OVARY
Mehmet Hanifi AKGÜN, Pınar COŞKUN, Hilal ÖZÇİFLİKÇİ

INVESTIGATION OF THE ANTIOXIDANT CONTENT OF ISABELLA
GRAPE (VITIS LABRUSCA L.)
Semanur BAYBURTLU, Tevhide SEL

ANTIOXIDANT CONTENT OF CARDAMOM OIL EXTRACT AND
ITS EFFECTS ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS
(CLL)
Sena TIRAŞ, Nazlı ERCAN, Tevhide SEL

04.09.2025
15:30-15:45

COFFEE BREAK

1st In-person Panel

Büyük veri, Yapay zeka ve Biyokimya: Geleceğin Bilimsel Köprüsü
Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Merve ALPAY	
04.09.2025 16:00-16:45	Assoc. Prof. Dr. Özkan DURU Kırıkkale University
	Assoc. Prof. Dr. Burcu Menekşe BALKAN Burdur Mehmet Akif Ersoy University
	Assist. Prof. Dr. Tolgay ERCAN Sivas Cumhuriyet University
04.09.2025 16:45-17:00	COFFEE BREAK

4th In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Hasret YARDİBİ & Prof. Dr. Hacı Ahmet DEVECİ	
04.09.2025 17:00-17:30	NEOPTERIN AND PROCALSITONIN LEVELS IN SHEEP INFECTED WITH ECTHYMA <i>Kadir BOZUKLUHAN, Halil AYVAZOĞLU, Oğuz MERHAN</i>
	TOTAL SIALIC ACID AND PROCALCITONIN LEVELS IN CATTLE WITH PARATUBERCULOSIS <i>Halil AYVAZOĞLU, Kadir BOZUKLUHAN, Oğuz MERHAN</i>
	EFFECTS OF ORAL BORON AND VITAMIN D SUPPLEMENTATION ON BORON, IRON, COPPER, ZINC, AND VITAMIN D LEVELS IN RAT BRAIN TISSUE <i>Azra Mila ERYAVUZ, Mehmet BAŞEĞMEZ, Fahriye KAN, İsmail KÜÇÜKKURT, Abdullah ERYAVUZ</i>

5th In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Tevhide SEL & Prof. Dr. Feraye ESEN GÜRSEL	
04.09.2025 17:30-18:00	THE EFFECT OF INTERMITTENT FASTING AND EXERCISE APPLICATION ON SERUM IRISIN AND IGF-1 LEVELS IN RATS <i>Miray ACAR, Serap ÜNÜBOL AYPAK</i>
	CHANGES IN CIRCADIAN CLOCK-RELATED GENE PROTEINS DEPENDING ON MIGRATION PERIODS IN MIGRATORY BIRDS <i>Paria İSFENDİYAROĞLU, Cevat NİSBET</i>
	INFLAMMATION AND HYPOXIA IN OBSTRUCTIVE SLEEP APNEA: ANALYSIS OF IL-1 β AND HIF1 α N SERUM LEVELS <i>Sare UYURCA, Merve ALPAY</i>

Dinner

04.09.2025, 18:30-21:00

6th In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Ayşegül BİLDİK & Prof. Dr. Hasan AKŞİT

05.09.2025
09:00-09:30

EVALUATION OF THE ANTIOXIDANT COMPOSITION OF ROYAL JELLY AND ITS EFFECTS ON COLORECTAL ADENOCARCINOMA AND BREAST CANCER CELL LINES

Merve EKREN, Yeliz KAYA KARTAL, Berrak KURT

LACTOFERRIN AS A NUTRITIONAL AND THERAPEUTIC BIOMOLECULE: FROM IRON METABOLISM TO CANCER

Tuba EKER, Mert PEKCAN

ASSESSMENT OF OXIDANT-ANTIOXIDANT BALANCE AND THIOL-DISULFIDE HOMEOSTASIS ALTERATIONS INDUCED BY MONOCROTALINE IN MICE

Ali ŞENOL, Alparslan Kadir DEVRİM, Miyase ÇINAR, Mahmut SÖZMEN, Hacı ÖCAL, Elisha Apatewen AKANBONG

EXPLORING THE IMPACT OF TIME-RESTRICTED FEEDING ON OXIDATIVE STRESS IN WISTAR ALBINO RATS

Özkan DURU, Elisha Apatewen AKANBONG, Ali ŞENOL, Hacı ÖCAL, Safiye ARZİK

7th In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Fatmagül YUR & Prof. Dr. İraz AKIŞ AKAD

05.09.2025
09:40-10:20

EVALUATION OF SERUM VITAMIN A AND E LEVELS IN CATTLE VACCINATED WITH FOOT-AND-MOUTH DISEASE VACCINE AND EXPERIENCED FOOT-AND-MOUTH DISEASE

Halil YAVUZ, Alparslan AYAZ, Ömer Barış İNCE

CD44-TARGETED AR INHIBITION IN PROSTATE CANCER CELLS

Sinan VICIL, Rıza SERTTAŞ, Suat ERDOĞAN

DISACCHARIDE INTERACTIONS WITH OUTER MEMBRANE PROTEINS OF BRUCELLA S19 VACCINE STRAIN: MOLECULAR MODELING FOR LYOPROTECTANT SELECTION

Gülseren YILDIZ ÖZ

05.09.2025
10:20-10:30

COFFEE BREAK

8th In-person Session

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Oğuz MERHAN & Doç. Dr. Meryem ŞENTÜRK

05.09.2025
10:30-11:30

EFFECT OF CAFFEIC ACID PHENETHYL ESTER AGAINST α -CYPERMETHRIN TOXICITY

Hacı Ahmet DEVECİ, Pınar AKSU KILIÇLE, Gökhan NUR, Ayla DEVECİ

ANTIOXIDANT AND ANTIDIABETIC EFFECTS OF ROSEMARY, SUMACH AND ZAHTER

Hacı Ahmet DEVECİ, Ayman KHOSRUF, Ayla DEVECİ

EVALUATION OF LIPID PROFILE, CHICKEN PLATELET-ACTIVATING FACTOR ACETYLHYDROLASES AND OXIDATIVE STRESS PARAMETERS LEVELS IN BROILER CHICKENS WITH DIFFERENT SLAUGHTERING AGES

Efe KURTDEDE, Ömer Faruk ALTINOK, Damla Nur ÇOBAN, Doğu TURGUT

EFFECTS OF DIALYL DISULFIDE ADMINISTRATION IN RATS WITH AN EXPERIMENTAL COLON CANCER MODEL

Beyza SUVARİKLİ ALAN, Özgür ÖZDEMİR, Zafer BULUT

ASSESSMENT OF THE IMPORTANCE OF REGULATORY COMPLIANCE OF MEDICAL DEVICES BETWEEN COUNTRIES IN TERMS OF GLOBAL HARMONIZATION

Tolgay ERCAN

2nd In-person Panel

What Should Undergraduate Veterinary Biochemistry Cover in "Metabolism"? How Much Should Students Know?

Sivas Cumhuriyet University, 100th Year Cultural Center

Moderator: Prof. Dr. Adil HİŞMİOĞULLARI

05.09.2025
11:30-12:30

Prof. Dr. Tayfun GÜLDÜR

İnönü University

Prof. Dr. Miyase ÇINAR

Kırıkkale University

Prof. Dr. Hasret YARDİBİ

Istanbul University - Cerrahpasa

Prof. Dr. Pınar COŞKUN

Hatay Mustafa Kemal University

Closing Ceremony & Oral and Poster Award Ceremony

Sivas Cumhuriyet University, 100th Year Cultural Center

05.09.2025, 12:30-13:00

Lunch

05.09.2025, 13:00-14:00

Social Program

05.09.2025, 14:00-21:00 City Trip and Dinner

06.09.2025, 07:00-21:00 Divriği Trip (Breakfast & Lunch)

Workshop

Sivas Cumhuriyet University, Experimental Animals Application and Research Center

05.09.2025, 09:30-13:30

Poster Presentations

Sivas Cumhuriyet University, 100th Year Cultural Center

(Only for 4th September)

EVALUATION OF APELIN, CHEMERIN, LEPTIN, RESISTIN AND VASPIN LEVELS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA SYNDROME

Eren Kırdar ÖZTÜRK, Merve Akıs YILMAZ, Nurhan SARIOĞLU, A. Adil HİŞMİOGULLARI

THE EVALUATION OF THE LEVELS OF GALECTIN-3, SIRT 1, THIOREDOXIN, NITRIC OXIDE IN OBSTRUCTIVE SLEEP APNEA SYNDROME

Betül KÖPRÜ, Nurhan SARIOĞLU, A. Adil HİŞMİOGULLARI

DETERMINATION OF GENE POLYMORPHISMS IN KITLG AND KISS1 GENE REGIONS EFFECTING THE REPRODUCTIVE TRAITS OF HAIR, ANGORA, AND KILIS GOATS

Atila ATEŞ, Berk Özcan ATALAY

IMPORTANCE OF BIOCHEMICAL, HEMATOLOGICAL AND URINE ANALYSIS IN LABORATORY DIAGNOSIS

Hasan AKŞİT

TRACE ELEMENT PROFILE IN HYPOTHYROIDISM

Çağlar Kaan BOZBEK, Meryem ŞENTÜRK

THE ROLE OF EXOSOMES AND MIRNAS IN CLINICAL BIOCHEMISTRY

Neslihan ORMANCI, Sedat ORMANCI

FERROPTOSIS: A REGULATED CELL DEATH PATHWAY IN CANCER

Sare UYURCA, Öğünç MERAL

INVESTIGATION OF THE EFFECTS OF ELECTRO-FREQUENCY WATER TREATMENT SYSTEM ON PERFORMANCE PARAMETERS IN POULTRY FARMING

Ebru EKİZCE, Prof. Dr. Berrin SALMANOĞLU

DETOXIFICATION STRATEGIES OF FOOD CONTAMINANTS BY PROBIOTICS

Soner TUTUN, Betül KARAPINAR, Tuğba DEMİR

Online Session-1, Hall-1

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Dr. Ayfer FINDIK PAKROOH

05.09.2025
12:30-14:30

EFFECT OF FUCOIDAN ON SOME BIOCHEMICAL PARAMETERS
IN CARBON TETRACHLORIDE-INDUCED NEPHROTOXICITY IN
RATS

Alper Cenk KAPLAN, Handan MERT, Nihat MERT

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF
MESENCHYMAL STEM CELL EXOSOMES IN KIDNEY DISEASES

Turgay GÜMÜŞ, Özkan DURU

THE RELATIONSHIP BETWEEN SOME BIOCHEMICAL
PARAMETERS AND BENIGN PAROXYSMAL POSITIONAL
VERTIGO: POTENTIAL MECHANISMS INFLUENCING ONSET
AND RECURRENCE

Ömer Faruk DEMİR, Abdulsamed KÜKÜRT

HEMATOLOGICAL, BIOCHEMICAL & IMMUNOLOGICAL
ALTERATIONS IN ACUTE COVID-19, LONG-COVID SYNDROMES
AND POST-VACCINATION SYNDROME

Ayfer FINDIK PAKROOH

4-HYDROXY-2-NONENAL MECHANISM AND ITS RELATIONSHIP
WITH CANCER

Doğuş ÖZALKAN, Burcu MENEKŞE BALKAN

A REVIEW ON APOPTOSIS AND APOPTOSIS MECHANISMS

Gökhan KOÇAK

Online Session-1, Hall-2

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Assoc. Prof. Dr. Semih YAŞAR

THE IMPORTANCE OF PHYTOCHEMICALS AND ANTIOXIDANTS
IN FOOD BIOCHEMISTRY: ANALYTICAL APPROACHES USING
CHROMATOGRAPHY TECHNIQUES

Ebubekir İZOL, Esma KAYAOĞLU

USE OF PLANT-BASED ANTIOXIDANTS IN MEAT PRODUCTS:
EVALUATION OF SYNTHETIC AND NATURAL SUBSTANCES IN
TERMS OF TBARS

Esma KAYAOĞLU, Ebubekir İZOL

THE EFFECT OF LOW-DOSE PRUNUS SPINOSA FRUIT EXTRACT
ON OXIDATIVE STRESS PARAMETERS IN A549 CELLS TREATED
WITH BENZO[A]PYRENE

Hakan ODUNCU, Ahmet BÜYÜKBEN

HEALTH EFFECTS AND SUPPLEMENTATION POTENTIAL OF
HESPERIDIN

Semih YAŞAR

IN VITRO DIAGNOSTIC (IVD) DEVICES: NEW CLASSIFICATION
AND RISK MANAGEMENT

Adem ÜNAL, Şükran Melda ESKİTOROS TOĞAY

COMPARATIVE ANALYSIS OF GALECTIN-3, IMA, MPO, AND
PON-1 SERUM LEVELS IN COVID-19 PATIENTS: POTENTIAL
DIAGNOSTIC BIOMARKERS

Ahmet DÜNDAR, Songul ÇETİK YILDIZ, Mehmet KABAK, Barış CIL

05.09.2025

12:30-14:30

Online Session-1, Hall-3

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: SAYERH Fatima Zahra

ANTICANCER EFFECTS OF CREPIDIUM ACUMINATUM ON
MULTIPLE IN VITRO CELL LINES

K.R.Padma

ANTIOXIDANT AND ANTI-INFLAMMATORY MECHANISMS OF
AFRICAN SPICES AND HERBS

Ahmed ATTAHIRU

NATURAL PRODUCT PROTEIN INTERACTIONS: IMPLICATIONS
FOR DRUG

Ahmed ATTAHIRU

PREVALENCE OF ESCHERICHIA COLI O157:H7 IN WELL WATER:
ZERO DETECTION AND RESISTANCE PATTERNS OF NON
O157:H7 STRAIN IN OKEHI LOCAL GOVERNMENT AREA, KOGI
STATE NIGERIA

***Muhammed Mustapha ADEBOYE, Lukman UPAHI, Daniel
Ojochenemi APEH, Yusuf A. EKEYI, Olutayo Adesewa AINA***

TARGETED DRUG DELIVERY VIA OCULAR, PULMONARY AND
NASAL ROUTES USING SOLID LIPID NANOPARTICLES

Vijay Kumar, Rahmuddin khan, Zeenat Iqbal, M.Aamir mirza

ESSENTIAL OILS FROM AROMATIC AND MEDICINAL PLANTS:
EXTRACTION TECHNIQUES AND MULTIFUNCTIONAL
BIOACTIVE AGENTS

***Hicham EL-BOUKHARY, Hamza ELKISSANY, Adil LAAZIZ,
Abdelouahed HAJJAJI***

DECODING HOSPITAL WASTEWATER PROFILES: INTEGRATING
PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS AND
AI-BASED CLASSIFICATION

SAYERH Fatima Zahra

ROLE OF DIFFERENT SURFACTANTS (TX-100 AND TWEEN-80) IN
ENHANCING THE SOLUBILITY OF ANTIBIOTIC DRUGS
(LEVOFLOXACIN) FOR BETTER DRUG DELIVERY SYSTEM

Madiha Anwar, Muhammad Usman, Ali Naqi, Hassan Manzoor

05.09.2025

12:30-14:30

Online Session-1, Hall-4

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Ferial HENNAOUI

NEW PROTEOMIC ANALYSES FOR CANCER BIOMARKERS:
ADVANCING PRECISION ONCOLOGY THROUGH MOLECULAR
PROFILING

Saloni Sharma, Suhani Sharma

ENHANCING THERAPEUTIC POTENTIAL OF MIDAZOLAM BY
SYNTHESIZING ITS NANOPARTICLES

***Huma Ikram, Sonia Ali, Muhammad Raza Shah, Darakhshan Jabeen
Haleem***

A FACILE SYNTHESIS OF 1,3,4-OXADIAZOLE-BASED
CARBAMOTHIOATE MOLECULES: ANTISEIZURE POTENTIAL,
EEG EVALUATION AND IN-SILICO DOCKING STUDIES

Zainib Razzaq, Samreen Gul Khan, Fozia Anjum, Naheed Akhtar

ALTERATIONS IN 5HT-2C RECEPTOR RESPONSIVENESS
FOLLOWING REPEATED MIDAZOLAM TREATMENT

Huma Ikram, Shahla Perveen, Sonia Ali, Darakhshan J. Haleem

THE BIOLOGICAL BASIS OF FATIGUE AND ENDURANCE:
MECHANISMS AND IMPLICATIONS

Saloni Sharma, Suhani Sharma

ASSESSMENT OF DIETARY SUPPLEMENT CONSUMPTION AND
ANALYSIS OF THEIR TOXICITY IN CONSUMERS

Ferial HENNAOUI, Amel BERREBBAH ALIOUA

GUAVA (PSIDIUM GUAJAVA L.): A GLORIOUS PLANT WITH
CANCER PREVENTIVE AND THERAPEUTIC POTENTIAL

Keerthana.V, Meenatchi.S, Kishore.R, Dr.R.Saravanan

POTENTIAL OF POMEGRANATE (Punica granatum L.) PEEL AND
ARIL EXTRACTS AS LIPID-LOWERING AGENTS IN A RAT MODEL
OF HYPERLIPIDEMIA

***Lamiae BENCHAGRA, Mhamed RAMCHOUN, Abdelouahed
HAJJAJI, Samira BOULBAROUD, Abdelouahed KHALIL, Hicham
BERROUGUI***

A COMPARATIVE ANALYSIS OF CLINICAL AND BIOCHEMICAL
PARAMETERS IN SCREW-RETAINED SINGLE IMPLANT CROWNS
OF DIFFERENT DESIGNS

***Andrea Luzi, Alessandro Auletta, Arianna Antonelli, Francesco Evolo,
Nicholas Prosperi, Gualtiero Gherardi, Valeria Martini, Alessio Zanza,
Sofia Leporale, Marta Cianfriglia, Luca Testarelli, Rodolfo Reda***

05.09.2025

12:30-14:30

Online Session-1, Hall-5

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Shirinboyeva Dilrabo Komilovna

EFFICACY AND APPLICATION OF A BIOPREPARATION BASED ON AN ASSOCIATION OF CELLULOLYTIC, PROBIOTIC BACTERIA

Shirinboyeva Dilrabo Komilovna, Kutliyeva Guzal Dzhumaniyozovna, Daminov Asadullo Suvonovich

ETHNOVETERINARY APPROACH FOR THE CONTROL OF SUBCLINICAL MASTITIS IN BUBALINE

Farrah Deebe, Muhammad Huzaifah Khalid, Anas Sarwar Qureshi, Muhammad Adil

HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES OF A NATURAL AFRICAN SWINE FEVER INFECTION IN PIGS

Simeon Chibuko OKAFOR, Uju Catherine OKAFOR, Donatus Lotanna OBINWOGU, John Ikechukwu IHEDIOHA

ACARICIDAL AND ANTIOXIDANT ACTIVITIES OF XYLOPIA AETHIOPICA CRUDE METHANOL EXTRACT AGAINST TICKS OF CATTLE AND DOG

Yemisi D. ADEOYE, Adegbolagun T. ADEOYE, Onyeka C. NWUFOH, Victor O. OYEKUNLE, Abibat O. ALADESOYIN

THE IMPORTANCE OF CARBOHYDRATES AND VITAMINS IN FEEDING PUPPIES (literature review)

Nina DANKEVYCH, Yelyzaveta GLUSHCHENKO, Artem DANKEVYCH

OBESITY IN DOMESTIC ANIMALS (Literature Review)

Kateryna Hornostaieva, Nina Dankevych

GINGER (ZINGIBER OFFICINALE) ESSENTIAL OIL AS A PHYTOGENIC MODULATOR OF IMMUNE AND OXIDATIVE STRESS RESPONSES IN WHITE FULANI DAIRY COWS

Emmanuel U. ANASO

COMPARATIVE IN VITRO ANTIOXIDANT AND TRYPANOCIDAL ACTIVITIES OF N-HEXANE AND ETHYLACETATE EXTRACTS OF AZADIRACHTA INDICA ON TRYPANOSOMA BRUCEI BRUCEI

Busari M. B., Yusuf R. S., Yunusa I. O., Yahaya A. S., Mohammed A. M., Adegboye, A. S.

RADIOGRAPHIC, CLINICAL, AND BIOCHEMICAL ASSESSMENT OF SCREW-RETAINED VERSUS CEMENT-RETAINED CR-CO-CERAMIC IMPLANT-SUPPORTED SINGLE CROWNS: A COHORT STUDY

F.Evolo, L.Adamo, S.Leporale, A.Antonelli, N.Carrai, G.Ferri, L.Testarelli, M.Galli, G. Miccoli, R. Palumbo, L. Lucente, R. Reda

05.09.2025

12:30-14:30

Online Session-2, Hall-1

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Doç. Dr. Mert PEKCAN

INVESTIGATION OF THE EFFECT OF DIFFERENT NUTRITIONAL GROUPS ON BEE VENOM PRODUCTION CAPACITY AND COMPOSITION UNDER IN VITRO CONDITIONS

Seyit Hasan ÖZTÜRK, Cevat NİSBET

CLINICAL BIOCHEMICAL EFFECTS OF STRESS, NUTRITION, AND ENVIRONMENTAL FACTORS IN BLACK SEA TROUT (*Salmo trutta labrax*)

Utku DURAN, Esen KULAÇ POLAT, Büşra ŞAHİN

ENVIRONMENTAL BIOMONITORING, BLOOD BIOCHEMISTRY, OXIDATIVE STRESS, AND APOPTOSIS MECHANISMS IN FISH

Esen KULAÇ POLAT, Utku DURAN

ISCHEMIA-MODIFIED ALBUMIN AS AN EARLY BIOMARKER FOR ABORTION IN SHEEP

Songül EKEN, Beran YOKUŞ

POLYCYCLIC AROMATIC HYDROCARBONS IN FOODS: SOURCES, EXPOSURE AND EFFECTS ON HUMAN HEALTH

İrem KOÇ & Seyda ŞAHİN

EFFECTS OF LAVANDULA STOECHAS ESSENTIAL OIL AND CYCLOPHOSPHAMIDE ON MEC-1 CELL LINE

Recep BOZKURT, Güzin ÖZKURT, Tevhide SEL, Yaren CEYRAN

05.09.2025

15:00-17:00

Online Session-2, Hall-2

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Major Gheorghe GIURGIU

MICROBIOTA MODULATION AS THERAPEUTIC APPROACH IN THE NEUROPATHIC PAIN IN DOG WITH SPINAL CORD INJURY: IMPACT OF POLENOPLASMIN

Major Gheorghe GIURGIU, Prof dr med Manole COJOCARU

HEAVY METAL AND PROXIMATE COMPOSITIONS OF DISCARDED FISH PARTS FROM AWURU RIVER AT RETAIL OUTLET IN NIGER STATE

Alfa Asmau Iyanuoluwa, A. Abubakar, Folashade F. Alfa

MULTICOMPONENT REACTION APPROACH FOR POTENTIAL BIOLOGICALLY ACTIVE THIOPHENE DERIVATIVES

Tanzeela Riaz, Sana Aslam, Matloob Ahmad, Muhammad Zubair

PREDICTION OF MANGO QUALITY AND DISEASE DETECTION USING DEEP LEARNING

K. Thejas kumar, A. Shobana, K. Sujatha, N.P.G. Bhavani, Tatireddy Omsai Reddy, D. Kabilan

DIVERSITY OF MICROBIAL ENDOPHYTES, THEIR ASSOCIATION WITH PLANTS, AND ROLE IN SECONDARY METABOLITE PRODUCTION: A REVIEW

Hamza EL KISSANY, Hicham EL-BOUKHARY1, Hicham EL KISSANI, Adil LAAZIZ, Abdelouahed HAJJAJI

BIOCHEMICAL EFFECTS OF NOVEL FOOD COMPOUNDS ON HUMAN HEALTH (Systematic Review)

Adegbola Oluwatosin Oluwabukola

NUTRITIONAL INTERVENTION FOR MITOCHONDRIAL DYSFUNCTION

Adegbola Oluwatosin Oluwabukola

MODELING OF MACRONUTRIENT METABOLISM AND ITS IMPACT ON GLYCEMIC CONTROL IN TYPE 2 DIABETES

GANIYU, Barakat Abiola, AKANDE, Sikirulai Abolaji, Obatusin Esther Iretholuwa

MULTI-OBJECTIVE OPTIMIZATION OF ENZYME KINETICS IN VETERINARY DRUG METABOLISM USING NSGA-II

AKANDE, Sikirulai Abolaji, RAUF Quadri Opeyemi, GANIYU, Barakat Abiola, MUSA, Ojeba Innocent, POPOOLA, Biliquees Oluwakemi, JOB, Samuel Oloruntoba

05.09.2025

15:00-17:00

Online Session-2, Hall-3

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Muhammad FAISAL

EXPLORING THE INTERNATIONAL LEGAL AND ETHICAL STANDARDS FOR RESEARCH AND MEDICAL PRACTICE IN CLINICAL BIOCHEMISTRY: A HUMAN RIGHT PERSPECTIVE

Priya Chaudhuri, Tuhina Sinha

EXPANDING HORIZONS IN VETERINARY AND CLINICAL BIOCHEMISTRY: BUSINESS OPPORTUNITIES IN PAKISTAN

Muhammad FAISAL

EXPLORING ANTICANCER CANDIDATES FROM MOROCCAN MARRUBIUM VULGARE L. THROUGH IN SILICO APPROACHES: BREAST AND PROSTATE CANCER

Amal Bouribab, El Mehdi KARIM, Abdelkbir ERROUGUI, Samir CHTITA

EMERGING TRENDS IN BIOLOGIC AND BIOSIMILAR DRUGS

Puniparthi SUNITHA, K. POOJA, M.K. VIJAYALAKSHMI

ADVANCEMENTS IN EARLY CANCER DETECTION: LIQUID BIOPSY AND GENOMIC PROFILING

Puniparthi SUNITHA, K. POOJA, M.K. VIJAYALAKSHMI

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN PHARMACEUTICAL QUALITY CONTROL

Puniparthi SUNITHA, K. POOJA, M.K. VIJAYALAKSHMI

ROLE OF EXTREMOPHILES IN BIOTECHNOLOGY

Dr. M.K. VIJAYALAKSHMI, K. POOJA, Puniparthi SUNITHA

INTEGRATION OF CLINICAL BIOCHEMISTRY AND MACHINE LEARNING IN IMPROVING DIAGNOSTIC ACCURACY OF HUMAN LIVER DISORDERS

Habib Olaniyi ALIU, Rotimi Jamiu OLASINA

05.09.2025
15:00-17:00

Online Session-2, Hall-4

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Mithila Sen

05.09.2025
15:00-17:00

NEW METHODS IN EARLY DETECTION OF SUBCLINICAL
MASTITIS IN DAIRY FARMS

Kave Koorehpaz

ARTIFICIAL INTELLIGENCE-DRIVEN SURVEY TECHNIQUES
FOR DECREASING TOTAL SOMATIC CELL COUNT IN DAIRY
FARMS

Kave Koorehpaz

HISTOLOGICAL AND BIOCHEMICAL EVALUATION OF 2-(2-
NITROPHENYL)-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID ON
BLOOD PROFILE AND HEART TISSUES IN ROTENONE INDUCED
PD RAT MODEL

Shazia Perveen, Shaheen Kousar, Sumaira Kanwal, Iraj Ajmal

DIGITAL TECHNOLOGIES IN MONITORING ANIMAL HEALTH
AND ENVIRONMENTAL POLLUTION: A CASE STUDY IN
KOSOVO

Selvije Lajqi, Soreja Adzajlic

JUVENILE GULSHA TENGRA (MYSTUS CAVASIUS) EXHIBITED
BETTER GROWTH PERFORMANCE IN PROBIOTIC TREATMENT
BY ENHANCING INTESTINAL MORPHOLOGY

Mithila Sen

EVALUATING THE GROWTH-ENHANCING EFFECTS OF LAPSI
FRUIT (Choerospondia axillaris) AS A DIETARY SUPPLEMENT FOR
RAINBOW TROUT (Oncorhynchus mykiss)

Hari Lama, Dr. Naveen Kumar Thoila, Dr. Archana Prasad

INTENSIVE AQUACULTURE AND CLIMATE WARMING: DRIVERS
OF GENETIC DIVERSITY IN MEDITERRANEAN MONOGENEANS

Sarra FARJALLAH

TABA HEAL CAT

A NOVEL APPROACH FOR STRAY CAT HEALTH AND
ENVIRONMENTAL PROTECTION

Tabarek Abdulridh Haji AL-FARISEE

Online Session-2, Hall-5

Zoom ID: 883 9498 5602 / Zoom Passcode: 040404

Moderator: Souvik Halder

05.09.2025
15:00-17:00

EVIDENCE-GROUNDED SENTIMENT GRAPHS FOR BIOCHEMICAL HYPOTHESES ACROSS TEXT, SPECTRA, AND IMAGING

Souvik Halder

CROSS-DOMAIN MULTIMODAL SENTIMENT ANALYSIS FOR BIOCHEMICAL LITERATURE, SEQUENCES, AND ASSAY DATA

Souvik Halder

INTEGRATED COMPUTATIONAL APPROACH FOR DESIGNING NOVEL PYRIDONE-BASED α -GLUCOSIDASE INHIBITORS: INSIGHTS FROM 2D-QSAR, MOLECULAR DOCKING, MOLECULAR DYNAMICS SIMULATIONS AND MM-GBSA ANALYSIS

Bouchra Rossafi, Oussama ABCHIR, Mhammed ELKOUALI, Samir CHTITA

ADVANCES IN PHARMACOTHERAPY FOR CANCER TREATMENT

R.Vidhyalakshmi, Saraswathi.V, E.Elavarasi

OXIDATIVE STRESS AND ITS IMPACT ON HUMAN HEALTH

Saraswathi.V, R. Vidhyalakshmi, Kowsika.M, Dhanush kumar.S, Sastha.K, Prashanth.S, Dr.W.Helen, Dr.R.Srinivasam

SUSTAINABLE POLYPROPYLENE COMPOSITES: PHYSICO-CHEMICAL INSIGHTS INTO INJECTION-MOLDED MATERIALS WITH SPENT COFFEE GROUNDS.

Zineb El Hamri, M. Karaoui, M. Alami, M. Assouag

POLLUTANTS REMOVAL FROM WASTEWATER THROUGH BIOFILM SYSTEM

Zanna Mustapha, Moses NyoTonglo Arowo, Muhammad Abbagoni Abubakar, Archana Kasinath, Abdulhalim Musa Abubakar

SYNTHESIS AND PHOTOPHYSICAL PROPERTIES OF NOVEL TRICYCLIC PYRIMIDO[5,4-H]QUINAZOLINES

Kirti, Taro Udagawa, Yasuhiro Kubota, Toshiyasu Inuzuka, G Krishnamoorthy, Kazumasa Funabiki

PHOTO GALLERY











Observer hall-1

Özkan Duru

Slide 1 / 26

MEZENKİMAL KÖK HÜCRE EKSOZOMLARININ BÖBREK HASTALIKLARINDAKİ TANI VE TEDAVİ POTANSİYELİ

4.Uluslararası Veteriner Biyokimya ve Klinik Biyokimya Kongresi

TURGAY GÜMAŞ, ÖZKAN DURU

TUBİTAK

Observer hall-1

1303 5.09.2025

Observer hall-1

4. ULUSLARARASI VETERİNER BİYOKİMYA VE KLİNİK BİYOKİMYA KONGRESİ

4-HİDROKSİ-2-NONENAL ve KANSERLE İLİŞKİSİ

Doktora Öğrencisi Doğuş ÖZALKAN
Doç. Dr. Burcu MENEKŞE BALKAN

MEHMET AKİF ERSOY ÜNİVERSİTESİ VETERİNER FAKÜLTESİ

1339 5.09.2025

zoom Workplace

Toplantı

Hall-2 Esmâ Kayaoglu adlı kişinin

Giriş yapın

Kaydediliyor...

Görüntüle

4.Uluslararası Veteriner Biyokimya ve Klinik Biyokimya Kongresi
4-7 Eylül 2025 / Sivas, Türkiye





**GIDA BİYOKİMYASINDA FİTOKİMYASALLARIN
VE ANTİOKSİDANLARIN ÖNEMİ:
KROMATOĞRAFI TEKNİKLERİ KULLANILAN
ANALİTİK YAKLAŞIMLAR**

Dr. Öğr. Üyesi Ebubekir İZOL^{1,2}, Esmâ KAYAOĞLU^{3,4}

¹Bingöl Üniversitesi, Arı ve Doğal Ürünler Ar-Ge ve Ürün Geliştirme Uygulama ve Araştırma Merkezi, Bingöl, Türkiye
²Bingöl Üniversitesi, Doğa Bilimleri Enstitüsü, Arıcılık ve Arı Ürünleri Bölümü, Bingöl, Türkiye
³Bingöl Üniversitesi, Veteriner Fakültesi, Gıda Hijyeni ve Teknolojisi Bölümü, Bingöl, Türkiye
⁴Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara, Türkiye

Hall-2 Esmâ Kayaoglu

Observer hall 2

HALL-2 Songul

HALL-2 Songul ÇETİK YILDIZ

Hall-2 Adem Ünal

Windows'u Etkinleştir

Windows'u etkinleştirmek için Ayarlar'a gidin

Ses

Video

Katılımcılar

Sohbet

Tepki ver

Paylaş

Toplantı bilgileri

Uygulamalar

Kayıt duraklat/durdur

Ara odalar

Daha fazla

zoom Workplace

Toplantı - HALL 2

Giriş yapın

Kaydediliyor...

Görüntüle

Hiçbir şey duymuyor musunuz? [Ses düzeyini artır](#)

Observer hall 2

Hall-2 Esmâ Kayaoglu

Observer hall 2

semih yaşar

Hall-2 Adem Ünal

HALL-2 Songul ÇETİK YILDIZ

Online Session-1, Hall-2, Dr. Ahmet BUYUKBEN

Windows'u Etkinleştir

Windows'u etkinleştirmek için Ayarlar'a gidin

Ses

Video

Katılımcılar

Sohbet

Tepki ver

Paylaş

Toplantı bilgileri

Uygulamalar

Kayıt duraklat/durdur

Ara odalar

Daha fazla

zoom Workplace

Toplantı

semih yaşar adlı kişinin ekranı

Giriş yapın

Kaydediliyor...

Görüntüle



Hesperidin

Hesperidin'in kimyasal yapısı

Hesperidin, flavanon glikozid yapısında olup, hesperetin ve rutinose şekerinden oluşmaktadır

Observer hall 2

semih yaşar

Hall-2 Adem Ünal

HALL-2 Songul ÇETİK YILDIZ

Online Session-1, Hall-2, Dr. Ahmet BUYUKBEN

Hall-2 Esmâ Kayaoglu

Windows'u Etkinleştir

Windows'u etkinleştirmek için Ayarlar'a gidin

Ses

Video

Katılımcılar

Sohbet

Tepki ver

Paylaş

Toplantı bilgileri

Uygulamalar

Kayıt duraklat/durdur

Ara odalar

Daha fazla

zoom Workplace

H 2 Gheorghe Giurgiu adlı kişinin

Giriş yapın

Kaydediliyor...

Görüntüle

Deniplant

Remedi naturale alimentare

centrul medical aide sante

Laboratorul de analize medicale

Centrul de Biomedicina

MICROBIOTA MODULATION AS THERAPEUTIC APPROACH
IN THE NEUROPATHIC PAIN IN DOG WITH SPINAL CORD INJURY:
IMPACT OF POLENOPLASMIN

Major Gheorghe Giurgiu¹, Prof dr med Manole Cojocaru^{2,3}

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4th INTERNATIONAL CONGRESS OF VETERINARY BIOCHEMISTRY AND CLINICAL BIOCHEMISTRY
Sivas Cumhuriyet University, Sivas, Turkey, September 4-7, 2025

Observer hall

Observer hall 2

Tanzeela Riaz

Tanzeela Riaz

3 atanmamış katılımcı

Windows'u Etkinleştir

Windows'u etkinleştirmek için Ayarlar'a gidin

Ses

Video

Katılımcılar

Sohbet

Tepki ver

Paylaş

Toplantı bilgileri

Uygulamalar

Kaydı duraklat/durdur

Ara odalar

Daha fazla

TUR

zoom Workplace

Tanzeela Riaz adlı kişinin ekranı

Giriş yapın

Kaydediliyor...

Görüntüle

Multicomponent Reaction Approaches
for Potential Biologically Active
Thiophene Derivatives

Tanzeela Riaz
Government College University Faisalabad, Pakistan

Observer hall 2

Observer hall 2

Tanzeela Riaz

OMSAI REDDY

OMSAI REDDY

Windows'u Etkinleştir

Windows'u etkinleştirmek için Ayarlar'a gidin

Ses

Video

Katılımcılar

Sohbet

Tepki ver

Paylaş

Toplantı bilgileri

Uygulamalar

Kaydı duraklat/durdur

Ara odalar

Daha fazla

TUR

Observer hall-1

13:22
5.09.2025



GIDALARDA POLİSİKLIK AROMATİK HİDROKARBONLAR: KAYNAKLARI, MARUZİYET VE İNSAN SAĞLIĞINA ETKİLERİ

POLYCYCLIC AROMATIC HYDROCARBONS IN FOODS: SOURCES, EXPOSURE AND EFFECTS ON HUMAN HEALTH

İrem KOÇ¹, Seyda ŞAHİN²

¹Sivas Cumhuriyet Üniversitesi, Sağlık Bilimleri Enstitüsü, Gıda Hijyeni ve Teknolojisi Anabilim Dalı,
²Sivas Cumhuriyet Üniversitesi, Veteriner Fakültesi, Gıda Hijyeni ve Teknolojisi Anabilim Dalı, Sivas, Türkiye.

4. Uluslararası Veteriner Biyokimya ve Klinik Biyokimya Kongresi

4-7 Eylül 2025
Sivas, Türkiye

Katılımcılar (10)

Katılımcı	Statü
OH Observ... (Ortak oturum sahibi, ben)	✓
I İrem Akveran Koç	✓
B Büğra Şahin	✓
DS Dr. Songül Eken-HALL 1	✓
H1 Hall 1Yaren Ceyran	✓
HS Hall-1 Seyit Hasan ÖZTÜRK	✓
R Hall-1, Recep Bozkurt	✓
SD S2-H1 Dr. Ulku DURAN	✓
SD S2-Hall-1 Dr.Esen KULAÇ POLAT	✓
SH Session-2, Hall-1 Mert Pekcan	✓

SİKLOFOSFAMİD ETKİ MEKANİZMASI

- CP'nin biyoaktivasyonunda hepatik sitokrom P450 (CYP) enzimleri rol oynamaktadır. Bu enzimler CYP2A6, CYP2B6, CYP3A4, CYP3A5, CYP2C9, CYP2C18 ve CYP2C19'dur. Bunların içerisinde en büyük aktiviteye CYP2B6 ve CYP2C19 sahiptir.
- CP bu enzimlerin etkisiyle iki metabolit üretir. İlk metabolit fosforamid hardalı olarak bilinen bir azot hardalı bileşiktir. Bu bileşik DNA alkilleme aktivitesine sahip olduğu için terapötik olarak aktif metabolit olarak kabul edilir. Diğer metabolit 4-OHCP aktivasyonu esnasında oluşan akroleindir.
- Siklofosfamid metabolitlerinden fosforamid hardal antikanser özelliklere sahipken, akrolein apoptoz ve nekroz gibi sitotoksikite gösterir.
- Genel olarak siklofosfamidin etki mekanizması, hücre apoptozuna yol açan guanin N-7 pozisyonlarındaki iki DNA ipliği arasında veya içinde geri dönüşümsüz bağlanma yoluyla gerçekleşir.

Katılımcılar (11)

Katılımcı	Statü
OH Observ... (Ortak oturum sahibi, ben)	✓
R Hall-1, Recep Bozkurt	✓
B Büğra Şahin	✓
DS Dr. Songül Eken-HALL 1	✓
ES Esma Akşakal	✓
H1 Hall 1Yaren Ceyran	✓
HS Hall-1 Seyit Hasan ÖZTÜRK	✓
I İrem Akveran Koç	✓
SD S2-H1 Dr. Ulku DURAN	✓
SD S2-Hall-1 Dr.Esen KULAÇ POLAT	✓
SH Session-2, Hall-1 Mert Pekcan	✓

zoom Workplace Toplantı - HALL 4 Giriş yapın Kaydediliyor... Görüntüle

Observer Hall-4

Hall_4 archana...

Session1 Hall4 Feriel HENNAOUI Observer Hall-4 H4.Zainib Razzaq

Hall-4, Session 1, BENCHAGRA Lamiar saloni sharma Hall_4 archana, prasad 1 atanmamış katılımcı

Ses Video Katılımcılar 6 Sohbet Tepki ver Paylaş Toplantı bilgileri Uygulamalar Kaydı duraklat/durdur Ara odalar Daha fazla Odadan çık

28°C Kışın güneşi Web'de &... 12:41 5.09.2025

zoom Workplace Toplantı - HALL 4 Giriş yapın Kaydediliyor... Görüntüle

Observer Hall-4

Soreja Mithila Sen (ses...

H4-Tabarek Observer Hall-4 kave koorehpaz s2h4

Soreja Mithila Sen (session-2, Hall-4) 3 atanmamış katılımcı

Ses Video Katılımcılar 5 Sohbet Tepki ver Paylaş Toplantı bilgileri Uygulamalar Kaydı duraklat/durdur Ara odalar Daha fazla Odadan çık

28°C Güneşli Web'de &... 14:59 5.09.2025

zoom Workplace Toplantı - HALL 5 Giriş yapın Kaydediliyor... Görüntüle

Observer Hall-5

Abdullahi Yahaya

Hall-5 Shirinboyeva Dilrabo Komilovna Observer Hall-5 Dr. Emmanuel Anaso Dr. M.B. BUSARI Abdullahi Yahaya

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AGED GARLIC EXTRACT AND INHIBITION OF PLATELET AGGREGATION, THE INSIDE STORY!

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ABSTRACT

Increased platelet aggregation is strongly associated with cardiovascular disease (CD). Platelet aggregation involves signalling molecules such as protein kinase C, cyclic adenosine monophosphate (cAMP), cyclic guanine monophosphate (cGMP) and nitric oxide (NO). Aged garlic extract (AGE) inhibits platelet aggregation both *in vitro* and *in vivo*, however the mechanisms remain unknown. In this study we have investigated the mechanisms by which AGE inhibits platelet aggregation. Human platelets were stimulated with ADP in the presence of either 3-morpholinosydnonimine (Sin-1) or sodium nitroprusside (SNP) both nitric oxide (NO) donors. In both cases inhibition of platelet aggregation was observed with nitric oxide (NO) being identified as the reactive species. When AGE was combined with Sin-1 and SNP, a much stronger inhibitory response was observed. The inhibitory effect of nitric oxide is linked to an increase in intracellular cGMP. Subsequent experiments using specific inhibitors targeting soluble guanylyl cyclase and phosphodiesterase activity were undertaken and demonstrated that NO donors were largely responsible for the increase in cGMP within the platelets, whereas AGE is largely responsible for an increase in intracellular cAMP. This explains why a synergistic inhibitory effect is observed when AGE is combined with either NO donor. AGE also inhibited platelet aggregation *in vivo* via an increase in cAMP, furthermore, AGE also inhibited the binding of activated platelets to fibrinogen and prevented the shape change in ADP-induced platelet aggregation. This work indicates that AGE may have a therapeutic role in reducing the risk of thrombotic episodes in individuals with advanced CD and exerts its effects via multiple pathways. Such studies are important for the scientific validation of nutraceuticals for their use in the prevention of chronic diseases.

CALIXARENE AS A NEW APPROACH FOR GLOBAL HEALTH

Prof. Dr. Omar M. ATROOZ

Mutah University & Al-Ahliyya Amman University

ABSTRACT

Calixarenes have emerged as powerful tools in both environmental and pharmaceutical applications due to their unique molecular recognition properties. Their ability to form stable complexes with biomolecules makes them highly effective in selective extraction and drug delivery systems. In environmental analysis, calixarene-based techniques facilitate the monitoring and remediation of pollutants, contributing to sustainable practices and ecological protection. These adaptable systems are also pivotal in isolating bioactive natural products, supporting efficient drug discovery. Hence, their continued exploration reinforces their vital role in advancing both environmental sustainability and biomedical research.

Keywords: Calixarene, Biomedical Application, Natural Products, Calixarene Derivatives

CELL BIOLOGY OF GALECTIN-3

Prof. Dr. Ralf JACOB

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ABSTRACT

In the galectin family, a group of lectins is united by their evolutionarily conserved carbohydrate recognition domains. These polypeptides play a role in various cellular processes and are implicated in disease mechanisms such as cancer, diabetes, fibrosis, infection and inflammation. Following synthesis in the cytosol, manifold interactions of galectins have been described both intra- and extracellularly. We found that galectin-3 is secreted by recruitment into multivesicular bodies through direct interaction with the ESCRT-I component Tsg101. This direct interaction depends on a highly conserved P(S/T)AP late domain motif in the amino-terminal region of galectin-3. Specific knockdown of Tsg101 or mutation of the late domain motif prevents galectin-3 release. Following fusion with the plasma membrane multivesicular bodies release their cargo enriched within extracellular vesicles. These vesicles provide excellent communication platforms between cells or even organs circulating in a living organism.

EPİGENETİK VE D VİTAMİNİN EPİGENETİK ETKİLERİ

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ÖZET

D vitamini, kalsiyum homeostazı ve kemik metabolizmasının çok ötesinde pleiotropik etkiler gösteren, bağışıklık tepkilerini, kardiyovasküler sağlığı ve kanser de dahil olmak üzere çeşitli kronik hastalık riskini etkileyen bir sekosteroid hormondur. Güneş ışığına maruz kalma, mevsim, enlem, diyet, yaş, cinsiyet ve vücut kitle indeksi gibi çevresel belirleyicilerin iyi bilinen rolüne rağmen, D vitamini düzeylerindeki bireyler arası değişkenlik, alta yatan genetik ve epigenetik faktörler dikkate alınmadan tam olarak açıklanamaz. Epigenetik, DNA dizisinde değişiklik olmaksızın meydana gelen, mitoz ve/veya mayoz yoluyla iletilen gen ifadesinde veya hücre fenotipinde kalıtsal değişiklikleri ifade eder ve DNA metilasyonu, histon modifikasyonları ve kodlamayan RNA'ları kapsar. Bu derlemede, D vitamini metabolizmasının altında yatan genetik ve epigenetik mekanizmalar, bu faktörlerin dolaşımdaki D vitamini konsantrasyonlarını bireysel düzeyde nasıl etkilediğini açıklığa kavuşturmak için kapsamlı bir şekilde incelenmiştir. İkiz çalışmaları ve genom çapında ilişki çalışmalarından elde edilen kanıtlar, D vitamini durumunun güçlü bir şekilde kalıtsal olduğunu göstermektedir; tahminler %29 ile %86 arasında değişmektedir. GC, CYP2R1, CYP24A1, CYP27B1, VDR ve DHCR7 gibi genler, sentez, aktivasyon, taşıma ve katabolizmayı etkileyen D vitamini metabolizmasının temel düzenleyicileri olarak tanınmaktadır. Daha da önemlisi, GC ve VDR genlerindeki tek nükleotid polimorfizmleri (SNP'ler) (örn. rs2282679, rs1155563, rs731236 ve rs7116978) dolaşımdaki 25-hidroksivitamin D düzeylerinin modülatörleri ve D vitamini takviyesine verilen farklı yanıtların öngörücüleri olarak tanımlanmıştır. Epigenetik mekanizmalar başka bir düzenleme katmanı ekler; Örneğin, CYP2R1 ve CYP24A1'in promotör bölgelerindeki DNA metilasyonu serum 25(OH)D ile ters orantılıyken, VDR metilasyonu D vitamini durumu ile pozitif korelasyon gösterebilir ve bu da D vitamini ile epigenom arasındaki çift yönlü etkileşimi vurgular. Bu bulgular bir araya geldiğinde, D vitamini eksikliğinin yalnızca yetersiz alım veya sınırlı güneş ışığına maruz kalmanın bir sonucu olmadığını, aynı zamanda karmaşık genetik ve epigenetik etkileşimlerin de bir sonucu olduğunu vurgulamaktadır. Gelecekteki araştırmalar, kişiselleştirilmiş takviye stratejilerinin geliştirilmesine bilgi sağlamak için genetik varyasyonun, çevresel maruziyetlerin ve epigenetik modifikasyonların D vitamini metabolizması üzerindeki birleşik etkisini araştırmalıdır. Genetik ve epigenetik taramanın klinik pratiğe entegre edilmesi, nihayetinde D vitamini eksikliğine ve ilişkili hastalıklara karşı daha etkili müdahalelere olanak tanıyabilir ve hassas beslenme ve kişiye özel sağlık hizmeti yaklaşımları için bir temel sağlayabilir.

Anahtar Kelimeler: Epigenetik, D vitamini metabolizması, genetik varyasyon

EPIGENETICS AND THE EPIGENETIC EFFECTS OF VITAMIN D

ABSTRACT

Vitamin D is a secosteroid hormone that exerts pleiotropic effects far beyond calcium homeostasis and bone metabolism, influencing immune responses, cardiovascular health, and the risk of several chronic diseases including cancer. Despite the well-established role of environmental determinants such as sun exposure, season, latitude, diet, age, sex, and body mass index, interindividual variability in vitamin D levels cannot be fully explained without considering underlying genetic and epigenetic factors. Epigenetics refers to heritable changes in gene expression or cellular phenotype that occur without alterations in the DNA sequence, transmitted through mitosis and/or meiosis, and it encompasses DNA methylation, histone modifications, and non-coding RNAs. In this review, the genetic and epigenetic mechanisms underlying vitamin D metabolism were comprehensively examined to clarify how these factors influence circulating vitamin D concentrations at the individual level. Evidence from twin studies and genome-wide association studies indicates that vitamin D status is strongly heritable, with estimates ranging from 29% to 86%. Genes such as GC, CYP2R1, CYP24A1, CYP27B1, VDR, and DHCR7 are recognized as key regulators of vitamin D metabolism, affecting synthesis, activation, transport, and catabolism. Importantly, single nucleotide polymorphisms (SNPs) in the GC and VDR genes (e.g., rs2282679, rs1155563, rs731236, and rs7116978) have been identified as modulators of circulating 25-hydroxyvitamin D levels and predictors of differential responses to vitamin D supplementation. Epigenetic mechanisms add another layer of regulation; for example, DNA methylation in the promoter regions of CYP2R1 and CYP24A1 is inversely associated with serum 25(OH)D, while VDR methylation may positively correlate with vitamin D status, highlighting the bidirectional interaction between vitamin D and the epigenome. Together, these findings emphasize that vitamin D deficiency is not solely a consequence of insufficient intake or limited sun exposure, but also the result of complex genetic and epigenetic interactions. Future research should investigate the combined influence of genetic variation, environmental exposures, and epigenetic modifications on vitamin D metabolism to inform the development of personalized supplementation strategies. Integrating genetic and epigenetic screening into clinical practice could ultimately enable more effective interventions against vitamin D deficiency and its associated diseases, providing a foundation for precision nutrition and individualized healthcare approaches.

Keywords: Keywords: Epigenetics, vitamin D metabolism, genetic variation

1. GİRİŞ

Epigenetik terimi ilk olarak 1940'larda Conrad Waddington tarafından ortaya atılmış ve “genler ile gen ürünleri arasındaki nedensel etkileşimleri inceleyen ve bu etkileşimlerin fenotipi nasıl ortaya çıkardığını açıklayan biyoloji dalı” olarak tanımlanmıştır. Bu dönemde epigenetik, genotipin belirli bir fenotipe dönüşümünü sağlayan çeşitli moleküler mekanizmaları kapsayan kapsamlı bir kavram olarak ele alınmaktaydı.

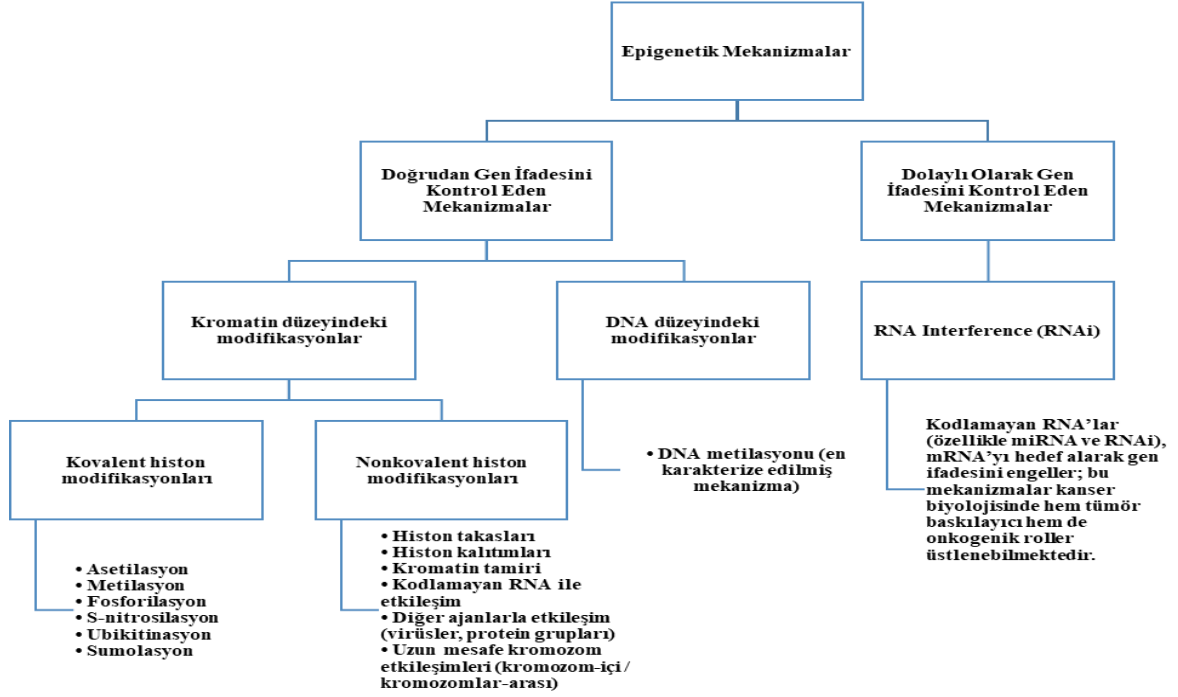
Bununla birlikte, genetik ve moleküler biyolojideki hızlı gelişmeler sonucunda terimin kapsamı giderek daralmış; geniş orijinal anlamından uzaklaşarak, DNA dizisinde herhangi bir değişiklik olmaksızın ortaya çıkan gen ifadesindeki kalıtsal değişikliklere odaklanan daha spesifik bir tanımlamaya dönüşmüştür.¹

Gen ifadesinden kaynaklanan kalıtsal bilgiler, ‘epigenetik’ olarak tanımlanır. Genetik bilginin aksine epigenetik, doğrudan gen dizisiyle ilişkili değildir. Özetle epigenetik, DNA dizisinde herhangi bir değişiklik olmaksızın, hücre bölünmesi (mitoz ve/veya mayoz) süresince bir nesilden diğerine aktarılabilen gen ekspresyonu ya da hücrel fenotip değişikliklerini ifade eder. Genetik ve epigenetik arasındaki fark, bir kitabın yazılması ve okunması arasındaki ilişkiye benzetilebilir. Kitap aynı kalmasına rağmen, farklı okuyucular farklı yorumlar geliştirir ve farklı duygular hisseder. Benzer şekilde, epigenetik de sabit bir şablonun (yani kitabın veya genetik kodun) değişen koşullar altında farklı çıktılarla yorumlanmasını sağlamaktadır.² Benzer şekilde DNA, sabit genetik bilgiyi taşıyan bir nota dizisi gibi düşünülebilirken, epigenetik bu bilginin nasıl ‘okunup’ yorumlandığını belirleyen düzenleyici süreçleri temsil etmektedir; bu durum, bir tarifi farklı aşçılarca çevresel koşullara göre farklı şekillerde pişirilmesi, genlerin bir dimmer anahtarı gibi ayarlanabilmesi ya da Waddington’un peyzaj metaforunda hücrel kaderin yönünü tayin etmesiyle örneklendirilmektedir.³ Epigenetik değişikliklerin, spesifik genlerin baskılanmasıyla karakterize edilen gen susturmayla sonuçlandığı gösterilmiştir. Benzer biçimde, genetik mutasyonlar veya delesyonlar da genin işlevsel inaktivasyonuna eşdeğer kabul edilmektedir. Bu süreç içerisinde erişkinlerde hücre yenilenmesi, X kromozom inaktivasyonu gibi olaylar gerçekleşmektedir. Eğer bu süreç bozulursa kanser, otoimmün hastalıklar ve nörolojik hastalıkların ortaya çıkması söz konusudur. Ancak epigenetik değişimler geri dönüşümlü oluşları ve DNA’nın baz dizisinde bir değişime neden olmamaları gibi özellikleriyle genetik değişimlerden ayrılırlar.

¹ Dupont, C., Armant, D., & Brenner, C. (2009). Epigenetics: Definition, Mechanisms and Clinical perspective. *Seminars in Reproductive Medicine*, 27(05), 351–357. <https://doi.org/10.1055/s-0029-1237423>

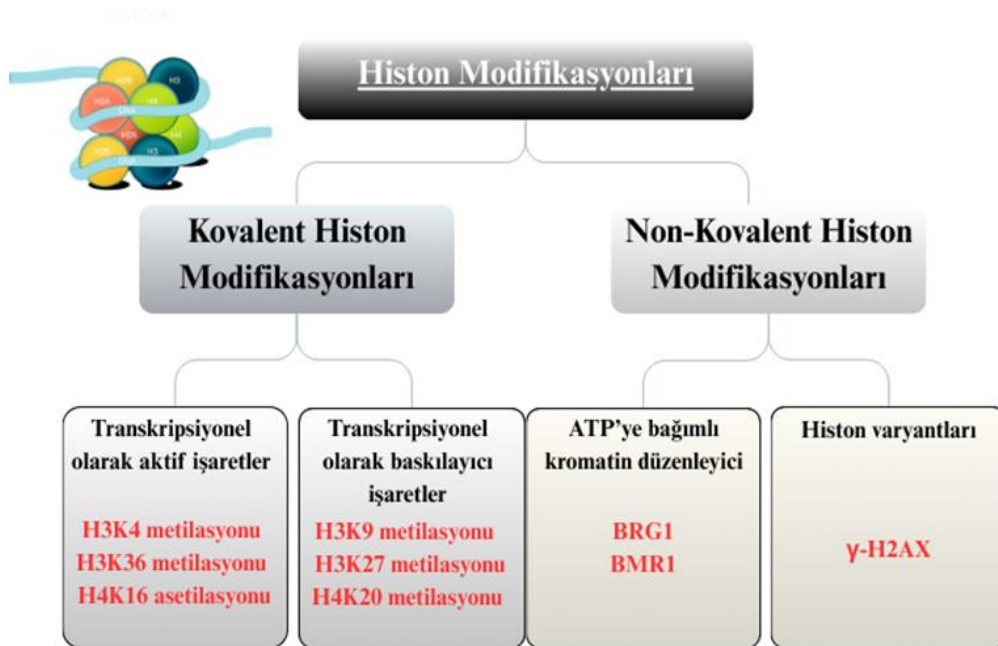
² Thomas Jenuwein: Histone lysine methylation and a new era in epigenetics,” *Research Institute of Molecular Pathology*, Vienna, IMP, erişim tarihi: 29.08.2025

³ Raz, A., Pontarotti, G., & Weitzman, J. B. (2019). Epigenetic metaphors: an interdisciplinary translation of encoding and decoding. *New Genetics and Society*, 38(3), 264–288. <https://doi.org/10.1080/14636778.2019.1601009> <



I. Kromatin Düzeyindeki Modifikasyonlar

Modifikasyonlar, bağ oluşumunun spesifik doğasına bağlı olarak kovalent veya kovalent olmayan olarak sınıflandırılabilir. Kromatin seviyesindeki modifikasyonlar, bağ oluşumunun spesifik doğasına bağlı olarak kovalent veya kovalent olmayan olarak sınıflandırılabilir.



IA) Kovalent Histon Modifikasyonları

Kovalent histon modifikasyonları, kromatin yapısını ve DNA erişilebilirliğini kritik bir şekilde düzenleyen histon proteinlerinde transkripsiyon sonrası oluşan kimyasal değişikliklerdir.⁴ Bu modifikasyonlar çoğunlukla histon kuyruklarının lizin ve arginin kalıntıları üzerinde meydana gelmekte ve asetilasyon, metilasyon, ubiquitinasyon, glikasyon, asilasyon ve lipidasyonu içermektedir.⁵ Bu modifikasyonlar, nükleozom sıkışmasını doğrudan değiştirebilmekte veya efektör proteinleri harekete geçirmek için moleküler sinyaller olarak görev yaparak transkripsiyonu, replikasyonu ve DNA onarımını etkileyebilmektedir. Birçok işaret, özelleşmiş "yazıcı" ve "silici" enzimler tarafından biriktirilip kaldırılırken, metabolik yan ürünler tarafından yönlendirilen enzimatik olmayan kovalent modifikasyonlar da histon modifikasyon profillerini şekillendirmektedir.⁵ Histon-kod hipotezine göre, bu modifikasyonların kombinasyonel örüntüsü, gen ifadesi sonuçlarını belirleyen düzenleyici bir dil oluşturmaktadır. Histon modifikasyonlarının hatalı yazılması, yanlış yorumlanması veya uygunsuz şekilde silinmesi, onkogeneze ve çeşitli patolojik süreçlerin gelişiminde önemli rol oynamaktadır.⁴

IB) Non-Kovalent Histon Modifikasyonları

Non-kovalent histon modifikasyonları veya kovalent olmayan histon modifikasyonları, histon amino asitlerinin kimyasal modifikasyonu olmaksızın nükleozom kompozisyonunu, konumunu veya etkileşimlerini değiştiren kromatin düzenleyici mekanizmaları ifade etmektedir. Bu mekanizmalar arasında, kanonik histonların yerini alan ve böylece kromatin stabilitesini, erişilebilirliğini ve transkripsiyonel dinamiklerini düzenleyen histon varyantlarının (örneğin, H2A.Z, H3.3, macroH2A) dahil edilmesi yer almaktadır.⁶ Örneğin, histon varyantı H2A.Z'nin transkripsiyon başlangıç bölgelerinin yakınındaki +1 nükleozomunda yer aldığı ve burada kromatini transkripsiyonel aktivasyon için hazırlayabildiği veya heterokromatin sınırlarını etkileyebildiği gösterilmiştir.⁷ Bir diğer önemli kovalent olmayan mekanizma ise ATP'ye bağımlı kromatin yeniden şekillenmesidir; burada çoklu alt birim yeniden şekillenme kompleksleri, düzenleyici DNA elemanlarının erişilebilirliğini artırmak veya engellemek amacıyla nükleozomların yeniden konumlandırılmasını sağlamak ve böylece transkripsiyon faktörlerinin ve RNA polimerazın erişimini kontrol etmektedir.⁶ Ek olarak, histon-protein ve histon-DNA etkileşimleri kovalent olmayan düzenlemeye katkıda bulunmaktadır. Histon şaperonları nükleozom montaj ve demontajını yönlendirirken, efektör proteinler ("okuyucular") veya uzun kodlamayan RNA'lar histon kimyasını değiştirmeden kromatin durumlarını düzenlemek için histonlara bağlanmaktadır. Pozitif yüklü histon kuyrukları ile negatif yüklü DNA arasındaki elektrostatik etkileşimler de kromatin yoğunlaşmasını ve erişilebilirliğini düzenleyen temel bir kovalent olmayan mekanizmayı temsil etmektedir.⁸

⁴ Chi, P., Allis, C. D., & Wang, G. G. (2010). Covalent histone modifications: Miswritten, misinterpreted and misread in human cancers. *Nature Reviews Cancer*, 10(7), 457–469. <https://doi.org/10.1038/nrc2876>

⁵ Maksimovic, I., & David, Y. (2021). Non-enzymatic covalent modifications as a new chapter in the histone code. *Trends in Biochemical Sciences*, 46(9), 718–730. <https://doi.org/10.1016/j.tibs.2021.04.004>

⁶ Talbert, P. B., & Henikoff, S. (2017). Histone variants on the move: Substrates for chromatin dynamics. *Nature Reviews Molecular Cell Biology*, 18(2), 115–126. <https://doi.org/10.1038/nrm.2017.26>

⁷ Atanasoff-Kardjaleff, A. K., Berger, H., Steinert, K., Janevska, S., Ponts, N., Humpf, H.-U., Kalinina, S., & Studt-Reinhold, L. (2024). Incorporation of the histone variant H2A.Z counteracts gene silencing mediated by H3K27 trimethylation in *Fusarium fujikuroi*. *Epigenetics & Chromatin*, 17(7). <https://doi.org/10.1186/s13072-024-00532-y>

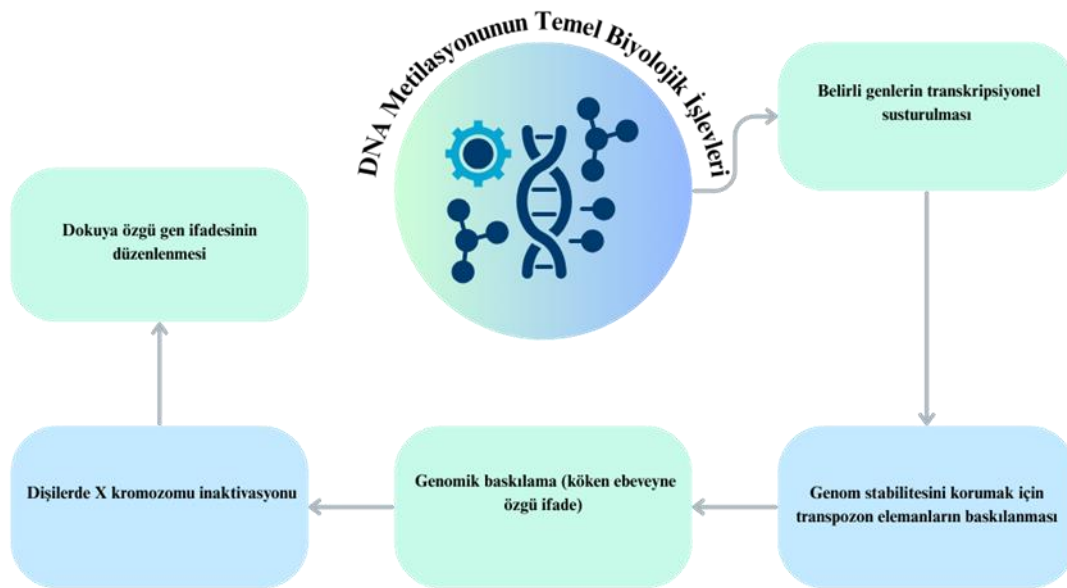
⁸ Zhou, H.-X., & Pang, X. (2018). Electrostatic interactions in protein structure, folding, binding, and condensation. *Chemical Reviews*, 118(4), 1691–1741. <https://doi.org/10.1021/acs.chemrev.7b00305>

II. DNA Düzeyindeki Modifikasyonlar

DNA metilasyonu, DNA düzeyinde en kapsamlı şekilde incelenen ve işlevsel açıdan en önemli modifikasyondur. Altta yatan nükleotid dizisini değiştirmeden tersine çevrilebilen kalıtsal bir kimyasal değişikliktir ve bu nedenle epigenetik bir mekanizma olarak sınıflandırılmaktadır. Omurgalılarda DNA metilasyonu, öncelikle DNA metiltransferazlar (DNMT'ler) tarafından katalize edilen CpG dinükleotidleri içindeki sitozin kalıntılarına bir metil grubunun eklenmesiyle gerçekleşir. Metil grubu, S-adenosil metioninden (SAM) sitozinin pirimidin halkasının beşinci karbonuna aktarılır ve 5-metilsitozin (5mC) üretilir.⁹

İşlevsel Sonuçlar

5mC'nin varlığı, transkripsiyonel baskılama ile güçlü bir şekilde ilişkilidir. Gen promotörlerindeki, özellikle CpG adalarındaki metilasyon, mitoz yoluyla gen susturulmasının istikrarlı bir biçimde devamını sağlamaktadır. Yetişkin somatik dokularda, DNA metilasyonu ağırlıklı olarak CpG bölgeleriyle sınırlıyken, CpG dışı metilasyon (örneğin, CpA, CpT, CpC) embriyonik kök hücrelerde zenginleştirilmiştir. İnsan genomundaki tüm CpG bölgelerinin yaklaşık %70-80'i metillenmiştir.



⁹ Li, T., Vu, T. H., Ulaner, G. A., Littman, E., Ling, J. Q., Chen, H. L., Hu, J. F., Behr, B., Giudice, L., & Hoffman, A. R. (2005). IVF results in de novo DNA methylation and histone methylation at an Igf2-H19 imprinting epigenetic switch. *Molecular Human Reproduction*, 11(9), 631–640. <https://doi.org/10.1093/molehr/gah230>

DNA Metilasyonu ve Kanser

Anormal DNA metilasyonu, kanser gelişiminin bir işaretidir. Bu durumda iki ana tip değişiklik gözlemlenir:

1. **Genomik hipermetilasyon**, genellikle tümör baskılayıcı genlerin promotör CpG adalarını etkiler ve bunların sessizleşmesine yol açar.
2. **Genomik hipometilasyon**, genellikle tekrarlayan DNA dizilerinde meydana gelir ve genomik instabiliteye ve onkogen aktivasyonuna katkıda bulunur.⁹

DOLAYLI OLARAK GEN İFADESİNİ ETKİLEYEN MEKANİZMALAR

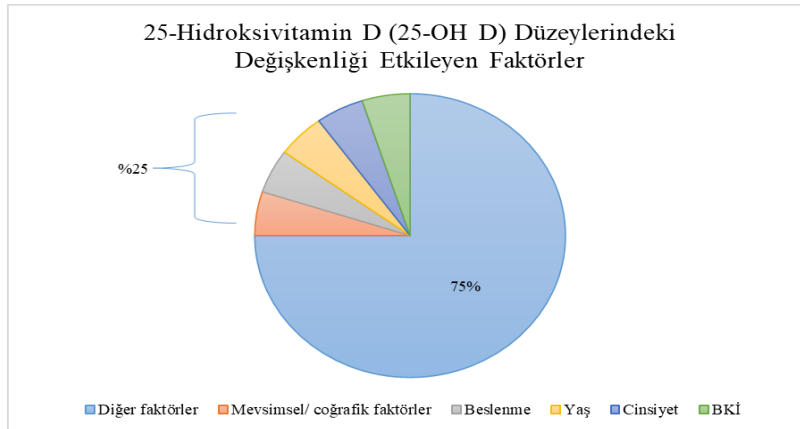
Post-transkripsiyonel Mekanizmalar

RNA interferansı (RNAi), transkripsiyon tamamlandıktan sonra (yani DNA'dan RNA sentezinin gerçekleşmesinden sonra) işlev gören mekanizmalardan biridir. Özellikle kodlamayan RNA'ların (örn. RNAi) kodlayıcı RNA'yı (mRNA) hedef alarak protein sentezini baskılaması bu süreçte kritik rol oynamaktadır. RNA interferansı, çift zincirli RNA (double-stranded RNA; dsRNA) aracılığıyla başlatılan, diziye özgü bir post-transkripsiyonel gen susturma mekanizmasıdır. Yapılan araştırmalar, *mikroRNA (miRNA)* genlerinin %50'den fazlasının kanserle ilişkili genomik bölgelerde veya fragil sitelerde lokalize olduğunu göstermektedir. Bu moleküller, bağlama ve hedefleme özelliklerine bağlı olarak iki yönlü rol üstlenebilir:

1. Tümör baskılayıcı işlev görerek onkogenlerin ekspresyonunu inhibe edebilirler.
2. Onkogen gibi davranarak tümör baskılayıcı genleri susturabilirler.¹⁰

D VİTAMİNİ EKSİKLİĞİNDE GENETİK FAKTÖRLERİN ROLÜ

Genler, direncimiz ve çoğu hastalığa yatkınlığımızı belirleyen güçlü ve zayıf biyolojik faktörleri oluşturmaktadır. Bu sebeple genetiğimizin D vitaminin işlenmesindeki rolünü anlamak önemlidir. D vitamini eksikliğinin etiyolojisi doğada genetikdir. Gözlenen yüksek kalıtım derecesi, genetik faktörlerin süreçteki potansiyel rolünü ortaya koymaktadır. Tahmini kalıtım oranının %53'e varabileceği düşünülmektedir. D vitamini durumunun yaygın olarak tanınan bir biyobelirteci olan 25-hidroksivitamin D'nin (25-OH D) serum seviyeleri, yaklaşık% 25'i tanımlanabilir faktörlere atfedilebilen önemli bireyler arası değişkenlik sergilemektedir.



¹⁰ Qian, Y., Liu, C., Zeng, X., & Li, L. C. (2025). RNAa: Mechanisms, therapeutic potential, and clinical progress. *Molecular Therapy – Nucleic Acids*, 36(2), 102494. <https://doi.org/10.1016/j.omtn.2025.102494>

Bununla birlikte, genetik yapı kritik bir faktör olmaya devam etmektedir. İkiz ve bağlantı çalışmaları, 25(OH)D düzeyleri için %28 ile %80 arasında değişen kalıtım tahminleri bildirmiş ve genetik etkinin önemli olduğunu vurgulamıştır. Uluslararası araştırma konsorsiyumları tarafından yürütülen büyük, çok merkezli genom çapında ilişki çalışmaları (GWAS), gelişmiş ülkelerdeki sağlıklı yetişkinlerin neredeyse yarısını etkileyen bir durum olan D vitamini eksikliği ile ilişkili dört genetik varyant tanımlamıştır. Bu varyantlar sadece kas-iskelet sağlığının bozulmasına katkıda bulunmakla kalmamakta, aynı zamanda diyabet, kardiyovasküler hastalıklar ve birkaç yaygın kanser türünün riskini de artırabilmektedir.^{11,12}

Vitamin D Metabolizmasında Genetik Varyantların Önemi

Dolaşımdaki 25-hidroksivitamin D [25(OH)D] düzeylerini etkileyen yaygın genetik varyantlar, vitamin D eksikliği riski taşıyan bireyleri belirlemek ve vitamin D durumu ile bir dizi hastalık arasında gözlemlenen ilişkileri açıklığa kavuşturmak için önemlidir. En sık bildirilen varyantlar GC, CYP2R1, VDR, DHCR7 ve CYP24A1 genlerinde bulunmaktadır.^{11,12,13}

Spesifik Genler Tarafından Kodlanan Proteinler

- DHCR7 geni tarafından kodlanan 7-dehidrokolesterol redüktaz (DHCR7), 7-dehidrokolesterolün kolesterole dönüşümünü katalize etmektedir.
- CYP2R1 geni tarafından kodlanan sitokrom P450 2R1 (CYP2R1), vitamin D'nin ilk aktivasyon aşamasını temsil eden 25-hidroksivitamin D'nin oluşumunu katalize etmektedir.
- CYP27B1 geni tarafından kodlanan sitokrom P450 27B1 (CYP27B1, 1-alfa-hidroksilaz olarak da bilinir), aktif formdaki vitamin D olan 1,25-dihidroksivitamin D₃'ün böbreklerde sentezini katalize etmektedir.
- CYP24A1 geni tarafından kodlanan sitokrom P450 ailesi 24 alt ailesi A üyesi 1 (CYP24A1), aktif vitamin D metabolitlerini inaktive eden hidroksilasyon reaksiyonlarını katalize ederek vitamin D katabolizmasını düzenlemektedir.
- GC geni tarafından kodlanan vitamin D bağlayıcı protein (DBP), vitamin D metabolitlerini deri, karaciğer ve böbrek arasında taşır ve ardından bunları hedef dokulara ulaştırmaktadır.
- VDR geni tarafından kodlanan vitamin D reseptörü (VDR), bağırsak ve böbrekte kalsiyum ve mineral homeostazında rol oynayan genlerin transkripsiyonunu

D Vitamini Durumu ile İlişkili Tek Nükleotid Polimorfizmleri (SNP'ler)

Vitamin D durumundaki değişkenliğin %50'ye kadarı genetik polimorfizmlerden kaynaklanabilmektedir.

Önemli SNP'ler şunlardır:

GC: rs2282679, rs4588, rs1155563, rs7041

¹¹ Karcioğlu Batur, L., Özaydın, A., Maviş, M. E., Gürsu, G. G., Harbige, L., & Hekim, N. (2021). Vitamin-D binding protein gene polymorphisms and serum 25-hydroxyvitamin-D in a Turkish population. *Metabolites*, 11(10), 696. <https://doi.org/10.3390/metabo11100696>

¹² Wang, T. J., Zhang, F., Richards, J. B., Kestenbaum, B., van Meurs, J. B., Berry, D., Kiel, D., Streeten, E. A., Ohlsson, C., Koller, D. L., & others. (2010). Common genetic determinants of vitamin D insufficiency: A genome-wide association study. *The Lancet*, 376(9736), 180–188. [https://doi.org/10.1016/S0140-6736\(10\)60588-0](https://doi.org/10.1016/S0140-6736(10)60588-0)

¹³ Tomei, S., Singh, P., Mathew, R., Mattei, V., Garand, M., Alwakeel, M., Sharif, E., & Al Khodor, S. (2020). The role of polymorphisms in vitamin D-related genes in response to vitamin D supplementation. *Nutrients*, 12(9), 2608. <https://doi.org/10.3390/nu12092608>

CYP2R1: rs10741657, rs10766197, rs2060793

VDR: rs7975232, rs2228570, rs1544410, rs731236^{11,13}

Bu SNP'ler kesin bir öngörü değeri sağlamasa da, dolaşımdaki 25(OH)D'deki farklılıklarla ilişkilidir. Örneğin, GC rs4588 polimorfizmi, Türk popülasyonunda 25(OH)D'nin biyoyararlanımıyla bağlantılıdır. Dolaşımdaki D vitamini, bağlı ve serbest formlarda bulunmaktadır. 25(OH)D'nin yaklaşık %85-90'ı DBP'ye, %10-15'i albümine bağlanmakta ve sadece ~%1'i serbest formda dolaşıma katılmaktadır.^{14,15} Albümine bağlı ve serbest 25(OH)D'nin toplamı "biyoyararlanabilir vitamin D" olarak adlandırılmaktadır. DBP'yi kodlayan GC geni de dahil olmak üzere, vitamin D sentezi, taşınması ve aktivasyonundan sorumlu proteinlerdeki genetik varyasyon, serum 25(OH)D konsantrasyonları ile tutarlı bir şekilde ilişkilendirilmiştir.^{16, 17, 18}

DBP Fenotipleri ve Genetik Varyantlar

GC SNP'leri rs7041 ve rs4588'in kombinasyonları, 25(OH)D'ye farklı afiniteleri olan üç ana DBP fenotipik grubunu tanımlamaktadır: Gc1s, Gc1f ve Gc2¹⁹

Özellikle:

Gc1f (1f): rs7041 (T) + rs4588 (C)

Gc1s (1s): rs7041 (G) + rs4588 (C)

Gc2 (2): rs7041 (T) + rs4588 (A)

Bununla birlikte, GC geni diploid olduğundan, altı olası DBP fenotipi tanımlanabilmektedir: Gc1f-1s, Gc1f-1f, Gc1f-2, Gc1s-2, Gc1s-1s ve Gc2-2.

¹⁴ Powe, C. E., Ricciardi, C., Berg, A. H., Erdenesanaa, D., Collerone, G., Ankers, E., Wenger, J., Karumanchi, S. A., Thadhani, R., & Bhan, I. (2011). Vitamin D–binding protein modifies the vitamin D–bone mineral density relationship. *Journal of Bone and Mineral Research*, 26(7), 1609–1616. <https://doi.org/10.1002/jbmr.387>

¹⁵ Bikle, D. D., & Schwartz, J. (2019). Vitamin D binding protein, total and free vitamin D levels in different physiological and pathophysiological conditions. *Frontiers in Endocrinology*, 10, 317. <https://doi.org/10.3389/fendo.2019.00317>

¹⁶ Berry, D., & Hyppönen, E. (2011). Determinants of vitamin D status: Focus on genetic variations. *Current Opinion in Nephrology and Hypertension*, 20(4), 331–336. <https://doi.org/10.1097/MNH.0b013e328346d6ba>

¹⁷ Wang, T. J., Zhang, F., Richards, J. B., Kestenbaum, B., van Meurs, J. B., Berry, D., Kiel, D., Streeten, E. A., Ohlsson, C., Koller, D. L., & others. (2010). Common genetic determinants of vitamin D insufficiency: A genome-wide association study. *The Lancet*, 376(9736), 180–188. [https://doi.org/10.1016/S0140-6736\(10\)60588-0](https://doi.org/10.1016/S0140-6736(10)60588-0)

¹⁸ Al-Daghri, N. M., Mohammed, A. K., Bukhari, I., Rikli, M., Abdi, S., Ansari, M. G. A., Sabico, S., Hussain, S. D., Alenad, A., Al-Saleh, Y., & others. (2019). Efficacy of vitamin D supplementation according to vitamin D-binding protein polymorphisms. *Nutrition*, 63–64, 148–154. <https://doi.org/10.1016/j.nut.2019.02.003>

¹⁹ Chun, R., Percy, B. E., Orwoll, E. S., Nielson, C. M., Adams, J. S., & Hewison, M. (2014). Vitamin D and DBP: The free hormone hypothesis revisited. *Journal of Steroid Biochemistry and Molecular Biology*, 144, 132–137. <https://doi.org/10.1016/j.jsbmb.2013.09.012>

D Vitamini Eksikliği ve Yaygınlığı

Düşük D vitamini alımı ve/veya takviye eksikliği, D vitamini eksikliği riskinin 2 ila 5 kat artmasıyla ilişkilidir (<20 ng/mL). Bildirilen yaygınlık, kullanılan eşik değerine göre değişmektedir (<20 ng/mL ile ≤30 ng/mL). Tıp Enstitüsü (IOM), neredeyse tüm bireylerde optimal kemik sağlığı için en az 20 ng/mL serum konsantrasyonunun gerekli olduğu sonucuna varmıştır.²⁰ D vitamini eksikliğinin dünya çapında yaygın olduğu bildirilmektedir; ancak, Türk yetişkinlerin durumu hakkındaki veriler sınırlıdır. Türkiye'de kıyı şeridi dışında, kentsel bir ortamda yaşayan yetişkinlerde yapılan bir çalışmada, eksikliğin yaygınlığı yüksek olarak bildirilmiştir. Bu çalışmanın amacı, sağlıklı Türk yetişkinlerdeki düşük serum 25(OH)D konsantrasyonlarının, DBP'yi kodlayan GC gen polimorfizmleriyle açıklanıp açıklanamayacağını araştırmak olarak belirlenmiştir. Araştırmacılar, serbest 25(OH)D'nin doğrudan ölçülmesinin, Türk popülasyonundaki GC polimorfizmlerinin dağılımı ile birlikte değerlendirildiğinde daha doğru prevalans tahminleri sağlayabileceği hipotezini ortaya atmışlardır. İncelenen Türk popülasyonunda, rs7041'de en yaygın genotip, kohortun %49'unda bulunan GT olarak tespit edilmiştir. Bu genotip, özellikle hesaplanan serbest 25(OH)D konsantrasyonları yüksek ancak biyoyararlanabilir 25(OH)D düzeyleri düşük olan bireylerde gözlenmiştir. Rs4588'de, AC genotipi, özellikle biyoyararlanabilir 25(OH)D düzeyi düşük olan bireylerde sıklıkla tespit edilmiştir. En yaygın fenotipler Gc1s/2 (%35,3) ve Gc1s/1s (%31,4) iken, Gc1f/1f nadir görülmüştür (%5,9). Sağlıklı Türk bireylerde serbest ve biyoyararlanabilir 25(OH)D düzeyleri ile toplam 25(OH)D konsantrasyonlarındaki farklılıklar, GC gen polimorfizmlerine atfedilmiştir. Bu nedenle, serum toplam 25(OH)D'nin geleneksel ölçümüne ek olarak, sağlıklı Türk popülasyonları için serbest ve biyoyararlanabilir 25(OH)D'nin (GC polimorfizmleri ile ilişkili olarak) klinik değerlendirmesi önerilmiştir Birleşik Krallık'ta COVID-19 prevalansının, beyaz, Çinli ve Güney Asyalı popülasyonlara kıyasla, daha önce D vitamini eksikliği tespit edilmiş siyahi bireyler arasında en yüksek seviyede olması nedeniyle bu bulguların, SARS-CoV-2 pandemisi ve D vitamininin rolü ile de ilgili olabileceği belirtilmiştir.¹¹

D VİTAMİNİ MEKANİZMALARI VE EPİGENETİK MODİFİKASYONLAR

D Vitamini Durumunun Genetik ve Çevresel Belirleyicileri

Genetik Faktörler

- D vitamini sentezi öncüllerinin (örneğin, kolesterolden türetilen D3) biyokimyasal yollarını etkileyen genetik varyantlar, vitamin D metabolizmasını etkileyebilmektedir.
- CYP2R1, CYP27B1, CYP27A1 ve CYP24A1 dahil olmak üzere vitamin D metabolizmasında rol oynayan enzimlerdeki polimorfizmler, dolaşımdaki 25-hidroksivitamin D [25(OH)D] düzeylerini değiştirebilmektedir.
- GC genindeki varyantlar (vitamin D bağlayıcı protein, DBP'yi kodlayan) vitamin D'nin bağlanması, taşınması ve dağılımını etkiler ve böylece serum konsantrasyonlarını etkilemektedir.

¹¹ Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., Durazo-Arvizu, R. A., Gallagher, J. C., Gallo, R. L., Jones, G., & others. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. *Journal of Clinical Endocrinology & Metabolism*, 96(1), 53–58. <https://doi.org/10.1210/jc.2010-2704>

- Genetik etkiler, vitamin D düzeylerinde bireyler arası geniş bir varyasyona katkıda bulunmaktadır, ancak eksiklik riskini bağımsız olarak belirlememekte; genetik yatkınlık genellikle çevresel maruziyetlerle etkileşime girmektedir.

Çevresel Faktörler

- UVB güneş ışığına maruz kalma sonrası deride vitamin D3 sentezi; mevsim, coğrafi enlem, rakım, giysi örtüsü ve güneş kremi kullanımından etkilenmektedir.
- D vitamini alımı diyetle sınırlıdır ve yağlı balıklar başlıca doğal kaynağıdır; birçok ülkede, takviye edilmiş gıdalar ve takviyeler gereklidir.
- Yaş, cilt pigmentasyonu, vücut yağı (yağ depolama) ve bağırsak emilim verimliliği, D vitamininin biyoyararlanımını etkilemektedir.
- Hastalıklar ve ilaçlar (örn. antikonvülsanlar, glukokortikoidler, bariatrik cerrahi) D vitamini metabolizmasını bozabilmektedir.

D VİTAMİNİNİN EPIGENETİK ROLÜ

Vitamin D ve VDR Yolu

Vitamin D, etkilerini büyük ölçüde, hedef gen transkripsiyonunu düzenlemek için genomik bölgelerle etkileşime giren vitamin D reseptörüne (VDR) bağlanarak göstermektedir. VDR bağlanması, promotör bölgelerindeki epigenetik imzaları, histon asetilasyonu ve DNA metilasyonu gibi, etkileyebilmektedir. VDR bağlanması, histon asetilasyonu ve DNA metilasyonu gibi promotör bölgelerindeki epigenetik imzaları etkileyebilmektedir.

Epigenetik Etkilerin Örnekleri

VDR tarafından düzenlenen genlerin DNA metilasyon paternleri ile bunların ekspresyon düzeyleri arasında korelasyonlar bildirilmiştir. Bazı dokulardaki D vitamini eksikliği, metilasyon durumlarındaki değişikliklerle ilişkilendirilmiştir. Histon modifikasyonlarının, kromatin yeniden yapılanmasını aracılık ederek VDR'nin gen ekspresyonunu düzenlemesine olanak sağladığı gösterilmiştir. Kodlamayan RNA'lar (örn. miRNA'lar, lncRNA'lar) da vitamin D sinyal ağlarının modülasyonunda rol oynayabilmektedir. Klinik ve Biyolojik Önemi Epigenetik mekanizmalar, bağışıklık modülasyonu ve inflamasyonla ilişkili genlerin düzenlenmesi dahil olmak üzere vitamin D eksikliğinin uzun vadeli etkilerinin altında yatan neden olabilmektedir. Bu etkiler yaş, cinsiyet, doku türü ve bireysel genetik/epigenetik geçmişe göre değişiklik göstermektedir.

D VİTAMİNİ DÜZEYLERİNDE GENETİK FAKTÖRLER

İkiz ve aile çalışmaları, dolaşımdaki 25-hidroksivitamin D [25(OH)D] konsantrasyonlarının önemli bir kalıtsal bileşene sahip olduğunu ve genetik katkının %29 ile %86 arasında değiştiğini göstermiştir. Bu kanıtlara dayanarak, genom çapında ilişki çalışmaları (GWAS), vitamin D yetersizliğinin ortak genetik belirleyicilerini tanımlamıştır. Bu çalışmaların en önemlisi, Wang ve arkadaşları tarafından yapılan ve vitamin D metabolizmasıyla ilişkili tek nükleotid polimorfizmleri (SNP'ler) üzerine sonraki araştırmaların temelini oluşturan çığır açıcı çalışmadır. Bu çalışma, vitamin D metabolizmasıyla ilişkili tek nükleotid polimorfizmleri (SNP'ler) üzerine yapılan sonraki araştırmaların temelini oluşturmuştur. Aday gen çalışmaları, vitamin D sentezi, taşınması, aktivasyonu ve sinyal iletiminde merkezi rol oynayan belirli lokusların, özellikle GC (vitamin D bağlayıcı proteini kodlayan), CYP2R1, CYP24A1, CYP27B1 ve VDR'nin önemini daha da vurgulamıştır.

GC rs2282679 ve rs1155563 gibi varyantlar, dolaşımdaki 25(OH)D konsantrasyonlarındaki farklılıklar ve takviyeye yanıtta bireyler arası değişkenlikle ilişkilendirilmiştir, ancak bu etkilerin çoğu düşük düzeyde olup kalıcı değildir. Genel olarak, bu bulgular genetik varyasyonun vitamin D durumunu değiştiren bir faktör olarak önemini vurgulamakta ve aynı zamanda bu tür etkilerin çevresel faktörlerle etkileşime girerek hem klinik sonuçları hem de müdahaleye verilen yanıtları şekillendirdiğini göstermektedir.^{20,21,22}

D VİTAMİNİ METABOLİZMASINDA ANAHTAR GENLER VE ENZİMLER

Birkaç anahtar gen, sentez, taşıma, aktivasyon ve inaktivasyon yolları aracılığıyla D vitamini metabolizmasını düzenlemektedir. D vitamini bağlayıcı proteini (DBP) kodlayan GC, taşıma ve dağılımı etkilemektedir; rs2282679 ve rs1155563 gibi SNP'ler serum 25(OH)D düzeyleri ve takviye yanıtı ile bağlantılıdır.^{20, 23, 24}

Karaciğer 25-hidroksilazını kodlayan CYP2R1, dolaşımdaki 25(OH)D konsantrasyonlarıyla ilişkili varyantlara (örn. rs10741657, rs10766197) sahiptir.²⁵ CYP24A1, vitamin D'yi inaktif metabolitlere dönüştürerek katabolizmayı yönetmektedir, CYP27B1 ise aktif form olan 1,25(OH)₂D'nin üretilmesinden sorumlu böbrek 1- α -hidroksilazı kodlamaktadır.²⁶

²¹ Theodoratou, E., Palmer, T., & Zgaga, L. (2012). Instrumental variable estimation of the causal effect of plasma 25-hydroxy-vitamin D on colorectal cancer risk: A Mendelian randomization analysis. *PLoS ONE*, 7(6), e37662. <https://doi.org/10.1371/journal.pone.0037662>

²² Saggese, G., Vierucci, F., Prodam, F., Cardinale, F., Cetin, I., Chiappini, E., De' Angelis, G. L., Massari, M., Miraglia Del Giudice, E., Miraglia Del Giudice, M., Peroni, D., Terracciano, L., Agostiniani, R., Careddu, D., Ghiglioni, D. G., Bona, G., Di Mauro, G., & Corsello, G. (2018). Vitamin D in pediatric age: Consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians. *Italian Journal of Pediatrics*, 44(1), 51. <https://doi.org/10.1186/s13052-018-0488-7>

²³ Thongthai, P., Chailurkit, L., Chanprasertyothin, S., Nimitphong, H., Sritara, P., Aekplakorn, W., & Ongphiphadhanakul, B. (2015). Vitamin D binding protein gene polymorphism as a risk factor for vitamin D deficiency in Thais. *Endocrine Practice*, 21(3), 221–225. <https://doi.org/10.4158/EP14266.OR>

²⁴ Szili, B., Szabó, B., Horváth, P., Bakos, B., Kirschner, G., Kósa, J. P., Toldy, E., Putz, Z., Lakatos, P., Tabák, Á. G., & Takács, I. (2018). Impact of genetic influence on serum total- and free 25-hydroxyvitamin-D in humans. *Journal of Steroid Biochemistry and Molecular Biology*, 183, 62–67. <https://doi.org/10.1016/j.jsbmb.2018.05.007>

²⁵ Jiang, X., O'Reilly, P. F., Aschard, H., Hsu, Y.-H., Richards, J. B., Dupuis, J., Ingelsson, E., Karasik, D., Pilz, S., Berry, D., Kestenbaum, B., Zheng, J., Luan, J., Sofianopoulou, E., Streeten, E. A., Albanes, D., Lutsey, P. L., Yao, L., Tang, W., ... Hyppönen, E. (2018). Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nature Communications*, 9, Article 260. <https://doi.org/10.1038/s41467-017-02662-2>

²⁶ Constantinou, C., & Kolokotroni, O. (2019). *Vitamin D: A multi-faceted role for the prevention, management, survivorship, and palliative care of breast cancer*. *World Journal of Breast Cancer Research*, 2(1), Article 1011.

VDR geni, vitamin D'nin genomik etkilerine aracılık etmekte ve rs731236 gibi polimorfizmler, yeterlilik ve takviye sonuçlarını etkilemektedir.²⁷ Son olarak, 7-dehidrokolesterol redüktazı kodlayan DHCR7, vitamin D sentezi için öncü maddelerin kullanılabilirliğini etkilemektedir.^{24,25} Özetle, bu lokuslardaki polimorfizmler, vitamin D durumundaki ve takviyeye verilen yanıtlardaki bireyler arası farklılıkları açıklamaya yardımcı olmaktadır.

Gen	Fonksiyon	Anahtar Polimorfizmler	D vitamini Metabolizmasındaki Rol
GC	DBP'yi kodlar, D vitamininin taşınmasını sağlar	rs2282679, rs1155563	Taşıma ve dağıtım
CYP2R1	Karaciğer 25-hidroksilasyonu	rs10741657, rs10766197, rs2060793	Birincil aktivasyon adımı
CYP24A1	D vitamini metabolitlerinin inaktivasyonu	Çeşitli SNP'ler	Katabolizma
CYP27B1	Renal 1- α -hidroksilasyon	-	Aktivasyon
VDR	D vitamini reseptörü, genomik düzenleme	rs731236	Hücre sel yanıt
DHCR7	Öncü metabolizma (kolesterol)	Çeşitli varyantlar	Sentez

D VİTAMİNİNİN TAKVİYESİNE GENETİK TEPKİLER

Bireylerin genetik yapısına dayalı olarak kişiselleştirilmiş vitamin D takviye protokollerinin geliştirilmesi, geleceğin terapötik yaklaşımları açısından büyük önem taşımaktadır. Ancak, bazı çalışmalarda, yaygın SNP'lerin etkisinin zaman veya doz bağımlı olduğu ve uzun vadeli takviyede bu etkilerin doygunluğa ulaşarak önemsiz hale geldiği gözlemlenmiştir⁴. Dolayısıyla, genetik arka planın klinik uygulamalarda kullanımının, özellikle kısa süreli tetkiklerde dikkatle değerlendirilmesi gerekmektedir. Genetik varyasyonlar, vitamin D metabolizmasına doğrudan müdahale ederek;

- Takviye sonrasında 25(OH)D artışında hız ve miktar
- Hücre sel yanıtların şiddeti ve süresi
- Bireysel yanıtın değerlendirilmesinde klinik kritiklik

gibi konularda etkili olmaktadır. Bu bağlamda, genetik testlerin, bireysel takviye planlarının belirlenmesinde rehberlik edebileceği öne sürülmektedir.^{12,27}

313 sağlıklı yetişkini kapsayan VIDARIS çalışması (Vitamin D ve Akut Solunum Enfeksiyonları Çalışması) gibi randomize kontrollü çalışmalar, GC, CYP2R1 ve CYP27B1'deki SNP'lerin takviye yanıtlarındaki farklılıklarla ilişkili olduğunu göstermiştir;

²⁷ Bouillon, R. (2009). *Vitamin D and human health* [Editorial]. *Presse Médicale*, 38(1), 3–6. <https://doi.org/10.1016/j.lpm.2008.11.001>

ancak GC varyantlarının (rs2282679, rs1155563) etkilerinin küçük ve geçici olduğu tespit edilmiştir.²⁵ Benzer şekilde, Orta Doğu'daki bir kohorttan elde edilen kanıtlar, VDR (rs731236) ve CYP2R1 (rs7116978) polimorfizmlerinin vitamin D durumunu önemli ölçüde etkilediğini göstermiştir. rs731236 GG ve rs7116978 CC genotiplerini taşıyan bireyler, daha yüksek vitamin D yeterliliği ve daha güçlü takviye yanıtları sergilemiş, böylece vitamin D sonuçlarını modüle etmede genetik varyasyonun rolünü güçlendirmiştir.

SONUÇ

Bu makalede, D vitamini düzeylerinde gözlemlenen bireysel farklılıkların genetik temelleri şu şekildedir. İncelenen kaynaklardan aşağıdaki temel bulgular öne çıkmaktadır:

- **Genetik Etki:** Dolaşımdaki 25(OH)D düzeylerinin belirlenmesinde genetik faktörlerin etkisi, ikiz ve aile çalışmalarından genom çapında ilişkilendirme çalışmalarına kadar geniş bir yelpazede ortaya konmuştur³.
- **Anahtar Genler:** GC, CYP2R1, CYP24A1, CYP27B1, VDR ve DHCR7 gibi genler, vitamin D metabolizmasının farklı aşamalarında kritik roller oynamakta ve bu genlerdeki polimorfizmler vitamin D düzeyleri üzerinde belirleyici etkilere sahiptir⁴⁵.
- **Takviye Yanıtları:** İntervensiyon çalışmalarında, özellikle GC ve VDR ile ilişkili SNP'lerin vitamin D takviyesine verilen yanıtı modüle ettiği; ancak bu etkinin zaman ve doz faktörlerine bağlı olarak sınırlı kaldığı gözlemlenmiştir⁴.
- **Epigenetik Mekanizmalar:** DNA metilasyonu gibi epigenetik düzenleyici mekanizmaların, gen ekspresyonu aracılığıyla vitamin D düzeylerini etkileyebileceği ileri araştırmalara açıktır.
- **Klinik Uygulama:** Genetik varyasyonların belirlenmesi, gelecekte kişiselleştirilmiş vitamin D takviyesi protokollerinin geliştirilmesinde önemli bir role sahip olacaktır.

Aşağıda, bu sonuçların özetlendiği ve araştırmanın anahtar noktalarının listelendiği tablo yer almaktadır:

Ana Bulgular	Detaylar
Genetik Etki	D Vitamini düzeylerinin %29-86 oranında genetik faktörlerden etkilendiği gösterilmiştir.
Anahtar Genler	GC, CYP2R1, CYP24A1, CYP27B1, VDR, DHCR7 genleri vitamin D metabolizmasında rol oynamaktadır.
Takviye Yanıtları	Özellikle GC ve VDR genlerinde bulunan belirli SNP'ler, vitamin D takviyesi yanıtını modüle etmektedir.
Epigenetik Düzenlemeler	DNA metilasyonu ve diğer epigenetik mekanizmalar vitamin D gen ekspresyonunu düzenleyebilir.
Kişiselleştirilmiş Tedavi Yaklaşımı	Genetik ve epigenetik verilerin, kişiye özel vitamin D takviye planlarının oluşturulmasında kullanılması.

AKUT KORONER SENDROMLU HASTALARDA TROPONİN İLE ADAMST ARASINDAKİ İLİŞKİ

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ÖZET

Amaç: Bu çalışmanın amacı akut koroner sendromlu (AKS) hastalarda troponin ve ADAMTS proteinleri arasındaki ilişkiyi belirlemektir. Kardiyak troponinler, kalp kası hasarında hassas ve yararlı parametreler olarak kullanılır. Bu çalışmada, AKS'li hastalarda troponin düzeyleri ile ADAMTS-4 ve ADAMTS-9 protein düzeyleri arasındaki ilişki araştırılmıştır.

Materyal ve Metot: Çalışmamız, deney grubunda AKS tanısı almış 25 yaş üstü 14 kadın ve 30 erkek birey ile kontrol grubunda 19 sağlıklı kadın ve 26 sağlıklı erkek birey ile yürütülmüştür. Kan örneklerinde troponin düzeyi yüksek olan AKS tanısı almış hastalar çalışmaya dahil edilmiştir. Hasta ve kontrol gruplarının antropometrik ölçümleri yapılarak kan örnekleri alınmıştır. Kan örneklerinden serum ayırıştırılmıştır. Daha sonra ADAMTS-4 ve ADAMTS-9 protein düzeyleri ELISA yöntemi ile ölçülmüştür.

Bulgular: Hasta grubunda troponin düzeyleri kontrol grubuna göre anlamlı derecede yüksek bulunmuştur ($p<0,001$). Benzer şekilde, ADAMTS-4 düzeyleri hasta grubunda kontrol grubuna kıyasla anlamlı derecede yüksekti ($p<0,001$). Ancak, iki grup arasında ADAMTS-9 protein düzeyleri açısından anlamlı bir fark bulunmadı ($p=0,519$).

Sonuç: Çalışmamız, troponin düzeyleri gibi ADAMTS-4 proteininin de AKS'de biyobelirteç olarak kullanıma potansiyeline sahip olduğunu göstermektedir. Ancak, ADAMTS-9'un süreçteki rolü ve işlevleri için daha detaylı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Troponin, Akut Koroner Sendrom, ADAMTS-4, ADAMTS-9.-

Etik Kurul Onayı: Balıkesir Üniversitesi, Tıp Fakültesi, Klinik Araştırmalar Etik Kurulu Başkanlığı: 22/03/2017-2017/23

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THE RELATIONSHIP BETWEEN TROPONIN AND ADAMTS IN PATIENTS WITH ACUTE CORONARY SYNDROME

ABSTRACT

Objective: The aim of this study was to determine the relationship between troponin and ADAMTS proteins in patients with acute coronary syndrome (ACS). Cardiac troponins are used as sensitive and useful parameters in cardiac muscle damage.

In this study, the relationship between troponin levels and the levels of ADAMTS-4 and ADAMTS-9 proteins in patients with ACS was investigated.

Material and Methods: Our study was conducted with 14 female and 30 male individuals over 25 years of age diagnosed with ACS in the experimental group and 19 healthy female and 26 healthy male individuals in the control group. Patients diagnosed with ACS with elevated troponin levels in blood samples were included in the study. Anthropometric measurements of the patient and control groups were made and blood samples were taken. Serum was separated from the blood samples. Then, ADAMTS-4 and ADAMTS-9 protein levels were measured by ELISA method.

Results: Troponin levels were significantly higher in the patient group compared to the control group ($p<0.001$). Similarly, ADAMTS-4 levels were significantly higher in the patient group compared to the control group ($p<0.001$). However, no significant difference was found between the two groups in terms of ADAMTS-9 protein levels ($p=0.519$).

Conclusion: Our study shows that ADAMTS-4 protein, like troponin levels, has the potential to be used as a biomarker in ACS. However, more detailed studies are needed for the role of ADAMTS-9 and its functions in the process.

Keywords: Troponin, Acute Coronary Syndrome, ADAMTS-4, ADAMTS-

NaF'UN HÜCRE İÇİ METABOLİZMASI

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ÖZET

Flor; yüksek elektronegativitesi nedeniyle biyolojik sistemlerde güçlü etkili ve doğada farklı bileşikler halinde bulunan önemli bir elementtir. Uzun süre yüksek dozda flor maruziyeti sonucunda gelişen florozis, ülkemizde ve bölgemizde hem insan hem de hayvan sağlığını tehdit eden yaygın bir toksikasyon tablosudur ve aynı zamanda ciddi ekonomik kayıplara da yol açmaktadır. Sınırlı tedavi olanakları ve çok yönlü komplikasyonları, florozisi önemli bir halk sağlığı sorunu haline getirmektedir.

NaF'un deneysel çalışmalarda flor kaynağı olarak kullanıldığı bilinmektedir. Çeşitli araştırmalarda farklı sonuçlar elde edilmiş olsa da, özellikle artan dozların hücre proliferasyonu üzerinde baskılayıcı etki gösterdiği ortaya konmuştur. Bu bağlamda, florun hücre içi metabolizmasının ayrıntılı olarak aydınlatılmasının, florozise bağlı fizyopatolojik mekanizmaların anlaşılmasına ve buna yönelik yeni tanı, takip ve tedavi yaklaşımlarının geliştirilmesine katkı sağlaması beklenir.

Flor toksisitesinin moleküler düzeyde incelenmesi amacıyla kullanılan deneysel modeller, özellikle deney hayvanları ve hücre kültürü uygulamaları, klinik komplikasyonların anlaşılmasında önemli rol üstlenmektedir. Bu çalışmada, anabilim dalımız laboratuvarında gerçekleştirilen araştırmalara dayalı olarak, florun neden olduğu sitotoksitenin moleküler mekanizmaları ve bu mekanizmaların düzenlenmesinde etkili olabileceği öngörülen bazı biyolojik ajanların rolleri değerlendirilmiştir. Ayrıca, NaF'un hücre içi metabolizmasına ilişkin güncel bilgiler sunulurken literatüre katkı sağlanması amaçlanmıştır.

Anahtar Kelimeler: NaF, moleküler yolaklar, hücre içi metabolizma

INTRACELLULAR METABOLISM OF NaF

ABSTRACT

Fluoride is an element with very high electronegativity, and it is found in nature in different compounds. Long-term exposure to high levels of fluoride can cause fluorosis, which is a common toxic condition. Fluorosis is seen in both humans and animals in our country and region, and it creates serious health problems as well as economic losses.

Because treatment options are limited and complications are diverse, fluorosis is an important public health issue.

Sodium fluoride (NaF) is widely used as a source of fluoride in experimental studies. Although different results have been reported, it is well known that higher doses of NaF reduce cell proliferation. For this reason, understanding the intracellular metabolism of fluoride is important. This knowledge can help us explain the mechanisms of fluorosis and may also provide new approaches for diagnosis, monitoring, and treatment.

Experimental models used to study fluoride toxicity at the molecular level, especially animal studies and cell culture systems, are important for understanding clinical complications. In this study, research from our departmental laboratory was used to evaluate the molecular mechanisms of fluoride-induced cytotoxicity and the possible roles of some biological agents that may help regulate these mechanisms. In addition, updated information about the intracellular metabolism of NaF is presented to contribute to the scientific literature.

Keywords: NaF, molecular pathways, intracellular metabolism

GİRİŞ

Florozis olarak bilinen yüksek flor toksisitesi, uygun beslenme stratejileri, erken tanı yöntemleri ve etkili koruyucu sağlık politikalarıyla büyük ölçüde önlenabilir ve yönetilebilir bir halk sağlığı sorunudur. Türkiye’de florozis gerek çevresel koşullar gerekse endüstriyel kirlenme nedeniyle özellikle Van Gölü havzası, Tendürek Volkanı çevresi, Isparta-Denizli-Eskişehir bölgeleri gibi alanlarda endemik görünümde olup içme sularında yüksek flor konsantrasyonu gibi riskler devam etmektedir.

Bununla birlikte, florozise bağlı sistemik komplikasyonların uzun vadeli izlenmesi ve tedavisi için yeni yaklaşımların geliştirilmesi önemlidir. Son yıllarda florun hücre içi metabolizmasının incelenmesi, toksisitenin moleküler mekanizmalarının daha iyi anlaşılmasında ve olası koruyucu ya da tedavi edici stratejilerin geliştirilmesinde kilit roller üstlenmektedir. Bu derlemede, florun hücre içi metabolizmasına dair güncel bilimsel verileri, florozisin fizyopatolojisi ve klinik etkilerinin aydınlatılması konuları dikkate alınarak, Van Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı laboratuvarımızda bu konuda gerçekleştirilen çalışmalar ve geleceğe yönelik araştırma önerileri hakkında kısaca bilgiler sunulmaktadır.

Florozis, insanlarda ve hayvanlarda ciddi sağlık sorunlarına ve ekonomik kayıplara yol açan yaygın bir toksik tablodur; mevcut tedavi seçeneklerinin sınırlılığı ve çeşitli komplikasyonların ortaya çıkması bu durumun ciddiyetini artırmaktadır. Sodyum florür (NaF) kullanılarak yapılan deneysel çalışmalarda, doz arttıkça hücre proliferasyonunun baskılandığı, dokulara ve hücrelere zarar veren etkilerin ortaya çıktığı bildirilmiştir. Sodyum florür (NaF), flor toksisitesini araştırmak amacıyla deneysel ve klinik çalışmalarda en sık kullanılan model bileşiktir. Bunun başlıca nedenleri; NaF, su ve biyolojik sıvılarda kolayca çözünmesi, kimyasal olarak kararlı olması, istenilen konsantrasyonlarda florür iyonlarının hassas bir şekilde uygulanmasına imkân tanınması, NaF maruziyetinin toksikolojik sonuçları, insan ve hayvanlarda gözlenen florür toksisitesinin genel mekanizmalarını yansıtmasıdır. Bu özellikleri nedeniyle NaF, florürün hücresel ve sistemik etkilerini anlamaya yönelik araştırmalarda standart bileşik olarak kullanılmaktadır.

Bu nedenle, florun hücre içi metabolizmasının ayrıntılı biçimde anlaşılması, toksisite mekanizmalarının çözülmesi ve yeni tedavi stratejilerinin geliştirilmesi bakımından büyük önem taşımaktadır.

Deneyisel modeller (hücre kültürü ve hayvan çalışmaları) florozisin komplikasyonlarının araştırılmasında önemli araçlar sağlamakta, mekanizmaların aydınlatılmasına ve klinik uygulamaya geçişte temel bilgi oluşturmaktadır (Yur ve ark., 2013; Dede ve ark., 2016; Kurtdeve ve ark., 2017; Dede, 2018; Anonim, 2024; Dede, 2025; WHO, 2002; 2017; 2019a; 2019b).

Flor, uzun yıllar boyunca diş çürüklerini önleme özellikleri nedeniyle yaygın biçimde faydalı bir element olarak değerlendirilmiş; diş macunları, ağız gargaraları ve içme suyuna katkı gibi uygulamalarla kullanılmıştır. Ancak, yüksek düzeyde flor alımı diş (dental florozis), iskelet florozisi ve yumuşak dokularda toksik etkilere yol açabilir. Tarihsel olarak, flor toksisitesi çürük önleyici özelliklerin gölgesinde kalmış, geniş halk kitlelerinin sağlık etkilerine dair çalışmalar son yıllarda artmıştır. Yapılan deneyisel ve klinik çalışmalar, florun sınırlı dozlarda bile oksidatif stres indüksiyonu, mitokondriyal işlev bozuklukları, DNA hasarı, apoptozis ve enerji metabolizmasında aksamalar gibi hücresel düzeyde olumsuz etkiler oluşturabileceğini göstermektedir. Bu nedenle, florozisin fizyopatolojisini tam olarak anlamak ve florun hücre içi etkilerini ortaya koymak için oksidatif stres yolları, mitokondriyal biyogenez, DNA tamiri ve enerji metabolizması gibi mekanizmaların daha ayrıntılı araştırılması kritik önemdedir (Dede ve ark., 2016; Ergin ve Eden, 2017; Tian ve ark., 2017; Dede, 2025; Sneha ve ark., 2023).

Florun hücresel düzeydeki metabolizmasını ortaya koyan çalışmalarda, oksidatif stresin indüklediği, hücre içi redoks homeostazının bozulabildiği, lipid peroksidasyonu ve protein karbonil içeriğinin modüle edildiği, ayrıca gen ekspresyonunu değiştirilebildiği ve apoptoza yol açabildiği gösterilmiştir. Apoptozun tetiklenmesinde mitojenle aktive olan protein kinaz (MAPK) kaskadının aktivasyonu yoluyla oksidatif stresin artışı önemli bir rol oynamaktadır (Gutiérrez-Salinas ve ark., 2013; Tian ve ark., 2017). NaF'e bağlı olarak oluşan hasarda, inflamatuvar yanıtın ve fibrotik değişimlerin düzenlenmesinde, oksidatif stres (NOX4, p38 α MAPK), inflamasyon (NF- κ B, COX-2, HO-1), apoptozis (cleaved caspase-3 p20, PARP1, ayrıca Hsp27 ve Hsp60 gibi stresle ilişkili proteinler) ve fibrozis (TGF- β 1, pSmad3, Coll α 1, hidroksiprolin düzeyi) gibi biyobelirteçlerin ölçülmesi yoluyla patolojik süreçlerin aydınlatıldığı bildirilmiştir (Ameeramja ve Perumal, 2018).

Aynı şekilde, NaF'un apoptozu indükleyerek mitokondriyal yolları aktive edebildiği de çeşitli çalışmalarda gösterilmiştir (Yan ve ark., 2017; Li ve ark., 2018). Mitokondriyal reaktif oksijen türlerinin (mROS) birikimi, florozisin patogeneğinde kritik bir mekanizma olarak öne çıktığı ve NaF verilmesinin mitokondriyal ultrastrüktürü bozarak TCMK-1 hücrelerinde hücre canlılığını azalttığı ve apoptozu artırdığı rapor edilmiştir (Song ve ark., 2017).

Flor kaynaklı nefrotoksisiteye yönelik araştırmalarda, özellikle çocukluk döneminde yüksek flor maruziyetinin böbrek histolojisinde hasara yol açtığı ve *in vivo* koşullarda apoptozun belirgin şekilde arttığı bildirilmiştir (Quadri ve ark., 2018). Ölüm reseptörü ve mitokondriyal yollar üzerinden gelişen apoptozis ile arasındaki ilişkiyi değerlendiren bir çalışmada ise, farklı dozlarda NaF uygulanan ratlarda apoptozis düzeylerinin kontrol gruplarına göre anlamlı olarak arttığı gösterilmiştir. Bu gruplarda kaspaz-3, -8, -9 ile sitokrom C'nin ortalama optik yoğunluklarının kontrol grubuna kıyasla daha yüksek olduğu tespit edilmiş, özellikle orta ve yüksek dozlarda bu artış belirgin hale gelmiştir (Gu ve ark., 2016). Ayrıca NaF uygulanan rat böbreklerinde sitoplazmada artan sitokrom C miktarına bağlı olarak kaspaz aracılı apoptozun şiddetlendiği bildirilmiştir (Song ve ark., 2014). Bununla birlikte, florun başlattığı apoptozda otofajinin rolü araştırılmış ve bazı bulgular, otofajinin indüksiyonu yoluyla flor kaynaklı apoptoza karşı hücresel düzeyde koruyucu bir yanıtın gelişebileceğini düşündürmektedir (Gu ve ark., 2016).

Moleküler Mekanizmayı Belirlemek İçin Biz Ne Yaptık?

Laboratuvarımızda bu konuda yürütülen çalışmalarda, NaF'un hücresel düzeydeki sitotoksik etkilerinin incelenmesi ve bu etkilerin antioksidanlar aracılığıyla ne şekilde modüle edilebileceğinin ortaya konulması hedeflenmektedir. Bu doğrultuda farklı hücre serilerine NaF uygulanarak; hücre proliferasyonu, oksidatif stres parametreleri ve apoptozis vs mekanizmaları ayrıntılı biçimde değerlendirilmiştir.

Elde edilen bulgulara göre, flor doz ve zaman bağımlı olarak hücre proliferasyonunu baskılamakta, sitotoksikiteye yol açmaktadır. Bu etkiler böbrek ve kemik hücrelerinde özellikle belirgin olarak saptanmıştır. Antioksidan kapasitenin yetersiz olduğu koşullarda florun toksik etkileri artmakta; vitamin C, flavonoidler gibi antioksidanların kullanımı bu zararlı etkileri önemli ölçüde hafifletebilmektedir.

Deneyisel modeller kapsamında hem hayvan çalışmaları hem de hücre kültürü uygulamaları değerlendirilmiş; bu çerçevede NaF'un neden olabileceği hücresel hasarın moleküler mekanizmaları ayrıntılı olarak incelenmeye çalışılmıştır. Bu tür araştırmaların, kemik ve böbrek dokularında flor kaynaklı hasarların ve olası komplikasyonların anlaşılması, tedaviye yönelik stratejilerin geliştirilmesi ve bazı prognostik göstergelerin şekillenmesi açısından katkılar sağlayabileceği düşünülmektedir.

Elde edilen bulguların yeni araştırma projelerine zemin oluşturabileceği ve bazı ön sonuçların ulusal ve uluslararası kongrelerde paylaşılmış olması, konunun önemini vurgulanması açısından önemlidir. Genel hatlarıyla, NaF'un moleküler düzeyde sitotoksikite ve apoptozu tetikleyebildiği; bu etkinin antioksidanlar, vitaminler ve mineraller aracılığıyla belirli ölçüde düzenlenebileceği gözlemlenmiştir. Bu çerçevede, ülkemizde hem endemik hem de endüstriyel kaynaklı florozisin biyokimyasal temellerine ilişkin daha kapsamlı ve derinlemesine verilerin elde edilebileceği düşünülmektedir.

Şimdiye kadar yapılan çalışmalarımızdan elde edilen sonuçları aşağıdaki şekilde özetleyebiliriz:

1. Apoptozis ve Oksidatif Stres

NaF uygulamalarının hücre proliferasyonu üzerinde doza ve süreye bağımlı baskılayıcı etkiler oluşturduğu, oksidatif stres ve apoptosis parametrelerinde belirgin değişikliklere yol açtığı ortaya konmuştur (Yüksek ve ark., 2017a, 2017b, 2017c, 2017d; Çetin, 2017b). NRK-52E ve hFOB 1.19 hücre hatlarında yapılan çalışmalarda, kaspaz-3, -8, -9 gibi apoptotik belirteçlerin yanı sıra 8-OHdG ve M30 düzeylerinin arttığı gösterilmiştir (Yüksek, 2017). Vitamin A'nın NaF ile birlikte verildiğinde ise apoptozis oranlarını azalttığı saptanmıştır. Bu durum, florun hücre ölümünde yalnızca klasik apoptotik yolların değil, farklı mekanizmaların da rol oynadığını düşündürmektedir. Vitamin D'nin NaF ile muamele edilen NRK-52E hücrelerinde TAS düzeyini artırdığı, TOS ve OSI indeksini azalttığı rapor edilmiştir (Dede ve ark., 2016; Kömüroğlu ve ark., 2017; Usta ve ark., 2017; Dede, 2018). Osteoblast hücre hattında yapılan çalışmalarda ise NaF uygulamasının TAS seviyelerini azaltıp, TOS'u artırdığı; vitamin ve mineral takviyelerinin bu dengeyi yeniden düzenlediği görülmüştür (Çetin ve ark., 2017a; Yüksek ve ark., 2017d). Ayrıca karaciğer ve böbrek dokularında yapılan çalışmalarda apoptotik gen ekspresyonlarında (caspase-3, -8, -9; Bcl-2, Bax, Bak, Bim) dengesizlikler; oksidatif stresle ilişkili genlerde ise (Cygb, Gstp1, Gpx1, Sod1, Sod2, Ncf1) anlamlı artışlar ve azalışlar saptanmıştır (Efe ve ark., 2021). Bu veriler florozisin DNA bütünlüğünü bozduğu ve hem apoptozis hem de oksidatif stres mekanizmalarını birlikte aktive ettiği yönünde güçlü kanıtlar sunmaktadır (Dede ve ark., 2023).

2. DNA Hasarı ve Onarım Mekanizmaları

Karaciğer ve böbrek dokularında yapılan analizlerde, genotoksisite göstergesi olarak DNA hasarı saptanmıştır. NaF'un genetik yapıya yönelik zararlı etkileri comet testi ve 8-OHdG analizleri ile doğrulanmış, NaF uygulanan gruplarda DNA hasarında anlamlı artış gözlenmiştir (Çetin, 2017a, 2017b; Çetin ve ark., 2017a, 2017b; Yüksek ve ark., 2020a). Vitamin D₃, vitamin E, selenyum ve antioksidan kombinasyonlarının DNA hasarını azalttığı, ayrıca DNA tamir genlerinin (Ku70, Ku80, PARP-1) ekspresyonunu artırdığı belirlenmiştir (Usta ve ark., 2019; Yüksek ve ark., 2018, 2020b). Bu bulgular, vitaminlerin ve antioksidanların flor kaynaklı genotoksisiteye karşı koruyucu rolünü vurgulamaktadır. Bu bulgular florozis DNA bütünlüğünü bozduğu yönünde güçlü kanıtlar sunmaktadır (Dede ve ark., 2023).

3. Nekroz ve Otofaji

NaF'un hücresel ölüm mekanizmaları yalnızca apoptozis ile sınırlı kalmamış, nekroz ve otofajiyolaklarının da aktive olduğu ortaya konmuştur. Ripk1 ve Ripk3 gen ekspresyonlarının NaF uygulamasıyla anlamlı şekilde arttığı ve zaman/konsantrasyon bağımlı olarak yukarı regüle olduğu bildirilmiştir. Özellikle renal epitelyal hücrelerde Ripk3 düzeyindeki artış, NaF'a bağlı nekroptozis mekanizmasının önemli bir göstergesi olmuştur (Urut ve ark., 2018; Usta ve ark., 2019; Çetin ve ark., 2020; Urut ve ark., 2021). NaF, osteoblastlarda (MC3T3-E1 hücre hattı) oksidatif stres ve endoplazmik retikulum (ER) stresini tetiklediği, ayrıca otofajiye aracılık eden ATG5, BECN1 gibi genlerin aktivitesini artırarak, hücre ölüm yollarını aktive ettiği (örneğin kaspaz-3 artışı) ortaya konulmuştur (Yüksek ve ark., 2023).

4. Vitaminler, Mineraller ve Antioksidanların Koruyucu Rolü

Laboratuvarımızda yürütülen çalışmalarda, vitamin A, C, D ve E'nin yanı sıra selenyum, magnezyum ve flavonoidlerin NaF toksisitesine karşı koruyucu etkileri araştırılmıştır. Vitamin E ve C'nin tek başına veya kombinasyon halinde uygulandığında florozise karşı biyokimyasal koruma sağladığı gösterilmiştir (Öner ve ark., 2017a, 2017b; Öner ve ark., 2020). Minerallerin NaF ile birlikte uygulanması, oksidatif stres azaltarak hücre canlılığını artırmış, apoptozis ve DNA hasarını ise azaltmıştır (Çetin, 2017; Çetin ve ark., 2019).

Likopen uygulamalarında ise doza bağlı çift yönlü etki gözlenmiştir: düşük dozlarda NaF toksisitesini baskımlarken, yüksek dozlarda toksisiteyi artırmıştır (Çetin ve ark., 2017a; Çetin, 2017b, 2021). Bu durum, antioksidanların doz ve süreye bağlı olarak prooksidan özellikler de gösterebileceğini düşündürmektedir.

SONUÇ VE ÖNERİLER

NaF'un hücresel metabolizmasının daha ayrıntılı biçimde anlaşılması, florozisin toksisite mekanizmalarının çözülmesinde önemli bir anahtar olabilir. Laboratuvarımızda yürütülen çalışmalar, NaF'un oksidatif stres yanıtını tetikleyebildiğini, DNA hasarına yol açabileceğini ve apoptozis ile nekroptoz gibi farklı hücre ölümü mekanizmalarını aktive edebileceğini düşündürmektedir. Ayrıca bu süreçlerde vitaminler, mineraller ve antioksidanların belirli ölçülerde düzenleyici bir rol üstlenebileceğine dair bulgular da elde edilmiştir. Bununla birlikte, mevcut veriler flor toksisitesinin yalnızca tek bir yolakla açıklanamayacağını, farklı hücresel mekanizmaların birbiriyle etkileşim içinde olabileceğini göstermektedir.

Şimdiye kadar laboratuvarımızda redoks homeostazı, kaspaz aktivasyonu, DNA hasarı ve onarım mekanizmaları ile Ripk1/Ripk3 aracılı nekroptoz gibi yollaklarda dikkate değer sonuçlara ulaşılmıştır.

Ancak bu süreçlerin daha kapsamlı ve bütüncül bir şekilde ele alınması, farklı moleküler mekanizmalar arasındaki ilişkilerin daha net ortaya konması ve bu bulguların klinik yansımalarının aydınlatılması için ileri düzey araştırmalara ihtiyaç duyulmaktadır.

Gelecek çalışmalarda neler yapılacaktır?

Gelecek çalışmalarda, NaF'un mitokondriyal fonksiyonlar üzerindeki etkilerinin, özellikle enerji metabolizması, ROS üretimi ve mitokondriyal membran bütünlüğü açısından daha kapsamlı biçimde incelenmesi planlanmaktadır. Bunun yanı sıra, hücrel stres yanıtında rol alan MAPK, PI3K/Akt, NF-κB ve Nrf2/Keap1 gibi sinyal yollarının ayrıntılı olarak değerlendirilmesi hedeflenmektedir. Ayrıca, otofaji ve ferroptoz gibi alternatif hücre ölümü mekanizmalarının NaF metabolizması ile ilişkili olup olmadığının araştırılması önem arz etmektedir. Epigenetik düzeyde ise NaF maruziyetine bağlı miRNA profilleri, DNA metilasyonu ve histon modifikasyonlarının aydınlatılması, flor toksisitesinin moleküler mekanizmalarının daha kapsamlı şekilde anlaşılmasına katkı sağlayacaktır.

Genel Değerlendirme

Elde edilen bulgular, florozisin çok boyutlu bir biyokimyasal süreç olduğunu açıkça göstermektedir. Hücrel metabolizmaya dair çalışmalarımız bu sürecin önemli bileşenlerini ortaya koymuş olsa da, mekanizmaların bütüncül biçimde anlaşılabilmesi için ileri düzey araştırmalara gereksinim vardır.

Hücre içi metabolizmanın tam olarak aydınlatılması, florozisin yol açtığı sistemik komplikasyonların anlaşılmasını kolaylaştıracak; daha etkili koruyucu ve tedavi edici stratejilerin geliştirilmesini mümkün kılacaktır.

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EFFECTS OF NON-THERMAL PLASMA APPLICATION ON AIR QUALITY AND PERFORMANCE PARAMETERS IN BROILER PRODUCTION

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ABSTRACT

Air quality in intensive broiler production systems is a decisive factor for animal welfare, respiratory health, and production efficiency. Elevated concentrations of ammonia (NH₃) and carbon dioxide (CO₂) lead to respiratory tract irritation, weakened immune responses, and decreased feed efficiency, ultimately reducing growth performance. For this reason, alternative methods to improve house air quality are of considerable interest. The present study aimed to investigate the effects of non-thermal plasma (NTP) application on gas concentrations, feed efficiency, and production parameters in broilers.

The trial was carried out in spring 2025 in Alaçam, Samsun Province (Türkiye), using the slow-growing Hubbard Red JA57 genotype. Two groups were formed: control (no application) and experimental (NTP applied). The production period lasted 76–79 days. Throughout the trial, NH₃ and CO₂ concentrations, feed conversion ratio (FCR), live weight, carcass weight, dressing percentage, mortality, and carcass uniformity were monitored.

Results demonstrated that the experimental group had a 55% reduction in NH₃ and a 45% reduction in CO₂ compared to the control. Furthermore, FCR improved by 7.33%, dressing percentage increased by 12.92%, carcass weight by 9.73%, and live weight by 0.25%. Mortality was lower and carcass uniformity was superior in the NTP group.

In conclusion, NTP application not only improved air quality but also contributed positively to feed efficiency, growth performance, and overall flock health. Thus, non-thermal plasma can be considered a promising biotechnological approach to achieving sustainable and welfare-oriented poultry production.

Keywords: Non-thermal plasma; broiler; air quality; animal welfare

**BEYOND ANTIOXIDANTS: APOPTOTIC PATHWAY MODULATION BY
ONOPORDUM ACANTHIUM IN CANCER CELL LINES AND COMPUTATIONAL
MODELS**

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ABSTRACT

Introduction and Purpose: This research was carried out to investigate the synergistic and anticancer properties of methanol extract of the seed part of *Onopordum acanthium* L. in combination with cisplatin against A549, Caco-2 and MDA-MB-231 cancer cells in vitro and in silico.

Materials and Methods: The cytotoxicity of the plant's methanol extract and cisplatin at different doses was monitored in A549, Caco-2, and MDA-MB-231 cells using the MTT assay. After calculating IC₅₀ values, the synergistic effect was examined using the Chou Talalay method. DPPH, total phenolic, and total flavonoid levels were assessed for the plant's antioxidant properties. Caspase-3 and caspase-8 were measured using ELISA using lysates obtained from 24-hour treatment. In silico, ligand-protein interaction scores were calculated using the AutoDock Vina program.

Results: Application of different doses of cisplatin and methanol extract of the plant for 24 and 48 hours showed antiproliferative effects. IC₅₀ values were determined as 200 µg/ml, 189 µg/ml and 135 µg/ml in A549, Caco-2 and MDA-MB-231 cell lines, respectively (p<0.05). 24-hour treatment led to significant increases in caspase-3 and -8 compared to control groups. In silico analysis, molecular docking showed strong binding scores in its interaction with caspase-3 and -8.

Discussion and Conclusion: The methanol extract of the plant has promising anticancer activity affecting apoptosis pathways in selected cell lines.

Key Words: *Onopordum acanthium* L., Apoptotic Pathway, Computational Models

OVARYUMUNDA İSKEMİ/REPERFÜZYON HASARINA KARŞI RUTİN VE TAKSİFOLİNİN OLASI KORUYUCU ETKİNLİĞİNİN ARAŞTIRILMASI

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ÖZET

Giriş ve Amaç: Ovaryumda kan akışının bozulması (iskemi) sonucu oksidatif stres açığa çıkar. İskemi sonrası oluşan reperfüzyon, oksidatif stresin şiddetini artırabilir. Reperfüzyon sürecinde açığa çıkan reaktif oksijen türleri dolaşıma katılabilir. Sonuç olarak doku ve organ hasarı şekillenebilir. Rutin ve taksifolin gıdalarda bol miktarda bulunan ve kimyasal yapısı nedeniyle güçlü antioksidan maddelerdir. Çalışmada ratlarda ovaryum torsiyon/detorsiyon modelinde, rutin ve taksifolinin olası iyileştirici etkilerinin biyokimyasal ve histolojik analizler ile araştırılması amaçlanmıştır.

Materyal ve Metod: Çalışmada 70 adet Sprague-Dawley rat; kontrol, sham, torsiyon (T), torsiyon/detorsiyon (T/D), torsiyon/detorsiyon+dimetilsülfoksit (T/D+DMSO), torsiyon/detorsiyon+rutin (T/D+DMSO+RUT, 80 mg/kg), torsiyon/detorsiyon+taksifolin (T/D+DMSO+TAX, 40 mg/kg) olmak üzere 7 gruba ayrılmıştır. Plazma örneklerinde glutatyon (GSH), malondialdehit (MDA) düzeyleri ve glutatyon peroksidaz (GPx) aktiviteleri manuel metot ile nitrik oksit (NO) düzeyleri ise ticari kit ile spektrofotometrik olarak ölçülmüştür. Ovaryum dokularında, histolojik muayeneler ve biyokimyasal ölçümler (MDA, serüloplazmin (Cp) ve GSH düzeyleri ile GPx aktiviteleri) spektrofotometrik olarak belirlendi.

Sonuç: Tüm DMSO gruplarında T/D grubuna kıyasla doku MDA düzeyleri azaldı. T/D+DMSO grubunda, T/D grubuna kıyasla plazma GSH düzeyleri ($p<0,001$) ve doku GSH düzeylerinde ($p<0,05$) artış olduğu belirlendi. T/D+DMSO+RUT ve T/D+ DMSO+TAX gruplarında, T/D+DMSO grubuna göre önemli bir azalma gözlenmiştir ($p<0,05$). T/D+DMSO+RUT grubunda, T/D+DMSO grubuna kıyasla doku Cp düzeyleri önemli ölçüde düşüktür ($p<0,01$).

T grubunda foliküler yapılanmada bozulmalar ve atretik folikül tespit edilmiştir. Ek olarak, intersitisyel alanlar ile corpus luteum ve çevresinde hemoraji ve hiperemi gözlenmiştir. T/D grubunda, T grubuna kıyasla daha yoğun bir hiperemi ayrıca yaygın bir hemoraji gözlenmiştir. T/D+DMSO grubunun, folikül yapılarındaki bozulmalar ve doku bütünlüğü açısından bütün T/D gruplarına benzer olduğu görülmüştür. T/D+DMSO grubunda hemorajik ve hiperemik alan yoğunluğunun sham ve T/D+ DMSO+TAX grubuna yakın derecede olduğu gözlenmiştir.

Tartışma ve Sonuç: Güncel çalışmalar sınırlı olmakla birlikte, T/D+DMSO grubunda, T/D grubuna kıyasla, doku MDA düzeylerinin düşük ve doku ile plazma GSH düzeylerinin yüksek olması, DMSO içinde çözündürülen rutin ve taksifolinin doku GSH düzeylerini T/D+DMSO grubuna göre düşürmesi ile histolojik bulgular birlikte değerlendirilmiştir.

DMSO'nun ovaryum T/D hasarında olumlu etkiye sahip bir ajan olabileceği, rutin ve taksifolinin uygulanan dozda ve yöntemle (DMSO ile birlikte verildiğinde) prooksidan etkinlik göstermiş olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: İskemi/Reperfüzyon, Ovaryum, Rutin, Taksifolin, Torsiyon/ Detorsiyon

INVESTIGATION OF THE POSSIBLE PROTECTIVE EFFICACY OF RUTIN AND TAXIFOLIN AGAINST ISCHEMIA/REPERFUSION INJURY IN THE RAT OVARY

ABSTRACT

Introduction and Purpose: Impaired blood flow to the ovary (ischemia) leads to oxidative stress. Subsequent reperfusion can intensify this oxidative stress. During reperfusion, reactive oxygen species are released into the circulation, which may cause tissue and organ damage. Rutin and taxifolin are potent antioxidants abundant in food, thanks to their chemical structure. This study aimed to investigate the potential therapeutic effects of rutin and taxifolin on biochemical and histological parameters in a rat model of ovarian torsion/detorsion.

Materials and Methods: Seventy Sprague-Dawley rats were divided into seven groups: control, sham, torsion (T), torsion/detorsion (T/D), torsion/detorsion+dimethyl sulfoxide (T/D+DMSO), torsion/detorsion+rutin (T/D+DMSO+RUT, 80 mg/kg), and torsion/detorsion+taxifolin (T/D+DMSO+TAX, 40 mg/kg). Plasma levels of glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx) activity were measured manually, while nitric oxide (NO) levels were assessed spectrophotometrically using a commercial kit. In ovarian tissues, histological examinations and biochemical measurements (MDA, ceruloplasmin (Cp) and GSH levels, and GPx activity) were also conducted spectrophotometrically.

Results: Tissue MDA levels decreased in all DMSO groups compared to the T/D group. The T/D+DMSO group showed increased plasma GSH levels ($p<0.001$) and tissue GSH levels ($p<0.05$) compared to the T/D group. A significant decrease was observed in the tissue GSH levels of the T/D+DMSO+RUT and T/D+DMSO+TAX groups compared to the T/D+DMSO group ($p<0.05$). Additionally, tissue Cp levels were significantly lower in the T/D+DMSO+RUT group compared to the T/D+DMSO group ($p<0.01$).

Histologically, follicular damage and atretic follicles were noted in the T group. Hemorrhage and hyperemia were also observed in interstitial areas and around the corpus luteum. The T/D group exhibited more intense hyperemia and widespread hemorrhage than the T group. The T/D+DMSO group showed similar follicular damage and tissue integrity to the other T/D groups, but the intensity of hemorrhagic and hyperemic areas was closer to that of the sham and T/D+DMSO+TAX groups.

Discussion and Conclusion: Although current studies are limited, a combined evaluation of the findings suggests that DMSO may act as a beneficial agent in ovarian torsion/detorsion injury. This is supported by the lower tissue MDA levels and higher tissue and plasma GSH levels in the T/D+DMSO group compared to the T/D group. Furthermore, rutin and taxifolin, when administered with DMSO at the given doses, may have exhibited a pro-oxidant effect, as evidenced by the decrease in tissue GSH levels compared to the T/D+DMSO group.

Keywords: Ischemia/Reperfusion, Ovary, Rutin, Taxifolin, Torsion/Detorsion

INVESTIGATION OF THE ANTIOXIDANT CONTENT OF ISABELLA GRAPE (VITIS LABRUSCA L.)

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ABSTRACT

Objective: Isabella grape (*Vitis labrusca* L.) is a grape variety widely cultivated in the Black Sea Region, notable for its high polyphenol content and bioactive compounds. There is an inverse relationship between diets rich in antioxidants and the incidence of chronic diseases. Bioactive compounds found in natural products help prevent these conditions by scavenging reactive oxygen species responsible for disease incidence. This study aimed to investigate the total phenolic and flavonoid contents as well as the antioxidant capacities of different extracts obtained from the pulp and skin of Isabella grape.

Material and Methods: Mature *Vitis labrusca* grapes grown in Ankara were used in this study. The pulp and skin portions of the grapes were extracted using water, methanol, and ethanol as solvents. Each extraction was performed in triplicate, and DPPH radical scavenging activity, total polyphenol, and flavonoid contents were analyzed spectrophotometrically.

Results: The phenolic contents of grape skin in ethanolic, methanolic, and aqueous extracts were found to be 2.42 ± 0.02 mg/g GAE, 2.09 ± 0.18 mg/g GAE and 1.13 ± 0.10 mg/g GAE respectively; whereas in the pulp they were 1.04 ± 0.20 mg/g GAE, 0.55 ± 0.20 mg/g GAE and 0.35 ± 0.06 mg/g GAE. The flavonoid contents of grape pulp in ethanolic, methanolic, and aqueous extracts were 1.42 ± 0.29 mg/g RE, 0.86 ± 0.17 mg/g RE and 0.37 ± 0.15 mg/g RE, respectively; while in the skin they were 3.54 ± 0.29 mg/g RE, 2.98 ± 0.23 mg/g RE and 1.18 ± 0.08 mg/g RE. DPPH radical scavenging activity for grape pulp in ethanolic, methanolic, and aqueous extracts was 345.87 ± 92.83 μ g/g GAE, 204.93 ± 28.71 μ g/g GAE and 100.78 ± 15.90 μ g/g GAE respectively; and for the skin, 692.46 ± 32.94 μ g/g GAE, 559.93 ± 21.30 μ g/g GAE and 283.09 ± 33.11 μ g/g GAE. It was determined that the grape skin exhibited higher total phenolic content and antioxidant activity, with the ethanolic extract showing the strongest radical scavenging capacity.

Conclusion: *Vitis labrusca* possesses strong antioxidant properties, particularly due to the polyphenolic compounds concentrated in its skin. It may play a supportive role as a functional food in the prevention of chronic diseases associated with oxidative stress. Considering the notably high antioxidant potential of *V. labrusca*, further studies are needed to analyze its phenolic components and to evaluate the effects of its ethanolic extracts in experimental animal models.

Keywords: *Vitis labrusca*, antioxidant capacity, polyphenol, flavonoid

INTRODUCTION

In recent years, the health problems caused by oxidative stress not only reduce individual quality of life but also increase global healthcare expenditures.

Oxidative stress occurs as a result of free radicals and reactive oxygen species (ROS) attacking cellular components, leading to serious issues such as lipid peroxidation of cell membranes, protein oxidation, and DNA damage (Halliwell & Gutteridge, 2015). Health problems associated with oxidative stress include cardiovascular diseases, cancer, diabetes, neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), and premature aging (Lobo et al., 2010).

Vitis labrusca L., commonly known as fox grape, is a rich source of natural antioxidants such as phenolic compounds, flavonoids, and anthocyanins. However, the biochemical properties and health benefits of this natural resource have not been sufficiently investigated in Türkiye, which limits efforts to fully reveal its potential. Although studies have demonstrated the ability of this grape species to neutralize free radicals and combat oxidative stress, further research is needed to clarify its phenolic profile and biological activities in comparison with other grape species.



Figure 1. *Vitis labrusca*

Vitis labrusca L. is an important species widely used in the production of wine, fruit juice, jam, and similar products. In addition to its strong aromatic characteristics, this grape species is notable for its resilience (Da Silva & Bordignon-Luiz, 2010). Being rich in bioactive compounds such as phenolic substances, flavonoids, and anthocyanins renders it a natural source of antioxidants.

The grape species known as “fragrant grape” is widely cultivated in the Black Sea Region of Türkiye and is also referred to by various names depending on regional differences, such as Isabella, black grape, strawberry grape, or American grape. Provinces such as Samsun, Bartın, Ordu, and Zonguldak provide suitable climatic conditions for its cultivation (Koca et al., 2021). This variety is also naturally grown in humid and cold climate regions such as the United States, Brazil, Russia, Canada, the United Kingdom, Japan, and Germany, and is distinguished by its resistance to fungal diseases. In Türkiye, beyond fresh consumption, it is traditionally utilized in the preparation of products such as molasses (pekmez), fruit leather (pestil), and pepeçura (a regional grape-based dessert) (Ertaş & Karadağ, 2013).

Different anatomical parts of *Vitis labrusca*, including the skin, seeds, and pulp, contain high levels of phenolic compounds and other bioactive substances. These features contribute to its strong antioxidant capacity and highlight its potential to provide various health benefits (Da Silva & Bordignon-Luiz, 2010).

The antioxidant capacity of *Vitis labrusca* grapes is influenced by various factors such as geography, soil characteristics, agricultural practices, climate, ripening stage, harvest time, and genotype, which lead to significant differences in the phenolic content and biochemical profiles of grapes and other plants (Domingues Neto et al., 2024).

Therefore, in this study, the antioxidant capacity of *V. labrusca* grapes grown in the Ankara region will be compared, and the effects of different extraction solvents (water, methanol, ethanol) on phenolic and flavonoid contents as well as radical scavenging activities will be evaluated.

MATERIAL AND METHOD

In this study, mature *Vitis labrusca* grapes cultivated in Ankara province were used as the experimental material. Following harvest, the fruits were promptly transported to the laboratory and initially stored at +4 °C to prevent spoilage. After separation of the pulp and skins, the samples were homogenized and stored at -20 °C. Extraction procedures were carried out using water, methanol, and ethanol as solvents, and each extraction was performed in three parallel replicates. The antioxidant potential of the obtained extracts was evaluated based on DPPH radical scavenging activity (Shirazi et al., 2014), the total phenolic content was determined according to the Folin–Ciocalteu method (Shirazi et al., 2014), and the total flavonoid content was quantified spectrophotometrically by employing a modified version of the method described by Boateng et al. (2008).

Statistical analyses were conducted in two different ways. To determine whether the solvents used in the extraction methods caused significant differences in the extraction contents, a one-way ANOVA test was applied. Since the variances were distributed homogeneously in pairwise comparisons, the Tukey test was used. To evaluate the statistical difference between the peel and pulp, each analysis was separately analyzed using an independent samples t-test. Differences were considered statistically significant when the p-value was less than 0.05. All statistical analyses were performed using the SPSS 21.00 software.

RESULTS

In the skin, phenolic content of ethanolic, methanolic, and aqueous extracts was 2.42 ± 0.02 mg/g GAE, 2.09 ± 0.18 mg/g GAE and 1.13 ± 0.10 mg/g GAE, respectively; whereas in the pulp it was 1.04 ± 0.20 mg/g GAE, 0.55 ± 0.20 mg/g GAE and 0.35 ± 0.06 mg/g GAE. Flavonoid content in the pulp was 1.42 ± 0.29 mg/g RE, 0.86 ± 0.17 mg/g RE and 0.37 ± 0.15 mg/g RE; while in the skin it was 3.54 ± 0.29 mg/g RE, 2.98 ± 0.23 mg/g RE and 1.18 ± 0.08 mg/g RE. DPPH radical scavenging activity of the pulp was 345.87 ± 92.83 µg/g GAE, 204.93 ± 28.71 µg/g GAE and 100.78 ± 15.90 µg/g GAE, whereas in the skin it was 692.46 ± 32.94 µg/g GAE, 559.93 ± 21.30 µg/g GAE and 283.09 ± 33.11 µg/g GAE. These findings indicate that grape skin contains higher levels of phenolics, flavonoids and exhibits stronger antioxidant activity, with the ethanolic extract showing the highest radical scavenging capacity.

In the comparison between the peel and pulp, the phenolic content of the peel was found to be significantly higher than that of the pulp. In the peel, there was no significant difference between the methanolic and aqueous extracts, while both methanolic and aqueous extracts differed significantly from the ethanolic extract ($p < 0.05$).

Regarding the flavonoid content of the fruit, all three extraction methods (MeOH, EtOH, and water) showed statistically significant differences. In addition, the comparison between the peel and pulp revealed that the flavonoid content of the peel was significantly higher than that of the pulp. For the peel, no significant difference was observed between the aqueous and methanolic extractions, whereas significant differences were detected between ethanolic–methanolic and ethanolic–water extractions. In the fruit, the aqueous extraction yielded significantly lower results compared to both ethanolic and methanolic extractions, while no significant difference was found between ethanolic and methanolic ($p > 0.05$).

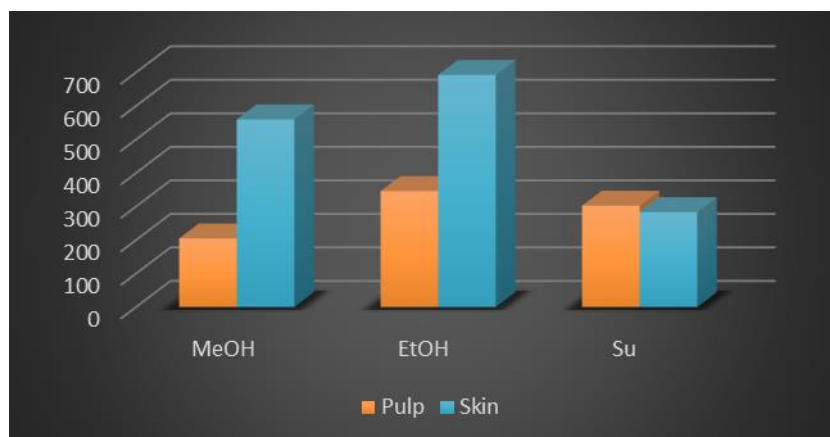


Figure 1. DPPH ($\mu\text{g/g}$)

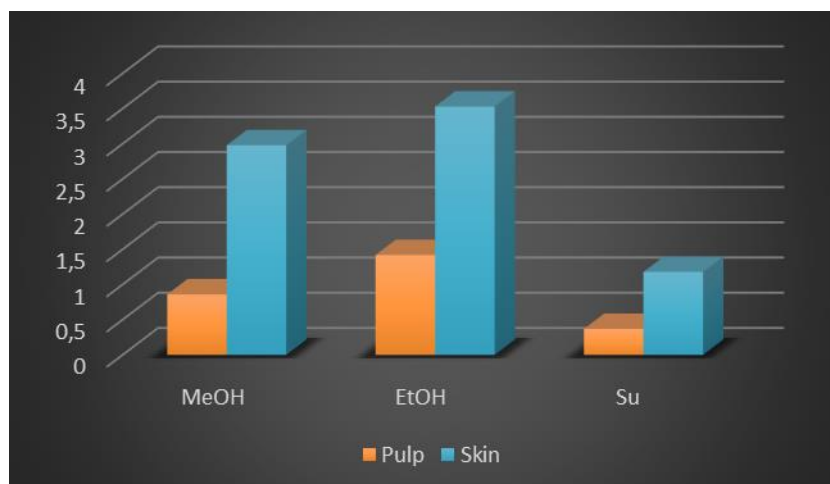


Figure 2. Total Flavonoid Content (mg/g)

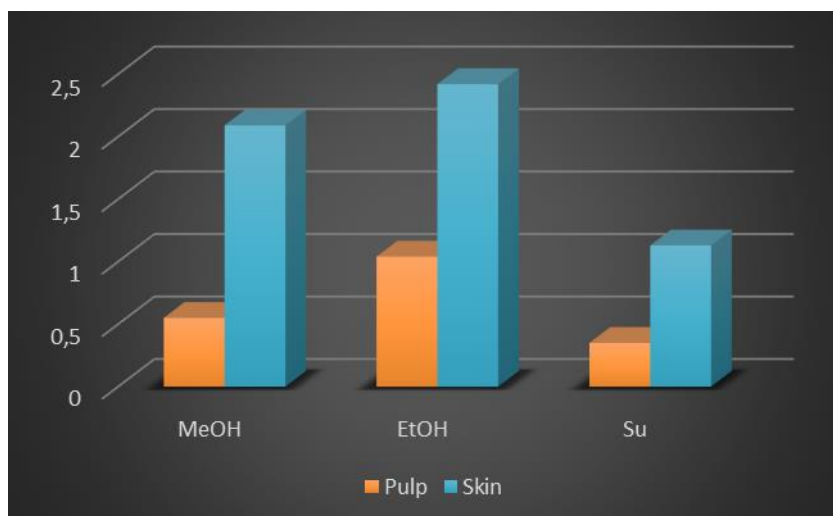


Figure 3. Total Phenolic Content (mg/g)

DISCUSSION AND CONCLUSION

Rockenbach et al. (2011) reported that grape seeds generally exhibited higher total phenolic content, flavanol levels, and antioxidant capacity compared to grape skins. However, a different trend was observed in Isabel grapes (*Vitis labrusca*). In this variety, the skin demonstrated antioxidant activity levels, particularly in terms of DPPH and FRAP (Ferric Reducing Antioxidant Power) values, that were comparable to or even higher than those of the seeds. This finding suggests that grape varieties derived from *Vitis labrusca* may possess phytochemical profiles distinct from those of *Vitis vinifera*.

In the study by Do et al. (2014) on *Limnophila aromatica*, the authors emphasized that solvent selection plays a decisive role in the amount of compounds extracted and in the antioxidant activity. They demonstrated that ethanol can be as effective as methanol, and in some cases even superior, in extracting phenolic and flavonoid compounds.

In their study on different grape genotypes (Cabernet Sauvignon, Kalecik Karası, Öküzgözü, Alicante Bouschet, table grape varieties such as Alphonse Lavallée, Hafızali, and Trakya İlkeren, as well as five different wild grapevines), Yeğin and Uzun (2018) reported that the highest phenolic content and antioxidant activity were found in the seed fractions, followed by the skins.

In the case of *Vitis labrusca*, it was observed that the skins, in particular, exhibited strong antioxidant effects due to their high concentration of polyphenolic, flavonoid compounds. Among the solvents used for extraction, the highest antioxidant potential values were obtained with ethanol extractions. *V. labrusca* may play a supportive role as a functional food in the prevention of oxidative stress-related chronic diseases. Considering its particularly high antioxidant potential, further studies are needed to analyze the phenolic compounds of *V. labrusca* and to investigate the effects of its ethanol extracts in animal models.

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ANTIOXIDANT CONTENT OF CARDAMOM OIL EXTRACT AND ITS EFFECTS ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS (CLL)

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ABSTRACT

Introduction and Purpose: Cardamom is a spice belonging to the Zingiberaceae family and contains bioactive compounds such as limonene, cymene, pinene, linalool, borneol, cardamonin, indole-3-carbinol, diindolylmethane, α -terpenyl acetate and 1,8-cineole. It is known to provide numerous health benefits such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and cancer risk reducing potential due to the bioactive compounds it contains. This investigation focused on the evaluation of total phenolic and flavonoid content, antioxidant capacity, and the anticancer activity of cardamom oil in the MEC-1 cell line.

Materials and Methods: The DPPH radical scavenging activity, total polyphenol and flavonoid content of cardamom oil methanolic extract were analyzed spectrophotometrically. The effects of cardamom oil on MEC-1 cell line were evaluated using MTT method.

Results: In the methanol extract of cardamom oil, total flavonoid content was found as 20.92 mg/g RE (Rutin Equivalent), total polyphenol substance content as 4.05 mg/g GAE (Gallic Acid Equivalent), and DPPH radical scavenging activity as 0.01 ng/g GAE. For MTT analysis, cardamom oil was applied to the MEC-1 cell line at different concentrations (100 - 0 mg/ml). The most pronounced cytotoxic effect was observed at a concentration of 100 mg/ml, resulting in a reduction in cell viability to 43.99%. Following application of cardamom oil at a concentration of 50 mg/ml, 53.325% cell viability was observed in the MEC-1 cell line. A dose-dependent decrease in cell proliferation was observed.

Discussion and Conclusion: The dose-dependent reduction observed in cancer cell proliferation following cardamom oil treatment is a promising indicator of its anticancer activity and is consistent with previous studies reporting the cytotoxic effects of cardamom oil on various cancer cell lines. Further research is needed to fully explore the therapeutic potential of cardamom oil, particularly given its anticancer effects. In conclusion, this study is significant in demonstrating the health benefits of cardamom oil, especially its therapeutic potential in cancer treatment.

Keywords: Anti-cancer, cardamom, chronic lymphocytic leukemia, MEC-1, MTT.

INTRODUCTION

Cardamom is a plant belonging to the Zingiberaceae family that is widely used in traditional medicine (Ahamad, 2024). Due to its therapeutic benefits, it is used in the treatment of various conditions such as stomach, heart, kidney, tooth and gum infections, asthma, cataracts, and diarrhea. It is also commonly used in the kitchen as a spice and seasoning to enhance the flavor and aroma of various food products (Ashokkumar et al., 2020; Kumar and Kumari, 2021).

Cardamom contains various bioactive compounds such as limonene, simene, pinene, linalool, borneol, cardamone, indole-3-carbinol, diindolylmethane, α -terpinyl acetate, and 1,8-cineole (Kumar et al., 2005; Savan and Küçükbay, 2013; Husain and Ali, 2014; Abdullah Asghar et al., 2017; Özkan et al., 2018; Vukovic et al., 2022; Tarfaoui et al., 2022; Sreedharan et al., 2023). Cardamom is known to exhibit antioxidant (Elguindy et al., 2018), antimicrobial, anti-inflammatory (Souissi et al., 2020), antidiabetic (Yahyazadeh et al., 2021), and anticancer (Manjunath et al., 2021; Zengin et al., 2022; Ahamad, 2024) effects because of the compounds it contains.

The chemical compounds contained in cardamom oil primarily target the lignin-1 gene, which plays a role in programmed cell death, which is more prevalent in cancer cells than in healthy cells (Bano et al., 2024). The main components of cardamom essential oil, 1,8-cineole and limonene, block cyclooxygenase-2 and cytochrome P450 activities and are known to downregulate various signaling molecules (Vutakuri and Somara, 2018).

Phytochemicals such as diindolylmethane (DIM) and indole-3-carbinol (I3C), obtained from plants, affect cancer pathways. I3C is reported to be a phytochemical found in black cumin that reduces metastasis, tumor formation, and immunomodulation and triggers the apoptotic pathway (Aggarwal and Ichikawa, 2005). Studies have shown that I3C induces the apoptotic pathway in the MCF-7 cell line (Weng et al., 2008). Studies have reported that cardamom induces apoptosis by affecting the Wnt/ β -catenin, NF- κ B, and PI3K/Akt signaling pathways; it also exhibits properties that inhibit cell proliferation, migration, and invasion (Ramchandani et al., 2020).

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries and is a highly heterogeneous disease. Immunoglobulin heavy chain variable region (IGHV) mutation status, cytogenetic alterations, and CD38 and ZAP-70 expression levels contribute to this heterogeneity. Disease control is particularly challenging in CLL patients with 17p deletion. Therefore, this study investigated the effects of cardamom oil on cell proliferation in the human CLL cell line MEC-1, which carries a p53 deletion/mutation (Liu et al., 2013).

This investigation focused on the evaluation of total phenolic and flavonoid content, antioxidant capacity, and the anticancer activity of cardamom oil in the MEC-1 cell line.

MATERIALS and METHODS

DPPH* Radical Scavenging Activity Analysis

The free radical scavenging activity analyses of the methanolic extract of cardamom oil were performed using the 2,2-diphenyl-1-picrylhydrazil (DPPH*) scavenging activity method, according to the method of Shirazi et al. (2014). Prior to analysis, the DPPH* solution and gallic acid stock solution were freshly prepared and read at 515 nm using a UV-spectrophotometer.

Total Polyphenol Content Analysis

The determination of the total phenolic content of the methanolic extract of cardamom oil was performed according to the method of Shirazi et al. (2014). Prior to analysis, the Folin-Ciocalteu reagent, Na_2CO_3 , and gallic acid stock solution were freshly prepared, and readings were taken at 760 nm using a UV-spectrophotometer.

Total Flavonoid Content Analysis

The determination of the total flavonoid content in the methanolic extract of cardamom oil was performed according to the method of Boateng et al. (2008). Prior to analysis, Sodium Nitrite (NaNO_2), Aluminum Chloride (AlCl_3), Sodium Hydroxide (NaOH), and Rutin stock solutions were freshly prepared and measured at 510 nm using a UV-spectrophotometer.

Total phenolic content, total flavonoid content, and free radical scavenging activity analyses were performed in triplicate on the methanolic extract of cardamom oil.

Antiproliferative Effect Analysis

Cardamom oil was dissolved in DMSO and then diluted with RPMI medium to concentrations ranging from 0 to 100 mg/ml for cell culture applications. The effects of cardamom oil on the MEC-1 cell line were determined using the MTT assay (Meral, 2018), and IC_{50} values were determined.

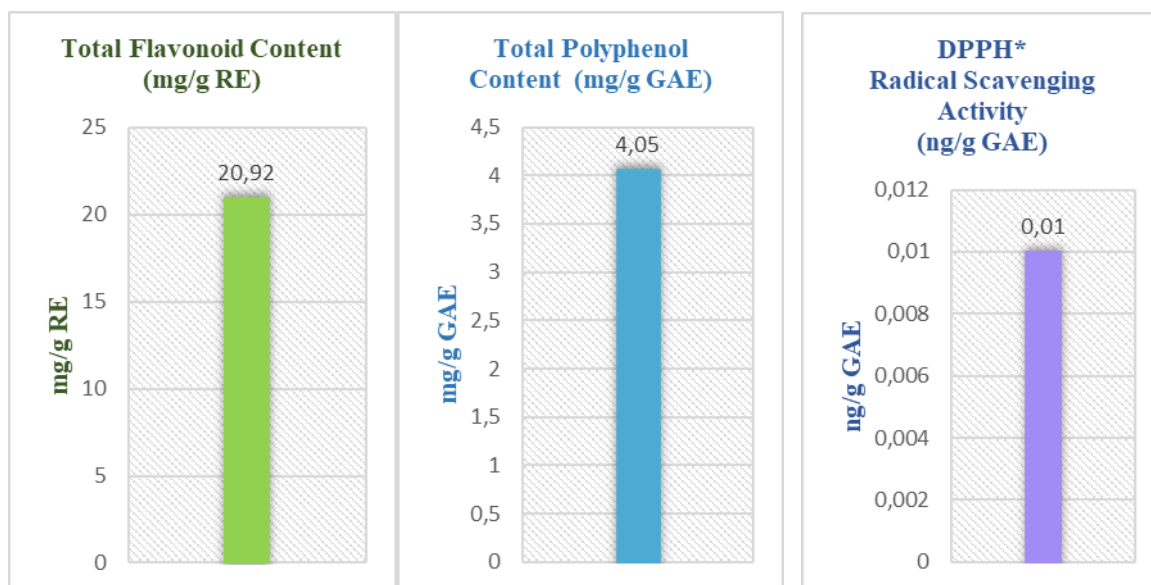


Figure 1. The total flavonoid content in the methanol extract was determined to be 20.92 mg/g RE (Rutin Equivalent), the total polyphenol content was 4.05 mg/g GAE (Gallic Acid Equivalent), and the DPPH radical scavenging activity was 0.01 ng/g GAE.

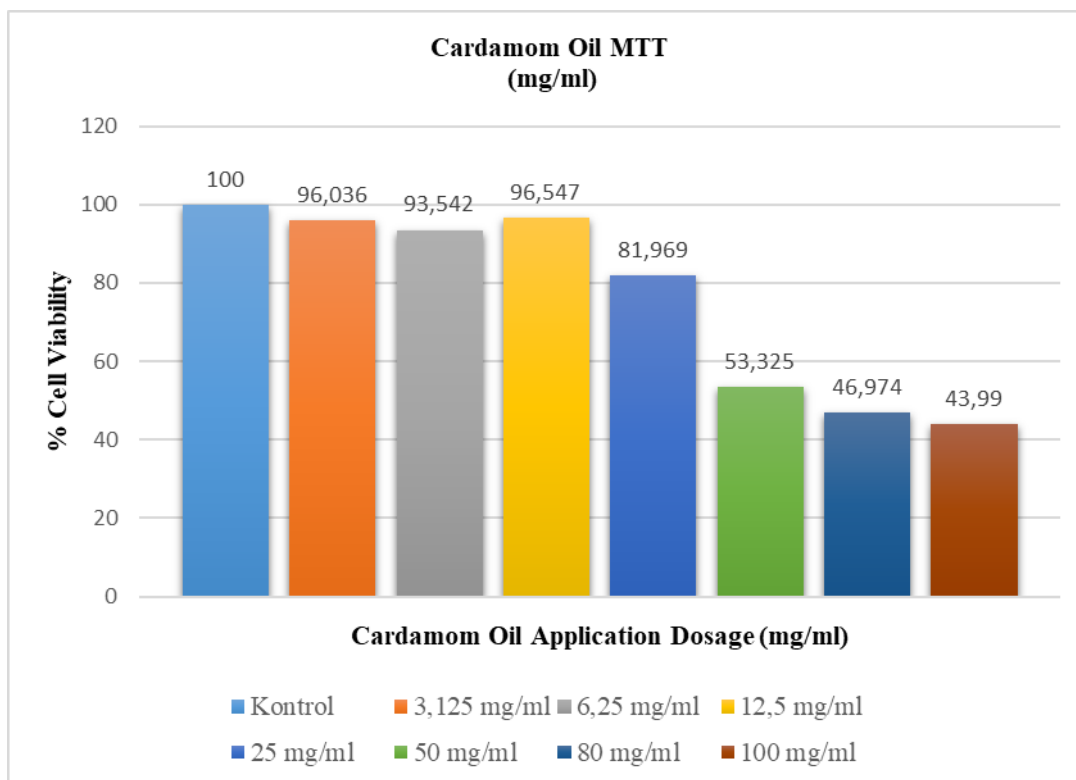


Figure2. In the MTT assay, cell viability decreased to 43.99% following the application of 100 mg/mL cardamom oil. At a concentration of 50 mg/mL, cell viability was observed to be 53.33%. A dose-dependent decrease in cell proliferation was detected in relation to cardamom oil.

CONCLUSION AND DISCUSSION

In the literature, chromatographic techniques have generally been preferred for determining the levels of volatile oil, phenolic compounds, and flavonoids in cardamom. However, in this study, a content analysis focusing on total phenolic and flavonoid compound levels was performed to evaluate the general presence of these compounds. Previous studies have shown that cardamom contains phenolic compounds such as luteolin and gallic acid, as well as various flavonoids such as quercetin, catechin, myricetin, and apigeninidin. In addition, major volatile oil components such as 1,8-cineole and α -terpinyl acetate have also been identified (Alkhalifah et al., 2022; Ahmad, 2024).

In a study conducted by Ahmad (2024), the anticancer activity of cardamom essential oil was evaluated using the MTT assay on MDA-MB-231, U-87, and HEK293 cell lines. Specifically, its main components, 1,8-cineole and α -terpinyl acetate, revealed cardamom oil to be a potential source of natural compounds with anticancer properties. These findings are consistent with previous studies reporting the cytotoxic effects of cardamom essential oil on various cancer cell lines. The study reported that cardamom essential oil exhibited significant cytotoxic effects against MDA-MB-231 and HEK293 cell lines, but this effect was limited in U87 glioblastoma cells. It was suggested that this situation may be related to the low sensitivity, aggressive biological behavior, and resistance to many chemotherapeutic agents of glioblastoma cells. The cytotoxic effect results obtained in this study are generally consistent with this study.

However, factors such as the difference in the cell lines used, the geographical region where cardamom essential oil was obtained, the cultivation conditions, the extraction method, and the variability in the ratios of bioactive compounds it contains may explain the observed differences in effect. The findings support the evaluation of cardamom essential oil as a potential anticancer agent.

In a study conducted by Manjunath and Mahurkar (2021), the in vitro cytotoxic effects of cardamom, lemon, and jasmine oils on A431, MKN-45, and U-87 MG cancer cell lines were investigated. Phytochemical analyses, which focused particularly on cardamom oil, detected the presence of carbohydrates and flavonoids. Flavonoids were analyzed using the Shinoda test, and the oils were dissolved in 70% ethanol for content analysis. According to the MTT test results, a decrease in cell proliferation was observed with increasing doses in all three cell lines. These findings are generally consistent with this study. Of course, differences in the cell lines used, the extraction method, and the content analysis methods can be considered factors affecting the observed results.

In a study conducted by Lio et al. (2019), the effects of cardamonin, an active compound found in cardamom, were evaluated on the WEHI-3 murine leukemia cell line. The study showed that cardamonin reduced cell viability by inducing apoptotic cell death. Furthermore, treatment with cardamonin was shown to increase intracellular reactive oxygen species (ROS) levels and calcium (Ca^{2+}) production, thereby triggering ROS-mediated apoptotic pathways. Additionally, a decrease in mitochondrial membrane potential and upregulation of caspase-3, caspase-8, and caspase-9 enzymes were observed. The decrease in cell viability reported in this study is generally consistent with the findings of this research. In particular, the cytotoxic effects and potential apoptosis-inducing properties of cardamom essential oil are supported by this literature. Despite methodological differences, such as the use of a different cell line and the application of an isolated compound (cardamom), the main findings are consistent.

The dose-dependent decrease in cell proliferation observed after cardamom oil application indicates the anticancer potential of cardamom oil and is consistent with previous studies reporting the cytotoxic effects of cardamom oil on various cancer cell lines. The therapeutic potential of cardamom oil, particularly in the context of cancer treatment, requires more comprehensive investigation. This study is significant in that it highlights the positive effects of cardamom oil on health, particularly its anticancer properties.

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NEOPTERIN AND PROCALCITONIN LEVELS IN SHEEP INFECTED WITH ECTHYMA

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ABSTRACT

Objective: Ecthyma is a viral infection of sheep and goats that is common all over the world. Ecthyma, which is especially common in lambs and kids with low immune system, is a zoonotic disease that can also be seen in humans if contact with infected animals. It is more common in young animals than in adults. Although morbidity is high, mortality rate is low. Biomarkers used in the diagnosis and prognosis evaluation of diseases are biological indicators that reveal physiological and pathological changes in the organism. Neopterin and procalcitonin, which are routinely used in human medicine, have found wide use and are widely used in the diagnosis and prognosis of many diseases. For these reasons, this study was conducted to determine neopterin and procalcitonin levels in sheep infected with ecthyma.

Materials and Methods: A total of 25 clinically diagnosed ecthyma-infected sheep and 15 healthy sheep were used. The samples taken from the vena jugularis of the sheep in tubes without anticoagulant were centrifuged at 3000 rpm for 15 minutes and serum was obtained. Samples were stored at -20 °C until analysis.

Results: When ecthyma-infected animals were compared with healthy sheep, serum neopterin and procalcitonin levels were found to be increased.

Conclusion: It was concluded that neopterin and procalcitonin can be used as inflammation markers.

Keywords: Ecthyma, Neopterin, Procalcitonin

GİRİŞ

Ektima (contagious ecthyma; orf, soremouth), küçük ruminantlarda -özellikle kuzu ve oğlaklarda- yaygın olarak görülen, dermatotropik bir Parapoxvirus'un neden olduğu akut veya subakut püstüler dermatit tablosudur (Kumar ve ark. 2015). Hastalık tipik olarak dudaklarda, ağız ve burun mukozasında, püstül veya boz esmer renkte karnabakar görünümlü kabuk oluşumu, ateş, yeme güçlüğü gibi semptomlara neden olmaktadır (Teshale ve Alemayehu 2018). Klinik seyir genellikle kendi kendini sınırlayıcı olmakla birlikte (Özellikle tek başına viral etken varlığında), lezyonların ağırlı olması nedeniyle beslenme bozuklukları, kilo kaybı ve üretim kayıpları görülebilir. Ayrıca lezyon sahalarında gelişen sekonder bakteriyel enfeksiyonlar morbiditeyi artırır. Ektimanın dünyada yaygın olması, sürü yönetimi ve halk sağlığı açısından önemini artırmaktadır, çünkü hastalık zoonotik olup enfekte hayvanlarla veya kontamine kabuklarla temas yoluyla insanlara da geçebilir.

Etiyolojik ajan olan Orf virüsü (Parapoxvirus familyası), epitel hücrelerine tropizmi olan ve epiderminin yeniden yapılanma fazındaki keratinositlerde replike olma eğiliminde bir virüstür. Virüsün epitelyal hücrelerdeki replikasyonu ve lezyon oluşturmaları, doku hasarı ile birlikte lokal inflamatuvar yanıtı tetikler. Bu yanıt hem hücresel hem de humoral bileşenleri içermektedir. Ayrıca, lezyonların kabuklanması ve dış ortamda uzun süre canlılığını koruyabilen viral partiküller, sürü içi yayılım ve fomite aracılığıyla bulaşma riskini yükseltir. Bu faktörler, ektimanın kontrolünü zorlaştırmakta ve sürülerde tekrarlayan enfeksiyon odaklarına neden olabilmektedir (Teshale ve Alemayehu 2018, Karki ve ark. 2019).

Klinik tanı; karakteristik lezyon görünümü ve risk faktörleri ile konulabilse de kesin tanı moleküler yöntemler (PCR) veya virüs izolasyonu ile yapılmaktadır. Tanı ve ayırıcı tanıda lezyonların bakteriyel püstüller dermatitlerden, veziküler hastalıklardan veya diğer dermatozlardan ayırt edilmesi gerekir. Hastalığın kontrolü; iyi sürü yönetimi, enfekte bireylerin izolasyonu, hijyen önlemleri, yara bakım uygulamaları, gerektiğinde sekonder bakteriyel enfeksiyonlara yönelik antibakteriyel tedaviler ve riskli durumlarda aşı uygulamaları ile sağlanmaktadır (Hajkazemi ve ark. 2016).

Ektimanın patofizyolojisi temel olarak lokal viral replikasyon ve buna eşlik eden yerel inflamasyon olsa da lezyonlarda gözlenen sekonder bakteriyel enfeksiyonlar veya sistemik etkilenme durumlarında konak immün yanıtının niteliği ve şiddeti klinik seyri belirleyen önemli faktörlerdir. Bu nedenle, enfeksiyon sırasında konak immün yanıtını kantitatif olarak değerlendirebilecek güvenilir biyobelirteçlerin belirlenmesi hem hastalığın şiddetinin objektif saptanması hem de sekonder bakteriyel enfeksiyonların ayırt edilmesi açısından önem taşır. Geleneksel hematolojik ve akut faz parametrelerinin yanında immünolojik göstergeler (Örneğin hücresel aktivasyonu ve interferon- γ yolağını yansıtan ölçütler) ile sistemik inflamasyon belirteçlerinin birlikte değerlendirilmesi, hastalığın fizyopatolojisini daha iyi anlamaya yardımcı olur. Neopterin; insan ve hayvan immünolojisinde hücresel immün yanıtın (Özellikle Th1 tipi yanıt ve interferon (IFN)- γ aracılı aktivasyonun) duyarlı bir biyokimyasal göstergesi olarak kabul edilen bir pteridin türevidir. Makrofaj ve monositlerin IFN- γ uyarısıyla tetiklenmesi sonucunda artan neopterin üretimi, hücresel bağışıklığın aktivasyonunu dolaylı olarak yansıtır (Michalak ve ark. 2017). Veteriner pratikte neopterin düzeyleri; viral, bakteriyel veya paraziter enfeksiyonlar sırasında, immün sistem aktivasyonunu değerlendirmek ve hastalık seyrini izlemek amacıyla çalışılmıştır. Bunun yanı sıra neonatal sepsis, solunum yolu enfeksiyonları ve diğer sistemik enflamatuvar durumlarda neopterin artışı bildirilmiştir. Bu özellikleri nedeniyle neopterin, ektima gibi lokal ve sistemik immün yanıtı uyarabilen viral enfeksiyonlarda hücresel immün aktivitenin değerlendirilmesi için uygun bir aday biyobelirteç olarak öne çıkmaktadır. Prokalsitonin (PCT) ise tiroid C-hücrelerinde üretilen kalsitonin hormonunun öncül peptidi olup sistemik bakteriyel enfeksiyon ve sepsiste plazma düzeyleri belirgin şekilde yükselme eğilimindedir. İnsan hekimliğinde PCT, bakteriyel ile viral enfeksiyonların ayırt edilmesinde ve sepsis tanı/tedavi takibinde yaygın şekilde kullanılan bir biyomarkerdir (Lee 2013). Veteriner alanda da son yıllarda prokalsitoninin hayvansal enfeksiyonların şiddetini değerlendirmede ve bakteriyel komplikasyonları saptamada faydalı olabileceğini gösteren çalışmalar artmaktadır. Koyunlarda solunum yolu enfeksiyonları gibi durumlarda PCT ve diğer immün belirteçlerin diagnostic/prognostik değerleri araştırılmıştır. Ektimada primer etken viral olmakla birlikte lezyonlarda sık görülen sekonder bakteriyel kolonizasyon ve yaygın inflamasyon, PCT'nin sistemik yansımalarını tetikleyebilir. Bu nedenle PCT ölçümü sekonder bakteriyel enfeksiyonların ve sistemik inflamasyonun göstergesi olarak değerli bilgiler sağlayabilir. Bu nedenlerden dolayı çalışma ektima ile enfekte koyunlarda neopterin ve prokalsitonin düzeyinin belirlenmesi amacıyla yapıldı.

MATERYAL VE YÖNTEM

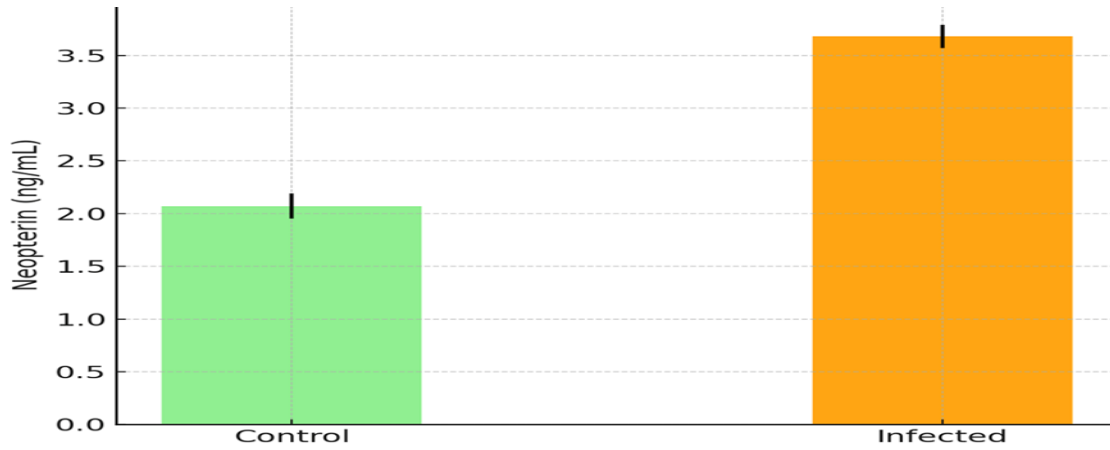
Çalışmada 25 adet ektimalı ve 15 adet sağlıklı koyun kullanıldı. Hayvanların *vena jugularis*'inden kan örnekleri antikoagülsüz tüplere alındı. Daha sonra alınan kan örnekleri at 3000 rpm'de 15 dakika santrifüj edilerek serumları elde edildi. Elde edilen serumlar analiz yapılncaya kadar -20 °C'de saklandı. Çıkarılan serum örneklerinde neopterin ve prokalsitonin (Mybiosource ELISA kit) düzeyi kolorimetrik olarak belirlendi.

ARAŞTIRMA BULGULARI VE TARTIŞMA

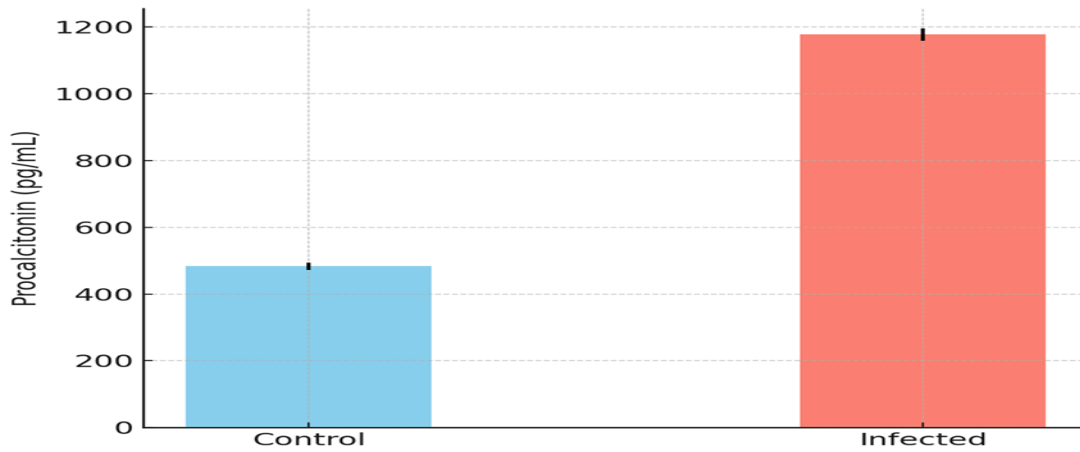
Ektima ile enfekte hayvanlar ile sağlıklı koyunlar karşılaştırıldığında serum neopterin ve prokalsitonin düzeylerinin arttığı saptandı.

Tablo 1. Klinik olarak sağlıklı ve ektima ile enfekte koyunlarda prokalsitonin ve neopterin düzeyleri

Parametreler	Kontrol	Enfekte	P
Neopterin (ng/mL)	2,07±0,12	3,68±0,11	P<0,001
Prokalsitonin (pg/mL)	483,27±11,71	1177,23±18,52	P<0,001



Şekil 1. Ektima ve sağlıklı koyunlarda serum neopterin düzeyi (P<0,001).



Şekil 2. Ektima ve sağlıklı koyunlarda serum prokalsitonin düzeyi (P<0,001).

Yapılan klinik muayene sonucu ektima ile enfekte hayvanlarda dudaklarda, ağız ve burun mukozasında, püstül veya boz esmer renkte karnabahar görünümlü kabuk oluşumu, ateş, yeme güçlüğü gibi semptomlar belirlendi.

Yangı, doku hasarı ve enfeksiyon sonucunda karaciğerde yangısal proteinler sentezlenmektedir. Bu proteinlerden olan ve beşeri hekimlikte rutinde kullanılan neopterin ve prokalsitonin geniş kullanım alanı bulmuş ve birçok hastalığın tanısı ve prognozunun belirlenmesinde yaygın olarak kullanılmaktadır.

Özellikle viral enfeksiyonlar başta olmak üzere, karaciğer, üriner ve solunum sistemi gibi birçok sistem hastalıkları neopterin düzeyinin yükselmesine neden olmaktadır (Ünüvar ve Aslanhan 2019). Yapılan çalışmalarda; omfalitisli buzağılarda (Bozukluhan ve ark. 2023) ve aspirasyon pnömonili buzağılarda (Akyüz ve ark. 2022) neopterin düzeyinin arttığı bildirilmektedir. Çalışmada da kontrol grubuna göre ektimalı grupta neopterin düzeyinin artması mononükleer fagositik hücrelerin uyarılması kaynaklı olabilir.

Prokalsitonin, özellikle bakteriyel enfeksiyonlarda oluşan yangısal cevabı izlemede kullanılan bir akut faz proteindir. Prokalsitonin, insan hekimliğinde bakteriyel ve non-bakteriyel enfeksiyonların ayırımında diagnostik, hastaların izlenmesinde prognostik bir parametre olarak kullanılabileceği bildirilmiştir (Kollçaku ve ark. 2022). Neonatal sepsisli (Akyüz ve Gökce 2021) ve omfalitisli buzağılarda (Bozukluhan ve ark. 2023), sistemik inflamatuvar yanıt sendromlu sığırlarda yapılan başka bir çalışmada (Başbuğ ve ark. 2020) prokalsitonin konsantrasyonunun sığırlarda akut yangının bir göstergesi olabileceği ifade edilmiştir. Çalışmada da prokalsitonin düzeyi kontrol grubuna artmış olup bu artış enfeksiyona karşı oluşan yangısal reaksiyon sonucu hücrelerden salınan yangı doğurucu sitokinlerin senteziyle bağlantılı olduğu düşünülmektedir.

SONUÇ

Sonuç olarak, neopterin ve prokalsitoninin yangı belirteci olarak kullanılabileceği ve bu konuda daha detaylı çalışmaların yapılması kanısına varıldı.

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TOTAL SIALIC ACID AND PROCALCITONIN LEVELS IN CATTLE WITH PARATUBERCULOSIS

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ABSTRACT

Objective: Paratuberculosis is a chronic disease characterized by chronic enteritis, weight loss, and thickening of the intestinal wall, which causes economic losses such as reduced meat and milk production and infertility in cattle, sheep, and goats. Early detection of systemic inflammation is essential for designing and implementing an effective treatment plan for the disease. Therefore, in recent years, research into early markers of inflammation has gained importance in both human and veterinary medicine. One such marker, sialic acid, plays a significant role in the early and accurate diagnosis of diseases, as well as in the implementation of effective treatment and preventive measures. Another marker, procalcitonin, which has a wide range of applications in human medicine, is commonly used in the diagnosis and prognosis of many diseases. The aim of this study is to determine the levels of total sialic acid and procalcitonin in cattle with paratuberculosis.

Materials and Methods: A total of 30 cattle were used in the study, including 15 clinically healthy cattle and 15 paratuberculosis-infected cattle as the control group. Blood samples were taken from the *Jugular vein* for biochemical analysis, and serum was separated.

Results: The analyses revealed that total sialic acid and procalcitonin levels were elevated in paratuberculosis-infected cattle compared to the control group.

Conclusion: It is thought that the levels of sialic acid and procalcitonin, which are indicators of the acute phase response, are increased in paratuberculosis-infected cattle and that these findings can be used to determine the severity of inflammation in the disease.

Keywords: Cattle, Paratuberculosis, Procalcitonin, Total sialic acid

GİRİŞ

Paratüberküloz, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) etkeninin neden olduğu, evcil ve vahşi ruminantlarda görülen, kronik ve ilerleyici enterit tablosu ile karakterize, dünya genelinde yaygın bir enfeksiyöz hastalıktır. Hastalık, özellikle süt ve et veriminde azalma, üreme performansında bozulma ve sürülerin ekonomik değer kaybı ile önemli bir hayvancılık sorunu olarak karşımıza çıkmaktadır. Hastalık, sığır yetiştiriciliğinde üretim kayıplarının yanı sıra zoonotik potansiyeli nedeniyle de ayrı bir önem arz etmektedir. MAP'n kronik seyirli olması sonucu, tanı ve kontrolün güçleşmesine yol açmakta; dolayısıyla hastalığın erken dönemde biyokimyasal belirteçlerle izlenebilmesi, günümüzde araştırmacılar için dikkat çekici bir konu haline gelmiştir.

Etken hasta ve taşıyıcı hayvanların dışkıları ile çevreye yayılarak yem ve suları kontamine etmektedir. Kontamine yem ve suların hayvanlar tarafından alınmasıyla enfeksiyon bulaşmaktadır. Etken önce bağırsak mukozasına sonra kan yoluyla tüm vücuda yayılmaktadır. Hastalık kronik seyirli olduğundan kuluçka süresi çok uzundur. İlk olarak ishal başlar, deri elastikiyetini kaybeder ve kıllar kurur. Hayvan sürekli olarak zayıflar, dışkı sulu köpüklü ve bağırsak mukozası içerir (Hasonova ve Pavlik 2006, Baumgartner ve Khol 2016, Constable ve ark. 2017). Klinik ve nekropsi bulgularıyla hastalık teşhis edilebilir. Subklinik olgularda ise teşhis PCR ve ELISA gibi laboratuvar testleriyle yapılmaktadır (Civelek 2017).

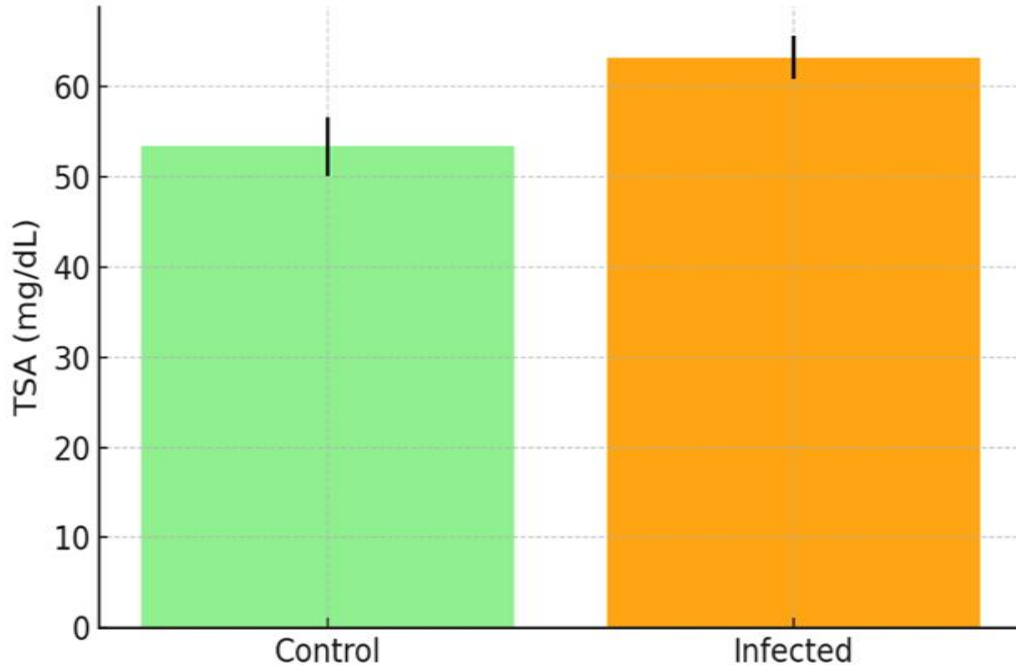
Siyalik asit, glikoproteinlerin, glikolipidlerin ve mukoproteinlerin terminal pozisyonunda bulunan, dokularda ve dolaşımda serbest veya protein bağlı şekilde yer alan asidik monosakkaritler grubudur (Traving ve Schauer 1998). Total siyalik asit (TSA), serbest formda bulunan, proteinlere ve lipidlere bağlı olan siyalik asitlerin toplamıdır (Merhan ve Özcan 2004). Hücre membranı stabilitesi, hücre-hücre etkileşimi, reseptör tanınması ve immün yanıtın düzenlenmesinde rol oynamaktadır. Patolojik durumlarda, özellikle kronik inflamasyon, malignite ve enfeksiyon süreçlerinde, serum ve plazmada siyalik asit düzeylerinde artış gözlenmektedir. Bu nedenle, TSA konsantrasyonunun belirlenmesi, organizmadaki inflamatuvar yükün ve immün yanıt aktivitesinin dolaylı bir göstergesi olarak kabul edilmektedir (Uzlu ve ark. 2010). Prokalsitonin (PCT), sağlıklı bireylerde oldukça düşük düzeylerde bulunan, kalsitonin hormonunun öncül peptididir. Fizyolojik koşullarda tiroid parafoliküler C hücrelerinden sentezlenirken, sistemik inflamatuvar yanıt ve özellikle bakteriyel enfeksiyonlar sırasında monositler ve makrofajlar tarafından sentezlenmekte ve dolaşıma salınmaktadır (Matur ve ark. 2017). PCT, bakteriyel kaynaklı enfeksiyonları viral veya aseptik inflamasyonlardan ayırt etmede kullanılan güvenilir bir biyomarker olarak tanımlanmıştır. Sığır hastalıklarında da, mastitis, metritis ve sepsis gibi durumlarda PCT düzeylerinin yükseldiği bildirilmiştir (Arslan ve ark. 2018). Dolayısıyla, paratüberkülozda TSA ve PCT düzeylerinin birlikte değerlendirilmesi, enfeksiyonun immünolojik ve biyokimyasal dinamiklerini anlamada önemli ipuçları sunabilir. TSA artışı, dokulardaki glikokonjugat yıkımı ve kronik inflamatuvar aktiviteye işaret ederken; PCT düzeylerindeki değişimler, bakteriyel enfeksiyona karşı sistemik yanıtın karakterini ortaya koyabilir. Bu nedenle, her iki parametrenin incelenmesi, paratüberkülozun biyokimyasal patofizyolojisinin aydınlatılması ve olası erken biyobelirteçlerin tanımlanması açısından değerli bir yaklaşım olarak değerlendirilmektedir.

MATERYAL ve YÖNTEM

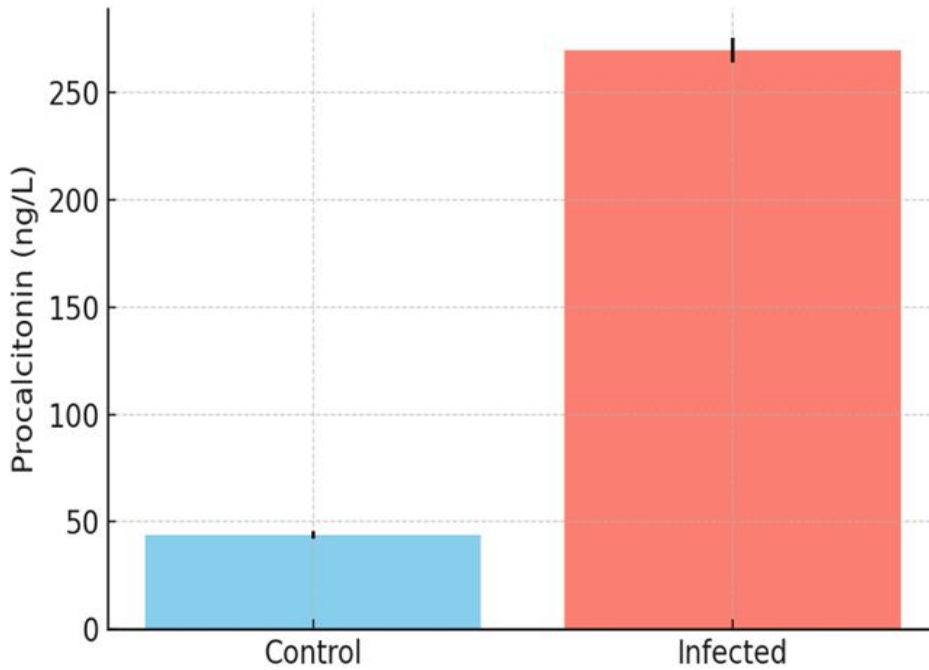
Toplam 30 adet (15 tanesi sağlıklı ve 15 tanesi ise paratüberküloz ile enfekte) sığır kullanıldı. Hastalığın tanısı *Mycobacterium paratuberculosis* Antibody Test Kit, 06-07130-26, IDEXX ELISA kiti ile konuldu. *Vena jugularis*’ten alınan kan örneklerinden serumlar ayrıldı. Elde edilen örneklerde TSA analizi Sydow (1985)’un bildirdiği kimyasal yöntemle, prokalsitonin analizi ise ticari test kiti (BT LAB) ile kolorimetrik olarak yapıldı.

Tablo 1. Klinik olarak sağlıklı ve paratüberkülozlu sığırlarda TSA ve prokalsitonin düzeyleri

Parametreler	Kontrol	Enfekte	P
TSA (mg/dL)	53,38±3,22	63,27±2,37	P<0,05
Prokalsitonin (ng/L)	44,07±1,79	269,80±5,66	P<0,001



Şekil 1. Paratüberkülozlu sığırlarda TSA düzeyleri ($P<0,05$).



Şekil 2. Paratüberkülozlu sığırlarda prokalsitonin düzeyleri ($P<0,001$).

ARAŞTIRMA BULGULARI VE TARTIŞMA

Yapılan analizler sonucunda paratüberkülozisli sığırlar ile kontrol grubu karşılaştırıldığında total siyalik asit ve prokalsitonin düzeyinin arttığı belirlendi. TSA yangısal hastalıkların prognozunda ve teşhisinde önemli bir biyobelirteçtir (Uzlu ve ark. 2010).

Yapılan çalışmalarda neonatal ishal, hidatik kist, hipoderma ve aspirasyon pnömonisi gibi hastalıklarda TSA düzeyinin arttığı bildirilmiştir (Uzlu ve ark. 2010, Merhan ve ark. 2020, Akyüz ve ark. 2022, Doğan ve Bozukluhan 2024). Çalışmada TSA düzeyi artmış olup artışın nedeni yangı ve doku hasarına bağlı olarak karaciğerdeki glikokonjugat yıkımı ve kronik inflammatuar aktivite artışı kaynaklı olabilir.

Prokalsitonin, özellikle bakteriyel enfeksiyonlarda oluşan yangısal cevabı izlemeye, insan hekimliğinde diagnostik/prognostik bir parametre olarak kullanılabileceği bildirilmiştir. Neonatal sepsisli (Akyüz ve Gökce 2021), akciğer hidatik kistli sığırlarda (Akyüz ve ark. 2025), omfalitisli buzağılarda (Bozukluhan ve ark. 2023) ve sistemik inflammatuar yanıt sendromlu sığırlarda yapılan başka bir çalışmada (Basbuğ ve ark. 2020) prokalsitonin sığırlarda akut yangının bir belirtecidir. Çalışmada prokalsitonin düzeyi kontrol grubuna artmış olup bu artış enfeksiyona karşı oluşan yangısal reaksiyon sonucu hücrelerden salınan yangı doğurucu sitokinlerin senteziyle bağlantılı olduğu düşünülmektedir.

SONUÇ

Sonuç olarak, akut faz yanıtın bir göstergesi olan siyalik asit ve prokalsitonin düzeyi paratüberkülozisli sığırlarda arttığı ve elde edilen bu bulguların ise hastalıkta yangı şiddetinin belirlenmesinde kullanılabileceği düşünülmektedir.

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SIÇANLARDA ORAL BOR VE D VİTAMİNİ TAKVİYESİNİN BEYİN DOKUSUNDAKİ BOR, DEMİR, BAKIR, ÇİNKO VE VİTAMİN D DÜZEYLERİNE ETKİSİ

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ÖZET

Amaç: Bor ve D vitamininin normal beyin fonksiyonu için gerekli mikro besinler arasında yer aldığı bilinmektedir. Son araştırmalar, bor ve D vitamininin ayrı ayrı takviyesinin sinir hücrelerine fayda sağlayabileceğini ve nörodejeneratif hastalıklarda nöroprotektif bir etkiye sahip olabileceğini göstermektedir. Bununla birlikte, bor ve D vitamininin birlikte takviyelerinin beyin bor ve D vitamin düzeylerine etkisine yönelik veri bulunmamaktadır. Bu nedenle bu çalışmada Bor ve D vitamininin birlikte takviyesinin ayrı ayrı takviyelerine göre sıçanların beyin dokusundaki bor, demir, bakır, çinko ve D vitamini konsantrasyonları üzerindeki etkileri değerlendirildi.

Materyal-Metot: Çalışmada 28 adet erkek Wistar albino sıçan (200-250 g) 4 gruba ayrıldı. Deney grupları şu şekildeydi; Kontrol grubu, Bor grubu (20 mg/kg/gün borik asit gastrik gavaj), D vitamini grubu (0.025 mg/kg/gün D vitamini gastrik gavaj), Bor+ D vitamini grubu (20 mg/kg/gün borik asit +0.025 mg/kg/gün D vitamini gastrik gavaj). Araştırmanın 30 gün süren deneysel aşaması boyunca bor ve D vitamini oral olarak verildi. Çalışma sonunda anestezi altında sakrifiye edilen sıçanlardan beyin doku örnekleri alınarak dokudaki bor, demir, bakır, çinko ve D vitamini düzeyleri belirlendi.

Bulgular: Çalışmada; bor takviyesinin beyin dokusunda bakır düzeyini artırırken, çinko düzeyini düşürdüğü ($p<0.05$) bulundu. D vitamini takviyesinin; beyin dokusu bor, demir ve çinko düzeylerini düşürdüğü, bakır düzeylerini ise artırdığı ($p<0.05$) gözlemlendi.

Bor + D vitamininin birlikte takviyesinin ise beyin dokusu bor ve bakır düzeylerini artırdığı, demir ve çinko düzeylerini düşürdüğü ($p<0.05$) tespit edildi. Beyin dokusu D vitamini düzeylerine uygulamaların etkisinin olmadığı ($p>0.05$) gözlemlendi.

Sonuç: Sonuç olarak borun D vitamini ile birlikte takviyesi, beyin dokusu bor düzeylerini sadece bor takviyesine göre daha fazla artırmaktadır. Bu bulguların beyin fonksiyonlarında daha iyi metabolik kontrol sağlamada ve nörodejeneratif bozukluklara bağlı komplikasyonları azaltmada etkili bor kullanımına yönelik yeni stratejilerin geliştirilmesine katkı sağlayacağı sonucuna varıldı.

Anahtar Kelimeler: Bor, D vitamini, Demir, Bakır, Çinko, Beyin.

ETİK ONAY: Çalışma Afyon Kocatepe Üniversitesi Deney Hayvanları Yerel Etik Kurulu tarafından 25/03/2024 tarih ve 49533702/174 sayılı kurul etik onayı alınarak yapılmıştır.

TEŞEKKÜR: Bu sunumdaki veriler Afyon Kocatepe Üniversitesi Bilimsel Araştırma Projeleri Komisyonu (Proje no: 24.VF.06) tarafından desteklenen araştırmadan alınmıştır.

EFFECTS OF ORAL BORON AND VITAMIN D SUPPLEMENTATION ON BORON, IRON, COPPER, ZINC, AND VITAMIN D LEVELS IN RAT BRAIN TISSUE

ABSTRACT

Introduction and Purpose: Boron and vitamin D are known to be among the micronutrients necessary for normal brain functions. Recent researches indicate that supplementation with boron or vitamin D individually may benefit nerve cells and exert neuroprotective effects in neurodegenerative diseases. However, there is no data on the effects of combined supplementation with boron and vitamin D on brain boron and vitamin D levels. Therefore, in this study, the effects of combined boron and vitamin D supplementation, compared to their individual supplementation, on boron, iron, copper, zinc, and vitamin D concentrations in the brain tissue of rats were evaluated.

Materials and Methods: A total of 28 male Wistar albino rats (200–250 g) were divided into four groups: Control group, Boron group (20 mg/kg/day boric acid via gastric gavage), Vitamin D group (0.025 mg/kg/day vitamin D via gastric gavage), and Boron + Vitamin D group (20 mg/kg/day boric acid + 0.025 mg/kg/day vitamin D via gastric gavage). During the 30-day experimental period, boron and vitamin D were administered orally. At the end of the study, brain tissue samples were collected from the rats sacrificed under anesthesia, and tissue levels of boron, iron, copper, zinc, and vitamin D were determined.

Results: Boron supplementation was found to increase copper levels and decrease zinc levels in brain tissue ($p<0.05$). Vitamin D supplementation decreased brain tissue boron, iron, and zinc levels while increasing copper levels ($p<0.05$). Combined boron + vitamin D supplementation increased boron and copper levels, while decreasing iron and zinc levels in brain tissue ($p<0.05$). No significant effects on brain tissue vitamin D levels were observed for any of the treatments ($p>0.05$).

Conclusion: In conclusion, supplementation with boron in combination with vitamin D increases brain tissue boron levels to a greater extent than boron supplementation alone. These results suggest that boron may contribute to the development of novel strategies aimed at improving metabolic control in brain functions and reducing complications associated with neurodegenerative disorders.

Keywords: Boron, Vitamin D, Iron, Copper, Zinc, Brain.

ETHICAL STATEMENT: This study was conducted with the approval of the Afyon Kocatepe University Local Animal Ethics Committee (Approval date: 25/03/2024, Approval No: 49533702/174).

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RATLARDA ARALIKLI AÇLIK VE EGZERSİZ UYGULAMASININ SERUM İRİSİN VE IGF-1 DÜZEYLERİ ÜZERİNE ETKİSİ

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ÖZET

Amaç: Bu çalışma, aralıklı açlık ve egzersizin serum irisin ve insülin benzeri büyüme faktörü-1 (IGF-1) düzeyine etkisini değerlendirmek amacıyla planlanmıştır.

Gereç ve Yöntem: Çalışmada, 48 erkek Sprague Dawley rat kullanılmıştır. Gruplar, G1 (Kontrol 1); *ad libitum* yem ve su erişimi olan grup, G2 (Kontrol 2); *ad libitum* yem ve su erişimi olan ve egzersiz yapan grup, G3; 09:00–17:00 saatleri arasında sınırlı yem ve su erişimi olan grup, G4; 09:00–17:00 saatleri arasında sınırlı yem erişimi, suya *ad libitum* erişimi olan grup, G5; 09:00–17:00 saatleri arasında sınırlı yem ve su erişimi olan ve egzersiz yapan grup, G6; 09:00–17:00 saatleri arasında sınırlı yem erişimi, suya *ad libitum* erişimi olan ve egzersiz yapan grup, şeklinde oluşturulmuştur. Aralıklı açlık modeli olarak 16:8 zaman kısıtlı beslenme düzeni ve yüzme egzersizi protokolü uygulanmıştır. Dört haftalık denemenin sonunda serum örneklerinde irisin, IGF-1, glukoz, üre azotu, kreatinin, ürik asit, AST, ALT, total kolesterol, trigliserit, HDL-C, LDL-C düzeyleri ölçülmüştür.

Bulgular: Çalışmada, irisin düzeylerinin kontrol gruplarına göre G3, G4 ve G5 gruplarında belirgin artışlar olmakla birlikte özellikle G6 grubunda anlamlı şekilde arttığı görülmüştür ($P<0,001$). IGF-1 düzeyleri açısından en yüksek ortalama değer G5 grubunda gözlemlenmiş olmakla birlikte gruplar arasındaki farkın istatistiksel olarak anlamlı olmadığı görülmüştür ($P>0,05$). Biyokimyasal parametrelerden glukoz, üre azotu, kreatinin, AST, trigliserit ve HDL düzeylerinde ise istatistiksel olarak anlamlı farklılıklar saptanmıştır ($P<0,05$).

Sonuç: Aralıklı açlık ve egzersiz kombinasyonunun, metabolik iyileşmeyi destekleyen güvenli ve etkili bir yöntem olduğu görülmüştür. Bazı parametrelerde gözlemlenen anlamlı olmayan değişikliklerin, uygulama süresi uzunluğu, bireysel ve biyolojik farklılıklar nedeniyle meydana gelmiş olabileceği düşünülmektedir.

Key Words: Aralıklı açlık, Egzersiz, İnsülin benzeri büyüme faktörü-1 (IGF-1), İrisin

Etik Beyan: Çalışmanın başlatılmasından önce, Aydın Adnan Menderes Üniversitesi Yerel Hayvan Deneyleri Etik Kurulu'ndan 64583101/2023/127 protokol numarası ile etik onay alınmıştır.

Teşekkür: Bu çalışma, Aydın Adnan Menderes Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (BAP Proje No: VTF-24020).

THE EFFECT OF INTERMITTENT FASTING AND EXERCISE APPLICATION ON SERUM IRISIN AND IGF-1 LEVELS IN RATS

ABSTRACT

Objective: This study was designed to evaluate the effects of intermittent fasting and exercise on serum levels of irisin and insulin-like growth factor-1 (IGF-1).

Materials and Methods: A total of 48 male Sprague Dawley rats were used in the study. The animals were divided into six groups as follows: G1 (Control 1); *ad libitum* access to food and water, G2 (Control 2); *ad libitum* access to food and water, G3; restricted access to food and water between 09:00 and 17:00, G4; restricted access to food between 09:00 and 17:00 with *ad libitum* access to water, G5; restricted access to food and water between 09:00 and 17:00 with exercise, G6; restricted access to food between 09:00 and 17:00 with *ad libitum* access to water and exercise. A 16:8 time-restricted feeding model was used to implement intermittent fasting and a swimming exercise protocol was applied. At the end of the four-week trial, serum levels of irisin, IGF-1, glucose, blood urea nitrogen (BUN), creatinine, uric acid, AST, ALT, total cholesterol, triglycerides, HDL-C, and LDL-C were measured.

Results: In the study, although irisin levels showed notable increases in the G3, G4, and G5 groups compared to the control groups, a statistically significant increase was particularly observed in the G6 group ($P<0.001$). Although the highest mean IGF-1 level was observed in the G5 group, the differences between the groups were not statistically significant ($P>0.05$). Among the biochemical parameters, statistically significant differences were found in glucose, blood urea nitrogen, creatinine, AST, triglyceride, and HDL levels ($P<0.05$).

Conclusion: The combination of intermittent fasting and exercise appears to be a safe and effective approach in supporting metabolic improvements. The non-significant changes observed in some parameters may be attributed to the duration of the intervention as well as individual and biological variations.

Keywords: Exercise, Insulin-like growth factor I (IGF-1), Intermittent fasting, Irisin

Ethical Statement: Prior to the initiation of the study, ethical approval was obtained from the Local Ethics Committee for Animal Experiments of Aydın Adnan Menderes University under the protocol number 64583101/2023/127.

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CHANGES IN CIRCADIAN CLOCK-RELATED GENE PROTEINS DEPENDING ON MIGRATION PERIODS IN MIGRATORY BIRDS

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ABSTRACT

Introduction and Purpose: Cryptochrome (CRY) proteins are involved in detecting Earth's magnetic field and regulating circadian rhythms, yet their role in the migratory metabolism of birds remains unclear. This study aimed to quantitatively assess seasonal variations in plasma CRY1 and CRY2 protein levels in the Alpine Swift (*Tachymarptis melba*), a long-distance migratory species, during premigration, breeding, and postmigration periods.

Materials and Methods: The study involved 45 adult individuals sampled during three periods: postmigration (5–9 April), breeding (19–23 June), and premigration (12–15 September). Plasma concentrations of CRY1 and CRY2 were measured in triplicate using commercial ELISA kits. Data were analyzed using one-way ANOVA and Tukey post-hoc tests.

Results: CRY1 levels were 42.6 ± 23.0 ng/mL postmigration, 61.8 ± 15.3 ng/mL during breeding, and 119.9 ± 13.3 ng/mL premigration, with significant differences observed among periods ($p < 0.01$). CRY2 concentrations were 988.0 ± 147.2 ng/mL postmigration, 544.3 ± 98.6 ng/mL during breeding, and 1721.5 ± 210.4 ng/mL premigration, again showing significant inter-period differences ($p < 0.01$).

Discussion and Conclusion: The marked elevation of CRY1 during premigration likely reflects circadian and metabolic adaptations for migration. The moderate stability during breeding could be explained by hormonal regulation and behavioral changes, while the postmigration decline suggests entry into a refractory phase after migration. High CRY2 levels during premigration and postmigration indicate a systemic timing role, potentially linked to rhythmic secretion from extraocular sources, such as the Harderian gland.

This study provides the first evidence that plasma CRY1 and CRY2 levels significantly in Alpine Swift, a long-distance migratory bird, vary significantly depending on the seasonal migration cycle. CRY1 appears critical in migration preparation and refractory phases, whereas CRY2 may coordinate processes surrounding migration. These findings contribute to our molecular understanding of avian migration and lay the groundwork for future studies on tissue-specific expression and behavioral correlations.

Keywords: Alpine Swift (*Tachymarptis melba*), Bird Migration, Cry1, Cry2, Circadian Clock.

INFLAMMATION AND HYPOXIA IN OBSTRUCTIVE SLEEP APNEA: ANALYSIS OF IL-1 β AND HIF1 α N SERUM LEVELS

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ABSTRACT

Introduction and Purpose: Obstructive Sleep Apnea Syndrome (OSAS) is a common sleep disorder characterized by recurrent upper airway obstructions during sleep, resulting in intermittent hypoxia. This process is thought to induce oxidative stress and a systemic inflammatory cascade. Considering the interplay between hypoxia and inflammation, exploring potential regulatory mechanisms appears important. This study aimed to compare serum levels of interleukin 1-beta (IL-1 β) and hypoxia inducible factor 1 alpha subunit inhibitor (HIF1 α N) between patients with OSAS and healthy controls.

Materials and Methods: The study included 9 patients with OSAS and 9 healthy individuals as a control group. Serum IL-1 β and HIF1 α N levels were measured using enzyme-linked immunosorbent assay (ELISA). Differences between the two independent groups were analyzed using the Mann-Whitney U test.

Results: In OSAS patients, IL-1 β levels were approximately 2-fold increased and HIF1 α N levels were approximately 2-fold reduced compared with controls. However, it was found that there was no statistically significant difference in IL-1 β and HIF1 α N levels between the patient and control groups ($p > 0.05$).

Discussion and Conclusion: Sleep apnea affects over one billion adults worldwide and predisposes them to various health conditions, particularly cardiovascular, metabolic, and neurocognitive disorders. Despite the well-documented increase in IL-1 β and HIF-1 α in OSAS, the role of HIF1 α N remains unexplored. Our findings suggested that IL-1 β levels were higher in the patient group, consistent with the pathophysiology of OSAS. Comparison of median values between the patient and control groups revealed increased IL-1 β and decreased HIF1 α N levels in the patient group. However, the observed differences were not statistically significant. This result may be attributable to our relatively small sample size, which limited the statistical power of the study. These preliminary findings could pave the way for more comprehensive studies on the subject.

Keywords: Obstructive Sleep Apnea Syndrome, IL-1 β , HIF1 α N, Inflammation, Hypoxia

EXPLORING THE IMPACT OF TIME-RESTRICTED FEEDING ON OXIDATIVE STRESS IN WISTAR ALBINO RATS

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ABSTRACT

Introduction and Purpose: Modern lifestyle habits, such as excessive food consumption and decreased physical activity, are becoming increasingly prevalent worldwide. This trend contributes to the rise in metabolic disorders, including type 2 diabetes, cancer, and immune system dysfunction due to obesity. Intermittent fasting (IF) protocols might be a promising approach for weight loss and improving blood chemistry in individuals who can safely tolerate them. Additionally, IF could serve as a sustainable and effective method for promoting overall health by reducing oxidative stress. Thus, this study investigates and aims to contribute to the existing literature on the impact of IF, particularly time-restricted feeding (TRF), on oxidative stress.

Materials and Methods: To achieve this aim, we used 48 male Wistar albino rats, aged 8 to 12 weeks and weighing between 250 and 300 grams. The rats were housed in a controlled environment with a 12-hour light/dark cycle at a temperature of $24\pm 2^{\circ}\text{C}$. Following a 14-day adaptation period, the rats were randomised into four groups of 12. Feeding times varied between groups, but all groups had *ad libitum* access to water. The control group had unrestricted access to food, while the experimental groups had limited feeding times. Group 2 was fed one meal a day (23 hours of fasting and 1 hour of eating), group 3 was fed two meals a day (18 hours of fasting, with each meal 1 hour within a 6-hour window), and group 4 was also fed two meals a day (14 hours of fasting, with each meal 1 hour within a 10-hour window). At the end of the 90-day study, the rats were anaesthetised and sacrificed, and liver and kidney samples were collected for the determination of nitric oxide levels, total antioxidant status (TAS), and total oxidant status (TOS) using spectrophotometric methods with commercial test kits. The oxidative stress index (OSI) was also calculated.

Results: Although not statistically significant, the TAS in the liver for groups 2 and 4 was relatively higher than in the control group, and the OSI for group 4 was the lowest. Nitric oxide levels in the experimental groups were significantly ($p=0.05$) lower than in the control group, with group 4 having the lowest levels. Additionally, TOS varied significantly among the groups in the kidneys ($p=0.032$), with group 4 having the lowest levels.

Conclusion: IF, particularly time-restricted feeding (TRF), appears beneficial for mitigating metabolic disorders and oxidative stress-related conditions. Notably, a fasting period of 14 hours with two meals a day seems to be the most appropriate pattern. Future research on this fasting duration would provide critical data.

Key Words: Intermittent Fasting, Time-restricted Feeding, Oxidative Stress, Metabolic Disorders

ETHICAL STATEMENT : An ethical committee decision was obtained for this study from the Kırıkkale University Local Ethics Committee for Animal Experiments (Date: 02.07.2025, Decision No: E-60821397-045.99-346496).

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EVALUATION OF THE ANTIOXIDANT COMPOSITION OF ROYAL JELLY AND ITS EFFECTS ON COLORECTAL ADENOCARCINOMA AND BREAST CANCER CELL LINES

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ABSTRACT

Introduction and Aim: Royal Jelly is a natural product rich in antioxidant content. Due to its natural antioxidant properties, it is considered to have therapeutic or protective potential in chronic diseases like cancer or diabetes and in various diseases. The aim of this study is to determine the antioxidant content of royal jelly, and to investigate the effect on colorectal adenocarcinoma and breast cancer cell lines in a dose dependent manner.

Materials and Methods: Total phenolic and flavonoid content and DPPH scavenge activity of the methanolic, ethanolic and aqueous extractions of royal jelly were measured spectrophotometrically. Royal jelly ethanolic and aqueous extracts were applied to colorectal adenocarcinoma and breast cancer cell lines at different concentrations (0, 3.125, 6.5, 12, 25, 50, 100, and 200 mg/mL) after being diluted with cell culture media. The MTT test was used to measure the effect on cell viability at 24 and 48 hours.

Results: The phenolic content (2.69 ± 0.09 mg/g) and DPPH scavenging activity (348.68 ± 20.84 μ g/g) of the royal jelly aqueous extract were statistically different from those of the methanolic (0.44 ± 0.11 mg/g; 470.60 ± 53.30 μ g/g) and ethanolic (0.35 ± 0.05 mg/g; 452.59 ± 30.65 μ g/g) extracts. There was no statistically significant difference between the flavonoid content of the methanolic (0.28 ± 0.11 mg/g), aqueous (0.21 ± 0.04 mg/g), and ethanolic (0.37 ± 0.17 mg/g) extracts. In colorectal adenocarcinoma and breast cancer cells, the ethanolic extract had no effect on cell proliferation at 24 hours; however, in colorectal adenocarcinoma cells, an IC₅₀ value of 100 mg/mL was found at 48 hours, but not in breast cancer cells. Royal jelly aqueous extracts had a dose-dependent proliferative effect on breast cancer cells but no effect on colorectal adenocarcinoma cells.

Discussion and Conclusion: Only the ethanolic extract of royal jelly showed antiproliferative effects on colorectal cancer cell lines, despite the aqueous extract having a higher total phenolic content. It has been noted that various cell lines have distinct proliferation responses from the same natural ingredients. Research into metabolic pathways and studies in which it is used in combination with anticancer drugs may help clarify the possible impacts and mechanisms of action of royal jelly against cancer.

Keywords: Cancer, flavonoid, HT-29, MDA-MB-231, phenolic

INTRODUCTION

Royal Jelly (RJ) is a biologically rich secretion produced by the mandibular and hypopharyngeal glands of worker honeybees. It plays a crucial role in the differentiation of larvae into either queen bees or worker bees. Larvae fed with RJ over an extended period develop into queens rather than workers. The composition of RJ consists of approximately 60–70% water, 12–15% protein, 3–8% lipids, 10–16% carbohydrates, and 3–6% lipids. In addition, it is also rich in polyphenols and vitamins (Botezan et al., 2023; Guo et al., 2021; Nabas et al., 2014).

Royal Jelly (RJ) exhibits antibacterial, anti-inflammatory, vasodilatory, hypotensive, and disinfectant effects. In addition, studies have demonstrated its antioxidant, anti-hypercholesterolemic, and antitumor activities. These findings have led to the use of RJ in traditional medicine, initially in China and Japan, and later worldwide. Today, RJ is consumed not only as a pharmaceutical and dietary supplement but is also widely incorporated into the cosmetic industry as a commercial product (Ramadan and Al-Ghamdi, 2012; Hu et al., 2017). The major fatty acid in royal jelly, and the most important parameter determining its quality, is recognized as trans-10-hydroxy-2-decenoic acid (10-HDA). Recent studies have demonstrated that 10-HDA promotes the proliferation of T lymphocyte subsets and supports interleukin production. Furthermore, it has been reported to exhibit immunoregulatory and anticancer activities. In addition, 10-HDA has been suggested as a beneficial therapeutic agent for aging, hypoglycemia, and tumors. It has also been identified as playing a crucial role in the development and balance of both the hormonal and immune systems. Therefore, 10-HDA is considered to hold significant value in the fields of medicine and healthcare (Barnutiu et al., 2011; Kamyab et al., 2020). But the antioxidant activity of RJ is not only connected to 10-HDA. The rich polyphenolic content of RJ can increase the anticancer activity.

Cancer is a chronic disease and the World Health Organization defines cancer as a genetic term for a group of diseases characterized by the abnormal growth of cells beyond their usual boundaries, which can invade adjacent tissues and/or spread to other organs. Cancer may originate in virtually any part of the body and can arise from all types of cells and tissues. It affects individuals across all age groups and socioeconomic backgrounds, including the elderly and the young, the poor and the wealthy, as well as men, women, and children (WHO, 2022). Breast cancer is one of the most common malignancies among women and represents a major cause of morbidity and mortality worldwide. Based on molecular and histological evidence, breast cancer is classified into three major groups: (1) hormone receptor-positive (HR⁺) tumors, characterized by the expression of estrogen receptor (ER⁺) and/or progesterone receptor (PR⁺); (2) human epidermal growth factor receptor 2-positive (HER2⁺) tumors; and (3) triple-negative breast cancer (TNBC), which lacks the expression of ER, PR, and HER2 (ER⁻/PR⁻/HER2⁻). Therapeutic approaches in breast cancer largely rely on these molecular subtypes (Nardin et al., 2020; Lehmann et al., 2011).

Colorectal cancer is a disease characterized by the uncontrolled growth of cells in the colon and rectum. Abnormal growths, known as polyps, can develop in the colon or rectum. Over time, these polyps may undergo malignant transformation (Faber et al., 2014). Colorectal cancer is classified according to the depth of local invasion (T stage), the lymph node involvement (N stage), and the presence of distant metastasis (M stage) (Sobin & Gospodarowicz, 2009).

Bee products like RJ are important natural antioxidants due to their biologically active compounds and the use of these products against chronic diseases like diabetes and cancer remains under investigation.

The aim of this study was to determine the polyphenolic content, flavonoid content and the dpph scavenger activity of RJ and the effect on colorectal (HT-29) cancer and breast cancer (MDA-MB-231) cell lines.

MATERIALS AND METHODS

This study was carried out at the Department of Biochemistry, Faculty of Veterinary Medicine, Ankara University.

Chemicals

In table 1, the chemicals used in this study are given together with their manufacturers and catalog numbers. Royal Jelly was applied from a market in Ankara province.

Table 1. Chemical compounds, manufacturer and cat no

Name of the chemical compound	Manufacturer	Catalog nr
2,2-Diphenyl-1-picrylhydrazyl (DPPH•), 1g	SIGMA	D9132-1G
AlCl ₃ .6H ₂ O	SIGMA	A0718-500G
Dimethyl sulfoxide (DMSO)	SIGMA	D2650-5X10
RPMI	Gibco	A1049101
DPBS	SIGMA	D8537-500ML
Ethanol	SIGMA Aldrich	LOT: SZBCI810V
Gallic Acid	Sigma Aldrich	G7384-100G
Gentamisin	Sigma	G1264-50MG
Hidrojen Peroksit	MERCK	108556
Hidroklorik Asit	MERCK	K25993914
Phosphoric acid	Riedel-De Haen	LOT: 51120
Lithium sulfate	Sigma	CAS 10377-48-7
Methanol	Merck	LOT: I0896109729
MTT	Sigma	M2128-1G
Rutin Trihydrate %97 100 mg	Alfa Aesar	LOT:10181593
Sodium Hydroxide (NaOH)	Merck	B0316462
Sodium doedecyl sulfate 500g	SIGMA	L3771-500G
Sodium Molybdate Dihydrate	Merck	CAS 10102-40-6
Sodium Nitrite (NaNO ₂)	Merck	0090815
Sodium Carbonate (Na ₂ CO ₃)	Merck	S033173
Sodium Tungstate Dihydrate	Merck	CAS: 10213-10-2

Extraction of RJ

Modified method of Agcam and Akyildiz (2015) was used. Fresh Royal Jelly (2 g) completed to 10 mL with methanolic, ethanolic and aqueous extraction solution, which contained HCl for MeOH and EtOH extracts and cytric acid for aqueous extracts (MeOH: 0.1 N HCl; EtOH: 0.1 N HCl; aqueous: 0.1 N cytric acid, 85:15 (v/v)) (Bronnum-Hansen et al., 1985). The extract was incubated for 20 minutes in room temperature and in dark and then was vortexed for 30 seconds. After the incubation time, extractions were centrifuged in 4000 rpm for 15 minutes and the upper phase, known as supernatant, was collected and used for further analyses. For cell culture the ethanolic and aqueous extractions of RJ were evaporated and dissolved in DMSO (1%) and then diluted with the culture media (RPMI).

Before using, the extractions were sterilised with a sterile filter and the highest concentration (200 mg/mL) was serially diluted to 3,125 mg/mL.

Polyphenol analysis

The same extraction was used and 100 μ L of sample was mixed with 500 μ L distilled water + 100 μ L Folin-Ciocalteu reagent + 1000 μ L 7% of Na_2CO_3 and again with 500 μ L distilled water and was incubated for 90 minutes in dark (Ullah Shirazi et al., 2014). The absorbance of the mix was measured in 760 nm spectrophotometrically. As a standard serial dilutions between 0.05 to 0.75 mg/mL of gallic acid solution (prepared in methanol) was used. Gallic acid stock solution was prepared as 10 mg/mL concentration. The results are given as mg per gram gallic acid equivalent (GAE).

DPPH analysis

In DPPH all of the extraction methods were measured and the inhibition % of DPPH was calculated given in the formula; $(1 - (\text{Abs of sample} / \text{Abs of control})) \times 100$. Sample volume was 50 μ L, mixed with 3 mL of DPPH (0.002%) and incubated in room temperature for 15 minutes. The incubated samples were measured spectrophotometrically in 515 nm. Alone DPPH was used as control (Ullah Shirazi et al., 2014).

Flavonoid Analysis

Samples (500 μ L) were mixed with 2 mL of distilled water and 150 μ L NaNO_2 (5%) and incubated for 5 minutes. Then 150 μ L of AlCl_3 (10%) was added and incubated in room temperature for 5 minutes again. After the incubation 4% of NaOH was added in a volume of 1 mL on the mixture and the mixture was vortexed and the results were measured spectrophotometrically in 510 nm. As a standard rutin trihydrate was used between 0 to 1 mg/mL and the results were given as mg/g Rutin equivalent (RE). The standard stock solution concentration was 10 mg/mL (Boateng et al., 2008).

Preparation of Cancer Cells

Cell lines were cultured at 37°C and in CO₂ (5%) incubators. A T75 cm³ flask was filled with cell lines prior to incubation, and 10% fetal bovine serum in RPMI with 1% of nonessential amino acids was added to boost the cell count. The media contained gentamycin, streptomycin, and penicillin to prevent infection of the cell.

Dimethylthiazol Diphenyltetrazolium Bromide (MTT) Assay

The principle of the assay is as following; proliferating cells, through increased dehydrogenase enzyme activity, reduce tetrazolium salt (MTT, yellow) to insoluble formazan crystals (purple), and the resulting color change is measured colorimetrically (Van Merloo et al., 2011).

Statistical Analysis

The statistical differences between the extraction methods in antioxidant content analysis and antioxidant activity was measured according to the one way ANOVA test. P value less than 0.5 means that there is a statistical difference between the extractions (Field, 2013). Results are given as ME $\pm$ SD. Statistical analysis was done with the SPSS 21.00 programme.

RESULTS

Polyphenolic content of RJ

There was a statistically significant difference in the total phenolic content among the extraction methods ($p < 0.05$). Post hoc analysis indicated that the aqueous extract (2.69 ± 0.07 mg/g GAE) contained significantly higher phenolic content compared to both the EtOH (0.35 ± 0.04 mg/g GAE) and MeOH (0.44 ± 0.09 mg/g GAE) extracts, while no significant difference was observed between EtOH and MeOH (Figure 1).

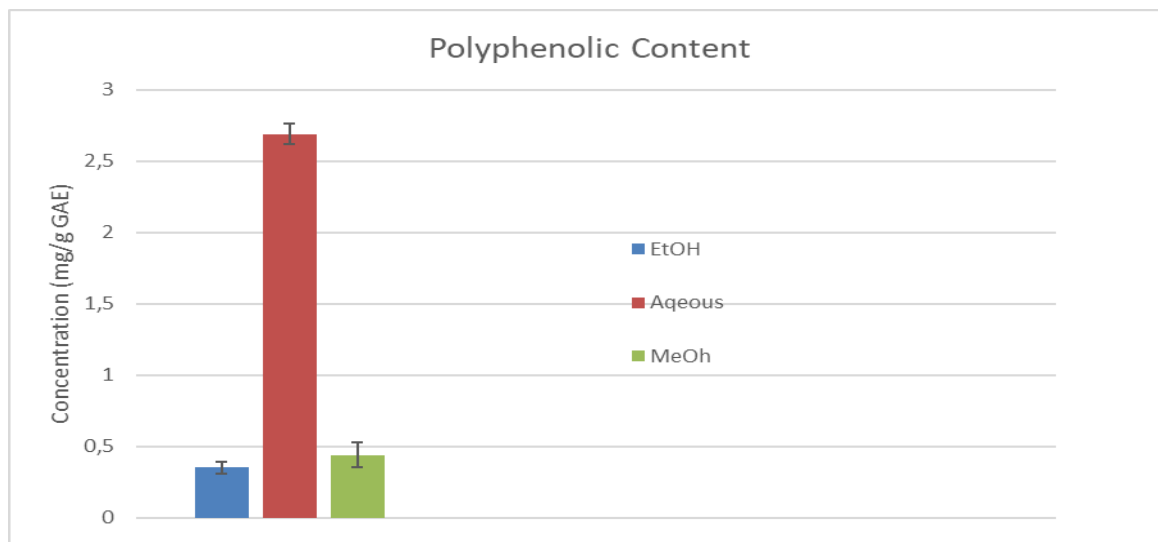


Figure 1. The total phenolic content of all three extraction methods (*= There is a statistical difference between aqueous and ethanolic; aqueous and methanolic extractions).

Flavonoid Content of RJ

No statistically significant difference in the total flavonoid content among the extraction methods were found ($p > 0.05$). Although the EtOH extract (0.37 ± 0.14 mg/g RE) exhibited slightly higher values compared to MeOH (0.28 ± 0.09 mg/g RE) and aqueous extraction (0.20 ± 0.03 mg/g RE), the differences were not significant due to the overlapping standard deviations (Figure 2).

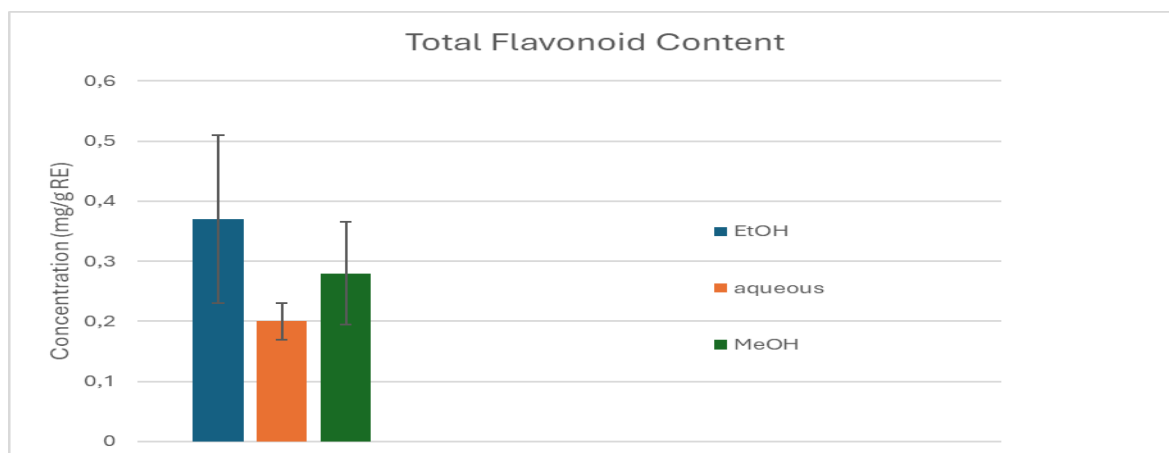


Figure 2. The total flavonoid content of RJ extractions

DPPH Scavenger Activity Of RJ

Statistically significant difference in the DPPH radical scavenging activity among the extraction methods were observed ($p < 0.05$). MeOH ($470.61 \pm 43.52 \mu\text{g/g GAE}$) and EtOH ($452.59 \pm 25.03 \mu\text{g/g GAE}$) extracts exhibited significantly higher scavenging activity compared to the aqueous extract ($348.68 \pm 17.02 \mu\text{g/g GAE}$), whereas no significant difference was found between the MeOH and EtOH extracts (Figure 3).

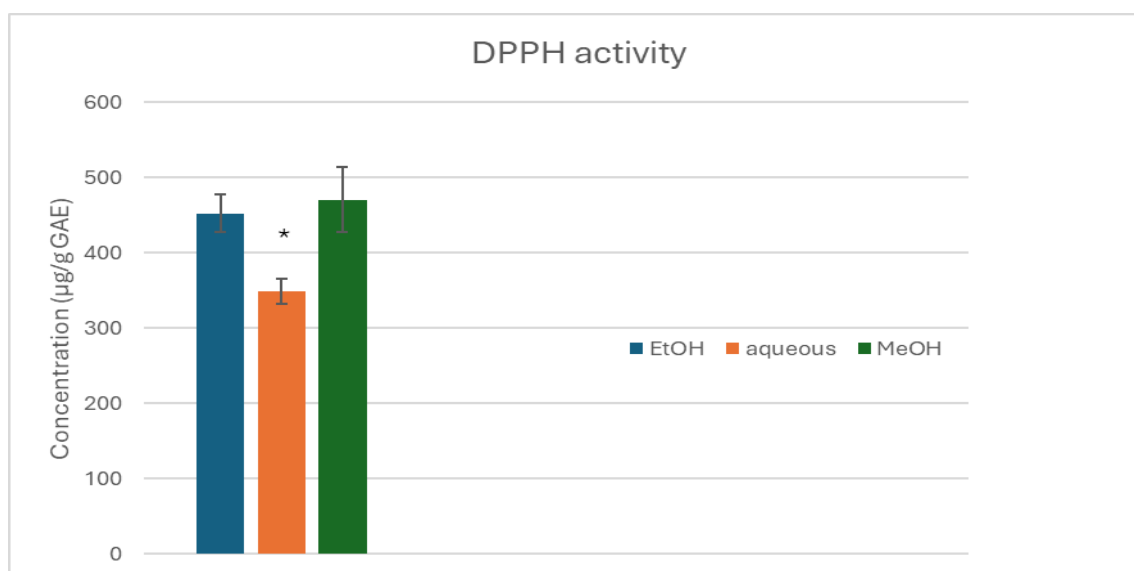


Figure 3. DPPH scavenge activity of the extracts of RJ (* means there is a significant difference between aqueous and methanolic and aqueous and ethanolic extractions).

MTT Results of HT-29 cell line

In HT-29 cell lines the IC₅₀ value of 24 h results could not be detected and the aqueous extract showed a proliferative effect at higher doses. In 48 h, the viability of the cell line decreased to 50% in 100 mg/mL in ethanolic extraction. In the aqueous extraction of RJ, there was a decrease in the cell viability up to 50 mg/mL; however further increases in doses resulted in a recovery of cell viability (Figure 4).

The cell viability for the applied doses are; 100% for control group (Cancer cell), 83% for 3.125 mg/mL, 92% for 6.25 mg/mL, 83% for 12.50 mg/mL, 87% for 25.00 mg/mL, 72% for 50.00 mg/mL, 84% for 100.00 mg/mL and at least 86% for 200 mg/mL concentrations of the ethanolic extract of RJ in 24 h MTT result. In 48 h on the other side the cell viability changed as following; 95% for 3.125 mg/mL, 64% for 6.25 mg/mL, 61% for 12.50 mg/mL, 58% for 25.00 mg/mL, 51% for 50 mg/mL, 50% for 100 mg/mL and at least the viability increased to 52% at 200 mg/mL concentration.

The aqueous extraction of RJ in HT-29 cell lines showed a proliferative effect. The results in 24 h were as following; 84% for 3.125 mg/mL, 97% for 6.50 mg/mL, 86% for both 12.50 and 25.00 mg/mL, 79% for 100 mg/mL and 114% for 200 mg/mL concentrations. In the 48-hour results, cell viability was determined as 105%, 88%, 76%, 75%, 69%, 75% and at least 88% at the increasing concentrations of RJ, respectively.

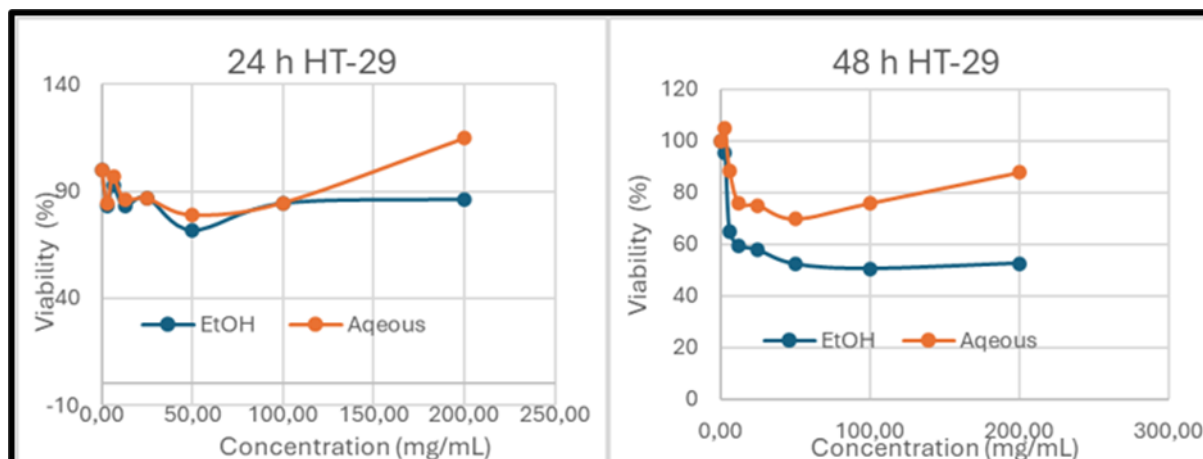


Figure 4. The effect of RJ on HT-29 cell lines in 24 and 48 hours.

MTT Results of MDA-MB-231 cell line

In breast cancer the 24 h results of MTT for both of the extractions were close and showed a proliferative effect in a dose dependent manner. In 48 h results the aqueous extract showed a proliferative effect again but in ethanolic extract there was a decrease in cell viability but the IC₅₀ values could not be detected between 0 and 200 mg/mL concentrations (Figure 5).

The cell viability for the applied doses are; 100% for control group (Cancer cell), 102% for 3.125 mg/mL, 97% for 6.25 mg/mL, 91% for 12.50 mg/mL, 94% for 25.00 mg/mL, 85% for 50.00 mg/mL, 97% for 100.00 mg/mL and at least 107% for 200 mg/mL concentrations of the ethanolic extract of RJ in 24 h MTT result. In 48 h the cell viability changed as following; 80% for 3.125 mg/mL, 70% for 6.25 mg/mL, 76% for 12.50 mg/mL, 68% for 25.00 mg/mL, 66% for 50 mg/mL, 82% for 100 mg/mL and at least the viability increased to 89% at 200 mg/mL concentration.

The aqueous extraction of RJ in MDA-MB-231 cell lines showed a proliferative effect. The results in 24 h were as following; 103% for 3.125 mg/mL and 6.50 mg/mL concentrations, 108% for 12.50 mg/mL, 105% for 25.00 mg/mL, 104% for 50 mg/mL, 123% for 100 mg/mL and 161% for 200 mg/mL concentrations. In the 48-hour results, cell viability was determined as 87%, 96%, 95%, 89%, 92%, 105% and at least 137% at the increasing concentrations of RJ, respectively.

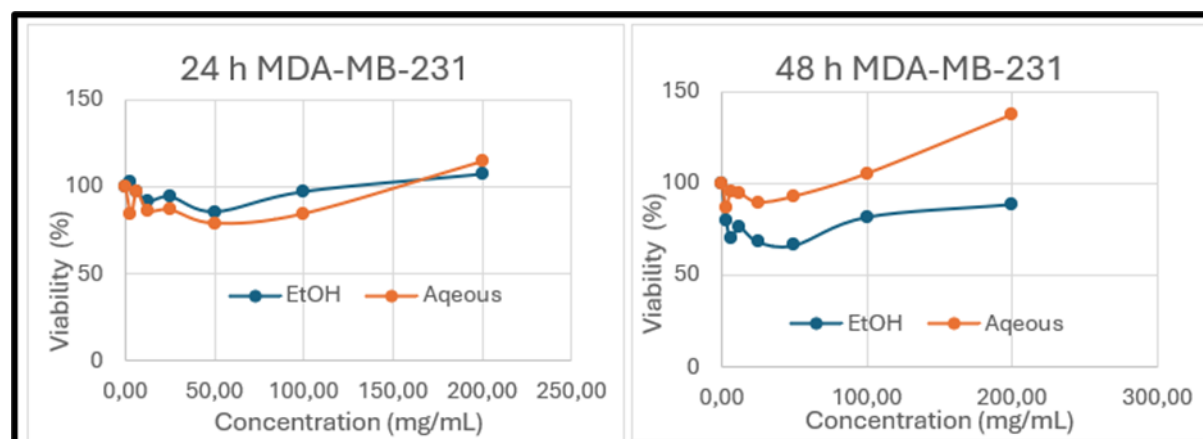


Figure 5. The proliferative effect of RJ in a dose dependent manner in MDA-MB-231 cell line.

DISCUSSION

The polyphenolic content of the aqueous extract of RJ showed a statistically higher result than the methanolic and ethanolic extracts of RJ. However according to the MTT results aqueous extractions showed a proliferative effect in both of the cancer cells. The polyphenolic or flavonoid contents of methanolic and ethanolic extractions of RJ were less than expected but still showed a cytotoxic effect especially on HT-29 cell line.

Balkanska et al (2017) investigated the antioxidant activity and phenolic content of different RJ products, which were obtained from different experimental apiaries. The inhibition activity of the aqueous extract of RJ was similar to our study but total phenolic content showed 10 times higher concentration compared to the current study. The antioxidant contents of RJ samples from different countries (Morocco, Portugal, Spain) were investigated and it was found that the region is important in antioxidant content and activity (El-Guendouz et al., 2020). Due to the importance of the region, RJ collected from Turkey, a concentration of 0.59 ± 0.02 mg/g GAE was found for the methanolic extraction of RJ and the DPPH inhibition % was less than our study (Özkök and Silici, 2017). These differences can be due to the extraction method. But the extraction method is not the only reason of these differences. The floral origin of the bee products, temperature, moisture, bee breeds, climate, rainfall and sunlight are the other reasons of the antioxidant content and activity of RJ. In a study fermented milks antioxidant content and activity was measured. Milks with RJ showed a higher inhibition compared to milks without RJ (Nabas et al., 2014). This shows us the antioxidant quality of RJ.

The study of Khoob et al (2022) found the IC₅₀ concentration in HT-29 cell lines as 1.781 mg/mL. In this study the extraction method was different, the fresh RJ was dissolved in DMSO and diluted with the culture media. In our study first an extraction method was done and then the extract was evaporated. This difference could be resulted in a higher content of RJ and a less IC₅₀ concentration. A study purified 10-HDA from RJ and investigated the antiproliferative effect on WiDr cell line, which is a derivative of HT-29 cell line. When the dose was increased from 3 mM to 5 mM, cell viability suddenly dropped from around 80% to 20% (Yang et al., 2018). There has been a growing interest in studies on bee products in recent years. Different bee products have different effects on cancer cell lines. The study of Azarshinfam et al (2021) investigated the effect of propolis on HT-29 colorectal adenocarcinoma cell lines. The IC₅₀ concentration of propolis was found 250 µg/mL concentration in 48 h. According to the studies it can be said that the polyphenolic and flavonoid composition of propolis is higher than RJ (Kocot et al., 2018).

There are different colorectal cell lines, which are used in cell culture. A study conducted by Javanovic et al (2018) different colorectal adenocarcinoma and carcinoma cell lines (SW-480 and HCT-116) were used and the cytotoxic effect of RJ could not be detected in 24, 48 and 72 hours results. HT-29 cancer cell line has a p53, APC and BRAF V600E mutation, while SW-480 cell line has an additional KRAS mutation but not a BRAF mutation. HCT-116 cell line on the other side is the wild type colorectal cancer according to p53 mutation. However there is a mutation in KRAS and APC status. A mutation in BRAF status results with more aggressive tumour growth. Yet, the metastatic potential of HT-29 is lower than SW-480 and HCT-116 cell lines (ATCC, 2025).

In breast cancer cell line the IC₅₀ value could not be detected according to the current study and in the study of Moubarak et al (2021) the IC₅₀ value could not be detected in MDA-MB-231 cell lines. The highest concentration in the study was 200 µg/mL while in our study the concentration was 1000 times higher. The combination of RJ and Thymoquinone showed an increased antiproliferative effect.

This result is an important research information due to the investigations of combination therapies in chronic diseases. Another cell line (MCF-7) of breast cancer, which is an estrogen responsive cell line, the use of RJ did not show any significant changes in the cell viability (Ayna and Darendelioglu, 2022). Studies showed that RJ has estrogenic effects and this could be the reason of its proliferative effect on MCF-7 cell lines. A study revealed that RJ alone has a proliferative effect on MCF-7, while it has an antiproliferative effect when it is used in combination with Tamoxifen, an estrogen antagonist (Mishima et al., 2005).

The estrogenic activity of royal jelly, and its consequent effect on increasing cell viability in MCF-7 cells, can be considered a primary reason; however, this cannot account for the same effect in MDA-MB-231 cell lines. The MDA-MB-231 cell line is highly aggressive and classified as triple-negative breast cancer (TNBC), exhibiting hormone-insensitive characteristics. In this context, the primary reason for royal jelly's proliferative effect on MDA-MB-231 cells may be the high metastatic potential and aggressive nature of the cell line. The use of RJ in in-vivo studies showed that the tumor weight decreased between 0.5 to 1.5 g/kg usage of RJ. But the decrease was not significantly different compared to control group (Zhang et al., 2017). To see the effect of RJ in breast cancer on mouse models, further research is needed.

As a conclusion, aqueous extract of RJ showed a higher polyphenolic content while it showed a proliferative effect on cancer cell lines. This could be due to the prooxidant effects and different biological active compounds in aqueous extracts. In this study the extraction solution contained 0.1 N HCl/citric acid in a 15% of volume, this could be the reason of the differences in antioxidant content and MTT test results. It has been noted that various cell lines have distinct proliferation responses from the same natural ingredients. Research into metabolic pathways and studies in which it is used in combination with anticancer drugs may help clarify the possible impacts and mechanisms of action of royal jelly against cancer.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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KESİM YAŞLARI FARKLI OLAN ETLİK PİLİÇLERDE LİPİD PROFİLİ, TAVUK TROMBOSİT-AKTİVE EDİCİ FAKTÖR ASETİLHİDROLAZ VE OKSİDATİF STRES PARAMETRELERİ DÜZEYLERİNİN DEĞERLENDİRİLMESİ

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ÖZET

Giriş ve Amaç: Bu çalışmada, insanlar için beslenme zincirinin önemli bir ögesi olan broylerlerde hematolojik profilin, lipit profilinin, oksidatif stresi ifade eden parametrelerin ve trombosit-aktive edici faktör asetilhidrolaz düzeylerinin değerlendirilmesi amaçlandı. Etlik piliç yetiştiriciliğinde kümeslerde seyreltmeye ve/veya kesime başlama için sıklıkla seçilen ve ekonomik olarak ideal olduğu kabul edilen günler, yetiştirme periyodunun 35. 42. ve 49. günleridir.

Materyal ve Metot: Çalışmamızda ticari bir etlik piliç yetiştiriciliğinde 35, 42 ve 49 gün sürelerle beslenen broylerler arasından rastgele seçilen ve her gün için 10'ar adet olmak üzere toplam 30 piliç kullanıldı. Broylerlerden elde edilen kan örneklerinde her bir kesim gününe göre belirlenen biyobelirteçlerin düzeyleri arasındaki değişiklikler değerlendirildi.

Kan örneklerinde hematolojik profil, serum örneklerinde alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), total protein, albümin, C-reaktif protein (CRP), trigliserit, LDL ve HDL ve trombosit-aktive edici faktör asetilhidrolaz düzeyleri belirlendi. Oksidatif stres profilini belirlemek için serum toplam antioksidan (TAS) ve toplam oksidan (TOS) durumları, oksidatif stres indeksi (OSI) ve malondialdehit (MDA) düzeyleri saptandı.

Sonuçlar: Çalışmamızda, 35 gün süreyle beslenen broyler örneklerinden toplanan kan örneklerinde, 42 ve 49 gün sürelerle beslenen broyler örneklerinden toplanan kan örneklerine göre, TAS ($p < 0,001$) ve CRP ($p < 0,05$) değerlerinin anlamlı düzeylerde düşük, TOS, OSI ($p < 0,001$), trombosit-aktive edici faktör asetilhidrolaz, total tiol ve disülfid ($p < 0,001$) değerlerinin ise anlamlı düzeyde yüksek olduğu bulundu. Oksidatif stres profilinin değerlendirildiği parametrelerdeki değişiklikler, 35 gün süreyle beslenen broylerlerde ortaya çıkan oksidatif stresin 42 ve 49 gün sürelerle beslenen broylerlere göre daha ciddi boyuta ulaştığını gösterdi.

Tartışma ve Sonuç: Broyler yetiştiriciliğinde seyreltme ve/veya kesim günü farklılığına göre bazı serum biyobelirteçlerinin düzeylerinde ortaya çıkan değişikliklerin sonraki çalışmalarda yolak düzeyinde yapılacak değerlendirme çalışmalarıyla gerçekleştirilmesi planlanmaktadır.

Anahtar Kelimeler: Etlik piliç, trombosit-aktif edici faktör asetilhidrolaz, oksidatif stress profili

EVALUATION OF LIPID PROFILE, CHICKEN PLATELET-ACTIVATING FACTOR ACETYLHYDROLASES AND OXIDATIVE STRESS PARAMETERS LEVELS IN BROILER CHICKENS WITH DIFFERENT SLAUGHTERING AGES

ABSTRACT

Introduction and Purpose: This study aimed to evaluate the hematological profile, lipid profile, parameters indicating oxidative stress, and platelet-activating factor acetylhydrolase levels in broilers, a crucial element of the human food chain. In broiler farming, the most frequently chosen and economically optimal days for initiating thinning and/or slaughtering in poultry houses are days 35, 42, and 49 of the rearing period.

Materials and Methods: In our study, a total of 30 broilers were randomly selected from broilers fed for 35, 42, and 49 days in a commercial broiler farm. Ten broilers were used for each day of rearing. Changes in the levels of biomarkers determined by each day of slaughter were evaluated in blood samples obtained from the broilers. Hematological profiles were determined in blood samples, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, C-reactive protein (CRP), triglycerides, LDL and HDL, and platelet-activating factor acetylhydrolase levels were determined in serum samples. Serum total antioxidant (TAS) and total oxidant (TOS) status, oxidative stress index (OSI), and malondialdehyde (MDA) levels were determined to determine the oxidative stress profile.

Results: In our study, blood samples collected from broilers fed for 35 days were significantly lower in TAS ($p<0.001$) and CRP ($p<0.05$), and significantly higher in TOS, OSI ($p<0.001$), platelet-activating factor acetylhydrolase, total thiol, and disulfide ($p<0.001$) compared to blood samples collected from broilers fed for 42 and 49 days. Changes in the parameters assessing the oxidative stress profile indicated that oxidative stress in broilers fed for 35 days was more severe than in broilers fed for 42 and 49 days.

Discussion and Conclusion: The changes in the levels of some serum biomarkers, depending on the differences in dilution and/or slaughter day in broiler farming, are planned to be evaluated at the pathway level in future studies. Keywords: Broiler chicken, platelet-activating factor acetylhydrolase, oxidative stress

Keywords: Broiler chicken, platelet-activating factor acetylhydrolase, oxidative stress profile

ŞAP HASTALIĞI AŞISI İLE AŞILANAN VE ŞAP HASTALIĞI GEÇİREN SIĞIRLARDA SERUM A VE E VİTAMİNİ DÜZEYLERİNİN DEĞERLENDİRİLMESİ

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ÖZET

Amaç: Bu çalışma, şap hastalığı geçirmiş ve şap hastalığına karşı aşılanmış sığırlarda serum A ve E vitamini düzeylerini belirlemek ve karşılaştırmalı olarak değerlendirmek amacıyla yürütülmüştür.

Materyal ve Yöntem: Çalışma materyalini 17'si daha önce şap hastalığı tanısı konmuş, 17'si ise aşılanmış olmak üzere toplam 34 sığır oluşturmuştur. Serum örnekleri, Eylül 2019 ile Mayıs 2021 yılları arasında Karaman ve Konya illerindeki sığır işletmelerinde 6–12 ay, 12–36 ay ve ≥ 36 ay olmak üzere 3 farklı yaş gruplarındaki sığırlardan elde edilmiştir. Hayvanların aşılanma durumu, yaşı, cinsiyeti, beslenme şekli ve genel sağlık durumuna ilişkin epidemiyolojik veriler kaydedilmiştir. Serum A ve E vitamini düzeyleri biyokimyasal yöntemlerle ölçülmüştür. Epidemiyolojik veriler ve biyokimyasal sonuçlar Microsoft Excel programına kaydedilmiş, istatistiksel analizler için SPSS yazılımına aktarılmıştır. Değişkenler arasındaki ilişkilerin değerlendirilmesinde Ki-kare testi kullanılmıştır.

Bulgular: Analiz sonuçlarına göre, 6-12 aylık yaş grubundaki aşıllı hayvanlarda serum E vitamini düzeyleri istatistiksel olarak anlamlı derecede düşük bulunmuştur ($p < 0.05$). Hem şap hastalığı geçiren hem de aşılanan gruplarda serum A vitamini düzeylerinde belirgin azalma saptanmıştır.

Sonuç: Şap hastalığı enfeksiyonu ve aşılanmanın, sığırlarda serum A ve E vitamini düzeylerini düşürdüğü belirlenmiştir. Elde edilen bulgular, antioksidan vitaminlerin hem enfeksiyon sürecinde hem de aşılama sonrasında metabolik denge ve bağışıklık yanıtının korunmasında kritik rol oynadığını göstermektedir. Bu nedenle şap hastalığı ile mücadelede destekleyici vitamin uygulamalarının, hayvan sağlığı ve bağışıklık sisteminin güçlendirilmesi açısından önemli katkılar sağlayabileceği düşünülmektedir.

Anahtar Kelimeler: Şap hastalığı, Sığır, A Vitamini, E Vitamini, Oksidatif stres, Aşılama.

**EVALUATION OF SERUM VITAMIN A AND E LEVELS IN CATTLE
VACCINATED WITH FOOT AND MOUTH DISEASE VACCINE AND
EXPERIENCED FOOT AND MOUTH DISEASE**

ABSTRACT

Aim: This study was conducted to determine and comparatively evaluate serum vitamin A and E levels in cattle that had foot and mouth disease and were vaccinated against foot and mouth disease.

Materials and Methods: The study material consisted of 34 cattle, 17 previously diagnosed with the disease and 17 vaccinated. Serum samples were obtained from cattle in 3 different age ranges: 6-12 months, 12-36 months, and ≥ 36 months in Karaman and Konya provinces between September 2019 and May 2021. Epidemiological data on vaccination status, age, sex, diet, and general health performance of the animals were collected. Serum vitamin A and E levels were measured using biochemical methods. Epidemiological data and biochemical results were recorded in Microsoft Excel and transferred to SPSS for analysis. The chi-square test was used to evaluate the relationships between variables.

Results: Based on the analysis performance, serum vitamin E levels were significantly lower in vaccinated animals aged 6–12 months ($p < 0.05$). Serum vitamin A levels were significantly reduced in both the FMD infected and vaccinated groups.

Conclusion: It was determined that FMD infection and vaccination decreased serum vitamin A and E levels in cattle. The findings indicated that antioxidant vitamins play a critical role in maintaining metabolic balance and immune response both during the infection process and after. It has been determined that foot and mouth disease infection and vaccination reduce serum vitamin A and E levels in cattle. The findings obtained show that antioxidant vitamins play a critical role in the main immune system. The findings obtained show that antioxidant vitamins play a critical role in maintaining metabolic balance and immune response both during the infection process and after vaccination. Therefore, supplemental vitamin administration in the fight against foot-and-mouth disease is thought to make significant contributions to animal health and immune system strengthening.

Keywords: Foot and Mouth Disease, Cattle, Vitamin A, Vitamin E, Oxidative stress, Vaccination.

PROSTAT KANSERİ HÜCRELERİNDE CD44 HEDEFLİ AR İNHİBİSYONU

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ÖZET

Giriş ve Amaç: Prostat kanserinde (PCa) androjen reseptörü (AR) sinyali ve kök-hücrelik programı (CD44/SOX2/OCT3/4/NANOG) tümör progresyonu ve tedavi direncinde belirleyicidir. Bu çalışmada, hyaluronik asit kaplı selenyum nanoparçacıkları-apigenin kombinasyonunun (HA-SeNP-Api) CD44 aracılı hedefleme ile AR eksenini baskılama ve apoptozu güçlendirme potansiyeli, LNCaP prostat kanseri hücrelerinde ve CD44⁺ alt popülasyonda değerlendirildi.

Yöntem: SeNP indirgeme yöntemiyle sentezlendi; apigenin eklenerek HA ile kaplandı (HA-SeNP-Api). LNCaP hücreleri beş gruba ayrıldı: Kontrol, Apigenin (6,25 µM), Enzalutamid (6,25 µM), SeNP (100 µM) ve HA-SeNP-Api (100 µM SeNP + 6,25 µM apigenin + %0,1 HA). Uygulamalar 72 saat sürdü. CD44⁺ alt popülasyon MACS ile izole edildi. Canlılık/sitotoksosite, AR/PSA mRNA (RT-qPCR, $2^{-\Delta\Delta Ct}$) ve PSA proteini; apoptoz belirteçleri (p53, BAX, CASP8, CASP3) ve kök-hücrelik belirteçleri (CD44, NANOG, SOX2, OCT3/4) analiz edildi. İstatistikler tek yönlü ANOVA ve Tukey testi ile yapıldı (p < 0,05).

Bulgular: CD44⁺ hücre canlılığında tüm tedaviler kontrolüne göre anlamlı azalma oluşturdu (p < 0,0001). Azalma oranları sırasıyla Apigenin %46,77, Enzalutamid %44,96, SeNP %80,76 ve HA-SeNP-Api %84,40 idi. Kombinasyon, tekli ajanlardan daha güçlü etki gösterdi. HA-SeNP-Api, AR ve PSA mRNA düzeylerini ve PSA proteinini anlamlı biçimde düşürdü. Aynı grupta p53 ve BAX artışı ile mitokondriyal (intrinsik) yol, CASP8 artışı ile ekstrinsik yol aktive oldu ve CASP3 düzey/aktivitesi güçlendi. Kök-hücrelikle ilgili olarak CD44, NANOG, SOX2 ve OCT3/4 transkriptleri belirgin şekilde azaldı.

Tartışma ve Sonuç: CD44 hedefli HA-SeNP-Api, LNCaP modelinde AR/PSA eksenini çok katmanlı baskılarken hem intrinsik hem ekstrinsik apoptozu eşzamanlı tetiklemekte ve kök-hücrelik programını zayıflatmaktadır. Bulgular, kombinasyonun özellikle CD44⁺ ve tedaviye dirençli alt popülasyonlara karşı umut verici bir aday olduğunu düşündürmektedir. Çoklu hücre hattı, protein-düzeyi doğrulamalar ve in vivo etkinlik-güvenlilik çalışmalarıyla teyit edilmesi önerilir.

Anahtar Sözcükler: Prostat kanseri, androjen reseptörü, CD44, hyaluronik asit, selenyum nanoparçacıkları, apigenin, apoptoz, kanser kök hücresi.

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CD44-TARGETED AR INHIBITION IN PROSTATE CANCER CELLS

ABSTRACT

Introduction and Purpose: Androgen receptor (AR) signaling and the stemness program (CD44/SOX2/OCT3/4/NANOG) drive progression and therapy resistance in prostate cancer (PCa). We evaluated whether hyaluronic acid-coated selenium nanoparticles loaded with apigenin (HA-SeNP-Api) can leverage CD44-mediated targeting to suppress AR signaling and potentiate apoptosis in LNCaP cells and their CD44⁺ subpopulation.

Materials and Methods: SeNPs were synthesized by reduction, loaded with apigenin, and coated with hyaluronic acid (HA-SeNP-Api). LNCaP cells were assigned to five groups: Control, Apigenin (6.25 µM), Enzalutamide (6.25 µM), SeNP (100 µM), and HA-SeNP-Api (100 µM SeNP + 6.25 µM apigenin + 0.1% HA) for 72 h. CD44⁺ cells were isolated by MACS. Viability/cytotoxicity were assessed; AR/PSA mRNA (RT-qPCR, 2^{-ΔΔCt}) and PSA protein, apoptosis markers (p53, BAX, CASP8, CASP3), and stemness markers (CD44, NANOG, SOX2, OCT3/4) were quantified. One-way ANOVA with Tukey post hoc was used (p < 0.05).

Results: All treatment strategies significantly reduced CD44⁺ cell viability vs control (p < 0.0001): Apigenin 46.77%, Enzalutamide 44.96%, SeNP 80.76%, and HA-SeNP-Api 84.40% reduction; the combination outperformed single agents. HA-SeNP-Api decreased AR and PSA mRNA and PSA protein. It increased p53 and BAX (intrinsic apoptosis), elevated CASP8 (extrinsic pathway), and enhanced CASP3, indicating convergence at the execution phase. Stemness transcripts (CD44, NANOG, SOX2, OCT3/4) were concomitantly downregulated.

Discussion and Conclusion: CD44-targeted HA-SeNP-Api multi-layered suppression of AR/PSA and dual activation of intrinsic/extrinsic apoptosis, while weakening the stemness program, in LNCaP cells. The data support HA-SeNP-Api as a promising candidate against CD44⁺, therapy-resistant PCa subpopulations. Validation across additional cell lines, protein-level assays, and in vivo efficacy/safety studies is warranted.

Keywords: Prostate cancer; androgen receptor, CD44, hyaluronic acid, selenium nanoparticles, apigenin, apoptosis, cancer stemness.

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GİRİŞ

Prostat kanseri (PCa) erkeklerde en sık teşhis edilen kanser olup, kansere bağlı ölümlerde ikinci sıradadır.¹ Hastalık, prostat epitel hücrelerinin kontrolsüz proliferasyonu ile gelişerek alt üriner sistem semptomları ve ileri evrede kemik-lenf nodu metastazları ile seyreder.² Klinik olarak androjen bağımlı erken evre ve androjen bağımsız/dirençli ileri evre biçiminde sınıflandırılır. Androjen ablasyon tedavisi başlangıçta etkili olsa da kısa sürede direnç gelişimi tipiktir. Bu bağlamda androjen reseptörü (AR), hem androjen bağımlı hem de bağımsız tümör progresyonunda merkezî bir düzenleyici olarak kabul edilir.³

AR, nükleer steroid reseptör ailesinden ligand-bağımlı bir transkripsiyon faktörüdür. İnaktif halde sitoplazmada şaperon proteinleriyle kompleks oluşturur, androjen bağlanmasıyla homodimerize olup nükleusa taşınır ve ARE dizilerine bağlanarak AKT, COX-2 ve MMP9 gibi hedef genlerin transkripsiyonunu düzenler. Bu eksen, proliferasyon ve invazyonu destekler. İleri evrede AR, sitokin/büyüme faktörü yollarıyla androjenden bağımsız biçimde de etkinleşebilir. Güncel stratejiler, androjen sentezinin ve AR sinyalinin eşzamanlı baskılanmasını hedefler.

Ayrıca AR'nin ubikuitin-proteazom sisteminde yıkıma yönlendirilebildiği, AKT ve MDM2'nin AR ile kompleks oluşturup fosforilasyon, ubikuitinasyon ve proteazomal yıkımı kolaylaştırdığı gösterilmiştir.^{4,5}

Nanoteknoloji, kemoterapötiklerin biyoyararlanım ve stabilitesini artırırken hedef dışı dağılımın yol açtığı toksisiteyi azaltma potansiyeli sunar.⁶ Kanser hücrelerinde yüksek eksprese edilen TfR1 ve CD44 gibi reseptörlere özgü ligandlarla işlevselleştirilen nanotaşıyıcılar, aktif tümör hedefleme olanağı sağlar. Hyaluronik asit (HA) biyouyumlu, biyobozunur bir polisakkarittir ve CD44'e özgül bağlanması nedeniyle aktif hedefleme için yaygın biçimde kullanılmaktadır.^{7,8} Nitekim HA kaplı selenyum nanoparçacıklarıyla (HA-SeNP) paklitaksel'in birleştirildiği bir sistemde, artmış hücresel alım, kontrollü salım ve üstün in vivo antitümör etkinlik bildirilmiştir.⁶

Selenyum nanoparçacıkları (SeNP), inorganik/organik Se formlarına kıyasla daha düşük toksisite ve daha iyi emilim profiliyle öne çıkar. PCa bağlamında SeNP'lerin, AR transkripsiyonu ve protein düzeylerini aşağı düzenlediği, AKT/MDM2 yolunu aktive ederek AR fosforilasyonu, ubikuitinasyonu ve proteazomal yıkımı tetiklediği gösterilmiştir.⁹ Böylece SeNP'ler, hem AR sinyalinin transkripsiyonel baskılanması hem de reseptör proteolizinin hızlandırılması ile çift yönlü bir antitümör mekanizma sergiler.

Öte yandan, polifenolik/flavonoid bileşikler geniş spektrumlu antikanser etkiler oluşturabilirken, düşük çözünürlük ve biyoyararlanım gibi kısıtlar taşır. Nano-tabanlı taşıma sistemleri, bu sınırlılıkları aşarak hedefe yönelim ve farmakokinetiği iyileştirebilir.^{10,11} Bu çerçevede apigenin, proliferasyonun baskılanması ve apoptozun başlatılması dahil çeşitli onkostatik etkiler gösterse de formülasyonel engeller nedeniyle nano-taşıyıcı yaklaşımlarıyla desteklenmesi önerilmektedir.^{12,13}

Bu hususlar dikkate alındığında, CD44-hedefli HA tabanlı sistemlerle yönlendirilen SeNP'lerin, AR eksenini çok katmanlı biçimde baskılama potansiyeli, polifenolik bir eş-ajanın nano-taşımasıyla birleştirildiğinde, androjen bağımlı ve bağımsız PCa alt tiplerinde direnç mekanizmalarını aşmaya aday bir yaklaşım sunabilir.^{3,6,8,9} Bu çalışma, prostat kanseri hücrelerinde CD44 hedefli AR inhibisyonu odağında söz konusu biyolojik ve nano-teknolojik temelleri birleştiren bir stratejiyi esas almaktadır.

MATERYAL VE METOD

Nanopartikül Sentezi (HA-SeNP-API)

Nanoselenyum partikülleri (SeNP), indirgeme yöntemi ile sentezlendi. Bunun için 10,6 mM Na₂SeO₃ ve 13 mM askorbik asit çözeltileri eşit hacimlerde alınarak manyetik karıştırıcıda 12 saat (h) karıştırıldı. Reaksiyondaki renk değişimi SeNP oluşumunu gösterdi. Bu karışımdan 1,89 mL alınarak 10 mL'ye tamamlanarak 1 mM stok SeNP elde edildi. Apigenin ise 62,5 µM olacak şekilde bu karışıma eklendi ve 24h karıştırma devam ettirildi. 24h sonunda %0,1 HA çözeltisinden 500 µL ilave edilip tekrar 24h karıştırılarak HA-SeNP-API yapısı elde edildi.

Hücre Kültürü

İnsan prostat kanseri hücre serisi LNCaP (ATCC, Manassas, VA) DMEM (10% FBS, %1 penisilin-streptomisin, 2 mM L-glutamin) ortamında 37 °C'de, %5 CO₂ içeren nemli inkübatörde kültüre edildi.

CD44⁺ Kök Hücre Elde Edilmesi

LNCaP prostat kanseri hücrelerinden gruplara göre yapılan uygulamalar sonunda CD44⁺ kök hücre alt popülasyonu manyetik hücre ayırıştırma yöntemi (MACS) ile ayırıştırıldı.

Bu amaçla, üretici firmanın (Miltenyi Biotec, DE) protokolüne uygun şekilde CD44 antijenine spesifik manyetik boncuklar kullanıldı. Her gruptan 10 µL hücre süspansiyonu alınarak hemositometrede sayım yapıldı. Boyanmış (ölü) ve boyanmamış (canlı) hücreler ayrı ayrı sayılarak, canlı hücre yüzdesi ve toplam hücre sayısı belirlendi.

Gen Ekspresyonu (RT-qPCR)

Toplam RNA ticari kit ile izole edildi, cDNA sentezi sonrası seçili genler RT-qPCR ile analiz edildi. GAPDH referans gen olarak kullanıldı, sonuçlar $2^{-\Delta\Delta C_t}$ yöntemi ile değerlendirildi.

PSA Düzeylerinin ELISA ile Analizi

PSA düzeyi ticari sandviç ELISA kiti kullanılarak üretici protokolüne (SunRed, CN) göre homojenatta analiz edildi. Ölçümler 450 nm’de yapıldı ve ng/mL olarak ifade edildi.

Deney Grupları ve Tedaviler

Deneylerde LNCaP hücreleri beş gruba ayrıldı:

Kontrol: İşlem görmemiş hücreler.

Apigenin: 6.25 µM apigenin uygulanmış hücreler.

Enzalutamid: 6.25 µM enzalutamid uygulanmış hücreler (kontrol ajanı).

SeNP: 100 µM selenyum nanoparçacıkları ile muamele edilen hücreler.

HA-SeNP-Api: 100 µM selenyum nanoparçacıkları, 6.25 µM apigenin ve %0,1 hyaluronik asit kombinasyonu ile muamele edilen hücreler.

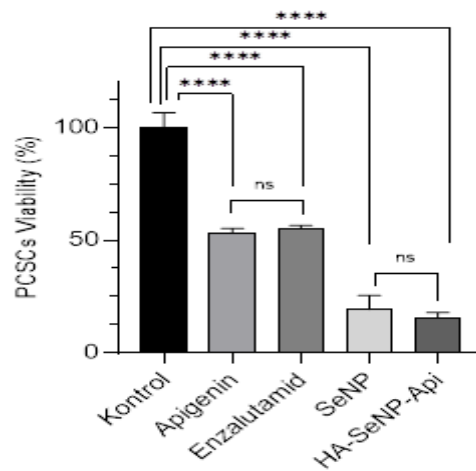
Tüm uygulamalar 72 saat sürdürüldü; ardından sitotoksite, ELİZA ve qPCR analizleri yapıldı.

İstatistiksel Analiz

Gruplar arası farklar tek yönlü ANOVA ve Tukey post-hoc testi ile değerlendirildi. $p<0,05$ değeri istatistiksel anlamlılık sınırı olarak kabul edildi.

BULGULAR

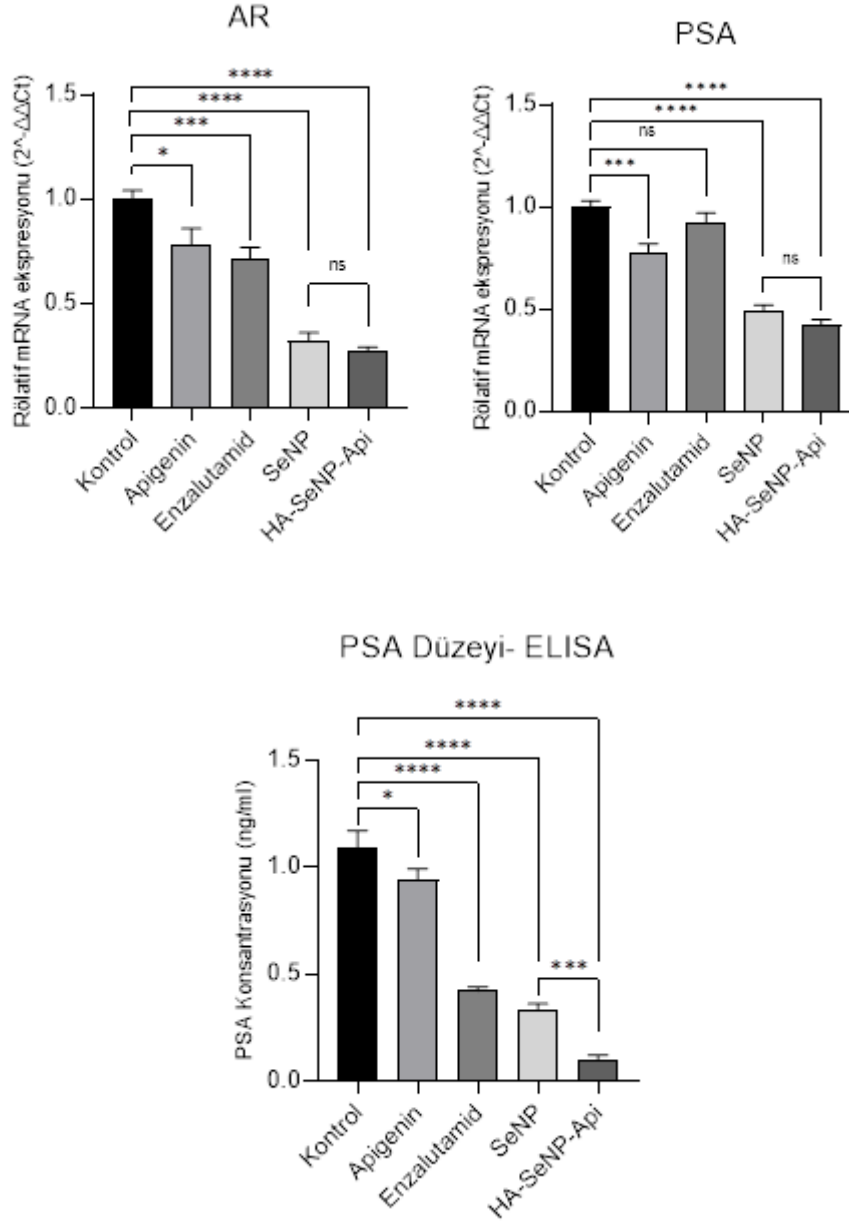
HA-SeNP-Api Kombinasyonunun Prostat Kanseri Kök Hücre Canlılığına Etkisi



Şekil 1. Prostat kanseri kök hücrelerinin canlılık analizinde, HA-SeNP-Api grubunda tekli uygulamalara kıyasla en güçlü azalma gözlenmiştir. PCSCs: Prostate Cancer Stem Cells.

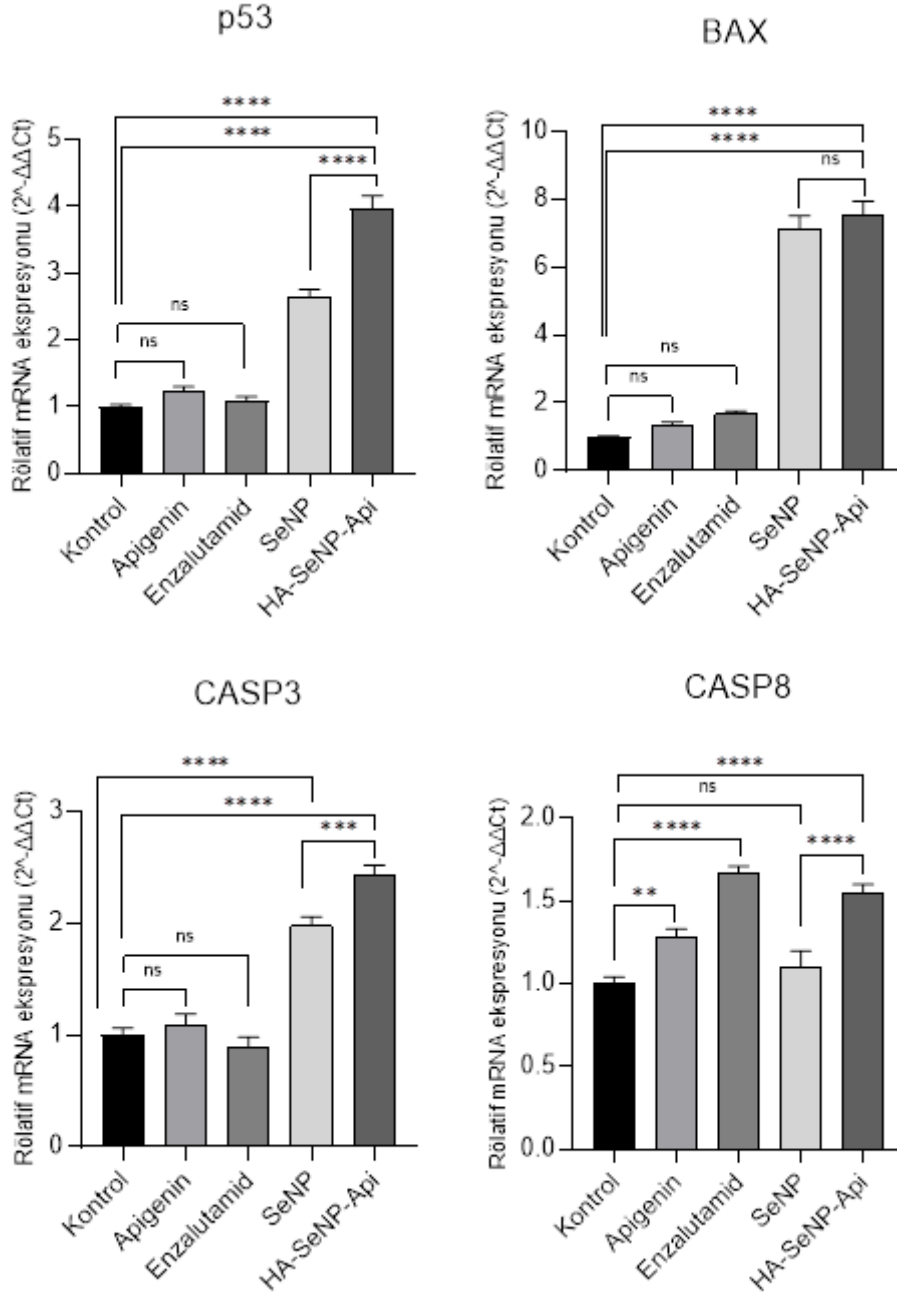
(****= <0.0001), ns: not significante

AR/PSA mRNA Ekspresyonu ve PSA Protein Düzeylerinin HA-SeNP-Api ile Baskılanması



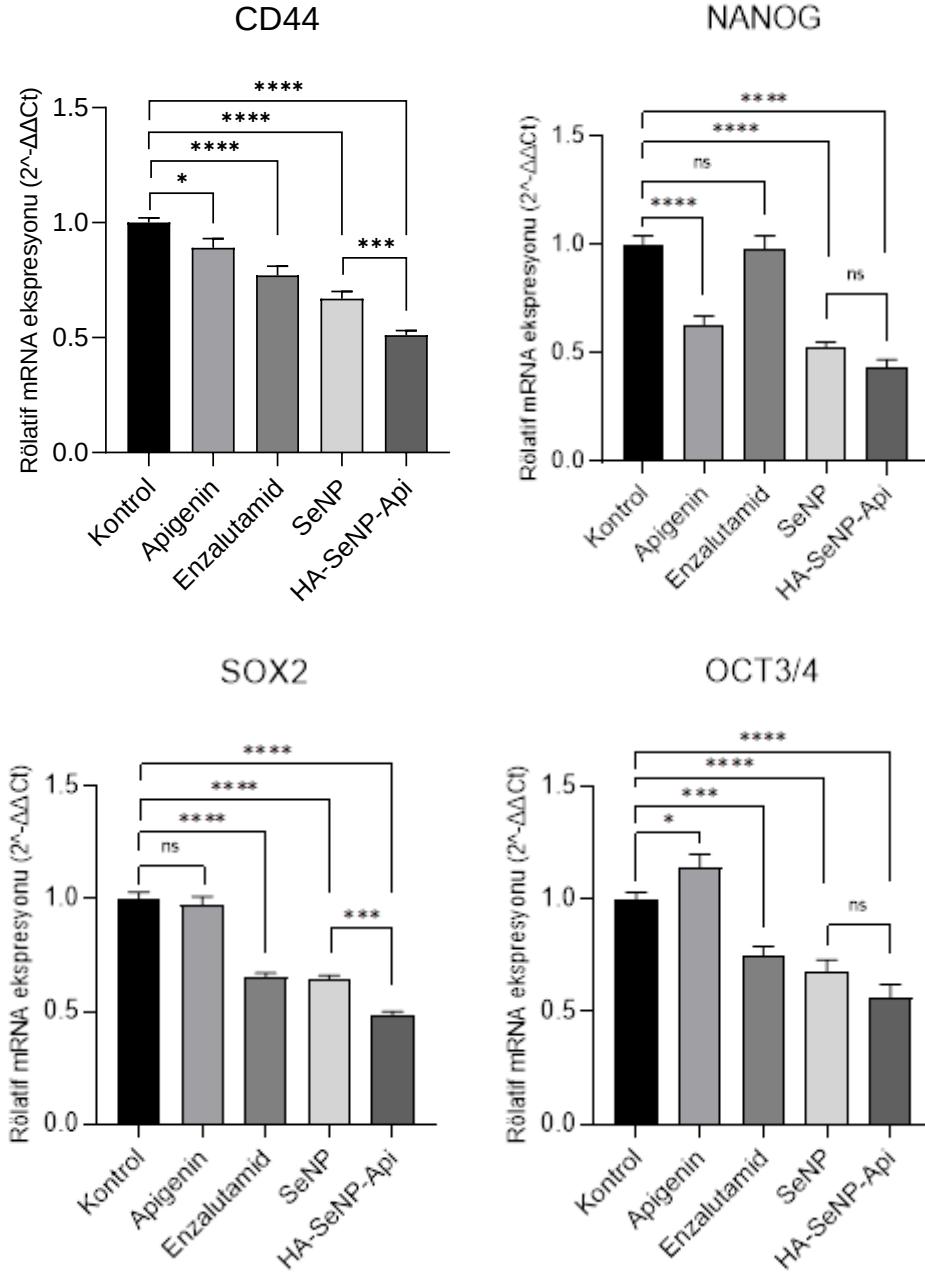
Şekil 2. Androjen reseptörü (AR), androjen sinyal yolunun yukarı akışında yer alan temel düzenleyicidir. PSA ise bu yolun aşağı akışındaki biyobelirteçlerden biridir ve AR aktivitesinin bir göstergesi olarak kabul edilmektedir. Sonuçlar, HA-SeNP-Api kombinasyonunun hem AR mRNA düzeylerini hem de PSA ekspresyonunu ve protein seviyelerini belirgin biçimde baskıladığını göstermektedir. AR (**=0.0030, ***=0.0003, ****<0.0001) PSA (**=0.0091, ***=0.0003, ****<0.0001) PSA-ELISA (*=0.0170, ***=0.0008, ****<0.0001), ns: not significant.

p53–BAX ve CASP8/CASP3 mRNA Ekspresyonlarının HA-SeNP-Api ile Düzenlenmesi



Şekil 3. HA-SeNP-Api kombinasyonu, p53 ve BAX ekspresyon düzeylerini artırarak mitokondriyal apoptoz yolunu, kaspaz-8'i yükselterek ölüm reseptörü aracılı apoptoz yolunu, kaspaz-3'ü aktive ederek her iki yolu birlikte güçlü biçimde tetiklemiştir. *p53* (****<0.0001), *BAX* (****<0.0001), *CASP3* (***=0.0005, ****<0.0001), *CASP8* (**=0.0015, ****<0.0001) ns: not significant.

Kök Hücre Belirteçlerinin HA-SeNP-Api ile baskılanması



Şekil 4. HA-SeNP-Api, kök hücrelikle ilişkili dört temel ekseni (CD44–adezyon, NANOG–pluripotensi, SOX2–tümör başlatıcı, OCT3/4–yenilenme) aynı anda baskılayarak prostat kanseri kök hücrelerinin adezyon, yenilenme ve tümör başlatma potansiyelini zayıflatır. Bu etki tekli tedavilerin üzerinde ve kombinasyon lehinedir. CD44 (*=0.0105, ***=0.0007, ****<0.0001), NANOG (****<0.0001), SOX2 (***=0.0002, ****<0.0001). OCT3/4 (*=0.0378, ***=0.0007, ****<0.0001) ns: not significant.

TARTIŞMA

Bu çalışmada CD44 aracılı hedeflemeyi kullanan HA–SeNP–Api nanoformülasyonun androjen sinyaline (AR/PSA), apoptoz yollarına ve kök-hücrelik üzerine etkisi LNCaP modelinde incelendi. Bu doğrultuda uygun kontroller eşliğinde tekli ajanlar (apigenin, SeNP, enzalutamid) ile kombinasyon tasarımları uygulandı. Hücresel düzeyde canlılık değerlendirmeleri yapılarak, moleküler düzeyde AR ve PSA için mRNA/protein analizleri, apoptoz paneli (p53, BAX, CASP3, CASP8) ve kök-hücrelik belirteçleri (CD44, NANOG, SOX2, OCT3/4) nicel olarak incelendi.

MACS yöntemi ile izole edilen CD44⁺ prostat kanseri kök hücrelerinin canlılık oranlarında, kontrol grubuyla karşılaştırıldığında tüm tedavi gruplarında istatistiksel olarak anlamlı azalmalar gözlemlendi ($p < 0.0001$). Tek başına apigenin ve enzalutamid uygulamaları %46,77 ve %44,96'lık bir düşüş sağlarken, SeNP ve HA–SeNP–Api kombinasyonu sırasıyla %80,76 ve %84,40'lık daha güçlü bir inhibisyona yol açtı. Apigenin ile enzalutamid grubu karşılaştırıldığında fark anlamlı bulunmadı ($p > 0.05$), bu da her iki ajanın benzer derecede sitotoksik etkiye sahip olduğunu göstermektedir. Ancak SeNP'nin apigenine kıyasla belirgin daha fazla canlı hücre azalması sağlaması ($p < 0.0001$), SeNP'nin bu hücre hattı üzerinde daha etkin bir ajan olduğunu ortaya koymaktadır. En etkili sonuç ise HA–SeNP–Api kombinasyonu ile elde edilmiş olup, bu grup kontrol grubuna kıyasla %84,40 oranında canlılık kaybı sağlamış ve bu etkinin hem tekli hem de ikili uygulamalara kıyasla daha belirgin olduğu görüldü (Şekil 1). Bu bulgular, SeNP'nin ve HA–modifiye kombinasyonların CD44⁺ prostat kanseri kök hücrelerinde sitotoksik etkiyi anlamlı düzeyde artırabileceğini göstermektedir. Ayrıca, hyaluronik asit modifikasyonu sayesinde CD44 hedeflemesi yoluyla hücre alımın artması, terapötik etkinliği yükseltmiş olabilir.

HA–SeNP–Api kombinasyonu LNCaP hücrelerinde AR ile PSA mRNA düzeylerini ve PSA proteinini anlamlı biçimde azalttı (Şekil 2). Bu etki, SeNP'lerin prostat kanserinde Akt–MDM2–AR eksenini baskılayarak büyümeyi durdurabildiğini gösteren çalışmalara paraleldir ve biyojenik SeNP'lerin LNCaP-FGC'de AR/PSA ekspresyonunu düşürdüğünü bildiren verilerle uyumludur.^{9, 14} Apigenin'in androjen-duyarlı LNCaP'de intraselüler ve salgılanan PSA düzeylerini azalttığı daha önce gösterildiğinden, kombinasyonda apigenin bileşeninin AR/PSA eksenindeki baskıya katkısı beklenebilir.¹⁵ HA kaplama, CD44 aracılı hedeflemeyle hücre içine alımı artırabilen bir stratejidir; bu nedenle kombinasyonun tek ajanlara kıyasla daha düşük AR/PSA düzeyleri oluşturması biyolojik olarak tutarlıdır.¹⁶ Enzalutamid grubunda gözlenen baskılama da ilacın AR bağlanmasını ve nükleer translokasyonu engelleyen mekanizmasıyla örtüşmektedir.¹⁷

Apoptotik yollar açısından HA–SeNP–Api kombinasyonu LNCaP hücrelerinde p53 ve BAX transkriptlerini anlamlı biçimde yükseltirken, CASP3 (yürütücü kaspaz) ve CASP8 (ekstrinsik yol başlatıcı kaspaz) ekspresyonlarını da artırdı. Bu durum hem mitokondriyal (intrinsik) hem de ölüm reseptörü aracılı (ekstrinsik) apoptozun eşzamanlı olarak devreye girdiğini düşündürmektedir (Şekil 3). Selenyum bileşikler prostat kanserinde ROS–p53–Bax eksenini aktive ederek mitokondriyal yolun açılmasını ve kaspaz-9/3 kaskadını tetiklediği, LNCaP'de sodyum selenitin, p53'ün Ser15 fosforilasyonu ile Bax artışını ve mitokondriyal translokasyonunu indükleyip sitokrom-c salınımı ve kaspaz-9/3 aktivasyonuna yol açtığı bildirilmektedir.¹⁸ Bu bulgu, SeNP'lerin prostat kanseri hücrelerinde apoptozu artırdığı ve kaspaz ailesi ile p53'ü düzenlediğini bildiren çalışmalarla da uyumludur.^{9, 19}

Apigenin tarafında, androjen-duyarlı prostat kanseri hücrelerinde ROS-bağımlı p53 aktivasyonu, Bax ekspresyon artışı ve kaspaz aktivasyonu ayrıntılı olarak gösterilmiştir. p53 inhibisyonu ve genel kaspaz inhibitörleri apigenin kaynaklı apoptozu belirgin biçimde azaltır.¹⁵

Ayrıca apigenin, PC-3 ve CD44⁺ prostat kanseri kök hücrelerinde ekstrinsik yol bileşenlerini (örn. CASP8, CASP3, TNF- α) yukarı regüle ederek kaspaz-8 aracılı apoptozu tetikler. Bu durum bizim bulgularımızda da olan CASP8 artışı ile uyumludur.²⁰ Kombinasyon halinde gözlenen CASP3 artışı, intrinsik-ekstrinsik yolların kaspaz-3 üzerinde birleştiğini ve çok bileşenli formülasyonun yürütücü basamakta etkileri potansiyel olarak güçlendirdiğini düşündürebilir (Şekil 3). Toplamda, verilerin hem SeNP (p53/Bax-mitokondriyal eksen) hem apigenin (p53 ve/veya CASP8'li ekstrinsik yol) üzerinden apoptozu güçlendirdiğini, kombinasyonun ise bu mekanizmaları sinerjistik biçimde birleştirerek CASP3 aktivasyonu yönünde yoğunlaştırdığını gösterdiği değerlendirilebilir.

HA-SeNP-Api kombinasyonu LNCaP hücrelerinde kök hücrelikle ilişkili CD44, NANOG, SOX2 ve OCT3/4 transkriptlerini belirgin biçimde azalttı (Şekil 4). Bu sonuç, prostat kanserinde kök-benzeri fenotipi tanımlayan çekirdek pluripotensi ağının (SOX2/OCT4/NANOG) tümör başlatma ve tedavi direncindeki rolüne ilişkin bulgularla uyumludur. SOX2/OCT4-yüksek alt popülasyonun seçici eliminasyonu tümör gelişimini engelleyip tedavi yanıtını artırır.²¹ CD44, hem prostat kanserinde CSC belirteci hem de HA'nın doğal ligandı olduğundan, HA-kaplı nanoformülasyonla CD44⁺ hücrelere hedefli alım, bu eksenindeki baskıyı güçlendirmiş olabilir.^{22, 23} Apigenin'in prostat kanseri CD44⁺ kök hücrelerinin canlılığını ve göçünü PI3K/Akt/NF- κ B yolunu baskılayarak azalttığı, ayrıca Oct3/4 proteinini düşürdüğü gösterilmiştir.²⁰ Bu da kombinasyonda gözlenen OCT3/4 azalmasıyla tutarlıdır. SeNP'ler prostat kanserinde antitümör etki gösterir ve miR-16 gibi tümör baskılayıcı miRNA'ları yukarı düzenleyerek proliferasyon/göçü baskılar bu düzenlemelerin stemness ağını ikincil olarak zayıflatması beklenir.¹⁹ Toplu olarak, veriler kombinasyonun CD44 aracılı hedefleme ve SeNP+apigenin kaynaklı sinyal baskısı üzerinden kök-hücrelik programını çok-bileşenli biçimde düşürdüğünü düşündürmektedir.

SONUÇ

Bu çalışma, LNCaP hücrelerinde geliştirilen HA-SeNP-Api kombinasyonunun çoklu tümör-ilerleme eksenlerini eşzamanlı hedefleyerek belirgin antitümör etki oluşturduğunu göstermiştir. Kombinasyon hem androjen yolunu baskılayarak AR ve PSA mRNA düzeyleri ile PSA proteinini anlamlı biçimde düşürmüş, hem de p53 ve BAX artışıyla mitokondriyal (intrinsik) apoptozu, CASP8 düzeyi artışıyla ekstrinsik apoptozu devreye sokmuş ve yürütücü basamakta CASP3'ü güçlendirmiştir. Buna paralel olarak kök-hücre ile ilişkili CD44, NANOG, SOX2 ve OCT3/4 transkriptlerinin eşzamanlı azalması, kombinasyonun adezyon, yenilenme ve tümör başlatma kapasitesini destekleyen programı zayıflatmış olduğunu düşündürmektedir. Hücre sağkalım analizlerinde en düşük değerler kombinasyonda elde edilmekle birlikte SeNP ile fark istatistiksel olarak anlamlı değildir; yine de moleküler düzeydeki daha derin baskılama, HA'nın CD44 aracılı hedefleme potansiyeli ve apigeninin sinyal yollarına katkısıyla sinerjik bir etkiye işaret etmektedir. Bulgular, HA-SeNP-Api yaklaşımının prostat kanserinde, özellikle kök-hücre benzeri ve tedaviye dirençli alt popülasyonlara karşı umut verici bir aday olabileceğini, bunun doğrulanması için çoklu hücre hattı/protein düzeyi doğrulamaları ve in vivo etkinlik-güvenlilik çalışmalarının gerekliliğini ortaya koymaktadır.

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**ASSESSMENT OF OXIDANT–ANTIOXIDANT BALANCE AND THIOL–
DISULFIDE HOMEOSTASIS ALTERATIONS INDUCED BY MONOCROTALINE
IN MICE**

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ABSTRACT

Introduction and Purpose: Monocrotaline (MCT), an unsaturated pyrrolizidine alkaloid, is an important source of plant toxicity in humans and animals. Exposure to MCT-containing plants leads to the development of oxidative stress and, consequently, disturbances in the oxidant-antioxidant balance. The aim of this study was to evaluate the oxidant/antioxidant balance and thiol-disulfide homeostasis in blood of mice experimentally exposed to MCT.

Materials and Methods: Thirty male BALB/c mice were divided into three groups of 10 animals each: control (FTS, oral), acute toxicity (120 mg/kg single dose of MCT, oral), and subacute toxicity (120 mg/kg MCT, 3 times at 5-day intervals, oral). At the end of the experiment, blood samples were collected to determine blood oxidative status. Plasma TAS, TOS, TT, and NT levels were measured using commercial test kits and a microplate reader. OSI is expressed as the percentage of TOS values relative to TAS values. Dynamic disulfide levels were calculated from TT and NT values. A one-way ANOVA test was employed in the comparison including all groups.

Results: Plasma TAS, TOS, and OSI levels were found to be increased in the experimental groups compared to the control group ($P<0.05$).

Compared to the control group, an insignificantly increase in plasma TT levels was observed in the subacute intoxication group and decrease in the acute group. In both experimental groups, a decrease in plasma NT levels ($P<0.001$) and an increase in DD levels ($P<0.05$) were detected compared to the control group.

Discussion and Conclusion: The significant increase in TOS and OSI levels in the experimental groups compared to the control group is thought to be due to oxidative stress caused by inflammation and increased ROS levels resulting from MCT intoxication. The increase in TAS levels in the experimental groups compared to the control group is considered to be an indicator of the compensatory response developed by the organism against the increased oxidant load. The decrease in NT levels and increase in DD levels in the experimental groups compared to the control group indicate that MCT may weaken systemic and local antioxidant defense mechanisms and increase oxidative load by disrupting the thiol/disulfide balance. In conclusion, the findings indicate that MCT, administered to mice at a dose of 120 mg/kg in both acutely and subacutely, may alter the oxidant–antioxidant balance and thiol–disulfide homeostasis.

Keywords: Blood, Monocrotaline, Mice, Oxidative stress

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DISACCHARIDE INTERACTIONS WITH OUTER MEMBRANE PROTEINS OF BRUCELLA S19 VACCINE STRAIN: MOLECULAR MODELING FOR LYOPROTECTANT SELECTION

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ABSTRACT

Brucella abortus S19 is a live attenuated vaccine strain widely used in the control of brucellosis. Preserving the antigenic properties of the vaccine during lyophilization is critical for maintaining its stability and effectiveness. Disaccharides such as sucrose and trehalose are commonly used lyoprotectants to stabilize proteins during the freeze-drying process; however, their molecular level interactions with *Brucella* outer membrane proteins have not been fully elucidated in the literature. In this study, the binding behaviors of sucrose and trehalose to Omp2b and Omp25 proteins of *Brucella abortus* S19 were evaluated using molecular modeling and docking methods, with the aim of establishing a rational, structure-based approach to lyoprotectant selection. The protein structures were obtained from AlphaFold and UniProt. The molecular structures of trehalose and sucrose were retrieved from the PubChem database. Docking analyses were carried out using SwissDock, and visual analyses were performed using UCSF Chimera to evaluate binding affinities, interaction energies, and conformations. The results showed that both sucrose and trehalose preferentially bind to both proteins. However, when binding energy and interaction parameters were evaluated, the sucrose Omp25 complex was found to exhibit the strongest and most stable binding. Different binding models suggest that the effectiveness of lyoprotectants may be related to protein-disaccharide interactions and the accessibility of the binding site. These findings provide a rational basis for optimizing lyoprotectant selection specific to the target protein. This molecular modeling approach provides valuable insights for designing more effective lyoprotectants to enhance the formulation stability of *Brucella* vaccines. The obtained *in silico* findings indicate that this approach can be applied to stabilize other antigenic proteins and may offer improved protection during the lyophilization process of *Brucella* vaccines.

Keywords: Molecular Docking, Vaccine Stability, Sucrose, Trehalose, Omp2b, Omp25.

INTRODUCTION

Brucella abortus S19 is a live, attenuated vaccine strain still widely used to control brucellosis in animals. However, during freeze-drying of this strain, structural damage and loss of function can occur in sensitive biomolecules, especially membrane proteins. Much of this damage is associated with the breakdown of hydrogen bonds caused by water loss, phase transitions in membrane lipids, and stress during protein folding (Wang, 2000). During drying, and especially in lyophilization media, disaccharides such as sucrose and trehalose are widely used for protein stabilization, preserving membrane integrity, and reducing damage to intracellular structures. Leslei et al. (1995) demonstrated that trehalose and sucrose maintain both membrane and protein stability in bacterial cells during drying. In this study, trehalose and sucrose were found to inhibit the rise in T_m (critical temperature for denaturation). Similarly, Sampedro et al. (1998) compared the effectiveness of disaccharides (trehalose, maltose, sucrose, etc.) to protect the H^+ -ATPase enzyme in the *Kluyveromyces lactis* plasma membrane during lyophilization and rehydration processes; trehalose demonstrated the highest protective power.

Furthermore, mechanisms such as reducing water activity, vitrification, and reducing freezing/drying stress are involved in the protection of biomaterials and cells.

In *Brucella* S19 strain, the immunogenic potential of proteins such as Omp25 and Omp2b, particularly outer membrane proteins, as well as their functional importance in protective vaccine designs, have been emphasized in the literature. For example, studies on the Omp25/Omp31 family of *Brucella* species indicate that these proteins play critical roles in pathogenicity, host attachment, and interactions with the immune system. Additionally, there are recent studies demonstrating that the Omp25 antigen induces an immune response and provides a protective effect through recombinant vaccines or antigen presentation systems. (García-Carrillo, 1981)

In this context, it is valuable to examine the interactions of disaccharides such as sucrose and trehalose with outer membrane proteins in the *Brucella abortus* S19 vaccine strain before or during lyophilization and the effects of these interactions on structure stabilization using in silico models. This will increase the protective effect of the lyophilized vaccine while ensuring product stability without losing the immunogenic properties of the live vaccine strain.

This study aims to analyze the molecular interactions of the disaccharides sucrose and trehalose with key membrane proteins (e.g., Omp25 and Omp2b) of the *Brucella abortus* S19 strain using in silico docking methods. To determine which disaccharides have which binding sites, their binding energies, and possible stabilization mechanisms for this binding.

METHODOLOGY

Preparation of Protein Structures

In this study, the outer membrane proteins Omp2b and Omp25 from *Brucella abortus* S19 were selected as targets. Protein sequences were obtained in FASTA format from the UniProtKB database (<https://www.uniprot.org>). 3D structures were downloaded using the AlphaFold Protein Structure Database and optimized before use in analyses (Jumper et al., 2021).

Ligand Preparation

Sucrose (PubChem CID: 5988) and trehalose (PubChem CID: 7427) were selected as lyoprotectant candidates. 3D structures were downloaded from the PubChem database and converted into formats suitable for docking analyses using OpenBabel v3.1.1 software. As control groups, a randomly selected inert ligand (glycerol, PubChem CID: 753) was subjected to docking analysis using the same method, allowing for a comparative assessment of the binding energy specificity of positive candidates.

Molecular Docking Analyses

Disaccharide-protein interactions were evaluated using the SwissDock (rev. 2022 version) online platform. SwissDock calculates binding free energies (ΔG) based on the EADock DSS algorithm (Grosdidier et al., 2011). Default parameters were used in the docking process, and the search space was set to encompass the entire protein surface. Three independent docking runs were performed for each ligand to ensure repeatability.

Structural Analysis and Visualization

UCSF Chimera v1.16 (Pettersen et al., 2004) software was used for analysis and 3D visualization of the docking results. Protein-ligand interaction sites, hydrogen bonds, hydrophobic interactions, and binding surfaces were examined.

Root Mean Square Deviation (RMSD) values were evaluated for comparison of conformations; RMSD values below 2 Å were considered stable binding models.

The reliability of the results was ensured by evaluating the conformations with the lowest binding free energy and by the consistency of different docking iterations.

RESULTS

Molecular docking analyses revealed that both sucrose and trehalose exhibited significant binding energies with the *Brucella abortus* S19 outer membrane proteins Omp25 and Omp2b. Compared to the results of glycerol docking, used as a negative control, the high RMSD and low Sd-pG values confirm the specificity of the results. The obtained binding free energy (ΔG), polar and nonpolar interaction parameters, and RMSD values reveal the protein-specific interaction patterns of the disaccharides.

Table 1. Docking results

refname	treomp2b	sucomp2b	treomp25	sucomp25	glyomp25	glyomp2b
SP-dG	-6.128	-6.395	-6.473	-6.700	-5.209	-5.526
RMSD	24.407	13.876	19.004	17.867	47.723	55.779

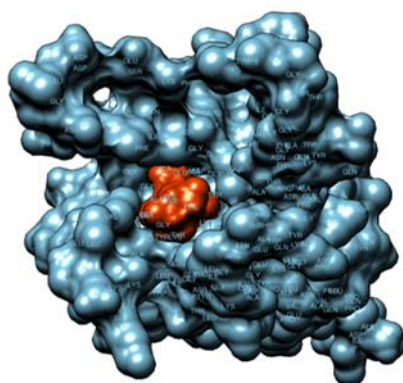


Figure 1. Trehalose-Omp25 Complex

Trehalose was found to be tightly packed into the central depression of the Omp25 protein. This binding location was found to be advantageous in terms of both hydrogen bonding and hydrophobic closure, suggesting that trehalose may have a high capacity to stabilize Omp25. This positioning also makes trehalose's protein-ligand interaction more specific and stable.

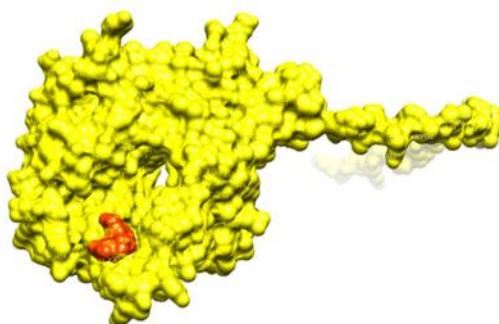


Figure 2. Trehalose-Omp2b Complex

Trehalose has been found to fit into a pocket structure within the Omp2b protein. This binding site provides better protection of the disaccharide from environmental factors, suggesting it may have a strong effect on protein stabilization. This binding, particularly in the inner regions, provides a mechanism to reduce the risk of denaturation from the external environment.

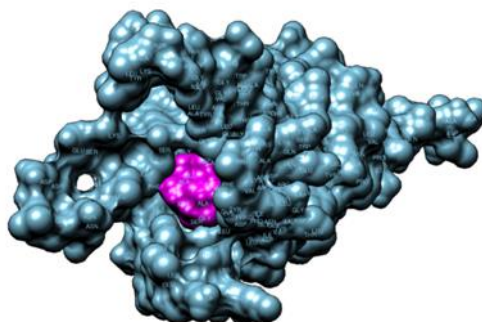


Figure 3. Sucrose-Omp25 Complex

Sucrose was observed to be positioned in a more open region on Omp25, but within a distinct groove. This binding model suggests that sucrose exhibits a compatible interaction geometry with Omp25 and that binding stability may be high. Furthermore, this binding model stood out as one of the models with the lowest ΔG values, consistent with the docking scores obtained.

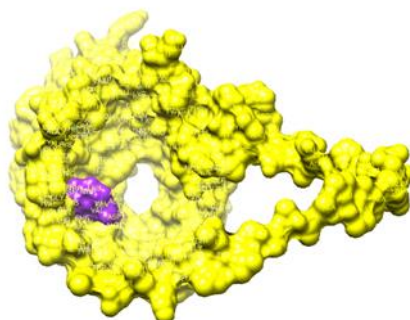


Figure 4. Sucrose-Omp2b Complex

It was determined that sucrose binds closer to the surface of the Omp2b protein. This binding, while more superficial than trehalose, allows sucrose to contact a larger surface area. This suggests that it may increase the strength of hydrophobic interactions, particularly in the presence of hydrophobic interactions.

Visual analyses reveal that sucrose and trehalose exhibit different binding sites and conformations on the same proteins. This suggests that disaccharides may have different protein-specific stabilizing capacities. Specifically, for Omp25, sucrose exhibits a more stable and energetically advantageous binding pattern. For Omp2b, trehalose appears to be superior in stabilization by occupying a more protected region of the protein.

These results are important for vaccine resolubilization after lyophilization, preservation of protein structure, and maintenance of antigenic properties. Disaccharide-protein interactions should be evaluated not only at the level of binding energy but also in terms of binding site and structural orientation.

CONCLUSION AND DISCUSSION

The findings of this study demonstrate that trehalose and sucrose exhibit significant binding energies with *Brucella abortus* S19 outer membrane proteins. The results are generally consistent when compared with data reported in the existing literature. In particular, the protective properties of both sucrose and trehalose for membrane and protein stability in bacterial cells were previously demonstrated by Leslie et al. (1995) and are consistent with the findings of our study. Furthermore, a study by Sampedro et al. (1998) reported that H⁺-ATPase enzyme activity was completely preserved in the presence of trehalose during lyophilization and rehydration processes, supporting the possibility that disaccharide-mediated prevention of protein structural damage. Trehalose's ability to support the vitrification process, thereby reducing damage due to water crystallization, and limiting intraprotein mobility are also important mechanisms explaining the protective role of disaccharides (Crowe et al., 1998).

When evaluated in the context of *Brucella*, the Omp25 protein has been shown to have the potential to elicit immunogenic responses in various antigen presentation systems. For example, the immune response elicited by expressing Omp25 in transgenic plant systems highlights the importance of this protein in vaccine development. Furthermore, multi-epitope vaccine designs based on Omp25 and Omp19 proteins have been reported to possess potent immunogenic and immunostimulatory properties (Jubier-Maurin et al., 2001; Li et al., 2021). In this context, the binding patterns established by disaccharides with these proteins provide data that can contribute to the rational design of vaccine formulations by preserving epitope integrity and enhancing stability.

The *in silico* analyses used in this study offer significant advantages in terms of time and cost. They allow for the prediction of candidate disaccharide-protein interactions before experimental validation. The binding energies, binding sites, and interaction types (hydrogen bonds, van der Waals forces, hydrophobic interactions) obtained from docking results provide insights into the mechanisms of biological stabilization. In particular, the interaction of disaccharides with polar and hydrophobic regions on the protein surface plays a critical role in maintaining structural integrity during water loss.

However, the study has several limitations. First, the protein models used do not fully represent the membrane lipid environment; therefore, the complexity of water-lipid-protein interactions under experimental conditions cannot be fully reflected. Furthermore, docking scores and predicted interactions may not perfectly match stabilization data in real biological systems. Aggressive environmental factors such as protein folding, oxidative stress, and physical drying conditions can lead to structural deterioration despite disaccharide binding. Furthermore, disaccharide concentration, solution pH, ion presence, drying rate, and rehydration conditions are also variables that determine biological effects (Zhang et al. 2021).

Experimental validation studies are crucial to enhance the value of *in silico* findings. Stability tests should be performed on Omp25 and Omp2b proteins lyophilized in the presence of trehalose or sucrose, assessing structural integrity (e.g., CD spectroscopy, fluorescence spectroscopy), immunogenicity (antibody responses), and, if applicable, functional activities. Parameters such as disaccharide type and concentration, co-stabilizers (amino acids, polymers), pH, and ionic conditions should be systematically optimized. Furthermore, stability studies should be conducted for lyophilized products, taking into account long-term storage conditions (temperature, humidity, light). Finally, testing vaccine formulations for immune response and protection in animal models, particularly comparisons with existing live vaccines, will enhance the translational value of the data obtained.

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α -SİPERMETRİN TOKSİSİTESİNE KARŞI KAFEİK ASİT FENETİL ESTERİN ETKİSİ

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EFFECT OF CAFFEIC ACID PHENETHYL ESTER AGAINST α -CYPERMETHRIN TOXICITY

ABSTRACT

In this study, it was aimed to investigate the effects of caffeic acid phenethyl ester (CAPE) on biochemical parameters that may be caused by subacute alpha-cypermethrin (α -CYP) administration in rats. In the study, 24 Sprague-Dawley rats were divided into 4 equal groups as control group (C), 20 mg/kg α -CYP group (α -CYP), 10 μ mol CAPE group (CAPE), 20 mg/kg α -CYP +10 μ mol CAPE group (α -CYP + CAPE). Then, plasma was obtained from blood samples taken intracardially from the rats. Paraoxonase 1 (PON 1), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities and high-density lipoprotein (HDL) levels were analyzed in these plasma samples. When the analysis results were evaluated; It was determined that there was a significant decrease in PON 1 activity and HDL levels and a significant increase in AST, ALT and ALP activities in the α -CYP-applied group compared to the control group. In addition, while the biochemical parameters of the CAPE group were similar to those of the control group, there were decreases in AST, ALT, and ALP activity, and increases in PON activity and HDL levels compared to the α -CYP group. Accordingly, it was concluded that subacute α -CYP in rats may cause significant activity changes in biochemical parameters, especially liver enzymes, and CAPE may prevent these changes. This suggests that subacute α -CYP administration in rats can cause significant changes in biochemical parameters, particularly liver enzyme activity, and that CAPE can prevent these changes.

Keywords: Alpha cypermethrin, liver enzymes, paraoxonase activity, high density lipoprotein

GİRİŞ

Tarımsal alanlarda tarım zararlılarına karşı kullanılan pestisitler, tarımsal amaç dışında evlerde, bahçelerde, kırsal bölgelerde sivrisineklerle ve yabancı otlarla mücadelede de sıklıkla kullanılmaktadır (Deveci et al. 2015, Dogan et al. 2021, Deveci et al. 2021).

Pestisitlerin kullanımı tarım ürünlerinin ve gıda üretiminin artmasına, vektör kaynaklı hastalıkların önlenmesine katkı sunarak gelişen dünyanın ekonomik, sağlık ve sosyal refahını önemli ölçüde iyileştirmiştir. Ancak pestisitler besin zinciri yoluyla vücuda girererek insan ve hayvanlarda zehirlenmelere sebep olmakta, bunların yıkım ürünleri ise çeşitli doku ve organlarda istenmeyen hasarlara neden olmaktadır (Deveci et al., 2015; Deveci and Karapehlivan 2018, Dogan et al. 2021, Nur et al., 2021, Nur et al., 2023).

Alpha cypermethrin'in (α -CYP) üyesi olduğu piretroid insektisitlerin diğer grup insektisitlere karşı kullanım avantajları; böcekler karşı oldukça toksik olmalarına karşın, memelilerde metabolizmalarının farklılığından dolayı düşük toksisiteye sahip olmaları ve toprakta fazla birikmemeleridir (Soderlund, 2010, Öz et al. 2021, Nur et al., 2023). Piretroid insektisitlerin toksisitesinde asıl mekanizma, böceklerin sinir sistemine ait sodyum kanallarının açık kalmasına neden olarak böceklerin ölümüne yol açmalarıdır (Silver, 2014, İssi et al. 2014; Nur et al. 2023)

Propolisin aktif bir bileşeni olan Kafeik asit fenetil ester (CAPE) antienflamatuar, antimikrobiyal, immunmodülatör, farmakolojik, nöroprotektif ve antioksidan etkilerinin olduğu bir çok çalışmada gösterilmiştir (Öktem et al. 2005, Anwar 2012, Nur et al., 2018, Deveci and Kaparehlivan 2018, Nur et al. 2023, Deveci et al.2023).

Yapılan bu çalışmada tarım alanında zararlılarla mücadelede sıklıkla kullanılan α -CYP'nin hedef organizma dışındaki canlılarda oluşturabileceği toksisiteye karşı CAPE'nin etkinliğinin araştırılması amaçlandı.

MATERYAL ve METOD

Kafkas Üniversitesi Deney Hayvanları Araştırma Merkezinden temin edilen, ağırlıkları 220-260 gr. arasında değişen Spraque-Dawley ırkı 24 adet erkek sıçan kullanıldı. Çalışmanın başlangıcında, hayvanlar benzer ortalama ağırlıklara sahip olmaları koşuluyla rastgele her grupta 6 adet rat olacak şekilde 4 gruba ayrıldı. Tüm hayvanlar çalışmanın hem başında hem de sonunda tartıldı. Ortam sıcaklığı 21 °C olan ve günün yarısı aydınlık diğer yarısı karanlık ortamda tutulan hayvanlar standart sıçan yemi (%21 ham protein) ve musluk suyu ile beslendi. Tüm gruplara yapılan doz uygulamaları aşağıda **Tablo 1.**'de verildi.

Tablo 1. Tüm gruplara yapılan doz uygulamaları

Control Group	CAPE Group	α -CYP Group	α -CYP+ CAPE Group
1 ml/kg corn oil (Gavage)	10 μ mol/kg CAPE (Intraperitoneal)	20 mg/kg α -CYP (Gavage)	20 mg/kg α -CYP (Gavage) + 10 μ mol/kg CAPE (Intraperitoneal)

Tüm uygulamalar 30 gün boyunca sürdürüldü. Deney protokolü sonunda sıçanlar intra musküler (i.m) yolla ketamin hidroklorür/xylasin (80/10 mg/kg) anestezi yapılarak intrakardiyak kan örnekleri alındı.

Biyokimyasal Analiz

Plazma eldesi için alınan kan örnekleri 10 dakika 3000 rpm'de santrifüj edildi ve analize kadar -20 °C'de saklandı.

Plazma Paraoksonaz 1 (PON 1) aktivitesi Eckerson'un bildirdiği metoda göre, yüksek dansiteli lipoprotein (HDL) seviyesi, aspartat amino transferaz (AST), alanin amino transferaz (ALT) ve alkalin fosfataz (ALP) aktiviteleri ise ticari test kiti (Biolabo, Fransa) kullanılarak kolorimetrik olarak belirlendi.

İstatistiksel Analiz

Çalışma sonucunda elde edilen bulguların değerlendirilmesinde SPSS (Statistical Package for Social Sciences) 22.0 paket programı kullanıldı. Elde edilen kantitatif değerler istatistiksel veri programında tek yönlü varyans analiziyle (one-way ANOVA) yapıldı. Gruplar arasındaki istatistiksel anlamlılık Tukey HSD çoklu karşılaştırma testi ile yapıldı. Anlamlılık eşik değeri olarak $p < 0.05$ kullanıldı. Sonuçlar ortalama $\pm$ standart sapma olarak verildi.

BULGULAR

Biyokimyasal Bulgular

Çalışmamızda elde ettiğimiz biyokimyasal veriler değerlendirildiğinde kontrol ve CAPE gruplarındaki değerlerin birbirlerine yakın olduğu ve aralarında istatistiki bir fark olmadığı tespit edildi ($p > 0.05$). Ancak kontrol ve CAPE grupları diğer tüm gruplar ile kıyaslandığında aralarında anlamlı derecede fark olduğu belirlendi ($p < 0.01$). Çalışmadan elde edilen tüm gruplara ait PON 1, AST, ALT, ALP aktiviteleri ve HDL seviyeleri Tablo.2'de gösterildi.

Tablo 2. Tüm gruplara ait PON 1, AST, ALT, ALP aktiviteleri ile HDL seviyeleri ve gruplar arasında istatistiki fark

PARAMETRELER	GRUPLAR				P
	Kontrol Mean $\pm$ SD	CAPE Mean $\pm$ SD	α -CYP Mean $\pm$ SD	α -CYP+CAPE Mean $\pm$ SD	
PON 1 (U/L)	199,31 $\pm$ 28,11 ^a	202,44 $\pm$ 27,12 ^a	117,35 $\pm$ 20,69 ^c	151,16 $\pm$ 22,15 ^b	*
AST (U/L)	168,48 $\pm$ 30,70 ^c	173,43 $\pm$ 28,54 ^c	281,69 $\pm$ 50,05 ^a	228,03 $\pm$ 39,71 ^b	*
ALT (U/L)	58,47 $\pm$ 17,38 ^c	61,57 $\pm$ 20,23 ^c	112,04 $\pm$ 29,50 ^a	88,18 $\pm$ 22,00 ^b	*
ALP (U/L)	132,84 $\pm$ 26,07 ^c	140,50 $\pm$ 25,27 ^c	301,07 $\pm$ 46,24 ^a	231,88 $\pm$ 37,04 ^b	*
HDL (mg./dl)	56,22 $\pm$ 10,86 ^a	55,47 $\pm$ 11,17 ^a	34,17 $\pm$ 8,49 ^c	47,31 $\pm$ 10,41 ^b	*

*: $p < 0.01$ = İstatistik olarak anlamlı fark. ^{a,b,c}: Aynı satırda farklı harf taşıyan grup ortalamaları arası fark önemlidir.

TARTIŞMA ve SONUÇ

α -CYP, böcekler üzerinde yüksek toksisiteye sahipken, memeliler ve kuşlar üzerinde daha düşük toksisiteye sahip bir pestisittir. CYP'nin kolay yıkımlanması, toprak ve suda birikmemesi gibi avantajları nedeniyle çevre sağlığı, hayvan sağlığı ve tarım zararlılarıyla mücadelede sıklıkla kullanılmasına yol açmıştır. Bu durum CYP'nin bazı hedef olmayan organizmalarda bazı biyokimyasal parametrelerde önemli değişikliklere neden olabileceğini düşündürmektedir (Abdou et al. 2012, Corcellas et al. 2013, Alalwani, 2020, Nur et al. 2023).

Paraoksonaz 1 (PON 1); karaciğerde sentez edilen, serumda yüksek dansiteli lipoproteine (HDL) bağlı taşınan ve kalsiyuma bağımlı glikoprotein yapısında bir enzimdir. PON 1 organofosfatlı pestisitleri ve sinir gazlarını hidrolize edebilme özelliğine sahip olup, son yıllarda pestisitlerin etkileriyle ilgili araştırmalarda PON 1 aktivitesi önemli bir parametre olarak çalışılmaktadır. Daha önce yapılan çalışmalarda farklı pestisitlerin hem kan hem de doku PON 1 aktivitesinde inhibisyona neden olduğunu, HDL düzeylerinde ise önemli değişiklikler meydana getirdiği bildirilmiştir (Abdel-Salam et al. 2018, Deveci and Karapehlivan 2018, Hosseini et al. 2019, Deveci et al 2021).

Bizim yaptığımız çalışmada ise diğer çalışmalarını destekler şekilde önemli bir piretroid pestisit olan α -CYP'nin PON 1 aktivitesini ve HDL düzeyini düşürdüğü belirlendi. Buna göre α -CYP ile birlikte CAPE verilen gruplarda PON 1 aktivitesi ve HDL düzeyinin kontrol grubu ve CAPE grubundan daha düşük olduğu, sadece α -CYP verilen gruba göre ise anlamlı derecede yüksek olduğu ($p<0,01$) tespit edildi. Bu durum bize α -CYP maruziyetinin özellikle karaciğer dokusunda harabiyete neden olabileceğini ve serum HDL düzeyini olumsuz etkileyebileceğini, CAPE'nin ise hepatoprotektif etkisinin olabileceğini, HDL düzeyini ve HDL'ye bağlı taşınan PON 1 aktivitesini arttırabileceğini düşündürmektedir.

Yapılan bir çok çalışmada artan ALT, AST ve ALP aktivitelerinin karaciğer hasarının bir göstergesi olabileceği ve pestisitlerin karaciğerde detoksifikasyonu esnasında bu enzimlerin aktivitesini yükseltebileceği bildirilmektedir (Abdel-Salam et al. 2017; Deveci et al. 2021). α -CYP'nin tek başına veya bazı pestisitlerle kombine halinde verilmesiyle deney hayvanlarında AST, ALT ve ALP aktivitelerini arttırdığı, artan AST ve ALT aktivitelerinin hepatoselüler hasarın bir göstergesi olabileceği, artan ALP aktivitesinin ise hepatik kolestasis varlığına işaret edebileceği belirtilmektedir (Kanbur et al. 2008, Bhushan et al. 2013, Kanbur et al. 2016). Yapılan bir çalışmada farklı dozlarda (62.5, 125 ve 250 mg kg⁻¹) CYP uygulanan farelerde kontrol grubuna kıyasla karaciğer enzim aktivitelerinde (AST, ALT) ve oksidatif stres parametrelerinde bir artış olduğu bildirilmiştir (Seven et al., 2022).

Bizim yaptığımız çalışmada ise AST, ALT ve ALP aktivitelerinin kontrol grubu ve CAPE verilen grupta birbirine benzer olduğu, α -CYP verilen grupta ise anlamlı derecede yüksek olduğu tespit edildi ($p<0,01$). α -CYP ile birlikte CAPE verilen grupta ise kontrol ve CAPE grubuna göre AST, ALT ve ALP aktivitelerinin anlamlı derecede yüksek olduğu ($p<0,01$), ancak α -CYP verilen gruba göre de anlamlı derecede düşük olduğu ($p<0,01$) tespit edildi. Buna göre α -CYP'ye uzun süre ve fazla maruz kalındığında karaciğerin detoksifikasyon yeteneğinin azalabileceğini, karaciğerin hasarlanmasına bağlı karaciğer enzimlerinin (AST, ALT ve ALP) aktivitelerinin artabileceğini, CAPE'nin ise karaciğer üzerinde koruyucu bir etkisinin olabileceğini düşünmekteyiz.

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ANTIOXIDANT AND ANTIDIABETIC EFFECTS OF ROSEMARY, SUMACH AND ZAHTER

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ABSTRACT

Diabetes and its complications have become a highly concerning health problem worldwide. This complex disease, characterized by high blood sugar levels, encompasses numerous pathogenic processes in its etiology. While the molecular mechanisms behind it are not fully understood, it is accepted that oxidative stress, the accumulation of advanced glycation end products, and inflammation play a role in the development and progression of diabetes. With the increasing prevalence of the disease, studies on the development of new, effective, safe, and affordable therapeutic approaches have become imperative. In this context, phenolic compounds found in plants offer a valuable therapeutic perspective. In recent years, studies have focused primarily on the role of phenolic compounds in diabetes-related oxidative stress, glycation production, and inflammation. In particular, in vitro and in vivo studies on the antioxidant and antidiabetic effects of phenolic compounds remain relevant. This study will share current literature on the phenolic compound content, antioxidant effects, and uses of rosemary, sumac, and zahter, commonly used in the treatment of diabetes.

Keywords: Antioxidant/oxidant capacity, Diabetes mellitus, rosemary, sumach, zahter

LACTOFERRIN AS A NUTRITIONAL AND THERAPEUTIC BIOMOLECULE: FROM IRON METABOLISM TO CANCER

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ABSTRACT

Introduction and Purpose: Lactoferrin (Lf) is an iron-binding glycoprotein that belongs to the transferrin class. It is found in many biological and mucosal fluids, primarily in bovine and human milk. This protein plays a critical role in both nutritional and immunological processes. The primary biological functions of Lf include the regulation of iron metabolism, antibacterial, antiviral, antifungal and antiparasitic effects, and regulation of the innate immune response. Furthermore, it is regarded as a potential therapeutic agent in both infectious diseases and cancer biology due to its ability to reduce oxidative stress and its anti-inflammatory effects.

Materials and Methods: Recent studies and review articles have been evaluated using databases such as PubMed, Scopus, and Web of Science. A comprehensive evaluation of the structural and biochemical properties of Lf, its biological functions, and its therapeutic potential in both human and veterinary medicine has been provided.

Results: In the domain of cancer biochemistry, it has been demonstrated to be effective on cellular signalling pathways, apoptosis mechanisms and cell proliferation. Recent studies have indicated the potential use of Lf in both animal and human health. Due to its benefits and useful properties, Lf is a valuable biomolecule not only in terms of nutrition, but also in terms of the pharmaceutical and functional food industries.

Discussion and Conclusion: The aim of this review is to examine the structural and biochemical properties of Lf, its biological functions such as immunological, antimicrobial, and anticancer activities, along with current research findings in veterinary and human medicine; and to provide a comprehensive assessment of the potential applications and uses of this protein.

Keywords: Lactoferrin, Iron Metabolism, Immunomodulatory, Cancer

DENEYSEL KOLON KANSER MODELİ OLUŞTURULAN RATLARDA DİALİL DİSÜLFİT UYGULAMASININ ETKİLERİ

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ÖZET

Amaç: Kanser; anormal hücre oluşumu ve büyümesi ile vücut fonksiyonlarında değişikliğe sebep olan bir hastalıktır. Kolon kanseri de dünyada oldukça yaygın olarak görülen kanser türleri arasındadır. Bu amaçla insanlarda kolorektal karsinogenezin sürecini ortaya koymada azoksimetan ile deneysel olarak ratlarda kolon kanseri oluşturmak çok önemli katkı sağlamaktadır. Ayrıca miRNA'ların, hastalıkların erken teşhisinde ve tedavisinde yol gösterebileceği düşüncesiyle araştırma alanı günümüzde oldukça fazladır. Bu çalışmada ratlarda deneysel kolon kanser modeli oluşturulup ekspresyon farklılıkları incelenmiştir. Ayrıca yaptığımız çalışmada sarımsağın yağda çözünen bileşenlerinden biri olan dialil disülfid'in (DADS) etkinliği de değerlendirilmiştir.

Materyal ve Metot: Çalışmada 58 adet erkek Wistar albino ratlar 5 gruba ayrıldı: Sağlıklı kontrol (K), kanser kontrol (AOM), sağlıklı dialil disülfid (K + DADS), kanser DADS (AOM + DADS), Mısır yağı (K + mısır yağı). Kanser gruplarının tamamına ilk iki hafta azoksimetan (AOM, 15 mg/kg, sc, 1 hafta ara ile 2 defa) uygulandı. DADS gruplarına 15. haftadan itibaren 50 mg/kg DADS (oral) 3 hafta (haftada 5 gün) süresince verildi. Mısır yağı grubuna da 15. haftadan itibaren 50 mg/kg (oral) 3 hafta (haftada 5 gün) süresince mısır yağı verildi. 18 haftanın sonunda tüm ratlar ötanazi edilerek doku örnekleri alındı. Çalışmada Real Time Polimeraz Zincir Reaksiyonu ile Bcl-2 ve Bax, genlerinin düzeyleri ile miR 34a ekspresyonu ayrıca Elisa ile Bax, Bcl proteini seviyesi belirlendi. Ayrıca kolon dokusunda histopatolojik olarak anormal kript odakları oluşumu belirlendi.

Bulgular: Yapılan çalışmanın sonucunda kontrol grubuyla kıyaslandığında Bcl mRNA ve protein seviyesinde gruplar arasında herhangi bir fark olmadığı, Bax mRNA seviyesinde kontrol grubuna kıyasla diğer gruplarda yükselme olduğu ($P<0.05$), Bax protein seviyesinde ise istatistiksel fark olmadığı gözlemlendi. miR34a ekspresyonunda kontrole göre DADS ve AOM+DADS grubunda anlamlı yükselme ($P<0.05$) olduğu tespit edilmiştir.

Sonuç: Sonuç olarak primer tümörün yerleşimi, tümörün boyutu, hastalığın ilerlemesine bağlı Bcl-2 ve Bax ekspresyon sonuçlarının değişiklik gösterebileceği kanısına varıldı.

Anahtar Kelimeler: Dialil disülfid, Kolon kanseri, Rat, miRNA

ETİK BEYAN: Yapılan çalışma Selçuk Üniversitesi Deneysel Tıp Araştırma ve Uygulama Merkezi'nde (SUDAM) yapıldı. Etik onayı (2020/51) SUDAM'dan 30.11.2020 tarihinde alındı.

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EFFECTS OF DIALYL DISULFIDE ADMINISTRATION IN RATS WITH AN EXPERIMENTAL COLON CANCER MODEL

ABSTRACT

Objective: Cancer is a disease that causes changes in body functions due to abnormal cell formation and growth. Colon cancer is also one of the most common types of cancer worldwide. For this purpose, experimentally inducing colon cancer in rats using azoxymethane has made a significant contribution to elucidating the process of colorectal carcinogenesis in humans. Additionally, there is currently a great deal of research in the field of miRNAs, as it is believed that they may guide the early diagnosis and treatment of diseases. In this study, an experimental colon cancer model was created in rats, and expression differences were examined. Furthermore, the effectiveness of diallyl disulfide (DADS), one of the fat-soluble components of garlic, was also evaluated in our study.

Material and Method: In the study, 58 male Wistar albino rats were divided into 5 groups: healthy control (K), cancer control (AOM), healthy diallyl disulfide (K + DADS), cancer DADS (AOM + DADS), and corn oil (K + corn oil). All cancer groups were administered azoxymethane (AOM, 15 mg/kg, sc, twice with a one-week interval) for the first two weeks. DADS groups were administered 50 mg/kg DADS (oral) for three weeks (five days per week) starting from the 15th week. The corn oil group also received 50 mg/kg (oral) of corn oil for 3 weeks (5 days per week) starting from the 15th week. At the end of 18 weeks, all rats were euthanized, and tissue samples were collected. In the study, the levels of Bcl-2 and Bax genes and miR 34a expression were determined using real-time polymerase chain reaction, and Bax, Bcl protein levels were determined using ELISA. In addition, the formation of histopathologically abnormal crypt foci was determined in colon tissue.

Results: The results of the study showed that there was no difference between the groups in terms of Bcl mRNA and protein levels when compared to the control group, while Bax mRNA levels were elevated in the other groups compared to the control group ($P<0.05$), and there was no statistical difference in Bax protein levels. A significant increase in miR34a expression was detected in the DADS and AOM+DADS groups compared to the control group ($P<0.05$).

Conclusion: In conclusion, it was concluded that Bcl-2 and Bax expression results may vary depending on the location of the primary tumor, tumor size and disease progression.

Keywords: Diallyl disulfide, Colon cancer, Rat, miRNA

ETHICAL STATEMENT: The study was conducted at Selçuk University Experimental Medicine Research and Application Center (SUDAM). Ethical approval (2020/51) was obtained from SUDAM on 30.11.2020.

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ÜLKELER ARASINDAKİ TIBBİ CİHAZ YÖNETMELİK UYGULAMALARININ KÜRESEL UYUM AÇISINDAN ÖNEMİNİN DEĞERLENDİRİLMESİ

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ÖZET

Tıbbi cihazlar sağlık sektöründe çok önemli bir yere sahiptir. Hastalığın teşhisi, önlenmesi, izlenmesi, tahmini, prognozu, tedavisi veya hafifletilmesine yardımcı olmak için kullanılan tıbbi cihazların güvenliği ve etkinliği dünyada düzenleyici otoriterler tarafından sıkı ve ciddi kontrollere tabidir. Tıbbi cihazların geliştirilmesi, otoriterler tarafından onaylanması ve pazarlama sonrası takibine yönelik ülkeler arasında farklılıklar bulunmaktadır. Uluslararası ticarete pazarlanan tıbbi cihazlar hasta güvenliği ve cihaz etkinliği konusunda önemli bir konu olarak görülmektedir. Dolayısıyla tıbbi cihazların kullanımı konusunda ülkeler arasındaki yönetmeliklerin uyumlu hale getirilmesine yönelik politikaların geliştirilmesi önemli olmaktadır. Bu araştırmanın farklı ülkeler arasındaki tıbbi cihaz otoriterlerinin yönetmelikleri ve uygulamaları hakkında genel bir bilgi değerlendirmesinde bulunarak tıbbi cihazlar için uygulanan yönetmeliklere genel bir bakış açısı sunmaktır. Bu bağlamda tıbbi cihaz yönetmelikleri hakkında ileriye dönük stratejiler hakkında fikir vererek, ülkeler arasındaki farklılıkları belirterek gelecekte kullanımı artarak devam edecek olan tıbbi cihazların uygulamaların uygulamalarına yönelik anlamayı hedeflemektedir. Araştırmada, ülkeler arasında tıbbi cihazların kullanımına yönelik farklılıklar olmasına rağmen ortak yapılacak çalışmalar hem güvenlik hem de ticaretin kolaylaştırılması açısından fayda sağlayabilir.

Anahtar Kelimeler: Düzenlemeler, Küreselleşme, Tıbbi cihaz.

GİRİŞ

Evrensel sağlık kapsamında daha sağlıklı toplumlar için kaliteli, güvenli ve uygun fiyatlı tıbbi cihazlara erişim önemlidir. Tıbbi cihazlar hastalıkları teşhis etmek, tedavileri izlemek, engelli kişilere yardımcı olmak, hem akut hem de kronik hastalıklara müdahale ve tedavi etmek için kullanılır (<https://www.who.int>). Modern sağlık hizmetlerinde önemli bir rol oynayan tıbbi cihazlar hastaların iyileşme sürelerini kısaltır ve hastanede kalış sürelerini de kısaltır. Tıbbi cihazlar etkili koruyucu sağlık hizmeti için sağlık sistemi kapasitesini artırır ve sağlık sistemi maliyetlerini de düşürür (Snowdon et al., 2011). Bu kadar önemli ve ciddi şekilde faydası olan tıbbi cihazlar, basit aletlerden tıbbi testlere ve implantlara kadar uzanan geniş ve heterojen bir ürün grubunu oluşturur ve bu ürünlerin güvenliği ve etkinliği tüm gelişmiş ülkelerde sıkı bir şekilde düzenlenir. (Potockova 2020). Basit cihazlardan, son derece gelişmiş, karmaşık görüntüleme cihazlarına ve uzun süreli cerrahi implantlara kadar uzanan tıbbi cihazlar, tanı ekipmanları, cerrahi aletler, kateterler, koroner stentler, kalp pilleri, manyetik rezonans görüntüleme (MRG) cihazları, röntgen cihazları, protez uzuvları örnekler arasında belirtilebilir (Sastri, 2021; Cheng, 2003)

Hastalığın teşhisi, önlenmesi, izlenmesi, tahmini, prognozu, tedavisi veya hafifletilmesine yardımcı olmak için kullanılan tıbbi cihazlar güvenlik endişeleri nedeniyle dünyada düzenleyici otoriterler tarafından ciddi kontrollere tabidir (Gilbert 2023; Kasoju et al. 2023).

Tıbbi cihazların yasal olarak satılabilmesi ve kullanılabilmesi için onayı veya izni olması gerekir. Tıbbi cihazlar hastalar tarafından kullanılmadan önce güvenlik önlemlerinin alınması gerekmektedir. Tıbbi cihazların güvenliğini sağlamak için, geliştirme aşaması, düzenleyici otoriteler tarafından yapılan incelemeler ve pazarlama sonrası takibi risklerin uygun şekilde dağıtılması ve yönetilmesine yönelik önlemlerin belirlenmesi açısından önemlidir. Çünkü geliştirme aşamalarında kısıtlı hasta sayısından dolayı pazarlama sonrası yaygın olarak görülen advers olayları tahmin etmek neredeyse imkansızdır. (Mori vd., 2014). Bu yüzden sıkı kontrol ve denetlemelere tabi olan tıbbi cihazların üretimden düzenleyici hükümlere kadar kendine özgü kuralları vardır (Sapkota, 2022). Potansiyel sağlık riskleri ve tıbbi cihazların güvenlik ve etkinliğinin değerlendirilmesi nedeniyle, birçok ülke tıbbi cihaz denetim ve yönetimi için yönetmelikler oluşturmuştur. Tıbbi cihazlar, herhangi bir ülkede pazara sunulmadan önce güvenlik ve etkinlik prosedürlerinden geçerek onaylanır. (Zhang, 2016).

Küresel tıbbi teknolojiler pazarı, dünyanın en gelişmiş ülkeleri olan ABD, Avrupa ve Japonya'da yoğunlaşmıştır. Bu nedenle, yalnızca ulusal veya uluslararası standartlar tarafından onaylanması önemli olmaktadır (Lee & Park 2017; Hospodkova vd., 2018)

Çünkü Gelişmiş ve gelişmekte olan ülkeler arasındaki tıbbi cihaz güvenliği ve kalitesi en büyük zorluklardan biridir. Gelişmekte olan ülkelerdeki hastaneler en son teknolojilere sahipken, dünyada birçok sağlık tesisi hastaları tedavi etmek için en temel tıbbi cihazlardaki erişim ve güvenlikten yoksun kalmaktadır (Lynn 2018).

Dünya Sağlık Örgütü, sanayileşmiş ülkeler tarafından sağlanan tıbbi cihazların neredeyse dörtte üçünün düşük kaynaklara sahip topluluklara ulaştığında kullanılmadığını tespit etmiştir. Çoğu tıbbi cihaz elektrik dalgalanmaları, yüksek sıcaklıklar, yüksek nem, toz, böcek istilası, yedek parça bulunabilirliğinin düşük olması, yüksek maliyetli sarf malzemeleri gibi durumlarda etkili bir şekilde çalışmamaktadır. Dolayısıyla sağlam, güvenli ve iyi tasarlanmış tıbbi cihazların geliştirilerek uluslararası pazara sunulması önemli olmaktadır (Fisher & Johansen 2020). Tıbbi cihazlara yönelik yapılan düzenlemeler, mal, sağlık ve hatta can kaybının önlenmesini amaç edinerek kamuoyu güvenliğini sağlar. (Maci & Marešová 2022). Dolayısıyla tıbbi cihazlar risk yönetimi süreci bağlamında güvenlik önemli olmaktadır (De Jong, 2020). Dolayısıyla Tıbbi cihazlar (MD'ler), Günlük tıbbi uygulamalardaki önemleri göz önüne alındığında, üretim, pazarlama ve kullanım süreçleri her düzeyde ciddi şekilde düzenlenmektedir (Badnjević, 2022). Tıbbi cihaz sektörü sağlığı hem de ekonomiyi iyileştirerek ülkeye fayda sağlayan önemli ve kazançlı bir sektördür. Tıbbi cihazların onay ve gözetim sürecini ve halk sağlığını ve güvenliğini daha iyi korumak için süreci hem güçlendirebilecek hem de kolaylaştırabilecek ve aynı zamanda hastalara fayda sağlayacak cihazların geliştirilmesini ve pazarlanmasını teşvik edebilecek politikalar günümüzde önemli olmaktadır (Lind, 2017). Tıbbi cihazlara yönelik olarak yapılacak farklı uygulamalar pazarlama açısından firmalara da zorluklar yaşaracaktır (Smith, 2015).

Bu araştırmanın amacı gelişmiş ülkelerdeki tıbbi cihaz otoritelerinin yönetmelikleri ve uygulamaları hakkında genel bir bilgi değerlendirmesinde bulunarak, tıbbi cihaz yönetmeliklerinin ülkeler arasındaki uyumunun önemine değinerek, tıbbi cihazlar için yapılan uygulamalara genel bir bakış açısı sunmaktır. Bu bağlamda tıbbi cihaz yönetmeliklerinin stratejiler hakkında ileriye dönük fikir vererek, ülkeler arasındaki farklılıkları yakalayarak gelecekte kullanımı artarak devam edecek olan tıbbi cihaz uygulamalarındaki uyumu anlamayı hedeflemektedir.

MATERYAL VE METOT

Araştırmada dünyadaki en önemli ve yetkin olarak kabul edilen ülkelerin sağlık otoriterleri ile Türkiye değerlendirilecektir. Bu amaçla Amerika Birleşik Devletleri (ABD) sağlık otoritesine bağlı Food and Drug Administration (FDA), Avrupa Birliği denetiminden sorumlu bir ajans olan European Medicines Agency (EMA), Japonya Pharmaceuticals and Medical Devices Agency (PMDA) ve Türkiye'deki tıbbi cihaz yönetmelikleri ele alınarak küresel açıdan tıbbi cihaz yönetmeliklerindeki uyumun önemi değerlendirilecektir. Tıbbi cihazların onayına ilişkin yönetmelikler ilgili kurumların web sitelerinden elde edilmiştir.

Araştırmada konu edinilen üç düzenleyici kurum (FDA, EMA, PMDA) çerçevesinden incelenmesinin önemi ise; ABD ve Japonya'nın iki ileri sanayi ülkesi olması ve bölgesel bir blok olarak 27 üye devletten oluşan Avrupa Birliği'dir. Özellikle Amerika Birleşik Devletleri ve AB'nin tıbbi cihazları düzenlemedeki faaliyetleri, uluslararası sistemde ve küresel düzenlemelerde en fazla dikkate alınan ve kurumlar olmasıdır. Çalışmadaki yer alan otoriterlerin ülkelere baktığımızda endüstrileri dünyanın en üretken ve yenilikçi ürünler üreten ülkeleri arasında yer almaktadır. (Altenstetter, 2012). Türkiye'deki çalışmalar üç düzenleyici kurumun çalışmaları arasına alınarak araştırmanın Türkiye açısından da değerlendirilmesine olanak sağlamaktadır.

Çalışmanın verileri kamuya açık internet sitelerinden elde edilmiştir ve etik kurul onayı gerektirecek şekilde veriler toplanmadığından etik kurul onayı alınmamıştır.

SONUÇLAR

FDA, ABD'de tıbbi cihazların (tanı testleri dahil) satışını, güvenliğini ve yasaklama yetkisini izler. Bir tıbbi cihazın ABD'de yasal olarak satılabilmesi için, cihazı satmak isteyen kişi veya işletme FDA'dan onay alması gerekir. Onay alabilmek için, cihazın belirli bir kullanım için makul ölçüde güvenli ve etkili olduğuna dair kanıtlar sunması gerekir. FDA'nın gıda, insan, veteriner ilaçları, biyolojik ürünler, tıbbi cihazlar ve radyasyon yayan ürünler üzerindeki denetimi 1906 yılında başlamıştır. FDA, Tıbbi cihazlar güvenliği ve etkinliğini düşük riskli Sınıf I tıbbi cihazlar, orta riskli Sınıf II tıbbi cihazlar ve yüksek riskli Sınıf III tıbbi cihazlar olmak üzere üç farklı şekilde risk tabanlı sınıflandırma sistemi kullanmaktadır. (<https://www.fda.gov/>)

AB'de tıbbi cihazların düzenlenmesi, tıbbi cihazların piyasaya arzı, piyasada bulundurulması, hizmete sunulması, güvenliği, yan etki durumları, klinik çalışmalara ilişkin kurallar, üretici, dağıtım, yetkili sorumlulukları 5 Nisan 2017 tarihli yönetmelik ile tıbbi cihaz üreticilerinin uyması gereken standartları belirlenmiştir. Yönetmeliğe göre tıbbi cihazın uygunluğu CE işareti ile belirlenmektedir. Avrupa Birliği'nde (AB), güvenli olduklarını ve amaçlandığı gibi performans gösterdiklerini garanti altına almak için yasal gereklilikleri karşıladıklarını kanıtlamak üzere bir uygunluk değerlendirmesinden geçmeleri gerekir. AB Üye Devleti düzeyinde düzenlenirler, ancak düzenleme sürecinde Avrupa İlaç Ajansı (EMA) da yer alır. EMA, tıbbi ürün için merkezi prosedür uygulaması kapsamında tıbbi cihazla birlikte kullanılan ilaçların güvenliğini ve etkinliğini değerlendirir. Sınıflandırma ve uygunluk değerlendirmesine göre cihazların sınıflandırmasına bakıldığında kullanım amaçları ve içerdikleri riskler dikkate alınarak düşük riskli günlük cihazlar veya gereçler olarak kabul edilen Sınıf I cihazlar, düşük orta riskli olarak kabul edilen Sınıf II cihazlar ve yüksek riskli olarak Kabul edilen Sınıf III cihazlardır. (<https://www.ema.europa.eu/en/homepage>)

Japonya'da tıbbi cihazların düzenlenmesinden sorumlu olarak iki düzenleyici kurum ortak olarak çalışır. Sağlık, Çalışma ve Refah Bakanlığı (MHLW) ve PMDA. MHLW, İlaçlar ve Tıbbi Cihazlar Dahil ürünlerin kalite, etkinlik ve güvenliğinin sağlanmasına ilişkin olarak bir ürünün tıbbi cihaz olarak kabul edilip edilmediğine ilişkin değerlendirilmesini yapar. PMDA ise ilgili cihazın incelemesini yaparak pazara arz sonrası güvenlik önlemlerini üstlenir. Japonya'da tıbbi cihazlar, risk seviyelerine göre dört sınıfa ayrılır: Sınıf I (Son Derece Düşük Risk), Sınıf II (Düşük Risk), Sınıf III (Orta Risk) ve Sınıf IV (Yüksek Risk). Tıbbi cihazları Japonya'da pazarlayabilmek için, yabancı bir üreticinin, MHLW'nin kurallarına uyması, sınıflandırmaya uygun olarak onay ya da sertifika alması gerekmektedir. (<https://www.pmda.go.jp/english/>) Türkiye'de insan kullanımına yönelik tıbbi cihazların, aksesuarlarının piyasaya arz edilmesi, piyasada bulundurulması veya imalatçı yükümlülükleri ile ilgili usul ve esasları tıbbi cihaz yönetmeliği ile belirlenmiştir. Türkiye İlaç ve Tıbbi Cihaz Kurumu (TİTCK) yönetmeliğin uygulanması ile ilgili esaslarda belirleyici kurum olarak görev yapar. Türkiye'de tıbbi cihazlarda CE uygunluk işareti zorunludur. Tıbbi Cihazlara İlişkin Avrupa Veri Tabanı (EUDAMED), kuralları doğrultusunda Ulusal Ürün Takip Sistemi yükümlülüklerini uygular. Cihazlar; cihazların kullanım amacı ve yapısı gereği barındırdığı riskler dikkate alınmak suretiyle sınıf I, IIa, IIb veya III şeklinde sınıflandırılır(<https://www.titck.gov.tr>).

Tıbbi Cihaz Düzenlemelerinin Kurumsal Uyumun Önemi

Uluslararası ticaret ve özellikle sağlık gibi sektörlerde ürünlerin güvenliğini ve etkinliğini sağlamak için düzenlemelerin uyumlaştırılması önemli olmaktadır. Ülkeler arasındaki mevzuatın uyumlu hale getirilmesi ve standartlaştırılması sayesinde ticaret engelleri azaltılmakta ve tıbbi cihaz üreten firmaların küresel pazarlara daha kolay erişebilmesi sağlanabilir. Düzenleyici süreç kolaylaştırılabilir ve çeşitli bölge ve ülkelerdeki düzenleyici kurallar ve standartlar uyumlu hale getirilerek hastaların yüksek kaliteli tıbbi cihazlara erişimi iyileştirilebilir (Chettri, & Ravi, 2024). Bu bağlamda dünyada Tıbbi cihazları uluslararası standartlara uygun hale getirmek amacıyla kurulan Global Harmonization Task Force (GHTF) başta Avrupa Birliği , Amerika Birleşik Devletleri , Kanada , Japonya ve Avustralya olmak üzere farklı ülkelerdeki yönetmelikleri düzenlemeleri uyumlu hale getirerek tıbbi cihazların güvenliğini, etkinliğini/performansını ve uluslararası ticareti kolaylaştırmaya yönelik çalışmalar yapmaktadır (<https://www.imdrf.org/>) Yapay zekâ sistemlerinin önemli bir hale gelmesi tıbbi cihaz niteliğindeki yapay zekâ sistemlerinin güvenli bir şekilde piyasaya arz edilmeleri için uygun olup olmadığının değerlendirilmesi gerekir. bu bağlamda Tıbbi cihazlarla yönelik uyumlu çalışmalar önemli olmaktadır (Arık vd 2016).

SONUÇ ve TARTIŞMA

Sağlık hizmetlerinde yüksek hizmet beklentisini karşılayabilmek için iyi bir tıbbi cihaz yönetimine ihtiyaç duyulmaktadır (Uluçay & Fidan 2022).

Tıbbi cihaz düzenlemesi ABD, Japonya, Türkiye ve Avrupa Birliği'nde araştırılan bir konudur. (Altenstetter 2012; Maak & Wylie 2016; Kaya, 2022).

Tıbbi cihazlar öncelikle hasta sağlığını desteklemek ve korumak amacıyla tasarlanmıştır. Bu bağlamda, hasta güvenliği ve cihaz etkinliği başlıca endişe kaynaklarıdır. Pazarlanan tıbbi cihazların artan sayısı ve karmaşıklığı göz önüne alındığında, hasta güvenliği giderek daha önemli hale gelmiştir (Chai, 2000). Bu bağlamda tıbbi cihazlar hasta güvenliğine yönelik olarak risklerine göre sınıflandırılmaktadır.

Üretici firmalar düşük riskli cihazları kendi kurallarına göre kullanıma uygun olarak onaylarken, yüksek riskli cihazlar otoriterlerin klinik çalışmaları değerlendirmesi ve pazarlama sonrası çalışmaları kapsamaktadır. Çok yüksek riskli cihazlar ise kullanım uygunluğunu almadan dosası ciddi şekilde incelenerek uygunluk sertifikası almaktadır (Parvizi & Woods 2014).

Çalışmada elde edilen verilere göre tıbbi cihazlar ile ilgili yönetmeliklerin birbirinden ayrı farklılıkları olmasına rağmen bir çok ortak noktada buluştuklarını göstermektedir. Sağlık otoriterlerinin tıbbi cihazlarda aradıkları en önemli yanın güvenlik ve kalite olduğu görülmektedir. Hastaların daha güvenli ve kaliteli cihazlara erişiminin önemli olduğu söylenebilir. Güvenli ve kaliteli cihazlara erişimdeki en önemli yanın ise kurumlar arası ortak uyumda önemli olmaktadır. Özellikle düşük geri kalmış ülkelere pazarlanan tıbbi cihazların uluslararası pazarlarda güvenli ve etkili bir şekilde kullanılabilmesi için uluslararası kabul görmüş bilimsel verilere göre onaylanmış cihazların uygunluğu önemli olmaktadır. Bu sayede tıbbi cihazlar dünyada uluslararası standartlara kavuşarak ülkeler arası pazarlanması yönündeki ticaret engelleride ortadan kalkacaktır. Bunun için sağlık otoriterlerin ortak noktada buluşması ile düzenleyici süreçler daha kolay hale gelebilir. Bu durum hastaların tıbbi cihazlara erişimini de kolaylaştırabilir.

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OBSTRÜKTİF UYKU APNE SENDROMLU HASTALARDA APELİN, CEMERİN, LEPTİN, RESİSTİN VE VASPIN DÜZEYLERİNİN DEĞERLENDİRİLMESİ

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ÖZET

Amaç: Bu çalışmanın amacı, apelin, kemerin, leptin, rezistin, vaspin ve ileri glikasyon son ürünleri (AGE) serum düzeylerinin Obstrüktif Uyku Apne Sendromu (OUA) ile ilişkilerini araştırmaktır.

Materyal ve Metot: Bu çalışmaya 54 OSA hastası ve 34 sağlıklı kontrol katılmıştır. Serumdaki kemerin, leptin, rezistin, vaspin ve AGE düzeyleri ELISA yöntemi ile belirlenmiştir.

Bulgular: Hasta grubunda kontrol grubuna kıyasla kemerin medyan değerleri anlamlı derecede yüksek ($p = 0,012$) bulunmuştur. Buna karşılık, hasta grubundaki AGE ortalamaları kontrol grubuna kıyasla anlamlı derecede düşük bulunmuştur ($p = 0,036$). Kemerin ile apne-hipopne indeksi (AHİ) ($r=304$, $p=0,025$) ve oksijen desatürasyon indeksi (ODİ) ($r=0,278$, $p=0,042$) arasında pozitif bir korelasyon bulundu. Kemerin ile hemoglobin ($r=0,228$, $p=0,032$) ve demir ($r=0,339$, $p=0,001$) arasında da bir korelasyon gözlemlendi. Apelin ile C-reaktif protein (CRP) arasında pozitif bir korelasyon vardı ($r=0,226$, $p=0,034$). Leptin ise vücut kitle indeksi (VKİ), glikoz, insülin, HbA1c, hemoglobin ve demir ile pozitif korelasyon gösterdi.

Sonuçlar: Bu çalışmanın sonuçları, kemerin ve AGE'lerin OSA fizyopatolojisi ile ilişkili olduğunu göstermektedir. Bu iki parametre uyku apnesinin tanı ve ilerlemesinde yardımcı olabilir.

Anahtar Kelimeler: Obstrüktif Uyku Apnesi, apelin, kemerin, leptin, rezistin, vaspin.

Etik Kurul Onayı: Balıkesir Üniversitesi, Tıp Fakültesi, Klinik Araştırmalar Etik Kurulu Başkanlığı: 22/03/2017-2017/23

Bu çalışma Balıkesir Üniversitesi Araştırma Fonu tarafından desteklenmiştir (Proje numarası: BAP 2017/062).

EVALUATION OF APELIN, CEMERIN, LEPTIN, RESISTIN AND VASPIN LEVELS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA SYNDROME

ABSTRACT

Background The aim of this study was to investigate serum levels of apelin, chemerin, leptin, resistin, vaspin and advanced glycation end-products (AGEs) relationships with Obstructive Sleep Apnea Syndrome (OSA).

Methods 54 OSA patients and 34 healthy controls participated in this study. The levels of chemerin, leptin, resistin, vaspin and AGEs were determined in the serum by the ELISA method.

Results The chemerin median values were significantly higher ($p = 0,012$) in the patients group when compared with the control. In contrast, the mean of AGEs in the patient's group was significantly decreased compared to the control group ($p = 0.036$). A positive correlation was found between chemerin and apnea-hypopnea index (AHI) ($r=0.304$, $p=0.025$) and oxygen desaturation index (ODI) ($r=0.278$, $p=0.042$). A correlation was also observed among chemerin and haemoglobin ($r=0.228$, $p=0.032$) and iron ($r=0.339$, $p=0.001$). There was a positive correlation between apelin and C-reactive protein (CRP) ($r=0.226$, $p=0.034$). Leptin was positively correlated with body mass index (BMI), glucose, insulin, HbA1c, haemoglobin and iron.

Conclusions The result of this study demonstrate that chemerin and AGEs are related to OSA physiopathology. These two parameters may help in the diagnosis and progress of sleep apnea.

Keywords: Obstructive Sleep Apnea, apelin, chemerin, leptin, resistin, vaspin.

OBSTRÜKTİF UYKU APNE SENDROMUNDA GALEKTİN-3, SIRT-1, TİYOREDOKSİN, NİTRİK OKSİT DÜZEYLERİNİN DEĞERLENDİRİLMESİ

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ÖZET

Amaç: Bu çalışmamın amacı; OUAS tanılı alan bireylerde galektin-3 (GAL-3), sirtuin-1 (SIRT-1), tiyoredoksin (TRX), nitrik oksit (NO) düzeylerini tespit ederek bunların Obstrüktif Uyku Apne Sendromu (OUAS) patolojisiyle olan ilişkisini belirlemektir.

Materyal ve Metot: Çalışmamız; yaşları 25-70 arasında değişen, deney grubunda OUAS tanısı konulmuş 44 kişi, kontrol grubunda da sağlıklı 46 kişinin katılımıyla yapılmıştır. OUAS'lu bireylerden alınan kan örneklerinden hemolizat elde edildi ve GAL-3, SIRT 1, tiyoredoksin, NO düzeyleri, ELİSA yöntemi ile çalışıldı.

Bulgular: Çalışmamızın sonuçlarına göre; OUAS'lu hasta grubu ve kontrol grubu arasındaki GAL-3 değerleri karşılaştırıldığında $p=0,00001$ ($p<0,05$) değerini alarak istatistiksel olarak yüksek derecede anlamlı fark saptanmıştır. Diğer yandan SIRT-1 değeri karşılaştırıldığında; ($p<0,05$) değerini alarak, hemoglobin ise ($p<0,05$) değerini alarak istatistiksel olarak anlamlı fark saptanmıştır. NO ve TRX ortalamalarında fark gözlenirken, gruplar arasındaki NO değeri istatistiksel olarak karşılaştırıldığında $p=0,10237$ ($p>0,05$) değeriyle, TRX ise $p=0,14519$ ($p>0,05$) değeriyle, hasta ve kontrol grupları arasında anlamlı fark saptanmamıştır.

Sonuç: Çalışmamızda istatistiksel olarak anlamlı sonuçlar elde edilmiş olup hemoglobin, GAL-3, SIRT-1'in OUAS patolojisinden etkilendiği gözlenmiştir. Sonuç olarak; OUAS teşhis ve tedavisinde yer alması için bu çalışmaların yeni verilerle desteklenmesine ihtiyaç vardır.

Anahtar kelimeler: Obstrüktif Uyku Apne Sendromu, galektin-3, hemoglobin, SIRT-1, nitrik oksit, tiyoredoksin.

Etik Kurul Onayı: Balıkesir Üniversitesi, Tıp Fakültesi, Klinik Araştırmalar Etik Kurulu Başkanlığı: 01/03/2017-2017/18

Bu çalışma Balıkesir Üniversitesi Araştırma Fonu tarafından desteklenmiştir (Proje numarası: BAP 2017/062).

THE EVALUATION OF THE LEVELS OF GALECTIN-3, SIRT 1, THIOREDOXIN, NITRIC OXIDE IN OBSTRUCTIVE SLEEP APNEA SYNDROME

ABSTRACT

Background: Attempt was to determine the levels of galectin-3 (GAL-3), sirtuin-1 (SIRT-1), thioredoxin (TRX), nitric oxide (NO) in the individuals who diagnosed with Obstructive Sleep Apnea Syndrome (OSAS) and investigating their relationship in OSAS's pathology.

Materials and Methods: The current study involved 90 participants. The experimental group included 44 individuals diagnosed with OSAS, while 46 healthy individuals served as the control group. The hemolysate obtained from blood samples of individuals with OUAS was analyzed, and the levels of GAL-3, SIRT-1, TRX, and NO were measured using the ELISA method.

According to the results of our study; compared to GAL-3 values between patients with OUAS and the control group, there were significantly high differences ($p<0,05$). On the other hand, there were significant differences in SIRT-1 values ($p<0,05$) and haemoglobin ($p<0,05$) between the same groups. The significance was observed in the average values of NO and TRX. However, there was no statistically significant difference between the groups in the values of NO ($p>0.05$) and TRX ($p>0.05$).

In the current study, statistically significant results were obtained, and it was observed that haemoglobin, GAL-3, and SIRT-1 were affected by OUAS's pathology. In conclusion, these data should be validated by future studies for their consideration in OUAS's diagnosis and treatment.

Keywords: Obstructive Sleep Apnea Syndrome, galectin-3, haemoglobin, SIRT-1, nitric oxide, thioredoxin.

KIL, TIFTİK VE KİLİS KEÇİLERİNİN ÜREME ÖZELLİKLERİNİ ETKİLEYEN KITLG VE KISS1 GEN BÖLGELERİNİN POLİMORFİZMLERİNİN BELİRLENMESİ

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ÖZET

Giriş ve amaç: Bu çalışma, Kıl, Tiftik ve Kilis keçilerinin üreme özelliklerini etkileyen KITLG ve KISS1 gen bölgelerindeki polimorfizmlerin belirlenmesini amaçlamaktadır. KISS1 geni, üreme fonksiyonlarının düzenlenmesinde kritik bir rol oynayan Kisspeptin proteinini kodlarken, KITLG geni ise hücre büyümesi, farklılaşması ve migrasyonu üzerinde etkili olan bir ligand kodlamaktadır. Bu genlerdeki varyasyonlar, keçilerin üreme performansı ve verimliliği üzerinde önemli etkiler yaratabilir.

Materyal ve Metot: Bu amaçla çalışmaya dahil edilen üç keçi ırkından (n=50) 150 adet numune kullanılmıştır. Genomik DNA örneklerinde KITLG ve KISS1 genleri PZR yöntemiyle çoğaltılmıştır. KITLG ve KISS1 gen bölgelerindeki polimorfizmleri belirlemek amacıyla Polimeraz Zincir Reaksiyonu-Kısıtlama Parçacık Uzunluğu Polimorfizmi (PZR-RFLP) tekniği kullanılmıştır. Popülasyonlardaki genetik varyasyonu değerlendirmek amacıyla Hardy-Weinberg dengesi test edilmiştir.

Bulgular: KISS1 genindeki C2540T ve A2510G SNP'leri, tüm ırklarda Hardy-Weinberg dengesiyle uyumlu bulunmuş; genotip dağılımı dengeli, ırklar arası fark önemsizdir. Buna karşılık, KITLG genindeki g.12654G>A ve g.12829T>C varyantları, heterozigot genotip fazlalığı ve homozigot eksikliğiyle Hardy-Weinberg dengesinden anlamlı sapma göstermiştir. Bu durum, genetik sürüklenme, seçilim baskısı veya kapalı çiftleşme sistemlerini düşündürmektedir. Sadece g.12772G>A varyantında denge korunmuştur.

Tartışma ve Sonuç: Genel olarak, genetik çeşitlilik saptanmış ve SNP'lerin yerli keçi ırklarının ıslah ve genetik korunmasında marker olarak potansiyel taşıdığı belirlenmiştir. Bulgular, ileride yapılacak fenotipik veri destekli moleküler araştırmalara zemin oluşturacak niteliktedir.

Anahtar Kelimeler: Kıl Keçisi, Tiftik Keçisi, Kilis keçisi, KITLG, KISS1, SNP

DETERMINATION OF GENE POLYMORPHISMS IN KITLG AND KISS1 GENE REGIONS EFFECTING THE REPRODUCTIVE TRAITS OF HAIR, ANGORA, AND KILIS GOATS

ABSTRACT

Introduction and Aim: This study aims to identify polymorphisms in KITLG and KISS1 gene regions that influence the reproductive traits of Hair, Angora, and Kilis goats. The KISS1 gene encodes the Kisspeptin protein, which plays a critical role in regulating reproductive functions, while the KITLG gene encodes a ligand involved in cellular growth, differentiation, and migration. Variations in these genes may exert significant effects on reproductive performance and productivity in goats.

Materials and Methods: A total of 150 samples were collected from three goat breeds (n=50 per breed). Genomic DNA samples were amplified using the Polymerase Chain Reaction (PCR) technique. Polymorphisms in KITLG and KISS1 gene regions were identified via Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Genetic variation within populations was evaluated by testing for Hardy-Weinberg equilibrium.

Results: The C2540T and A2510G SNPs in the KISS1 gene were found to be in accordance with Hardy-Weinberg equilibrium across all breeds; genotype distribution was balanced and interbreed differences were not statistically significant. Conversely, the g.12654G>A and g.12829T>C variants in the KITLG gene showed significant deviations from Hardy-Weinberg equilibrium, characterized by an excess of heterozygous and a deficiency of homozygous genotypes. These deviations suggest possible influences of genetic drift, selection pressure, or closed mating systems. Only the g.12772G>A variant-maintained equilibrium.

Discussion and Conclusion: Overall, genetic diversity was detected, and the examined SNPs possess potential as genetic markers for breeding and conservation of indigenous goat breeds in Turkey. These findings provide a foundational framework for future molecular-level research supported by phenotypic data.

Keywords: Hair Goat, Angora Goat, Kilis Goat, KITLG, KISS1, SNP

BİYOKİMYASAL, HEMATOLOJİK VE İDRAR ANALİZLERİNİN LABORATUVAR TANIDA ÖNEMİ

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ÖZET

Laboratuvar tanı yöntemleri, hastalıkların erken saptanması, tedavi planının oluşturulması ve hastalık seyrinin izlenmesinde temel rol oynar. Biyokimyasal, hematolojik ve idrar analizleri, modern tıbbın vazgeçilmez tanı araçlarıdır. Biyokimyasal, hematolojik ve idrar analizleri organ fonksiyonlarını, metabolik bozuklukları, çeşitli hastalıkları ve enfeksiyonları değerlendirmek için kullanılan temel laboratuvar testleridir. Biyokimyasal analizlerden karaciğer, böbrek, kalp ve pankreas fonksiyon testleri; organ hasarını erken dönemde belirlemeye ve metabolik bozuklukları saptamaya yardımcı olur. Hematolojik analizler, kan hücrelerinin sayısal ve yapısal özelliklerini değerlendirir. Anemi, dehidrasyon, enfeksiyon, stres, pıhtılaşma bozuklukları ve hematolojik malignitelerin tanısında kritik öneme sahiptir. İdrar analizleri, böbrek fonksiyonlarının değerlendirilmesi, idrar yolu enfeksiyonlarının tespiti ve sistemik hastalıkların belirlenmesinde kullanılır. Bu üç analiz türü birlikte değerlendirildiğinde, hastalıkların nedenleri daha doğru şekilde belirlenebilir. Doğru numune alma, uygun laboratuvar teknikleri ve sonuçların klinik verilerle birlikte yorumlanması, güvenilir tanı için şarttır. Laboratuvar tanıda yanlış teşhis konulmaması için laboratuvar değerlerine etki eden faktörlere özellikle dikkat edilmelidir. Örneğin tok olarak alınan kanda glikoz ve trigliserit yüksek, fosfor düşük görülebilmektedir. Ayrıca total protein, bikarbonat ve demir artarken Cl azalır ve bu durum sanki ılımlı bir metabolik alkaloz varmış gibi düşündürür. Vakumlu tüplere kan alınırken vakum ortadan kalkıncaya kadar kan alınmaması yanlış yorumlamaya neden olacak şekilde total protein ölçümlerinin düşük çıkmasına neden olmaktadır. Hemolizli serumda kreatinin, potasyum, magnezyum, LDH, AST, ALT normalden daha yüksek çıkabilir. Stres altında alınan kandaki glikoz seviyesi normal değerlerin 5-10 katını bulabilir. Ayrıca hemogramda lenfosit, eozinofil seviyelerinin düşüp monosit düzeyinin arttığı unutulmamalıdır. Dehidrasyon durumu gözden kaçarsa artan üre ve kreatinin seviyesi hatalı olarak böbrek hasarı diye yorumlanabilir. Canlının yetersiz ve dengesiz beslenmesi albumin ve globulin düşüklüğüne neden olabilir ve yanlış olarak karaciğer, böbrek veya gastro intestinal sistem hasarları olarak yorumlanabilir. Sonuç olarak, biyokimyasal, hematolojik ve idrar analizleri; doğru, hızlı ve güvenilir laboratuvar tanısının temel taşlarıdır. Klinik uygulamalarda etkin kullanımları, hasta sağlığının korunması ve iyileştirilmesinde hayati öneme sahiptir.

Anahtar Kelimeler: Ayrıcı tanı; hemogram; klinik biyokimya; laboratuvar testleri

IMPORTANCE OF BIOCHEMICAL, HEMATOLOGICAL AND URINE ANALYSIS IN LABORATORY DIAGNOSIS

ABSTRACT

Laboratory diagnostic methods play a fundamental role in the early detection of diseases, the establishment of treatment plans, and the monitoring of disease progression.

Biochemical, hematological, and urinalysis tests are indispensable diagnostic tools in modern medicine. These tests are essential laboratory analyses used to evaluate organ functions, metabolic disorders, various diseases, and infections. Biochemical analyses, such as liver, kidney, heart, and pancreas function tests, help detect organ damage at an early stage and identify metabolic disorders. Hematological analyses assess the quantitative and structural characteristics of blood cells, and are of critical importance in the diagnosis of anemia, dehydration, infections, stress, coagulation disorders, and hematologic malignancies. Urinalysis is used to evaluate kidney function, detect urinary tract infections, and identify systemic diseases. When these three types of analyses are evaluated together, the causes of diseases can be determined more accurately. Correct sample collection, appropriate laboratory techniques, and the interpretation of results together with clinical data are essential for reliable diagnosis. To avoid misdiagnosis in laboratory diagnostics, particular attention should be paid to factors that can influence laboratory values. For example, blood collected in a non-fasting state may show high glucose and triglyceride levels, and low phosphorus levels. Additionally, total protein, bicarbonate, and iron levels may increase while chloride decreases, which could be misinterpreted as a mild metabolic alkalosis. When using vacuum tubes, failing to stop blood collection once the vacuum is depleted may lead to falsely low total protein measurements. In hemolyzed serum, creatinine, potassium, magnesium, LDH, AST, and ALT values may appear higher than normal. Blood drawn under stress may show glucose levels five to ten times above normal. Furthermore, hemograms may reveal decreased lymphocyte and eosinophil counts and increased monocyte levels. If dehydration is overlooked, elevated urea and creatinine levels may be incorrectly interpreted as kidney damage. Poor and unbalanced nutrition in the patient may cause low albumin and globulin levels, which could be mistakenly attributed to liver, kidney, or gastrointestinal damage. In conclusion, biochemical, hematological, and urinalysis tests are the cornerstone of accurate, rapid, and reliable laboratory diagnostics. Their effective use in clinical practice is of vital importance for maintaining and improving patient health.

Keywords: Differential diagnosis; hemogram; clinical biochemistry; laboratory tests

TRACE ELEMENT PROFILE IN HYPOTHYROIDISM

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ABSTRACT

Introduction and Purpose: Thyroid hormones are known to play a crucial role in the regulation of carbohydrate, lipid, and protein metabolism. Thyroid hormones affect many metabolic pathways and mediate important physiological processes such as growth, development and metabolism. Hypothyroidism, one of the most common endocrine disorders, develops in conditions where the synthesis, secretion, or peripheral action of thyroid hormones is impaired. This dysfunction may arise from primary thyroid gland disorders such as autoimmune thyroiditis, iodine deficiency, or post-surgical hypothyroidism; secondary causes related to pituitary insufficiency; or tertiary causes stemming from hypothalamic dysfunction. Given the multifactorial etiology and systemic implications of hypothyroidism, recent research has increasingly focused on the role of trace elements in its pathogenesis, diagnosis, and treatment. Therefore, this review aims to provide a comprehensive overview of the involvement of trace elements in the diagnostic and therapeutic processes of hypothyroidism.

The Role of Trace Elements in Hypothyroidism: Minerals are essential for health, and their deficiencies can lead to serious health problems. Certain elements are required for the normal function and metabolism of thyroid hormones. Changes in the concentrations of these elements or exposure to potentially toxic elements can negatively affect not only the body's overall systemic balance but also the synthesis of thyroid hormones, leading to disruptions in thyroid homeostasis, particularly hypothyroidism and hyperthyroidism. The most important trace elements in hypothyroidism include iodine (I), selenium (Se), zinc (Zn), manganese (Mn), chromium (Cr), copper (Cu), and iron (Fe). Trace elements can participate in cellular functions such as immune regulation, neurotransmission, muscle contractions, mitochondrial activity, and enzyme reactions through humoral and cellular mechanisms. It is emphasized that preventing trace element deficiencies can not only reduce the incidence of diseases related to these elemental deficiencies but also increase the bioavailability and metabolic efficiency of other trace elements. It has been determined that trace elements that play a role in neutralizing free radicals are also necessary for the proper functioning of thyroid hormone metabolism; their deficiency results in impaired thyroid functions.

Conclusion: A possible decrease in trace element levels may adversely affect thyroid metabolism, leading to hypothyroidism. Changes in trace element levels in hypothyroidism can also lead to changes in basal metabolic rate. Therefore, when studies revealing the role of trace elements in cases of hypothyroidism are examined, it can be recommended to take trace elements as supplements in a controlled manner.

Keywords: Hypothyroidism; Metabolism; Trace Element

EKSOZOMLAR VE MİRNA'LARIN KLİNİK BİYOKİMYADAKİ ROLÜ

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ÖZET

Ekstrasellüler veziküller (EV), hücreler arası iletişimin düzenlenmesinde rol alan biyolojik yapılardır. Bunlar arasında 40–120 nm çapındaki eksozomlar, taşıdıkları protein, lipid, DNA, mRNA ve özellikle mikroRNA (miRNA) içerikleri ile dikkat çekmektedir. İlk tanımlandıklarında hücresel atıkların uzaklaştırılmasında görevli oldukları düşünülse de, günümüzde gen ekspresyonunun post-transkripsiyonel düzeyde düzenlenmesinde ve uzak hücrelere biyolojik sinyal iletilmesinde işlev gördükleri anlaşılmıştır. Özellikle eksozomal miRNA'ların, stabil yapıları ve özgül biyobelirteç özellikleri nedeniyle klinik biyokimyada giderek artan bir öneme sahip oldukları bildirilmektedir.

İnsan tıbbında yapılan araştırmalar, kanser biyolojisi, kardiyovasküler bozukluklar, nörodejeneratif hastalıklar ve metabolik sendrom gibi birçok patolojik durumda eksozomal miRNA profillerinin değiştiğini göstermektedir. Bu bulgular, eksozomal miRNA'ların erken tanı, prognoz değerlendirmesi ve hedeflenmiş tedavi yaklaşımlarında kullanılabileceğini düşündürmektedir. Benzer şekilde, veteriner hekimlikte mastitis, viral enfeksiyonlar (ör. BVD, BLV), üreme bozuklukları ve metabolik hastalıklarda eksozomal miRNA'ların hem tanısal hem de prognostik potansiyeli rapor edilmiştir. Ayrıca köpek ve at gibi farklı türlerde yapılan çalışmalar, eksozomal miRNA'ların hastalık biyobelirteci olarak değerlendirilebileceğini ortaya koymaktadır.

Mevcut bilgiler ışığında eksozomal miRNA'lar, klinik biyokimya alanında umut vadeden biyobelirteçlerdir. Ancak rutin uygulamaya geçiş için izolasyon tekniklerinin standardizasyonu, analiz yöntemlerinin iyileştirilmesi ve türler arası karşılaştırmalı çalışmaların artırılması gerekmektedir. Bu derlemenin amacı, eksozomlar ve miRNA'ların biyokimya alanındaki önemini vurgulamak, mevcut literatürü özetlemek ve gelecekteki klinik uygulamalara yönelik potansiyellerine dikkat çekmektir.

Anahtar Kelimeler: Eksozomlar, MicroRNA, Biyobelirteçler, Klinik Biyokimya, Veteriner Hekimlik

THE ROLE OF EXOSOMES AND MIRNAS IN CLINICAL BIOCHEMISTRY

ABSTRACT

Extracellular vesicles (EVs) are biological structures that play a role in regulating intercellular communication. Among these, exosomes, which are 40–120 nm in diameter, are notable for their content of proteins, lipids, DNA, mRNA, and especially microRNA (miRNA). Although they were initially thought to be responsible for the removal of cellular waste, it is now understood that they function in the post-transcriptional regulation of gene expression and the transmission of biological signals to distant cells.

It is reported that exosomal miRNAs, in particular, are of increasing importance in clinical biochemistry due to their stable structures and specific biomarker properties.

Research in human medicine shows that exosomal miRNA profiles are altered in many pathological conditions such as cancer biology, cardiovascular disorders, neurodegenerative diseases, and metabolic syndrome. These findings suggest that exosomal miRNAs could be used for early diagnosis, prognosis assessment, and targeted treatment approaches. Similarly, in veterinary medicine, the diagnostic and prognostic potential of exosomal miRNAs has been reported for mastitis, viral infections (e.g., BVD, BLV), reproductive disorders, and metabolic diseases. Furthermore, studies in different species such as dogs and horses reveal that exosomal miRNAs can be evaluated as disease biomarkers.

In light of current knowledge, exosomal miRNAs are promising biomarkers in the field of clinical biochemistry. However, for their integration into routine practice, the standardization of isolation techniques, improvement of analysis methods, and an increase in cross-species comparative studies are required. The aim of this review is to emphasize the importance of exosomes and miRNAs in the field of biochemistry, summarize the current literature, and draw attention to their potential for future clinical applications.

Keywords: Exosomes, MicroRNA, Biomarkers, Clinical Biochemistry, Veterinary Medicine

FERROPTOSIS: A REGULATED CELL DEATH PATHWAY IN CANCER

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ABSTRACT

Objective: This study aims to examine the mechanism of ferroptosis, based on iron-mediated oxidation and lipid peroxidation, and to highlight its significance in cancer.

Material and Method: The relationship of ferroptosis with iron metabolism, lipid metabolism, and antioxidant defense systems was evaluated through a literature review, and the key regulatory pathways underlying this process were examined.

Results: Ferroptosis is defined as an iron-dependent form of regulated cell death. It is considered to be associated with various diseases, including neurodegenerative disorders, cancer, and chronic kidney disease. Ferroptosis is marked by intracellular iron accumulation, excessive generation of reactive oxygen species (ROS), reduction of glutathione, and intense lipid peroxidation of membrane lipids. During ferroptosis, excessive Fe^{2+} accumulation triggers the Fenton reaction, producing hydroxyl radicals that induce lipid peroxidation of polyunsaturated fatty acids (PUFAs) in the cell membrane. In this process, phospholipid-related PUFAs, phosphatidylethanolamine and phosphatidylcholine, are crucial in ferroptosis by functioning as critical substrates for lipid peroxidation. Lipoxygenases catalyze PUFA peroxidation, producing diverse lipid peroxides. Glutathione peroxidase 4 (GPX4) and system Xc⁻ (cystine/glutamate antiporter system) are the principal mediators of antioxidant metabolism; inhibition of system Xc⁻ leads to cysteine and glutathione (GSH) depletion, resulting in GPX4 inactivation. Disruption of iron homeostasis leads to free iron accumulation, which promotes the formation of lipid ROS and triggers ferroptosis.

Discussion and Conclusion: The tumor-suppressing mechanisms activated by ferroptosis limit cancer progression and act as a natural barrier. However, ferroptosis resistance induced by oncogenic signals can lead to proliferation, metastasis, and treatment resistance in tumor cells. Tumor cells increase their defense systems by regulating ferroptosis-related proteins to survive. However, ferroptosis is also a vulnerability in terms of oncological treatments and is therefore considered a promising target for treatment strategies.

Keywords: Ferroptosis, Iron Metabolism, Lipid Peroxidation, Cancer

INVESTIGATION OF THE EFFECTS OF ELECTRO-FREQUENCY WATER TREATMENT SYSTEM ON PERFORMANCE PARAMETERS IN POULTRY FARMING

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ABSTRACT

Introduction and Aim: Water quality is a critical factor directly affecting animal health, growth performance, and feed efficiency in poultry farming. This study aimed to investigate the effects of an electro-frequency water treatment system on performance parameters in commercial broiler production and to compare results with similar studies in the literature.

Materials and Methods: This comprehensive 13-month field study was conducted in a commercial poultry facility across 4 production periods, involving a total of 1,548,980 ROSS 308 chicks. The study compared control periods (1st and 2nd periods using standard municipal water) with experimental periods (3rd and 4th periods using water treated with Vulcan S10 Anti-Scale System). Key performance metrics including mortality rate, feed conversion ratio (FCR), performance index, and live weight were recorded and statistically analyzed.

Results: Significant improvements were observed following electro-frequency system implementation. Mortality rate decreased from $5.70 \pm 0.93\%$ to $3.07 \pm 0.74\%$ ($P < 0.001$), FCR improved from 1.681 ± 0.006 to 1.582 ± 0.050 ($P < 0.05$), performance index increased from 360 ± 1.5 to 395.0 ± 8.0 ($P < 0.01$), and live weight increased from 2.57 ± 0.001 kg to 2.67 ± 0.125 kg ($P < 0.05$). These results demonstrate both statistical significance and economic value.

Discussion and Conclusion: The electro-frequency impulse technology modifies water molecular structure without chemicals, enhancing mineral absorption and preventing biofilm formation. Our findings align with previous research on magnetic and ionized water treatments showing improved broiler performance. The technology presents a sustainable, environmentally friendly solution for poultry production, demonstrating commercial applicability through scientific validation. Further research across different and detailed biochemical analyses are recommended to understand the underlying mechanisms.

Keywords: Poultry, Electro-frequency, Drinking water, Performance, FCR, Water treatment

DETOXIFICATION STRATEGIES OF FOOD CONTAMINANTS BY PROBIOTICS

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ABSTRACT

Introduction and Purpose: At every stage of the food production chain, there is a risk of contamination from environmental or technological sources. Among the most common contaminants are mycotoxins, heavy metals, pesticides, and antibiotic residues, all of which present significant threats to public health. Conventional physical and chemical detoxification techniques often lead to a reduction in nutritional value, alter sensory qualities, and may pose economic challenges. In recent years, probiotic microorganisms have emerged as promising natural alternatives. This study explores the biochemical mechanisms, applied strategies, and potential applications of probiotics in the detoxification of food contaminants.

Materials and Methods: A comprehensive literature review was conducted, covering studies performed in vitro, in vivo, and within different food matrices. Comparative evaluation focused on contaminant-binding abilities, cell wall composition, metabolic enzyme activity, biotransformation capacity, and adaptation strategies of probiotic strains in various food systems.

Results: Species belonging to *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* demonstrated remarkable binding and detoxification potential, particularly against aflatoxins, patulin, lead, cadmium, and certain pesticides. Peptidoglycan, teichoic acids, and surface proteins in the bacterial cell wall were identified as critical components in the adsorption process. Furthermore, some probiotic strains produce enzymes capable of transforming mycotoxins into less toxic metabolites. These mechanisms provide a significant advantage in enhancing food safety without compromising nutritional quality.

Discussion and Conclusion: Probiotics hold considerable promise as biotechnological agents for the detoxification of food contaminants. Nonetheless, for successful commercial application, further research is needed to determine strain-specific performance, optimize dosage, integrate probiotics into various food matrices, and ensure compliance with regulatory frameworks.

Keywords: Probiotics; Food contaminants; Detoxification strategies; Biotransformation; Adsorption

EFFECT OF FUCOIDAN ON SOME BIOCHEMICAL PARAMETERS IN CARBON TETRACHLORIDE-INDUCED NEPHROTOXICITY IN RATS²⁸

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ÖZET

Fucoidan, kahverengi deniz yosunlarının hücre duvarlarında ve dış hücre boşluklarında bulunan sülfatlı polisakkaritlerden biridir. Bu çalışmada ratlarda karbon tetraklorür (CCl₄) ile oluşturulan nefrotoksisitede fucoidan kullanılmasının bazı biyokimyasal parametreler üzerine etkisi araştırılmıştır. Yaklaşık 7-8 haftalık, 200-250 g ağırlığındaki 32 adet Wistar Albino dişi rat rastgele olmak üzere her biri 8 rattan oluşan 4 gruba ayrıldı: Kontrol grubu (fizyolojik tuzlu su p.o 8 gün), fucoidan grubu (fucoidan 100 mg/kg/gün intragastrik gavaj), CCl₄ grubu grubu (serum fizyolojik p.o 8 gün + 8. gün CCl₄ 1 ml/kg i.p tek doz), fucoidan+CCl₄ grubu (fucoidan 100 mg/kg/gün intragastrik gavaj 8 gün+8. gün CCl₄ 1 ml/kg i.p tek doz). 8 günlük çalışma süresinden 24 saat sonra, yani 9. gün intraperitoneal olarak ketamin (90 mg/kg) enjekte edilerek kalpten kan örnekleri alındı. Tüm kanda GSH, plazmada ise MDA, AOPP, Na, Cl, K, kreatinin, üre düzeylerine bakıldı. MDA, GSH ve AOPP analizleri spektrofotometrik, diğer analizler otoanalizör ile gerçekleştirildi. Biyokimyasal analizler sonucu fucoidan+CCl₄ grubunda CCl₄ grubuna göre kreatinin (p<0.007), üre (p<0.001), MDA (p<0.001), AOPP (p<0.05) seviyeleri düşerken, GSH (p<0.004) düzeyinin yükseldiği görüldü. Sonuç olarak; antioksidan özelliğe sahip olduğu görülen fucoidanın, CCl₄ tarafından oluşturulan oksidatif stresi azaltarak nefrotoksik hasarı hafiflettiği ve böbrek üzerine koruyucu etkisinin bulunduğu söylenebilir.

Anahtar Sözcükler: Biyokimyasal parametreler, Fucoidan, Karbon tetraklorür, Nefrotoksisite

SUMMARY

Fucoidan is one of the sulfated polysaccharides found in the cell walls and extracellular spaces of brown seaweeds. This study investigated the effects of fucoidan on some biochemical parameters in carbon tetrachloride (CCl₄)-induced nephrotoxicity in rats. Thirty-two Wistar Albino female rats, approximately 7-8 weeks old and weighing 200-250 g, were randomly divided into 4 groups, each consisting of 8 rats: Control group (physiological saline p.o 8 days), fucoidan group (fucoidan 100 mg/kg/day intragastric gavage), CCl₄ group group (physiological saline p.o 8 days + 8th day CCl₄ 1 ml/kg i.p single dose), fucoidan+CCl₄ group (fucoidan 100 mg/kg/day intragastric gavage 8 days + 8th day CCl₄ 1 ml/kg i.p single dose).

²⁸This article is produced from the Master's Thesis of the first author.

After 24 hours of the 8-day study period, namely on the 9th day, ketamine (90 mg/kg) was injected intraperitoneally and blood samples were taken from the heart. GSH levels were measured in whole blood and MDA, AOPP, Na, Cl, K, creatinine, and urea levels were measured in plasma. MDA, GSH, and AOPP analyses were performed spectrophotometrically, while other analyses were performed with an autoanalyzer. As a result of biochemical analyses, it was observed that creatinine ($p<0.007$), urea ($p<0.001$), MDA ($p<0.001$), and AOPP ($p<0.05$) levels decreased in the fucoidan+CCl₄ group compared to the CCl₄ group, while GSH ($p<0.004$) levels increased. As a result; it can be said that fucoidan, which has antioxidant properties, alleviates nephrotoxic damage by reducing oxidative stress caused by CCl₄ and has a protective effect on the kidney.

Keywords: Biochemical parameters, Fucoidan, Carbon tetrachloride, Nephrotoxicity

GİRİŞ

Dört klor atomunun bir karbon molekülüne tetrahedral bir şekilde bağlanması ile oluşan karbon tetraklorür (CCl₄) apolar bir moleküldür ve organik bir çözücüdür. Kimyasal laboratuvarlarda, kuru temizleme gibi birçok sektörde yaygın olarak kullanılan CCl₄, uçucu özelliği ile çevresel olarak çok toksik kabul edilen bir maddedir. Farklı yollarla organizmalar tarafından alınması halinde farklı organ ve dokularda hasarlar oluşturur ki bunların başında karaciğer ve böbrekler gelmektedir. Karaciğerde steatoz, sentrilobuler nekroz, bu olayları takip eden siroz olguları gözlenirken, böbrekte akut tübüler nekroza kadar varan ciddi hasarlar oluşturur (Ruprah ve ark., 1985; Tombolini ve Cingolani, 1996).

CCl₄, birçok patofizyolojik durumda oksidatif stresi taklit eden deneysel modeller oluşturmak için bilimsel araştırmalarda yaygın olarak kullanılan en etkili toksinlerden biridir. CCl₄ tarafından üretilen toksisite, oksijenin varlığında triklorometil peroksil radikali (CCl₃O₂•) haline dönüşen triklorometil radikali (CCl₃•) nedeniyle ortaya çıkar ki bu, CCl₃• radikallerinden daha toksiktir. Bu radikaller, lipidler, proteinler ve DNA ile bağlanarak birçok dokunun peroksidatif dejenerasyonuna neden olur.

Böbrekler CCl₄'e oldukça duyarlıdır. CCl₄'e çok yüksek maruziyetten sonra hayatını kaybeden insanlarda genellikle böbrek yetmezliği ölümün ana nedenidir. CCl₄ toksisitesiyle ilgili insanlarda yapılan birçok çalışmada, böbrek hasarının karaciğer hasarına yol açan aynı maruziyet seviyelerinde görüldüğü rapor edilmiştir. Şiddetli vakalarda başlıca klinik belirtiler, oligüri veya anüri olup, bunun sonucunda azotemi ve ödem gelişir ve bunlar sırasıyla hipertansiyon ve pulmoner ödem ile sonuçlanır. Proksimal tübül hücreleri, büyük olasılıkla yüksek sitokrom P-450 içeriği nedeniyle CCl₄'den en çok zarar gören hücrelerdir. Hayvan çalışmalarında da böbrek hasarı gözlenmiştir (Faroon, 2005).

Fucoidan, kahverengi deniz yosunlarının (Fucus, Ascophyllum, Saccharina ve Sargassum gibi) hücre duvarlarında ve dış hücre boşluklarında bulunan L-fükoz ve sülfat gruplarından oluşan bir polisakkarittir. Fucoidanın aktivitesinin çoğunlukla molekül ağırlığı ve sülfatlı gruplarla ilişkili olduğu bildirilmiştir (Anastyuk ve ark., 2012). Biyolojik aktiviteleri arasında antitümör, antioksidan, antikoagülan, antitrombotik, immün düzenleyici, antiviral ve antiinflamatuvar etkiler bulunur. Son zamanlarda, fucoidanın metabolik sendromu hafiflettiği, gastrointestinal sistemi koruduğu, anjiyogeneze ve kemik sağlığına fayda sağladığı gösterilmiştir (Wang ve ark., 2019).

Doğal ürünler ve alternatif tıp mamulleri, insan hastalıklarını tedavi etmek ve yeni ilaçlar geliştirmek için önemli kaynaklardır. Fucoidan da hayvan deneylerinde kronik böbrek hastalıklarını tedavi edici etki gösteren çok az sayıdaki doğal ürünlerden biridir. Fucoidanın hücre ve hayvan çalışmalarında oksidatif stresi ve inflamasyonu iyileştirdiği gösterilmiştir

(Wang ve ark., 2020). Daha önce yapılan çalışmalarda, düşük moleküler ağırlıklı fucoidanın (oligo-fucoidan) kronik böbrek hastalığı semptomlarını hafiflettiği ve renal interstisyel fibrozu engellediği bulunmuştur (Chen ve ark., 2018).

Fucoidanın faydalı biyolojik özelliklerine rağmen CCl₄ nefrotoksisitesine karşı koruyucu etkisi şimdiye kadar araştırılmamıştır. Bu çalışmada, ratlarda CCl₄ verilerek oluşturulan nefrotoksisitede fucoidanın GSH, MDA, AOPP, Na, K, Cl, kreatinin ve üre seviyeleri gibi bazı biyokimyasal parametreler üzerine etkileri incelenmiştir. Böylece böbrek fonksiyon testleri ile beraber oksidatif stres parametreleri olan GSH, MDA, AOPP düzeylerine bakılarak fucoidanın antioksidan özelliği ile CCl₄ kaynaklı böbrek hasarına karşı koruyucu etkilerinin olup olmadığı araştırılmıştır.

MATERYAL METOT

Hayvan materyali

Çalışmada kullanılan hayvan materyali Van Yüzüncü Yıl Üniversitesi Deney Hayvanları Ünitesi'nden temin edildi. Yaklaşık 7-8 haftalık, 200-250 g ağırlığındaki 32 adet Wistar Albino dişi ratlar ile çalışma yapıldı. Deney öncesinde ratların 7 gün süreyle ortama adaptasyonu sağlandı. Ratlar 8 günlük deneme süresince 12 saat karanlık/aydınlatma uygulanarak sıcaklığı 22 ± 2°C olarak ayarlanmış odalardaki kafeslerde barındırıldı. Hayvanlara ticari rat yemi (pellet yem) ve içme suyu ad libitum verildi. Bu çalışma, Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 27/03/2025 tarih ve 2025/03-10 sayılı kararı ile tamamlandı.

Çalışma gruplarının hazırlanması

Çalışmada kullanılan ratlar rastgele olmak üzere her biri 8 rattan oluşan 4 gruba ayrıldı:

1. Kontrol grubu: Fizyolojik tuzlu su 8 gün süreyle p.o verildi.
2. Fucoidan grubu: Fucoidan 100 mg/kg/gün 8 gün süreyle intragastrik gavaj ile verildi.
3. CCl₄ grubu: 8 gün süreyle fizyolojik tuzlu su p.o. verildi, ilaveten 8. gün CCl₄ 1 ml/kg intraperitoneal olarak tek doz uygulandı.
4. Fucoidan+CCl₄ grubu: Fucoidan 100 mg/kg/gün intragastrik gavaj ile 8 gün verildi, ilaveten 8. gün CCl₄ 1 ml/kg intraperitoneal olarak tek doz uygulandı.

Örneklerin toplanması

8 günlük çalışma süresinden 24 saat sonra, yani 9. gün intraperitoneal olarak ketamin (90 mg/kg) enjekte edilerek anesteziye alınan hayvanların kalplerinden heparinli tüplere kanlar alındı. Kanların plazması 10 dakika 2500 rpm'de santrifüj edilerek çıkarıldı. Tüm kanda glutatyon, plazmada Na, Cl, K, kreatinin, üre, MDA ve AOPP düzeylerine bakıldı. Na, Cl, K, üre ve kreatinin düzeyleri otoanalizörde (Roche Modüler P 800), MDA (Yoshioka ve ark., 1979), GSH (Beutler ve ark., 1963) ve AOPP (Witko-Sarsat ve ark., 1999) seviyeleri ise spektrofotometrik yöntemler ile tayin edildi.

BULGULAR

Kontrol, fucoidan, CCl₄, fucoidan+CCl₄ grubu ratlara ait plazma Na, K, Cl, kreatinin, üre, MDA, AOPP ve tüm kan GSH düzeylerinin ortalamaları Tablo 1'de verildi.

Tablo 1. Kontrol, fucoidan, CCl₄, fucoidan+CCl₄ grubu ratlara ait bazı biyokimyasal parametrelerin düzeyleri

	Kontrol		Fucoidan		CCl ₄		Fucoidan+CCl ₄		
	n	X ± Sx	n	X ± Sx	n	X ± Sx	n	X ± Sx	p
Na (mmol/L)	8	141.38 ± 2.39 ^a	8	142.86 ± 1.95 ^a	8	142.25 ± 1.67 ^a	8	143.25 ± 0.46 ^a	0.191
K (mmol/L)	8	4.12 ± 0.36 ^a	8	5.05 ± 1.41 ^a	8	5.13 ± 0.48 ^a	8	4.54 ± 1.06 ^a	0.120
Cl (mmol/L)	8	101.25 ± 1.49 ^a	8	102.43 ± 2.99 ^a	8	101.38 ± 1.06 ^a	8	99.88 ± 1.13 ^a	0.072
Kreatinin (mg/dl)	8	0.40 ± 0.04 ^b	8	0.38 ± 0.07 ^b	8	0.56 ± 0.13 ^a	8	0.45 ± 0.10 ^b	0.007
Üre (mg/dl)	8	33.03 ± 3.76 ^c	8	31.92 ± 2.43 ^c	8	42.51 ± 2.62 ^a	8	38.08 ± 3.18 ^b	0.001
GSH (mg/dl)	8	32.91 ± 8.26 ^a	8	31.46 ± 8.89 ^a	8	20.44 ± 4.29 ^b	8	26.71 ± 3.51 ^a	0.004
MDA (mmol/L)	8	5.53 ± 1.06 ^b	8	7.72 ± 1.30 ^b	8	10.85 ± 1.39 ^a	8	8.70 ± 1.22 ^b	0.001
AOPP (mmol/L)	8	101.16 ± 29.37 ^b	8	104.90 ± 46.06 ^b	8	159.82 ± 37.52 ^a	8	93.39 ± 26.52 ^b	0.05

a, b, c: Aynı satırda farklı harfleri taşıyan grup ortalamaları arasındaki fark istatistiksel olarak önemlidir.

Biyokimyasal analizler sonucu fucoidan+CCl₄ grubunda CCl₄ grubuna göre kreatinin (p<0.007), üre (p<0.001), MDA (p<0.001), AOPP (p<0.05) seviyeleri düşerken, GSH (p<0.004) düzeyinin yükseldiği görüldü.

TARTIŞMA VE SONUÇ

CCl₄ deneysel çalışmalarda organ toksisitesini indüklemek için sıklıkla kullanılan bir toksindir. Kimya endüstrisinde yaygın kullanılan bir çözücü olan CCl₄'ün böbrekler, karaciğer, beyin ve akciğerler üzerinde zararlı etkileri olduğu belgelenmiştir. Bu nedenle ciddi sağlık riskleri oluşturur (Baig ve Khan, 2023; Mohammed et al., 2024). CCl₄'ün organlar üzerindeki olumsuz etkilerinin nedeni olarak çeşitli mekanistik değerlendirmeler yapılmıştır. CCl₄ tarafından üretilen radikallerin, lipid peroksidasyonu takiben biyomoleküllerden DNA ve protein hasarlarına ve fonksiyonel bozulmalara yol açtığı, böbrekte gelişen değişikliklere de oksidatif stresin neden olduğu şeklinde raporlar bildirilmiştir (Levine ve ark., 1990).

CCl₄ verilmesiyle şekillenen nefrotoksisitede anahtar rol oynayan enzim kortikal tübül hücrelerinde bulunan P450 sitokromdur ki sitokrom P450 tarafından üretilen serbest radikallerin katalitik aktivitelerde oksidatif inaktivasyona neden olması muhtemeldir. Bir diğer bulgu ise renal brush border'da artmış lipid peroksidasyonunun görülmesidir (Delane ve Karninski, 1993). CCl₄ uygulaması mitokondriyal membranda Ca akışını ve diğer bazı renal mitokondriyal faaliyetleri de etkilediği saptanmıştır (Paustenbach ve ark., 1986).

Böbrekler, hayvan vücudunun atık maddelerini filtreleme ve bertaraf sistemidir. Bu organlar aynı zamanda çok sayıda ksenobiyotik toksikanın da hedefidir (Yoshioka ve ark., 2016). CCl₄, antihelmintikler, tahıl fümigantları ve kloroflorokarbonların sentezinde ara maddeler olarak yaygın olarak kullanılan bir kimyasaldır. CCl₄ hepatotoksikant olarak en kapsamlı incelenen, mekanizması en iyi rapor edilen maddelerden biridir (Recknagel ve ark., 1989). Bununla birlikte, CCl₄'ün toksik etkileri böbrek, beyin, akciğer ve testis dahil olmak üzere birçok dokularda da saptanmıştır (Fadhel ve Amran, 2002; Manjrekar ve ark., 2008; Ganie ve ark., 2010; Yoshioka ve ark., 2016; Altinoz ve ark., 2018; Emam ve ark. 2020). Özellikle CCl₄ sistemik olarak uygulandığında karaciğere kıyasla böbreklere daha fazla miktarda CCl₄ dağıtılır (Tombolini ve Cingolani, 1996; Sanzgiri ve ark., 1997).

Organizmada farklı metabolik olaylar sırasında veya dışarıdan alınan maddelerin eliminasyonu sırasında oluşan oksidan maddeler, reaktif oksijen türlerini zehirsizleştirmek için veya onların sentezini inhibe etmek için enzimatik veya nonenzimatik birtakım maddeler antioksidan olarak görev yapar. Oksidan maddelerin çok az miktarları bazı yazarlar tarafından faydalı olduğu zikredilse de bunların fazla miktarda sentezlenmesi veya alınması sırasında oksidatif stres şekillenir. Bunlar birçok dejeneratif hastalıkların oluşumunda ana nedenlerden birisi olarak görülmektedir. Oksidatif maddelerin sentezinin uyarılmasında rol oynayan maddelerden birisi de CCl₄'dür. Bu madde serbest radikallerin oluşumunu artırarak hem hepatotoksisiteye ve hem de nefrotoksisiteye neden olmaktadır. Ayrıca antioksidan savunma sistemlerinde değişimler ve azlıklar varsa sıçanlarda potansiyel bir böbrek harabiyetine yol açmaktadır (Ohkawa ve ark., 1979; Levine ve ark., 1990; Sundari ve ark., 1997).

CCl₄, sitokrom P450 CYP2E1 aracılığıyla metabolize edilerek son derece toksik triklorometil serbest radikali ve/veya triklorometil peroksil serbest radikali üretir. Bu radikaller böbrek hasarına neden olur, çünkü serbest radikaller hücre içi proteinlere, hücre zarının lipidlerine özellikle hücre zarında bulunan fosfolipitlerin doymamış yağ asitlerine veya DNA'ya bağlanır. Bunun sonucunda da protein denatürasyonuna, hücre zarında lipid peroksidasyonuna, oksidatif DNA hasarına yol açar ve böbrek hücrelerinin ölümüne neden olur (Hismiogullari ve ark., 2015; Yoshioka ve ark., 2016).

Kahverengi deniz yosunu *Saccharina japonica*, Çin ve diğer birçok ülkelerde yaygın bir deniz ürünüdür ve bin yıldan uzun süredir geleneksel Çin tıbbında bir ilaç olarak yer almaktadır. Çok uzun süredir, Çinliler bunu böbrek hastalığının bir belirtisi olan ödem rahatsızlığını iyileştirmek için geleneksel bir ilaç olarak kullanmaktadır (Jia, 1996). Fucoidan da, *Saccharina japonica*, *Undaria pinnatifida* ve *Sargassum hemiphyllum* gibi çeşitli kahverengi deniz yosunu türlerinin ve deniz hıyarı gibi bazı hayvan türlerinin hücre duvarlarından izole edilen sülfatlanmış polisakkaritlerdir (Anastyuk ve ark., 2012; Aleissa ve ark., 2020; Abdel-Daim ve ark., 2021). Yüksek oranda L-fukoz-4-sülfat, daha az miktarda ise glukuronik asit, galaktoz, glukoz, mannoz, ksiloz, ramnoz ve arabinoz içerir (Wang ve ark., 2019; Abdel-Daim ve ark., 2021). Fucoidanın mükemmel bir doğal antioksidan olması ve potansiyel ROS temizleme aktivitesi üzerine çeşitli çalışmalar bulunmaktadır (Zhao ve ark., 2005; Wang ve ark., 2016, Wang ve ark., 2019).

Nefrotoksisite yaygın olarak bilinen bir sağlık sorunudur ve genellikle bazı toksik kimyasal maddeler veya ilaçlara maruz kalmanın sonucu olarak böbrek fonksiyonlarının bozulması ile karakterizedir. Nefrotoksisitede histopatolojik incelemelerin yanı sıra kanda üre, kreatinin, BUN, sistatin C gibi böbrek fonksiyonları hakkında bilgi veren parametrelerin düzeylerine bakılarak değerlendirilebilir (Başdınç, 2023). CCl₄ uygulaması, üre, kreatinin ve ürik asidin plazma seviyelerinde değişiklik oluşturur ve nefrotoksisiteye neden olur. Bu patolojik değişiklikler, CCl₄ tedavisi ile indüklenen karaciğer ve/veya böbreklerde potansiyel bir hasarı işaret eder (Ozturk ve ark., 2003; Ogeturk ve ark., 2005). Artan plazma kreatinin seviyesi, nefronun hasarlı yapısal bütünlüğüne bağlanabilir.

Makni ve ark. (2012) sıçanlarda CCl₄ vererek oluşturdukları nefrotoksisitede vanilin vererek üre, ürik asit ve kreatinin seviyelerini ölçmüşler, kontrol grubuyla karşılaştırıldığında CCl₄ grubundaki bu plazma parametrelerindeki önemli değişiklikleri tespit etmişlerdir. Sunulan bu çalışmada da; CCl₄ ile oluşturulan nefrotoksisitede böbrek fonksiyon testi için kullanılan rutin biyokimya analizlerinden plazma kreatinin ve üre düzeyleri değerlendirilmiştir. CCl₄ grubunun kreatinin ve üre seviyeleri diğer üç gruba göre istatistiksel olarak $p < 0.007$ ve $p < 0.001$ düzeyinde önemle yüksek hesaplanmıştır. CCl₄ ve fucoidanın birlikte verilmesiyle birlikte kreatinin ve üre düzeylerinin istatistiki önemle düştüğü tespit edilmiştir. Bu değerler kontrol grubuna ait düzeye doğru olumlu yönde değişmiş ve fucoidanın burada nefrotoksik hasarı azalttığı sonucuna varılmıştır. Böylece CCl₄ ile verilen fucoidanın; kreatinin ve üre gibi böbrek fonksiyon belirteçlerini normal seviyelere geri getirerek CCl₄'ün toksik etkilerini iyileştirdiği ortaya konulmuştur. Yine farklı maddeler verilip oluşturulan patolojik durumların fucoidan ile tedavilerinin araştırıldığı çalışmalarla da paralellik sağlanmış (Zhang ve ark., 2003; Wang ve ark., 2014, Al-Khatib ve ark. 2019; Aleissa ve ark., 2020), fucoidanın böbrek fonksiyonunda önemli iyileştirmeler sağlayabileceği bildirilmiştir.

Böbrekler, vücuttan toksik atık ürünlerin uzaklaştırılması, sıvıların, minerallerin ve elektrolitlerin fizyolojik seviyelerde tutulması için hayati öneme sahiptir (Wang ve ark., 2014). Bu sebeple böbrek yetmezliği olduğu zaman sıvı, elektrolit ve asit-baz dengesinde değişiklikler meydana gelebilir. Yapılan bu çalışmada CCl₄ verilmesiyle oluşan elektrolit değişimlerini incelemek amacıyla Na, K ve Cl düzeylerine bakılmıştır. Plazma Na, K ve Cl seviyeleri bakımından gruplar arası farkta istatistiksel olarak önem bulunmamıştır ($p > 0.05$). Normal olarak böbrek hastalıklarında elektrolitlerde beklenen değişiklikler bazen gözlenmeyebilir. Bunun nedeni beslenmeleri için laboratuvar hayvanlarına verilen standart yemlerde fazla miktarda elektrolit bulunması veya elektrolit dengesizliği durumuna yatkınlık oluşturan birtakım faktörlerin olmaması şeklinde yorumlanmaktadır (Bach ve Lock, 1987).

CCl₄ gibi toksinlerin doğrudan nefrotoksik etkileri ROS oluşumuyla sonuçlanır ve böylece oksidatif stresi artırarak proksimal tübüler toksisiteye neden olur (Hosohata, 2016). Bu nedenle reaktif oksijen metabolitlerinin CCl₄ nefrotoksisitesinin patogeneğinde rol oynadığı kabul edilmektedir (Rahmat ve ark., 2014; Emam ve ark., 2020). Hücrelerde serbest radikallerin birikmesi, lipid peroksidasyonunu uyarabilir ve membran çoklu doymamış yağlarının oksidatif parçalanması sonucu hücre membran geçirgenliğinde ve viskozitesinde değişiklikler oluşturabilir (Baud ve Ardaillou, 1986). CCl₄'ün böbrek korteksindeki oksitlenmiş glutatyon düzeylerini azalttığı, lipid peroksidasyonunu artırdığı ve lipid peroksidasyonunun azalmasına neden olacak enzimlerin aktivitesinde bir düşüşe neden olduğu gösterilmiştir (Khan ve Siddique, 2012). Yine CCl₄, böbrekte proksimal tübüler hasarı subletal olarak indükleyebilir (Rajesh ve Latha, 2004).

Pek çok araştırmada CCl₄ uygulanması ile serumda lipid peroksidasyon ürünü olan MDA düzeyinin yükseldiği, memeli hücrelerinde bulunan temel bir non-enzimatik antioksidan olan GSH seviyesinin ise düştüğü bildirilmiştir (Makni ve ark., 2012; Khan ve ark., 2009; Nomier ve ark., 2022). Bu çalışmada da CCl₄ verilerek oluşturulan nefrotoksisitede MDA düzeyi yükselmiş, GSH seviyesi düşmüş ve benzer çalışmalarla paralellik göstermiştir. Fucoidan verilmesiyle fucoidan+CCl₄ grubunda MDA seviyesi düşmüş, GSH seviyesi ise yükselmiştir. Böylece oksidatif stresin azaldığı saptanmış, fucoidan CCl₄ kaynaklı nefrotoksisiteye karşı önemli koruma ve iyileştirme sağlamıştır.

Böbreklerin çeşitli kimyasalların düzenlenmesinde çok önemli bir rol oynadığı iyi bilinmektedir. Terapötik kimyasal bileşiklerin varlığı nedeniyle, birçok tıbbi bitki böbrek rahatsızlıklarını tedavi etmek için kullanıldığında iyileştirici özellikleriyle bilinmektedir (Priyadarsini ve ark., 2012; Emam ve ark., 2020). Nefroprotektif özelliklere sahip tıbbi bitkiler çeşitli nefrotoksik ajanlarla birlikte uygulandığında, toksisiteyi azaltabilmektedirler (Lakshmi ve ark., 2012). Kahverengi alglerde bol miktarda bulunan sülfatlanmış polisakkaritler, özellikle antioksidan ajanlar ve güçlü serbest radikal temizleyicileri olarak geniş farmakolojik etkilere sahiptir (Xue ve ark., 2001; Balboa ve ark., 2013; Al-Khatib ve ark., 2019). Fucoidanın böbrek hastalıklarında oksidatif hasarı azaltarak böbrek koruyucu etkisinin bulunduğu pek çok çalışmada gösterilmiştir. Bunlar arasında STZ (Aleissa ve ark., 2020; Wang ve ark. 2014), aflatoksin B1 (Aleissa ve ark., 2020; Abdel-Daim ve ark., 2021), siklosporin A (Al-Khatib ve ark., 2019), gentamicin (Yılmaz ve ark., 2018) ile böbrek hasarı oluşturulmuş ve fucoidanın antioksidan etkisi vurgulanmıştır.

Protein oksidasyonunun markerları olarak bilinen AOPP'ler, monositlerin aktivasyonunu uyurabildikleri için proinflamatuvar araçlar olarak da değerlendirilir. Yapılan değerlendirmelerde AOPP'lerin malondialdehit ile pozitif ve antioksidan glutatyon peroksidaz ile negatif korelasyon gösterdiği rapor edilmiştir. AOPP'ler, plazma albumini ile klorlu oksidanlar arasındaki bir reaksiyonun son ürünleri olup, hipokloröz asit ve miyeloperoksidaz yoluyla da oluşur. Protein karbonilleri, proteinlerin yapısındaki amino asit rezidülerinin oksidasyonu ile şekillenir. AOPP oluşumundan hemen sonra, antioksidan/pro-oksidan dengesizliğin neticesi olarak doku harabiyeti ve reaktif oksijen türlerinin birikmesi gözlenir. ROS ölçümü, formlarının çeşitliliği ve yarı ömürlerinin kısa olması gibi olumsuz özelliklere sahip olmaları nedeniyle dışlanmış, yerine AOPP'ler oksidasyon aracılı protein hasarının biyobelirteçleri olarak tercih edilmiştir. AOPP düzeyleri, ekstrasellüler sıvıdaki oksidatif stres durumunu yansıtır ki, bilindiği üzere oksidatif stres, çeşitli mekanizmalar yoluyla birçok kronik ve dejeneratif hastalıkların oluşumunda primer bir faktördür (Meza ve ark. 2019). Üremi, diabetes mellitus, romatoid artrit, yanık ve koroner arter hastalarında AOPP'lerin seviyesindeki artış rapor edilmiştir (Mert ve ark., 2021).

Witko-Sarsat ve ark. (2003), AOPP konusunda detaylı derleme ve bilimsel araştırma yapmış ve bu konuda otorite sahibi olmuşlardır. Organizmada gelişen oksidatif stres, kronik böbrek yetmezliği ve hemodiyalizle ilişkili olarak inflamatuvar sendromun oluşmasında önemli göreve sahip olduğu görüşünden yola çıkarak, AOPP'nin diyaliz hastalarının plazmasında varlığını ispat etmişlerdir. Bu maddeyi böbrek hastalıklarının veya oksidatif stresle bağlantılı gelişen hastalıkların tanımlanmasına yardım edecek yangıyı belirleyen önemli potansiyel bir belirteç olarak önermişlerdir. Bu açıdan AOPP'nin fagosit kaynaklı oksidatif stres belirteci olarak değerlendirilmesinin gerektiğini ve kronik böbrek yetmezliği ve diyalizle bağlantılı oluşan yan etkilerin fizyopatolojisinde önemli rollerinin bulunduğunu bildirmişlerdir.

Araştırmacılar ayrıca AOPP'lerin nötrofiller ve monositlerdeki oksidatif patlamayı ve inflamatuvar sitokinlerin üretiminin başlamasını uyardığını ve böylelikle gerçek inflamatuvar mediatörler gibi davrandığını belirtmişlerdir. Burada AOPP'nin klorlanmış oksidanlar ve plazma proteinleri arasındaki tepkimelerden oluşmuş proinflamatuvar etkilere sahip yeni üremik toksinler oluşturduğunu rapor etmişlerdir. Bu ifadeler sunulan çalışmada nefrotoksisitenin saptanmasında AOPP'nin belirteç olarak seçilmesinin ne kadar isabetli olduğunu göstermektedir.

Üremi ve AOPP düzeyleri konusunda yapılan bir diğer çalışmada (Descamps-Latscha ve Witko-Sarsat, 2001) kronik üreminin, böbrek yetmezliğinin erken döneminde ortaya çıkan, üreminin ilerlemesiyle belirginleşen ve bakım diyaliz tedavisiyle sonuçlanan çok faktörlü bir immünoinflamatuvar sendromla ilişkili olduğuna dair önemli kanıtlar elde etmişler ve bu maddenin AOPP olduğunu belirtmişlerdir. Böbrek patolojilerinin takibinde de güvenilen bir parametre olduğunu ileri sürmüşlerdir. Sunulan bu çalışmada AOPP düzeylerinde gruplar arasında AOPP değişimleri gözlenmiş, CCl₄ grubu ile diğer üç grup arasında istatistiksel önem saptanmıştır ($p < 0.05$). CCl₄ verilen grupta en yüksek bulunan AOPP seviyesi, fucoidan+CCl₄ grubunda fucoidan verilmesiyle düşmüş, böylece fucoidan verilmesinin protein oksidasyonunu dolayısıyla oksidatif stresi azattığını söylemek mümkündür. Fucoidanın nefrotoksisitede olumlu yönde katkı sağlayacak bir madde olabileceğini göstermektedir.

CCl₄'e maruz kalmış sıçanlarda karaciğer, pankreas, dalak, böbrek gibi farklı organlarda oluşmuş histolojik değişiklikler Doi ve ark. (1991) tarafından incelenmiştir. Böbreğe ait bulguları değerlendirdikleri zaman proksimal ve distal tübüllerde glomerüler nekroz ve histolojik değişiklikler saptamışlardır. Ayrıca tübüler nekroza karşılık gelen vakuolizasyon, atrofi ve epitel hücrelerinin ayrılması gibi değişiklikleri tespit etmişlerdir. Yine uzun süre CCl₄'e maruz kalan sıçanlarda fokal segmental glomerüloskleroz ve idrar tübüllerinin epitelinde fokal dejenerasyon gibi benzer bulgular gözlemişlerdir. Bu histolojik değişiklikleri lipid peroksidasyonu ve membran yapısının bozulması sonucuna bağlamışlardır. Araştırmacıların histopatolojik incelemeleri sunulan bu çalışmada yapılmaya bile mikroskopik olarak elde ettikleri bulgular biyokimyasal bulgularını desteklemekte ve çalışmamızda analizi yapılan MDA, GSH ve AOPP seviyelerindeki değişimi de (Tablo 1) açıklar niteliktedir.

Araştırmadan elde edilen sonuçlar değerlendirildiğinde, ratlara CCl₄ verilerek oluşturulan nefrotoksisitede bazı biyokimyasal analizler yapılarak fucoidanın böbrek hasarı üzerinde olumlu etkisi gözlenmiştir. Elektrolit olarak incelenen Na, K, Cl düzeylerinde CCl₄ verilmesini takiben bazı değişimler saptanmış ancak istatistiksel önem bulunmamıştır. Böbrek fonksiyon testi olarak rutin yapılan kreatinin ve üre analizleri sonucu, CCl₄ verilen ratlarda bu parametrelerin düzeylerinin arttığı tespit edilmiştir. Fucoidan+CCl₄ grubunda ise kreatinin ve üre seviyeleri olumlu yönde, istatistiki önemle azalmıştır. Oksidatif stres parametreleri olarak kabul edilen GSH, MDA, AOPP düzeyleri incelendiğinde fucoidan+CCl₄ grubunda CCl₄ grubuna göre MDA, AOPP seviyeleri istatistiksel önemle düşerken, GSH düzeyinin istatistiksel önemle yükseldiği ve böylece oksidatif stresin azaldığı saptanmıştır. Bu nedenle antioksidan özelliğe sahip olduğu görülen fucoidanın, CCl₄ tarafından uyarılmış nefrotoksisite sonucu oluşan hasarların giderilmesi ve oksidatif stresle ilişkili böbrek bozukluklarının iyileştirilmesi için kullanılabilecek potansiyel terapötik bir ajan olarak kabul edilebilir. Sonuç olarak; CCl₄ ile oluşturulan nefrotoksisitede incelenen biyokimyasal parametrelerin değişimlerine bakarak fucoidan verilmesinin CCl₄ tarafından oluşturulan nefrotoksik hasarı azalttığı ve böbrek üzerine koruyucu etkisinin bulunduğu söylenebilir.

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MEZENKİMAL KÖK HÜCRE EKSOZOMLARININ BÖBREK HASTALIKLARINDAKİ TANI VE TEDAVİ POTANSİYELİ

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ÖZET

Akut böbrek hasarı (ABH) ve kronik böbrek hastalığı (KBH), dünya genelinde artan prevalansı ve yüksek morbidite-mortalite oranları ile önemli bir sağlık sorunudur. Mevcut tedavi yöntemleri olan diyaliz ve böbrek nakli, yüksek maliyet, donör yetersizliği ve yan etki riskleri nedeniyle sınırlı başarı göstermektedir. Mezenkimal kök hücre (MSC) temelli tedaviler umut vaat etse de, uzun süreli kültürlerde tümörleşme, immün yanıt ve patojen bulaşma riskleri alternatif arayışlarını hızlandırmıştır. Bu bağlamda, MSC kaynaklı eksozomlar hücresiz tedavi yaklaşımı olarak öne çıkmakta, hem tanısal biyobelirteç hem de terapötik ajan olarak potansiyel taşımaktadır. Bu derleme çalışmasında, ABH ve KBH patofizyolojisinde eksozomların rolü, idrar kökenli eksozomların tanısal kullanımı ve MSC-eksozomlarının rejeneratif potansiyelleri üzerine son beş yıl içinde yayımlanan yüksek etki faktörlü dergilerdeki araştırmalar incelenmiştir. Literatür taraması PubMed, Web of Science ve Scopus veri tabanlarında, “exosome”, “kidney disease”, “diagnostic biomarker” ve “mesenchymal stem cell” anahtar kelimeleri ile gerçekleştirilmiştir. İdrar eksozomlarının moleküler kargo içeriğindeki değişimler, ABH ve KBH’nin erken tanısında yüksek duyarlılık göstermektedir. Örneğin, miR-9a, miR-200a, miR-200c artışı iyileşme evresini; miR-21 artışı ve miR-29c düşüşü renal fibrozisi işaret etmektedir. MSC-eksozomları ise anti-apoptotik, anti-fibrotik ve immünmodülatör etkiler göstererek, hem ABH’de iskemi/reperfüzyon hasarını azaltmakta hem de KBH’de fibrozis ve inflamasyonu baskılamaktadır. Çeşitli hayvan modellerinde, MSC-eksozomlarının kreatinin ve BUN düzeylerini düşürdüğü, oksidatif stresi azalttığı ve rejeneratif süreçleri aktive ettiği bildirilmiştir. Eksozomlar, stabil yapıları ve hedefe yönelik biyolojik kargo taşımaları sayesinde böbrek hastalıklarında hem invaziv olmayan tanısal biyobelirteçler hem de güvenli terapötik ajanlar olarak önem kazanmaktadır. Ancak klinik uygulamaya geçiş için izolasyon ve standardizasyon tekniklerinin geliştirilmesi, biyobelirteç referans aralıklarının belirlenmesi ve geniş ölçekli klinik çalışmaların yapılması gerekmektedir. Gelecekte MSC-eksozomlarının hücresiz tedavi stratejilerinde kullanımı, böbrek hastalıklarının yönetiminde yenilikçi ve etkili bir yaklaşım sağlayabilir.

Anahtar Kelimeler: Eksozom, böbrek hastalığı, mezenkimal kök hücre, biyobelirteç, rejeneratif tıp

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL EXOSOMES IN KIDNEY DISEASES

ABSTRACT

Acute kidney injury (AKI) and chronic kidney disease (CKD) are significant health problems with increasing prevalence and high morbidity and mortality rates worldwide. Current treatment methods, dialysis and kidney transplantation, have limited success due to high costs, donor shortages, and risks of side effects. While mesenchymal stem cell (MSC)-based therapies offer promise, the risks of tumorigenicity, immune responses, and pathogen transmission in long-term cultures have accelerated the search for alternatives. In this context, MSC-derived exosomes are emerging as a cell-free treatment approach and hold potential as both a diagnostic biomarker and therapeutic agent. This review examines research published in high-impact journals over the past five years on the role of exosomes in the pathophysiology of AKI and CKD, the diagnostic utility of urine-derived exosomes, and the regenerative potential of MSC-exosomes. A literature search was conducted in PubMed, Web of Science, and Scopus databases using the keywords "exosome," "kidney disease," "diagnostic biomarker," and "mesenchymal stem cell." Changes in the molecular cargo content of urinary exosomes demonstrate high sensitivity in the early diagnosis of AKI and CKD. For example, increases in miR-9a, miR-200a, and miR-200c indicate the recovery phase, while increases in miR-21 and decreases in miR-29c indicate renal fibrosis. MSC-exosomes exhibit anti-apoptotic, anti-fibrotic, and immunomodulatory effects, reducing ischemia/reperfusion injury in AKI and suppressing fibrosis and inflammation in CKD. In various animal models, MSC-exosomes have been reported to lower creatinine and BUN levels, reduce oxidative stress, and activate regenerative processes. Exosomes, thanks to their stable structure and targeted biological cargo delivery, are gaining importance as both non-invasive diagnostic biomarkers and safe therapeutic agents in kidney diseases. However, their transition to clinical practice requires the development of isolation and standardization techniques, the establishment of biomarker reference ranges, and large-scale clinical trials. In the future, the use of MSC-exosomes in cell-free therapeutic strategies may provide an innovative and effective approach to the management of kidney diseases.

Keywords: Exosome, kidney disease, mesenchymal stem cell, biomarker, regenerative medicine

GİRİŞ

Böbrek hastalıkları, dünya çapında artış gösteren ciddi bir halk sağlığı sorunudur. Bu hastalıklar, genellikle akut böbrek hasarı (ABH) ve kronik böbrek hastalığı (KBH) olarak iki ana kategoriye ayrılır.¹ Akut böbrek hasarı; ani böbrek fonksiyon kaybıyla ortaya çıkan, 7-90 gün süren bir durumdur ve glomerüler filtrasyon hızında, idrar çıkışında ve serum kreatinin seviyelerinde düşüşle karakterizedir.² Kronik böbrek hastalığı ise, üç aydan uzun süren yapısal ve fonksiyonel anormalliklerle tanımlanır ve sıklıkla son dönem böbrek hastalığına (SDBH) ilerler.³ Hastalığın şiddeti ve seyri; etiyolojiye, fizyopatolojik süreç ve bireysel yanıtla bağlı olarak değişir.

Günümüzde böbrek yetmezliğinin başlıca tedavileri diyaliz ve böbrek naklidir. Ancak bu yöntemler, yaşam kalitesini olumsuz etkileyebilir ve sınırlı hayatta kalma avantajı sunar. Diyaliz, yüksek mortalite oranı, sık hastaneye yatış ve psikososyal sorunlarla ilişkilidir.⁴

Böbrek nakli ise uygun donör bulma güçlüğü, enfeksiyon, tümör gelişme riski ve şiddetli immünolojik reaksiyonlar gibi dezavantajlara sahiptir.⁵ Bu nedenle, daha yenilikçi ve etkili tedavi yöntemlerine olan ihtiyaç giderek artmaktadır.⁶ Son yıllarda yapılan çalışmalar, kök hücre naklinin böbrek fonksiyonlarını iyileştirebildiğini göstermektedir.⁷ Düşük tümör riski, immünsüpresif etkileri ve etik açıdan sağladığı avantajlar nedeniyle mezenkimal kök hücreler (MKH) böbrek rejenerasyonu için umut verici hücreler olarak değerlendirilmektedir.⁸ Bununla birlikte, MKH temelli tedavilerde; kültürlerde uzun süreli farklılaşma, morfolojik değişiklikler, viral veya prion kontaminasyonu ve immünolojik yanıtlarla ilgili sorunlar yaşanabilmektedir.⁹ Bu durum, özellikle eksozomlar olmak üzere hücresiz tedavilere olan ilginin artmasına yol açmıştır.^{6,10} Eksozomlar, çoklu veziküler cisimler (MVB) içinde oluşan 30-150 nm boyutunda nanoyapılar olup, hücreler arası iletişimi kolaylaştırırlar. MKH'lerden elde edilen eksozomlar protein, mRNA ve mikroRNA içerikleri aracılığıyla MKH'lerin rejeneratif etkilerini taklit eder ve tümör riski olmaksızın hücresel iletişimi geliştirirler.^{11,12} Bu özellikler, eksozomları doku mühendisliği ve rejeneratif tıp alanında, hücre nakline göre daha avantajlı bir alternatif haline getirmektedir.⁷ İdrar eksozomları, serum kreatinin ve idrar çıkışının yanı sıra ABH ve KBH için benzersiz ve hassas bir tanı aracı olarak değerlendirilmektedir. Böbrek, prostat ve mesane hücrelerinden salınan eksozom moleküllerindeki farklılıklar, hastalık durumunu doğrudan yansıtabilir. Eksozomlar, lipid zarları sayesinde kargolarını yapısal ve enzimatik yıkımdan korur; bu sayede hastalıkla ilişkili biyobelirteçler güvenle izole edilebilir ve ölçülebilir.¹³ Örneğin, iskemi-reperfüzyon modelinde, idrar eksozom mikroRNA'ları böbrek hasarını ve fibrozisini gösterebilmektedir.¹⁴

Akut Böbrek Hasarı

Akut böbrek hasarı (ABH), böbrek fonksiyonunda ve yapısında ani bozulmaya neden olmaktadır. Klinik belirtiler arasında serum kreatinin düzeyinde ani artış, idrar çıkışında azalma ve glomerüler filtrasyon hızında (GFR) düşüş yer alır.^{2,15} Gelişmiş ülkelerde, yaşlı ve klinik olarak ağır hastaların ABH geliştirme olasılığı daha yüksekken, gelişmekte olan ülkelerde sepsis ve nefrotoksik ilaç kullanımı başlıca nedenlerdir. Kırsal bölgelerde ise toplum kaynaklı ABH genellikle enfeksiyonlar ve ishalden kaynaklanmaktadır.¹⁶ Yapılan bazı çalışmalara göre, dünya genelinde her beş yetişkinden biri ve her üç çocuktan biri hastaneye yatış sırasında ABH geliştirmektedir.¹⁷

Kronik Böbrek Hastalığı

Kronik böbrek hastalığı (KBH), üç aydan uzun süren böbrek yetmezliği veya GFR'nin 60 mL/dakika/1.73 m²'nin altında olması ile karakterizedir.¹⁵ Küresel KBH prevalansı giderek artmakta olup % 13.4'e ulaşmıştır.⁶ Son yıllarda, 4.9 ile 7 milyon arasında kişinin son evre böbrek hastalığına (SEBH) yakalandığı bildirilmiştir.⁶ KBH'nin başlıca nedenleri arasında akut böbrek hasarı (ABH), hipertansiyon, toksik madde maruziyeti, diyabet, yaşlanma, obezite ve nefrektomi yer almaktadır.³ KBH, klinik ve patolojik açıdan heterojen bir hastalıktır ve bazı patolojik süreçleri ABH ile ortaktır. Ayrıca KBH; perisit göçü ve fenotipik değişiklik, mikrovasküler kayıp, kronik tübüler hipoksi, hücresel yaşlanma, kollajen birikimi, miyofibroblast proliferasyonu, tübüler atrofi, kollajenle yer değiştirme, kronik lökosit infiltrasyonu ve SEBH gelişimi ile ilişkilidir.¹⁴ Son evre böbrek hastalığı (SEBH), böbrek fonksiyonunun ileri derecede bozulduğu KBH'nin son aşamasıdır ve yaşamın sürdürülebilmesi için diyaliz veya böbrek nakli gereklidir.⁴

Diyabet, KBH'nin en yaygın nedenidir. Hiperglisemi, böbrek dokusunda inflamatuvar ve oksidatif stres oluşturarak hasara yol açar.

Bu süreç, diyabetik nefropati (DN) gelişimine neden olan proinflatuar sitokinlerin salınımını tetikler. DN; glomerüler bazal membran kalınlaşması, glomerüler skleroz, GFR’de azalma, albüminüri ve proteinüri gibi yapısal ve fonksiyonel değişikliklere yol açarak SEBH’ye ilerleyebilir.^{6,18} KBH, genellikle asemptomatik seyretmesi nedeniyle erken tanı güçleşir ve tedavi gecikir.¹³

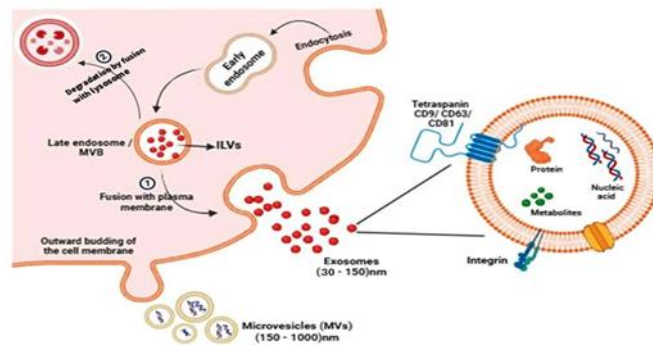
Eksozomlar

Eksozomların ilk bilimsel gözlemi, 1938 yılında transferrin reseptörleri taşıyan yaklaşık 50 nm çapındaki zar veziküllerinin retikülositlerden salınmasıyla yapılmıştır. Bilimsel literatürde “eksozom” terimi, bu hücre dışı vezikülleri tanımlamak amacıyla ilk kez 1987 yılında kullanılmıştır.¹⁹ Çift katmanlı lipid zarına sahip, içbükey yapıda olan eksozomlar, 30-100 nanometre çapındadır. Nöronlar, sitotoksik T hücreleri, trombositler ve mezenkimal kök hücreler (MKH) eksozom salgılar. Ayrıca sperm, kan, idrar ve amniyotik sıvı da eksozom içermektedir.^{8,20}

Eksozom Oluşumu

Erken endozomlar, eksozom oluşumu sürecinde geç endozomlara veya çok veziküllü cisimlere (MVB) olgunlaşır. Geç endozom zarlarının içe doğru tomurcuklanması, hücre içi materyalin intraluminal veziküller (ILV) içerisinde hapsedilmesini sağlar. MVB’ler, lizozomlarla birleşerek içeriklerini parçalayabilir veya plazma zarıyla kaynaşarak ILV’leri eksozomlar şeklinde hücre dışına salabilirler.⁶ Araştırmalar, ILV oluşumunun iki temel yolla gerçekleştiğini göstermektedir.

Eksozom oluşumunun ana yollarından biri olan ESCRT mekanizması, ESCRT-0, ESCRT-I, ESCRT-II ve ESCRT-III adlı dört kompleksin düzenli iş birliğiyle gerçekleşir. Bu proteinler, hücre zarlarındaki ubikuitinle etiketlenmiş proteinleri tanıyarak, içeriye doğru tomurcuklanma yöntemiyle intraluminal veziküller (ILV) oluşturur. Diğer bir yol olan ESCRT’den bağımsız mekanizma ise, sfingomiyelinazlar ve tetraspaninler gibi özel moleküllerin yoğunlaştığı lipid salı mikrodomanleri aracılığıyla çalışır.¹⁰ Aşağıdaki şekilde eksozom oluşumu aşamaları görseli verilmiştir.⁶



Şekil 1. Eksozom oluşumu görseli

Eksozom Bileşenleri

Eksozomlar, hücre tipine bağlı olarak farklılık gösteren karmaşık yapılardır. Temel bileşenleri; lipidler, proteinler ve nükleik asitlerden oluşur. Tetraspanin proteinleri (CD63, CD81, CD82, CD9), ısı şoku proteinleri (HSP70, HSP90), ESCRT kompleksi, Alix, TSG101, GTPazlar, anneksinler ve fosfolipazlar, eksozomların yaygın yüzey belirteçleri arasında yer alır.⁶ Eksozomlar, alıcı hücre biyolojisini etkileyebilen çeşitli RNA türlerini taşır.¹⁰

Bu RNA'ların büyük çoğunluğunu miRNA'lar oluşturur.⁸ Lipit bileşenleri arasında sfingomiyelin, prostaglandinler, kolesterol, fosfatidilserin ve yağ asitleri bulunur.²¹

Eksozom İzolasyonu

Eksozomların izole edilmesi için çeşitli yöntemler mevcuttur. En yaygın kullanılanı diferansiyel ultrasantrifüjlemedir.¹⁰ Bu yöntem, düşük maliyetli ve hızlıdır, ancak sınırlı seçiciliği nedeniyle kontaminasyon riski taşır. Sükroz tamponu, özellikle idrar ve serum gibi karmaşık biyolojik sıvılardan elde edilen eksozomların saflaştırılmasında önerilmektedir.¹⁶ Ultrafiltrasyon, belirli gözenek boyutuna sahip membranlar kullanarak küçük kirleticileri hızlı ve verimli bir şekilde uzaklaştırır, ancak benzer boyuttaki partiküller filtrasyonu geçebilir.²² Yüksek performanslı sıvı kromatografisi (HPLC) ile son derece saf eksozomlar elde edilebilir. Ancak yöntemin yüksek maliyeti ve büyük ölçekli üretimdeki sınırlamaları dezavantaj oluşturmaktadır.²³ İmmünoafiniteye dayalı yakalama, CD63 ve CD81 gibi eksozom belirteçlerine bağlanan antikorlar kullanarak yüksek seçicilik sağlar, ancak maliyetli ve verimi düşüktür.²⁴

Eksozomların Tanımlanması

İzolasyonun ardından, eksozomların yapısal ve moleküler olarak karakterize edilmesi gerekir. İmmünositokimyasal işaretleme, Western blotting ve akış sitometrisi, CD9, CD63, CD81 ve CD82 gibi yüzey belirteçlerinin doğrulanmasında kullanılır.²⁰ Morfoloji ve boyut analizi için atomik kuvvet mikroskobu (AFM), taramalı elektron mikroskobu (SEM) ve geçirimli elektron mikroskobu (TEM) gibi görüntüleme tekniklerinden yararlanılır.¹⁰ Dinamik ışık saçılımı (DLS) ve nanoparçacık izleme analizi (NTA), eksozomların konsantrasyonunu ve boyut dağılımını ölçmek için kullanılır.²² Yüzey belirteçlerinin hızlı ve uygun maliyetli kantifikasyonu için ELISA altın standart yöntem olarak kabul edilmektedir.¹⁶ Son yıllarda, eksozom benzeri veziküllerin hassas ve seçici analizine olanak tanıyan yüzey geliştirilmiş Raman spektroskopisi (SERS) teknolojisinin geliştirilmesi dikkat çekmektedir.²¹

Tablo 1. Eksozom Özellikleri, İzolasyon ve Tanımlama Yöntemlerinin Özeti

Değişkenler	Eksozom Özellikleri
Çap ve Şekil	30 - 100 nm, kase (içbükey) şekli
Köken	Çoklu veziküler cismin plazma zarıyla kaynaşmasından sonra eksozomların hücre dışı matrise salınması
Protein İçeriği	CD63, CD81, CD82, CD9, HSP70, HSP90, ESCRT kompleksi, Alix, TSG101, GTPazlar, anneksinler, fosfolipaz ve lipit ile ilgili proteinler
Lipit İçeriği	Fosfatidilserin, çeşitli yağ asitleri, lökotrienler, sfingomiyelin, prostaglandinler ve kolesterol
Nükleik Asitler	miRNA, mRNA, DNA ve kodlama yapmayan RNA
İzolasyon Teknikleri	Ultrasantrifüjleme, ultrafiltrasyon, immünoafinite yakalama, yüksek basınçlı sıvı kromatografisi ve çöktürme
Tanımlama Teknikleri	İmmünositokimyasal analiz, Western blot, akış sitometrisi, mikroskobik analiz, ELISA, dinamik ışık saçılımı, nanoparçacık izleme analizi, yüzeyde güçlendirilmiş Raman spektroskopisi

Eksozomların İşlevleri

Eksozomlar; bağışıklık yanıtlarını, inflamasyonu, anjiyogenezi, koagülasyonu, hücreler arası iletişimi ve patojenlerin taşınmasını modüle edebilirler.^{6,10,20} Ayrıca hastalık tespitinde, hücre dışı tedavilerde ve gen iletiminde potansiyel araçlar olarak kullanılabilirler.

MSC-Eksozomları

Mezenkimal kök hücrelerin (MKH) doku onarımı üzerinde parakrin etkiler gösterdiği ve iskemi/reperfüzyon hasarı fare modelinde doku yenilenmesini destekleyen eksozomlar salgıladığı ilk kez gösterilmiştir.²⁵ MSC eksozomlarının biyolojik işlevleri, köken aldıkları hücrelere benzerdir. Hasarlı hücre ve dokuları onarabilir, immün ve inflamatuvar yanıtları düzenleyebilir ve apoptozu baskılayabilirler.⁸ MSC eksozomları, yaklaşık 2000 farklı proteinin yanı sıra mikroRNA'lar (miRNA), prostaglandinler ve lökotrienler gibi çeşitli biyomoleküller içerir.^{21,10} MSC eksozomlarını terapötik araçlar olarak cazip kılan çeşitli faktörler vardır. Avantajları arasında MKH'lerin yaygın bulunabilirliği ve büyük ölçekli eksozom üretimine elverişliliği yer alır.^{22,23} MSC eksozom izolasyonu, MSC izolasyonuna kıyasla daha hızlı, daha düşük maliyetli ve daha uygulanabilir bir yöntemdir.¹⁰ Eksozomlar bölünemez ve immünojenik yük taşımazlar; bu nedenle bağışıklık tepkisine yol açma olasılıkları düşüktür.⁸ Ayrıca MSC eksozomları, canlı hücre varlığı gerektirmediği ve -20 °C'de yapısal bütünlüğünü koruyabildiği için hücresiz tedavi yaklaşımları açısından önemli bir aday olarak değerlendirilmektedir.⁶

İdrar Eksozomlarının Böbrek Hastalıklarının Teşhisindeki Rolü

Eksozomlar, hücrelerin biyokimyasal özelliklerini yansıtan karmaşık yapıları sayesinde biyolojik fonksiyonların izlenmesinde değerli araçlardır. Güncel araştırmalar, özellikle böbrek tübül lümeni epitelinden kaynaklanan idrar eksozomlarının böbrek fonksiyonunu yansıtabileceğini ortaya koymaktadır. Bu doğrultuda, idrar eksozomlarına dayalı biyobelirteçler, akut böbrek hasarı (ABH) ve kronik böbrek hastalığının (KBH) teşhisinde kullanılabilmektedir.⁶

Akut Böbrek Hasarı (ABH) Tanısı

Akut böbrek hasarı (ABH), idrar analizi, idrar çıkışının izlenmesi ve kan testleri ile teşhis edilir. İdrar eksozomları, hastalık tespitinde daha hassas ve hızlı bir biyolojik materyal olarak değerlendirilmektedir. Bir çalışmada, idrarla atılan eksozomlar hasar evresini yansıtırken; miR-9a, miR-141, miR-200a, miR-200c ve miR-429 iyileşme evresini, miR-125 ile miR-351 ise iskemi/reperfüzyon kaynaklı ABH'nin geç fibrotik evresini göstermiştir.²⁶ Başka bir araştırmada, sıçanlarda sisplatinin erken ve geç dönemde indüklediği ABH'yi tespit etmek amacıyla idrar eksozomlarındaki akvaporin-1 (AQP-1) ve akvaporin-2 (AQP-2) seviyeleri ölçülmüştür. Bulgulara göre, böbrek yetmezliği; 24. saatte dış medullada AQP-1 düzeyinde artış, 168. saatte ise azalma ile; ayrıca 168. saatte iç medullada AQP-2 düzeyinde düşüş ile karakterizedir.¹⁴ İdrar eksozomlarında aktivasyon transkripsiyon faktörü 3 (uATF3) seviyesindeki artış, sepsis kaynaklı ABH modelleri için önemli bir biyobelirteç olarak tanımlanmıştır.¹⁷ Ayrıca artmış miR-30c-5p ve miR-192-5p seviyeleri, iskemi/reperfüzyon hasarına bağlı erken evre böbrek lezyonlarının tespitinde yardımcı olabilmektedir.¹⁰

Kronik Böbrek Hastalığı (KBH) Tanısı

İdrar eksozom mikroRNA biyobelirteçlerinin profillenmesi ve veritabanı analizleri, kronik böbrek hastalığı (KBH) araştırmalarında yaygın olarak kullanılmaktadır.

Böbrek fibrozisi olan hastaların idrar eksozomlarında miR-21 düzeyinin artması ve miR-29c düzeyinin azalması, düşük maliyetli ve duyarlı bir tanı aracı olarak tanımlanmıştır.⁶ Tip 2 diyabetik nefropati olgularında, idrar eksozomlarında miR-29c-5p ve miR-15b-5p düzeyleri azalırken, let-7c-5p düzeyinin artması, bu moleküllerin potansiyel biyobelirteç olabileceğini düşündürmektedir.¹⁶ Biyoinformatik analizler, tip 2 diyabetik nefropatinin miR-30a, miR-133b ve miR-342'nin yüksek ekzosomal mikroRNA seviyeleri ile ilişkili olabileceğini ortaya koymuştur.²⁷ İdrar eksozomlarında miR-21 artışı, böbrek fonksiyonuna zarar veren invaziv olmayan bir belirteç olarak değerlendirilmektedir.¹³ Öte yandan, proksimal olmayan tübül kaynaklı idrar eksozomlarında miR-200b düzeyinin azalması, KBH'de böbrek fibrozisinin teşhisinde yardımcı olabilir.²⁸ Ayrıca miR-451 düzeyindeki artış, erken böbrek hücresi hasarına yanıt olarak ortaya çıkar ve KBH hastalarında hücre stresin göstergesi olabilir.²⁹

MSC'lerden Elde Edilen Eksozomlarla Böbrek Hastalığı Tedavisi

Mezenkimal kök hücreler (MKH), anti-apoptotik, antioksidan, anti-fibrotik, anti-inflamatuar ve immünomodülatör özellikleri sayesinde terapötik potansiyele sahiptir. MKH eksozomları, bu fizyolojik işlevlerin büyük bölümünü taklit edebilir. Hücrelerinin immünomodülatör ve hücre koruyucu aktivitelerini taşıyarak hasarlı hücrelere veya dokulara rejeneratif sinyaller iletebilirler.⁶

Akut Böbrek Hasarı (ABH) Tedavisinde MSC Eksozomları

Sisplatin kaynaklı bir ABH modelinde, IGF-1 içeren insan kemik iliği kökenli MKH eksozomları, fare proksimal tübüler epitel hücrelerinde (PTEC) hasarlı hücre proliferasyonunu artırmıştır.³⁰ Başka bir çalışmada, insan göbek kordonu kökenli MKH'lerden elde edilen eksozomlar, sisplatin kaynaklı ABH fare modellerinde hücre proliferasyonunu artırmak amacıyla ERK1/2 sinyal yolunu aktive etmiş; kan üre nitrojeni (BUN) ve kreatinin düzeylerini, tübüler proteinüriyi ve epitel nekrozunu azaltmış; ayrıca anti-apoptotik ve antioksidan etkiler göstermiştir.³¹ Renal iskemi/reperfüzyon hasarı olan sıçanlarda, miR-199a-3p ile zenginleştirilmiş insan kemik iliği kökenli MKH eksozomları güçlü anti-apoptotik etkiler sergilemiştir. Bu etki, Sema3A, kısmi kaspaz-3 ve Bax düzeylerini azaltan, Bcl-2 düzeyini ise artıran ERK ve AKT yollarının aktivasyonuna bağlanmıştır.³² Bir diğer çalışmada, insan göbek kordonu kökenli MKH eksozomları (hUCMSC-Exo), mTOR sinyal yolunu inhibe ederek LC3B otofagozom belirtecini ekspresyonunu artırmış; inflamasyonu ve apoptozu azaltarak böbrek rejenerasyonunu desteklemiştir.³³ ATP eksikliği bulunan bir böbrek iskemi/reperfüzyon modelinde, MKH kaynaklı ekstraselüler veziküller hipoksi, apoptoz ve iskelet yeniden modellemesi ile ilişkili genlerin (CASP3, CASP7, SHC1, SMAD4) ekspresyonunu baskılamak amacıyla PTEC mikroRNA profillerini modüle etmiştir.³⁴ İskemi/reperfüzyon kaynaklı böbrek hasarında, CCR2 açısından zengin kemik iliği kökenli MKH eksozomları (BMMSC-Exo), CCL2 proteinine bağlanarak makrofaj ve monositlerin böbreğe göçünü engellemiş ve dokuyu korumuştur.³⁵ Son olarak yapılan bir çalışmada, akut iskemi/reperfüzyon modelinde adipoz doku kökenli MKH'ler (ADMSC) ile bunlardan elde edilen eksozomların sinerjik etkilerini incelemiştir. ADMSC'ler ve eksozomları, oksidatif stresi, inflamasyonu, fibrotik ve apoptoz göstergelerini azaltırken; anjiyogenezi ve anti-apoptotik belirteçleri artırmıştır.³⁶

Kronik Böbrek Hastalığı Tedavisinde MSC Kaynaklı Eksozomlar

Renal arter stenozuna bağlı kronik böbrek hastalığı (KBH) domuz modellerinde, mezenkimal kök hücrelerden (MKH) elde edilen eksozomların onarıcı etkileri araştırılmıştır.

Çalışmada, IL-10 ile zenginleştirilmiş MKH eksozomlarının proinflatuar sitokinleri (IL-1 α , IL-1 β ve TNF- α) azaltarak inflamasyonu baskıladığı, medüller oksijenasyonu artırdığı ve fibrozisi azalttığı gösterilmiştir.⁶ Tek taraflı üreteral obstrüksiyon (UUO) modeli oluşturulan farelerde, miRNA-let-7c'yi aşırı ifade eden genetik olarak mühendislik uygulanmış MKH'lerden elde edilen eksozomlar kullanılmıştır. Bu eksozomlar, kolajen IV α 1, α -SMA, TGF- β R1 ve TGF- β 1 dahil olmak üzere fibrozis ile ilişkili genlerin ekspresyonunu azaltmış ve fibrotik süreci hafifletmiştir.³⁷ Yapılan bir araştırmada, UUO modeli farelerde böbrek kaynaklı MKH eksozomlarını test etmiş ve bu tedavinin endotel hücre proliferasyonunu, makrofaj infiltrasyonunu (F4/80 pozitif) ve böbrek fibrozisini artırırken, endotel-mezenkimal geçişi (EndoMT) ve apoptozu azalttığını bildirmiştir.³⁸ Başka bir çalışmada, anjiyogenez ile ilişkili genler ve proteinler içeren MKH eksozomlarının mikrovasküler yapıyı düzenleyerek böbrek fonksiyonunu ve onarımını artırdığı, endotel hücre ölümünü ise azalttığı gösterilmiştir.¹⁶

SONUÇ

Sonuç olarak, eksozomlar yapısal olarak dayanıklıdır ve lipid çift katmanlı zarları sayesinde içeriklerini enzimatik parçalanmadan koruyarak erken tanı ve tedavi için değerli araçlar haline gelmektedirler. Ancak, MSC eksozomlarının böbrek hastalıklarının tedavisindeki etkinliğini kanıtlayacak yeterli sayıda klinik çalışma bulunmamaktadır. Ayrıca, eksozomların izolasyonu ve saflaştırılması için daha hızlı ve daha etkili yöntemlere ihtiyaç duyulmaktadır. Laboratuvar ortamında renal tanı amacıyla kullanılabilecek güvenilir eksozomal biyobelirteçlerin sınırlı olması ve referans değerler hakkındaki belirsizlikler, bu alandaki tanı standartlarının hâlen gelişim aşamasında olduğunu göstermektedir. Eksozom temelli tanı uygulamalarının yaygınlaşabilmesi için biyobelirteç indekslerinin doğru şekilde tanımlanması ve güvenilir biçimde yorumlanması gereklidir. Gelecekteki hedefler arasında, hücre içermeyen tedavi yaklaşımlarında MSC eksozomlarının kullanımının araştırılması, hastaların yaşam kalitesini iyileştirme potansiyeli ve tedaviye bağlı olumsuz etkileri azaltmaya yönelik yöntemlerin geliştirilebilmesi yer almaktadır.

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**THE RELATIONSHIP BETWEEN SOME BIOCHEMICAL PARAMETERS AND
BENIGN PAROXYSMAL POSITIONAL VERTIGO: POTENTIAL MECHANISMS
INFLUENCING ONSET AND RECURRENCE**

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ABSTRACT

Benign Paroxysmal Positional Vertigo (BPPV) is the most common peripheral vestibular disorder, characterized by brief episodes of vertigo triggered by changes in head position. The pathophysiology is associated with displacement of otoconia from the utricle into semicircular canals. Recently, biochemical factors such as vitamin D deficiency and altered serum trace elements (calcium, magnesium, zinc, copper) have been investigated as possible contributors to otoconia degeneration, recurrence, and susceptibility to BPPV. The aim of this study is to explore the relationship between biochemical parameters and BPPV, highlighting potential mechanisms that influence its onset and recurrence. This study is based on a review of clinical and laboratory studies that evaluated serum vitamin D levels, calcium metabolism, and trace elements in patients with BPPV compared with healthy controls. Methods typically included audiovestibular assessment, biochemical blood tests, and statistical comparisons between groups. Several studies demonstrated that patients with recurrent BPPV often exhibit significantly lower serum vitamin D levels compared to controls. Similarly, alterations in trace elements such as decreased magnesium and zinc, and occasionally elevated or imbalanced copper levels, have been associated with impaired otoconial stability and vestibular dysfunction. Correction of vitamin D deficiency or mineral imbalance has been shown to reduce recurrence rates in some patients. The findings suggest that biochemical factors play a relevant role in the pathogenesis and recurrence of BPPV. Vitamin D deficiency may compromise calcium homeostasis, while abnormal serum trace element levels could influence otolith metabolism. Identifying and correcting these biochemical abnormalities may improve treatment outcomes, reduce recurrence risk, and provide new preventive strategies. Further controlled trials are required to clarify causal mechanisms and optimize management.

Keywords: BPPV, Vitamin D, Serum Trace Elements, Otoconia, Vestibular Disorders

INTRODUCTION

Vertigo is clinically defined as the perception that one's surroundings are rotating relative to oneself (objective vertigo) or that one is spinning within the environment (subjective vertigo). Although the term is frequently misapplied as a general descriptor for dizziness, true vertigo is characterized by a distinct rotational sensation, either of the self or of the external space. This condition most commonly originates from pathologies affecting the vestibular system. Etiologies may include disorders of the inner ear or disruptions within the central nervous system's vestibular centers or neural pathways.

Such causative factors encompass Meniere's disease, cerebral arteriosclerosis, intracranial lesions, cranial trauma, motion sickness, and significant, Benign Paroxysmal Positional Vertigo (BPPV), abrupt changes in atmospheric pressure (Thomson & Amedee, 2009).

Vertigo and dizziness rank among the most prevalent clinical presentations, constituting a significant reason for patients to seek medical attention and accounting for a notable proportion of emergency department visits. The lifetime prevalence of these conditions is substantial, with studies reporting rates between 20% and 30% in the general population (Kroenke & Price, 1993; Yardley et al., 1998). Recent investigations have further quantified a high one-year prevalence for specific symptomatology, noting that vertigo, unsteadiness, and dizziness frequently present in combination rather than in isolation. Demographically, these conditions demonstrate a clear predisposition, with a consistently observed female preponderance and a strong correlation with advanced age, indicating that their prevalence is significantly higher among elderly populations (Kurre et al., 2012).

BPPV is among the most prevalent disorders of the vestibular system and constitutes the leading source of peripheral vertigo. Epidemiological studies estimate that approximately 2.4% of the general adult population will experience this condition during their lifetime, with a pronounced gender differential—women are affected at nearly twice the rate of men (3.2% versus 1.6%). Annual incidence rates approach 0.6%, reflecting the considerable number of adults diagnosed each year. Although BPPV most frequently presents in individuals between 50 and 70 years of age, it may occur in adults of any age (von Brevern et al., 2007).

The condition is clinically defined by sudden, short-lived episodes of vertigo—often lasting less than one minute—precipitated by specific changes in head position, such as turning while recumbent or tilting the head backward. Accompanying symptoms commonly include a spinning sensation, postural imbalance, nausea, and, in certain cases, oscillopsia. These manifestations can considerably impair daily functioning, leading to self-imposed activity limitations, heightened fall-related anxiety, and, for some, absenteeism from occupational responsibilities due to symptom severity.

The pathophysiology BPPV involves the dislodgement of otoconia, which are calcium carbonate crystals, from the otolithic organs within the utricle. These crystals migrate into one or more of the semicircular canals. Their aberrant location disrupts the hydrodynamic properties of the endolymphatic fluid within the canals. This interference leads to aberrant deflection of the cupula during head movement, generating inappropriate neural signals in the vestibular nerve that are interpreted by the brain as intense, rotatory vertigo.

In most clinical presentations, the posterior semicircular canal is affected, accounting for 80–90% of cases. The horizontal canal is involved less frequently (5–15% of cases), while involvement of the anterior canal is the rarest (1–2%) (Power et al., 2020). From an etiological perspective, a substantial proportion of cases (50–70%) are classified as idiopathic, arising without a discernible cause (Parnes et al., 2003). However, BPPV can also occur secondary to other conditions, including head trauma—which represents the most common identifiable cause—as well as vestibular neuritis, Ménière's disease, migraine, or surgical procedures involving the inner ear (Katsarkas, 1999).

Furthermore, BPPV is recognized for its notable tendency to recur. Literature generally reports that between approximately one-third to one-half of affected patients will experience recurrence during their lifetime, with recurrence rates varying from around 13% up to 50% or more depending on the length of follow-up. Most recurrences tend to occur within the first year after initial treatment, although risk persists for several years (Pan et al., 2025).

BPPV is a common but mostly unknown vestibular disorder that significantly impacts many people's quality of life due to recurrent vertigo episodes. While BPPV is classically linked to mechanical dislodgement of otoconia in the inner ear, this review focuses on the relationship between various biochemical parameters and BPPV onset and recurrence. Key biochemical factors explored include calcium metabolism, vitamin D deficiency, and trace elements such as copper and zinc, which may affect otoconial stability and antioxidant defenses. By synthesizing clinical and experimental evidence, this review aims to highlight potential biochemical mechanisms influencing BPPV and their implications for future diagnosis and therapy.

CONCEPTUAL FRAMEWORK

Benign Paroxysmal Positional Vertigo (BPPV); Definition and Pathophysiology

Benign Paroxysmal Positional Vertigo (BPPV) is the predominant form of peripheral vestibular dysfunction. It is clinically defined by transient episodes of rotational vertigo induced by changes in head position. Affected individuals commonly report abrupt, severe sensations of spinning that persist for seconds to minutes, frequently accompanied by nausea and postural unsteadiness. While the condition is not life-threatening, it can substantially disrupt activities of daily living and elevate the likelihood of falls, particularly in older adults (Bhattacharyya et al., 2017).

Benign Paroxysmal Positional Vertigo (BPPV) is explained by two main mechanisms: canalithiasis and cupulolithiasis. Canalithiasis occurs when calcium carbonate crystals (otoconia) detach from the utricular otolithic membrane and enter a semicircular canal; with head movements, their gravitational displacement produces abnormal endolymph flow and brief, delayed-onset nystagmus, most often in the posterior canal, which accounts for approximately 93% of BPPV cases (House & Honrubia, 2003; Solomon, 2000). In cupulolithiasis, dislodged otoconia adhere to the cupula, altering its density and leading to persistent abnormal stimulation of hair cells, typically in the horizontal canal, resulting in longer-lasting nystagmus that gradually diminishes through central vestibular adaptation (Psillas et al., 2011; Nuti et al., 2016). Both mechanisms ultimately produce vertigo and nystagmus through abnormal stimulation of the semicircular canals in response to positional changes.

Although the aberrant migration of otoconia into the semicircular canals provides a well-established mechanistic explanation for the symptomatic episodes of BPPV, the fundamental etiological factors predisposing to otoconial degeneration, detachment, or impaired dissolution remain incompletely understood. Contemporary research posits that the pathophysiology may extend beyond purely biomechanical models. Emerging evidence indicates that systemic biochemical dysregulation, specifically concerning calcium and vitamin D homeostasis, alongside imbalances in essential trace elements, may constitute a significant pathogenic contributor to otoconial instability and the subsequent development of BPPV.

Role of Biochemical Parameters

The conceptual framework for understanding the relationship between systemic biochemical parameters and BPPV is grounded in the principle of otoconial homeostasis. Otoconia, the calcium carbonate (CaCO_3) crystals located within the utricle and saccule, are essential for detecting linear acceleration and gravitational forces. Their structural integrity and stability are profoundly influenced by the body's systemic mineral and metabolic equilibrium.

The core hypothesis posits that a dysregulation in this systemic balance—particularly of critical minerals and nutrients—can lead to the development of structurally fragile otoconia that are prone to detachment, thereby initiating the onset of BPPV (Vibert et al., 2003).

Calcium serves as the fundamental mineral constituent of otoconia, which are comprised of crystalline calcite. The biogenesis, preservation, and physiological turnover of these crystalline structures are intimately linked to systemic calcium homeostasis. The precise regulation of calcium balance is essential for the integrity of all calcified tissues, such as bone and otoconia. Dysregulation in calcium metabolism, as observed in conditions like osteoporosis and other disorders of bone remodeling, may similarly affect the otoconial matrix. Such systemic metabolic impairment can adversely affect the structural quality and density of otoconia, heightening their fragility and raising the likelihood of their fragmentation and displacement from the utricular macula. Multiple investigations have demonstrated a significant epidemiological correlation between osteoporosis and BPPV, especially among elderly and postmenopausal populations, supporting the notion that systemic mineral deficiency may represent a risk factor for the disorder (Jeong et al., 2013).

Vitamin D is integral to the regulation of calcium metabolism, enhancing intestinal calcium absorption and supporting its physiological integration into mineralized structures. The hormonally active metabolite, 1,25-dihydroxyvitamin D, mediates these functions through binding to vitamin D receptors (VDRs), which are present in numerous tissues, including components of the inner ear such as the vestibular apparatus. This localization implies a direct influence of vitamin D on otoconial maintenance and vestibular function. Inadequate vitamin D levels may disrupt normal otoconial biomineralization, resulting in the production of mechanically inferior or hypoplastic crystals that exhibit a greater tendency to become dislodged. Current meta-analyses and systematic reviews have substantiated a connection between vitamin D insufficiency and BPPV, although the mechanistic pathways remain under investigation. Evidence increasingly indicates that while vitamin D deficiency may not invariably precipitate the initial onset of BPPV, it is more strongly and consistently correlated with the recurrence of the disorder. This pattern implies that suboptimal vitamin D concentrations may hinder the natural repair and resorptive processes necessary for otoconial clearance, thereby elevating the probability of symptomatic relapse (Wood et al., 2024).

While calcium and vitamin D are central to otoconial health, emerging research suggests that other trace elements may also play a contributing role in the etiology of BPPV. As a group, these micronutrients are essential cofactors for a wide array of enzymatic reactions that maintain cellular integrity and metabolic function. For instance, serum trace elements are critical antioxidants, and a deficiency could lead to increased oxidative stress in the inner ear, potentially damaging the delicate structures that anchor the otoconia (Günizi & Savaş, 2023). Similarly, magnesium is deeply involved in calcium metabolism and is vital for neurological and neuromuscular function, hinting at a potential indirect influence on the integrity of the otoconial matrix and the overall vestibular system (Yu et al., 2014). The collective influence of these trace elements underscores the complexity of inner ear health and points to the potential for a broader nutritional basis for BPPV pathogenesis.

Potential Mechanisms Linking Biochemical Changes to BPPV

The relationship between systemic biochemical changes and BPPV is not merely correlational. Several potential mechanisms exist that could drive this connection, influencing both the initial onset and subsequent recurrence of the condition.

Otoconial Biomineralization Defect: This is the most widely supported mechanism. Deficiencies in calcium and vitamin D, and potentially other trace elements, can lead to the formation of degraded or brittle otoconia. This biomineralization defect results in the creation of amorphous or structurally flawed crystals that are inherently unstable and more likely to break off and become displaced into the semicircular canals (Jeong et al., 2013).

Systemic Mineral Dysregulation as a Proxy for Otolith Health: The otoconial complex shares biochemical similarities with bone tissue and undergoes a continuous process of remodeling. Conditions of systemic mineral dysregulation, such as osteoporosis, may therefore serve as a proxy for similar metabolic dysfunction affecting the otoconial matrix. This systemic link highlights why treatments for osteoporosis, such as vitamin D and calcium supplementation, may also be effective in reducing BPPV recurrence (Lee et al., 2017).

METHODOLOGY

Data Collection And Examination Of Ethical Principles

This review was designed as a structured narrative synthesis incorporating systematic elements to ensure methodological rigor. Data collection was conducted through comprehensive electronic searches of major scientific databases, including PubMed/MEDLINE, Scopus, Web of Science, and Embase, covering literature published between January 2000 and July 2025. The selection of this timeframe was based on the recognition that research exploring biochemical parameters in relation to BPPV is relatively recent, with the earliest seminal reports appearing in the late 1990s. Search terms combined both MeSH headings and free-text keywords such as “benign paroxysmal positional vertigo,” “BPPV,” “calcium metabolism,” “vitamin D,” “trace elements,” and “recurrence.” Boolean operators and truncation were applied to maximize sensitivity and specificity, and reference lists of relevant articles were manually screened to identify additional eligible studies.

The ethical principles of openness, justice, beneficence, and non-maleficence guided the data collection process. Only peer-reviewed, ethically approved, and publicly accessible studies were included, thereby ensuring respect for research participants’ rights and confidentiality. No unpublished or patient-identifiable data were accessed. The review avoided bias by evaluating studies from multiple geographic regions and diverse populations, thereby supporting justice and inclusivity. Beneficence was addressed by synthesizing evidence that may ultimately inform preventive and therapeutic strategies in BPPV, while non-maleficence was observed by avoiding misrepresentation of data or overgeneralization of findings. As no new human or animal subjects were directly involved in this review, formal ethical approval was not required.

A Structured Approach for Evidence Synthesis

The analysis of the research problem followed a structured, multi-step framework designed to systematically explore the complex relationship between systemic biochemical parameters and BPPV. The process began by delineating the research scope, which involved identifying BPPV as a vestibular disorder with mechanical origins and then expanding the focus to include systemic biochemical influences, thereby defining the critical variables of the research system. This was followed by a critical assessment of threats to research validity, encompassing both biological confounding factors and methodological vulnerabilities such as small sample size or heterogeneous diagnostic criteria. To control for these risks, the study then operationalized its variables, prioritizing studies that used validated diagnostic protocols and standardized biochemical assays to ensure evidence quality.

A detailed impact analysis was conducted to evaluate how nutritional deficiencies could influence otoconial degeneration and clinical recurrence. Finally, the evidence from these domains was synthesized and evaluated to construct an integrated conceptual model of BPPV onset and recurrence, with inconsistent findings highlighted as key areas for future research, thus refining the model for a more holistic understanding of the condition.

CONCLUSION AND DISCUSSION

The accumulated evidence examined in this review indicates that benign paroxysmal positional vertigo (BPPV) ought to be conceptualized not merely as a biomechanical anomaly, but also as a condition with considerable metabolic determinants, fundamentally linked to systemic mineral and nutrient regulation. The functional integrity of the vestibular system is inherently dependent on the structural soundness of otoconia—calcium carbonate crystals—whose stability is intimately connected to the body's general metabolic state. This relationship is reinforced by documented correlations between BPPV and several systemic disorders, particularly those affecting bone metabolism, suggesting a common pathogenic pathway in which broader homeostatic disturbances may adversely affect inner ear integrity.

The interplay of calcium, vitamin D, and trace elements appears to be a crucial determinant of otoconial health, affecting both the *onset* and subsequent *recurrence* of BPPV. Systemic calcium dysregulation is believed to weaken the structural integrity of otoconia. This otoconial biomineralization defect can make the crystals inherently fragile and more prone to fragmentation and detachment from the utricular macula, thus contributing to the initial onset of the condition (Jeong et al., 2013). This mechanism is further compounded by a deficiency in vitamin D, which is essential for proper calcium absorption and deposition, as well as for the active biomineralization process within the inner ear (Wood et al., 2024).

While these deficiencies may be a predisposing factor for the initial episode, their role in BPPV recurrence is even more profound and consistently supported by the literature. The inner ear possesses a natural, albeit slow, mechanism for the resorption and clearance of displaced otoconia. A chronic state of mineral and vitamin D deficiency may significantly impair this process. The lack of adequate vitamin D may hinder the body's ability to effectively resorb the dislodged crystalline debris, leading to its persistence within the semicircular canals and increasing the likelihood of recurrent episodes of vertigo (Wood et al., 2024). This is a critical distinction, as it suggests that while a sudden trauma or event may trigger the onset, the underlying nutritional status of an individual dictates the chronicity and recurrence of the disease.

The role of trace elements, while an emerging area of study, adds a layer of complexity to this metabolic hypothesis. Micronutrients such as zinc and magnesium, despite not being the primary components of otoconia, act as crucial cofactors in various biochemical pathways that support inner ear health. Zinc, for example, is a vital antioxidant that protects against cellular damage from oxidative stress. A deficiency could compromise the structural integrity of the otoconial membrane, contributing to both the *onset* of fragmentation and the difficulty of repair. Similarly, magnesium's central role in calcium metabolism and neuromuscular function suggests a plausible indirect influence on the stability of the otoconia and their supporting matrix, which could affect both the initial attachment and the long-term resilience of the vestibular system (Günizi & Savaş, 2023; Yu et al., 2014).

The evidence presented throughout this review provides a robust framework for understanding benign paroxysmal positional vertigo as a disorder with significant metabolic underpinnings.

While the primary treatment for BPPV remains physical repositioning maneuvers, the high rate of recurrence in many individuals underscores the need to address the underlying etiology. The consistent findings of associations between BPPV and deficiencies in calcium and vitamin D highlight a clear opportunity for targeted clinical intervention. Screening for and supplementing these nutrients in patients with recurrent BPPV represents a promising, non-invasive strategy to reduce the frequency of episodes.

Furthermore, this line of inquiry opens up new avenues for future research. The potential role of trace elements and the nuanced mechanisms linking systemic dysregulation to inner ear health require more rigorous investigation. While the current body of literature is primarily composed of observational studies, a shift towards prospective, randomized controlled trials will be essential to establish a definitive causal relationship and determine optimal supplementation dosages. Ultimately, by shifting the focus from a purely mechanical viewpoint to a more holistic understanding of otoconial homeostasis, we can pave the way for more effective preventative and therapeutic strategies for this common and debilitating condition.

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HEMATOLOGICAL, BIOCHEMICAL & IMMUNOLOGICAL ALTERATIONS IN ACUTE COVID-19, LONG-COVID SYNDROMES AND POST-VACCINATION SYNDROMES

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ABSTRACT

Introduction and Purpose: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its vaccines have profoundly influenced global health. Beyond respiratory illness, SARS-CoV-2 provokes systemic hematological, biochemical, and immunological disturbances. These alterations determine acute disease severity, drive long-term sequelae (long COVID), and contribute to rare post-vaccination syndromes. Clarifying their clinical significance is essential for establishing reliable biomarkers that enhance diagnosis, support risk stratification, and guide prognosis.

Methods: This review synthesizes peer-reviewed clinical and translational studies describing changes in blood indices, biochemical parameters, and immune signatures across acute COVID-19, long COVID, and vaccine-associated complications. A comparative framework highlights overlapping and distinct mechanisms to evaluate their utility as biomarkers.

Results: Acute COVID-19 is characterized by lymphopenia, neutrophilia, and elevated D-dimer, C-reactive protein (CRP), ferritin, and interleukin-6 (IL-6), alongside a hypercoagulable state that correlates with poor outcomes. Long COVID reflects persistent immune activation, chronic cytokine imbalance, endothelial dysfunction, autoantibody generation, and metabolic disruption. Post-vaccination syndromes, although rare, include hematological events such as vaccine-induced immune thrombotic thrombocytopenia, biochemical signs of hepatic or renal stress, and immune-mediated complications including myocarditis and pericarditis. Collectively, these profiles distinguish acute hyperinflammation, prolonged immune dysregulation, and focal post-vaccination reactions.

Discussion: Integrating hematological (lymphocyte and platelet counts), biochemical (D-dimer, ferritin, CRP), and immunological (cytokine and autoantibody) markers provides a practical framework for clinical decision-making. Such biomarkers hold potential for predicting severity, monitoring recovery, and anticipating late complications, thereby supporting precision medicine approaches.

Conclusion: Hematological, biochemical, and immunological alterations form a unifying perspective through which acute, chronic, and vaccine-associated SARS-CoV-2 outcomes can be interpreted. Employing these signatures as biomarkers may refine diagnosis, improve prognostication, and personalize management while informing vaccination safety and public health policy.

Keywords: HBI alterations, Acute COVID, Long COVID Syndrom, Post Vaccination Syndrom

4-HYDROXY-2-NONENAL MECHANISM AND ITS RELATIONSHIP WITH CANCER

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ABSTRACT

Introduction and Purpose: Free radicals are highly reactive molecules formed as a result of cellular metabolism and environmental factors. Under normal physiological conditions, these molecules are balanced by antioxidant defense systems; however, when this balance is disrupted, oxidative stress occurs. Oxidative stress has destructive effects on proteins, nucleic acids, and particularly on polyunsaturated fatty acids (PUFAs) in cell membranes. One of the most important biochemical events arising in this process is lipid peroxidation. During lipid peroxidation, cell membrane phospholipids are attacked by free radicals, and through intermediate products, various reactive aldehydes are formed. Among the products of lipid peroxidation, 4-hydroxy-2-nonenal (4-HNE) is one of the most biologically reactive aldehydes and is of great importance due to its toxic and signaling regulatory properties. Structurally, 4-HNE is an α,β -unsaturated hydroxyaldehyde that can form covalent bonds with nucleic acids, proteins, and enzymes, thereby directly modulating cellular functions. Through these properties, it plays a critical role in regulating fundamental biological processes such as cell proliferation, differentiation, apoptosis, and necrosis. Studies have shown that the levels of 4-HNE are elevated in cancer patients. Therefore, 4-HNE emerges as an important molecule in both understanding the mechanisms of cancer development and being investigated as a biomarker.

Keywords: Oxidative stress; PUFA; Lipid peroxidation; 4-hydroxy-2-nonenal; Cancer

1.INTRODUCTION

Oxidative stress is defined as an imbalance resulting from increased production of reactive oxygen species (ROS) and the insufficiency of antioxidant defense systems. This condition may cause damage to cellular structures and compromise tissue integrity. Cell membranes and organelle membranes are particularly vulnerable to ROS-induced damage due to their high content of polyunsaturated fatty acids (PUFAs); this process is commonly referred to as lipid peroxidation [1]. Among the products of lipid peroxidation, 4-hydroxynonenal (4-HNE) is biologically active and significant in terms of its effects. By interacting with various mitochondrial targets, it forms adducts, leading to mitochondrial dysfunction, cellular damage, and consequently, numerous pathological conditions. 4-HNE exhibits a dual role; while low concentrations are beneficial to cells by promoting proliferation, differentiation, and antioxidant defense, high concentrations activate pathways that result in cell demise. As a marker of lipid peroxidation, 4-HNE is closely associated with tumor size and stage. Tumors with higher degrees of malignancy tend to display lower levels of 4-HNE.

Accordingly, in contrast to the supportive role of elevated 4-HNE levels in carcinogenesis within healthy cells, mechanisms that reduce and detoxify excessive 4-HNE production in tumor tissues may contribute to the survival of tumor cells [2].

CONCEPTUAL FRAMEWORK

Free Radicals and Reactive Oxygen Species

Reactive free molecules are molecules or ions that contain unpaired electrons in their outer orbitals, a property that renders them extremely unstable and have a short half-lives. within biological system short-lived. The unpaired electrons in their structures drives these species to reach stable energy levels through electron pairing, a process that often initiates chain reactions and predisposes cells to damage [3]. Reactive free molecules can arise from both endogenous mechanisms within the organism and exogenous sources. From an endogenous perspective, free radicals are byproducts of normal cellular metabolism [3,4]. For example, during mitochondrial respiration, electrons leaking from the electron transport chain to oxygen molecules can result in the formation of the superoxide radical ($O_2^{\bullet-}$) [5]. In addition, several enzymatic reactions, where nitric oxide synthase, cytochrome P450, and xanthine oxidase are involved in the mechanism, contribute to free radical production [6]. Cells of the immune system, such as macrophages and neutrophils, also generate free radicals during a process known as the respiratory burst to defend against pathogens [7].

Exogenous sources include ultraviolet (UV) radiation, ionizing radiation, cigarette smoke, environmental pollutants, certain chemicals, and drugs [8]. The major groups of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3]. ROS play significant roles in both normal cellular physiological and pathological processes. The superoxide radical ($O_2^{\bullet-}$) and the hydroxyl radical ($\bullet OH$) are two the most important ROS [9]. The superoxide radical is the precursor of most ROS and is predominantly generated during mitochondrial processes. Despite its relatively weak reactivity, it can spontaneously or enzymatically undergo dismutation by the enzyme superoxide dismutase (SOD) to form hydrogen peroxide (H_2O_2) [10,11]. Although H_2O_2 is not a radical itself, reactions in the presence of transition metals such as iron or copper it can be converted into the extremely unstable hydroxyl radical via Fenton or Haber–Weiss [12].

The hydroxyl radical is the most reactive species among ROS. It can interact with all biomolecules, including lipids, proteins, and nucleic acids, and can damage these molecules. This extreme reactivity stems from its small size and lack of charge, which allow it to rapidly diffuse across cellular structures. The hydroxyl radical differs from other ROS. It directly attacks biomolecules, as a result widespread cellular damage because of the lipid peroxidation and DNA strand breaks occur.

Oxidative Stress

Cellular homeostasis is the effort of living cells to ensure stable internal environment within the cell to maintain normal cell functions and processes, representing a dynamic mechanism that ensures physiological integrity and health. In the context of oxidative homeostasis, this refers to the delicate balance between ROS and the antioxidant systems responsible for their elimination [13]. When the balance between ROS and antioxidant system disrupted in favor of ROS, it leads to the development of redox imbalance [14]. Under redox imbalance conditions, the suppression of antioxidant mechanisms allows ROS to readily attack cellular macromolecules such as DNA, proteins, and lipids.

During mild or moderate redox imbalance, ROS-mediated protein modifications are mostly reversible, contributing to the physiological regulation of intracellular signaling pathways [15–18]. However, under severe redox imbalance, ROS induced damage primarily results in cell demise; modifications of DNA bases promote chromosomal instability and mutations, while irreversible protein damage leads to the accumulation of aggregates that cannot be degraded by the proteasome or repaired [19,20].

Lipid Peroxidation and 4-HNE Formation

The lipid bilayer of the plasma membrane and the mitochondrial membrane is highly susceptible to the auto-catalytic chain reaction initiated by ROS, a process defined as lipid peroxidation [21,22]. This process can be examined in three stages: initiation, propagation, and termination. For instance, ROS such as the hydroxyl radical attack the carbon double bonds of polyunsaturated fatty acids (PUFAs), abstracting hydrogen and generating a lipid radical. This radical then combines with oxygen to form a peroxy radical. The peroxy radical, in turn, sustains the chain reaction by producing new lipid radicals and lipid hydroperoxides [23]. The end products of this mechanism include reactive aldehydes such as malondialdehyde, acrolein, and 4-hydroxynonenal (4-HNE) [22,24]. The recognition of lipid peroxidation and its aldehyde products as both a consequence and a driving factor in the pathogenesis of various diseases has heightened scientific interest in this process [25]. Among these, 4-HNE stands out as being more stable and possessing a longer half-life compared to ROS, allowing it to diffuse from its site of formation and affect targets distant from the initial site of free radical attack.

4-HNE Structure and Mechanism of Action

4-HNE contains three major functional groups: a carbonyl group (C=O) at the C1 position, a double bond (C=C) between C2 and C3, and a hydroxyl group at C4. These chemical features make 4-HNE one of the most reactive aldehyde products of lipid peroxidation. Its regulatory effects on various cellular processes depend on both its concentration and the cell type. At low concentrations, 4-HNE acts as a signaling molecule that regulates diverse (patho)physiological processes such as proliferation, differentiation, antioxidant capacity, and programmed cell death; however, at high concentrations, it can cause irreversible cellular damage, leading to uncontrolled cell damage [26–28]. Accordingly, 4-HNE and similar aldehydes are considered secondary messengers of free radicals, capable of exerting their effects even in the absence of excessive ROS production due to their strong affinity for protein binding. Two major types of chemical reactions characterize the interactions of 4-HNE with histidine, cysteine, lysine, and arginine residues in proteins: the formation of Michael adducts and Schiff bases [29]. These modifications of proteins and peptides by 4-HNE can alter their structure and function, representing the principal mechanism by which 4-HNE exerts its bioactive effects [30]. The electrophilic sites of 4-HNE promote the formation of Schiff bases between the carbonyl group at C1 and an amino group, and Michael adducts involving thiol or amino compounds at C3 [31–33]. The reactivity of 4-HNE toward amino acids has been reported in the order cysteine > histidine > lysine [34–36]. Initially, amino acids undergo Michael addition to the C=C double bond of 4-HNE. The susceptibility of the C2–C3 double bond to Michael addition confers rotational flexibility, facilitating secondary reactions between primary amines and the carbonyl group of 4-HNE, thereby promoting Schiff base formation [37].

The covalent modifications induced by 4-HNE on nucleophilic side chains of amino acids can regulate protein activation or inactivation, thereby altering cellular signaling pathways.

Consequently, many critical pathways associated with programmed cell death, cell cycle regulation, and redox imbalance are significantly influenced by 4-HNE, contributing to cellular toxicity [38–40]. Furthermore, 4-HNE inhibits the 20S proteasome, preventing the degradation of oxidatively damaged or modified proteins [41]. It has also been reported that 4-HNE adducts impair chaperone activities mediated by HSP-72 and HSP-90 [42,43]. In addition, 4-HNE directly interacts with guanine bases in DNA to form 1,N-2-propano-deoxyguanosine adducts, with a reported frequency of approximately 1.2 ± 0.5 per 10^7 nucleotides [44]. Moreover, reactions of 4-HNE with DNA nucleotides can generate etheno-DNA (ϵ -DNA) adducts, which have been shown to promote mutagenic alterations and increase the risk of cellular transformation into cancer [45].

4-HNE and Cancer

Metabolic reprogramming in cancer cells is a hallmark that emerges during carcinogenesis and tumor progression [46,47]. This phenomenon results in the abnormal accumulation of various metabolites [48]. 4-HNE reacts with nucleic acids and suppresses nucleotide excision repair, thereby leading to DNA damage and chromosomal abnormalities [49,50]. In addition, it forms covalent bonds with the tumor suppressor protein p53, contributing to the development of malignancy [51]. While 4-HNE suppresses programmed cell death at physiological levels, its excessive accumulation triggers programmed cell death [52–54]. Furthermore, nuclear factor erythroid 2–related factor 2 (Nrf2), which is activated by 4-HNE, regulates the expression of multiple genes, leading to alterations in cellular signaling pathways [55]. The accumulation of 4-HNE, with its cytotoxic properties, has been strongly associated with carcinogenesis [56]. Indeed, in colonic and pulmonary epithelial cells, 4-HNE has been shown to inhibit nucleotide excision repair of both benzo[a]pyrene diol epoxide (BPDE)- and ultraviolet (UV) induced DNA damage [57]. These findings demonstrate that 4-HNE not only damages the DNA molecule but also impairs DNA repair mechanisms, thereby contributing to tumor development. Clinically, it has been reported that 4-HNE accumulation is higher in nonalcoholic fatty liver disease compared with control groups, and it has been identified as a prognostic marker associated with poor outcomes in hepatitis C virus–mediated hepatocellular carcinoma [58,59]. Moreover, elevated 4-HNE levels have been detected in esophageal squamous cell carcinoma (ESCC), colon adenocarcinoma, lung cancer, and thyroid cancer [60–63]. In contrast, some studies have demonstrated reduced accumulation of 4-HNE in colorectal cancer, clear cell renal cell carcinoma, and urothelial carcinoma [64,65]. Variations in the accumulation levels of 4-HNE differ depending on the pathological condition and cancer type. Depending on the dose and the nature and functional importance of the target proteins within biological systems, 4-HNE exerts diverse pathophysiological effects, including both carcinogenic and anti-carcinogenic activities [66]. At bioactive low concentrations (in the range of 1–10 μ M), 4-HNE has been shown to induce programmed cell death in HT-29 cell lines and inhibit cancer cell growth by modulating cell cycle progression. It achieved this by suppressing c-myc and p21 gene expression [67], inhibiting telomerase activity [68], and enhancing the pro-apoptotic effect of TGF β 1 through the activation of c-Jun N-terminal kinase (JNK), which synergistically increased the activity of Smad4, a mediator of cytokine signaling [69]. By using antibody-based techniques directed against 4-HNE–protein adducts, persistently high levels of 4-HNE have been consistently detected in colorectal cancer (CRC).

The formation of 4-HNE–protein adducts in colon cancer tissues has been associated with a decrease in non-enzymatic antioxidants such as vitamin E and C and glutathione (GSH), linking these changes to CRC growth and progression [70].

4-HNE can act as an alarm signal against oxidative alterations by activating NRF2 in vascular cells [71]. NRF2, in turn, regulates the transcription of critical antioxidant and cytoprotective enzymes such as heme oxygenase-1 (HO-1) and thioredoxin reductase 1 (TrxR1). In fully transformed cancer cells, these enzymes may exert oncogenic effects, particularly by contributing to therapy resistance. These findings reveal that tumor cells undergo metabolic reprogramming by promoting the inactivation of oxidative mediators that would otherwise suppress growth. Taken together, these results suggest that physiological levels of 4-HNE stimulate antioxidant release and prevent carcinogenesis, whereas chronic exposure to elevated 4-HNE is associated with cancer growth and progression.

Conversely, low 4-HNE levels in fully transformed cancer cells may play a role in anticancer effects and resistance to therapies. Thus, 4-HNE levels in cancer cells are increasingly regarded as a therapeutic target. In a study by Zhang et al., 4-HNE expression levels were evaluated in ESCC patients, and in ESCC tissues compared with benign esophageal epithelial tissues, 4-HNE expression was found significantly higher. This finding suggests that 4-HNE may serve as a driving force in ESCC carcinogenesis. When patient survival times were analyzed, patients with 4-HNE positive tumors has significantly longer overall survival than patients with 4-HNE negative tumors (30,7 vs. 10.9 months). This result indicates that high 4-HNE levels are associated with improved prognosis in ESCC [72]. Similarly, Wang et al. reported elevated levels of CREB and glutathione peroxidase 4 (GPX4), an inhibitor of lipid peroxidation, in lung adenocarcinoma tissues, with correspondingly low levels of 4-HNE. Their study concluded that in lung adenocarcinoma low 4-HNE levels were to be found associated with more advanced tumor stages and larger tumor sizes [73]. In another study by Ma et al. on gastric cancer, 4-HNE levels were found to be significantly lower in the cancer group compared with controls, indicating that cancer patients are characterized by reduced lipid peroxidation. The authors further suggested that this reduction resulted from tumor cells excessively expressing antioxidant enzymes, thereby favorably influencing tumor aggressiveness [74].

CONCLUSION

At physiological levels, 4-HNE maintains homeostasis in healthy cells, supports cell proliferation and differentiation, and strengthens antioxidant defense. However, at high concentrations, it can bind to DNA and proteins, leading to mutations. These mutations can affect proteins involved in the cell cycle or apoptosis, resulting in resistance to cell death and uncontrolled proliferation, which can trigger cancer development. Variable levels of 4-HNE in cancer cells can alter signaling pathways that regulate fundamental processes such as inflammation, cell proliferation, and cell death. Therefore, it has been demonstrated that different 4-HNE levels in cancer cells have distinct effects and that this molecule has significant potential as both a biomarker and a therapeutic target.

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A REVIEW ON APOPTOSIS AND APOPTOSIS MECHANISMS

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ABSTRACT

Apoptosis is a complex process that leads to the programmed death of cells and is regulated by two main signaling pathways: the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway. Both pathways result in the activation of cysteine proteases called caspases, leading to the controlled disintegration of the cell. The intrinsic pathway is triggered by intracellular stress signals (e.g., DNA damage, oxidative stress, growth factor depletion, endoplasmic reticulum stress). In this pathway, mitochondria play a central role in the initiation of apoptosis. The extrinsic pathway is triggered by extracellular signals, namely, the activation of cell-surface death receptors (e.g., Fas/CD95, TNFR1, TRAIL receptors). Caspases are the central regulators and executioners of apoptosis. Caspases, belonging to the cysteine protease family, normally exist in the form of an inactive zymogen (procaspase). When activated by apoptosis signals, they cleave target proteins at specific aspartate residues, causing cell lysis and apoptosis. Advances in apoptosis research in recent years have provided a deeper understanding of the molecular details of this complex mechanism. Targeting apoptotic pathways, particularly in the treatment of diseases associated with apoptosis dysregulation, such as cancer, neurodegenerative diseases, and autoimmune disorders, has led to the development of new and promising therapeutic strategies. Studies on the apoptosis-inducing effects of natural compounds and medicinal plants have the potential to offer more effective treatment options with fewer side effects. Furthermore, recent studies have revealed the role of apoptosis in various cancer types and new molecular targets in this process.

Keywords: Apoptosis, Apoptotic Pathways, Caspase

GİRİŞ

Apoptozis, hücrelerin yaşam döngüsünün doğal bir parçası olarak, genetik olarak programlanmış ve enerjiye bağımlı şekilde gerçekleşen bir ölüm sürecidir. İlk kez 1972 yılında Kerr, Wyllie ve Currie tarafından tanımlanan bu mekanizma, hücre içi ve hücre dışı sinyallerin koordineli etkileşimiyle başlar. Sürecin en belirgin özelliği, nekrozdan farklı olarak inflamatuvar yanıt oluşturmaması ve hücre zarının bütünlüğünü korumasıdır (Kerr ve ark., 1972).

Tarihsel olarak kavram, morfolojik tanımla ortaya çıkmış, 1980’lerde *Caenorhabditis elegans* modeli üzerinde yapılan çalışmalarla genetik temelleri (*ced-3*, *ced-4*, *ced-9*) aydınlatılmış, 1990’lardan itibaren ise insanlarda Bcl-2 ailesi, kaspazlar ve ölüm reseptörleri gibi moleküler bileşenlerin tanımlanmasıyla biyokimyasal mekanizmaları anlaşılmıştır (Horvitz, 1999). Günümüzde apoptozis, yalnızca temel biyoloji açısından değil, kanser, nörodejeneratif hastalıklar, otoimmün bozukluklar ve enfeksiyon hastalıkları gibi geniş bir patolojik yelpazede rolü nedeniyle klinik araştırmaların da merkezinde yer almaktadır (Elmore, 2007).

Fizyolojik olarak apoptozis, embriyonik gelişim sırasında parmakların ayrılması, sinir sisteminin şekillenmesi, bağışıklık sisteminde otoreaktif lenfositlerin ortadan kaldırılması ve doku homeostazının sürdürülmesi gibi kritik görevler üstlenir. Bu süreçte, hücrelerin kontrollü bir şekilde ortadan kaldırılması, organizmanın bütünlüğünü ve sağlığını korur.

Ancak apoptozisin yetersiz olduğu durumlarda kanser, viral enfeksiyonlar ve otoimmün hastalıklar gelişebilirken; aşırı apoptozis, Alzheimer ve Parkinson gibi nörodejeneratif hastalıklar ile iskemik doku hasarına yol açabilir (Mattson, 2000).

Bu bağlamda apoptozis, hücre ölümü spektrumunda nekroz, nekroptoz, piroptoz ve ferroptoz gibi diğer mekanizmalarla birlikte değerlendirilir; ancak moleküler düzenleme, morfoloji ve biyokimyasal imzalar açısından kendine özgü bir konuma sahiptir. Araştırma ve klinik uygulamalar açısından apoptozis, hedefe yönelik kanser tedavilerinden nöroprotektif stratejilere, immünomodülatör yaklaşımlardan doku mühendisliğine kadar geniş bir yelpazede potansiyel sunar (Souers ve ark., 2013). Bu tür gelişmeler, apoptoz mekanizmalarının anlaşılmasının yalnızca temel bilim açısından değil, translasyonel tıp ve kişiselleştirilmiş tedavi yaklaşımları açısından da kritik olduğunu göstermektedir.

MORFOLOJİK VE BİYOKİMYASAL ÖZELLİKLER

Apoptozis, hem morfolojik hem de biyokimyasal düzeyde kendine özgü değişikliklerle tanımlanır ve bu özellikler, onu diğer hücre ölümü türlerinden ayıran temel kriterlerdir. Morfolojik olarak apoptozis, hücre küçülmesi (cell shrinkage) ile başlar. Hücre hacmi azalırken sitoplazma yoğunlaşır, organeller daha kompakt hale gelir. Bu sırada kromatin, nükleer zarın iç yüzüne doğru yoğunlaşır (kromatin kondensasyonu) ve bu yoğunlaşma genellikle yarım ay şeklinde veya nükleusun tamamını kaplayacak şekilde gözlenir (Wyllie ve ark., 1980).

İlerleyen aşamalarda nükleer zar parçalanır ve DNA, endonükleazlar tarafından oligonükleozomal boyutlarda fragmanlara ayrılır. Hücre zarı bütünlüğünü korumaya devam ederken, yüzeyinde belirgin membran çıkıntıları (bleb) oluşur. Bu blebler, sitoplazma ve organellerin küçük, zarla çevrili veziküller halinde paketlenmesine yol açar. Bu yapılar apoptotik cisimcikler olarak adlandırılır ve çevredeki fagositler tarafından hızla tanınıp ortadan kaldırılır. Bu hızlı temizlenme, apoptozisin inflamatuvar yanıt oluşturmamasının temel nedenlerinden biridir (Elmore, 2007).

Biyokimyasal düzeyde apoptozis, kaspaz adı verilen sistein proteazlarının ardışık aktivasyonu ile yürütülür. Başlatıcı kaspazlar (kaspaz-8, -9) spesifik adaptör proteinler aracılığıyla aktive olur ve yürütücü kaspazları (kaspaz-3, -6, -7) keserek aktif hale getirir. Yürütücü kaspazlar, hücre iskeleti proteinlerini, nükleer laminleri ve DNA onarım enzimlerini hedef alarak hücre sel yapının kontrollü bir şekilde parçalanmasını sağlar (Cohen, 1997).

Apoptozisin bir diğer biyokimyasal imzası, DNA'nın oligonükleozomal fragmentasyonu ve bunun jel elektroforezinde “merdiven” (ladder) görünümü oluşturmasıdır. Bu durum, endonükleazların (özellikle CAD – caspase-activated DNase) aktivasyonu ile gerçekleşir. Ayrıca fosfatidilserin (PS) normalde plazma zarının iç yaprağında bulunurken, apoptozis sırasında flipaz ve scramblaz enzimlerinin aktivasyonu ile dış yaprağa taşınır.

Mitokondriyal membran potansiyelinin kaybı da apoptozisin önemli biyokimyasal özelliklerinden biridir. Bu kayıp, mitokondri dış membran permeabilizasyonu (MOMP) ile ilişkilidir ve sitokrom c, Smac/DIABLO gibi pro-apoptotik faktörlerin sitoplazmaya salınmasına yol açar. Bu olaylar, intrinsik yolun başlatılmasında kritik rol oynar (Gurzov & Eizirik, 2011).

APOPTOZİS YOLAKLARI

Apoptozis, hücre ölümünün en iyi tanımlanmış ve en sık çalışılan biçimlerinden biridir. Hücreler, ölüm kararını iki ana sinyal yolundan biri aracılığıyla alır: intrinsik (mitokondriyal) yol ve ekstrinsik (ölüm reseptörü) yol. Bu iki yol, farklı tetikleyicilerle başlasa da yürütücü aşamada kaspaz aktivasyonu ile birleşir.

Ayrıca, aralarında çift yönlü iletişim sağlayan moleküler köprüler vardır. Bu bölümde her iki yol ayrıntılı olarak incelenecek, ardından aralarındaki etkileşim ve klinik önemi tartışılacaktır.

İntrinsik (Mitokondriyal) Yol

İntrinsik yol, hücre içi stres sinyalleri tarafından tetiklenir. DNA hasarı, oksidatif stres, hipoksi, büyüme faktörü eksikliği, endoplazmik retikulum stresi ve sitotoksik ilaçlar bu yolu aktive eden başlıca uyarılardır (Gurzov & Eizirik, 2011). Bu sinyaller, mitokondri dışı membran permeabilizasyonu (MOMP) ile sonuçlanan karmaşık bir moleküler zinciri başlatır.

Bcl-2 ailesi bu yolun merkezinde yer alır. Bu aile, üç fonksiyonel gruba ayrılır:

1. Anti-apoptotik üyeler (Bcl-2, Bcl-xL, Mcl-1) – MOMP’u engeller.
2. Pro-apoptotik efektörler (Bax, Bak) – MOMP’u doğrudan gerçekleştirir.
3. BH3-only proteinler (Bid, Bim, Puma, Noxa, Bad) – Anti-apoptotik proteinleri inhibe eder veya efektörleri aktive eder.

DNA hasarı gibi bir uyarın, p53 tümör baskılayıcı proteinin aktivasyonuna yol açar. p53, Puma ve Noxa gibi BH3-only proteinlerin transkripsiyonunu artırarak anti-apoptotik proteinleri baskılar ve Bax/Bak aktivasyonunu kolaylaştırır (Vousden & Lane, 2007). Bax ve Bak oligomerleşerek mitokondri dışı membranında porlar oluşturur. Bu porlardan sitokrom c, Smac/DIABLO ve Omi/HtrA2 gibi pro-apoptotik faktörler sitoplazmaya salınır.

Sitokrom c, sitoplazmada Apaf-1 ile birleşir ve ATP/dATP varlığında apoptosom adı verilen heptamerik bir kompleks oluşturur. Apoptosom, prokaspaz-9’u aktive eder; aktif kaspaz-9 ise yürütücü kaspazları (özellikle kaspaz-3 ve kaspaz-7) keserek aktive eder. Bu yürütücü kaspazlar, hücre yapısını hedef alarak ölüm sürecini tamamlar. Bax/Bak çift knock-out fare fibroblastlarının, DNA hasarına rağmen apoptozise dirençli olması, bu proteinlerin intrinsik yol için zorunlu olduğunu gösterir (Wei ve ark., 2001).

Ekstrinsik (Ölüm Reseptörü) Yol

Ekstrinsik yol, hücre dışı sinyallerle başlar. Bu yolun anahtar bileşenleri, ölüm reseptörleri olarak bilinen TNF reseptör süper ailesinin üyeleridir. En iyi çalışılmış örnekler Fas (CD95/APO-1), TNFR1 ve TRAIL reseptörleridir (Peter & Krammer, 2003).

Ligand (ör. Fas Ligand, TNF- α , TRAIL) reseptöre bağlandığında reseptör trimerleşir ve sitoplazmik ölüm alanı (death domain, DD) aracılığıyla adaptör protein FADD’i (Fas-associated death domain) bağlar. FADD, prokaspaz-8 veya prokaspaz-10’u toplayarak DISC (death-inducing signaling complex) kompleksini oluşturur. DISC içinde prokaspaz-8, dimerleşerek aktif forma dönüşür.

Aktif kaspaz-8 iki yolla ilerleyebilir:

1. Doğrudan yürütücü kaspazları (kaspaz-3, -7) aktive ederek hızlı hücre ölümünü başlatır.
2. Bid proteininin BH3-only formu olan tBid’e kesilmesini sağlayarak intrinsik yolu devreye sokar.

Fas mutasyonları, otoimmün lenfoproliferatif sendrom (ALPS) ile ilişkilendirilmiştir; bu durum, ekstrinsik yolun immün homeostazdaki kritik rolünü ortaya koyar (Rieux-Laucat ve ark., 1995).

Crosstalk ve Yolaklar Arası Etkileşim

İki yol arasındaki en önemli bağlantı, kaspaz-8’in Bid’i tBid’e dönüştürmesidir. tBid, mitokondriye giderek Bax/Bak aktivasyonunu tetikler ve MOMP’u başlatır. Böylece ekstrinsik yol, intrinsik yolu güçlendirebilir. Bu bağlantı özellikle “Tip II hücreler” olarak adlandırılan, ekstrinsik sinyalin tek başına yeterli olmadığı hücrelerde önemlidir (Scaffidi ve ark., 1998).

Ayrıca, hücre içi inhibitörler (ör. c-FLIP) DISC oluşumunu engelleyerek ekstrinsik yolu baskılayabilir. Benzer şekilde, anti-apoptotik Bcl-2 ailesi üyeleri intrinsik yolu bloke edebilir. Bu nedenle, hücre kaderi bu iki yolun sinyal yoğunluğu ve düzenleyici proteinlerin dengesi ile belirlenir.

Klinik Önemi

Kanser hücreleri sıklıkla bu yolaklardan birini veya her ikisini baskılayarak ölümden kaçır. Örneğin, Bcl-2 aşırı ekspresyonu intrinsik yolu engellerken, Fas reseptörünün kaybı ekstrinsik yolu devre dışı bırakır. Terapötik olarak, BH3 mimetikleri intrinsik yolu yeniden aktive ederken, TRAIL reseptör agonistleri ekstrinsik yolu hedefler (Ashkenazi, 2015).

DÜZENLEYİCİ AĞLAR VE MODÜLASYON

Apoptozis, yalnızca başlatıcı sinyallerin varlığıyla değil, aynı zamanda bu sinyallerin hücre içindeki düzenleyici protein ağları tarafından nasıl yorumlandığıyla da belirlenir. Bu ağlar, pro-apoptotik ve anti-apoptotik faktörler arasındaki hassas bir dengeye dayanır. Hücre kaderi, bu dengeyi belirleyen moleküler oyuncuların ekspresyon düzeyleri, post-translasyonel modifikasyonları ve hücre içi lokalizasyonları ile şekillenir.

Bcl-2 Ailesi: Hücre Kaderinin Anahtar Bekçileri

Bcl-2 ailesi proteinleri, intrinsik yolun merkezinde yer alır ve mitokondri dış membran permeabilizasyonunu (MOMP) kontrol eder. Bu aile üç fonksiyonel gruba ayrılır:

- Anti-apoptotik üyeler: Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1. Bu proteinler Bax ve Bak gibi efektörlerin oligomerleşmesini engelleyerek MOMP'u önler.
- Pro-apoptotik efektörler: Bax ve Bak, MOMP'u doğrudan gerçekleştirir.
- BH3-only proteinler: Bid, Bim, Puma, Noxa, Bad, Hrk, Bmf. Bu proteinler ya anti-apoptotik üyeleri inhibe eder ya da efektörleri doğrudan aktive eder.

Bcl-2 ailesi üyelerinin dengesi, hücreyi yaşam veya ölüm yönünde belirler. Örneğin, kanser hücrelerinde Bcl-2 veya Mcl-1'in aşırı ekspresyonu, apoptoz direncinin başlıca nedenlerinden biridir (Cory & Adams, 2002). Bu nedenle BH3 mimetikleri gibi ilaçlar, anti-apoptotik proteinleri hedefleyerek bu dengeyi pro-apoptotik yöne kaydırmayı amaçlar.

Apoptoz İnhibitörleri (IAP'ler)

IAP ailesi proteinleri (Inhibitor of Apoptosis Proteins), kaspazları doğrudan inhibe ederek apoptozun yürütücü aşamasını engeller. XIAP, cIAP1, cIAP2 ve Survivin bu ailenin en iyi bilinen üyeleridir. XIAP, kaspaz-3, -7 ve -9'u doğrudan bağlayarak inaktive eder (LaCasse ve ark., 2008).

Mitokondriden salınan Smac/DIABLO ve Omi/HtrA2, IAP'lere bağlanarak kaspazların üzerindeki baskıyı kaldırır. Bu mekanizma, intrinsik yolun etkinliğini artırır. Klinik olarak Smac mimetikleri, IAP'leri hedefleyerek apoptozu yeniden aktive etmeyi amaçlar (Fulda & Vucic, 2012).

p53 Tümör Baskılayıcı

p53, "genomun bekçisi" olarak bilinir ve DNA hasarına yanıt olarak hücre döngüsünü durdurur, onarım mekanizmalarını aktive eder veya hasar onarılamazsa apoptozu başlatır. p53, Puma, Noxa ve Bax gibi pro-apoptotik genlerin transkripsiyonunu artırır (Vousden & Lane, 2007). Ayrıca Fas reseptörü ve DR5 gibi ekstrinsik yol bileşenlerinin ekspresyonunu da uyarabilir.

p53'ün işlev kaybı, birçok kanser türünde apoptoz direncinin temel nedenlerinden biridir. Bu nedenle p53'ü yeniden aktive eden moleküller (ör. Nutlin-3, MDM2 inhibitörleri) kanser tedavisinde araştırılmaktadır.

Diğer Modülatörler

- c-FLIP: DISC kompleksinde kaspaz-8'in aktivasyonunu engelleyerek ekstrinsik yolu baskılar.
- NF-κB: Hücre sağkalımını destekleyen genlerin transkripsiyonunu artırır; ancak bağlama bağlı olarak pro-apoptotik genleri de aktive edebilir.
- MAPK Yolakları: JNK, p38 ve ERK gibi kinazlar, bağlama göre apoptozisi teşvik edebilir veya engelleyebilir.
- MikroRNA'lar: Bcl-2 ailesi üyelerinin veya kaspazların translasyonunu baskılayarak apoptoz regülasyonuna katkıda bulunur.

Klinik Önemi ve Terapötik Hedefler

Düzenleyici ağların bozulması, kanserden otoimmün hastalıklara kadar birçok patolojide rol oynar. Örneğin, Mcl-1 aşırı ekspresyonu akut miyeloid lösemide kötü prognozla ilişkilidir. BH3 mimetikleri, Smac mimetikleri ve p53 aktivatörleri bu ağları hedefleyen güncel terapötik stratejilerdir.

APOPTOZİS VE HASTALIKLAR

Apoptozis, fizyolojik koşullarda doku homeostazını koruyan ve hasarlı hücreleri ortadan kaldıran bir mekanizma iken, bu sürecin bozulması çok çeşitli patolojik durumların temelinde yer alır. Apoptozisin yetersizliği, hücrelerin ölümden kaçmasına ve kontrolsüz çoğalmasına neden olurken; aşırı apoptozis, doku kaybı ve organ fonksiyon bozukluğu ile sonuçlanır. Bu nedenle apoptozis, hem proliferatif hem de dejeneratif hastalıkların patogenezinde merkezi bir role sahiptir.

Kanser

Kanser, apoptozisin baskılanmasının en iyi bilinen sonuçlarından biridir. Normalde DNA hasarı, onkogen aktivasyonu veya hücre döngüsü bozuklukları gibi durumlar apoptozisi tetikler. Ancak kanser hücreleri, bu ölüm sinyallerini engelleyen çok sayıda mekanizma geliştirir. Bu mekanizmalarda genel olarak Anti-apoptotik proteinlerin aşırı ekspresyonu, Bcl-2, Bcl-xL, Mcl-1 gibi proteinler, intrinsik yolu bloke eder (Cory & Adams, 2002). Pro-apoptotik proteinlerin kaybı, Bax veya Bak mutasyonları, MOMP'un gerçekleşmesini engeller. p53 mutasyonları, DNA hasarına yanıt olarak apoptozisi başlatan p53'ün işlev kaybı, hücrelerin ölümden kaçmasına yol açar (Vousden & Lane, 2007). Ölüm reseptörü sinyal bozuklukları, Fas veya TRAIL reseptörlerinin kaybı, ekstrinsik yolun devre dışı kalmasına neden olur. BH3 mimetikleri, Smac mimetikleri ve TRAIL reseptör agonistleri, kanser hücrelerinde apoptozisi yeniden aktive etmeyi hedefler.

Nörodejeneratif Hastalıklar

Alzheimer, Parkinson, Huntington ve ALS gibi nörodejeneratif hastalıklar, aşırı veya uygunsuz apoptozis ile karakterizedir. Nöronlar post-mitotik hücreler olduğundan, kayıpları geri dönüşüzdür.

Kısaca özetleyecek olursak; Mitokondriyal disfonksiyon, Oksidatif stres ve kalsiyum dengesizliği, MOMP'u tetikler. Protein agregatları, β-amiloid (Alzheimer) veya α-sinüklein (Parkinson) gibi toksik proteinler, kaspaz aktivasyonunu artırır.

Eksitotoksiste, Glutamat aracılı kalsiyum aşırı yüklenmesi, intrinsik yolu aktive eder (Mattson, 2000). Kaspaz inhibitörleri ve mitokondriyi stabilize eden ajanlar, deneysel modellerde nöron kaybını azaltır.

Otoimmün Hastalıklar

Otoimmün hastalıklarda apoptozis hem yetersiz hem de aşırı olabilir. Yetersiz apoptozis, otoreaktif lenfositlerin ortadan kaldırılamamasına; aşırı apoptozis ise doku hasarına yol açar. Fas/FasL yolunun bozulması ile otoreaktif T hücrelerinin yaşam süresini uzatır. Apoptotik kalıntıların temizlenememesi durumunda hücre içi antijenlerin bağışıklık sistemine sunulmasına ve otoantikor üretimine neden olur (Gaipl ve ark., 2007). İmmünomodülatör ilaçlar, apoptozis dengesini yeniden kurmayı hedefler.

Enfeksiyon Hastalıkları

Birçok patojen, konak hücre apoptozisini manipüle ederek hayatta kalır. Bazı virüsler (ör. adenovirüs, poxvirüs) anti-apoptotik proteinler kodlayarak hücre ölümünü engeller; bazıları ise (ör. HIV) bağışıklık hücrelerinde aşırı apoptozis indükleyerek immün yetmezliğe yol açar (Best, 2008). Örneğin HIV enfeksiyonunda CD4+ T hücrelerinin büyük kısmı, doğrudan enfeksiyonla değil, bystander apoptozis ile kaybedilir. Terapötik olarak antiviral tedaviler, patojenin apoptozis manipülasyonunu engellemeyi hedefleyebilir.

TERAPÖTİK YAKLAŞIMLAR

Apoptozun moleküler mekanizmalarının anlaşılması, özellikle kanser ve dejeneratif hastalıklar gibi patolojilerde hedefe yönelik tedavi stratejilerinin geliştirilmesine olanak sağlamıştır. Bu stratejiler, hücre ölümünü yeniden aktive etmek veya aşırı apoptozisi baskılamak amacıyla tasarlanmıştır. Terapötik müdahaleler, genellikle Bcl-2 ailesi proteinleri, IAP'ler, kaspazlar ve p53 gibi düzenleyici molekülleri hedef alır. Ayrıca doğal bileşikler ve immün modülatörler de bu süreçte önemli rol oynar.

Bcl-2 İnhibitörleri ve BH3 Mimetikleri

Bcl-2 ailesi üyeleri, özellikle anti-apoptotik proteinler (Bcl-2, Bcl-xL, Mcl-1), kanser hücrelerinde sıklıkla aşırı eksprese edilir ve apoptoz direncinin temelini oluşturur. Bu nedenle bu proteinleri hedefleyen ilaçlar, apoptozun yeniden aktive edilmesinde kritik rol oynar.

BH3 mimetikleri, BH3-only proteinlerin işlevini taklit ederek anti-apoptotik proteinlerle etkileşime girer ve onların pro-apoptotik efektörleri baskılamasını engeller. Bcl-2'ye yüksek afinitesi sayesinde özellikle kronik lenfositik lösemi (CLL) ve akut miyeloid lösemi (AML) gibi hematolojik malignitelerde etkili bulunmuştur (Souers ve ark., 2013). CLL tedavisinde onaylanmış ve kombinasyon tedavilerinde remisyon oranlarını belirgin şekilde artırmıştır. Ancak Mcl-1 gibi diğer anti-apoptotik proteinlerin varlığı, direnç gelişimine neden olabilir. Bu nedenle S63845 gibi Mcl-1 inhibitörleri, dirençli tümörlerde araştırılmaktadır (Kotschy ve ark., 2016).

IAP İnhibitörleri ve Smac Mimetikleri

IAP proteinleri, kaspazları doğrudan inhibe ederek apoptozun yürütücü aşamasını engeller. Bu nedenle IAP'leri hedefleyen ilaçlar, kaspaz aktivasyonunu serbestleştirerek hücre ölümünü tetikleyebilir.

Smac mimetikleri, mitokondriden salınan Smac/DIABLO'nun işlevini taklit eder ve IAP'lerle etkileşerek onların kaspazları baskılamasını engeller.

Bu ilaçlar, özellikle TNF- α ile sinerjik etki göstererek nekroptoz ve apoptoz arasında geçişi modüle edebilir (Fulda & Vucic, 2012). cIAP1/2'yi hedefleyen bir Smac mimeti, solid tümörlerde ve hematolojik kanserlerde faz I/II klinik denemelere girmiştir. TRAIL reseptör agonistleri ile kombinasyonları, apoptozun ekstrinsik yolunu güçlendirebilir.

Kaspaz Modülatörleri

Kaspazlar, apoptozun yürütücü enzimleridir ve bu nedenle hem aktivatörleri hem de inhibitörleri terapötik olarak değerlendirilmektedir.

Kaspaz aktivatörleri, özellikle kanser hücrelerinde apoptozun yeniden başlatılması için kullanılır. Örneğin, Apoptin adlı viral protein, sadece tümör hücrelerinde kaspaz-3 aktivasyonunu tetikleyerek seçici hücre ölümü sağlar (Danen-Van Oorschot ve ark., 1999).

Kaspaz inhibitörleri, nörodejeneratif hastalıklar ve iskemik hasar gibi aşırı apoptozis ile ilişkili durumlarda kullanılır. zVAD-fmk gibi pan-kaspaz inhibitörleri, deneysel modellerde nöron kaybını azaltmıştır. Ancak sistemik kaspaz inhibisyonu, immün yanıtı ve doku yenilenmesini olumsuz etkileyebileceğinden dikkatle değerlendirilmelidir.

p53 Aktivatörleri ve MDM2 İnhibitörleri

p53'ün işlev kaybı, birçok kanser türünde apoptoz direncinin temel nedenidir. Bu nedenle p53'ü yeniden aktive eden moleküller, özellikle tümör baskılayıcı yolları restore etmek için geliştirilmiştir.

MDM2 ile p53 arasındaki etkileşimin enlenmesi p53'ün stabilizasyonunu sağlar. Böylece, özellikle p53'ü baskılayan MDM2'nin aşırı ekspresyon gösterdiği tümörlerde etkili bulunmuştur (Vassilev ve ark., 2004).

Bazı ilaçlar, mutant p53'ü yeniden katlanabilir hale getirerek fonksiyonunu restore etmeye çalışır. AML ve MDS gibi hastalıklarda faz II denemeleri sürmektedir.

Doğal Bileşikler ve Fitokimyasallar

Doğal bileşikler, düşük toksisite profilleri ve çoklu hedeflere etki potansiyelleri nedeniyle apoptoz modülasyonunda önemli bir araştırma alanıdır.

Flavonoidler (ör. quercetin, apigenin), Bcl-2 ailesi proteinlerinin ekspresyonunu modüle eder ve kaspaz aktivasyonunu artırır. Alkaloidler (ör. berberin), p53 aktivasyonunu tetikleyerek intrinsik yolu uyarır. Terpenoidler (ör. betulinik asit), mitokondriyal membran potansiyelini bozarak MOMP'u başlatır (Mosadegh ve ark., 2025).

Klinik potansiyel olarak bu bileşiklerin çoğu preklinik aşamadadır; ancak kombinasyon tedavilerinde kemoterapiye duyarlılığı artırma potansiyeli taşırlar.

Gelecek Perspektifler ve Kombine Yaklaşımlar

Apoptoz hedefli tedavilerin geleceği, kombine stratejiler ve kişiselleştirilmiş tıp yaklaşımları ile şekillenmektedir. Moleküler biyobelirteçlerin kullanımı, hangi hastaların hangi apoptoz modülatörlerinden fayda göreceğini öngörmeyi kolaylaştıracaktır. Ayrıca yapay zekâ destekli ilaç keşfi, yeni hedeflerin belirlenmesini hızlandıracaktır.

APOPTOZ VE DİĞER HÜCRE ÖLÜMÜ TÜRLERİ

Apoptozis, uzun yıllar boyunca hücre ölümünün tek düzenlenmiş biçimi olarak kabul edilmiştir. Ancak son on yıllarda yapılan araştırmalar, hücrelerin ölüm kararını farklı moleküler yollarla da alabileceğini ortaya koymuştur. Bu alternatif yollar arasında nekroptoz, piroptoz, ferroptoz ve otofajiye bağlı hücre ölümü gibi mekanizmalar yer alır.

Her biri kendine özgü tetikleyicilere, düzenleyici proteinlere ve morfolojik özelliklere sahiptir. Bununla birlikte, bu yollar arasında belirgin geçiş noktaları ve etkileşimler bulunur. Hücre kaderi, çoğu zaman bu yolların rekabeti veya iş birliği ile belirlenir.

Nekroptoz

Nekroptoz, kaspaz bağımsız ancak düzenlenmiş bir hücre ölüm şeklidir. Genellikle apoptozun engellendiği durumlarda devreye girer. Bu yol, Receptor-Interacting Protein Kinase 1 (RIPK1) ve RIPK3'ün aktivasyonu ile başlar. Bu kinazlar, Mixed Lineage Kinase Domain-Like protein (MLKL)'yi fosforile eder ve MLKL'nin plazma zarına translokasyonu ile hücre membranı bozulur (Vandenabeele ve ark., 2010).

Nekroptoz, inflammatuar bir yanıtla sonuçlanır; çünkü hücre içeriği çevreye salınır. Bu nedenle özellikle viral enfeksiyonlar ve immün yanıt bağlamında önemlidir. Apoptozun kaspaz-8 aracılığıyla baskılandığı durumlarda, RIPK1/RIPK3 kompleksi stabilize olur ve nekroptoz başlar. Bu durum, apoptoz ve nekroptoz arasında bir moleküler geçiş noktası olduğunu gösterir.

Piroptoz

Piroptoz, inflammatuar kaspazlar (özellikle kaspaz-1, -4, -5, -11) tarafından yürütülen, patojenlere karşı bağışıklık yanıtında görev alan bir hücre ölüm şeklidir. Bu yol, inflammasom adı verilen çok proteinli komplekslerin aktivasyonu ile başlar. Inflammasomlar, patojen veya hasar ilişkili moleküler desenleri (PAMP/DAMP) tanıyan sensör proteinler (ör. NLRP3) tarafından tetiklenir (Bergsbaken ve ark., 2009).

Kaspaz-1, Gasdermin D adlı proteini keserek aktif hale getirir. Gasdermin D'nin N-terminal bölgesi, plazma zarında porlar oluşturarak hücre içeriğinin salınmasına ve inflammatuar yanıtın başlatılmasına neden olur. Piroptoz, özellikle makrofaj ve dendritik hücrelerde görülür.

Ferroptoz

Ferroptoz, demir bağımlı lipid peroksidasyonu ile karakterize edilen, non-apoptotik bir hücre ölüm şeklidir. Bu yol, özellikle antioksidan savunma mekanizmalarının bozulduğu durumlarda devreye girer. Glutathione peroxidase 4 (GPX4)'ün inaktivasyonu, hücre zarındaki çoklu doymamış yağ asitlerinin oksidasyonunu hızlandırır ve hücre membranı bozulur (Dixon ve ark., 2012).

Ferroptoz, nörodejeneratif hastalıklar, iskemik hasar ve bazı kanser türlerinde rol oynar. Apoptozdan farklı olarak kaspazlar bu süreçte görev almaz. Ancak bazı stres koşullarında hücre, ferroptoz ve apoptoz arasında geçiş yapabilir.

Otofajiye Bağlı Hücre Ölümü

Otofaji, hücre içi organellerin ve proteinlerin lizozomal sistem aracılığıyla parçalanmasıdır. Genellikle hayatta kalma mekanizması olarak görev yapar. Ancak aşırı otofaji, hücre içeriğinin kritik düzeyde kaybına neden olarak hücre ölümüne yol açabilir.

Otofajiye bağlı hücre ölümü, apoptozdan morfolojik olarak farklıdır; kaspaz aktivasyonu gözlenmez. Bununla birlikte, bazı durumlarda otofaji ve apoptoz birlikte çalışabilir. Örneğin, otofaji apoptotik proteinlerin aktivasyonunu kolaylaştırabilir veya geciktirebilir.

Hücre Ölüm Yolları Arası Geçiş ve Etkileşim

Hücre ölüm yolları arasında geçiş, hücrenin çevresel koşullara ve içsel sinyal yoğunluğuna göre şekillenir.

Örneğin, kaspaz-8'in inhibisyonu nekroptozu tetiklerken; inflammasom aktivasyonu piroptozu başlatabilir. Ayrıca bazı moleküler oyuncular (ör. RIPK1, Gasdermin D, GPX4), birden fazla yolda görev alabilir.

Bu geçişler, özellikle kanser tedavisinde önemlidir. Apoptoz direnci gelişen hücrelerde alternatif yolların aktive edilmesi, tedavi başarısını artırabilir. Bu nedenle hücre ölüm yollarının bütüncül olarak değerlendirilmesi, terapötik stratejilerin tasarımında kritik rol oynar.

GELECEK PERSPEKTİFLER

Apoptozis, hücre yaşama ve ölüm arasındaki dengeyi sağlayan temel bir biyolojik süreç olarak hem temel bilim hem de klinik araştırmalar açısından önümüzdeki yıllarda da merkezi önemini koruyacaktır. Moleküler düzeydeki derinleşen anlayış, yeni terapötik hedeflerin belirlenmesini kolaylaştırmakta; teknolojik gelişmeler ise bu hedeflere yönelik ilaçların daha hızlı ve etkili biçimde tasarlanmasına olanak tanımaktadır. Bu bölümde, apoptoz araştırmalarının gelecekteki yönelimleri, klinik uygulamalara entegrasyon potansiyeli ve multidisipliner yaklaşımların katkısı ele alınacaktır.

Kişiselleştirilmiş Tıp ve Moleküler Biyobelirteçler

Kişiselleştirilmiş tıp, hastaların genetik, epigenetik ve moleküler profillerine göre tedavi stratejilerinin belirlenmesini amaçlar. Apoptozla ilişkili proteinlerin ekspresyon düzeyleri, mutasyon durumları ve fonksiyonel aktiviteleri, tedavi yanıtını öngörmede kullanılabilecek biyobelirteçler arasında yer alır.

Örneğin, Bcl-2 aşırı ekspresyonu gösteren hastalar, BH3 mimetiklere daha duyarlı olabilirken; p53 mutasyonu taşıyan tümörler, MDM2 inhibitörlerine direnç gösterebilir. Ayrıca kaspaz aktivitesi, Smac/DIABLO düzeyleri ve IAP ekspresyon profilleri, tedaviye yanıtı belirlemede kullanılabilecek parametrelerdir (Hanahan & Weinberg, 2011). Apoptoz biyobelirteçlerinin klinik test panellerine entegre edilmesi, tedavi seçiminde daha rasyonel kararlar alınmasını sağlayacaktır.

Yapay Zekâ Destekli İlaç Keşfi

Yapay zekâ (YZ) ve makine öğrenimi algoritmaları, ilaç keşfi sürecini dönüştürmektedir. Apoptozla ilişkili protein-protein etkileşim ağlarının modellenmesi, potansiyel inhibitör veya aktivatör moleküllerin belirlenmesinde YZ'nin katkısı büyüktür.

Özellikle kaspazların aktif bölgeleri, Bcl-2 ailesi üyelerinin bağlanma cepleri ve IAP'lerin ubiquitinasyon mekanizmaları gibi yapısal veriler, YZ algoritmaları ile analiz edilerek ilaç tasarımı hızlandırılabilir. Ayrıca klinik veri setlerinin analiziyle, apoptoz modulatorlerinin hangi hasta alt gruplarında etkili olabileceği öngörülebilir (Zhavoronkov ve ark., 2019). Bu teknolojik entegrasyon, hem prelinik hem de klinik aşamalarda zaman ve maliyet tasarrufu sağlayarak translaşyonel araştırmaları güçlendirecektir.

Kombine ve Çoklu Hedefli Tedavi Yaklaşımları

Apoptozun tek bir moleküler hedefe indirgenmesi, çoğu zaman yetersiz kalmaktadır. Hücre ölüm yolları arasındaki geçişler ve kompensatuar mekanizmalar, monoterapilere karşı direnç gelişimini kolaylaştırır. Bu nedenle gelecekteki tedavi stratejileri, birden fazla hücre ölüm yolunu hedefleyen kombinasyonlara yönelecektir.

Örneğin, BH3 mimetikleri ile Smac mimetiklerinin birlikte kullanımı, hem intrinsik hem de ekstrinsik yolun eşzamanlı aktivasyonunu sağlayabilir.

Ayrıca ferroptoz indükleyicileri ile kaspaz aktivatörlerinin kombinasyonu, apoptoz dirençli hücrelerde alternatif ölüm yollarını devreye sokabilir (Stockwell ve ark., 2017).

Bu çoklu hedefli yaklaşımlar, özellikle tedaviye dirençli tümörlerde ve heterojen hücre popülasyonlarında daha etkili sonuçlar verebilir.

Translasyonel Uygulamalar ve Klinik Entegrasyon

Apoptoz modülatörlerinin klinik uygulamaya geçişi, yalnızca moleküler etkinliklerine değil, aynı zamanda farmakokinetik özelliklerine, toksisite profillerine ve immün sistemle etkileşimlerine bağlıdır. Bu nedenle translasyonel araştırmalar, in vitro ve in vivo modellerin yanı sıra insan biyopsi örnekleri ve organoid sistemleri ile desteklenmelidir.

Ayrıca apoptoz hedefli tedavilerin immün sistem üzerindeki etkileri, özellikle immünoterapi ile kombinasyon potansiyeli açısından değerlendirilmelidir. Apoptotik hücrelerin fagositozu, antijen sunumu ve immün tolerans mekanizmaları ile doğrudan ilişkilidir (Green ve ark., 2009). Bu bağlamda, apoptoz modülatörlerinin immün kontrol noktası inhibitörleri, CAR-T hücre tedavileri ve tümör aşılıları ile birlikte kullanımı, gelecekteki klinik protokollerde yer alabilir.

SONUÇ

Apoptozis, çok hücreli organizmalarda yaşamın sürekliliği ve doku bütünlüğünün korunması için vazgeçilmez bir hücre ölüm mekanizmasıdır. Bu süreç, hem gelişimsel hem de patolojik bağlamda hücrelerin kaderini belirlerken; moleküler düzeyde oldukça karmaşık ve sıkı şekilde düzenlenmiş bir ağ tarafından kontrol edilir. İntrinsik ve ekstrinsik yollar, kaspazlar, Bcl-2 ailesi proteinleri, IAP'ler ve p53 gibi düzenleyici faktörler, hücre içi ve dışı sinyalleri entegre ederek ölüm kararını şekillendirir.

Apoptozisin bozulması, kanserden nörodejeneratif hastalıklara, otoimmün bozukluklardan enfeksiyonlara kadar geniş bir hastalık yelpazesinde temel patojenetik mekanizma olarak karşımıza çıkar. Bu nedenle apoptozun moleküler bileşenleri, terapötik hedefler olarak büyük bir potansiyel taşır. BH3 mimetikleri, Smac mimetikleri, kaspaz modülatörleri ve p53 aktivatörleri gibi ajanlar, bu süreci yeniden düzenleyerek hastalıkların kontrol altına alınmasında önemli rol oynayabilir.

Gelecekte, kişiselleştirilmiş tıp yaklaşımları, yapay zekâ destekli ilaç keşfi ve çoklu hücre ölüm yollarını hedefleyen kombinasyon tedavileri, apoptoz araştırmalarının klinik uygulamalara entegrasyonunu daha da güçlendirecektir. Bu bağlamda, apoptozun yalnızca bir hücre ölüm biçimi değil, aynı zamanda organizmanın sağkalım stratejilerinin temel bir bileşeni olduğu daha iyi anlaşılacaktır.

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THE IMPORTANCE OF PHYTOCHEMICALS AND ANTIOXIDANTS IN FOOD BIOCHEMISTRY: ANALYTICAL APPROACHES USING CHROMATOGRAPHY TECHNIQUES

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ABSTRACT

Food biochemistry is a scientific discipline that studies the chemical structures, biological functions, and metabolic effects of macro- and micro-components found in foods. In this context, phytochemicals, as plant-derived secondary metabolites, play important roles in protecting human health and preventing chronic diseases. Phytochemical groups such as flavonoids, phenolic acids, carotenoids, and alkaloids exhibit antioxidant, anti-inflammatory, anticancer, and cardioprotective effects. Antioxidant compounds prevent cellular damage by reducing oxidative stress caused by reactive oxygen species (ROS) and maintain redox balance in biological systems.

Advanced analytical methods are required for the isolation, identification, and quantitative analysis of these compounds from food matrices. Chromatography techniques, particularly high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS), provide high sensitivity and selectivity in the characterization of phytochemicals. Modern chromatography systems have become indispensable in the field of food biochemistry due to their short analysis time, high resolution, and ability to analyze multiple components.

Recent studies have revealed that elucidating the biochemical content of plant foods using chromatographic methods is of strategic importance in both nutritional science and functional food development. This review aims to evaluate the biochemical functions and antioxidant mechanisms of phytochemicals in light of the current literature using chromatography techniques.

Keywords: Food biochemistry, phytochemicals, antioxidants, chromatography, GC-MS, LC-MS/MS

INTRODUCTION

Food biochemistry is a scientific discipline that studies the chemical profile, biological activities, and metabolic transformations of foods. Phytochemicals, which are found in plant-based foods and contain secondary metabolites, have been the subject of intense research in recent years due to their protective effects on health.

Phytochemical compounds such as flavonoids, phenolic acids, carotenoids, alkaloids, and glucosinolates exhibit antioxidant, anti-inflammatory, anticancer, antimicrobial, and cardioprotective activities (Liu et al., 2024).

Antioxidants protect cellular redox balance by preventing oxidative damage caused by reactive oxygen species (ROS) and free radicals (İzol, 2025; İzol and Turhan, 2024; İzol et al., 2025a). From a food biochemistry perspective, the bioavailability of these compounds is influenced by factors such as food matrix structure, processing conditions, and storage time. Antioxidants contain natural substances that can serve as alternatives to chemicals used as preservatives in many foods. Due to the harmful effects of inorganic preservatives and synthetic antioxidants, natural antioxidants are receiving significant attention. This approach aims to both delay and prevent food spoilage while protecting against the harmful effects of synthetic substances (Gülçin, 2020; İzol, 2023; İzol et al., 2025b).

In this review, the biochemical functions of phytochemicals, antioxidant mechanisms, and chromatography techniques used for the analysis of these compounds are discussed in light of the current literature.

THE BIOCHEMICAL IMPORTANCE OF PHYTOCHEMICALS

Phytochemicals are compounds synthesized in plants as secondary metabolites that are not essential for nutrition but have positive effects on health. Biochemically, these compounds perform many important functions. Some of these include modulating cellular signaling pathways, affecting enzyme activities, and regulating gene expression (Martinez et al., 2023).

Main phytochemical groups:

- Phenolic compounds: Flavonoids, phenolic acids, stilbenes
- Carotenoids: β -carotene, lycopene, lutein
- Alkaloids: Caffeine, theobromine, capsaicin
- Sulfur-containing compounds: Glucosinolates, allicin
- Terpenoids: Monoterpenes, sesquiterpenes

Phenolic compounds can neutralize free radicals and prevent lipid peroxidation thanks to their phenolic hydroxyl groups. In particular, they eliminate free radicals with their hydroxyl groups and do not form radicals. Therefore, they play an important role in antioxidant activity (Gülçin et al., 2024; Yılmaz et al., 2024).

BIOCHEMICAL MECHANISMS OF ANTIOXIDANTS

Oxidative stress occurs as a result of an imbalance between free radical production and antioxidant defense systems. At the cellular level, it can result in DNA damage, protein oxidation, and lipid peroxidation (Yılmaz et al., 2023; Çelik et al., 2024; Yurt et al., 2024).

Antioxidant mechanisms include:

1. Radical scavenging
2. Metal ion chelation (binding of pro-oxidant metals such as Fe^{2+} and Cu^{2+})
3. Activation of enzymatic defense systems (SOD, catalase, glutathione peroxidase)
4. Breaking the lipid peroxidation chain

Phytochemicals provide biochemical protection by directly or indirectly supporting these mechanisms. For example, catechins and quercetin are both free radical scavengers and metal ion chelators (Zhao et al., 2025; İzol, 2023).

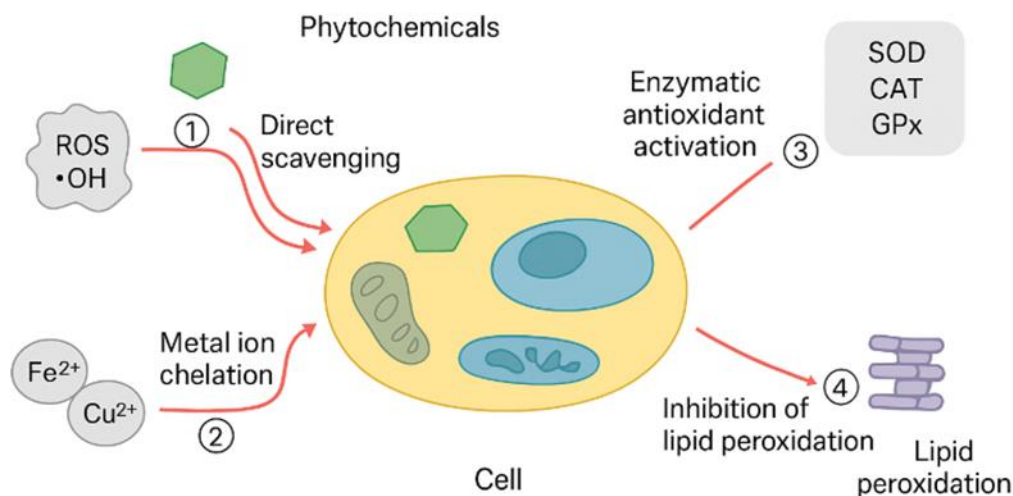


Figure 1. Schematic representation of antioxidant mechanisms of phytochemicals.

Phytochemicals act through multiple biochemical pathways to prevent oxidative damage: 1. Direct scavenging of reactive oxygen species (ROS) and free radicals, 2. Chelation of pro-oxidant metal ions such as Fe^{2+} and Cu^{2+} , 3. Activation of endogenous enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), 4. Inhibition of lipid peroxidation chain reactions in cell membranes.

CHROMATOGRAPHY TECHNIQUES AND ANALYTICAL APPROACHES

HPLC (High Performance Liquid Chromatography)

It is widely used in the quantitative analysis of phenolic compounds and flavonoids. However, since the measurement range cannot detect very low concentrations, it is primarily used for the analysis of components with high concentrations. It is also used in the analysis of amino acids and carbohydrates. The variety of columns and detectors varies depending on the type of analysis performed. Reverse phase C18 columns are used with UV-Vis, RID, or DAD detectors (İzol, 2023).

LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry)

It is an important chromatography technique that exhibits high sensitivity for structural elucidation and detection at low concentrations and is widely used in the detection of food components. It is ideal for determining the profile of secondary metabolites such as polyphenols, carotenoids, and alkaloids (Huang et al., 2023).

GC-MS (Gas Chromatography-Mass Spectrometry)

An important chromatography technique used in the analysis of volatile compounds and low molecular weight phytochemicals. In fatty acid analyses, it usually requires a derivatization step. It is actively used with the Headspace unit and SPME technique in the specific analysis of volatile components such as naphthalene (İzol et al., 2022).

Sample Preparation Techniques

In chromatography techniques, samples are generally subjected to a pretreatment stage. These pretreatment stages are mostly applied as extraction methods. Some important extraction methods are as follows:

Solid phase extraction (SPE)
Solid-phase microextraction (SPME)
Liquid-liquid extraction (LLE)
Ultrasound-assisted extraction (UAE)
Pressurized liquid extraction (PLE)

Table 1. Comparison of chromatographic techniques for phytochemical and antioxidant analysis

Technique	Main Application	Detection Limit	Advantages	Limitations
HPLC (High-Performance Liquid Chromatography)	Quantitative analysis of phenolics, flavonoids	µg/L	High resolution, widely available, suitable for thermolabile compounds	Requires solvents, time-consuming sample preparation
LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)	Structural elucidation, profiling of polyphenols, carotenoids	ng/L	High sensitivity and specificity, structural identification	High equipment cost, complex operation
GC-MS (Gas Chromatography–Mass Spectrometry)	Volatile and low-molecular-weight compounds	ng/L	High separation efficiency, robust database matching	Requires derivatization for non-volatile compounds
UPLC (Ultra-Performance Liquid Chromatography)	Rapid analysis of phytochemicals	µg/L	Faster analysis, higher resolution than HPLC	Higher operational pressure requirements

EVALUATION FROM THE PERSPECTIVE OF FOOD BIOCHEMISTRY

Chromatography techniques are indispensable for the separation and identification of complex components in food matrices. Understanding the biochemical effects of phytochemicals contributes to the development of functional food formulations. Studies conducted between 2023 and 2025 show that LC-MS/MS-based metabolomic analyses have become standard in food biochemistry. Quantitative analyses using chromatography techniques are used to evaluate which food components may be responsible for antioxidant activity.

CONCLUSION AND DISCUSSION

Phytochemicals and antioxidants are of critical importance in food biochemistry in terms of both health and product quality. Elucidating the biochemical mechanisms of these compounds is made possible by advanced chromatography techniques. In the future, it is expected that research in this field will deepen further with the use of high-resolution mass spectrometry, metabolomic approaches, and artificial intelligence-supported data analysis.

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USE OF PLANT-BASED ANTIOXIDANTS IN MEAT PRODUCTS: EVALUATION OF SYNTHETIC AND NATURAL SUBSTANCES IN TERMS OF TBARS

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ABSTRACT

Meat and meat products are rich in unsaturated fatty acids. This causes a significant sensitivity to lipid oxidation. Oxidative deterioration can cause a decrease in food value, loss of sensory properties, and the formation of toxicity. Therefore, antioxidants are one of the most commonly used substances in the food industry to prevent lipid oxidation. However, in recent years, consumer preferences have changed due to concerns about their health effects, and plant-derived natural antioxidants are now preferred over synthetic antioxidants.

In the review, the antioxidant effects of synthetic and natural (plant-derived) antioxidants were compared in terms of Thiobarbituric Acid Reactive Substances (TBARS), a biochemical indicator of oxidation-induced deterioration in meat products. A literature review revealed that plant-derived antioxidants are effective in reducing TBARS levels in many meat products. In particular, the rate of inhibition of TBARS by rosemary was found to be similar to or better than that of E320 (butylated hydroxyanisole-BHA) and E321 (butylated hydroxytoluene-BHT), which are known food additives. In addition, plant antioxidants continuously suppressed oxidative deterioration during storage. The antioxidant effect varies depending on the type of product, extraction technique, application dose, and polyphenol content, particularly secondary metabolites, in the plant.

In conclusion, this review aims to provide promising natural alternatives for preventing lipid oxidation in meat products and to contribute to the development of healthy and reliable solutions to the distrust of plant-based antioxidants as food additives.

Keywords: Natural antioxidants, Lipid oxidation, TBARS, Meat products.

INTRODUCTION

In recent years, consumer demand has stimulated the meat and meat products sector. Consumers are not only seeking quality in meat products offered for sale; they prefer healthy, natural and safe products. To supply high-quality meat products, the biological composition must remain intact. (1) Lipid oxidation is one of the primary causes of composition deterioration. The amount of malondialdehyde (MDA), a secondary oxidation product resulting from lipid oxidation, is measured using thiobarbituric acid (TBA) analysis. The results obtained indicate the degree of deterioration as the TBARS value. (2)

The formation process of oxidation products may be due to the processing method of meat and meat products, storage conditions, and the natural structure of the meat.

Synthetic food preservatives are used to prevent these adverse effects. However, some of these products may pose potential risks to consumer health. Caution should be exercised when using it due to its toxicological effects and regulatory restrictions. (3,4)

Food additives have been prepared within the framework of Regulation (EC) No 1333/2008 in the European Union. In our country, the Turkish Food Codex Food Additives Regulation has been prepared in accordance with this legislation. (5,6) The Codex GSFA (Codex Alimentarius – General Standard for Food Additives) (CXS 192) has separated the maximum levels of additives according to food groups. (7) For poultry products, meat and meat products (food category 08.3), the values for BHA (E320) and BHT (E321) are set at 200 mg/kg and 100 mg/kg respectively in the fats/oil-based and dried products category (food category 08.2). (8,9)

DEFINITION AND PROPERTIES OF SYNTHETIC AND NATURAL ANTIOXIDANTS

SYNTHETIC ANTIOXIDANTS

Synthetic antioxidants such as BHA and BHT, produced by chemical methods under laboratory conditions, are widely used in the food, pharmaceutical and cosmetic industries to protect products from oxidation-induced deterioration. Many synthetic antioxidants are phenolic compounds that carry a hydroxyl group in their benzene ring structure. These compounds are effective in extending the shelf life of fats, particularly by preventing the formation of free radicals. (10)

BHA is used in various food products, particularly in fats (butter and margarine) and baked goods, to prevent oxidative deterioration. BHT is a fat-soluble antioxidant used in processed meats, grain products and snacks. BHT neutralises and halts the chain reaction of lipid peroxidation by donating hydrogen atoms to free radicals. (11) Although its versatility, stable structure, affordability and ease of supply are reasons for its preference, its toxic effects, carcinogenic effects and harm to nature are considered disadvantages. (12,13)

Recently, high-quality food additives produced from natural sources or natural organisms have been used instead of synthetic additives such as preservatives and antioxidants. In the meat industry, health problems that synthetic antioxidants may cause have been taken into account, and natural antioxidants are preferred over synthetic ones (14).

NATURAL ANTIOXIDANTS

Compounds naturally synthesised from various plants, animals and microorganisms are called natural antioxidants. Phenolic compounds (tannins, flavonoids, phenolic acids), tocopherols-tocotrienols, and terpenoids/carotenoids, which exhibit antimicrobial and oxidative effects, are the most commonly used plant-based antioxidant compounds. (15,16).



Fruits (grapes, pomegranates, blackberries, strawberries, cranberries and plums), spices (cloves, sage, ginger, rosemary, green tea) and seed extracts from these plants are examples of natural antioxidants. (17) The mechanisms by which plant antioxidants inhibit oxidation in meat and meat products are neutralising free radicals, forming complexes with metal ions, and breaking the peroxidation reaction chain of lipids. (18-20)

TBARS ANALYSIS

Oxidative processes affect lipid, protein and vitamin components. Therefore, TBARS analysis is important in terms of quality and shelf life in meat products. TBARS analysis is performed by measuring the MDA–TBA complex using a spectrophotometric method commonly used to measure lipid oxidation. The results of this practical and inexpensive oxidation indicator are calculated in mg MDA/kg. TBARS is not evaluated on its own. As it may interact with other possible compounds besides MDA, it is evaluated in most studies using peroxide/anisidine value and antioxidant capacity tests. (21-25)

COMPARISON OF THE EFFECTS OF SYNTHETIC AND NATURAL ANTIOXIDANTS ON TBARS

A meta-analysis comparing the effects of natural and synthetic antioxidants on TBARS in beef products found no significant differences between groups in terms of protein carbonyl, peroxide value, methaemoglobin ratio, pH value, and colour parameter. In TBARS values, it was observed that natural antioxidants yielded lower results. (26)

In another study reported in the literature, extracts obtained from rosemary and mixtures of green tea or acerola positively affected the colour and flavour of meat/poultry products over time without any change in sensory parameters and extended the storage period. (27)

It has been reported that natural antioxidant extracts used in minced meat and fresh sausage-like products significantly slow down TBARS increase and, in some combinations, can be offered as an alternative to synthetic antioxidants. In a study conducted with tocopherols and grape seed extract, although BHA demonstrated the highest results in both tests, it exhibited high antioxidant effects in the DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging test and FRAP (Ferric Reducing Antioxidant Power) reduction test, which are chemical antioxidant tests. Analysis results on fresh sausages revealed that the peroxide values of tocopherols were lower and, when compared to the control group, showed TBARS or acidity levels comparable to BHA. (14, 24, 28)

In a study conducted on smoked sausages, it was determined that extracts of chokeberry (*Aronia melanocarpa*) and blackcurrant (*Ribes nigrum* L.) inhibited lipid peroxidation during a 25-day storage period. At the end of 25 days, it was observed that TBARS and peroxide values decreased compared to the control group, and some analysis samples also showed a decrease in microbial load. (29)

CONCLUSION AND DISCUSSION

The preference for using natural antioxidants in meat and meat products is a preferable approach in terms of TBARS compared to synthetic antioxidants. Natural antioxidants can demonstrate an effect equivalent to synthetic antioxidants in controlling lipid peroxidation. Green tea, rosemary and aronia melanocarpa, which are natural antioxidants, have been found to be effective in enhancing the antioxidant activity of meat products. However, further research is required into the cost and standardisation aspects for industrial sectors.

In future stages, expanding the use of synthetic and natural antioxidants could offer optimal solutions in terms of both economic and antioxidant effects.

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EFFECT OF LOW-DOSE PRUNUS SPINOSA FRUIT EXTRACT ON OXIDATIVE STRESS PARAMETERS IN BENZO[A]PYRENE-EXPOSED A549 CELLS

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ABSTRACT

While various environmental and genetic factors play a role in the growing of lung cancer and other respiratory diseases, one of the most significant risk factors is exposure to cigarette smoke containing Benzo[a]pyrene (BaP), which is a major carcinogen. Plants rich in polyphenols and their extracts are considered potential alternatives in treating inflammatory diseases due to their potent antioxidant properties and ability to suppress cytokine production. In this context, *Prunus spinosa* fruit, which has a high polyphenol content, has been widely used in traditional medicine because of its biological effects. In this study, different doses of *Prunus spinosa* extract were applied to BaP-treated A549 cell lines, and LC₀ and LC₁₀ doses were determined using the MTT assay. Experimental groups were formed based on these doses (Control, BaP, LC₀, LC₁₀, BaP+LC₀, and BaP+LC₁₀). The levels of oxidative stress index (OSI), total oxidant capacity (TOS), total antioxidant capacity (TAS), glutathione (GSH), and malondialdehyde (MDA) were analyzed in the cells to examine their effects on oxidative stress. It was observed that increasing extract concentrations exerted an antiproliferative effect and enhanced cell death in BaP-treated cells. MDA levels decreased in the treatment groups compared to both the BaP and control groups. Moreover, GSH levels showed a significant increase in the treatment groups (BaP+LC₀ and BaP+LC₁₀) compared to the BaP group. Although TAS levels in the treatment groups did not vary from those of the BaP group, TOS levels were statistically lower, indicating that OSI decreased in the BaP+extract groups; however, this reduction was not statistically significant. As in other cancer cell lines, in BaP-exposed A549 lung adenocarcinoma cells, the induction of oxidative stress via increased production of oxidant components such as reactive oxygen species is a desirable outcome. Reaching toxic oxidant levels in cancer cells enhances oxidative stress, and the apoptotic pathways induced by oxidative stress accelerate cancer cell destruction. Although this study showed that lipid peroxidation, an indicator of oxidative stress, was suppressed in the treatment groups and that antioxidant defenses were activated through elevated GSH levels, the lack of significant changes in OSI values suggests that oxidant production in A549 cells continues via other pro-oxidant mechanisms. Thus, the oxidant/antioxidant balance appears to be maintained by *Prunus spinosa* extract without a significant alteration in the oxidative stress state.

Keywords: A549, *Prunus spinosa*, Benzo[a]pyrene, Oxidative stress.

INTRODUCTION

According to the World Health Organization, cancer—affecting millions of individuals directly or indirectly each year—is recognized as one of the leading causes of mortality worldwide ¹. The lung cancer, with a higher mortality rate compared to prostate, pancreatic, and breast cancers, accounts for a substantial proportion of cancer-related deaths globally ².

Data from the Turkish Ministry of Health further indicate that lung cancer is the most frequently diagnosed malignancy in Turkey ³. Histologically, lung cancer is classified into two major groups: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) ⁴.

Among tobacco-specific compounds, nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and polycyclic aromatic hydrocarbons such as benzo[a]pyrene are potent inducers of carcinogenesis ⁵. Benzo[a]pyrene is classified as carcinogenic for humans based on strong and consistent evidence from both animal and human studies. This evaluation is supported by findings of increased cancer risk—particularly in the lungs and skin—in multiple animal species exposed through various routes, as well as in human populations exposed to polycyclic aromatic hydrocarbon mixtures containing benzo[a]pyrene ⁶.

Prunus spinosa (blackthorn), whose biological activities and health benefits have been documented in the literature, is a perennial, deciduous shrub that grows naturally across Europe, temperate regions of Asia, and the Mediterranean. Recent studies have provided significant insights into the chemical composition, antioxidant properties, and anti-inflammatory activities of *Prunus spinosa* extracts ⁷. Among its predominant constituents are anthocyanins and other phenolic compounds, which represent the most commonly consumed dietary antioxidants. These bioactive molecules enhance the nutraceutical potential of the plant and are thought to play an important role in preventing chronic diseases ⁸.

In the present study, A549 non-small-cell lung carcinoma cell lines treated with benzo[a]pyrene were exposed to different concentrations of *Prunus spinosa* extract, and cytotoxicity was assessed using the MTT assay. Based on the obtained data, LC₀ and LC₁₀ doses were determined; in cell lines treated with these doses, OSI, TOS, TAS, GSH and MDA levels were analyzed to evaluate the effects of low-dose *Prunus spinosa* extract on oxidative stress parameters.

MATERIALS AND METHODS

Extraction of Plant Material

Dried plant samples were ground into powder using a blender. For extraction, 10 g of plant powder was weighed and placed into a 250 mL borosilicate glass bottle. Subsequently, 100 mL of deionized water (60°C, 1:10 w/v ratio) was added. The suspension was incubated for 1 hour in an ultrasonic water bath at 60°C, followed by magnetic stirring at 600 rpm for 1 hour. After the water bath treatment, the suspension was kept at room temperature in the dark for 12 hours. The suspension was then filtered through filter paper to separate plant residues, and the filtrate was collected. The solvent was evaporated using a rotary evaporator to obtain the extract ⁹. Following extract preparation, the experimental phase was initiated.

MTT Cell Viability Assay

To investigate the effects of aqueous extracts of *Prunus spinosa* fruit on the viability of benzo[a]pyrene (BaP)-exposed A549 cells, 200 µL of medium was added to each well of 96-well flat-bottom culture plates. A549 cell suspension was prepared at a density of 1×10⁴ cells/mL, and 100 µL (≈2×10³ cells) of this suspension was added per well. Cells were incubated for 24 h to allow attachment. Once confluency reached at least 50%, the culture medium was replaced with fresh medium containing 20 µM BaP ¹⁰, and cells were incubated for another 24 h.

After incubation, *Prunus spinosa* extract dissolved in culture medium was applied at 25–2000 µg/mL concentrations to determine LC₀ and LC₁₀ doses.

The cells were incubated under 5% CO₂ at 37°C. After 24 h of incubation, MTT solution (5 mg/mL in PBS, pH 7.4) was added to each well at 10% of the well volume (22 µL). Cells were incubated for an additional 2–4 h at 37°C. The medium was then removed, and 200 µL of DMSO was added to each well to solubilize the formazan crystals, followed by a 10-minute incubation. Absorbance was measured at 540 nm using an ELISA plate reader. Cell viability of untreated control cells was considered 100%, and the viability of treated cells was expressed as a percentage relative to the control ¹¹.

LC₀ and LC₁₀ doses of *Prunus spinosa* extract were used for subsequent biochemical analyses. The LC₁₀ dose was defined as the concentration with no significant effect on cell viability compared to control, whereas the LC₁₀ dose was defined as the concentration that reduced cell viability by approximately 10%. After the determination of lethal concentrations, experimental groups were established as shown in Table 1, and in vitro applications were performed accordingly.

Table 1. Experimental groups and treatments applied

Groups	Treatments
Control Group	Vehicle control (solvents used to dissolve <i>Prunus spinosa</i> extract and BaP at appropriate volumes)
BaP Group	20 µM BaP solution
<i>Prunus spinosa</i> LC ₀ Group	LC ₀ dose (1 µg/mL) <i>Prunus spinosa</i> extract
<i>Prunus spinosa</i> LC ₁₀ Group	LC ₁₀ dose (5 µg/mL) <i>Prunus spinosa</i> extract
Treatment 1 (BaP + <i>Prunus spinosa</i> LC ₀) Grubu	20 µM BaP solution + LC ₀ dose (1 µg/mL) <i>Prunus spinosa</i> extract
Treatment 2 (BaP + <i>Prunus spinosa</i> LC ₁₀)	20 µM BaP solution + LC ₁₀ dose (5 µg/mL) <i>Prunus spinosa</i> extract

Preparation of Cell Lysates for Biochemical Analyses

For MDA, GSH, TAS, TOS, and OSI assays, A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1 mM sodium pyruvate, and 2 mM L-glutamine in 75 cm² flasks (three flasks per group). After 24 h of incubation at 37°C in a 5% CO₂ incubator, once cell confluency reached 50–60%, the medium was replaced with fresh medium containing 20 µM BaP (C₂₀H₁₂, purity >96%) for the BaP groups (Tampio et al., 2008). BaP was initially dissolved in DMSO, and the final concentration of DMSO in culture medium was kept below 0.1%. After 24 h, treatments described in Table 1 were applied, followed by 24 h incubation.

Cells were detached by adding 1 mL of 0.25% trypsin per flask and gently tapping. Suspensions were collected in 15 mL Falcon tubes and centrifuged at 400×g, 25°C for 5 min to obtain pellets. Pellets were washed with PBS, resuspended in lysis buffer (PBS, pH 7.4, supplemented with 1% Triton X-100 (v/v) and 8% protease inhibitor cocktail [aprotinin, PMSF, leupeptin, NaF]), pipetted, and sonicated on ice. Insoluble material was removed by centrifugation at 12,000×g, 4°C for 10 min, and supernatants were collected for biochemical analyses ¹¹.

Total Protein Analysis

Since the cell number may vary among samples, oxidative stress parameters were normalized to total protein levels. Total protein concentration in cell supernatants was determined using a commercial ELISA kit with bovine serum albumin as the standard ¹².

Analyses of Oxidative Stress Parameters

TAS levels were measured using commercial spectrophotometric kits (Rel Assay, Gaziantep, Turkey). Standard solutions (0.5 mmol, 1 mmol, and 2 mmol Trolox) provided with the kit were used for calibration, and TAS levels were expressed as mmol Trolox Equivalent/g-protein.

TOS levels were measured using commercial kits (Rel Assay, Gaziantep, Turkey). Standards provided (25, 50, and 100 $\mu\text{mol H}_2\text{O}_2$ equivalents) were used for calibration. Results were expressed as $\mu\text{mol H}_2\text{O}_2$ Equivalent/g-protein.

The oxidative stress index (OSI) was calculated as:

$$\text{OSI} = (\text{TOS level} / \text{TAS level}) \times 100$$

GSH levels were determined using the method of Beutler et al. ¹³, and MDA levels were measured at 540 nm after the reaction of one MDA molecule with two thiobarbituric acid (TBA) molecules in acidic conditions, producing a pink-colored product ¹⁴.

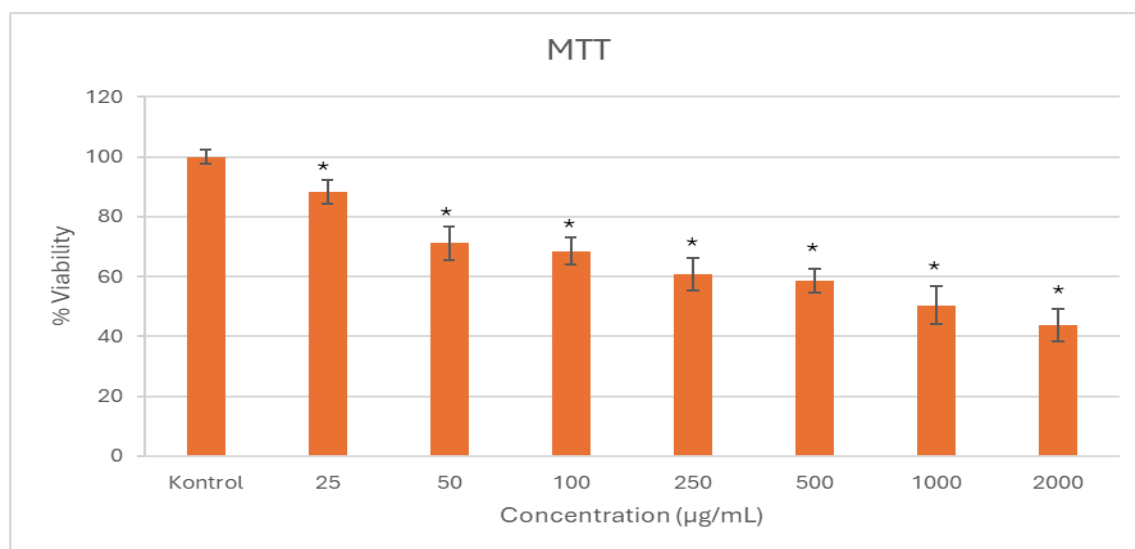
Statistical Analysis

All data were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using SPSS 20 software. One-way analysis of variance (ANOVA) was applied, followed by Duncan's post hoc test for multiple comparisons.

RESULTS

In the analysis phase, the cytotoxicity level of the *Prunus spinosa* extract and its LC_0 and LC_{10} doses were first determined in A549 cells. Based on these doses, MDA, GSH, TAS, TOS, and OSI analyses were subsequently performed on the cell lysates obtained from A549 cells following the experimental treatments.

The cytotoxicity of the aqueous extract of *Prunus spinosa* in A549 cells was determined using the MTT assay. For this purpose, the extract was dissolved in culture medium at seven different concentrations ranging from 25 to 2000 $\mu\text{g/mL}$ and applied to BaP-intoxicated cells. The analysis enabled the assessment of the effect of extract concentration on cell viability as well as the determination of lethal concentrations (Figure 1 and Table 2).



* Indicates a statistically significant difference in cell viability compared to the control group ($p < 0.05$).

Figure 1. Effect of different concentrations of aqueous *Prunus spinosa* extract on cell viability

MTT analysis revealed that all tested doses of aqueous *Prunus spinosa* extract exhibited cytotoxic properties compared to the control group ($p < 0.05$), with a dose-dependent increase in the rate of cell death (Figure 1).

Based on the data obtained from the MTT assay, probit analysis was performed via the SPSS 20 software package, and lethal concentrations with low cytotoxic effects (LC_0 and LC_{10}) were calculated (Table 2). These concentrations were subsequently used to evaluate the oxidative stress status in A549 cells subjected to BaP-induced toxicity.

Table 2. Lethal concentrations of *Prunus spinosa* extract

	Lethal concentrations of <i>Prunus spinosa</i> extract ($\mu\text{g/mL}$)
LC_0	1,23
LC_{10}	5,35
LC_{30}	115,34
LC_{50}	967,08

In this study, the effectiveness of *Prunus spinosa* extract doses with low cytotoxicity (LC_0 and LC_{10}) against oxidative stress was assessed in BaP-induced A549 cells by measuring MDA, GSH, TAS, TOS, and OSI parameters (Table 3).

Table 3. Effect of *Prunus spinosa* Extract on Oxidative Stress Parameters

	MDA $\mu\text{mol/L}$	GSH $\mu\text{g/mL}$	TOS $\mu\text{mol H}_2\text{O}_2$ Equiv./g- protein	TAS mmolTrolox Equiv./g-protein	OSI Arbitrary Unit
Control	0,133 $\pm$ 0,012 ^{b,c}	18,933 $\pm$ 0,667 ^c	0,385 $\pm$ 0,014 ^a	0,034 $\pm$ 0,005 ^a	1142,67 $\pm$ 138,27
BaP	0,146 $\pm$ 0,008 ^c	9,156 $\pm$ 0,770 ^a	0,561 $\pm$ 0,094 ^b	0,035 $\pm$ 0,005 ^a	1601,03 $\pm$ 316,20
LC_0	0,133 $\pm$ 0,005 ^{b,c}	11,266 $\pm$ 1,452 ^{a,b}	0,731 $\pm$ 0,076 ^c	0,056 $\pm$ 0,008 ^b	1306,15 $\pm$ 99,08
LC_{10}	0,114 $\pm$ 0,006 ^{a,b}	10,045 $\pm$ 1,678 ^a	0,703 $\pm$ 0,068 ^c	0,060 $\pm$ 0,009 ^b	1105,49 $\pm$ 105,35
BaP+ LC_0	0,115 $\pm$ 0,015 ^{a,b}	12,711 $\pm$ 2,037 ^b	0,449 $\pm$ 0,049 ^a	0,034 $\pm$ 0,007 ^a	1359,83 $\pm$ 153,69
BaP+ LC_{10}	0,103 $\pm$ 0,010 ^a	13,600 $\pm$ 0,647 ^b	0,426 $\pm$ 0,021 ^a	0,035 $\pm$ 0,006 ^a	1239,05 $\pm$ 205,79

* Data are presented as mean $\pm$ standard deviation (n=3).

^{a, b, c}: Means with different superscript letters within the same column are significantly different ($p < 0.05$).

MDA: Malondialdehyde, GSH: Glutathione, TOS: Total Oxidant Status, TAS: Total Antioxidant Status, OSI: Oxidative Stress Index

When examining MDA levels, the BaP group, which exhibited the highest levels, was found to differ significantly from the treatment groups (BaP+ LC_0 and BaP+ LC_{10}) ($p < 0.05$). Additionally, these treatment groups displayed lower MDA levels compared to the extract-only groups (LC_0 and LC_{10}) without BaP exposure.

Regarding GSH levels, a statistically significant decrease was observed in the BaP group compared to the control group ($p < 0.05$). Moreover, in the groups treated with *Prunus spinosa* extract following BaP-induced toxicity, GSH levels increased significantly compared to the BaP group ($p < 0.05$). Similar increases were observed between the BaP group and the LC_0 and LC_{10} extract-only groups, although these differences were not statistically significant.

Regarding TOS levels, BaP exposure resulted in a statistically significant increase compared to the control group ($p < 0.05$).

However, in the BaP+ LC₀ and BaP+ LC₁₀ groups, treatment with *Prunus spinosa* extract limited this increase, and a significant decrease was observed in these groups compared to the BaP group ($p<0.05$).

TAS levels in the LC₀ and LC₁₀ extract-only groups were significantly higher than in all other groups ($p<0.05$), while no other statistically significant differences were observed for TAS levels.

Among the groups, the highest oxidative stress index (OSI) was observed in the BaP group. Although therapy with *Prunus spinosa* extract reduced this level, no statistically significant change was recorded.

DISCUSSION AND CONCLUSION

Lung cancer, for which smoking is the most significant etiological factor, accounts for approximately one-third of all cancer-related deaths. Individuals who smoke have a 30-fold higher risk of developing lung cancer compared to non-smokers¹⁵. In addition to smoking, other risk factors include chronic obstructive pulmonary disease (COPD), recurrent lung inflammation, pulmonary scars, a family history of lung cancer, and exposure to carcinogens such as chromium, nickel, arsenic, polycyclic aromatic hydrocarbons (PAHs), and radioactivity¹⁶.

Benzo[a]pyrene (BaP), which is a polycyclic hydrocarbon compound, is an industrial pollutant with carcinogenic properties that primarily affects the lungs but can also impact various other tissues while suppressing the immune system¹⁷. BaP is released during petroleum and asphalt production, aluminum industry processes, and tobacco smoke¹⁸. Through metabolic activation by cytochrome P-450 enzymes, BaP is converted into highly reactive epoxides with cytotoxic and mutagenic effects on cells¹⁹. Furthermore, BaP interacts with membrane lipids, inducing lipid peroxidation and contributing to oxidative stress²⁰. Bioactive molecules involved in metabolic pathways may exert detrimental and beneficial effects, including anti-carcinogenic, anti-inflammatory, and antioxidant properties²¹. Compounds derived from plants, called phytochemicals, also exhibit bioactive properties. Primary metabolites essential for plant survival and secondary metabolites utilized for defense purposes constitute phytochemical species²².

The fruit of *Prunus spinosa*, a spiny shrub that grows wild in uncultivated areas of the Mediterranean, exhibits antioxidant activity due to its high polyphenolic content and vitamin C^{23,24}. Its polyphenols can counteract cellular degeneration, cancer, and cardiovascular diseases²⁵. Additionally, due to its antioxidant content, it can mitigate oxidative stress, which contributes to aging and tumor formation²⁶. The plant is also rich in anthocyanins²⁷. Studies have highlighted the anti-inflammatory and antibacterial properties of *Prunus spinosa* fruit. Traditionally used as a cough remedy, as well as a laxative, antispasmodic, diuretic and anti-inflammatory agent, *Prunus spinosa* has recently garnered scientific interest due to its potential biological activities^{28,29}.

The present study aimed to evaluate the cytotoxic effects of low-dose aqueous extracts of *Prunus spinosa* fruit on BaP-exposed A549 cells and to investigate the extracts' potential effects on oxidative stress induced by BaP toxicity. MTT assays were performed alongside MDA, GSH, TAS, TOS, and OSI analyses to assess these effects. In this way, the study sought to identify the potential benefits of *Prunus spinosa* extracts as a natural approach to mitigating BaP-induced toxicity.

A549 cells, one of the most commonly used cell lines in lung cancer research, were employed to investigate cytotoxicity and oxidative stress.

Previous studies on trigonocotone *Prunus spinosa* extracts reported that increasing concentrations reduced viability and increased apoptosis in various human cancer cell lines, including A549³⁰. Similarly, studies examining the effects of *Prunus spinosa* on autophagy found decreased cell viability with higher extract concentrations³¹. In line with these reports, the our study demonstrated an anti-proliferative effect and increased cell death in BaP-treated cells with rising extract concentrations (Figure 1).

Studies investigating the phenolic content of *Prunus spinosa* aqueous extracts identified caffeoylquinic acid as the primary component³². Other research indicated that caffeoylquinic acid exhibited cytotoxicity at 300 µg/mL³³. The present study suggests that combining these phenolic compounds with the toxic agent BaP further enhances cytotoxicity.

Polyphenol-rich foods in the diet, through their hydroxyl groups, can neutralize reactive oxygen species (ROS) and enhance the circulation of antioxidant compounds, thereby preventing oxidative stress-related diseases³⁴. Lipid peroxidation, inflammation, nitration of proteins and DNA damage can be prevented by polyphenols, which act as free radical scavengers, inhibitors of pro-inflammatory enzymes, and modulators of cellular signaling pathways³⁵. The study of Pinacho et al.²⁵ identified 26 different polyphenolic compounds in *Prunus spinosa* flowers and other parts, while another study reported approximately 60 compounds, mainly flavonoids and phenolic acids³⁵. Predominant polyphenols in *Prunus spinosa* fruit include rutin, quercetin, chlorogenic acid, and gallic acid³⁶.

In studies on diabetic rats induced by streptozotocin, *Prunus spinosa* leaf and flower extracts significantly reduced MDA levels and increased GSH levels³⁶. Research on wild Italian *Prunus spinosa* fruit demonstrated reduced MDA levels and increased GSH in rat liver and brain tissues²⁶. Furthermore, in vitro studies on oxidative/nitrosative modifications of human plasma components showed that *Prunus spinosa* flower extracts decreased TBARS levels, indicating reduced lipid peroxidation³⁵. Consistent with the literature, the present study observed decreased MDA levels in both BaP and control groups, with the most significant reduction in the BaP+LC₁₀ group, suggesting effective prevention of lipid peroxidation (Table 3).

Bioactive components in *Prunus spinosa*, such as proanthocyanidins and phenolic acids, regulate lipid metabolism³⁷⁻³⁹. This regulation scavenges oxidants, protects lipids, and reduces lipid peroxidation³⁵. Similar trends were observed for GSH levels, with significant increases in treatment groups (BaP+LC₀ and BaP+LC₁₀) compared to the BaP group (Table 3). The total antioxidant activity of *Prunus spinosa* extracts, measured by ferric reducing antioxidant power (FRAP), also increased in response to oxidative stress, consistent with TAS data for LC₀ and LC₁₀ groups (Table 3). These results indicate activation of both GSH-specific and total antioxidant defense mechanisms in A549 cells, including those exposed to BaP.

High TAS values in the LC₀ and LC₁₀ groups were paralleled by TOS parameters (Table 3). Some studies have reported that high doses of pure polyphenols or polyphenol-rich plant extracts can act as pro-oxidants^{40,41}. This observation suggests that *Prunus spinosa* extracts may also trigger pro-oxidant mechanisms in A549 cells. Nevertheless, along with GSH, other antioxidant systems were activated, lipid peroxidation decreased, and the balance between oxidant and antioxidant was maintained, effectively suppressing oxidative stress in A549 cells. Comparisons of OSI levels between control, LC₀, and LC₁₀ groups confirm this effect (Table 3). In the BaP+LC₀ and BaP+LC₁₀ treatment groups, TAS levels did not significantly differ from the BaP group, while TOS levels were statistically lower, indicating reduced oxidative stress indices, albeit not statistically significant (Table 3).

In conclusion, as observed in other cell lines, BaP-exposed A549 lung adenocarcinoma cells exhibited increased level of ROS and other oxidants, initiating oxidative stress—a favorable outcome in the context of cancer therapy⁴². Toxic levels of oxidants in cancer cells enhance oxidative stress, and stress-induced apoptotic pathways accelerate cancer cell death. In the present study, using low doses (LC₀ and LC₁₀) resulted in limited oxidative stress and, consequently, restricted apoptosis. These findings provide a foundation for future studies, in which higher doses of *Prunus spinosa* extract could be applied to BaP-exposed A549 lung adenocarcinoma cells to evaluate oxidative stress responses under these conditions.

*This study was derived from the first author's master's thesis.

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HESPERİDİNİN SAĞLIĞA ETKİLERİ VE TAKVİYE POTANSİYELİ

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ÖZET

Giriş ve Amaç: Bu bildiride Bir flavonoid olan Hesperidin'in çeşitli etkileri anlatılacaktır. Hesperidin, narenciye yetiştiriciliğinin bol miktarda ve düşük maliyetli bir yan ürünü olup, özellikle tatlı portakal ve limonun temel flavonoidi olarak dikkat çeker. Bunun yanı sıra, hesperidin, çay, zeytinyağı ve özellikle narenciye meyvelerinde doğal olarak bulunur.

Materyaller ve Yöntemler: Hesperidin, anti-oksidan, anti-bakteriyel, anti-mikrobiyal, anti-inflamatuar ve anti-karsinogenik özellikler dahil olmak pek çok yararlı özelliğe sahip bir maddedir. Sağlık üzerindeki bu olumlu etkileri nedeniyle gıda takviyesi olarak kullanımı günümüzde artmıştır.

Sonuçlar: Yapılan çeşitli klinik ve prelinik çalışmalarda hesperidin Kan basıncını düşürücü ve damar elastikiyetini artırıcı etkisi olduğu, Glikoz metabolizmasını düzenleyerek insülin direncini azalttığı, Alzheimer ve Parkinson gibi hastalıklarda oksidatif hasarı azaltıcı rol oynadığı, Anti-proliferatif ve apoptozis indükleyici etkileri olduğu, sitokin üretimini düzenleyerek enflamasyonu azalttığı görülmüştür. Antioksidan özellikleri sayesinde hücre membranlarının, proteinlerin ve DNA'nın oksidatif hasara uğramasını engelleyebilmekte ve. bu sayede yaşlanma sürecini yavaşlatıcı etkiler gösterebilmektedir. Ayrıca, inflamatuvar süreçleri baskılayarak kronik inflamatuvar hastalıkların gelişimini azaltabileceği düşünülmektedir. Bu etkileri ile günümüz takviye gıda endüstrisinde kendine sağlam bir yer edinmiştir.

Tartışma ve Sonuç: Sonuç olarak, bu kadar etkili bir maddenin farklı hastalıklar üzerindeki iyileştirici etkileri araştırmacılara geniş bir çalışma alanı sunmaktadır.

Anahtar Kelimeler: Antioksidanlar; Flanonidler; Hesperidin; Takviye gıda

HEALTH EFFECTS AND SUPPLEMENTATION POTENTIAL OF HESPERIDIN

ABSTRACT

Introduction and Purpose: This report will discuss the various effects of hesperidin, a flavonoid. Hesperidin is an abundant and low-cost byproduct of citrus cultivation, particularly notable as the primary flavonoid in sweet oranges and lemons. Additionally, hesperidin is naturally found in tea, olive oil, and citrus fruits in particular.

Materials and Methods: Hesperidin possesses numerous beneficial properties, including antioxidant, antibacterial, antimicrobial, anti-inflammatory, and anti-carcinogenic properties. Due to these beneficial health effects, its use as a dietary supplement has increased.

Results: Various clinical and preclinical studies have shown that hesperidin lowers blood pressure and increases vascular elasticity. It reduces insulin resistance by regulating glucose metabolism. It plays a role in reducing oxidative damage in diseases such as Alzheimer's and Parkinson's. It has anti-proliferative and apoptosis-inducing effects. It also reduces inflammation by regulating cytokine production. These effects have earned it a strong place in the current food supplement industry. Thanks to its antioxidant properties, it can prevent oxidative damage to cell membranes, proteins, and DNA, thus slowing the aging process.

It is also thought to reduce the development of chronic inflammatory diseases by suppressing inflammatory processes. Consequently, the potential therapeutic effects of such a potent substance in various diseases offer researchers a wide range of research opportunities.

Discussion and Conclusion: As a result, the healing effects of such an effective substance on different diseases offer researchers a wide field of study.

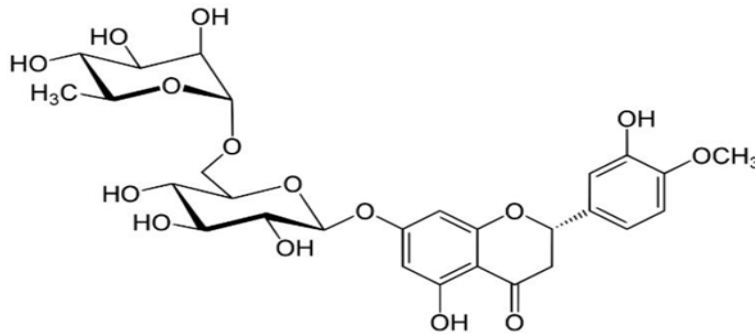
Key Words: Antioxidants; Flavonoids; Food supplements; Hesperidin

GİRİŞ

Flavonoidler, bitkilerin ikincil metabolitlerinden elde edilen polifenolik yapıya ve düşük moleküler ağırlığa sahip önemli biyoaktif bileşiklerdir. Flavonoidler yaygın olarak meyvelerde, sebzelerde, baharatlarda ve yağlarda bulunur (Panche vd., 2016). Sarı renklerinden dolayı "flavonoidler" olarak adlandırılırlar ve Latince "sarı" anlamına gelen "flavus" kelimesinden türetilmişlerdir. Tüm flavonoidler, omurgalarında iki adet altı üyeli halkadan ve çoğunlukla bunlara C6-C3-C6 olarak bağlanmış üç karbonlu bir birimden oluşan 15 karbon atomu içerir (Dias vd., 2021). Fenil gruplarını birbirine bağlayan üç karbondan oluşan yapı üçüncü bir halka oluşturmak amacıyla oksijenle çevrim yapar. Bu çekirdek yapı 2-fenilbenzopiranon şeklinde isimlendirilir. Flavonoidler genellikle O-glikozitler veya C-glikozitler şeklinde şekerlerle konjuge edilir. Aglikon olarak da bulunabilirler (Xie ve ark., 2022).

Bitkilerde flavonoidler UV filtreleri ve ROS tutucular olarak hareket edebilir ve kuraklık, ısı ve don toleransında çeşitli işlevlere sahiptir (Panche vd., 2016, Rodríguez vd., 2020). İnsanlarda bu bileşikler, antikanser, anti-enflamatuar, kardiyoprotektif, yaşlanma karşıtı, nöroprotektif, antidiyabetik, immünomodülatör, antibakteriyel, antiviral ve antiparazitik etkiler göstererek sağlık için faydalar sağlarlar (Panche vd., 2016, Rodríguez vd., 2020). -parazitik ve antiviral özellikler (Panche vd., 2016, Fraga vd., 2019, Jucá vd., 2020). Flavonoidler, serbest radikallerin temizlenmesi, peroksitlerin ve diğer reaktif oksijen türlerinin inaktivasyonu, metallerin şelasyonu ve ikincil lipid oksidasyon ürünlerinin söndürülmesi gibi çeşitli etki mekanizmalarına sahiptir (Dias ve ark., 2021, Beecher, 2003).

Hesperidin (C₁₆H₁₄O₆), turuncgillerde yüksek konsantrasyonlarda bulunan biyoflavonoidlerin alt sınıflarından biri olan bir flavanon bileşiğidir (Şekil 1) (Pyrzynska, 2022). İlk olarak 1827 yılında Fransız kimyager Lebreton tarafından hesperid ailesine ait olan ve albedo olarak adlandırılan portakal kabuğunun iç süngerimsi kısmından izole edilmiştir (Garg ve ark., 2001). Kullanımı, antioksidan, antibakteriyel, antimikrobiyal, anti-enflamatuar ve anti-kanser özellikleri de dahil olmak üzere birçok yararlı sağlık fonksiyonu ile ilişkilidir. Sağlık üzerindeki olumlu etkileri nedeniyle besin takviyesi olarak kullanımı artmıştır. Hesperidin antioksidan özellikleri, bağışıklık sistemini destekleyici etkisi ve damar sağlığına olumlu katkısı ile karakterize edilir (Li ve Schluesener, 2017).



Şekil 1. Hesperidin'in kimyasal yapısı

GELİŞME

Hesperidinin Kimyasal Yapısı ve Kaynakları

Hesperidin, hesperetin ve rutinoz şekerinden oluşan bir flavanon glikozittir (Manach ve ark., 2005). Hesperidin, narenciye üretiminde bol ve ucuz elde edilebilen bir yan üründür ve özellikle tatlı portakal ve limondaki ana flavonoid olarak dikkat çekmektedir. Genç, olgunlaşmamış portakalların taze ağırlığının %14'üne kadarı hesperidinden oluşabilir. Ayrıca hesperidin çayda, zeytinyağında ve özellikle turuncgillerde doğal olarak bulunur. Aynı zamanda geleneksel Çin tıbbında uzun zamandır kullanılan önemli bir bileşiktir (Pyrzynska, 2022). Gıda endüstrisinde hem biyolojik aktiviteye sahip bir bileşik hem de doğal bir antioksidan olarak değerlendirilir.

Hesperidinin Biyoyararlanımı

Ağız yoluyla alındığında hesperidin ince bağırsakta sınırlı ölçüde emilir. Bağırsak mikrobiyotası tarafından metabolize edilir ve aktif formu olan hesperetine dönüştürülür (Serra ve ark., 2013). Şu anda biyoyararlanımını artırmak için lipozomal formlar ve nanopartiküllü taşıyıcı sistemler üzerinde çalışmalar yürütülmektedir.

Antioksidan Etkileri

Hesperidin güçlü bir antioksidandır. Serbest radikallerin sebep olduğu oksidatif stresi azaltmada etkilidir. Antioksidan mekanizması şu şekilde açıklanmaktadır;

- Serbest radikallerin nötralizasyonu,
- Lipid peroksidasyonunun azaltılması,
- Glutasyon seviyelerinin korunması,
- Katalaz ve Süperoksit dismutaz gibi endojen antioksidan enzimlerin aktivitesini artırır (Garg ve ark., 2001).

Antioksidan özellikleri sayesinde hücre zarlarında, proteinlerde ve DNA'da oksidatif hasarı önleyebilir. Bu da yaşlanma sürecini yavaşlatabilir. Ayrıca, enflamatuar süreçleri baskılayarak kronik enflamatuar hastalıkların (örn. artrit, enflamatuar bağırsak hastalığı) gelişimini azaltabileceği bildirilmiştir (Parhiz ve ark., 2015).

Hesperidinin antioksidan kapasitesi tek başına etkili olabilir, ancak diğer polifenollerle sinerjik etkilere de sahip olabilir. Bu nedenle, genellikle kombine antioksidan takviyelerinde tercih edilir. Oksidatif stresi baskılayıcı etkisinin C vitamini ile birlikte kullanıldığında daha güçlü olduğu bulunmuştur (Parhiz ve ark., 2015).

Tablo 1. Hesperidinin Antioksidan Etki Mekanizmaları

Mekanizma	Etki
Serbest radikal tutucu	Oksidatif hasarın önlenmesi
Lipid peroksidasyonunun inhibisyonu	Hücre membranlarının korunması
Antioksidan enzimlerin aktivasyonu	SOD, katalaz, glutasyon peroksidaz aktivitesinde artış,
DNA ve proteinlerin korunması	Mutasyonların ve hücre yaşlanmasının önlenmesi

Klinik Çalışmalar ve Sağlık Üzerindeki Etkileri

Çeşitli klinik ve klinik öncesi çalışmalar hesperidinin faydalarını ortaya koymuştur:

- Kardiyovasküler sistem: Kan basıncının düşürülmesi ve damar esnekliğini artırıcı etkileri vardır (Rizza ve ark., 2011).
- Diyabet: Glikoz metabolizmasının düzenlenmesini sağlayarak insülin direncini azaltıcı etki gösterebilir (Osama ve ark., 2011).

- Nöroprotektif etkiler: Parkinson ve Alzheimer gibi hastalıklarda oksidatif hasarın azaltılmasında rol oynar (Spagnuolo ve ark., 2018).
- Kanseri: Apoptozisi indükleyici ve anti-proliferatif etkileri vardır (Roohbakhsh ve ark., 2015).
- Anti-Enflamatuar Etkiler: İnflamasyonu azaltmak için sitokin düzeylerini dengeler. . Bu etkisini öncelikle TNF- α ve IL-1 β gibi pro-inflamatuar araçları inhibe ederek gösterir (Horowitz ve Gentili, 1963).

Gıda Takviyesi Olarak Kullanım Alanları

1. Kardiyovasküler Sağlık

Hesperidin, kan damarlarının esnekliğini artıran ve endotel fonksiyonunu düzenleyen etkilere sahiptir. Klinik çalışmalar düzenli hesperidin takviyesinin kan basıncını düşürdüğünü ve enflamatuar belirteçleri azalttığını göstermiştir (Rizza ve ark., 2011). Bu nedenle yüksek tansiyondan korunmak ve damar sağlığını korumak için kullanılır.

2. Metabolik Sendrom ve Diyabet

Çalışmalar, hesperidin'in alındığında insülin direncini azaltıcı ve glikoz metabolizmasını düzenleyici etki gösterdiğini ortaya koymaktadır. Ayrıca trigliserit ve LDL kolesterol seviyelerini düşürerek lipid profilini iyileştirir (Osama ve ark., 2011).

3. Bağışıklık Sistemi ve Antioksidan Etkiler

Hesperidin serbest radikalleri nötralize eder ve böylece hücre hasarını azaltır. Antioksidan etkisi ile bağışıklık sistemi için destekleyici bir rol üstlenir. Özellikle vitamin C ile birlikte sinerjik bir etkiye sahip olduğu bildirilmiştir (Parhiz ve ark., 2015).

4. Cilt ve Yaşlanma Karşıtı Etkiler

Hesperidin, vücutta kolajen üretimini destekleyerek cildin elastikliğini artırır. Bu nedenle dermokozmetik ürünlerde ve yaşlanma karşıtı preparatlarda kullanılır (Roohbakhsh ve ark., 2015).

5. Sporcularda Kullanımı

Hesperidin takviyelerinin yoğun egzersiz sonrası oluşan oksidatif stresi azaltabileceği bildirilmiştir. Bu etki kas hasarını azaltabilir ve iyileşmeyi hızlandırabilir (Yáñez ve ark., 2012).

6. Venöz Yetmezlik ve Damar Sağlığı

Hesperidin, özellikle mikronize flavonoid fraksiyonu (diosmin ile) şeklinde, kronik venöz yetmezlik ve hemoroid tedavisinde yardımcı olarak kullanılır. Bu preparatlar damar geçirgenliğini azaltır, ödemi ve ağrıyı hafifletir (Yasım ve ark., 2019).

Güvenlik ve Dozaj

Hesperidin genellikle güvenli kabul edilir. Günlük 500-1000 mg dozunda besin takviyesi olarak kullanılabilir (Parhiz et al., 2015). Yüksek dozlarda nadiren gastrointestinal rahatsızlıklar bildirilmiştir. Hamilelerde ve kronik hastalığı olan kişilerde doktor tavsiyesi ile kullanılması önerilmektedir.

Tablo 2. Hesperidinin Gıda Takviyesi Olarak Kullanım Alanları

Kullanım Alanı	Amaç	Destekleyici Etki
Kardiyovasküler sağlık	Yüksek tansiyon ve damar sağlığı	Damar esnekliğinin artırılması
Bağışıklık sistemi	Serbest radikallerin azaltılması	Hastalıklara karşı direnç
Cilt sağlığı	Kolajen sentezi	Yaşlanma karşıtı etki
Sporcularda performans	Egzersiz sonrası stres azaltma	Kas iyileşmesi
Metabolik sendrom	İnsülin duyarlılığı artırma	Kilo kontrolü

SONUÇ

Hesperidin doğal kaynaklardan elde edilen güçlü bir antioksidandır ve besin takviyesi olarak alındığında sağlık açısından sayısız faydası vardır. Kardiyovasküler sistemin korunması, metabolik hastalıkların önlenmesi, nörolojik bozukluklardaki destekleyici rolü ve kanserle mücadeledeki potansiyeli hesperidini değerli bir biyoflavonoid haline getirmektedir. Gelecekte yapılacak randomize kontrollü çalışmalar klinik uygulama alanlarını daha da netleştirecektir.

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İN VİTRO TANI (IVD) CİHAZLARINDA YENİ SINIFLANDIRMA VE RİSK YÖNETİMİ

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ÖZET

Amaç: Bu çalışmanın amacı, güncel yönetmeliklere göre in vitro tanı (IVD) cihazlarının sınıflandırma sistemindeki değişiklikleri ve risk yönetimi süreçlerini inceleyerek, imalatçıların ve sağlık kuruluşlarının yükümlülüklerini vurgulamaktır. Özellikle, sağlık kuruluşlarında imal edilen (in-house) cihazlara ilişkin madde 6.5 hükümleri bağlamında uygulamaya yönelik değerlendirmeler yapılmıştır.

Materyal ve Metot: Çalışmada, TS EN ISO 14971:2019 ve ISO/TR 24971:2020 standartlarının risk yönetimi ilkeleri, “İn Vitro Tanı Amaçlı Tıbbi Cihaz Yönetmeliği (2017/745 IVDR)” ve eklerinde yer alan yeni sınıflandırma kriterleri incelenmiştir. Yönetmeliğin Ek I’de belirtilen Genel Güvenlilik ve Performans Gereklilikleri ile risk kontrol öncelikleri dikkate alınmıştır. Ayrıca madde 6.5 kapsamında, yalnızca ilgili sağlık kuruluşunda kullanılmak üzere imal edilen cihazlara yönelik mevzuat analizi yapılmıştır.

Bulgular: Yeni sınıflandırma, cihazları kullanım amacı, risk seviyesi ve teknik özelliklerine göre dört ana risk sınıfına ayırmaktadır. Daha önceki yönetmeliği göre ürünlerin yaklaşık 80’nin risk sınıfı yükselmiştir. ISO 14971 çerçevesinde risk yönetimi, tehlike tanımlama, risk tahmini, kontrol tedbirleri ve artık riskin değerlendirilmesi aşamalarını içermektedir. Madde 6.5 uyarınca, sağlık kuruluşu içinde imal edilen ve yalnızca bu kuruluştaki kullanılan cihazlar, belirli istisnalar dışında piyasaya arz gereklerinden muaf tutulmaktadır; ancak güvenlik, performans ve izlenebilirlik yükümlülükleri devam etmektedir.

Tartışma ve Sonuç: Yeni sınıflandırma, yüksek riskli IVD cihazlarda klinik kanıt ve performans doğrulamasını zorunlu kılmakta, risk yönetiminde ISO 14971’in sistematik yaklaşımını güçlendirmektedir. Sağlık kuruluşu bünyesindeki (in-house) üretimleri, esneklik sağlasa da kalite yönetimi, validasyon ve kayıt tutma zorunluluklarıyla desteklenmelidir. Bu, hasta güvenliği ile inovasyon arasında denge sağlamaktadır. Güncel düzenlemeler, IVD cihazlarının sınıflandırma ve risk yönetimi süreçlerini daha net ve katı hale getirmiştir. Sağlık kuruluşlarında imal edilen cihazlar da dahil olmak üzere, tüm üretimlerde sistematik risk yönetimi ve mevzuata uyum, güvenli ve etkili tanılmal uygulamalar için kritik önemdedir.

Anahtar Kelimeler: IVDR; IVD; Risk Yönetimi; Sınıflandırma

IN VITRO DIAGNOSTIC (IVD) DEVICES: NEW CLASSIFICATION AND RISK MANAGEMENT

ABSTRACT

Objective: The aim of this study is to examine the changes in the classification system of in vitro diagnostic (IVD) devices and risk management processes in accordance with current regulations, and to highlight the responsibilities of manufacturers and healthcare institutions. In particular, practical evaluations were made in the context of the provisions of Article 6.5 regarding devices manufactured in-house (in-house devices) in healthcare institutions.

Materials and Methods: The study examined the risk management principles outlined in TS EN ISO 14971:2019 and ISO/TR 24971:2020 standards, as well as the new classification criteria outlined in the “In Vitro Diagnostic Medical Devices Regulation (2017/745 IVDR)” and its annexes. The General Safety and Performance Requirements specified in Annex I of the Regulation and risk control priorities were taken into consideration. Additionally, a regulatory analysis was conducted for devices manufactured exclusively for use within the relevant healthcare facility, in accordance with Section 6.5.

Findings: The new classification divides devices into four main risk classes based on their intended use, risk level, and technical characteristics. Compared to the previous regulation, the risk class of approximately 80% of products has been elevated. Risk management within the framework of ISO 14971 includes the stages of hazard identification, risk estimation, control measures, and assessment of residual risk. In accordance with Section 6.5, devices manufactured within a healthcare facility and used exclusively within that facility are exempt from market authorization requirements, except for certain exceptions; however, obligations related to safety, performance, and traceability remain in effect.

Discussion and Conclusion: The new classification requires clinical evidence and performance verification for high-risk IVD devices and strengthens the systematic approach of ISO 14971 in risk management. Although in-house production provides flexibility, it must be supported by quality management, validation, and record-keeping requirements. This balances patient safety with innovation. Current regulations have made the classification and risk management processes for IVD devices clearer and more stringent. Systematic risk management and regulatory compliance are critical for safe and effective diagnostic applications, including devices manufactured within healthcare organizations.

Keywords: IVDR; IVD; Risk Management; Classification

GİRİŞ

Klinik laboratuvarlarda teşhis ve tedavi takibinde otoanalizörler, reaktifler, kitler, kontrol ve kalibratörler gibi birçok ürün kullanılmaktadır. Rutin çalışma hayatı içerisinde tüm bu ürünler farklı şekilde isimlendirilse de mevzuatta bunlar İn Vitro Tanı (IVD – In Vitro Diagnostic) cihazı tanımı altında toplanmaktadır.

IVD cihazların geçmişi oldukça uzun olmasına rağmen herhangi bir standart veya düzenlemeye tabi olmaları 20. yüzyılın sonlarına doğru olmuştur. Avrupa Birliği (AB), üye ülkeler arasında teknik düzenleme farklılıklarından kaynaklanan ticari engelleri kaldırmak üzere 1985 yılında yeni Yaklaşım (New Approach) olarak adlandırılan yeni bir standardizasyon politikası benimsemiştir. Burada amaç tüm üye ülkeler arasında ürünlerin belli bir standartta üretilmesi ve böylece malların rahatça AB sınırları içinde dolaşmasıydı. Bu kapsamda pek çok ürün grubunda standartlaşmayı sağlayacak direktifler yayınlanmaya başladı. İlk olarak 1990 yılında 90/385/EEC Vücuda Yerleştirilebilir Aktif Tıbbi Cihazlar Direktifi (AIMDD – Active Implantable Medical Devices Directive)¹ yayınlandı. Bu kapsamdaki cihazlar kalp ve beyin pili gibi vücuda takılan yüksek riskli ürünleri içeriyordu. Arkasından 1993 yılında 93/42/EEC Tıbbi Cihaz Direktifi (MDD – Medical Device Directive)² yayınlandı. Bu direktif AIMDD haricinde insan üzerinde kullanılan tüm tıbbi cihazlarla ilgili hususları düzenliyordu. Ancak IVD cihazlar bu iki direktifin de kapsamında değildi. 1998 yılına gelindiğinde 98/79/EC İn Vitro Tanı Tıbbi Cihazlar Direktifi (IVDMD – In Vitro Diagnostic Medical Devices)³ yayınlandı. Bu direktifle beraber klinik laboratuvarlarda kullanılan birçok ürünün üretiminden piyasaya arzına kadar pek çok hususta standardizasyon sağlanmış oldu.

Hem MDD hem de IVDMD teknolojik gelişmelere bağlı olarak bazı revizyonlar geçirerek yirmi yıldan fazla bir süre yürürlükte kalmıştır. Özellikle ürün güvenliği ile ilgili yaşanan ciddi sıkıntılar AB’ni daha yüksek güvenlik ve sağlık seviyesi sağlamak için şeffaf, öngörülebilir ve sürdürülebilir bir mevzuat hazırlamaya mecbur bırakmıştır. Bu doğrultuda hazırlanan mevzuat 2017 yılında;

- (EU) 2017/745 Medical Devices Regulation (MDR)4,
- (EU) 2017/746 In Vitro Medical Devices Regulation (IVDR)5 olarak yayınlanmıştır.

Hem tıbbi cihazlarda hem de IVD cihazlarda önceki düzenleme direktif seviyesindeyken yeni yapılan düzenleme ile bu durum regülasyon seviyesine yükselmiştir. Durumu ülkemiz mevzuatı ile anlatacak olursak daha önce yönetmelik ile düzenlenen konular kanun seviyesine geçilerek daha güçlü bir hale getirilmiştir.

AB, tıbbi cihaz ve IVD cihazları bu şekilde düzenlerken ülkemizde ise bu konuları ele alan derli toplu herhangi bir mevzuat yoktu. Türkiye ile AB arasında Gümrük Birliği’ni tesis eden 1/95 sayılı AB-Türkiye Ortaklık Konseyi Kararı6 (OKK)’nın 8 ilâ 11 inci maddeleri gereği, Türkiye’nin AB’nin ürünlere ilişkin teknik mevzuatını iç hukukuna dâhil etmesi hükmüne bağlanmıştır. Bu bağlamda; Ticaret Bakanlığı (Ekonomi Bakanlığı) tarafından hazırlanan 4703 sayılı "Ürünlere İlişkin Teknik Mevzuatın Hazırlanması ve Uygulanmasına Dair Kanun"7 11 Ocak 2002’de yürürlüğe girmiş ve mezkûr kanuna istinaden 97/9196 sayılı Bakanlar Kurulu Kararı8 gereğince Avrupa Birliği’nin tıbbi cihazlarla ilgili 3 temel Direktifinin uyumlaştırma çalışmaları Sağlık Bakanlığına yapılmıştır. İlgili çalışmalar sonucunda;

- 90/385/EEC sayılı Vücuda Yerleştirilebilir Aktif Tıbbi Cihazlar Direktifi uyumlaştırılarak 12 Mart 2002 tarihli ve 24693 sayılı Resmî Gazete’de “Vücuda Yerleştirilebilir Aktif Tıbbi Cihazlar Yönetmeliği”9,
- 93/42/EEC sayılı Tıbbi Cihaz Direktifi uyumlaştırılarak 13 Mart 2002 tarihli ve 24694 sayılı Resmî Gazete’de “Tıbbi Cihaz Yönetmeliği”10,
- 98/79/EC sayılı İn vitro Tıbbi Tanı Cihazları Direktifi uyumlaştırılarak 14 Ekim 2003 tarihli ve 25259 sayılı Resmî Gazete’de “Vücut Dışında Kullanılan (in vitro) Tıbbi Tanı Cihazları Yönetmeliği”11 yayımlanmıştır.

Her 3 yönetmelikte AB’nde direktiflerde yapılan güncellemeler ışığında 2007 ve 2011 yıllarında revizyona uğramıştır. Bu yönetmeliklerin yayımlanmasıyla birlikte tıbbi cihazların ülkemizde piyasaya arz edilmesinde AB’ndeki düzenlemelere uyum sağlanmıştır. Bu sayede global düzenlemelere uygun olarak imal edilen ürünlerin ülkemizde satışı mümkün olmuş buna ek olarak ülkemizde imal edilen tıbbi cihazların da herhangi bir ek belgelendirme ya da ruhsatlandırmaya tabi olmadan AB üyesi ülkelere ihraç edilebilmesinin önü açılmıştır.

AB’deki uygulamalara paralel olarak 02 Haziran 2021 tarihli ve 31499 mükerrer sayılı Resmî Gazete’de;

- (EU) 2017/745 Medical Devices Regulation (MDR) uyumlaştırılarak Tıbbi Cihaz Yönetmeliği12,
- (EU) 2017/746 In Vitro Medical Devices Regulation (IVDR) uyumlaştırılarak İn Vitro Tanı Amaçlı Tıbbi Cihaz Yönetmeliği13 yayımlanmıştır.

Her iki yönetmelikte aynı tarihte yayımlanmasına rağmen Tıbbi Cihaz Yönetmeliği 26.05.2021 tarihi itibarıyla yürürlüğe girerken İn Vitro Tanı Amaçlı Tıbbi Cihaz Yönetmeliği 26.05.2022 tarihi itibarıyla yürürlüğe girmiştir.

Yeni yayımlanan mevzuatla birlikte pek çok husus daha detaylı ve yüksek seviyede tanımlanmıştır. Bu hususlardan bir tanesi de tıbbi cihazların ve IVD cihazların risk yönetimidir. Tüm tıbbi cihazların risk yönetiminin genel çerçevesini “ISO 14971 Tıbbi cihazlar – Tıbbi cihazlara risk yönetimin uygulanması”14 standardı belirlemektedir.

Bu standart ilk olarak 1998 yılında Uluslararası Standartlar Örgütü (ISO – International Organization for Standardization) tarafından yayımlanmıştır. Teknolojik gelişmelere ve mevzuatta yapılan değişikliklere bağlı olarak 2000, 2007, 2012 ve son olarak 2019 yılında yenilenmiştir. Ayrıca ISO/TR 24971:202015 açıklayıcı dokümanı yayımlanmıştır. Açıklayıcı doküman, standardın her bir maddesinin nasıl uygulanacağını örneklerle anlatmaktadır. Ayrıca sadece IVD cihazlara spesifik riskleri anlatan bir ek içermektedir.

Gelişme

Yönetmelik İn vitro tanı cihazı (İn vitro tanı amaçlı tıbbi cihaz)’nı “Yalnızca veya esas olarak;

- 1) Fizyolojik veya patolojik bir süreç veya duruma ilişkin,
- 2) Konjenital fiziksel veya zihinsel bozukluğa ilişkin,
- 3) Tıbbi bir duruma veya hastalığa yatkınlığa ilişkin,
- 4) Potansiyel alıcılar için güvenlik ve uyumluluğu belirlemeye yönelik,
- 5) Tedavi cevabını veya reaksiyonlarını tahmin etmeye yönelik,
- 6) Tedavi tedbirlerini tanımlamaya veya izlemeye yönelik,

hususlardan biri veya daha fazlası hakkında bilgi sağlamak amacıyla, kan ve doku bağışları dâhil olmak üzere, insan vücudundan elde edilen numunelerin incelenmesinde imalatçı tarafından in vitro olarak kullanılması amaçlanan, gerek tek başına gerekse birlikte kullanılan, reaktif, reaktif ürün, kalibratör, kontrol materyali, kit, alet, aparat, ekipman parçası, numune kapları, yazılım veya sistem olan tıbbi cihazları,” şeklinde tanımlamaktadır.

Tanımda italik yazılan kısımlar IVDR ile değişen ve/veya eklenen kısımları göstermektedir. Tanımdan da anlaşılacağı üzere bir ürüne IVD diyebilmek için numunenin insan vücudundan elde edilmesi ve in vitro ortamda incelenmesi gerekmektedir. Görüldüğü üzere tanımda sıralanan amaçlardan herhangi bir veya birkaçını gerçekleştiren pek çok ürün IVD kapsamında girmektedir. IVDR ile birlikte tanıma eklenen önemli hususlardan bir tanesi de yazılımlarda artık IVD olarak kabul edilmektedir.

Hem MDR hem de IVDR risk bazlı düzenlemelerdir. Yani ürünler düşükten yükseğe doğru risk sınıflarına ayrılırlar. Bir ürünün risk sınıfı ne kadar yüksekse piyasaya arz edilebilmesi için karşılaması gereken gereklilikler o kadar yüksektir. Yönetmeliklere göre risk seviyesi yüksek olan ürünler gereklilikleri karşıladıklarını kanıtlamak için Onaylanmış Kurulardan (Notified Body) sertifika almak zorundadırlar.

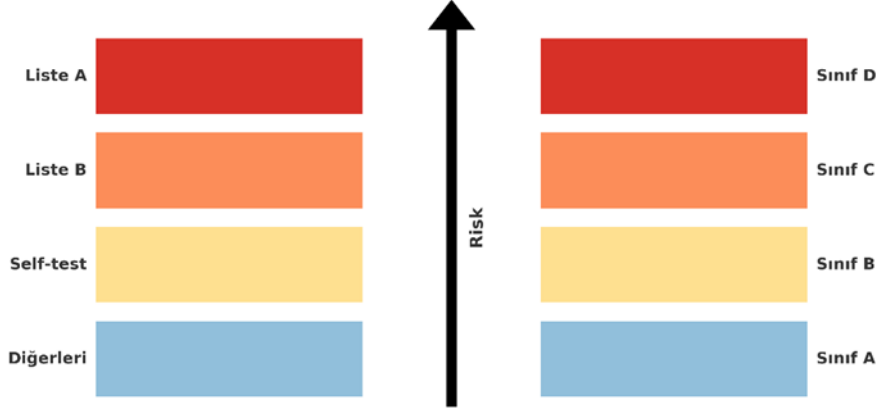
Eski düzenleme olan 98/79 EC IVDMD ürünleri 4 risk sınıfına ayırmaktaydı;

- Ek II A listesi,
- Ek II B listesi,
- Kişisel test amaçlı cihazlar,
- IVD diğer.

Bu sınıflandırmaya göre A ve B listesi, kişisel test amaçlı cihazlar ile diğer kapsamında giren çok az IVD cihaz onaylanmış kuruluştan sertifika almak zorundaydı.

IVDR ile birlikte risk sınıflandırma kuralları ve isimlendirmesi değişmiştir. 7 tane sınıflandırma kuralı ile A, B, C ve D olmak üzere 4 risk sınıfı belirlenmiştir. A, en düşük; D ise en yüksek risk sınıfını oluşturmaktadır. B, C ve D sınıfına giren ürünlerin piyasaya arz edilebilmesi için onaylanmış kuruluşa başvurması ve sertifika alması zorunludur. Yapılan bu değişiklikle eskiden IVD ürünlerin sadece %20’si onaylanmış kuruluştan sertifika almak zorunda iken bu sınıflandırmaya göre yaklaşık %80’i onaylanmış kuruluştan sertifika alması gereken daha yüksek risk sınıfına girmiştir.

98/79/EC IVDMD vs 2017/746 IVDR - Risk Sınıfları Karşılaştırması



Şekil 1.98/79 EC IVDMD ve (EU) 2017/746 IVDR risk sınıflandırmasının karşılaştırılması

Risk sınıfından bağımsız olarak tüm IVD cihazların yönetmeliğin Ek I'inde detaylandırılan Genel Güvenlilik ve Performans Gerekliliklerini karşılaması gerekmektedir. Bu Ek'e göre piyasaya arz edilen IVD cihazlar amaçlanan performansını gerçekleştirmek ve güvenli olmak zorundadır. İmalatçılar bu gerekliliği karşılamak için kalite yönetim sisteminin (KYS) bir parçası olarak veya KYS'den bağımsız bir risk yönetim sistemi kurmak zorundadır. Kurduğu bu sistem, ürettiği IVD cihazın risk sınıfına uygun olmalıdır.

İmalatçılar için IVD cihaz risklerini yönetmek zor olabilir. Çünkü IVD cihazların riskleri dolaylıdır ve genelde klinik laboratuvarlarda kullanıldığı için kullanıcıları laboratuvar personeli ve klinisyenler gibi eğitilmiş kişilerdir. İmalatçılar burada ISO 15189 Tıbbi Laboratuvarlar – Kalite ve Yeterlilik İçin Şartlar ve ISO 22367 Tıbbi laboratuvarlar – Tıbbi laboratuvarlara risk yönetimi uygulaması gibi standartlardan da yararlanabilirler.

Risk yönetimindeki ilk adım IVD cihazın kullanım amacı ve yeridir. Örneğin organ veya kan nakli uygunluğunu ölçen bir IVD ile idrar kabının risk seviyeleri birbirinden farklıdır. Aynı şekilde hastane ortamında veya evde kullanım senaryosunda ortaya çıkan risk değişecektir.

İmalatçı ürettiği IVD cihazın risklerini yönetirken analitik ve klinik kullanımını ile cihazın kullanıcı profilini iyi belirlemelidir. Çünkü klinik laboratuvarında analitik ve klinik kullanımı yapan kişiler ayrı iken söz konusu cihaz kişisel kullanıma yönelik bir IVD olduğunda (glukometre gibi) kullanıcı tek kişidir.

Aynı zamanda IVD cihazın kalitatif veya kantitatif ölçüm yapması karşılaması gereken zorunlulukları değiştirmektedir. IVD cihazın hem analitik hem de tanısal spesifikite ve sensitivite limitleri değişebilmektedir.

İçerisinde yazılım içeren IVD cihazlar (otoanalizörler vb.) veya kendi başına yazılım olan IVD cihazlar özel riskler taşımaktadırlar. Özellikle kişisel veri içeren durumlarda yazılımın hatasız çalışması, veri aktarımı ve depolanması gibi hususlar ön plana çıkmaktadır. Hasta sonuçlarını yorumlayan veya laboratuvar testleri ile ilgili genel eğilimleri belirleyen vb. alanlarda kullanılan yapay zeka yazılımlarının riskleri dikkatli ele alınmalıdır.

İmalatçı ürettiği IVD cihazla ilgili tüm tehlikeli durumları tanımlamalı ve öngörülebilir olay dizilerini / senaryolarını analiz etmelidir. Bunu yaparken kendi kontrolü altında olan ve olmayan faaliyetleri de dikkate almalıdır (imalatçının, IVD cihazı kullananın veya klinisyenin kontrolündeki).

Daha sonra riski hesaplamalı ve değerlendirmelidir. İmalatçı, daha önceden “düşük, orta, yüksek” veya “kabul edilebilir, azaltılmalı, kabul edilemez” gibi farklı risk değerlendirme kriterleri oluşturmaktadır.

Risk: Zararın meydana gelme olasılığı x Zararın şiddeti

RISK ASSESSMENT MATRIX					
PROBABILITY	Likely	Low	Medium	High	High
	Unlikely	Unlikely	Medium	High	Catast
	Rare	Low	Medium	Serious	Catastrophic
	Rare	Low	Low	Serious	Catastrophic
		Negligible	Minor	Serious	Catastrophic
		SEVERITY			

Şekil 2. Örnek risk matrisi

Ortaya çıkan risk düzeyi yüksekse, imalatçı riski düşürmek için çeşitli tedbirler alır. Tasarım değişikliği, ekstra güvenlik önlemleri, kalite kontrol prosedürleri, bilgilendirici dokümanlar veya kullanıcı eğitimleri bu tedbirlerden bazılarıdır. Tüm bu tedbirlerden sonra geride hala risk kalmış olabilir ve buna “artık risk” denir.

İmalatçı artık riskleri ve IVD cihazın faydalarını detaylandıran bir fayda – risk analizi yapar ve ürünün güvenli olduğunu, amaçlanan performansını gerçekleştirdiğini gösteren bir doküman hazırlar. Ürünü piyasaya arz ettikten sonra takip etmesi gereken hususlara yönelik dokümantasyonu hazırlamalıdır.

IVDR imalatçılara birçok sorumluluk yüklerken aynı zamanda sağlık kuruluşlarının da sorumluluklarını tanımlamaktadır. Yönetmeliğin “Piyasaya arz ve hizmete sunum” başlıklı 6. maddesinin 5. fıkrasında;

“Ek I’de belirtilen ilgili genel güvenlik ve performans gereklilikleri hariç olmak üzere, bu Yönetmeliğin gereklilikleri, aşağıdaki koşulların tümünün sağlanması şartıyla, sağlık kuruluşları bünyesinde imal edilen ve kullanılan cihazlara uygulanmaz:

a) Cihazların başka bir tüzel kişiliğe aktarılmaması.

b) Cihazların imalatı ve kullanımının uygun kalite yönetim sistemleri altında gerçekleştirilmesi.

c) Sağlık kuruluşuna ait laboratuvarın ilgili mevzuata, EN ISO 15189 standardına veya uygulanabildiği hallerde Türk Akreditasyon Kurumu tarafından belirlenen akreditasyonla ilgili hükümlere uyumlu olması.

ç) Sağlık kuruluşunun hedef hasta grubunun spesifik ihtiyaçlarının karşılanamadığını veya piyasada bulundurulmuş eş değer bir cihaz tarafından uygun performans seviyesinde karşılanamadığını kendi dokümantasyonunda değerlendirmesi.

d) Sağlık kuruluşunun, bu tür cihazların kullanımına ilişkin bilgiler ile cihazların imalatının, modifiye edilmesinin ve kullanılmasının gerekçesini içeren bilgileri, talebi halinde Kuruma sağlaması.

e) Sağlık kuruluşunun, aşağıdaki bilgileri içerecek şekilde kamuya açık bir beyan hazırlaması:

1) İmalat yapan sağlık kuruluşunun adı ve adresi.

2) Cihazları tanımlamak için gerekli ayrıntılar.

3) Cihazların Ek I'de belirtilen genel güvenlik ve performans gerekliliklerini karşıladığının beyanı ve uygulanabildiği hallerde makul bir gerekçeyle birlikte hangi gerekliliklerin tamamen karşılanmadığı hakkında bilgi.

f) Ek VIII'de belirtilen kurallar uyarınca sınıf D cihazlarla ilgili olarak sağlık kuruluşu tarafından; imalat tesisinin, imalat sürecinin, kullanım amacı da dâhil olmak üzere cihazların tasarım ve performans verilerinin anlaşılmasını mümkün kılan ve Kurumun Ek I'de belirtilen genel güvenlik ve performans gerekliliklerinin karşılandığını tespit etmesine imkân sağlayacak şekilde yeterince ayrıntılı dokümantasyon hazırlanması. Kurum, bu hükümleri Ek VIII'de belirtilen kurallar uyarınca sınıf A, B veya C cihazlara da uygulayabilir.

g) Sağlık kuruluşunun, tüm cihazların (f) bendinde atıfta bulunulan dokümantasyon uyarınca imal edilmesini sağlamak üzere gerekli bütün tedbirleri alması.

ğ) Sağlık kuruluşunun, cihazların klinik kullanımından kazanılan deneyimleri gözden geçirmesi ve gerekli tüm düzeltici faaliyetleri yürütmesi." hükmü yer alır.

Bu madde "in-house cihaz" olarak tanımlanan ve herhangi bir sağlık kuruluşunda (hastaneler, laboratuvar vb.) üretilen cihazları anlatmaktadır. Buna örnek olarak PCR ana karışımı (bir sağlık kuruluşu, bilimsel literatüre dayalı olarak primer sipariş eder ve insan DNA/RNA numuneleri üzerinde PCR testi yürütmek üzere tampon, primerler, dNTP'ler, kofaktörler ve enzimler içeren kendi in-house ana karışımını imal eder) verilebilir¹⁸.

Sağlık kuruluşları, in-house ürettikleri IVD cihazların, IVDR'ın Ek I'indeki ilgili genel güvenlik ve performans gereklilikleriyle uyumlu olmasını sağlamalıdır. Bunun için bir risk yönetim sistemi kurmalı ve fayda-risk analizi yapmalıdır. IVD cihazın tasarımı, imalatı, performansı ile ilgili gereklilikleri açıklamalı ve bunları nasıl gerçekleştirdiğini kayıt altına almalıdır. Ayrıca ürettiği bu in-house IVD cihazı başka bir tüzel kişiliğe devredemez.

Yönetmelik açıkça in-house cihazların imalatı ve kullanımının uygun bir KYS altında yapılması söyler. İşletilen KYS; klinik laboratuvarlarla ilgili mevzuata, ISO 15189 standardına veya Türk Akreditasyon Kurumu akreditasyon standartlarına uygun olmalıdır.

SONUÇ

İlgili maddenin 4 bendi hariç tüm hususları MDCG 2023-1 dokümanında belirtilen zaman çizelgesine göre yürürlüğe girmiştir. 26 Mayıs 2028 tarihi itibarıyla 6. madde tamamen yürürlüğe girecektir.

Bu şu anlama gelmektedir, herhangi bir klinik laboratuvar kendi bünyesinde IVD tanıma giren bir cihaz üretiyorsa (reaktif, kontrol, kalibratör, yazılım vb. gibi) yönetmeliğin gereklerine uymak zorundadır. Laboratuvarında sürekliliği olan bir KYS kurmalı ve işletmelidir. Ürettiği in-house IVD cihazın genel güvenlik ve performans gerekliliklerini karşılamasını sağlamalı, bununla ilgili tüm kanıtları oluşturmalı ve saklamalıdır. İlgili maddede detayları verilen kamuya açık bir beyan hazırlamalıdır. Gerekğinde Türkiye İlaç ve Tıbbi Cihaz Kurumuna sunulmak üzere ayrıntılı bir dokümantasyon hazırlamalıdır. İn-house ürettiği IVD cihazın performansını takip etmeli, verileri gözden geçirmeli ve gerekiyorsa düzeltici faaliyet yürütmelidir. Bu hususlar şu anda yürürlükte değildir.

"Hedef hasta grubunun spesifik ihtiyaçlarının karşılanamadığını veya piyasada bulunan eş değer bir cihaz tarafından uygun performans seviyesinde karşılanamadığını" gösterilmesi hususu henüz yürürlükte değildir. Ancak bu konudaki hazırlıkların ve dokümantasyon sürecinin en kısa sürede başlatılması sağlık kuruluşlarının yararına olacaktır.

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COVID-19 HASTALARINDA GALECTİN-3, İMA, MPO VE PON-1 SERUM DÜZEYLERİNİN KARŞILAŞTIRMALI ANALİZİ: POTANSİYEL TANISAL BİYOBELİRTEÇLER

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ÖZET

Amaç: Viral bir enfeksiyon hastalığı olan COVID-19, özellikle immün sistem aracılı inflamatuvar yanıtlar açısından miyokardit ile önemli patofizyolojik benzerliklere sahiptir. İMA, Galektin-3, PON-1 ve MPO gibi biyomarkerlar hem miyokardit hem de COVID-19’da hastalık süreçlerinin anlaşılması ve tanisal değerlendirmede kullanılabilmektedir. Dolayısıyla çalışmamızda miyokardit etiopatogenezinde kullanılan bu biyomarkerlarının COVID-19 hastalığı patofizyolojisi ile olan ilişkisini değerlendirmeyi amaçladık.

Materyal ve Metot: Bu çalışma COVID-19 servis ve yoğun bakımında yatan iki hasta grubuyla yapıldı; Grup I: COVID-19 tanısı alan hastalar (n=45), Grup II: Kontrol (n=30); herhangi bir ilaç kullanmayan sağlıklı kişilerden seçildi. COVID-19 hastalarından alınan kanlar biyokimyasal olarak incelendi. Serum İMA, MPO, Galektin-3 ve PON-1 analizleri ELISA yöntemiyle mikroelisa cihazında gerçekleştirildi. Oluşan enzimatik tepkime ürünü spektrofotometrik veya spektrofluorometrik olarak ölçüldü.

Bulgular: COVID-19 hasta grubunda serum Galektin -3, İMA , MPO düzeylerinin kontrol grubuna göre yükseldiği PON-1 düzeyini ise azaldığı ve istatistiksel olarak anlamlı olduğu tespit edildi. Serum Galektin-3, İMA, MPO, ve PON-1 düzeylerinin ağır COVID-19 hastalarında yüksek olduğu ve istatistiksel olarak anlamlı olduğu tespit edildi.

Sonuç: Çalışmamızda, COVID-19 hastalarında Galektin-3, İMA ve MPO düzeylerinin artarken PON-1 düzeylerinin azaldığı gözlemlenmiş, bu bulgular miyokarditte de benzer şekilde görülen biyobelirteç değişimleriyle örtüşmüştür. Bu durum, bu biyobelirteçlerin hem COVID-19’un hem de miyokarditin inflamatuvar ve immünolojik mekanizmalarını yansıttığını, dolayısıyla tanı ve hastalık şiddetinin belirlenmesinde ortak biyolojik göstergeler olarak kullanılabileceğini düşündürmektedir.

Anahtar Kelimeler: COVID-19, Miyokardit, İMA, Galektin-3, PON-1, MPO

ETİK BEYAN: Prospektif olan çalışmamız, Mardin Artuklu Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'ndan 2021/3 nolu onay alınarak başlatılmıştır. Ayrıca çalışmamız Helsinki Bildirgesi'ne uygundur ve tüm hastalardan aydınlatılmış onam formu (imzalı) alınmıştır.

COMPARATIVE ANALYSIS OF GALECTIN-3, IMA, MPO, AND PON-1 SERUM LEVELS IN COVID-19 PATIENTS: POTENTIAL DIAGNOSTIC BIOMARKERS

ABSTRACT

Objective: COVID-19, a viral infectious disease, shares significant pathophysiological similarities with myocarditis, particularly in terms of immune-mediated inflammatory responses. Biomarkers such as IMA, Galectin-3, PON-1, and MPO can be used in the understanding and diagnostic evaluation of disease processes in both myocarditis and COVID-19. Therefore, in our study, we aimed to evaluate the relationship between these biomarkers used in the etiopathogenesis of myocarditis and the pathophysiology of COVID-19.

Material and Method: This study was conducted with two patient groups hospitalized in the COVID-19 ward and intensive care unit: Group I: Patients diagnosed with COVID-19 (n=45), and Group II: Control (n=30), selected from healthy individuals not taking any medication. Blood samples from COVID-19 patients were biochemically analyzed. Serum IMA, MPO, Galectin-3, and PON-1 analyses were performed using the ELISA method on a microELISA device. The enzymatic reaction product was measured spectrophotometrically or spectrofluorometrically.

Results: Serum levels of Galectin-3, IMA, and MPO were found to be increased in the COVID-19 patient group compared to the control group, while PON-1 levels were decreased and statistically significant. Serum levels of Galectin-3, IMA, MPO, and PON-1 were found to be higher in severe COVID-19 patients and statistically significant.

Conclusion: In our study, we observed increased levels of Galectin-3, IMA, and MPO, while decreased levels of PON-1 in COVID-19 patients. These findings coincide with similar biomarker changes seen in myocarditis. This suggests that these biomarkers reflect the inflammatory and immunological mechanisms of both COVID-19 and myocarditis and can therefore be used as common biomarkers in diagnosis and determination of disease severity.

Keywords: COVID-19, Myocarditis, IMA, Galectin-3, PON-1, MPO

ETHICAL STATEMENT: Our prospective study was initiated after obtaining the necessary approval from the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (Non-Interventional Clinical Research Ethics Committee, numbered 2021/3). Furthermore, our study complies with the Declaration of Helsinki, and signed informed consent forms were obtained from all patients.

ANTICANCER EFFECTS OF CREPIDIUM ACUMINATUM ON MULTIPLE IN VITRO CELL LINES

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ABSTRACT

Introduction and Purpose: Cancer is a persistent global health challenge characterized by abnormal cell growth with uncontrolled proliferation and an inability to regulate cellular division. These malignant cells often form tumors with the potential to metastasize to distant organs. Current treatment modalities, such as chemotherapy, radiotherapy, and chemically synthesized drugs, can be effective but are frequently associated with significant side effects, imposing both physical and psychological stress on patients. In recent years, there has been growing interest in exploring natural plant products for anticancer therapy. The Orchidaceae family, the second-largest group of flowering plants, comprises approximately 900 genera and 35,000 species. Orchids have been valued for their medicinal properties since ancient times, with historical records from China, Theophrastus, and references in the Vedas and the Bible noting their therapeutic potential. Among these, *Crepidium acuminatum* produces a diverse range of bioactive compounds that could be a rich source of anticancer agents. The aim of this study was to investigate the potential anticancer activity of *C. acuminatum* extracts on multiple human cancer cell lines in vitro, with a focus on apoptosis induction.

Materials and Methods: Multiple human in vitro cancer cell lines were selected for the study, including: Hec1A (endometrial adenocarcinoma), HUT102 (T-cell leukemia), Colo205 (colon carcinoma), Karpas (B-cell lymphoma), LNCaP (prostate carcinoma), MCF-7 (breast adenocarcinoma), OSTR (osteosarcoma), YC (specific subtype not detailed). All cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and antibiotics under standard cell culture conditions. The herbal plant *Crepidium acuminatum* was processed to obtain extracts containing bioactive compounds. These extracts were applied to the cancer cell lines, and cell viability along with apoptosis induction was assessed using standard in vitro assays.

Results: Treatment with *C. acuminatum* extracts demonstrated significant apoptotic activity across multiple tested cell lines. The extent of cell death varied among cell types but consistently indicated a cytotoxic effect leading to programmed cell death rather than nonspecific necrosis.

Discussion: The observed apoptosis in diverse cancer cell lines suggests that *C. acuminatum* contains phytochemicals capable of interfering with malignant cell survival pathways. These findings align with historical records of orchids being used for medicinal purposes and support their potential as sources of novel anticancer agents. Given the limitations and side effects of conventional cancer treatments, plant-derived therapeutics such as those from *C. acuminatum* may provide alternative or complementary approaches. However, the exact molecular mechanisms behind the observed anticancer effects remain to be elucidated. Further studies, including compound isolation, molecular target identification, and in vivo evaluations, are necessary before clinical applications can be considered.

Conclusion: This study highlights the anticancer potential of *Crepidium acuminatum* extracts, which induced apoptosis in various human cancer cell lines in vitro.

These results provide preliminary evidence supporting further research into *C. acuminatum*-derived compounds as candidates for developing plant-based anticancer therapies.

Keywords: *Crepidium acuminatum*, Anticancer properties, Orchidaceae, Chemotherapy, Radiotherapy, Cancer

ANTIOXIDANT AND ANTI-INFLAMMATORY MECHANISMS OF AFRICAN SPICES AND HERBS

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ABSTRACT

Spices and herbs have long been an important part of African cuisine and ethnomedicine, offering not only flavor and fragrance but also bioactive compounds with potent health benefits. More and more, the antioxidant and anti-inflammatory characteristics of African spices and herbs are being found to be the key to their disease fighting and health promoting effects. This paper reviews and compares some key African spices and herbs such as *Zingiber officinale*, *Curcuma longa*, *Allium sativum*, *Capsicum annum*, *Piper guineense* and *Xylopia aethiopica*. Data were collated from peer-reviewed literature through PubMed, Scopus, and Web of Science with inclusion criteria being African spices and herbs with proven antioxidant and anti-inflammatory activity. Mechanistically, these botanicals exhibit antioxidant activity by scavenging free radicals, increasing endogenous antioxidant enzyme systems and chelating transition metals. Their anti-inflammatory activities are exerted by pro-inflammatory cytokine (e.g., TNF- α , IL-6) inhibition, nuclear factor kappa B (NF- κ B) suppression, cyclooxygenase-2 (COX-2) downregulation, and nitric oxide modulation. These two functions have consequences for addressing oxidative stress associated chronic diseases such as cardiovascular diseases, diabetes, arthritis, and neurodegenerative diseases. This paper underscores African spices and herbs as an untapped source of nutraceuticals of international importance. But clinical validation, dosage optimization, standardization, and mechanistic elucidation are areas where research gaps remain. Enhancing interdisciplinary research, omics integration, and policy advocacy for African nutraceuticals are key to unlocking their potential therapeutic and economic value.

Keywords: African spices, Antioxidants, Anti-inflammatory mechanism, Phytochemicals, Medicinal plants

INTRODUCTION

Spices and herbs have been central to African food culture and ethnopharmacology for centuries. Beyond their culinary applications, they are recognized for their diverse bioactive constituents, which include polyphenols, flavonoids, alkaloids, terpenoids, and organosulfur compounds (Akinmoladun et al., 2020). Oxidative stress and chronic inflammation are widely acknowledged as underlying mechanisms in the pathogenesis of non-communicable diseases (NCDs) such as cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders (Liguori et al., 2018). The ability of natural products to modulate these processes underscores their significance in preventive and therapeutic strategies.

African spices and herbs, including ginger, turmeric, garlic, chili pepper, West African black pepper, and Ethiopian pepper, are rich in phytochemicals that target oxidative and inflammatory pathways. Despite growing scientific interest, most research remains fragmented and underrepresented in global nutraceutical markets. This paper reviews the antioxidant and anti-inflammatory mechanisms of African spices and herbs, synthesizing evidence to highlight their health promoting potential and identify research gaps.

LITERATURE REVIEW

Antioxidants act by neutralizing free radicals, enhancing enzymatic defenses, and preventing biomolecular damage. Polyphenols such as curcumin, quercetin, and eugenol demonstrate free radical scavenging and lipid peroxidation inhibition (Rahman et al., 2021). In African herbs, these effects are complemented by anti-inflammatory mechanisms involving modulation of NF- κ B, COX-2, and inducible nitric oxide synthase (iNOS) pathways.

For instance, *Zingiber officinale* (ginger) contains gingerols and shogaols that inhibit prostaglandin and leukotriene biosynthesis, reducing inflammatory pain (Mashhadi et al., 2013). *Curcuma longa* (turmeric), though not indigenous to Africa, is cultivated in Nigeria and Ghana and widely used in traditional medicine. Its curcuminoids downregulate NF- κ B and reduce oxidative stress markers (Aggarwal & Harikumar, 2009). *Allium sativum* (garlic) exerts antioxidant effects via allicin and S-allyl cysteine, which enhance glutathione and superoxide dismutase activities (Bayan et al., 2014).

Indigenous African spices such as *Piper guineense* and *Xylopi aethiopica* are rich in alkaloids and essential oils with anti-inflammatory effects. Piperine from *P. guineense* improves bioavailability of other phytochemicals and inhibits pro-inflammatory mediators. *X. aethiopica* essential oils reduce edema and cytokine production in animal models (Okpe et al., 2020). Collectively, these botanicals contribute to a pharmacopeia of dietary antioxidants and anti-inflammatory agents with biomedical relevance.

METHODOLOGY

Study Design

This paper adopts a narrative review and comparative analysis approach, synthesizing existing literature on the antioxidant and anti-inflammatory mechanisms of African spices and herbs.

Data Sources and Search Strategy

Databases searched included PubMed, Scopus, Web of Science, and Google Scholar. Search terms used were: “African spices,” “antioxidant mechanisms,” “anti-inflammatory mechanisms,” “phytochemicals in African herbs,” “nutraceuticals,” “ginger,” “turmeric,” “garlic,” “*Piper guineense*,” “*Xylopi aethiopica*.”

Inclusion/Exclusion Criteria

- Inclusion: Peer-reviewed articles (2000–2025), studies focusing on African spices and herbs with reported antioxidant/anti-inflammatory activity, in vitro, in vivo, and clinical studies.
- Exclusion: Non-African species without African usage context, studies without mechanistic detail, non-English publications.

Data Extraction and Synthesis

Key information extracted included plant species, bioactive compounds, antioxidant mechanisms, anti-inflammatory pathways, and study models. Comparative synthesis identified overlapping mechanisms and highlighted research gaps.

RESULTS AND DISCUSSION

Antioxidant Mechanisms

African spices demonstrate antioxidant effects through:

- Free radical scavenging: Curcumin, gingerols, and quercetin neutralize reactive oxygen species (ROS).
- Enhancement of endogenous defenses: Garlic increases glutathione peroxidase and catalase activities.
- Metal chelation: Polyphenols bind iron/copper, reducing Fenton reaction induced ROS.

- Lipid peroxidation inhibition: Capsaicin and eugenol prevent oxidative damage to membranes.

Anti-inflammatory Mechanisms

Anti-inflammatory activities are mediated by:

- Cytokine modulation: Spices suppress IL-6, TNF- α , and IL-1 β production.
- NF- κ B inhibition: Turmeric and ginger block nuclear translocation of NF- κ B.
- COX-2/iNOS suppression: Gingerols and curcuminoids downregulate pro-inflammatory enzymes.
- Nitric oxide regulation: Garlic reduces excessive NO production, preventing tissue damage.

Comparative Insights

Table 1 summarizes representative African spices, bioactive compounds, and mechanistic activities. Indigenous species such as *Piper guineense* and *Xylopia aethiopica* demonstrate comparable activity to globally popular turmeric and ginger but remain under-researched. Figure 1 illustrates shared antioxidant and anti-inflammatory pathways, showing convergence on NF- κ B and ROS regulation.

Research Gaps

- Lack of clinical trials validating efficacy in African populations.
- Limited standardization of herbal formulations.
- Insufficient bioavailability and pharmacokinetic data.
- Underrepresentation of African spices in global nutraceutical markets.

Table 1. Selected African spices and herbs with antioxidant and anti-inflammatory properties

Plant (Common Name)	Key Compounds	Antioxidant Mechanisms	Anti-inflammatory Mechanisms	References
<i>Zingiber officinale</i> (Ginger)	Gingerols, shogaols	ROS scavenging, lipid peroxidation inhibition	COX-2 inhibition, NF- κ B suppression	Mashhadi et al., 2013
<i>Curcuma longa</i> (Turmeric)	Curcumin	Free radical neutralization, metal chelation	NF- κ B inhibition, cytokine downregulation	Aggarwal & Harikumar, 2009
<i>Allium sativum</i> (Garlic)	Allicin, S-allyl cysteine	Glutathione enhancement, catalase activation	iNOS suppression, NO regulation	Bayan et al., 2014
<i>Capsicum annuum</i> (Chili)	Capsaicin	Lipid peroxidation inhibition	TNF- α suppression, COX-2 inhibition	Rahman et al., 2021
<i>Piper guineense</i> (West African black pepper)	Piperine	ROS scavenging, bioenhancer	Cytokine suppression, NF- κ B inhibition	Okpe et al., 2020
<i>Xylopia aethiopica</i> (Ethiopian pepper)	Essential oils, alkaloids	ROS scavenging	Edema reduction, cytokine inhibition	Okpe et al., 2020

CONCLUSION & RECOMMENDATIONS

African spices and herbs constitute a valuable reservoir of antioxidant and anti-inflammatory agents with significant potential in disease prevention and therapy. Their mechanisms converge on the modulation of oxidative stress and inflammatory signaling pathways, positioning them as nutraceutical candidates against NCDs. To fully harness their potential, future research should prioritize clinical validation, pharmacokinetic profiling, standardization, and exploration of synergistic effects among spices. Policymakers and industry stakeholders should promote African nutraceuticals through supportive regulations, investment in biotechnological innovations, and global market integration.

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NATURAL PRODUCT PROTEIN INTERACTIONS: IMPLICATIONS FOR DRUG DISCOVERY

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ABSTRACT

Natural products have been at the foundation of modern drug discovery, with more than 50% of approved drugs being natural product derivatives or inspired by natural scaffolds. The medicinal value of natural products is, to a large extent, defined by their interactions with biomacromolecules, especially proteins, which are the predominant drug targets. This paper survey NP-PI with a focus on their impact for rational drug discovery and development. A narrative review and comparison using PubMed, Scopus, and Web of Science literature on natural product binding, structural diversity, and target modulation of proteins was conducted. Principal mechanisms feature covalent and noncovalent interactions (hydrogen bonding, hydrophobic effects, ionic interactions, π - π stacking), which account for affinity and specificity. Case studies feature clinically relevant drugs such as taxol (microtubule stabilizer), artemisinin (PfATP6), and curcumin (NF- κ B modulation). Structural biology, high throughput screening, molecular docking, and omics based approaches opened new horizons in understanding NP-PI and made it possible to identify new leads for antimicrobial, anticancer, and anti-inflammatory therapies. While natural products present issues of bioavailability, structural complexity, and target promiscuity, they continue to serve as an invaluable fountain of drug discovery motivation. New horizons are to merge computational biochemistry, AI, and synthetic biology to quicken the discovery of NP-PI. Above all, however, this review highlights the importance of natural product protein interactions at the interface of ancient wisdom and cutting edge pharma.

Keywords: Natural products, Protein interactios, Drug discovery, Molecular docking, Pharmacology

INTRODUCTION

Proteins are central to nearly all physiological and pathological processes, making them the most exploited targets in drug discovery. Natural products (NPs), derived from plants, microorganisms, and marine organisms, possess diverse chemical scaffolds that interact with proteins to modulate biological activity (Newman & Cragg, 2020). Historically, antibiotics such as penicillin, anticancer agents such as taxanes, and antimalarials such as artemisinin originated from natural product research.

Natural product protein interactions (NP-PI) determine pharmacological outcomes, influencing binding affinity, selectivity, and efficacy. Unlike synthetic libraries, natural products are pre-validated by evolution, having co-evolved with biological macromolecules to interact with protein pockets and domains (Harvey et al., 2015). This structural diversity makes them an invaluable resource for lead discovery.

This paper synthesizes evidence on NP-PI, exploring mechanisms, case studies, and emerging technologies, while also identifying challenges and future opportunities in drug discovery.

LITERATURE REVIEW

Mechanisms of NP Protein Interactions

NP-PI involve diverse binding modes:

- Noncovalent interactions: Hydrogen bonds, hydrophobic interactions, electrostatic forces, and van der Waals forces stabilize reversible binding.
- Covalent interactions: Certain NPs form irreversible bonds with protein residues, as in β -lactam antibiotics binding to penicillin-binding proteins.
- Allosteric modulation: Some NPs bind to regulatory sites, altering protein function without occupying the active site.
- Protein stabilization/destabilization: Taxol stabilizes tubulin filaments, while geldanamycin destabilizes Hsp90 client proteins.

Case Studies in NP PI and Drug Discovery

- Taxol (Paclitaxel): A diterpenoid from *Taxus brevifolia*, taxol binds tubulin and prevents microtubule depolymerization, revolutionizing chemotherapy (Rowinsky & Donehower, 1995).
- Artemisinin: From *Artemisia annua*, artemisinin interacts with *Plasmodium falciparum* ATPase 6 (PfATP6), causing parasite death (Eckstein-Ludwig et al., 2003).
- Curcumin: A polyphenol from turmeric, curcumin interacts with NF- κ B and COX-2, showing anti-inflammatory and anticancer potential (Gupta et al., 2013).
- Epigallocatechin gallate (EGCG): A green tea polyphenol, EGCG binds to multiple proteins, including Bcl-2 family proteins, influencing apoptosis (Khan & Mukhtar, 2018).

Emerging Insights

Recent studies emphasize network pharmacology and polypharmacology, recognizing that many natural products interact with multiple protein targets, which may underlie synergistic therapeutic effects but also raise challenges in specificity. Advances in proteomics, X-ray crystallography, cryo-electron microscopy, and molecular docking have deepened understanding of NP-PI.

METHODOLOGY

Study Design

A narrative review and comparative analysis were employed to synthesize available literature on natural product protein interactions and their implications in drug discovery.

Data Sources and Search Strategy

Databases searched included PubMed, Scopus, Web of Science, and Google Scholar.

Inclusion/Exclusion Criteria

- Inclusion: Peer-reviewed articles (2000–2025), studies on natural products with reported protein targets, mechanistic insights, or drug development potential.
- Exclusion: Non-protein targets, duplicate records, studies lacking mechanistic data, and non-English publications.

Data Extraction and Synthesis

Extracted data included natural product identity, protein targets, interaction mechanisms, and therapeutic implications. A comparative synthesis was used to highlight patterns and research gaps.

RESULTS AND DISCUSSION

Binding Mechanisms of Natural Products with Proteins

Natural products exert therapeutic effects by binding to protein targets via:

- Hydrogen bonding: Polyphenols (e.g., quercetin, EGCG) form hydrogen bonds with active site residues, stabilizing enzyme inhibition.
- Hydrophobic interactions: Terpenoids (e.g., taxanes) bind deep hydrophobic pockets, ensuring high affinity.
- Covalent modification: Alkaloids and β -lactams covalently modify protein residues, leading to irreversible inhibition.
- Allosteric binding: Flavonoids modulate enzyme kinetics by binding away from active sites.

Case Studies of NP-PI in Drug Discovery

Table 1 summarizes representative examples.

Table 1. Representative natural product protein interactions in drug discovery

Natural Product	Source	Protein Target	Mechanism of Interaction	Therapeutic Use
Taxol (Paclitaxel)	<i>Taxus brevifolia</i>	Tubulin	Stabilizes microtubules	Anticancer
Artemisinin	<i>Artemisia annua</i>	PfATP6	Alkylates and inhibits ATPase	Antimalarial
Curcumin	<i>Curcuma longa</i>	NF- κ B, COX-2	Inhibits transcription and enzymes	Anti-inflammatory
Quinine	<i>Cinchona spp.</i>	Heme detoxification enzyme	Inhibits heme polymerase	Antimalarial
EGCG	Green-tea (<i>Camellia sinensis</i>)	Bcl-2, proteasome	Induces apoptosis, inhibits proteasome	Anticancer, antioxidant
Geldanamycin	<i>Streptomyces hygroscopicus</i>	Hsp90 chaperone protein	Destabilizes client proteins	Anticancer

Implications for Drug Discovery

- Structural diversity: NPs expand chemical space beyond synthetic libraries.
- Polypharmacology: Many NPs interact with multiple targets, offering potential for complex diseases like cancer but complicating target validation.
- Lead optimization: Understanding NP-PI guides structural modifications to improve potency, selectivity, and pharmacokinetics.
- Computational approaches: Molecular docking, dynamics, and AI predict NP-PI with increasing accuracy, reducing experimental costs.

Challenges

- Bioavailability: Many NPs suffer from poor absorption and rapid metabolism.
- Complexity: Structural complexity complicates synthesis and modification.
- Target identification: Multiplicity of protein targets hinders mechanistic clarity.

- Underexplored biodiversity: African and tropical flora remain underutilized despite rich NP diversity.

Future Perspectives

- Integration of omics technologies (proteomics, metabolomics) for NP-PI mapping.
- Cryo-EM and X-ray crystallography for structural elucidation.
- AI-driven screening for NP-PI prediction.
- Harnessing African natural product diversity for novel protein modulators.
- Development of nanocarriers to improve NP bioavailability.

CONCLUSION & RECOMMENDATIONS

Natural product protein interactions form the molecular basis for many therapeutic effects and continue to inspire drug discovery. Through hydrogen bonding, covalent modifications, hydrophobic effects, and allosteric modulation, natural products regulate protein function with high specificity and biological relevance. Despite limitations in bioavailability and target complexity, natural products remain indispensable for modern pharmaceuticals. Future research should focus on expanding structural libraries of natural products, applying AI driven molecular docking to accelerate NP-PI discovery, and exploring underutilized biodiversity, particularly from Africa. Enhancing collaborations between traditional medicine practitioners, natural product chemists, and molecular biologists will unlock novel bioactive compounds and support global drug innovation.

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**PREVALENCE OF ESCHERICHIA COLI O157:H7 IN WELL WATER: ZERO
DETECTION AND RESISTANCE PATTERNS OF NON O157:H7 STRAIN IN
OKEHI LOCAL GOVERNMENT AREA, KOGI STATE NIGERIA**

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ABSTRACT

Introduction: This study investigated the presence and antibiotic susceptibility profile of *Escherichia coli* O157:H7 in well water samples collected from various locations in Okehi Local Government Area, Kogi State, Nigeria. The research aimed to assess water quality by evaluating well depth, water temperature, total bacterial and coliform counts, and identifying *E. coli* O157:H7 through biochemical methods.

Method: A total of 20 water samples in duplicates were analyzed for microbial contamination using standard techniques, depth was measured in cm by measuring the string down the well, distance of well from septic tank and temperature of water sample using thermometer.

Results: The results indicating high bacterial loads and the presence of coliforms in several samples. Temperature readings ranged from 28°C to 31°C, a range suitable for the survival of mesophilic pathogens such as *E. coli*.

Discussion and Conclusion: The proximity of many wells to septic tanks often less than the WHO recommended minimum distance of 30 meters was found to be a major risk factor for contamination. Biochemical tests confirmed the presence of *E. coli* in some samples however no *E. coli* O157:H7 detected. Antibiotic susceptibility testing of non O157:H7 revealed that the isolates were resistant to β -lactam antibiotics including amoxicillin and cefoxitin, but remained susceptible to fluoroquinolones (levofloxacin, ofloxacin, ciprofloxacin) and gentamicin. The study highlights the urgent need for improved sanitation infrastructure, safe well construction practices, routine water quality monitoring, and antibiotic resistance surveillance.

These findings underscore the public health risks associated with the use of untreated well water in rural communities and call for immediate intervention to prevent outbreaks of waterborne diseases.

Keywords: E coli o157:H7; Well Depth; Septic Tank; Temperature; Okehi

TARGETED DRUG DELIVERY VIA OCULAR, PULMONARY AND NASAL ROUTES USING SOLID LIPID NANOPARTICLES

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INTRODUCTION

Targeted drug delivery systems have revolutionized therapeutic outcomes by optimizing drug efficacy, reducing side effects, and improving patient adherence. Solid lipid nanoparticles (SLNs) have emerged as a promising carrier due to their biocompatibility, ability to load both hydrophilic and lipophilic drugs, and potential for controlled release. Their non-invasive routes, including ocular, pulmonary, and nasal delivery, offer direct access to systemic or local circulation, bypassing first-pass metabolism.

Materials and Methods

This review critically analyzed studies focusing on the design, functionalization, and therapeutic use of SLNs for targeted drug delivery. Data were collected from peer-reviewed articles highlighting modifications such as mucoadhesive polymers, ligand conjugation, and stimuli-responsive materials for enhanced targeting.

Results and Discussion

Ocular SLNs improved bioavailability, corneal penetration, and drug retention by overcoming tear drainage and conjunctival absorption barriers. Pulmonary delivery demonstrated deep lung deposition with sustained release, enabling localized treatment of respiratory diseases and systemic peptide/protein delivery. Nasal administration of SLNs facilitated rapid action and potential nose-to-brain targeting via the olfactory pathway. Despite these advancements, challenges such as large-scale manufacturing, regulatory standardization, and long-term safety persist, hindering clinical translation.

Keywords: Solid lipid nanoparticles (SLNs); Targeted drug delivery; Ocular delivery; Pulmonary delivery; Nasal delivery; Mucoadhesive polymers; Controlled release; Nose-to-brain delivery; Biocompatibility; Clinical translation.

ESSENTIAL OILS FROM AROMATIC AND MEDICINAL PLANTS: EXTRACTION TECHNIQUES AND MULTIFUNCTIONAL BIOACTIVE AGENTS

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ABSTRACT

Essential oils derived from aromatic and medicinal plants (PAMs) are complex mixtures of volatile secondary metabolites with remarkable biological activities. They exhibit antimicrobial, antioxidant, anti-inflammatory, and insecticidal properties, making them promising candidates for applications in pharmaceuticals, agro-alimentary sectors, and natural preservation. Their eco-friendly nature and wide spectrum of bioactivity provide sustainable alternatives to synthetic chemicals. Advances in extraction techniques and phytochemical analysis have enhanced the identification and valorization of bioactive constituents, supporting the growing interest in essential oils as natural resources for health and industry.

Keywords: Essential oils; Aromatic plants; Medicinal plants; Antimicrobial; Antioxidant; Biotechnology.

DECODING HOSPITAL WASTEWATER PROFILES: INTEGRATING PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS WITH AI-BASED CLASSIFICATION

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ABSTRACT

Introduction and Purpose: Hospital effluents represent a complex matrix containing pathogenic microorganisms, antibiotic residues, and various chemical pollutants, posing a threat to public health, food safety, and aquatic ecosystems. Their management remains a major environmental challenge due to their high load of chemical, biological, and pharmaceutical contaminants. The objective of this study is to characterize hospital effluents using an integrated approach combining physical-chemical and microbiological analyses with advanced modeling using supervised and unsupervised machine learning.

Materials and Methods: Samples of hospital effluents were analyzed for their physical, chemical, and microbiological parameters, then compared to international standards for direct and indirect discharge. The data were processed using supervised and unsupervised classification techniques, including Principal Component Analysis (PCA) and clustering methods. An environmental risk index was used to assess the hazardousness of the discharges.

Results and Discussion: The concentrations of several physical and chemical parameters greatly exceeded regulatory thresholds. Microbiological analysis revealed conditions conducive to the development of numerous pathogenic microorganisms. Machine learning techniques highlighted significant heterogeneity in the effluents, linked to variability in organic matter, nutrient, pharmaceutical residue, and pathogen content. PCA and clustering enabled the identification of significant patterns and the determination of the main factors influencing discharge quality.

Conclusion: This study demonstrates the importance of an integrated approach combining data science and environmental monitoring for the sustainable management of hospital effluents. The models developed pave the way for intelligent warning systems capable of quickly identifying hazardous effluents and adapting treatment strategies to reduce their toxicity and preserve the quality of aquatic environments.

Keywords: Hospital wastewater; Physico-chemical analysis; Microbiological analysis; Machine learning; Environmental risk

**ROLE OF DIFFERENT SURFACTANTS (TX-100 AND TWEEN-80) IN
ENHANCING THE SOLUBILITY OF ANTIBIOTIC DRUGS (LEVOFLOXACIN)
FOR BETTER DRUG DELIVERY SYSTEM**

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ABSTRACT

This study investigates the role of two commonly used surfactants, Triton X-100 (TX-100) and Tween-80, in enhancing the solubility of the antibiotic drug levofloxacin, a broad-spectrum fluoroquinolone. Levofloxacin's poor water solubility limits its bioavailability and therapeutic efficacy, presenting a challenge for effective drug delivery. Surfactants like TX-100, a nonionic surfactant with an aromatic ether group, and Tween-80, a nonionic surfactant composed of a polyoxyethylene sorbitan ester, are known for their ability to improve drug solubility by reducing surface tension and forming micelles. In this study, the solubility of levofloxacin was analyzed in aqueous solutions of varying concentrations of TX-100 and Tween-80. The results demonstrated a significant increase in levofloxacin solubility with the addition of both surfactants, with Tween-80 showing a more pronounced effect at lower concentrations. The enhancement in solubility is attributed to the formation of micelles by the surfactants, which encapsulate the hydrophobic drug molecules, thereby increasing their aqueous solubility. This research highlights the potential of TX-100 and Tween-80 as solubilizing agents in pharmaceutical formulations, offering a promising approach to improving the bioavailability of levofloxacin. The findings suggest that surfactant-based strategies could be effectively employed to enhance the therapeutic efficacy of poorly soluble drugs.

NEW PROTEOMIC ANALYSES FOR CANCER BIOMARKERS: ADVANCING PRECISION ONCOLOGY THROUGH MOLECULAR PROFILING

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ABSTRACT

The identification of reliable cancer biomarkers is critical for early detection, prognosis, and personalized treatment strategies. With advances in high-throughput proteomic technologies, researchers are now able to comprehensively analyze the protein expression profiles of tumor and biofluid samples, leading to the discovery of novel biomarkers with high diagnostic and prognostic potential. Unlike genomic approaches, which focus on static DNA mutations, proteomics reflects the dynamic functional state of cells and their response to environmental and pathological changes.

Recent innovations such as mass spectrometry-based proteomics, quantitative label-free analysis, isobaric tagging (iTRAQ, TMT), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have significantly improved the sensitivity, specificity, and throughput of protein detection. These techniques have enabled the discovery of key cancer-associated proteins involved in cell signaling, immune response, tumor microenvironment modulation, angiogenesis, and metastasis. In parallel, bioinformatics and machine learning tools are increasingly integrated into proteomic workflows to extract clinically relevant signatures from complex datasets.

Proteomic biomarkers are being explored for a range of malignancies including breast, lung, colorectal, pancreatic, and ovarian cancers, with several candidates undergoing validation in clinical trials. Moreover, extracellular vesicles(exosomes) and post-translational modifications (PTMs) such as phosphorylation and glycosylation offer new layers of biomarker insight. Despite promising advances, challenges remain in terms of sample standardization, reproducibility, and translation to clinical use. Continued integration of proteomics into multi-omics frameworks promises to accelerate the development of precision oncology and improve patient outcomes.

Keywords: Proteomics, Cancer Biomarkers, Mass Spectrometry (MS), LC-MS/MS, iTRAQ, TMT (Tandem Mass Tag), Early Cancer Detection, Quantitative Proteomics, Post-Translational Modifications (PTMs), Exosomes, Tumor Microenvironment, Precision Oncology, Bioinformatics, Systems Biology, Molecular Profiling

INTRODUCTION

Cancer remains a global health challenge, ranking among the leading causes of morbidity and mortality despite significant advances in prevention, diagnosis, and treatment. According to the World Health Organization, cancer accounted for nearly 10 million deaths in 2020, with projections suggesting a continued increase in incidence due to aging populations, lifestyle factors, and environmental exposures. Traditional diagnostic methods, such as imaging, histopathology, and serum-based assays, though indispensable, often lack the sensitivity and specificity required for early detection and accurate prognosis.

Moreover, conventional approaches are limited in their ability to predict therapeutic responses, contributing to treatment resistance and poor clinical outcomes in a significant subset of patients.

In recent years, the emergence of precision oncology has transformed cancer research and clinical practice. This paradigm shift emphasizes tailoring therapeutic interventions to the molecular landscape of an individual's tumor rather than applying uniform treatment strategies. While genomics-driven approaches have yielded important insights by identifying oncogenic mutations and molecular subtypes, they often provide a static snapshot of the cancer genome. Such genomic information, although critical, cannot fully capture the dynamic interplay of cellular signaling pathways, tumor microenvironmental changes, and adaptive responses to therapy.

Proteomics, the comprehensive large-scale study of proteins and their post-translational modifications, offers a complementary and functionally relevant perspective. Since proteins act as direct effectors of cellular function, their abundance, localization, and modifications provide real-time information about disease states and therapeutic responsiveness. Unlike genomic mutations, which may or may not translate into phenotypic consequences, proteomic alterations reflect the actual biochemical activities driving tumor growth, metastasis, and resistance. For instance, aberrant phosphorylation, glycosylation, or acetylation of proteins can modulate signaling cascades in ways that profoundly influence tumor progression, making proteomic analysis an indispensable tool in biomarker discovery.

Advancements in high-throughput proteomic platforms, such as mass spectrometry (MS)-based proteomics, protein microarrays, and next-generation antibody-based assays, have enabled unprecedented depth and resolution in characterizing the cancer proteome. These technologies facilitate the identification of diagnostic biomarkers for early detection, prognostic markers for disease progression, and predictive markers for treatment selection. Moreover, integrative approaches combining proteomics with genomics, transcriptomics, and metabolomics—collectively termed multi-omics—are now unraveling the complex molecular networks underlying cancer heterogeneity and therapeutic resistance.

Despite these advances, the clinical translation of proteomic biomarkers faces considerable challenges. Issues such as sample heterogeneity, variability in analytical workflows, standardization of methodologies, and regulatory hurdles continue to impede their routine application in oncology. Nonetheless, with ongoing technological innovations, machine learning-driven data integration, and large-scale biomarker validation studies, proteomics holds immense promise to revolutionize precision oncology by enabling more accurate, real-time, and patient-tailored interventions.

This article critically examines the progress and prospects of proteomics in cancer biomarker discovery. We explore the technological advancements driving the field, highlight successful applications in oncology, and discuss the major challenges and future directions required for their clinical implementation.

Proteomic Technologies in Biomarker Discovery

Mass Spectrometry-Based Proteomics

Mass spectrometry (MS) has become the cornerstone of proteomics due to its ability to identify and quantify thousands of proteins with high sensitivity and specificity. Modern workflows typically combine liquid chromatography with tandem mass spectrometry (LC-MS/MS), which separates complex protein mixtures and allows in-depth peptide sequencing.

Continuous advancements in resolution, dynamic range, and speed have made MS an indispensable tool for cancer biomarker research. For example, data-independent acquisition (DIA) strategies such as SWATH-MS have enabled reproducible quantification across large patient cohorts, making them suitable for translational studies. Additionally, targeted MS methods like multiple reaction monitoring (MRM) are now being applied in clinical laboratories to validate candidate biomarkers for diagnostic use.

Quantitative Proteomics

Accurate protein quantification is essential for identifying biomarkers that distinguish healthy from diseased states or predict treatment responses. Several quantitative strategies have emerged:

- **Label-Free Quantification (LFQ):** Relies on peptide ion intensities or spectral counting. It is cost-effective and suitable for large-scale studies, but variability in sample preparation can affect reproducibility. LFQ has been successfully used in breast and prostate cancer research to profile differential protein expression.
- **Isobaric Tagging (iTRAQ, TMT):** Involves labeling peptides with chemically identical but isotopically distinct tags, allowing multiplexing of up to 16 samples in a single MS run. This approach enhances throughput and comparability, making it ideal for large cohort studies. For example, TMT-based proteomics has identified protein signatures predictive of chemotherapy response in ovarian and colorectal cancers.
- **Stable Isotope Labeling (SILAC):** Incorporates heavy isotopes into proteins during cell culture, enabling highly accurate quantification. Though limited to cell-line or model system studies, SILAC provides mechanistic insights into cancer cell signaling and drug resistance pathways.

Post-Translational Modifications (PTMs)

Proteins undergo diverse PTMs that modulate their activity, localization, and interactions, profoundly influencing cancer biology. Proteomic technologies now allow systematic mapping of PTM landscapes, offering valuable biomarker candidates:

- **Phosphorylation:** Aberrant kinase signaling drives tumor proliferation and survival. Phosphoproteomics has revealed hyperactivation of PI3K/AKT and MAPK pathways in multiple cancers, which not only serves as biomarkers but also as therapeutic targets.
- **Glycosylation:** Altered glycosylation patterns influence cancer cell adhesion, immune evasion, and metastasis. Serum glycoproteomics has identified specific glycoforms of alpha-fetoprotein and mucins as diagnostic biomarkers in liver and pancreatic cancers.
- **Ubiquitination:** Dysregulated ubiquitin-proteasome signaling contributes to tumor progression and therapy resistance. Mapping ubiquitination sites helps identify novel druggable pathways in cancers such as multiple myeloma.
- **Acetylation & Methylation:** Epigenetic regulation through histone modifications alters chromatin accessibility, thereby driving oncogene activation or tumor suppressor silencing. Proteomic approaches to histone modification profiling are being actively pursued in hematological and solid tumors.

By integrating these PTM datasets with genomic and transcriptomic data, researchers are now developing multi-omics biomarker panels that provide a holistic understanding of cancer progression and treatment response.

Exosome Proteomics

Exosomes, a subtype of extracellular vesicles (30–150 nm in diameter), are secreted into body fluids such as blood, urine, saliva, and cerebrospinal fluid. They play a pivotal role in intercellular communication by transferring bioactive molecules—proteins, nucleic acids, and lipids—between tumor cells and the surrounding microenvironment. Because exosomal cargo reflects the molecular composition and functional state of the originating tumor, exosome proteomics has gained traction as a minimally invasive tool for cancer diagnosis, prognosis, and therapy monitoring.

- **Diagnostic Potential:** Exosomal proteins such as CD63, CD81, EpCAM, and integrins have been identified as surface markers that can distinguish cancer-derived vesicles from those secreted by healthy cells. For example, proteomic profiling of exosomes from pancreatic cancer patients revealed glypican-1 (GPC1) as a highly specific biomarker for early disease detection.
- **Prognostic Indicators:** Tumor-derived exosomes often contain proteins involved in metastasis and therapy resistance. In breast cancer, exosomal HER2 levels have been correlated with tumor burden and treatment outcomes, offering real-time insights into disease progression.
- **Therapeutic Monitoring:** Because exosomes are continuously secreted, their proteomic content can dynamically capture changes in tumor biology in response to treatment. For instance, longitudinal profiling of exosomal proteins has been used to track emerging resistance mechanisms in lung cancer patients undergoing tyrosine kinase inhibitor (TKI) therapy.
- **Advantages over Tissue Biopsies:** Unlike invasive tissue biopsies, exosome-based “liquid biopsies” are repeatable, scalable, and suitable for monitoring heterogeneous tumors. Moreover, exosomes protect their cargo from enzymatic degradation, improving biomarker stability.

Collectively, exosome proteomics bridges the gap between molecular tumor profiling and clinical utility, providing a pathway toward precision oncology through real-time and non-invasive monitoring.

Bioinformatics and Systems Biology Approaches

The vast complexity and high dimensionality of proteomic data demand advanced computational approaches to derive clinically actionable insights. Traditional statistical methods often fall short, making bioinformatics and systems biology essential in cancer biomarker discovery and validation.

- **Bioinformatics Pipelines:** Advanced algorithms are employed to handle raw proteomic data, including noise reduction, peak alignment, normalization, and protein identification. Machine learning models, such as support vector machines (SVMs) and random forests, are increasingly used to classify patient groups and predict treatment responses based on proteomic signatures. For example, ML-driven proteomics has identified predictive protein panels for immunotherapy responsiveness in melanoma.
- **Integration with Multi-Omics:** By combining proteomics with genomics, transcriptomics, and metabolomics, researchers can build multi-dimensional biomarker panels that provide a holistic view of tumor biology. This integration improves sensitivity and specificity compared to single-omics approaches.
- **Pathway and Network Analysis:** Systems biology approaches go beyond single-protein biomarkers by contextualizing them within broader biological networks. Proteins identified in cancer proteomics are mapped onto signaling pathways such as angiogenesis, immune regulation, epithelial–mesenchymal transition (EMT), and tumor-stroma interactions. This pathway-level insight helps uncover mechanistic underpinnings of cancer progression and potential therapeutic targets.

- Artificial Intelligence (AI): Deep learning approaches are now being applied to large-scale proteomic datasets for biomarker discovery, prognostic modeling, and drug sensitivity prediction. For example, convolutional neural networks (CNNs) trained on proteomic data have been shown to classify cancer subtypes with high accuracy, surpassing traditional statistical models.
- Clinical Translation: Bioinformatics-guided proteomics enables the identification of reproducible, clinically relevant biomarkers that can be validated across cohorts and implemented in diagnostic assays. These approaches also facilitate the development of predictive models for personalized treatment planning, ultimately bridging discovery research and bedside application.

Clinical Applications of Proteomic Biomarkers

Proteomic biomarkers have moved from experimental research into translational oncology, offering tools for early detection, prognosis, treatment stratification, and therapy monitoring. Their clinical applications span multiple cancer types, where proteomic profiling has uncovered disease-specific signatures with diagnostic and therapeutic potential.

Breast Cancer

- The discovery of HER2 (human epidermal growth factor receptor 2) as a proteomic biomarker revolutionized breast cancer management by enabling the development of HER2-targeted therapies such as trastuzumab.
- Beyond HER2, phosphoproteomic signatures provide insight into aberrant signaling pathways that underlie resistance to endocrine therapies and HER2 inhibitors.
- Proteomic approaches have also uncovered biomarkers such as mucin-1 (MUC1) and heat shock proteins, which serve as potential candidates for both prognosis and therapeutic targeting.

Lung Cancer

- Lung cancer, particularly non-small cell lung cancer (NSCLC), has benefited from proteomic advances in liquid biopsy technologies.
- Exosome-derived proteins, including annexins, integrins, and EGFR variants, have been identified as non-invasive indicators of tumor progression and resistance to targeted therapies.
- Proteomic panels are being developed to complement imaging modalities, allowing earlier detection of NSCLC and dynamic monitoring of treatment responses to tyrosine kinase inhibitors (TKIs) and immunotherapies.

Colorectal Cancer (CRC)

- Proteomic research has identified multi-protein panels that distinguish CRC patients from healthy individuals at an early stage, significantly improving screening accuracy compared to colonoscopy alone.
- Examples include panels enriched with proteins such as C-reactive protein (CRP), S100A9, and metabolic enzymes linked to tumor metabolism.
- Moreover, proteomic biomarkers provide prognostic information, helping to stratify patients for adjuvant chemotherapy based on recurrence risk.

Ovarian Cancer

- Ovarian cancer remains difficult to detect at early stages due to non-specific symptoms, making proteomic biomarkers especially valuable.
- Altered glycoprotein patterns, such as aberrant glycosylation of CA125 and haptoglobin, have been leveraged to improve differential diagnosis and distinguish malignant ovarian tumors from benign conditions.

- Additionally, proteomic profiling of ascites fluid has revealed markers of tumor aggressiveness and therapeutic resistance, guiding individualized management strategies.

Pancreatic Cancer

- Pancreatic ductal adenocarcinoma (PDAC) is notoriously aggressive and often diagnosed at advanced stages.

- Exosome proteomics has uncovered novel early-detection biomarkers such as glypican-1 (GPC1), which can be detected in circulating exosomes with high specificity.

- Proteomic profiling also provides insights into tumor–stroma interactions, which are critical in PDAC biology, thereby offering new avenues for both diagnostic and therapeutic innovation.

Clinical trials are currently underway to validate several candidate biomarkers, underscoring the translational potential of proteomics.

Challenges and Future Perspectives

Despite remarkable advances, the translation of proteomic biomarkers from bench to bedside faces multiple obstacles. These challenges arise from technical, clinical, and regulatory complexities that must be addressed before proteomics can be fully integrated into routine oncology practice.

Key Challenges

1. Lack of Standardization

- There is no universally accepted protocol for sample collection, processing, and storage, leading to significant variability in proteomic profiles across studies.

- Pre-analytical variables, such as differences in blood collection tubes, centrifugation speed, or storage temperature, can alter protein composition, undermining reproducibility.

2. Reproducibility Across Laboratories

- Proteomic platforms, including mass spectrometry and antibody-based methods, may yield inconsistent results depending on instrument sensitivity, operator expertise, and data analysis pipelines.

- This lack of cross-laboratory reproducibility hampers the validation of candidate biomarkers and delays clinical translation.

3. Scalability and Cost-Effectiveness

- Biomarker discovery often relies on resource-intensive technologies such as high-resolution mass spectrometry, which are not feasible for widespread clinical use.

- Transitioning from exploratory studies to affordable, high-throughput diagnostic assays remains a bottleneck.

4. Regulatory and Clinical Validation

- For a proteomic biomarker to be clinically adopted, it must undergo rigorous analytical validation, clinical validation, and regulatory approval.

- The pathway from discovery to approval is long and expensive, and many promising biomarkers fail during validation due to insufficient sensitivity or specificity.

Future Perspectives

1. Integration of Multi-Omics Approaches

- Combining proteomics with genomics, transcriptomics, metabolomics, and epigenomics provides a holistic view of tumor biology.
- Multi-omics integration can enhance biomarker accuracy, identify novel therapeutic targets, and support personalized treatment strategies.

2. Artificial Intelligence and Machine Learning

- AI-driven approaches can handle the complexity of proteomic datasets, identifying predictive protein signatures and stratifying patients into clinically relevant subgroups.
- Predictive models powered by machine learning are already being tested to forecast drug response, survival outcomes, and resistance mechanisms in cancer patients.

3. Organoid and Exosome-Based Proteomics

- Emerging technologies such as patient-derived organoids and exosome profiling provide platforms for real-time biomarker discovery and drug testing.
- These models bridge the gap between in vitro studies and patient-specific tumor biology, accelerating translational research.

4. Standardization and Clinical Pipeline Development

- International consortia, such as the Human Proteome Organization (HUPO), are working toward establishing standardized pipelines for proteomic analysis.
- Standardization will improve reproducibility, facilitate regulatory acceptance, and enable the development of clinically robust diagnostic assays.

5. Toward Precision Oncology

- Ultimately, the integration of validated proteomic biomarkers into clinical workflows will enable early detection, risk stratification, personalized therapies, and dynamic monitoring of treatment response.
- With continued technological advancements and interdisciplinary collaborations, proteomic biomarkers hold the promise of transforming cancer care into a truly precision-guided paradigm.

CONCLUSION

Proteomics has established itself as a cornerstone in modern cancer research, moving beyond its initial role as a discovery tool to becoming a key driver of translational oncology. Unlike genomics, which provides a static snapshot of genetic alterations, proteomics reflects the dynamic, real-time functional state of cancer cells, capturing signaling events, post-translational modifications, and tumor–microenvironment interactions. This ability to profile disease biology at the protein level gives proteomics a unique advantage in identifying biomarkers that are not only diagnostic but also prognostic and predictive of therapeutic response.

The rapid advancements in mass spectrometry technologies, such as data-independent acquisition (DIA) and high-resolution LC-MS/MS, coupled with innovations in quantitative proteomics and PTM mapping, have exponentially increased the depth and accuracy of proteome coverage.

At the same time, computational biology, machine learning, and systems-level analyses are enabling the interpretation of vast proteomic datasets, transforming raw protein signatures into clinically actionable insights. These developments have already led to significant breakthroughs, including the identification of exosome-based biomarkers for liquid biopsies, phosphoproteomic signatures for drug resistance prediction, and protein panels for early cancer detection.

However, for proteomic biomarkers to achieve their full potential in precision oncology, several hurdles must be addressed. Standardization of workflows, reproducibility across platforms, and cost-effective assay development remain pressing challenges. Overcoming these barriers will require interdisciplinary collaborations that unite clinicians, proteomics researchers, computational scientists, and regulatory authorities. Moreover, the integration of proteomics with genomics, metabolomics, and transcriptomics will create multi-omics frameworks capable of delivering a more holistic view of tumor biology, leading to more accurate biomarker discovery and personalized treatment strategies.

Looking ahead, the clinical application of proteomics is expected to transform cancer care in several dimensions:

- Early detection, through minimally invasive assays such as exosome-based liquid biopsies.
- Prognostic and predictive stratification, enabling physicians to tailor therapies to the individual molecular profiles of patients.
- Real-time treatment monitoring, allowing rapid identification of resistance mechanisms and dynamic adjustments in therapy.

In conclusion, proteomics represents not only a scientific advancement but also a paradigm shift in how we approach cancer diagnosis and treatment. With continued technological refinement, AI-powered data interpretation, and standardized clinical pipelines, proteomic biomarkers are on the path to becoming integral components of routine oncology practice. Their successful translation into the clinic will not only improve treatment precision and patient survival but also pave the way toward a future where cancer management is truly personalized, predictive, and preventive.

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ENHANCING THERAPEUTIC POTENTIAL OF MIDAZOLAM BY SYNTHESIZING ITS NANOPARTICLES

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ABSTRACT

Introduction and Purpose: Midazolam, an imidazobenzodiazepine, exhibits unique pharmacological properties compared to other benzodiazepines, including a rapid onset of action and high metabolic clearance. The use of midazolam nanoparticles (NPs) aims to bypass the blood-brain barrier, increase drug retention time, and improve its therapeutic index.

Materials and Methods: Rats were divided into five groups: (i) Saline (1ml/kg), (ii) midazolam (2.5mg/kg), (iii) midazolam-NPs (0.012mg/kg), (iv) midazolam-NPs (0.025mg/kg), and (v) midazolam-NPs (0.05mg/kg). Rats were treated with midazolam and midazolam-NPs intranasally on alternate days for two weeks. Behaviors in elevated plus maze, light dark activity box and forced swim test were monitored.

Results: The study evaluated the effects of intranasal midazolam nanoparticles (midazolam-NPs) on rat behavior, comparing them to conventional midazolam and saline controls. Key findings include stronger anxiety-reducing effects of midazolam-NPs moderate dose (0.025 mg/kg), as monitored in elevated plus maze, light dark activity box and forced swim test.

Discussion and Conclusion: Midazolam-NPs demonstrated superior efficacy in treating anxiety and depression-like behaviors at significantly reduced doses compared to standard midazolam. The nanoparticle delivery system likely improves brain targeting while minimizing systemic side effects. This approach holds promise for rapid, non-invasive treatment of neuropsychiatric disorders. Future research should explore long-term safety, clinical applications, and precise delivery mechanisms. Overall, midazolam-NPs represent a major advancement in neuropharmaceuticals, offering safer and more effective treatment options.

Keywords: midazolam, nanoparticles, drug delivery, therapeutic enhancement, bioavailability

A FACILE SYNTHESIS OF 1,3,4-OXADIAZOLE-BASED CARBAMOTHIOATE MOLECULES: ANTISEIZURE POTENTIAL, EEG EVALUATION AND IN-SILICO DOCKING STUDIES

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ABSTRACT

Introduction and Purpose: Pharmacoresistant epilepsy affects a substantial proportion of epileptic patients, leading to persistent seizures, neurological impairments, and socioeconomic burdens. The present study aimed to synthesize and evaluate novel 1,3,4-oxadiazole–carbamothioate hybrids for their potential antiseizure activity, focusing on GABAA and SV2A modulation.

Materials and Methods: Target compounds (7a–f) were synthesized by coupling an oxadiazole intermediate (4) with diverse electrophiles, yielding products in 84–88% yields. Structural elucidation was carried out using FTIR, ¹H NMR, ¹³C NMR, and physicochemical methods. In vivo anticonvulsant activity was evaluated in mice using 6 Hz (32 mA) and pentylenetetrazole (80 mg/kg) seizure models. EEG recordings were analyzed to assess the effect on epileptic spiking. Molecular docking was performed to predict blood–brain barrier permeability, binding affinities, and interactions with GABAA receptors and SV2A protein.

Results: Compounds 7b and 7c significantly reduced seizure severity, increased protection, and lowered mortality ($p < 0.05$) in both seizure models. EEG data revealed marked suppression of epileptic spikes in 7b-treated mice, while both 7b and 7c reduced overall seizure activity. Docking results indicated favorable BBB penetration, low binding energies, and strong receptor interactions.

Discussions and Conclusions: The findings demonstrate that 7b and 7c possess potent antiseizure activity, likely mediated through GABAA agonism and SV2A modulation. These compounds hold promise as lead candidates for developing safer and more effective therapies for pharmacoresistant epilepsy.

Keywords: Epilepsy, 1,3,4-Oxadiazole, Carbamothioate, Antiseizure activity, GABAA receptor; SV2A protein

ALTERATIONS IN 5HT-2C RECEPTOR RESPONSIVENESS FOLLOWING REPEATED MIDAZOLAM TREATMENT

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ABSTRACT

Introduction and Purpose: Repeated administration of midazolam, a benzodiazepine with potent anxiolytic properties, has been associated with alterations in 5-hydroxytryptamine (5-HT; serotonin) neurotransmission, particularly involving the serotonin 5-HT_{2C} receptors. This study aimed to investigate the responsiveness of 5-HT_{2C} receptors in rats repeatedly treated with midazolam.

Materials and Methods: Adult male Albino Wistar rats were administered midazolam (2.5 mg/kg, oral) or vehicle once daily for 12 days. Following the treatment period, 5-HT_{2C} receptor responsiveness was assessed using a combination of behavioral and neurochemical techniques, post- meta-Chlorophenylpiperazine (mCPP) challenge.

Results: Behavioral assessments included the conditioned place preference test and light-dark box activity. Neurochemical analyses involved estimations of 5HT, dopamine, and metabolites in the midbrain region using High-Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC). Results indicated that repeated midazolam treatment led to alterations in 5-HT_{2C} receptor responsiveness, including changes in anxiety-like behavior, hypophagic effects, place preference, and biogenic amines and metabolites.

Discussion and Conclusion: These findings suggest that chronic midazolam exposure can induce adaptations in the serotonergic system, particularly involving 5-HT_{2C} receptors, which may contribute to the development of behavioral and neurochemical changes associated with benzodiazepine use. Further investigation into the mechanisms underlying these adaptations is warranted to better understand the implications for benzodiazepine pharmacology and potential therapeutic interventions.

Keywords: midazolam, meta-Chlorophenylpiperazine, 5-HT_{2C}, addiction, 5-HT, dopamine, conditioned place preference, midbrain

THE BIOLOGICAL BASIS OF FATIGUE AND ENDURANCE: MECHANISMS AND IMPLICATIONS

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ABSTRACT:

Fatigue and endurance are fundamental biological processes that determine human performance, both in athletic and everyday contexts. The biological basis of fatigue involves complex interactions between the muscular, cardiovascular, and nervous systems, alongside metabolic and biochemical pathways. Peripheral fatigue is largely attributed to the depletion of muscle glycogen, accumulation of lactate and hydrogen ions, and impaired calcium handling in skeletal muscle. Central fatigue, on the other hand, is influenced by neurotransmitter dynamics, reduced motor unit recruitment, and altered brain–muscle communication. Endurance capacity depends on aerobic metabolism, efficient oxygen delivery, mitochondrial density, and the ability to regulate oxidative stress. Emerging research highlights the role of genetics, hormonal regulation, and the gut microbiome in modulating both fatigue and endurance. Understanding these mechanisms not only advances sports science but also offers insights into clinical conditions associated with chronic fatigue and reduced physical capacity. This paper presentation explores the physiological and molecular determinants of fatigue and endurance, while emphasizing their applications in sports training, rehabilitation, and public health interventions aimed at enhancing quality of life.

Keywords: Fatigue, endurance, metabolism, exercise physiology, central fatigue, mitochondrial function

INTRODUCTION

Fatigue is a complex, multidimensional phenomenon that arises from the interplay of physiological, biochemical, and psychological factors. Rather than being a simple feeling of tiredness, fatigue reflects a measurable decline in the ability of biological systems to sustain force, endurance, or cognitive function. It manifests at multiple levels—from cellular bioenergetics and neurotransmission to systemic cardiovascular and musculoskeletal performance—making it a central limiting factor in both physical activity and daily functioning. Endurance, in contrast, is the ability to sustain prolonged physical or cognitive activity and is shaped by the efficiency of oxygen transport, energy metabolism, neuromuscular coordination, and adaptive plasticity of multiple organ systems. The balance between fatigue and endurance represents a fundamental determinant of human performance, with implications extending from elite athletics to clinical rehabilitation and chronic disease management.

Biologically, fatigue can be categorized into peripheral and central domains. Peripheral fatigue arises from metabolic disturbances within skeletal muscles, such as impaired ATP resynthesis, accumulation of lactate and hydrogen ions, ionic imbalances (e.g., calcium, potassium, sodium dysregulation), and mitochondrial dysfunction. These processes compromise excitation–contraction coupling and force generation.

Central fatigue, on the other hand, originates in the central nervous system (CNS), where altered neurotransmitter dynamics (serotonin, dopamine, norepinephrine), reduced motor neuron firing, and impaired cortical excitability limit voluntary drive to the muscles. Both components interact dynamically, and their relative contribution varies across tasks, intensity, and individual physiology.

Endurance capacity is heavily influenced by intrinsic physiological factors such as VO_2max , mitochondrial density, capillary perfusion, glycogen storage, and the efficiency of oxidative phosphorylation. Equally important are extrinsic modulators, including training regimens, nutritional strategies, sleep quality, hydration status, and psychosocial stressors. At a systems biology level, endurance reflects an integrated response of the cardiovascular system (oxygen delivery), musculoskeletal system (energy conversion and mechanical efficiency), and the nervous system (neurocognitive resilience and motor control).

The significance of understanding fatigue and endurance extends beyond the domain of sports science. In clinical contexts, chronic fatigue is a hallmark of multiple disorders—including chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), multiple sclerosis, cancer-related fatigue, and cardiovascular disease—where maladaptive metabolic and neuroimmune processes disrupt normal performance. Likewise, rehabilitation protocols for post-stroke patients, individuals recovering from trauma, or those living with neuromuscular disorders rely on mechanistic insights into fatigue to optimize recovery and functional independence.

Recent advances in molecular biology, neuroimaging, and metabolomics have shed light on novel mechanisms underlying fatigue, including alterations in mitochondrial biogenesis, inflammatory cytokine signaling, central autonomic regulation, and brain–muscle communication. Moreover, emerging integrative approaches such as systems physiology modeling, artificial intelligence-driven performance analytics, and personalized medicine strategies are providing deeper understanding of how individuals experience and adapt to fatigue.

Thus, fatigue and endurance are not binary opposites but interconnected phenomena shaped by molecular, physiological, and environmental determinants. A comprehensive understanding of their biological basis is essential not only for enhancing athletic performance but also for developing targeted interventions in clinical medicine, improving public health outcomes, and addressing global challenges associated with sedentary lifestyles, aging, and chronic disease.

Mechanisms of Fatigue

Fatigue emerges from an intricate interplay between peripheral (muscle-related) and central (nervous system-related) mechanisms. These processes rarely occur in isolation; instead, they interact dynamically, with the relative contribution of each depending on exercise intensity, duration, and individual physiology.

Peripheral Fatigue

Peripheral fatigue originates within the contracting skeletal muscles and reflects biochemical, ionic, and structural perturbations that impair force generation. Key contributors include:

1. Energy Depletion

- ATP and phosphocreatine (PCr): Sustained exercise depletes intramuscular ATP and PCr reserves, limiting immediate energy availability for cross-bridge cycling.
- Muscle glycogen depletion: Reduced glycogen compromises glycolytic and oxidative phosphorylation pathways, leading to inadequate ATP resynthesis.

- Mitochondrial dysfunction: Impaired oxidative phosphorylation lowers endurance capacity, particularly in prolonged exercise or metabolic disorders.

2. Metabolite Accumulation

- Lactate and hydrogen ions (H^+): Anaerobic glycolysis increases lactate and H^+ concentration, reducing intracellular pH. This acidosis interferes with glycolytic enzyme activity, calcium binding to troponin, and cross-bridge kinetics.
- Inorganic phosphate (P_i): Elevated P_i from ATP hydrolysis disrupts actomyosin interactions and reduces sarcoplasmic reticulum (SR) calcium release.

3. Calcium Dysregulation

- SR calcium release: Prolonged activity leads to oxidative stress and P_i accumulation, impairing ryanodine receptor (RyR) function and reducing calcium release.
- Calcium reuptake: Dysfunction in SR Ca^{2+} -ATPase (SERCA) slows reuptake, prolonging relaxation times and impairing subsequent contractions.
- Mitochondrial calcium handling: Disturbances in mitochondrial Ca^{2+} buffering exacerbate energy deficits and oxidative stress.

4. Oxidative Stress and Muscle Damage

- Accumulation of reactive oxygen species (ROS) during intense exercise damages proteins, lipids, and DNA, further impairing mitochondrial and contractile function.
- Microstructural damage to sarcomeres and membranes (e.g., t-tubules) reduces efficiency of excitation–contraction coupling.

Central Fatigue

Central fatigue is rooted in the central nervous system (CNS) and reflects limitations in neural drive from the motor cortex to skeletal muscles. It encompasses neurotransmitter dynamics, cortical excitability, and neuroimmune interactions.

1. Neurotransmitter Imbalance

- Serotonin hypothesis: Elevated brain serotonin (5-HT) levels during prolonged exercise increase perceptions of effort and lethargy.
- Dopamine and norepinephrine: Reduced dopamine availability decreases motivation, arousal, and reward signaling; norepinephrine decline impairs vigilance and attention.
- Glutamate/GABA balance: Excess inhibitory GABAergic activity reduces cortical excitability and voluntary drive.

2. Reduced Motor Unit Recruitment

- The CNS strategically reduces recruitment of high-threshold motor units as a protective mechanism against muscle damage and metabolic exhaustion.
- Altered corticospinal excitability leads to reduced firing rates of alpha motor neurons, limiting muscle output despite sufficient peripheral energy availability.

3. Brain–Muscle Communication

- Afferent feedback: Group III/IV muscle afferents sense metabolites and mechanical stress, signaling fatigue to the CNS, which then downregulates motor output.

- Central governor model: Proposed regulatory mechanism in which the brain subconsciously modulates performance to preserve homeostasis and prevent catastrophic failure (e.g., cardiovascular collapse, hyperthermia).
- Neuroinflammation: Prolonged exercise elevates pro-inflammatory cytokines (IL-6, TNF- α), influencing neurotransmitter metabolism and contributing to central fatigue.

Determinants of Endurance

Endurance—the capacity to sustain prolonged physical or cognitive activity—is governed by a complex interplay of metabolic, cardiovascular, neuromuscular, hormonal, genetic, and environmental factors. Understanding these determinants provides insight into performance optimization, rehabilitation strategies, and individualized training protocols.

1. Aerobic Metabolism

- Oxidative Phosphorylation: Endurance performance depends on the efficiency of mitochondria in converting substrates (carbohydrates, fats, and proteins) into ATP via oxidative phosphorylation.
- Substrate Utilization: Trained endurance athletes exhibit enhanced fat oxidation, preserving glycogen stores and delaying fatigue.
- Metabolic Flexibility: The ability to switch efficiently between carbohydrate and fat metabolism during prolonged exercise is crucial for sustained energy supply.
- Mitochondrial Enzyme Activity: High activity of enzymes like citrate synthase and succinate dehydrogenase enhances electron transport and ATP yield.

2. Oxygen Delivery

- Cardiovascular Capacity: Cardiac output, stroke volume, and heart rate adaptation determine the rate at which oxygen-rich blood reaches skeletal muscles.
- Hemoglobin and Myoglobin Levels: Hemoglobin concentration in blood and myoglobin content in muscle fibers directly influence oxygen transport and storage, ensuring a steady supply during prolonged exertion.
- Capillarization: Higher capillary density around muscle fibers improves oxygen diffusion, nutrient delivery, and metabolite clearance.
- Blood Flow Regulation: Efficient redistribution of blood to active tissues through vascular adaptations supports endurance performance.

3. Mitochondrial Density and Function

- Mitochondrial Biogenesis: Training-induced upregulation of PGC-1 α and other transcription factors increases mitochondrial content and oxidative capacity in skeletal muscles.
- ATP Generation Efficiency: Greater mitochondrial density enhances sustained ATP production while minimizing reactive oxygen species (ROS) accumulation.
- Resistance to Fatigue: Well-developed mitochondria improve endurance by maintaining energy homeostasis and supporting recovery during repeated bouts of activity.

4. Hormonal Influence

- Cortisol: Regulates glucose availability via gluconeogenesis during prolonged exercise but chronic elevation can impair muscle recovery.
- Catecholamines (Adrenaline, Noradrenaline): Increase heart rate, cardiac output, and lipolysis, mobilizing energy substrates for prolonged activity.

- Thyroid Hormones (T3, T4): Modulate basal metabolic rate and mitochondrial efficiency, affecting energy production and endurance capacity.
- Insulin and Glucagon: Maintain glucose homeostasis during prolonged exertion by balancing substrate utilization.

5. Genetics and Epigenetics

- Muscle Fiber Composition: Genetic variants determine the proportion of Type I (slow-twitch) fibers, which are fatigue-resistant and optimized for oxidative metabolism, versus Type II (fast-twitch) fibers.
- Mitochondrial Enzyme Polymorphisms: Variations in genes coding for components of the electron transport chain and oxidative enzymes influence ATP production efficiency.
- Epigenetic Modifications: DNA methylation, histone modifications, and microRNAs regulate gene expression in response to training, environmental stress, and nutrition, affecting long-term endurance adaptation.

6. Gut Microbiome

- Energy Harvesting: Certain gut bacteria enhance extraction of energy from dietary substrates, increasing availability for prolonged physical activity.
- Inflammation Modulation: Microbiota-derived short-chain fatty acids (SCFAs) regulate systemic inflammation, reducing fatigue-related signaling.
- Nutrient Absorption: Gut flora modulates absorption of amino acids, vitamins, and minerals essential for muscular and mitochondrial function.
- Neuromodulation: Emerging evidence suggests gut–brain axis communication influences central fatigue and endurance perception via neurotransmitters and neuroimmune signaling.

Integration of Determinants

Endurance is not determined by a single factor but by the synergistic interaction between energy production, oxygen delivery, hormonal regulation, genetic predisposition, and systemic modulators such as the gut microbiome. Training interventions, nutritional strategies, and personalized medicine approaches can optimize these determinants, enhancing both performance and resilience against fatigue.

Implications for Sports and Health

Understanding the biological basis of fatigue and determinants of endurance has far-reaching implications across sports performance, rehabilitation, public health, and clinical medicine. Translating mechanistic insights into practical strategies can optimize outcomes at both individual and population levels.

1. Sports Training

- Personalized Endurance Programs: Knowledge of peripheral and central fatigue mechanisms allows coaches and exercise physiologists to design individualized training regimens that optimize mitochondrial density, capillarization, and neuromuscular efficiency.
- Periodization and Load Management: Manipulating training intensity, duration, and recovery periods helps delay the onset of fatigue and prevents overtraining syndromes.
- Nutritional Strategies:
 - Carbohydrate Loading: Enhances glycogen stores in skeletal muscle, prolonging ATP availability during prolonged or high-intensity activity.

- Antioxidant Supplementation: Mitigates oxidative stress-induced peripheral fatigue, preserving mitochondrial and contractile function.
- Protein and Amino Acids: Support muscle repair, mitochondrial biogenesis, and neurotransmitter synthesis, reducing both peripheral and central fatigue.
- Recovery Modalities: Techniques such as cryotherapy, massage, and active recovery facilitate metabolic clearance, reduce inflammation, and restore neuromuscular function.

2. Rehabilitation and Clinical Applications

- Post-Stroke and Neuromuscular Disorders: Insight into central and peripheral fatigue allows therapists to tailor rehabilitation programs that gradually increase motor unit recruitment, improve cardiovascular conditioning, and enhance neuroplasticity.
- Chronic Illness Management: Patients with conditions such as heart failure, chronic obstructive pulmonary disease (COPD), or multiple sclerosis often experience exercise intolerance. Understanding fatigue mechanisms guides graded activity programs that improve endurance while minimizing symptom exacerbation.
- Integration with Assistive Technologies: Wearable sensors and biofeedback systems can monitor fatigue in real-time, allowing dynamic adjustments in therapy intensity and duration.

3. Public Health and Lifestyle Interventions

- Cardiovascular Health: Regular endurance-based exercise enhances VO_2max , cardiac output, and vascular endothelial function, reducing the risk of hypertension, coronary artery disease, and stroke.
- Metabolic Disease Prevention: Improved mitochondrial function and energy metabolism mitigate risks of obesity, type 2 diabetes, and metabolic syndrome.
- Mental Health Benefits: Physical activity modulates neurotransmitter balance, reduces central fatigue, and enhances cognitive function, contributing to better mental well-being and resilience against stress.
- Quality of Life: Sustained physical activity improves functional independence, mobility, and overall healthspan, particularly in aging populations.

4. Chronic Fatigue Syndromes (CFS/ME)

- Mechanistic Insights: Understanding the interplay of central neurotransmitter imbalance, immune dysregulation, mitochondrial dysfunction, and impaired neuromuscular signaling provides a framework for targeted interventions.
- Clinical Management Strategies:
 - Graded Exercise Therapy (GET): Incremental physical activity adapted to patient tolerance can improve functional capacity without exacerbating symptoms.
 - Cognitive Behavioral Therapy (CBT): Addresses the perception of fatigue, motivational decline, and central fatigue-related behavioral adaptations.
 - Metabolic and Nutritional Interventions: Supporting mitochondrial function through targeted nutrition or pharmacological agents may alleviate peripheral fatigue.
- Personalized Medicine Approaches: Integrating biomarkers of central and peripheral fatigue could enable patient-specific therapeutic strategies, improving prognosis and quality of life.

Integration Across Domains

- Fatigue and endurance research bridges sports physiology, clinical rehabilitation, and public health, emphasizing the continuum from elite performance to daily functional capacity.
- Mechanistic understanding allows interventions to be tailored, evidence-based, and multidimensional, targeting not just the muscular system but also cardiovascular, neural, endocrine, and metabolic networks.

- Emerging technologies, including wearable sensors, metabolomics, and AI-driven predictive models, further enhance the ability to monitor, predict, and optimize endurance and recovery in both healthy and diseased populations.

CONCLUSION

Fatigue and endurance are complex, multifactorial phenomena shaped by an intricate network of metabolic, neuromuscular, cardiovascular, and neuroendocrine mechanisms. Fatigue arises from both peripheral factors—including energy substrate depletion, metabolite accumulation, calcium dysregulation, and oxidative stress—and central factors, such as neurotransmitter imbalances, reduced motor unit recruitment, and altered brain–muscle communication. These processes interact dynamically, and their relative contributions vary according to task intensity, duration, and individual physiology.

Endurance, in contrast, reflects the capacity of the body to sustain prolonged activity and depends on the efficiency of oxidative metabolism, mitochondrial density and function, oxygen transport, cardiovascular adaptations, and hormonal regulation. Emerging evidence highlights the significant roles of genetic predisposition, epigenetic modifications, and the gut microbiome in modulating individual endurance potential and susceptibility to fatigue. Such integrative understanding underscores that endurance is not simply a property of muscle, but the output of coordinated systemic responses spanning multiple organ systems.

Recent advances in molecular biology, metabolomics, wearable technologies, and computational modeling have provided unprecedented insights into these mechanisms, enabling more precise monitoring of fatigue and performance. Translating this knowledge into sports science, rehabilitation, and clinical medicine offers tangible benefits: optimizing training regimens, enhancing recovery protocols, managing chronic fatigue syndromes, and improving overall health outcomes.

Ultimately, the interplay between fatigue and endurance represents a delicate balance between physiological capacity, environmental challenges, and adaptive plasticity. Continued research integrating molecular, systemic, and behavioral approaches holds promise for personalized interventions that enhance human performance, resilience, and quality of life. Understanding these mechanisms not only informs athletic and therapeutic strategies but also advances broader public health initiatives aimed at promoting functional capacity and mitigating disease-related fatigue across the lifespan.

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ASSESSMENT OF DIETARY SUPPLEMENT CONSUMPTION AND ANALYSIS OF THEIR TOXICITY IN CONSUMERS

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ABSTRACT

Introduction and Purpose: A dietary supplement is a food whose purpose is to provide additional nutrients or substances having a nutritional or physiological effect (vitamins, minerals, fatty acids or amino acids) missing or in insufficient quantity in the normal diet of a person. They may contain nutrients, plants, substances for nutritional or physiological purposes, traditional substances, additives, flavorings and technological aids whose use is authorized in human nutrition.

Materials and Methods: We conducted a retrospective descriptive survey on the use and consumption of dietary supplements by the population of Oran. The methodology used to carry out this study was based on a survey, followed by a statistical analysis of the collected data. The collected information included a wide range of questions about dietary supplements, structured into five different types of questionnaires designed for 20 doctors, 13 pharmacists, 33 athletes, 49 patients, and 41.

Results: The rate of dietary supplement (DS) consumption among women particularly in the study conducted on patients and students is higher than that of men. DS can be valuable allies in helping women maintain hormonal balance over time, especially during periods of hormonal changes such as menstruation, pregnancy, and menopause. On the other hand, among athletes, our survey showed that men consume more DS than women. Athletes involved in endurance sports are proportionally higher consumers. Dietary supplements are also widely used in strength training and bodybuilding.

Discussion and Conclusion: Our results show that the age of dietary supplement (DS) consumers ranges from 4 to 60 years, with the majority falling between 18 and 45 years old. Moreover, females are the predominant consumers of DS compared to males: 69.39% of patients who consume DS are women, and 58.59% of student DS users are also female. On the other hand, 66.67% of athletes who consume DS are men, generally using them for bodybuilding purposes.

Keywords: Deficiency; dietary supplement; tiredness; medicine; overdose.

GUAVA (PSIDIUM GUAJAVA L.): A GLORIOUS PLANT WITH CANCER PREVENTIVE AND THERAPEUTIC POTENTIAL

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ABSTRACT

Guava (*Psidium guajava* L.) tree (Myrtaceae family) bears fruit rich in vitamins, fiber, and other nutrients. While native to Latin America, guava is grown in many tropical and subtropical regions across the globe where it has long been used in traditional medicine to treat a myriad of ailments. Guava has been shown to exhibit a number of biological and pharmacological activities, such as antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, antidiabetic, and anticancer properties. Several parts of the plant, including the leaves, fruits, seeds, peels, pulp, bark, and oil, produce phytochemicals with medicinal properties. Emerging research has found that guava bioactive phytochemicals exert antitumorigenic effects against various human malignancies through multiple mechanisms. This review aims to present a concise summary of the in vitro and in vivo studies investigating the anticancer activity of the plant against various human cancer cell lines and animal models, including the identified phytochemicals that contributes to their activity via the different mechanisms. In vitro growth and cell viability studies, such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the sulforhodamine B (SRB) assay, and the trypan blue exclusion test, were conducted using *P. guajava* extracts and their biomolecules to assess their effects on human cancer cell lines. Numerous studies have showcased that the *P. guajava* plant and its bioactive molecules, especially those extracted from its leaves, selectively suppress the growth of human cancer cells without cytotoxicity against the normal cells.

Keywords: *Psidium guajava*; cancer; cytotoxicity; flavonoids. apoptosis; cancer; guava; molecular mechanisms; phytochemicals; prevention; proliferation

**POTENTIAL OF POMEGRANATE (*Punica granatum* L.) PEEL AND ARIL
EXTRACTS AS LIPID-LOWERING AGENTS IN A RAT MODEL OF
HYPERLIPIDEMIA**

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ABSTRACT

Introduction and Purpose: *Punica granatum* L. is claimed to provide several health benefits. Some studies have reported a relationship between pomegranate consumption and the prevention of oxidative stress, diabetes, inflammation, cancer, and the development of cardiovascular disease. This study aims to evaluate the antioxidant properties of phenolic-rich extracts from pomegranate peels (PPPE) and arils (PAPE) collected in the central area of Morocco (plans of Tadla, Beni Mellal, Morocco).

Materials and Methods: We analyzed the antioxidant activity, total phenolic content, and flavonoids of the different secondary metabolites of pomegranate, as well as its phytochemical screening. The hypolipidemic activity was studied in a rat model that was injected intraperitoneally with Triton WR-1339. The animals were grouped as follows: a normolipidemic control group, a hyperlipidemic group, a simvastatin (reference) group, and a phenolic extract-treated group. After 24 hours of treatment, serum lipid profiles were investigated using commercially available kits.

Results: The total phenolic and flavonoid content were 283.86 ± 17.89 and 166.90 ± 18.10 mg GAE/g dw and 185.37 ± 3.05 and 57.43 ± 0.41 mg QE/g dw, respectively, for the peels and arils. The antioxidant activity was evaluated using DPPH and hydrogen peroxide scavenging assays, and the ferric reducing antioxidant power (FRAP). The IC₅₀ value for DPPH and FRAP inhibition activity was highest for the peel extract compared to the aril extract. Similarly, peel extract exhibited a more potent antioxidant effect than aril extract in terms of its capacity to scavenge hydrogen peroxide radicals.

Administering the peel and aril extract significantly ($p < 0.001$) reduced the serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), as well as the atherogenic index (AI). However, the concentration of high-density lipoprotein (HDL) was significantly higher ($p < 0.001$) in the treated groups than in the hyperlipidemic control rats induced by Triton WR-1339 after 24 hours of treatment.

Discussion and Conclusion: These results reflect the protective effects that pomegranates can provide against metabolic disorders such as atherosclerosis and cardiovascular disease. Due to these properties, studies can be extended to explore their potential applications in food preservation and as health supplements and nutraceuticals.

Keywords: Antioxidant activities, flavonoids, hypolipidemic activity, polyphenols, *Punica granatum* L.

**A COMPARATIVE ANALYSIS OF CLINICAL AND BIOCHEMICAL
PARAMETERS IN SCREW-RETAINED SINGLE IMPLANT CROWNS OF
DIFFERENT DESIGNS**

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ABSTRACT

Background: The gold standard of implant-retained crowns has for many years been the metal-ceramic cement-retained crowns on a screwed-abutments. Today it represents an alternative if the implant positioning does not allow a screw-retained crown. The possibilities currently offered for this kind of rehabilitation in the posterior areas are represented by a chromium-cobalt/ceramic or a titanium/zirconia crown. These two solutions allow different productions workflows and generate different prosthetic interfaces at the implant abutment level.

The aim of the study is to verify a significant difference in tissue response can be defined between these two different rehabilitations, 18 months after delivery.

Materials and methods: A total of 20 participants were included in the study, each of whom had at least two implants in the posterior sectors prosthetically treated with one of the two solutions previously illustrated. The values of IL-1 β , IL-6, IL-8 were evaluated, together with the periodontal indices plaque index (PI), gingival index (GI), probing depth (PD), bleeding on probing (BOP), gingival recession (REC).

Results: During the time of clinical observation, at t0 (1 month from the delivery), t1 (9 months after the delivery) and t2 (18 months after the delivery), both types of crowns performed satisfactorily from a clinical point of view. In light of the results obtained, it is possible to define that there are no statistically significant differences between the two techniques, although Cr-Co/Cer has slightly higher values than the other.

Conclusions: It is possible to hypothesize, and confirm through subsequent or longer-term studies, that this difference could lead to a clinically visible difference between these two prosthetic solutions in the longer term. The single crown in Cr-Co shows a different interface with the titanium implant connection compared to the titanium-titanium contact of the connection between the implant and the Ti-Zir crown.

The ceramic that is layered directly on the Cr-Co does not show a connection interface with it, other than the areas hand-finished by the technician, vice versa, the Ti-Zir crown shows an area in which the milled zirconia product is bonded onto the titanium abutment with a resin-based cement.

The causes of this slight difference could be either the better tissue response to titanium and zirconia, or an increased level of inflammation from the Cr-Co/Ti connection.

Keywords: Cr-Co; Implants; Titanium; Resin; Screw-retained; Milling; Zirconia.

EFFICACY AND APPLICATION OF A BIOPREPARATION BASED ON AN ASSOCIATION OF CELLULOLYTIC, PROBIOTIC BACTERIA

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ABSTRACT

Introduction and Purpose: This article investigates the effectiveness of a biopreparation developed from an association of cellulolytic bacteria capable of degrading cellulose and probiotic bacteria that restore the microbiological balance of the organism. The physiological effects of the biopreparation on animals, its role in improving digestion, and its impact on the immune system were studied. Experimental results demonstrated the high biological activity and environmental safety of this biopreparation. Biopreparations developed from associations of these two bacterial types contribute to improving animal health and productivity.

Materials and Methods. Strains of *Bacillus subtilis* (with cellulolytic activity) and *Lactobacillus acidophilus* (with probiotic properties) were selected for the study. The biopreparation was prepared as an association and tested on Karakul sheep. The evaluation included clinical and biochemical parameters, weight gain, feed conversion ratio, intestinal microflora composition, and general health status.

Results: In the experimental group that received the biopreparation: weight gain was 12–15% higher compared to the control group; digestibility coefficient increased by 8–10%; positive changes were observed in leukocyte and hemoglobin levels; the number of lacto- and bifidobacteria in the intestinal microflora increased, while pathogenic microflora levels decreased. The differences between the experimental and control groups are presented in the table below:

Indicators	Control Group	Experimental Group
Weight gain (%)	100.0	115.0
Digestibility (%)	100.0	110.0
Leukocyte count ($10^9/l$)	7.2	8.5
Hemoglobin (g/l)	110.0	125.0
Increase in beneficial microflora (%)	100.0	135.0
Reduction in pathogenic microflora (%)	100.0	70.0

Discussion and Conclusion: Biopreparations developed through the combined application of probiotics and cellulolytic bacteria exhibit multifaceted effects, improving the digestive and immune functions of farm animals and enhancing their overall productivity. Moreover, they offer a cost-effective alternative to expensive imported products.

Keywords: Biopreparation; Cellulolytic bacteria; Probiotics; Symbiotic effect; Efficiency ; Microflora.

ETHNOVETERINARY APPROACH FOR THE CONTROL OF SUBCLINICAL MASTITIS IN BUBALINE

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ABSTRACT

Introduction and Purpose: Sub-clinical mastitis is an inflammatory disorder affecting parenchymal cells of the mammary gland and is a major challenge in the dairy industry. This study examined the efficacy of Centratherum anthelminticum in sub-clinical mastitis and its therapeutic value to improve the quality of milk in buffalo.

Materials and Methods: Thirty lactating buffaloes were screened for sub-clinical mastitis using the California Mastitis Test (CMT), identifying nine CMT-positive animals. These were randomly assigned into three groups: G1 received Tylosin, G2 received a combination of Tylosin and Centratherum anthelminticum, and G3 received Centratherum anthelminticum alone for seven consecutive days. Therapeutic response was assessed by repeating the CMT post-treatment. Standard Plate Count (SPC) determines bacterial load in milk. Milk composition, including solids-not-fat (SNF), lactose, fat, and protein, was analyzed using a milk analyzer. Hematological parameters, including white blood cell (WBC) and red blood cell (RBC) counts, were measured using an automated hematology analyzer to evaluate systemic inflammatory status.

Results: After the post-treatment, all buffaloes in G1 and G2 tested negative on the CMT, whereas one buffalo in Group 3 remained positive. Standard plate count analysis revealed a pronounced reduction in bacterial load across all groups, with the greatest decline in Group 2. SNF, lactose, milk fat and milk protein levels showed no statistically significant differences ($p > 0.05$) between groups, although a slight increase in milk fat was observed in G2. Total WBC counts decreased significantly ($p < 0.05$) in G1 and G2, indicating reduced systemic inflammation, while RBC counts increased significantly in G2, suggesting enhanced erythropoietic activity.

Discussion and Conclusion: The combination of Tylosin and Centratherum anthelminticum (G2) appeared to be the most effective in treating sub-clinical mastitis in buffaloes but remained non-significant in improving milk quality. Further detailed studies are recommended to investigate the potential of Centratherum anthelminticum in enhancing milk quality and production in dairy animals.

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Keywords: Subclinical mastitis, Centratherum Anthelminticum, lactose, fat, RBC, WBC

HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES OF A NATURAL AFRICAN SWINE FEVER INFECTION IN PIGS

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ABSTRACT

Introduction and Purpose: African swine fever (ASF) is a contagious viral disease that affects pigs of all ages, inducing hemorrhagic fever with high mortality and severe threat to pig production. This study investigated the hematological and serum biochemical abnormalities associated with a natural African swine fever virus (ASFV) infection in pigs.

Materials and Methods: A total of 100 serum samples of pigs from piggery suspected of ASF infection were screened for antibodies by ELISA. Thirty two blood samples from serologically positive pigs and 32 negative pigs were subjected to hematological and serum biochemical analyses following standard procedures.

Results: The mean values of the red blood cell (RBC) count, total white blood cell (TWBC) count, absolute lymphocyte count, absolute monocyte count, serum total protein (TP) and globulin were significantly ($p < 0.05$) lower while the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), absolute neutrophil count and serum gamma glutamyl transferase (GGT) were significantly ($p < 0.05$) higher in the infected than the healthy pigs. There were no significant differences ($p > 0.05$) in the mean values of the packed cell volume (PCV), hemoglobin concentration, absolute eosinophil count, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities between the infected and healthy pigs.

Discussion and Conclusion: Hence, natural ASF infection may have caused alterations in the hematological and serum biochemical parameters in the infected pigs. The generated data could complement the existing laboratory diagnostic techniques such as polymerase chain reaction (PCR), direct fluorescence antibody test (FAT), indirect fluorescent antibody test (IFA) and ELISA in the diagnosis of ASF in pigs.

Keywords: African swine fever, Biochemical analysis, Hematological analysis, Pigs.

ACARICIDAL AND ANTIOXIDANT ACTIVITIES OF XYLOPIA AETHIOPICA CRUDE METHANOL EXTRACT AGAINST TICKS OF CATTLE AND DOG

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ABSTRACT

Introduction and Purpose: Ticks are significant ectoparasites that transmit a variety of pathogenic agents to livestock, companion animals, and humans, causing substantial health and economic losses. The emergence of resistance to synthetic acaricides has prompted the search for eco-friendly and sustainable alternatives. This study investigated the acaricidal and antioxidative effects of crude methanol extract of *Xylopiya aethiopica* on cattle (*Rhipicephalus microplus*) and dog (*Rhipicephalus sanguineus*) ticks.

Materials and Methods: Fresh *X. aethiopica* leaves were collected, authenticated, and subjected to methanol extraction. Phytochemical screening revealed the presence of bioactive compounds in varying concentrations. Acaricidal efficacy was assessed by evaluating adult oviposition and egg hatchability at different extract concentrations, while antioxidative effects were examined through in vitro assays of glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) activities.

Results: From the results showed that the crude methanol extract significantly reduced oviposition and egg hatchability in both cattle and dog ticks, with a more pronounced effect observed in dog ticks. The extract also caused notable reductions in GST, CAT, and SOD activities, suggesting interference with oxidative stress regulation as a possible mechanism of acaricidal action. These findings indicate that *X. aethiopica* possesses both potent acaricidal and antioxidative properties, making it a promising candidate for developing plant-based tick control agents.

Discussion and Conclusion: Crude methanol extract of *X. aethiopica* offers a viable natural alternative for tick management, particularly in the face of increasing acaricide resistance.

Further studies are recommended to isolate and characterize the specific bioactive components responsible for these effects, as well as to evaluate their safety, environmental impact, and potential integration into sustainable livestock and companion animal health programs.

Keywords: *Xylopi* *aethiopica*; Acaricidal activity; Antioxidative effect; Cattle ticks; Dog ticks

THE IMPORTANCE OF CARBOHYDRATES AND VITAMINS IN FEEDING PUPPIES (literature review)

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ABSTRACT

Introduction. Feeding puppies plays a key role in their growth and development. The balance of the diet determines the physiological condition of the animals, their resistance to diseases, and the formation of a healthy organism. The most important nutritional components for puppies are carbohydrates and vitamins, which provide energy and regulate metabolic processes.

Objective. To determine the importance of carbohydrates and vitamins in the diet of puppies and to identify their role in maintaining health and preventing pathologies.

Materials and Methods. An analysis of literature sources dedicated to puppy nutrition, carbohydrate and vitamin requirements, and the effects of their deficiency or excess on physiological functions was conducted.

Results and Discussion. Carbohydrates are the main source of energy. The daily requirement for puppies is up to 16 g/kg of body weight. The main sources include cereals, bran, honey, vegetables, and fruits. Carrots and beets are especially beneficial. Fresh bread is undesirable, as it causes digestive disorders, but rusks are acceptable from two months of age. Semolina is not recommended, and legumes should be excluded from the diet, except for soy in the form of flour. Plant products such as tomatoes contribute to normal coat pigmentation and tooth cleaning; however, citrus fruits and grapes are contraindicated. Vitamins play a crucial role in puppy development. Vitamin A is necessary for the normal function of the digestive, respiratory, and reproductive systems. Vitamin D deficiency causes rickets, while its excess leads to pathological bone development. B vitamins regulate liver function, hematopoiesis, coat condition, and body weight. Vitamin C enhances immunity, while Vitamin E affects coat elasticity and pigmentation. During periods of hypovitaminosis, especially in spring and autumn, the diet should be enriched with fresh greens (onion, parsley, nettle, dandelion, lettuce), pre-treated with boiling water to remove bitterness.

Conclusion. A balanced diet with sufficient carbohydrates and vitamins plays an important role in forming a healthy puppy organism. Their deficiency or excess leads to pathologies and developmental delays. The inclusion of vitamin supplements and the correct choice of carbohydrate sources ensure harmonious growth, stronger immunity, and the prevention of diseases in young dogs.

Keywords: carbohydrates, vitamins, puppies, balanced diet

**OBESITY IN DOMESTIC ANIMALS
(Literature Review)**

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ABSTRACT

Introduction: Obesity is one of the most widespread metabolic disorders in domestic animals, affecting up to 40% of dogs and cats worldwide. Excess body weight reduces life expectancy, lowers quality of life, and predisposes animals to numerous diseases, including diabetes mellitus, hepatic lipidosis, osteoarthritis, cardiovascular and respiratory disorders, and endocrine pathologies.

Objective: To analyze the significance of obesity in domestic animals, identify its main risk factors, and outline preventive and corrective measures to improve animal health and longevity.

Methods: A review of current literature and clinical observations was conducted, focusing on the prevalence, etiology, clinical consequences, and management of obesity in dogs and cats. Results. The analysis revealed that the main risk factors for obesity include insufficient physical activity, overfeeding, inappropriate diet composition (high-fat feeds in cats, excessive treats in dogs), neutering, as well as breed and sex predisposition. Obese animals demonstrate higher incidence of metabolic and systemic disorders, with clinical evidence showing that even moderate weight loss significantly improves physiological parameters and overall quality of life.

Conclusion: Obesity in domestic animals is a serious health problem requiring preventive strategies and timely correction. Diet regulation, caloric control, reduced use of high-calorie treats, and regular physical activity are essential measures. Proper weight management not only reduces the risk of life-threatening diseases but also extends the active lifespan of companion animals.

Keywords: obesity, domestic animals, prevention, weight management.

GINGER (ZINGIBER OFFICINALE) ESSENTIAL OIL AS A PHYTOGENIC MODULATOR OF IMMUNE AND OXIDATIVE STRESS RESPONSES IN WHITE FULANI DAIRY COWS

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ABSTRACT

Phytogenic feed additives have emerged as sustainable alternatives to conventional growth promoters in livestock production, with the potential to enhance productivity, health, and product quality. Among these, ginger (*Zingiber officinale*) essential oil (ZEO) has gained attention due to its antioxidant, antimicrobial, and immunomodulatory properties. This study aimed to evaluate the effects of dietary ZEO supplementation on biochemical and antioxidant responses in early lactating White Fulani dairy cows. Thirty multiparous White Fulani dairy cows with comparable initial body weights (310.65 ± 0.80 kg) were randomly assigned to three dietary groups ($n = 10$ per group) in a completely randomized design. The feeding trial lasted for 50 days. Cows in the control group were offered a standard basal diet, while those in the treatment groups received the basal diet supplemented with either 5 mL/kg or 10 mL/kg ZEO. Blood samples were analyzed to determine serum biochemical indices and antioxidant status. ZEO supplementation significantly modulated several blood biochemical and oxidative stress parameters. Specifically, cows receiving ZEO diets exhibited reduced serum globulin concentrations and increased albumin:globulin ratios, high-density lipoprotein, total lipids, and total antioxidant capacity compared with the control group. Conversely, serum glucose, total protein, albumin, cholesterol, low-density lipoprotein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea concentrations were not significantly affected ($P > 0.05$).

The findings demonstrate that dietary inclusion of ZEO up to 10 mL/kg diet is safe for lactating cows and beneficial to lipid metabolism and oxidative balance without impairing overall biochemical health indices. These results suggest that ZEO holds promise as a phytogenic feed additive for improving metabolic resilience in dairy cattle. However, further studies involving extended feeding durations, different physiological stages, and possible synergistic interactions with other phytogenics are recommended to better elucidate its long-term role in enhancing dairy cow performance under tropical production systems.

Keywords: White Fulani dairy cows, *Zingiber officinale* essential oil, phytogenic feed additives, oxidative stress, antioxidant capacity, biochemical indices

INTRODUCTION

Over the past decade, the application of essential oils (EOs)—plant-derived extracts with distinctive aromatic properties—has gained increasing attention in animal health and production (Kholif et al., 2018; Anaso et al., 2025a; Anaso et al., 2025b; Anaso et al., 2025c). These bioactive compounds, predominantly polyphenolics, possess diverse physiological functions and have been shown to modulate ruminal fermentation, thereby enhancing nutrient utilization and improving overall feed efficiency in ruminant systems (Anaso et al., 2024c; Emmanuel, 2025).

Recent studies emphasize the role of EOs as viable natural alternatives to synthetic growth promoters.

In particular, Anaso (2023a) and Anaso et al. (2025c) underscored their antimicrobial, aromatic, and biofunctional properties, which contribute to improved animal performance and health. With the global challenge of antimicrobial resistance and the increasing restrictions on antibiotic use in livestock production, the search for sustainable phytogetic feed additives has intensified (Anaso, 2023b).

Among these, *Zingiber officinale* (ginger) has attracted considerable research interest for its potential to enhance productivity and health in various livestock species. However, despite evidence of its beneficial effects in other animals, little is known about its capacity to modulate immune status and oxidative stress responses in White Fulani dairy cows. The present study was therefore designed to address this knowledge gap.

MATERIALS AND METHODS

Collection of *Zingiber Officinale* Rhizomes and Extraction of the Essential Oil

Mature ginger (*Zingiber officinale*) rhizomes were harvested at 8–10 months after planting from local farms in North central Nigeria, harvest was done at full maturity to ensure optimal aroma and bioactive content, ensuring that rhizomes were carefully dug up to minimize physical damage, then thoroughly washed and rinsed to remove soil and debris. Rhizomes were peeled and cut into small uniform pieces to ensure representative sampling and efficient drying for further storage and processing. Subsequently, the samples were air-dried in a forced-air oven at precisely 50 ± 5 °C until residual moisture content reached 12%, a temperature range shown to preserve essential volatile compounds effectively. After drying (approximately 3–4 days), samples were stored in airtight plastic containers with silica gel and kept at 4 °C until extraction. The final essential oil extraction was performed by hydrodistillation using a Clevenger-type apparatus, following established procedures where the dried ginger (100 g) was combined with distilled water at a ratio of 1:5 (w/v) in a 1 L flask. The mixture was heated to gently boil and maintained under distillation for 4–6 hours to maximize yield. The condensate was collected; the oil layer was separated, dried with anhydrous sodium sulfate to remove moisture, and stored in amber vials at 4 °C until further analysis. Residual water was removed using anhydrous sodium sulfate, and the volume (or mass) of the extracted oil was recorded to calculate yield percentage relative to dry sample weight. The extracted GEO was stored under refrigeration at 4 °C in amber glass vials to preserve its composition until incorporation into feeding trials as described by Emmanuel (2025).

Experimental site

In collaboration with the Co-farms Green aid Revolution Ruminant Research Unit, the current experimental study was conducted at the Morugo Farms. Between latitudes 08051' and 09037'N and longitudes 007020' and 007051' E is where the project site is located. Rainfall varies from 1,145 to 1,631 mm each year. During the rainy season, temperatures range from 25.8 to 30.2°C, whereas during the dry season, they range from 36 to 42°C. According to Anaso (2025a and b), the relative humidity is roughly 60% during the rainy season and 30% during the dry season.

Experimental Animals, Management And Diets

The control group was fed a diet of 13 kg of concentrate and 40 kg of *Panicum maximum* hay per head per day without any supplementation. The second group received the same control diet supplemented with 5 mL of ginger EO (ZEO) per head per day. The third group was fed the control diet supplemented with 10 mL ZEO per head per day. The dosage of each oil or their blend was added and manually mixed to the feed before morning feeding at 06:00 h daily.

Cows were housed in open semi-shaded barns with free access to fresh water. Cows were fed three times daily at 06:00, 12:00 and 18:00 h. The concentrate mixture and Panicum maximum hay were used to formulate diets to provide cows with their requirements plus 10% margin (NRC, 2001). Feed intake was individually recorded daily as the difference between the offered concentrate mixture and forage and refusal.

Blood Biochemical Parameters

Blood samples were collected biweekly from cows via tail vein puncture without the use of anticoagulants for biochemical analysis. The samples were centrifuged at 3000 rpm for 20 min to separate the serum, which was subsequently stored at -20°C until analysis. Biochemical parameters, including total protein, albumin, urea, total cholesterol, low-density lipoprotein cholesterol (LDL-C), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total lipids, and total antioxidant capacity, were determined colorimetrically using commercial diagnostic kits (Biodiagnostic Co., Giza, Egypt) in accordance with the manufacturer's instructions and measured with a visible light spectrophotometer. Serum globulin concentration was calculated as the difference between total protein and albumin values, while high-density lipoprotein cholesterol (HDL-C) was derived by subtracting LDL-C from total cholesterol. The albumin-to-globulin (A/G) ratio was obtained by dividing serum albumin by serum globulin.

Statistical Analysis

The data was statistically analyzed using statistical package for social sciences (SPSS®, version 2016).

Results and Discussions

Cows fed diets supplemented with Zingiber officinale essential oil (ZEO) showed a reduction in serum globulin concentrations alongside increases in the albumin-to-globulin (A/G) ratio, high-density lipoprotein cholesterol (HDL-C), total lipids, and total antioxidant capacity compared with the control group. In contrast, serum glucose, total protein, albumin, cholesterol, low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea concentrations were not significantly influenced ($P > 0.05$). The present findings indicate that EO supplementation did not alter serum total protein, albumin, glucose, urea, AST, or ALT, which is consistent with the observations of Elazab et al. (2025). The decline in globulin concentration aligns with the results of Kholif et al. (2012), who reported that supplementation of goats with ginger essential oil (2 mL/head/day) reduced serum globulin, possibly due to alterations in ruminal microbial protein synthesis. Conversely, Anaso et al. (2025a) reported decreased concentrations of total protein, cholesterol, and the A/G ratio with EO additives, suggesting that such responses may vary depending on species, dosage, or oil type.

Although serum cholesterol and LDL-C remained unaffected in the current trial, the significant increase in HDL-C with ZEO supplementation highlights a potential health-promoting effect, as HDL plays a crucial role in cardiovascular protection by reducing risks of atherosclerosis, stroke, and heart disease. Moreover, the marked elevation of serum total lipids and total antioxidant capacity in ZEO-fed cows corroborates the findings of El-Essawy et al. (2019), who observed that clove supplementation (either at 5% of diet DM or 2 mL/day of clove EO) significantly enhanced serum HDL-C, total lipids, and antioxidant activity.

Collectively, these results underscore the potential of ZEO as a natural feed additive that can modulate lipid metabolism and enhance antioxidant defense mechanisms in dairy cattle.

Table 1. Blood biochemical parameters White Fulani Dairy Cows supplemented Ginger (*Zingiber Officinale*) essential oil

Parameter	T1	T2	T3	SEM
Total protein (g/dL)	5.77	5.29	5.29	0.54
Albumin (g/dL)	2.64	2.68	2.70	0.24
Globulin (g/dL)	3.13 ^a	2.61 ^b	2.59 ^b	0.37
Albumin globulin ratio (g/dL)	0.85 ^b	0.99 ^a	1.01 ^a	0.06
Glucose (mg/dL)	60.48	60.51	60.53	6.08
Cholesterol (mg/dL)	165.11	165.95	166.01	2.10
High density lipoprotein (mg/dL)	15.86 ^b	16.91 ^a	17.00 ^a	0.16
Low density lipoprotein (mg/dL)	140.08	140.06	140.07	3.48
Total lipids	200.16 ^b	209.99 ^a	210.08 ^a	3.86
Aspartate transaminase (u/L)	109.84	109.91	109.99	3.60
Alanine transaminase (u/L)	25.61	25.84	25.91	2.26
Urea (mg/dL)	14.56	14.63	14.71	0.23
Total antioxidant capacity	0.59 ^b	0.68 ^a	0.70 ^a	0.46

CONCLUSIONS

The results of this study indicate that dietary supplementation with *Zingiber officinale* essential oil (ZEO) at levels up to 10 mL/kg diet is safe for lactating cows and exerts beneficial effects on lipid metabolism and oxidative balance, without compromising key biochemical health parameters. These findings highlight the potential of ZEO as a phytogetic feed additive capable of enhancing metabolic resilience in dairy cattle. Nonetheless, further research is warranted to assess its efficacy over longer feeding periods, across different physiological stages, and in combination with other phytogetic compounds, in order to fully establish its long-term role in optimizing dairy cow performance under tropical production systems.

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**COMPARATIVE IN VITRO ANTIOXIDANT AND TRYPANOCIDAL ACTIVITIES
OF N-HEXANE AND ETHYLACETATE EXTRACTS OF AZADIRACHTA INDICA
ON TRYPANOSOMA BRUCEI BRUCEI**

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ABSTRACT

The parasitic disease known as trypanosomiasis, caused by protozoa of the genus *Trypanosoma*, remains a major health issue in Sub-Saharan Africa, affecting both humans and animals. In this study, phytochemical analysis, antioxidant analysis via ferric reducing antioxidant power, 2, 2-diphenyl-1-picrylhydrazyl and Inhibition of lipid peroxidation) as well as in vitro trypanocidal activities of n-hexane and ethylacetate extracts of *Azadirachta indica* (NAI and EAI) were carried out using standard methods. For in vitro analysis blood obtained from infected donor mice was diluted with glucose phosphate buffered saline to contain 1×10^3 parasites. Exactly 20 μL of the blood sample contained parasite was incubated with varying concentrations of 62.5 $\mu\text{g/mL}$, 125 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ of n-hexane and ethylacetate extract of *Azadirachta indica* in separate microtiter plates in triplicate for 190 minutes. The mixture was observed under microscope for parasite motility at 10 minutes intervals. Quantity of the phytochemicals are present in the following order; phenol > saponins > tannins > alkaloids > flavonoids in both samples while EAI showed high antioxidant activities in all concentrations. Complete cessation of the parasite motility was observed for all test concentrations within 190 minutes of the study. The most active extract was ethylacetate extract of *Azadirachta indica* which causes complete cessation of the parasite motility within 50 minutes in 500 $\mu\text{g/mL}$ concentration. Whereas NAI caused complete cessation of the parasite motility within 90 minutes in 500 $\mu\text{g/mL}$ concentration when compared with berenil that occurred within 10 minutes at 500 $\mu\text{g/mL}$. These results showed that EAI has high trypanocidal and antioxidant activities when compared with NAI. Hence, EAI could explored further for the treatment of African Animal Trypanosomiasis.

Keywords: *Azadirachta indica*, Trypanosomiasis, Antioxidant, Trypanocidal activities.

INTRODUCTION

Trypanosomiasis also known as sleeping sickness is a parasitic disease caused by protozoan parasites of the genus *Trypanosoma* (Ibrahim et al., 2022). The disease is endemic to Sub-Saharan Africa and is transmitted primarily by the bite of infected tsetse flies (*Glossina* species). Human African Trypanosomiasis (HAT) manifests in two forms: the chronic form caused by *Trypanosoma brucei gambiense*, and the acute form caused by *Trypanosoma brucei rhodesiense* (Ibrahim et al., 2022). This disease poses a significant public health challenge in many African countries, where poverty, limited healthcare infrastructure, and remote populations hinder effective treatment and control (Kayode et al., 2020).

In livestock, trypanosomiasis, also known as Animal African Trypanosomiasis (AAT), leads to significant economic losses due to reduced productivity, weight loss, and mortality in cattle, sheep, goats, and other animals. The disease burden in both humans and livestock not only affects individual health but also hampers agricultural productivity, contributing to food insecurity and poverty in affected regions (Maichomo et al., 2021).

The current treatment options for trypanosomiasis are limited to a few drugs such as suramin, pentamidine, melarsoprol, and eflornithine, all of which have significant drawbacks. These drugs are associated with severe side effects, including toxicity, and the need for prolonged hospitalization for their administration. Moreover, drug resistance has emerged as a growing concern, further limiting the effectiveness of these treatments (Kayode et al., 2020). As a result, there is an urgent need to explore alternative therapies that are safe, effective, and affordable for populations in endemic areas.

In recent years, researchers have turned their attention to natural products and medicinal plants as potential sources of new trypanocidal agents. Plants have been used in traditional medicine for centuries and are known to contain bioactive compounds that possess antimicrobial, antifungal, antiviral, and antiparasitic properties. One such plant is *Azadirachta indica*, commonly known as neem. Neem has been widely used in Ayurvedic and African traditional medicine to treat a variety of ailments, including parasitic infections (Wylie and Merrel, 2021). Neem leaves contain a wide range of bioactive compounds, including limonoids, flavonoids, triterpenoids, and azadirachtin, which have shown promise in preclinical studies for their antimicrobial and antiparasitic activities. Neem (*Azadirachta indica*), a plant with a long history of medicinal use, presents a promising alternative due to its rich phytochemical composition. Various parts of the neem tree, including the leaves, seeds, and bark, contain bioactive compounds that have been shown to possess antiparasitic, antimicrobial, and anti-inflammatory properties (Wasim et al., 2023). Given the rich phytochemical profile of neem, its potential as a trypanocidal agent is an area of increasing interest (Wylie and Merrel, 2021). This study focuses on the in vitro trypanocidal activities of neem leaf extracts obtained using two solvents: n-hexane and ethylacetate, which are known for their efficacy in extracting different classes of bioactive compounds.

MATERIALS AND METHODS

Materials

Plant Material

Fresh *Azadirachta indica* leaves (neem) were obtained from Federal University of Technology, Gidan Kwano Campus, Minna, Niger State, Nigeria in July, 2023 and authenticated by Dr. O.A.Y. Daudu of the department of Biological Sciences, Federal University of Technology, Minna with voucher number FUT/PLB/MEL/001. The plant sample was deposited in the herbarium of the department of Plant Biology, Federal University of Technology, Minna.

Chemicals and Reagents

The chemicals and reagents used includes: ethylacetate, n-hexane, dimethyl sulfoxide (DMSO), folin-ciocalteu's reagent, sodium carbonate, gallic acid, absolute methanol, aluminum chloride, sodium acetate, distilled water, folin-denis' reagent, standard tannic acid, petroleum ether, acetone-ethanol, ferrous sulphate, sulphuric acid, standard saponins, formaldehyde, acetate buffer, TPTZ solution, and ferric chloride solution, DPPH solution, linoleic acid, thiobarbituric acid, diminazene aceturate were products of Sigma Chemical Co., USA.

Parasites

The parasite was sourced from stablites preserved at the Nigerian Institute of Trypanosomiasis Research (NTR) in Kaduna, Kaduna State. It was maintained in the Animal House of the Department of Biochemistry, Federal University of Technology, Minna, through continuous.

Site of Experiment

The experiment was conducted at the Centre of Genetic Engineering and Biotechnogy, Federal University of Technology, Minna, Bosso Campus. Minna is situated within the Southern Guinea Savanna region of Nigeria, at 9°33' N latitude and 9°37' E longitude.

Methods

Preparation of *Azadirachta indica* Leaves

The *Azadirachta indica* leaves were thoroughly washed with tap water to remove dirt and dried at 30 °C. Thereafter, the plant leaves were reduced to powder using the Silver Crest 2L Industrial 8500W electric blender, and the powder form was kept inside a tight covered plastic container.

Extraction of Bioactive Compounds

Extraction of *Azadirachta indica* leaf was done as reported by Busari et al. (2025). Briefly, exactly 250 grams of *Azadirachta indica* leaf powder was weighed into 1000 mL volumetric flask. Afterwards, 750 mL of each solvent was separately poured into flask and mixture was properly agitated and left for 72 hours with occasional vortexing each day. After the 72 hours, the solvent was evaporated using a water bath under reduced pressure before finally lyophilized with freeze drier. The freeze-dried sample was stored in tight covered glass containers and refrigerated at 10°C.

Phytochemical Screening (Quantitative Test)

Determination of phenolic content

The n-hexane plant extract was dissolved in 0.01 g of 10 cm³ distilled water, and 0.5 cm³ of this solution was oxidized using 2.5 cm³ of 10% Folin-Ciocalteu reagent. The solution was neutralized with the addition of 2 cm³ of 7.5% sodium carbonate. This mixture was then incubated at 45°C for a duration of 40 minutes. Following incubation, its absorbance was measured at 765 nm using a UV spectrophotometer. A calibration curve was generated using standard gallic acid (Singleton et al., 1999). This process was also carried out for the ethylacetate plant extract.

Determination of Flavonoid Content

In a test tube, 1.5 cm³ of absolute methanol was combined with 0.1 cm³ of 10% aluminum chloride, 0.1 cm³ of 1 M sodium acetate, and 2.8 cm³ of distilled water. Then, 0.5 cm³ of the plant extract in n-hexane was added.

The resulting mixture was incubated at room temperature for 30 minutes, after which its absorbance was measured at 415 nm using a UV spectrophotometer. The above process was also carried out for the ethylacetate extract. Standard quercetin was used to prepare the calibration curve. (Chang et al., 2002).

Determination of Tannin Content

A 0.2 g sample of the plant extract in n-hexane was placed in a 50 cm³ beaker, to which 20 cm³ of 50% methanol was added. The beaker was then covered with parafilm and heated in an 80°C water bath for 1 hour. After heating, the mixture was thoroughly shaken to ensure uniform distribution and then filtered into a 100 cm³ volumetric flask. To the filtered solution, 20 cm³ of distilled water, 2.5 cm³ of Folin-Denis reagent, and 10 cm³ of sodium carbonate were added and mixed thoroughly. After standing at room temperature for 20 minutes, the solution developed a bluish-green color. The absorbance was taken at 760 nm using a UV-spectrophotometer. The same process was also carried out for the ethylacetate extract. Standard tannic acid was used to prepare the calibration curve.

Determination of Saponin Content

A 0.5 g sample of the plant extract in n-hexane was weighed and dissolved in 20 cm³ of 1N HCl, then heated in a water bath at 80°C for 4 hours. Once cooled, the mixture was filtered, and 50 cm³ of petroleum ether was added. The ether layer was then separated and evaporated to dryness. The resulting dry residue was combined with 5 ml of a 1:1 acetone-ethanol mixture, 6 cm³ of ferrous sulfate, and 2 cm³ of concentrated sulfuric acid, and allowed to stand for 10 minutes. Absorbance was recorded at 490 nm. The same procedure was conducted for the ethylacetate extract, using standard saponins to establish the calibration curve (Oloyed, 2005).

Determination Of Alkaloid Content

A 0.5 g sample of the n-hexane extract was weighed and dissolved in 5 cm³ of a 1:1 mixture of 96% ethanol and 20% H₂SO₄, then filtered. One cm³ of the filtrate was added to a test tube with 5 cm³ of 60% H₂SO₄ and left to stand for 5 minutes. Following this, 5 cm³ of 0.5% formaldehyde was added, and the mixture was kept at room temperature for 3 hours. Absorbance was measured at 565 nm. The same procedure was applied to the ethyl acetate extract, using the extinction coefficient of vincristine (E₂₉₆, EtOH = 15136 M⁻¹ cm⁻¹) as a reference for alkaloids (Oloyed, 2005).

Antioxidants Activities Assay

Ferric Reducing Antioxidant Assay

The FRAP reagent was prepared by mixing acetate buffer, TPTZ solution, and ferric chloride solution. The *Azadirachta indica* n-hexane extract was then added to the FRAP reagent, incubated for 30 minutes, and its absorbance was measured at 593 nm using a spectrometer. The antioxidant capacity was assessed by comparing the change in absorbance to that of the standard. The same procedure was followed for the ethylacetate extract of *Azadirachta indica*.

The 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging

A DPPH solution was prepared in methanol, to which the n-hexane extract was added and thoroughly mixed. The mixture was then incubated in the dark for 30 minutes, followed by absorbance measurement at 517 nm. This process was also applied to the ethyl acetate extract. The percentage of radical scavenging activity was calculated in comparison to a control.

The Inhibition of Lipid Peroxidation

Linoleic acid was homogenized mixed with the n-hexane extract. The mixture was incubated at 37 °C for 30 minutes. Thiobarbituric acid (TBA) reagent was added to the mixture, the mixture was then heated to form a colored MDA-TBA complex (Malondialdehyde – Thiobarbituric). The percentage of lipid peroxidation inhibition was calculated compared to a control.

Trypanocidal Study

Test Organism

The *Trypanosoma brucei brucei* (Federa strain) was sourced from stablites maintained at the Nigerian Institute of Trypanosomiasis Research (NITR) in Kaduna, Nigeria, and kept in the Animal House through repeated passaging in mice until required. Passages were conducted when parasitemia levels reached between 16 and 32 parasites per field, generally occurring 3 to 5 days post-infection. For each passage, 1×10^3 parasites in 0.1 to 0.2 mL of blood were injected intraperitoneally into mice that had been acclimated to laboratory conditions for two weeks. (Abedo et al., 2013).

In vitro Trypanocidal Activity

The in vitro trypanocidal activity assessment was conducted in quadruplicate using 96-well microtiter plates (Tewabe et al., 2014). A 0.5 g sample of the n-hexane plant extract was dissolved in 10 ml of distilled water to create a 0.05 g/ml stock solution (equivalent to 50,000 µg/ml). From this stock, solutions of 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml were prepared, resulting in four different concentrations. The same procedure was applied to the ethylacetate plant extract.

A 20 µl blood sample containing approximately 20–25 parasites per microscopic field was mixed with 5 µl of each test concentration, specifically 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml, respectively. To verify that any effects observed were specifically from the extract, two control setups were established. The first control used Berenil (Diminazene aceturate, a reference drug) as the positive control, while the second control consisted of blood mixed with glucose-phosphate buffered saline as the negative control. Berenil was prepared to match the concentrations of the test solutions.

Each test mixture was incubated for 5 minutes in sealed microtiter plates at 37°C. Then, 2 µl of each test mixture was placed on separate microscope slides, covered with 7×22 mm cover slips, and the parasites were observed every 10 minutes for a total of 60 minutes, monitoring for any cessation or reduction in motility, using a 400 x objective lens (Tewabe et al, 2014).

Results

Quantitative Phytochemical Screening

The table below shows the phytochemical analysis of *Azadirachta indica* (neem) extracts. The analysis revealed significant differences in the concentrations of bioactive compounds between the n-hexane and ethylacetate extracts. The ethylacetate extract exhibited higher levels of phenols (134.49 ± 0.7 mg/g), tannins (42.81 ± 0.5 mg/g), saponins (101.85 ± 0.8 mg/g), and alkaloids (5.85 ± 0.2 mg/g) compared to the n-hexane extract. These compounds are known for their potent antioxidant, antiparasitic, and membrane-disrupting properties, which may explain the higher trypanocidal activity observed in the ethylacetate extract.

While the n-hexane extract had higher flavonoid content (3.33 ± 0.4 mg/g), the overall lower concentrations of key phytochemicals suggest that the ethylacetate extract contains more bioactive compounds responsible for its stronger antitrypanosomal effects.

Table 1. Quantitative phytochemical composition of N-hexane and ethylacetate extract of *Azadirachta indica*

Sample	Concentration (mg/100g)				
	Phenols	Flavonoids	Tannins	Saponins	Alkaloids
AIN	114.73±0.6	3.33±0.4	30.43±0.5	87.01±0.2	4.05±0.8
AIE	134.49±0.7	1.39±0.5	42.81±0.5	101.85±0.8	5.85±0.2

Note: AIN = n-hexane extract of *Azadirachta indica*, AIE = ethylacetate extract of *Azadirachta indica*

Antioxidants Activities of N-Hexane And Ethylacetate Extracts of *Azadirachta Indica* **Ferric Reducing Antioxidant Power of N-Hexane and Ethylacetate Extracts of *Azadirachta Indica***

The antioxidant capacities of the n-hexane and ethylacetate extracts of *Azadirachta indica* were evaluated using the FRAP assay at various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml), as presented in the table below. The results indicate that the ethylacetate extract exhibited stronger ferric reducing antioxidant power (FRAP) at all tested concentrations compared to the n-hexane extract. At the highest concentration (500 µg/ml), the ethylacetate extract showed the highest FRAP value (73.08 ± 0.1), while the n-hexane extract displayed a lower value (65.45 ± 0.3). As the concentration decreased, the FRAP values for both extracts reduced accordingly, with the ethylacetate extract maintaining higher activity even at the lowest concentration (62.5 µg/ml), where it recorded a FRAP value of 19.02 ± 0.2 compared to 15.93 ± 0.2 for the n-hexane extract. This suggests that the ethylacetate extract possesses superior antioxidant capacity, which may contribute to its higher trypanocidal activity.

Table 2. Ferric Reducing Antioxidant Power of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	Concentrations			
	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml
AIN	65.45±0.3	51.20±0.3	31.82±0.2	15.93±0.2
AIE	73.08±0.1	59.21±0.3	44.93±0.2	19.02±0.2

Note: AIN = n-hexane extract of *Azadirachta indica*, AIE = ethylacetate extract of *Azadirachta indica*

The 2,2-Diphenyl-1-picrylhydrazyl of N-hexane and Ethylacetate extracts of *Azadirachta indica*

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was performed to assess the free radical scavenging capabilities of the n-hexane and ethylacetate extracts of *Azadirachta indica* at various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml).

The ethylacetate extract showed stronger antioxidant activity across all concentrations compared to the n-hexane extract. At the highest concentration (500 µg/ml), the ethylacetate extract exhibited the highest DPPH radical scavenging activity (62.81±0.2), while the n-hexane extract had a lower activity (54.54±0.2). As the concentration decreased, the free radical scavenging ability of both extracts diminished. At the lowest concentration (62.5 µg/ml), the ethylacetate extract still showed higher activity (9.77±0.2) compared to the n-hexane extract (3.02±0.2). These results indicate that the ethylacetate extract possesses better antioxidant activity, which may contribute to its stronger trypanocidal efficacy.

Table 3. The 2,2-Diphenyl-1-picrylhydrazyl of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	Concentration			
	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
AIN	54.54±0.2	41.21±0.3	22.96±0.1	3.02±0.2
AIE	62.81±0.2	47.66±0.2	29.71±0.3	9.77±0.2

Note: AIN = n-hexane extract of *Azadirachta indica*, AIE = ethylacetate extract of *Azadirachta indica*

The Inhibition of Lipid Peroxidation of N-hexane and Ethylacetate extracts of *Azadirachta indica*

The ILP (Inhibition of Lipid Peroxidation) assay was performed to assess the ability of n-hexane and ethylacetate extracts of *Azadirachta indica* to prevent lipid peroxidation at different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml). The ethylacetate extract exhibited stronger inhibitory effects on lipid peroxidation across all concentrations compared to the n-hexane extract. At 500 µg/ml, the ethylacetate extract demonstrated the highest inhibition (50.33±0.2), while the n-hexane extract showed lower inhibition (42.31±0.7). As the concentrations decreased, the inhibition of lipid peroxidation declined for both extracts. Even at the lowest concentration of 62.5 µg/ml, the ethylacetate extract maintained a higher inhibition value (3.12±0.2) compared to the n-hexane extract (1.54±0.1). These results indicate that the ethylacetate extract is more effective in preventing lipid peroxidation, likely due to its higher content of bioactive compounds, contributing to its overall antioxidant and trypanocidal activities.

Table 4. The Inhibition of Lipid Peroxidation of N-hexane and Ethylacetate extracts of *Azadirachta indica*

Sample	Concentration			
	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml
AIN	42.31±0.7	32.91±0.2	19.32±0.2	1.54±0.1
AIE	50.33±0.2	37.29±0.2	26.22±0.2	3.12±0.2

Note: AIN = n-hexane extract of *Azadirachta indica*, AIE = ethylacetate extract of *Azadirachta indica*

Effect Of Different Concentrations Of N-Hexane And Ethylacetate Extracts Of *Azadirachta Indica* On Motility Of *T. Brucei Brucei*

The Table (5) presents the effects of different concentrations of n-hexane and ethylacetate extracts of *Azadirachta indica* on the motility of *Trypanosoma brucei brucei*.

At 500 µg/ml, the n-hexane extract ceased the parasite's motility after 90 minutes, while the ethylacetate extract was more effective, ceasing motility in 50 minutes. As the concentrations decreased, the time taken for motility cessation increased for both extracts but less effective than diminazene Aceturate which took 10 minutes cessation at the same concentration.

Table 5. Effect of Different Concentrations of N-hexane and Ethylacetate extracts of *Azadirachta indica* on Motility of *T. brucei brucei*

Test Substance	Time (Minutes) after which motility ceased with different concentrations of extracts Parasite motility (µg/ml)			
	500 µg/ml	250 µg/ml 62.5 µg/ml	125 µg/ml	
AIN	90.40 ± 8.30	130.20 ± 10.60	180.40 ± 12.10	240.60 ± 15.50
AIE	50.40 ± 5.10	90.30 ± 6.10	150.40 ± 8.10	190.70 ± 10.10
Diminazene Aceturate	10.20 ± 1.15	10.50 ± 1.20	10.60 ± 1.30	30.80 ± 2.90

Value was expressed as the mean of triplicate ± standard deviation

AIN = n-hexane extract of *Azadirachta indica*, AIE = ethylacetate extract of *Azadirachta indica*

DISCUSSION

The findings showed higher antioxidant activates in AIE might be as a result of content of phenols, tannins and flavonoids. These compounds contain hydroxyl groups that can serve as reactive oxygen species quenchers (Busari et al., 2021). The same effect that was revealed by AIE on higher exhibition of trypanocidal effects might be due to higher phytochemicals such as flavonoids, tannins, phenolics, and alkaloids, which have been reported to possess strong antiprotozoal and antiparasitic properties (Rahman et al., 2020). These phytochemicals are known for their ability to induce oxidative stress in parasites, disrupt cellular processes, and inhibit enzyme activities essential for parasite survival. The higher trypanocidal activity observed in the ethylacetate extract may be attributed to these compounds ability to interfere with the metabolism and reproduction of the parasite, ultimately leading to its death (Patel et al., 2022).

Although, ability of NAI to show less trypanocidal activities than the ethylacetate extract suggests that less polar solvent could still extract potent compounds that can still play a role in combating *T. brucei brucei* existence. However, the superior performance of the ethylacetate extract suggests that the polar solvent could extract compounds from *A. indica* that may have a stronger affinity for intracellular targets within the parasite. This finding is consistent with previous research indicating that *A. indica* extracts contain a variety of bioactive compounds with potential antitrypanosomal properties. This study reinforces the idea that *Azadirachta indica* could be a promising natural source of trypanocidal agents, with the ethyl acetate extract showing particularly strong activity in this regard (Rahman et al., 2020).

Hence, in vivo antioxidants, trypanocidal and toxicities studies of ethylacetate extract should be carried out to affirm the antioxidant and trypanocidal effects as well as safety of the extract.

The active compound(s) should also be isolated in order to obtain the trypanocidal drug candidate as well as to unravel the mechanism through which the isolated compound(s) exhibit their trypanocidal activities.

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**RADIOGRAPHIC, CLINICAL, AND BIOCHEMICAL ASSESSMENT OF SCREW-
RETAINED VERSUS CEMENT-RETAINED CR-CO-CERAMIC IMPLANT-
SUPPORTED SINGLE CROWNS: A COHORT STUDY**

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ABSTRACT

Background: The analysis that was conducted in this study, comparing two different crown-retention solutions, considered periodontal indices and standardized radiographic measurements and the indices of possible damage which now represent a concrete help in diagnosis and prognosis.

Materials and Methods: The present study was designed to compare peri-implant soft and hard tissue response after 2 years of functional loading between direct screw-retained posterior single

crowns in Cr-Co/Ceramic or cement-retained Cr-Co/Ceramic on titanium abutment. 24 non-smoking partially edentulous patients, requiring implant therapy for a prosthetic rehabilitation, were included in the study. The values of IL-1 β , IL-6, IL-8 were evaluated, together with the periodontal indices PI, GI, PD, BOP, REC. The PICF was collected with standardized paper strips. Repeated measure ANOVA was used between groups months at 1, 3, and 12 months.

Results: During the time of clinical observation, at t0 (1 month), t1 (3 months) and t2 (12 months), both types of crowns performed satisfactorily from a clinical point of view. In light of the results obtained, it is possible to define that screwed Cr-Co/Cer has slightly higher values than the other.

Conclusions: It is possible to hypothesize, that this difference could lead to a clinically visible difference between these two prosthetic solutions in the longer term. The single screwed crown in Cr-Co shows a different interface with the titanium implant connection compared to the titanium-titanium contact of the connection between the implant and the titanium abutment for the cement-retained prosthetic solution.

Keywords: Cr-Co; Implants; Titanium; Cement-retained; Screw-retained; Ceramic.

INVESTIGATION OF THE EFFECT OF DIFFERENT NUTRITIONAL GROUPS ON BEE VENOM PRODUCTION CAPACITY AND COMPOSITION UNDER IN VITRO CONDITIONS

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ABSTRACT

Introduction and Purpose: In recent years, alongside conventional treatments, various supportive therapeutic methods have become widely used to complement them. One of these supportive treatment types is apitherapy. Although apitherapy products are generally honey, pollen, propolis, and royal jelly, in recent years, bee venom has gained significant importance. Bee venom is a very valuable product. Increasing the production of bee venom will facilitate access to this product and increase its use against diseases. Bee venom has been tested on a wide range of diseases and has yielded very interesting results. However, its use is limited by the fact that some people show allergic reactions to this product. Therefore, to obtain the desired result more effectively, the component in bee venom that is to be used against a specific disease needs to be in a high ratio. For these reasons, studies have been conducted to investigate whether the content of the venom changes, and if so, to what extent, by feeding bees with certain syrups under in-vitro conditions.

Materials and Methods: All of our studies were conducted under in-vitro conditions. The materials used in our studies consisted of bees all born on the same day. The bees were placed into cages, with five cages allocated to each group, and each cage containing 150 bees. These bees were fed with glucose, fructose, sucrose, invert sugar, and honey for 20 days. Honey was used as the control group. On the 21st day, all bees were placed in a deep freezer, and their venom sacs were removed along with their stingers. These sacs were analyzed for apamin, melittin, and phospholipase A2 at Muğla Sıtkı Koçman University using an HPLC device.

Results: Based on the results obtained, although there was a positive increase in quantity in all groups, the feeding with invert sugar was the one that increased the quantity the most. As a result of the chemical analyses, the change in melittin was not statistically significant in any group. When examined for apamin, the invert sugar and sucrose groups were found to be significantly different. For phospholipase A2, glucose, fructose, and invert sugar feedings showed a significant difference..

Discussion and Conclusion: More effective bee venom for treating diseases could be possible through purpose-driven feeding of bees.

Keywords: In vitro, apamin, melittin, Phospholipase A2, nutrition

**CLINICAL BIOCHEMICAL EFFECTS OF STRESS, NUTRITION, AND
ENVIRONMENTAL FACTORS IN BLACK SEA TROUT (*Salmo trutta labrax*)**

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ABSTRACT

Introduction and Purpose: *Salmo trutta labrax* is an economically significant species that excels in the Black Sea environment both in its natural stock and aquaculture yield. Farming success and health of the species depend on understanding its metabolic and biochemical responses. Fish stress physiology, dietary effects on biochemical parameters, and biochemistry-mediated metabolic reactions to environmental stimuli are all important areas of research for aquaculture professionals and veterinary biochemistry researchers. In particular, the fluctuating Black Sea environmental conditions, such as water temperature, salinity, and environmental contaminants, have a direct impact on the biochemical profile of the fish. Experimental works have found that acute stress conditions cause significant elevations of the key stress markers such as blood cortisol and glucose, and thus temporarily alter the metabolic state of the fish. Immunological markers such as lysozyme activity also react sensitively to stress. Nutritional intervention has been found to directly affect fish growth performance and feed conversion ratio, albeit much less so on general health parameters such as condition factor. Furthermore, environmental pollutants and heavy metals have been reported to cause effects of sub-lethal physiological debilitation through suppressing the activity of important enzymes for vital functions such as fish respiration and ion balance.

Discussion and Conclusion: Clinical biochemical indices such as biochemistry in blood (glucose, lactate, cortisol), markers of oxidative stress (MDA, SOD, CAT, GPx), and immunological variables (cytokines, complement system) are valuable parameters for health checks of aquaculture. These variables not only provide critical insights into the stress physiology of *S. trutta labrax* but also reflect the adaptability of the species to environmental changes and anthropogenic stressors. The review will involve biochemical responses of *Salmo trutta labrax*, changes in metabolism due to stress and diet, and adaptive mechanisms it gains against environmental poisons. Lastly, the economic and biotechnological implications of such findings on the producers, particularly the Black Sea coastal producers of Turkey, will be discussed.

Keywords: *Salmo trutta labrax*, Black Sea, Biochemistry, Stress, Oxidative Stress, Turkey, Aquaculture

INTRODUCTION

The Black Sea trout (*Salmo trutta labrax*) is a native species unique to the Black Sea and holds significant economic value both within the natural ecosystem and in aquaculture 1. The physiological condition of this species is closely affected by many factors, such as environmental stress factors, feeding regime, and water quality. As production targets in aquaculture continue to rise and the need to protect natural populations increases, monitoring the biochemical and physiological changes in *S. trutta labrax* is crucial for tracking environmental changes and evaluating aquaculture conditions.

Stress in fish is a complex process involving a series of physiological and biochemical changes. The activation of primary stress markers, cortisol and glucose, defines an acute stress response 2. For example, significant increases in cortisol and glucose levels have been recorded in Black Sea trout exposed to acute thermal stress 2,3. Long-term stress has been shown to have more serious consequences such as immunosuppression, reduced growth rate, and negative effects on reproductive performance 4. The physiological response of fish to stress factors may vary depending on the species, age, sex, intensity, and duration of the stress factor 4,5. Oxidative stress is one of the most prominent biochemical responses in fish following exposure to environmental pollutants or other stress factors 6,7. Biomarkers of oxidation levels, such as antioxidant enzyme activity and lipid peroxidation, have been key parameters for assessing oxidative stress status 7,8.

Nutrition is closely related to fish growth, development, reproduction, and disease resistance. Ensuring sufficient levels of protein, lipids, carbohydrates, vitamins, and minerals in feed has a significant effect on fish metabolism and clinical biochemical parameters 9. For example, the effect of diets with different protein content on the growth performance and intestinal villus morphology of Black Sea trout has been investigated 10. The hematological and biochemical parameters of fish can be improved with functional feeds and dietary supplements, which also increases stress resistance 9,11,12. Adding antioxidants to the diet, especially vitamins such as vitamin E, may positively affect the reproductive performance of Black Sea trout broodstock 11. Additionally, alternative protein sources such as insect meal may provide some benefits on the growth performance, microbiota, and blood biochemical parameters of Black Sea trout fry 13.

The physical and chemical properties of the marine environment where Black Sea trout live can have a significant impact on the physiological condition of the fish. Water temperature, dissolved oxygen, pH, salinity, and the presence of pollutants are some of the physical and chemical factors that affect the biochemical responses of fish 14,15. For example, water temperature is an important environmental factor that affects all biochemical and physiological processes in fish 14. The effects of hydroelectric power plants (HES) on rivers in the Eastern Black Sea may have negative impacts on aquatic ecosystems and Black Sea trout populations. Decreased compensation water has led to habitat loss, higher water temperatures, and algal colonization, contributing to the decline of trout populations 16. Heavy metals and other chemicals can cause oxidative stress and biochemical abnormalities in different organs of fish 17. These environmental challenges can exceed the adaptive capacity of fish, disrupting their normal functioning and reducing their health and survival rates.

Biochemical parameters in fish are influenced by physiological and metabolic responses and the interactions between these factors, determining the overall health status of the fish. Further research is needed to understand these interactions and propose management strategies aimed at protecting and improving Black Sea trout fishing and aquaculture.

In particular, the interaction of various stress factors, the long-term effects of dietary regimes on physiological responses, and genetic adaptation mechanisms to environmental changes represent promising areas for research. This review aims to provide a comprehensive approach to the effects of stress, nutrition, and environmental factors on clinical biochemistry in Black Sea trout.

Stress Physiology in Fish

Stress in fish is defined as a series of physiological and behavioral responses that occur when an environmental or internal factor disrupts or threatens to disrupt the animal's normal physiological balance 18. These responses are adaptive mechanisms that have evolved to help the organism cope with stressors and survive 19. The stress response is generally examined in three stages: primary, secondary, and tertiary responses. The primary response involves the activation of the hypothalamic-pituitary-adrenal (HPI) axis upon perception of the stressor and the release of stress hormones such as cortisol into the bloodstream 20. This hormonal signal triggers secondary responses. Secondary responses include metabolic and physiological adaptations such as increased blood glucose levels, changes in ion transport in the gills, and alterations in blood circulation. If the stressor persists for a long time or is very severe, tertiary responses emerge. This stage is characterized by adverse effects at the organism level, such as slowed growth, reduced reproductive performance, and suppression of the immune system (Figure 1). This situation can lead to the fish becoming more susceptible to disease and a deterioration in the overall health of the population 21. Especially in aquaculture conditions, stock density, changes in water quality, handling, and transport can be chronic sources of stress for fish. Therefore, understanding stress physiology is of fundamental importance for ensuring fish welfare and increasing aquaculture productivity.

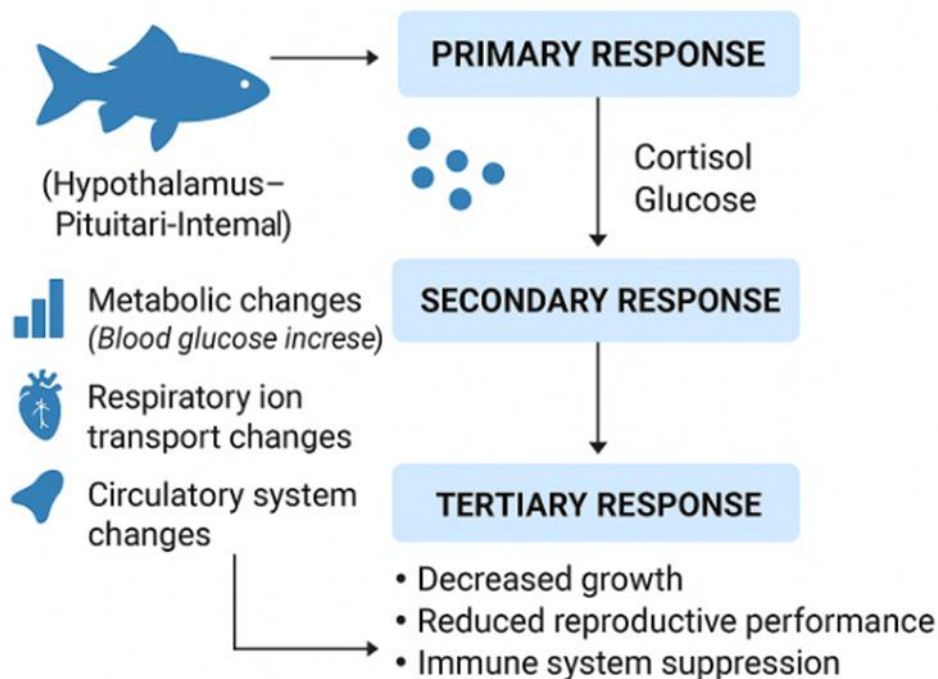


Figure 1. Stress and physiological response mechanisms in fish

Another important aspect of the stress response is oxidative stress. Under stressful conditions, the production of reactive oxygen species (ROS) increases in cells. Normally, antioxidant defense systems (enzymes such as superoxide dismutase - SOD, catalase - CAT, glutathione peroxidase - GPx) neutralize these molecules. However, when stress causes excessive ROS production that exceeds antioxidant capacity, oxidative stress occurs 22. This situation leads to the peroxidation of lipids in cell membranes and damage to proteins and DNA. Oxidative stress is associated with many pathological conditions, such as impaired immune function and cellular aging 21,23. Many processes in aquaculture cause stress in fish 24. In economically important species such as *Salmo trutta labrax*, monitoring the physiological and biochemical changes caused by both acute and chronic stress provides critical biomarkers for protecting population health and optimizing aquaculture management strategies. For example, sudden increases in blood cortisol and glucose levels indicate acute stress, while decreased growth rates, weakened immune parameters, and chronic increases in oxidative stress markers may signal prolonged suboptimal conditions. The comprehensive evaluation of these biomarkers plays a key role in understanding the adaptive capacity of *Salmo trutta labrax* to environmental changes and farming conditions. This facilitates the monitoring of fish health in aquaculture while also serving as a bio-monitoring tool for tracking environmental changes.

Primary Stress Responses in Fish

The first and fastest responses observed in fish exposed to stress factors are called primary responses and primarily involve activation of the endocrine system 4. When a stressor is perceived, the hypothalamus in the brain releases corticotropin-releasing hormone (CRH). CRH stimulates the pituitary gland, causing the release of adrenocorticotrophic hormone (ACTH) into the bloodstream. The ACTH entering the circulation reaches the interrenal tissue, which is part of the adrenal glands, triggering the synthesis and release of glucocorticoid hormones, particularly cortisol. This hypothalamic-pituitary-interrenal (HPI) axis is the fundamental stress response axis in fish 25. Cortisol is considered the primary indicator of stress in fish, and an increase in its levels is a sign that the organism has begun to mobilize metabolic resources to cope with stress. For example, sudden and significant increases in blood cortisol levels have been observed in Atlantic salmon (*Salmo salar*) exposed to acute heat stress 26. Similarly, it has been reported that plasma cortisol levels are significantly elevated in rainbow trout (*Oncorhynchus mykiss*) fed a diet with electrolyte imbalance or exposed to acute handling stress 21. In addition to cortisol, catecholamines (adrenaline and noradrenaline) are also rapidly released into the bloodstream with the activation of the sympathetic nervous system. These hormones increase blood pressure and heart rate, ensuring that more oxygen and nutrients are transported to the tissues. Another important component of primary responses is rapid changes in energy metabolism. Under the influence of cortisol and catecholamines, the breakdown of glycogen stored in the liver into glucose (glycogenolysis) and the synthesis of new glucose (gluconeogenesis) accelerate. As a result, blood glucose levels rise. This hyperglycemic response is an adaptive mechanism aimed at providing immediate energy substrates to critical tissues such as muscles and the brain to meet the increased energy demand during stress. Elevated blood glucose levels have been reported in Atlantic salmon 26 under acute heat stress and in rainbow trout (*Oncorhynchus mykiss*) populations exposed to different salinity levels 27. These primary responses are a series of short-term, vital hormonal and metabolic adjustments that organize the fish's "fight or flight" response.

Secondary and Tertiary Responses (Immunity, Growth, Reproductive Performance)

Secondary responses triggered by primary stress responses lead to more extensive changes in the fish's physiology and metabolism. These responses involve the redistribution of energy and resources mobilized by primary responses. One of the most prominent secondary responses is changes in blood chemistry and hematological parameters. In addition to the hyperglycemia caused by cortisol, blood lactate levels, an indicator of anaerobic metabolism, may also increase. This occurs particularly during behaviors such as escape that require intense physical activity, due to oxygen deficiency in the muscles 26. Additionally, the permeability of the gill epithelium increases, which can lead to ion and osmoregulation disturbances. This causes the fish to expend more energy to maintain the ion concentration of its body fluids 27.

Under acute stress conditions, hematocrit and hemoglobin levels increase in rainbow trout. In a study conducted by Parlak (2019), rainbow trout exposed to temefos, an insecticide commonly used to control mosquito larvae, showed dose-dependent increases in hematological parameters, particularly hemoglobin (Hb), hematocrit (Hct), MCV, and MCH levels, and these changes were reported to be related to the fish's stress response 28. This increase is related more to a decrease in plasma volume than to a change in red blood cell count. Thus, the oxygen-carrying capacity of the blood temporarily increases. While the red blood cell count generally remains constant unless there is an agent causing red blood cells to break down, a significant decrease is seen in white blood cells, particularly lymphocytes. This indicates suppression of the immune system. In conclusion, the acute stress response is characterized by an increase in cortisol and glucose levels, an increase in hematocrit, and a decrease in white blood cell count. This situation is an indicator of the suppressing effect of stress on the immune system.

When stressors are prolonged or repetitive, tertiary responses occur at the organism level. These responses generally have negative effects on the animal's overall performance and health. Growth is one of the first physiological processes to be suppressed due to energy resources being directed toward survival-focused emergency functions under stress. It has been observed that growth ceases and even weight loss occurs in rainbow trout exposed to low-temperature stress 23. Similarly, reproductive functions are also negatively affected by stress because they are energy-intensive processes. Stress can reduce gamete development, ovulation behavior, and ultimately reproductive success by inhibiting the production and secretion of reproductive hormones 29. One of the most critical consequences of tertiary responses is the suppression of the immune system. Therefore, plasma cortisol and catecholamines are considered reliable biomarkers of acute stress in fish 30. Adrenaline is defined as a stress hormone, while cortisol is defined as an adaptive hormone. The effects of cortisol on energy metabolism and other physiological functions are well known in fish; this hormone helps the organism cope with stress by stimulating the release of energy substrates into the bloodstream (secondary stress response), the glycolysis process, and other metabolic pathways 31. However, chronically elevated cortisol levels exert a suppressive effect on the immune system; they reduce lymphocyte production and activity, decrease antibody production, and weaken natural immune elements such as lysozyme activity. This renders fish much more vulnerable to pathogens and diseases. Consequently, tertiary stress responses pose serious and long-term consequences that threaten the overall sustainability of the population by jeopardizing the individual health, growth, and reproduction of fish.

Oxidative Stress Biomarkers (MDA, SOD, CAT, GPx)

Fish possess a complex antioxidant defense system to protect against oxidative damage. This system consists of enzymatic and non-enzymatic components. Among the enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play key roles. SOD converts the highly reactive superoxide radical into the less reactive hydrogen peroxide. CAT and GPx then break down hydrogen peroxide into water and oxygen, rendering it harmless. Changes in the activities of these enzymes are an indicator of the fish's response to oxidative stress. Under stress conditions, the activities of these enzymes may initially increase, functioning as a protective mechanism. However, if stress becomes chronic or too severe, the synthesis of antioxidant enzymes may be suppressed or they may be inactivated by excessive ROS, leading to a collapse of the defense system and increased cellular damage. Therefore, the combined assessment of damage markers such as MDA and antioxidant enzyme activities such as SOD, CAT, and GPx provides a comprehensive and reliable approach to understanding the physiological effects of stress and the adaptive capacity of *Salmo trutta labrax* populations.

In a comprehensive study conducted by Yousefi and colleagues (2024) on rainbow trout (*Oncorhynchus mykiss*), it was observed that plasma cortisol, glucose, hepatic glutathione peroxidase, glutathione reductase, and catalase activities significantly increased under thermal stress conditions 32. This increase has been interpreted as a defense response to the organism's increased ROS production. In a study by Duran and Çenesiz (2025), it was reported that oxidative damage increased in rainbow trout transferred to tanks with different dissolved oxygen levels, and that this situation was manifested by changes in SOD, CAT, and GPx activities, particularly accompanied by an increase in MDA levels. Furthermore, the intensity of the oxidative response varied depending on the intensity of the applied stressor 24. Similarly, in a molecular-level study conducted by Olsvik and colleagues (2005) on Atlantic salmon (*Salmo salar*), it was reported that the mRNA expression levels of SOD, CAT, and GSH-Px genes changed in fish exposed to hyperoxic conditions and that these changes could be used as indicators of oxidative stress 33. This situation demonstrates that the antioxidant defense system varies depending on the duration and intensity of the stressor, playing a protective role under acute stress but becoming insufficient under chronic stress conditions, potentially leading to oxidative damage.

Although MDA, the end product of lipid peroxidation, is not a standalone indicator of cellular damage, it can be accepted as an indicator of cellular damage because it arises from the oxidative degradation of polyunsaturated fatty acids in the cell membrane by ROS. Wang et al. (2024) reported that hydrogen peroxide-induced oxidative stress increased liver MDA content in *Lateolabrax maculatus* fish, indicating severe cellular damage 34. In a study conducted by Tuna Keleştemur et al. (2010), the transplantation procedure significantly increased malondialdehyde (MDA) levels in the serum, muscle, liver, and kidney tissues of rainbow trout (*Oncorhynchus mykiss*) significantly increased malondialdehyde (MDA) levels in serum, muscle, liver, and kidney tissues, indicating cellular damage caused by lipid peroxidation 35. These findings indicate that cellular damage is inevitable when the antioxidant defense capacity is overwhelmed.

In conclusion, the biochemical changes that develop in fish in response to oxidative stress provide valuable information about how the organism copes with environmental challenges.

The activities of antioxidant enzymes such as SOD, CAT, and GPx, along with the levels of lipid peroxidation products such as MDA, play a critical role in assessing the health of aquatic ecosystems and the physiological status of fish populations when used as biomarkers. Studies confirm that these parameters vary depending on the type, duration, and severity of stress, but when evaluated as a whole, they provide a reliable picture of oxidative stress and its consequences. Therefore, regular monitoring of these biomarkers is essential for developing conservation strategies and sustainable aquaculture practices.

The Effect of Nutrition on Biochemical Parameters and Its Relationship with Stress Physiology

Salmo trutta labrax aquaculture in Türkiye is concentrated mainly in the Eastern Black Sea region. It is cultivated in marine and land facilities in Trabzon, Rize, and Artvin 1,36. Although being a species native to this region provides adaptability to environmental conditions, the farming methods and nutritional factors applied in aquaculture can create additional stress loads. Fish nutrition is directly related to the organism's nutrient utilization processes. Therefore, it is one of the most critical issues in the aquaculture industry 37. Adequate nutrition is essential for healthy growth and maintaining physiological balance. In recent years, there has been increased interest in nutritional strategies that support the immune system and enhance disease resistance 38–41. This approach, particularly in aquaculture operations, envisions the use of nutrition as a preventive rather than a curative tool.

Feeding programs are tailored to the species' requirements. The protein requirement for *Salmo trutta labrax* ranges from 42–48%, and this ratio is adjusted based on water temperature. For example, a protein level of 45% is considered appropriate at 12–16°C, and 42% at 8–12°C. The lipid ratio is maintained between 18–22%, and the omega-3/omega-6 ratio is targeted at 3:1 42. These strategies are important not only for growth but also for regulating stress responses. Vitamins and minerals are essential for maintaining immune function. Deficiencies suppress the immune response and increase the risk of disease. For this reason, they are added to most commercial feed formulations 43. Antioxidant vitamins, in particular, have a direct protective effect against oxidative stress. Vitamin C reduces MDA accumulation by supporting SOD, CAT, and GPx activities. Vitamin E prevents lipid peroxidation in cell membranes 44. Serezli et al. (2010) demonstrated that adding vitamin E to the diet improved reproductive performance in *S. trutta labrax* broodstock 11. These results indicate that nutrition plays a protective role under stress conditions. Minerals also play an important role in stress responses. Iron deficiency reduces hemoglobin synthesis and erythropoiesis, thereby decreasing oxygen-carrying capacity. This increases the metabolic stress load 45. Magnesium is a cofactor for many enzymes. Its deficiency slows down the necessary metabolic adaptations during times of stress 46. Zinc is a critical mineral in natural immune mechanisms such as lysozyme activity and macrophage function. These elements directly affect the effectiveness of stress responses.

Nutrition in salmonids is a powerful tool for mitigating the negative effects of stress. Studies on rainbow trout and Atlantic salmon show that antioxidants, particularly vitamins C and E, reduce oxidative damage under stress and mitigate the cortisol response 44,47. Omega-3 fatty acids increase resilience under high temperature and low oxygen conditions by protecting cell membranes 48, Probiotics and prebiotics support stress tolerance by strengthening immunity 49. Although studies directly related to feeding and stress on *Salmo trutta labrax* are limited, these findings obtained from salmonids are indicative for the species. Therefore, the use of antioxidant vitamins, omega-3 fatty acids, and functional feed additives can be considered an effective strategy for stress management in Black Sea trout 50.

The feeding strategies of Black Sea trout affect not only growth but also stress tolerance. The right proteins, vitamins, and minerals reduce oxidative stress and strengthen immunity. Vitamins C and E, in particular, increase the fish's resistance to environmental challenges. Omega-3 fatty acids and minerals support cell health and metabolism. These findings show that nutrition is not just protective but also an important tool in stress management.

CONCLUSION

The Black Sea trout (*Salmo trutta labrax*) is a valuable species for the region, both in its natural habitat and in aquaculture. However, environmental changes, feeding conditions, and various stress factors directly affect the biochemical balance of this fish. Stress responses develop gradually. In acute situations, cortisol, glucose, and lactate rise rapidly, while antioxidant enzymes such as SOD, CAT, GPx, and MDA levels change depending on the duration and severity of stress. These parameters can be used as reliable biomarkers for understanding the effects of both environmental pressures and farming practices on fish health. Nutrition plays a critical role in fish farming. The balanced use of protein, lipids, vitamins, and minerals not only supports growth but also strengthens immunity and limits oxidative damage. Antioxidants such as vitamin C, vitamin E, and omega-3 fatty acids, in particular, contribute to making fish more resistant to harsh environmental conditions. Water temperature, dissolved oxygen, pollutants, and habitat changes are also key factors determining biochemical responses. Therefore, addressing environmental conditions and feeding practices together is essential for both maintaining healthy populations and sustainable farming. In conclusion, a better understanding of the interactions between stress, nutrition, and environmental factors in Black Sea trout will contribute to both the conservation of natural stocks and the efficient and sustainable continuation of production. New research in this species will pave the way for aquaculture to be built on more conscious and resilient systems in the future.

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ENVIRONMENTAL BIOMONITORING, BLOOD BIOCHEMISTRY, OXIDATIVE STRESS, AND APOPTOSIS MECHANISMS IN FISH

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ABSTRACT

Introduction and Purpose: Increased pollution in aquatic ecosystems threatens the cellular and physiological health of fish. Fish are valuable biological indicators for environmental biological monitoring, and exposure to heavy metals, pesticides, and other toxic chemicals can cause many undesirable effects, such as metabolic disorders, suppression of the immune system, and cell damage. Blood biochemistry parameters such as glucose, enzyme activity, and protein levels, along with oxidative stress markers like MDA, SOD, and CAT, are frequently used in veterinary medicine to assess fish health. These parameters have the ability to indicate the physiological effects of environmental stress at an early stage and provide information about fish adaptability. Oxidative stress caused by the toxic effects of environmental pollutants triggers cell death mechanisms through lipid peroxidation in the cell membrane, DNA damage, and protein structure disruption. Here, apoptosis is a critical process in regulating cellular damage under environmental stress. Apoptosis, or programmed cell death, plays a role in the regular removal of cells and the maintenance of tissue homeostasis. Oxidative stress caused by environmental pollution leads to cellular dysfunction and immune system weakening by initiating apoptosis pathways. Pathways such as the mitochondrial pathway and caspase activation are the primary mechanisms responsible for the toxic effects that lead to apoptosis. In this study, the role of environmental bio-monitoring blood biochemistry, oxidative stress, and apoptosis parameters, as well as their applications in veterinary medicine, will be discussed.

Discussion and Conclusion: To protect aquatic ecosystems and ensure the sustainability of fish health, early diagnosis and effective intervention must be carried out through a multidisciplinary approach. The active participation of veterinarians in this process is critical for ecosystem health and the continuity of fish populations. This review investigates the role of blood biochemistry, oxidative stress, and apoptosis markers in environmental biomonitoring and their importance in veterinary medicine applications. Early diagnosis and successful intervention for the protection of aquatic ecosystems and the sustainability of fish health require the development of multidisciplinary approaches. The direct participation of veterinarians in this process is crucial for the health of ecosystems and the sustainability of fish populations.

Keywords: Apoptosis, Fish health, Environmental biomonitoring, Blood biochemistry, Oxidative stress

INTRODUCTION

Water ecosystems have become polluted to an alarming degree in recent years due to the accumulation of industrial, agricultural, and domestic waste 1.

This pollution poses a serious threat to aquatic organisms due to toxic substances such as heavy metals, pesticides, and persistent organic pollutants². Fish are considered key bioindicators in the assessment of aquatic ecosystems because they reflect changes in their environment biochemically¹. Their direct exposure to pollutants leads to the transfer of these toxic substances accumulated in their bodies to organisms higher up the food chain, including humans². Therefore, monitoring fish health is critically important for both ecosystem and public health. The most effective tools used to assess the effects of environmental stressors on fish are biochemical biomarkers. Biomarkers are measurable parameters that reflect an organism's response to environmental effects and indicate physiological changes caused by exposure to pollutants². Among these markers, blood biochemistry parameters are widely used in the early detection of fish health and stress adaptation ability. Changes in glucose, protein levels, and various enzyme activities are important indicators of metabolic imbalance³. One of the most fundamental cellular mechanisms caused by pollutants is oxidative stress.

Oxidative stress arises from the disruption of the balance between the production of reactive oxygen species (ROS) and antioxidant defense systems, leading to lipid peroxidation in cell membranes, damage to protein structures, and DNA damage⁴. This process can be monitored by increases in lipid peroxidation products such as malondialdehyde (MDA) and changes in the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT)³.

Oxidative stress-induced cellular damage can activate apoptosis, known as programmed cell death, to maintain tissue homeostasis⁵. Environmental stressors activate mitochondrial pathways, triggering apoptosis-related mechanisms such as caspase, resulting in the controlled elimination of cells, and consequently tissue damage and organ dysfunction⁶. In this context, mitochondrial pathways play a critical role in regulating cellular health under environmental stress conditions and are important for understanding fish responses to stressors.

In conclusion, the early diagnosis and effective monitoring of damage caused by pollutants in aquatic ecosystems contributes to the development of preventive strategies for environmental and human health, along with fish health management. The comprehensive assessment of blood biochemistry, oxidative stress, and apoptosis biomarkers in fish provides a powerful tool for understanding the biological effects of environmental pollutants. The discipline of veterinary medicine assumes an important responsibility in this process, both in terms of environmental biomonitoring and preventive medicine for aquatic animal health. This review aims to highlight the role of blood biochemistry, oxidative stress, and apoptosis mechanisms in environmental biomonitoring and the importance of these parameters in fish health management.

Environmental Pollutants and Routes of Exposure in Fish

Water ecosystems have become a accumulation point for various chemical pollutants resulting from human activities. These pollutants include synthetic compounds such as pesticides, heavy metals, antibiotics, industrial chemicals, and pharmaceuticals⁷. These substances enter water bodies from a wide variety of sources, including agricultural and industrial waste, mining activities, and domestic discharges. Some substances, such as persistent organic pollutants (POPs) and heavy metals, do not break down easily in nature and persist in aquatic environments for long periods^{2,7}. These pollutants enter the aquatic food chain and accumulate from the lowest trophic levels to the highest. This process is known as bioaccumulation and biomagnification⁶.

Fish are exposed to these pollutants in multiple ways. The most fundamental route of exposure is direct absorption from the water through the gills.

Gills, with their large surface area and dense network of blood vessels, serve as a highly efficient gateway for the direct passage of dissolved toxins in water into the bloodstream 1,8. A second pathway is the digestive system, which occurs through the ingestion of contaminated water or contaminated food (small fish, plankton, or benthic organisms) 6. In addition, some lipophilic (fat-soluble) pollutants can also enter the body through the fish's skin. These toxins that enter the body are transported by the bloodstream to detoxification organs such as the liver and kidneys; but when the capacity of these organs is exceeded, they accumulate in other tissues such as muscle, brain, and adipose tissue, negatively affecting the fish's physiology, immune system, and overall health ⁹.

Blood Biochemical Parameters in Fish and Assessment of Fish Health

Blood biochemical parameters play an important role as sensitive and reliable biomarkers in assessing the physiological status of fish 10. These parameters provide important information about the fish's nutritional status, metabolic balance, and responses to environmental stressors. Since blood is a biological indicator reflecting metabolic changes in fish, changes in blood parameters are considered early warning signals for fish health ¹¹. Therefore, blood analyses are widely used in aquaculture and ecotoxicological research for the diagnosis and prognosis of diseases and for monitoring the effects of environmental pollution ¹².

Blood glucose level is one of the most commonly used parameters in fish blood biochemistry analyses. Glucose is a fundamental indicator of energy metabolism in fish, and significant changes in its level are observed in situations such as environmental stress, hypoxia, or exposure to toxic substances 10. Hyperglycemia is commonly observed in stressed fish, usually associated with cortisol release 5. However, fish carbohydrate metabolism differs from that of mammals, and carnivorous species in particular may exhibit “glucose intolerance” to high-carbohydrate diets, which can lead to feed-induced metabolic problems ¹¹.

Total protein (TP), albumin (ALB), and globulin (GLB) are also critical protein parameters in assessing fish health. A decrease in total protein levels (hypoproteinemia) usually indicates liver damage, malnutrition, or kidney dysfunction, while an increase in globulin levels may indicate that the immune system is responding to an infection or inflammation 10. Similarly, liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are among the most sensitive markers of hepatocellular damage. An increase in the levels of these enzymes in the blood as a result of exposure to toxic substances suggests that damage has developed in the liver cells. ¹².

Regular monitoring of these parameters is an important tool in the health management of fish populations and for early intervention against potential threats. Different studies examining the relationship of these parameters with environmental pollutants are summarized in Table 1.

Table 1. Effects of Different Pollutants and Stress Factors on Biochemical Parameters in Fish

Author / Year	Fish Species	Examined Parameters	Experimental Condition / Pollutant	Main Findings
Bojarski et al., 2025	<i>Cyprinus carpio</i> (common carp)	Glucose, TP, ALT, AST	Heavy metal (cadmium) exposure	Glucose ↑, TP ↓, ALT/AST ↑; indicators of liver damage
Ahmed et al., 2022	<i>Oreochromis niloticus</i> (tilapia)	Glucose, LDH, ALP, Protein	Pesticide (organophosphate) exposure	Increase in glucose and enzymes; decrease in protein levels
Israel et al., 2019	<i>Clarias gariepinus</i> (African catfish)	TP, ALB/GLB ratio, AST, ALT	Glyphosate-based herbicide	Decrease in protein levels, increase in liver enzymes
Salah & El-Murr, 2023	Various species	ALT, AST, ALP, LDH	Clinical pathology study	ALT/AST sensitive for liver function; ALP sensitive for kidney function
Chen et al., 2024	<i>Danio rerio</i> (zebrafish)	ALT, AST, LDH	Hypoxia stress	Increased ALT/AST activity; impaired metabolic adaptation
Banaee et al., 2024	<i>Oreochromis niloticus</i>	Glucose, TP, ALP, LDH	Cigarette butt leachate	Glucose ↑, LDH ↑, ALP ↓, TP ↓
Ghelichpour et al., 2020	<i>Cyprinus carpio</i>	ALT, AST, ALP, LDH, TP	Pesticide (lufenuron) exposure	ALT/AST/ALP ↑, TP ↓
Firat et al., 2011	<i>Oreochromis niloticus</i>	ALT, AST, ALP, LDH, Glucose, TP	Copper, lead, and cypermethrin exposure	Glucose ↑, ALP/LDH ↑, TP ↓
Mirghaed et al., 2018	<i>Oncorhynchus mykiss</i> (rainbow trout)	ALT, AST, ALP, LDH, Glucose, TP	Silver nanoparticles (Ag-NP)	Increase in all enzymes; glucose ↑, protein ↓
Atli et al., 2015	<i>Oreochromis niloticus</i>	ALT, AST, ALP, LDH, Glucose, TP	Cd and Pb exposure	ALT, AST, LDH ↑, ALP ↓, Glucose ↑
Tunçsoy & Erdem, 2021	<i>Oreochromis niloticus</i>	ALT, AST, ALP, LDH, Glucose, TP	CuO nanoparticles and CuSO ₄	ALT, AST, ALP, LDH ↑, Glucose ↑
Motamedi-Tehrani et al., 2025	<i>Oreochromis niloticus</i>	ALT, AST, ALP, LDH, Glucose, Protein	Ammonia and salinity stress	ALT, AST, ALP, LDH ↑, Protein ↓

Canli et al., 2018	<i>Oreochromis niloticus</i>	Glucose, ALT, ALP	Al ₂ O ₃ , CuO, TiO ₂ nanoparticles	Glucose and ALT ↑, ALP variable
Duran & Çenesiz, 2024	<i>Oncorhynchus mykiss</i> (rainbow trout)	LDH, Glucose, Cortisol, TOS, TAS, OSI	Oxygen concentration changes (hypoxic, normoxic, hyperoxic conditions)	LDH and glucose ↑ in hypoxia and hyperoxia; antioxidant mechanism suppressed, oxidative stress ↑

Mechanisms of Oxidative Stress and Biomarkers in Fish

Fish are exposed to a wide variety of stress factors in aquaculture systems, including environmental, chemical, physical, and social factors. These stress factors disrupt the fish's homeostasis, triggering a series of responses. The stress response occurs in three stages: primary, secondary, and tertiary. The primary response involves the activation of the hypothalamic-pituitary-adrenal (HPI) axis and the release of glucocorticoids such as cortisol. The secondary response occurs at the metabolic level as a result of these hormonal changes and leads to alterations in blood biochemistry (e.g., glucose and lactate levels) and the hematological profile. If the stressful situation persists, it causes tertiary responses at the organism level, such as decreased growth rates, reduced reproductive capacity, and decreased resistance to disease ¹³.

One of the most important cellular mechanisms underlying these stress responses is oxidative stress. Oxidative stress arises from the disruption of the balance between the production of reactive oxygen species (ROS) and the organism's antioxidant defense systems. Contaminants such as nanoplastics and gemfibrozil found in aquaculture environments are among the main factors that can trigger oxidative stress in fish. In fish exposed to these pollutants, immune and antioxidant responses have been observed to be triggered. Gills, in particular, are primary target organs because they are in direct contact with pollutants, and this can lead to disruptions in gas exchange and osmo-ionic regulation ¹³.

Various biomarkers are used to measure the presence and level of oxidative stress. These markers play a critical role as early warning signals in monitoring fish health and welfare. The main oxidative stress biomarkers are as follows:

Total Oxidative Status (TOS) and Total Antioxidant Capacity (TAC): These two parameters indicate the total levels of oxidants and antioxidants in the organism, respectively. Studies have found that TAC levels in the gills of crucian carp (*Carassius auratus*) exposed to polystyrene nanoplastics (PS-NPs) increased significantly, indicating accelerated production of antioxidant compounds. This is a defense mechanism to counteract the adverse effects of ROS ¹³.

Enzymatic Activities:

Esterase Activity (EA): The activity of this enzyme, which is associated with immune and antioxidant responses, increased in the gills of fish exposed to PS-NPs, while it decreased in their brains. This indicates that the gills serve as the primary defense line against pollutants and that the immune system is more active here.

Acetylcholinesterase (AChE): Changes in the activity of this enzyme, which is an indicator of neurotoxicity, point to neurological problems. The absence of significant changes in this enzyme upon exposure to nanoplastics suggests that there is no significant neurotoxicity under the tested conditions.

Creatine Kinase (CK): This is an indicator of muscle damage. The absence of changes in the level of this enzyme indicates that no serious damage occurred in the muscle tissue of the fish. **Adenosine Deaminase (ADA):** This enzyme, which plays a role in immune response and oxidative stress management, did not show any significant changes in nanoplastics exposure, suggesting that the relevant mechanisms were not significantly affected 13.

Metabolomic Changes: Oxidative stress also affects energy metabolism and amino acid balance. For example, increased levels of glutamate, glycerol, and formate, and decreased levels of valine were observed in the plasma of sea bream (*Sparus aurata*) exposed to polystyrene nanoplastics. Glutamate is necessary for the synthesis of glutathione, which is both a neurotransmitter and an antioxidant. An increase in its levels may indicate a disrupted metabolic process or a disruption in the synthesis of other neurotransmitters such as GABA. The increase in glycerol reflects metabolic stress resulting from the breakdown of triglycerides, while changes in other metabolites such as proline and formate indicate impairments in cellular functions and the utilization of energy sources 13.

In conclusion, oxidative stress in fish is central to the cellular response to environmental pollutants. Although antioxidant defense mechanisms (enzymatic and non-enzymatic) attempt to establish a balance against this stress, chronic or high-level exposure overwhelms these defense systems, leading to cellular damage, physiological disorders, and ultimately, deterioration in the fish's overall health and welfare. Therefore, monitoring oxidative stress biomarkers is a vital tool for understanding the effects of pollution in aquatic ecosystems and protecting fish health.

Apoptosis Mechanisms and Environmental Triggers in Fish

Apoptosis is a genetically programmed cell death mechanism that regulates vital processes such as maintaining tissue integrity and eliminating damaged cells in multicellular organisms. Unlike necrosis, defined as traumatic cell death, apoptosis is a programmed process involving the controlled shrinkage of the cell, condensation of chromatin, fragmentation of DNA, and finally transformation into “apoptotic bodies” that can be engulfed by neighboring cells 14. This allows unnecessary or dangerous cells to be eliminated without causing inflammation. In fish, apoptosis plays a critical role in a wide range of physiological processes, from embryonic development to immune responses, tissue regeneration, and reproductive cycles, just as it does in mammals 15.

The fundamental mechanism of apoptosis operates through two evolutionarily conserved pathways: the intrinsic and extrinsic pathways. The intrinsic pathway is triggered by intracellular stress signals such as DNA damage, oxidative stress, or exposure to toxins. These signals increase the permeability of the mitochondrial outer membrane, leading to the release of apoptotic factors such as cytochrome c into the cytoplasm 16. In the cytoplasm, cytochrome c binds to the Apaf-1 protein to form the “apoptosome” complex, which activates the initiator caspase-9. Caspases are a family of enzymes that are the executioner molecules of apoptosis, carrying out cell death by cleaving specific proteins 17. Initiator caspases, such as caspase-9, initiate the apoptosis cascade (chain reaction) by activating executor caspases, such as caspase-3 and caspase-7. This process is regulated by the Bcl-2 family, which consists of anti-apoptotic and pro-apoptotic proteins that determine whether the cell survives or dies (Schmidt and Eckersall, 2015) (Figure 1).

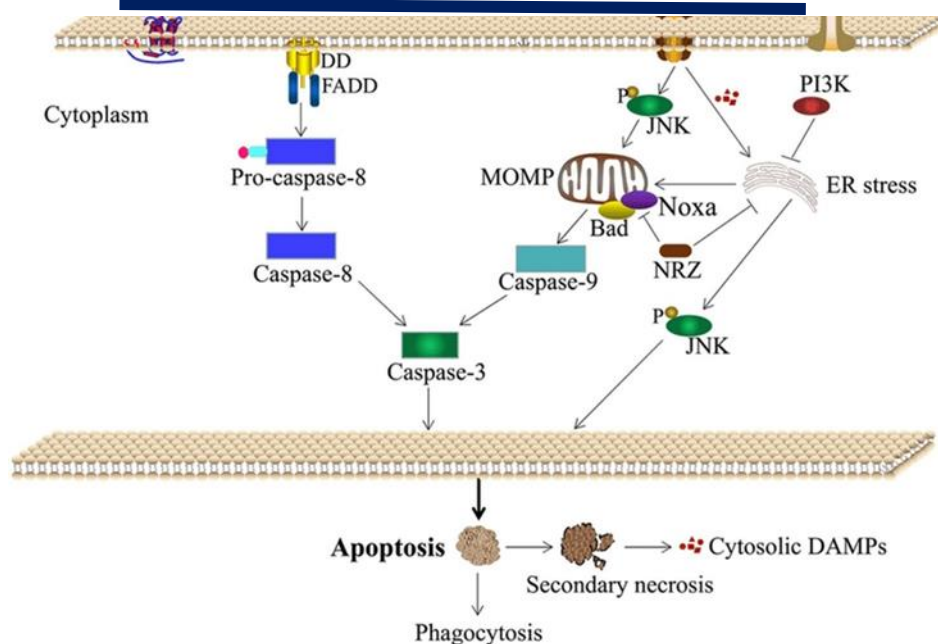


Figure 1. Mechanism of apoptosis

Fish are directly exposed to environmental toxins due to their aquatic environment and these toxins can trigger apoptosis. For example, heavy metals such as cadmium and organic pollutants such as tributyltin chloride have been scientifically proven to cause apoptosis in fish cells by activating the mitochondrial pathway and increasing caspase activity 17. Therefore, apoptosis is considered a reliable biomarker for assessing the biological effects of environmental pollution 15.

Various histopathologic and molecular methods are used to detect and monitor apoptosis in the laboratory. Histopathological examinations by electron microscopy directly show the characteristic morphological changes (chromatin condensation, apoptotic bodies, etc.) of apoptosis 14. Molecular markers provide biochemical evidence of the process. The TUNEL assay makes apoptotic cells visible by marking breaks in DNA fragmented during apoptosis. DNA laddering analysis shows the organized fragmentation of DNA as a ladder-like band pattern on gel electrophoresis 14. The Annexin V assay detects phosphatidylserine molecule export from the cell membrane, an early sign of apoptosis. These methods are powerful tools to both prove the existence and elucidate the mechanisms of apoptosis.

CONCLUSION

This review shows that pollution in aquatic ecosystems has multifaceted effects on fish health. Blood biochemistry, oxidative stress and apoptosis mechanisms are reliable biomarkers for early detection of environmental stress. The findings reveal that pollutants cause metabolic imbalances, cellular damage and programmed cell death in fish. The evaluation of these biomarkers is crucial for the protection of fish health, early diagnosis of diseases and the regular conduct of environmental biomonitoring studies and the detection of the presence of environmental pollutants. Maintaining fish health is vital for the sustainability of aquatic ecosystems. Therefore, it is necessary to monitor water quality, develop early warning systems, control pollution sources and implement regular biomonitoring programs. The evaluation of blood biochemistry, oxidative stress and apoptosis parameters together allows for a more accurate and holistic assessment of the environmental stress to which fish are exposed.

Thus, the data obtained can serve as a reference both in academic research and field applications. In this respect, the study is an important resource that will contribute to the development of science-based strategies for the conservation and sustainable management of aquatic ecosystems.

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ISCHEMIA-MODIFIED ALBUMIN AS AN EARLY BIOMARKER FOR ABORTION IN SHEEP

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ABSTRACT

Introduction and Purpose: This study aimed to evaluate the clinical utility of Ischemia-Modified Albumin (IMA) as an early diagnostic biomarker for abortion in sheep. Since oxidative stress is a major factor affecting reproductive health, the relationship between IMA and oxidative stress markers was also investigated.

Materials and Methods: Serum samples were collected from 148 Akkaraman sheep, including 22 that experienced abortion, 24 healthy pregnant controls, and 13 non-pregnant animals. IMA and IMA-to-Albumin Ratio (IMAR) were measured using ELISA kits. Additionally, Total Antioxidant Status (TAS), Total Oxidative Stress (TOS), and Oxidative Stress Index (OSI) were evaluated. Statistical analyses were performed using SPSS 13.0.

Results: IMA and IMAR levels were significantly higher in sheep that experienced abortion ($p < 0.05$). These increases were accompanied by elevated TOS and OSI levels and reduced TAS levels ($p < 0.001$), indicating a significant association with oxidative stress.

Conclusion: Elevated maternal serum IMA levels appear to be a promising early biomarker for abortion in sheep. This is the first study to investigate IMA in ovine pregnancy and suggests its potential role in veterinary reproductive diagnostics.

Keywords: Sheep; Abortion; Ischemia-Modified Albumin; Oxidative Stress; Biomarker

GIDALARDA POLİSİKLIK AROMATİK HİDROKARBONLAR: KAYNAKLARI, MARUZİYET VE İNSAN SAĞLIĞINA ETKİLERİ

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ÖZET

Polisiklik aromatik hidrokarbonlar (PAH), organik maddelerin tam yanmaması sonucu oluşan, toksik ve karsinojenik potansiyele sahip organik bileşiklerdir. Doğada 100'den fazla PAH tanımlanmış olmakla birlikte, uluslararası otoriteler tarafından 16'sı öncelikli kirleticiler olarak değerlendirilmektedir. Bu derlemede, PAH'ların gıdalardaki oluşum kaynakları, insan maruziyet yolları, biyotransformasyon mekanizmaları ve sağlık üzerindeki olası etkileri literatür verileri doğrultusunda değerlendirildi. İnsanlarda başlıca maruziyet yolları; kontamine gıdaların tüketimi, kirli havanın solunması ve tütün dumanına maruz kalmadır. Özellikle et ve balık ürünlerinin yüksek sıcaklıkta pişirilmesi, tütsülenmesi ve kavrulması PAH oluşumuna yol açmakta; yağ oranı yüksek gıdalarda bu seviyeler daha da artmaktadır. PAH'lar; alındıktan sonra Faz I (oksidasyon, redüksiyon ve hidroliz), Faz II (konjugasyon) ve Faz III (atılım) metabolik süreçlerinden geçmektedir. İdrarda ölçülebilen 1-hidroksipiren gibi metabolitler, maruziyetin biyobelirteçleri olarak kullanılmaktadır. Son yıllarda yapılan çalışmalar, diyet alışkanlıkları, sigara kullanımı, trafik yoğunluğu ve pişirme yöntemlerinin idrar PAH metabolit düzeylerini etkilediğini ortaya koymaktadır. Risk değerlendirmeleri, benzo[a]piren ve naftalin gibi bazı PAH'ların yüksek tüketim düzeylerinde kanser dışı sağlık riskleri oluşturabileceğini göstermektedir. Kronik maruziyet; solunum sistemi, kardiyovasküler ve üreme sağlığı üzerinde olumsuz etkilere yol açabilmektedir. Koruyucu önlemler arasında doğrudan ateş yerine dolaylı pişirme yöntemlerinin tercih edilmesi, yağ içeriğinin azaltılması, marinasyon ve pişirme parametrelerinin kontrolü öne çıkmaktadır. Türk Gıda Kodeksi ve Avrupa Birliği mevzuatında gıdalardaki benzo[a]piren ve dört temel PAH için maksimum sınır değerler bulunmaktadır. PAH maruziyetinin azaltılmasında Tek Sağlık yaklaşımı doğrultusunda gıda güvenliği, çevre ve halk sağlığı boyutlarının birlikte değerlendirilmesi, ayrıca tüketici farkındalığının artırılması büyük önem taşımaktadır.

Anahtar Kelimeler: Polisiklik aromatik hidrokarbonlar, gıda güvenliği, maruziyet, insan sağlığı, risk değerlendirmesi, tek sağlık

POLYCYCLIC AROMATIC HYDROCARBONS IN FOODS: SOURCES, EXPOSURE, AND EFFECTS ON HUMAN HEALTH

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds formed as a result of incomplete combustion of organic materials, possessing toxic and carcinogenic potential.

Although more than one hundred PAHs have been identified in nature, sixteen of them are considered priority contaminants by international authorities. In this review, the sources of PAHs in foods, human exposure pathways, biotransformation mechanisms, and possible health effects were evaluated based on current literature data. The main routes of human exposure are consumption of contaminated foods, inhalation of polluted air, and exposure to tobacco smoke. High-temperature cooking of meat and fish products, smoking, and roasting contribute significantly to PAH formation, with higher levels observed in high-fat foods. Once ingested, PAHs undergo metabolic processes including Phase I (oxidation, reduction, and hydrolysis), Phase II (conjugation), and Phase III (excretion). Metabolites such as 1-hydroxypyrene, which can be detected in urine, are used as biomarkers of exposure. Recent studies have shown that dietary habits, smoking, traffic density, and cooking methods influence urinary PAH metabolite levels. Risk assessments indicate that certain PAHs, such as benzo[a]pyrene and naphthalene, may pose non-carcinogenic health risks at high intake levels. Chronic exposure has been associated with adverse effects on the respiratory, cardiovascular, and reproductive systems. Preventive measures include the use of indirect instead of direct flame cooking, reduction of fat content, margination, and control of cooking parameters. The Turkish Food Codex and European Union regulations have set maximum permissible levels for benzo[a]pyrene and the sum of four key PAHs in foods. In order to reduce PAH exposure, it is essential to evaluate food safety, environmental, and public health aspects together within the framework of the One Health approach, while increasing consumer awareness also plays a critical role.

Keywords: Polycyclic aromatic hydrocarbons, food safety, exposure, human health, risk assessment, one health

GİRİŞ

Polisiklik Aromatik Hidrokarbonlar (PAH), çeşitli karbon bazlı maddelerin (fosil yakıtlar ve gıda kaynakları dahil) eksik yanması veya pirolitik ayrışması sonucu meydana gelen, karsinojenik potansiyele sahip organik bileşiklerdir (Kılıç ve ark., 2017). PAH'lar, birbirine bağlı aromatik karbon ve hidrojen halkalarından oluşan kimyasal bileşiklerdir ve çoğunlukla beyaz, açık sarı veya renksiz katı formdadır (Mallah ve ark., 2022).

PAH Kaynakları ve Gıdalarda Oluşumu

Doğal yollarla ortaya çıkan PAH kaynakları sınırlıdır ve genellikle volkanik patlamalar, orman yangınları veya yıldırım sonucu meydana gelen bataklık yangınları gibi olaylarla ilişkilidir. Ancak PAH kirliliğinde esas belirleyici faktörler, insan faaliyetlerinden kaynaklanan kirliliklerdir (Mallah ve ark., 2022). Endüstriyel süreçlerden kaynaklanan atıklar, pestisitler, evsel çöpler, tütün dumanı ve sanayi bacalarından çıkan gazlar gibi farklı kaynaklardan çevreye yayılabilen bu bileşikler; hava, su, toprak ve gıda zincirine karışarak insan sağlığı açısından ciddi risk oluşturan önemli çevresel kirleticiler arasında yer almaktadır (Günç Ergönül & Kaya, 2015). Kahve çekirdeklerinin yüksek sıcaklıkta kavrulması, et ve balık ürünlerinin tütülenmesi ya da et ve yağın mangalda pişirilmesi sırasında gerçekleşen yanma reaksiyonları, özellikle benzo[a]piren başta olmak üzere farklı düzeylerde PAH oluşumuna yol açar (Şahin ve ark., 2020; Şahin, 2022). Gıdanın doğrudan kömür üzerinde pişirilmesi durumunda, etin yağ oranına bağlı olarak benzo[a]piren miktarı yaklaşık 0,5-12 µg/kg aralığında değişebilir. Mangalda pişirilen etlerde yağ oranı arttıkça PAH seviyeleri de yükselmektedir. Bunun temel nedeni, eriyen yağların ateşe damlayarak yanması ve bu süreçte benzo[a]pirenin yanı sıra daha yüksek miktarda PAH'ların açığa çıkmasıdır. Oluşan bu bileşikler, pişen etin yüzeyine geçerek kontaminasyona neden olmaktadır (Şahin ve ark., 2020; Erol, 2022).

Doğada 100'den fazla PAH türü tanımlanmış olmakla birlikte, toksisite ve kanserojenite potansiyeli yüksek olan 16'sı, çevresel açıdan öncelikli kirleticiler olarak değerlendirilmektedir. Öncelikli kirletici olarak değerlendirilen 16 polisiklik aromatik hidrokarbon bileşiği şunlardır: Naftalin (Np), Asenaftelen (Anp), Asenaften (Ane), Floren (Flr), Fenantren (Phe), Antrasen (An), Floranten (Flu), Piren (Py), Benzo[a]antrasen (BaA), Krisen (Chr), Benzo[b]floranten (BbF), Benzo[k]floranten (BkF), Benzo[a]piren (BaP), Dibenzo[a,h]antrasen (DahA), İndeno[1,2,3-cd]piren (IcdP) ve Benzo[g,h,i]perilen (BgHiPy)'dir (Alver ve ark., 2012). Bu derlemede, PAH'ların gıdalardaki oluşum kaynakları, insan maruziyet yolları, biyotransformasyon mekanizmaları ve sağlık üzerindeki olası etkileri güncel literatür verileri doğrultusunda değerlendirildi.

PAH'lar: İnsan Maruziyeti ve Biyotransformasyonu

PAH'lar, ekosistemler ve insan sağlığı üzerinde zararlı etkiler gösteren bileşiklerdir; bu durum, bu maddelerin mikrobiyal dönüşümü üzerine araştırmaları artırmıştır. Son yıllarda, PAH'ların mikrobiyal biyotransformasyon mekanizmaları, katabolik enzim sistemleri ve genetik düzenlemeler detaylı şekilde incelenmiş; genomik, proteomik ve metabolomik yaklaşımlar ise çevresel matrislerde PAH metabolizmasının anlaşılmasında güçlü araçlar olarak kullanılmaktadır. Bu bilgiler, hem biyokimyasal düzeyde hem de biyoremediasyon uygulamaları açısından PAH yönetimi için temel oluşturmaktadır (Sakshi & Haritash, 2020). İnsanlarda PAH maruziyeti temel olarak kontamine gıdaların tüketimi, sigara kullanımı ve kirli havanın solunması yoluyla gerçekleşmektedir. Özellikle kok fırını işçileri yüksek risk grubunda yer almakta olup, bu nedenle koruyucu stratejiler büyük önem taşımaktadır. Vücutta metabolize edilen PAH'lar reaktif ara ürünler oluşturarak Deoksiribonükleik asit (DNA) hasarına neden olmakta ve yüksek maruziyet koşullarında çeşitli sağlık sorunlarına neden olmaktadır (Montano ve ark., 2025).

PAH ve türevlerinin insan vücudunda nasıl metabolize edildiğinin ayrıntılı olarak anlaşılması, etkili korunma yöntemleri ve hedefe yönelik sağlık müdahalelerinin geliştirilmesi açısından büyük önem taşır. Bu bileşikler, organizmada çeşitli enzimler ve biyokimyasal yolların rol aldığı çok aşamalı bir biyotransformasyon sürecinden geçer ve süreç genellikle üç fazda incelenir. Faz I aşamasında, başta sitokrom P450 (CYP) enzimleri olmak üzere oksidasyon, redüksiyon ve hidroliz reaksiyonları gerçekleşir. Bu basamakta monohidroksiller, dioller, diol-epoksitler ve kinonlar gibi ara ürünler oluşur. Özellikle diol-epoksitler, DNA ile kovalent bağ kurarak genotoksik etki gösterebilir. Faz II aşamasında ise glukuronidasyon, sülfatlama ve glutatyon konjugasyonu gibi reaksiyonlar aracılığıyla metabolitlerin suda çözünürlüğü artırılır ve böylece organizmadan uzaklaştırılmaları kolaylaşır. Faz III aşamasında, detoksifiye edilmiş bu metabolitler aktif taşıma mekanizmalarıyla safra veya idrar yoluyla vücuttan atılır. Böylece PAH birikimi engellenmiş olur. PAH metabolizması sırasında ortaya çıkan bazı metabolitler, maruziyetin değerlendirilmesinde biyobelirteç olarak kullanılabilir. Örneğin idrarda ölçülen 1-hidroksipiren gibi monohidroksi PAH türevleri, çevresel veya mesleki PAH maruziyetini belirlemede güvenilir göstergeler arasında yer almaktadır. Bununla birlikte, PAH'ların kimyasal yapılarındaki farklılıklar nedeniyle metabolik dönüşüm mekanizmaları değişkenlik göstermektedir. Bu durum, hem maruziyetin doğru ölçülmesi hem de olası sağlık risklerinin ayrıntılı olarak değerlendirilmesi için her bir PAH türünün ayrı ayrı ele alınmasını gerekli kılmaktadır (Qian ve ark., 2025). Gıda kaynaklı PAH'ların alımı gastrointestinal sistem yoluyla gerçekleşmekte ve yağ dokusunda depolanmaktadır. Yağda çözünürlüğü nedeniyle bu bileşiklerin atılımı zordur (Zhao ve ark., 2023).

PAH'ların insanlardaki emilim, dağılım, metabolizma ve atılım süreçlerine ilişkin veriler sınırlıdır. Choi ve ark. (2023) tarafından yürütülen çalışmada, deuterium-etiketli dört PAH bileşiği (naftalen, florene, fenantren ve piren) sağlıklı gönüllülere tek doz oral olarak uygulanmış ve bu bileşiklerin toksikokinetik özellikleri değerlendirilmiştir. Bulgular, PAH'ların hızlı şekilde sistemik dolaşıma geçtiğini (T max: 0,25-1,5 saat) ve kısa yarı ömürle (1-3 saat) elimine edildiğini göstermiştir. Metabolitlerin ise idrarla %1,8-11,3 oranında ve daha uzun sürede atıldığı bildirilmiştir. İdrarda PAH metabolitleri, vücuttaki PAH maruziyetini gösteren biyobelirteçlerdir. Wuhan-Zhuhai çalışmasında yer alan 4092 katılımcının sigara kullanımı, yemek pişirme, trafik maruziyeti ve beslenme alışkanlıkları değerlendirilmiş olup, 12 idrar monohidroksile PAH metaboliti (OH-PAH) düzeyi ölçülmüştür. Analizler, sigara kullanımı, diyetle yüksek PAH alımı, trafikte uzun süre bulunma ve evde yemek pişirmenin bazı idrar PAH metabolitlerini artırdığını; buna karşılık iyi mutfak havalandırmasının düşük molekül ağırlıklı PAH maruziyetini azalttığını göstermiştir. Bu bulgular, özellikle kendi yemeğini pişiren bireylerde düşük molekül ağırlıklı PAH maruziyetini etkili şekilde azalttığını göstermektedir (Cao ve ark., 2020). PAH'lar, besin ve içecekler aracılığıyla vücuda ağız yoluyla alınabilmektedir. Yağın ısınması sırasında ortaya çıkan duman, PAH'ları taşıyabilir ve bu bileşikler gıdalara geçebilmektedir (Rigi ve ark., 2025). PAH'ların gıdalardaki maruziyet yolları ve dağılımı, literatürde öne çıkan araştırma konuları arasındadır. İşlenmiş etlerde PAH oluşumunda maillard reaksiyonunun etkili olduğu, özellikle yağ oranı yüksek olan gıdalarda PAH seviyelerinin daha yüksek bulunduğu bildirilmektedir (Lu ve ark., 2024). 2011-2023 yılları arasında yayımlanan 42 çalışmayı kapsayan bir meta-analizde, kırmızı etten yapılan kebaplarda 16 öncelikli PAH düzeyi incelenmiştir. En yüksek ortalama konsantrasyonların piren (359,32 µg/kg) ve naftalin (263,20 µg/kg) için belirlendiği, benzo[a]pirenin ise daha düşük seviyelerde (1,79 µg/kg) tespit edildiği bildirilmiştir. Risk değerlendirmesi sonuçlarına göre, özellikle BaP ve Np için hesaplanan tehlike katsayısının (HQ) 1'in üzerinde olması yüksek tüketim düzeylerinde non-kanserojen etkiler açısından risk oluşturabileceğini göstermektedir. Bununla birlikte, tüm PAH'lar için yaşam boyu kanser riski (LTCR <10⁻⁶) ABD Çevre Koruma Ajansı (United States Environmental Protection Agency-USEPA) kriterlerine göre ihmal edilebilir bulunmuştur. Çalışma, PAH maruziyetinin azaltılması için güvenli pişirme yöntemlerinin tercih edilmesi ve tüketici farkındalığının artırılması gerektiğini vurgulamaktadır (Gholami-Borujeni ve ark., 2025). Brezilya'da yapılan başka bir çalışmada, işlenmiş et ürünlerinde PAH seviyelerinin yüksek olabileceği belirlenmiştir. Bu durum, olası sağlık risklerinin azaltılması için PAH düzeylerinin düzenli olarak izlenmesi gerektiğini vurgulamaktadır (da Silva ve ark., 2024). PAH'ların gıdalardaki varlığı, hem Avrupa Birliği Mevzuatı (EC, 2023) hem de Türk Gıda Kodeksi Bulaşanlar Yönetmeliği (TGK, 2023) kapsamında sınırlandırılmıştır. 2023 yılında yapılan güncellemeyle, Benzo[a]piren (BaP) için en yüksek izin verilen miktar 2.0 µg/kg, dört temel PAH bileşiğinin (Benzo[a]piren, Benzo[a]antrasen, Benzo[b]floranten ve Krisen) toplamı için ise 12 µg/kg olarak belirlenmiştir (EC, 2023). Tütsülenmiş et ve tütsülenmiş et ürünlerinde önceki düzenlemelerde BaP için 5.0 µg/kg ve toplam dört PAH için 30.0 µg/kg sınırı uygulanmıştır (EC, 2011). Ancak Avrupa Gıda Güvenliği Otoritesi (EFSA, 2008), BaP'in tek başına güvenilir bir indikatör olmadığını bildirmiş; bunun yerine BaP, Krisen (Chr), Benzo[a]antrasen (BaA) ve Benzo[b]floranten (BbF) toplamının değerlendirilmesinin daha uygun olduğunu vurgulamıştır. Sınırlı sayıda araştırmada, Türk Gıda Kodeksi Bulaşanlar Yönetmeliği ve Avrupa Birliği Mevzuatı ile belirlenen PAH limit değerlerinin, bazı döner pişirme yöntemleri ve kullanılan parametrelere bağlı olarak aşılabildiği ortaya konmuştur.

Ayrıca, son dönem çalışmalar pişirme sırasında tercih edilen odun türündeki farklılıkların da oluşan PAH seviyeleri üzerinde etkili olduğunu göstermektedir (Turp ve Atakan, 2022). Gıdalarda ölçülen PAH düzeylerinin, yaşanan bölgedeki çevresel kirlilik koşullarıyla (hava, toprak ve su kalitesi gibi) yakından ilişkili olduğu bildirilmektedir. Çevresel ortamlarda biriken bu bileşikler, besin zinciri aracılığıyla gıdalara taşınarak insanlar tarafından doğrudan alınabilmektedir. Bu durum; gelişimsel anormalliklerin ortaya çıkmasına, kanser gelişme olasılığının artmasına ve genetik mutasyonların tetiklenmesine katkıda bulunabilmektedir. Ayrıca, beslenme alışkanlıklarını anlamak amacıyla yapılan diyet araştırmaları ve rehberler, farklı bölgelerde geleneksel yemek kültürü, tat tercihleri ve beslenme biçimlerinin değişiklik gösterdiğini ortaya konulmuştur (Zhao ve ark., 2023).

PAH'ların İnsan Sağlığı Üzerindeki Etkileri

PAH'lar insan sağlığı üzerindeki fizyolojik etkileri çok yönlü olup farklı organ ve sistemleri etkilemektedir. PAH'lara akut maruziyet; göz irritasyonu, bulantı, kusma ve diyare gibi semptomlarla ilişkilendirilmektedir (Feng ve ark., 2025). Kronik maruziyet ise solunum sistemi hastalıklarının yanı sıra kardiyovasküler bozukluklar ve immün sistemin baskılanmasıyla bağlantılı olup, ayrıca kanser gibi ciddi hastalıklarından bir nedeni olarak değerlendirilmektedir (Montano ve ark., 2025). Havada buhar fazında ya da partiküllere bağlı halde bulunan PAH'lar, atmosferik hareketler ve rüzgâr etkisiyle uzak mesafelere taşınabilmektedir. Bu bileşikler solunum yoluyla akciğerlere ulaşabildiği gibi, deri temasıyla da insan vücuduna giriş yapabilmektedir (Yatmaz, 2021). İnhalasyon yoluyla alınan PAH'ların astım, bronşit ve akciğer fonksiyonlarında bozulmaya neden olmaktadır (Sombri ve ark., 2024). Özellikle PAH düzeylerinin yüksek olduğu kentlerde kronik obstrüktif akciğer hastalığı (KOA) alevlenmelerinin artabileceği bildirilmektedir (Venkatraman ve ark., 2024). Ayrıca bazı PAH türlerinin karsinojenik özellikleri nedeniyle akciğer kanseri riskini artırdığı gösterilmiştir (Sombri ve ark., 2024). PAH'lar, kardiyovasküler sistem üzerinde çeşitli olumsuz etkiler gösterebilmektedir. Bunlar arasında ateroskleroz, tromboz, hipertansiyon ve miyokard enfarktüsü (MI) gibi hastalıklara neden olabilmektedir. Preklinik araştırmalar, PAH maruziyetinin oksidatif stres düzeylerini artırarak ateroskleroz oluşumunu tetikleyebileceğini göstermektedir (Mallah ve ark., 2022). Bağışıklık sisteminde baskılanmaya ve hormonal dengede bozulmalara yol açabilen bu bileşikler için, gıdalarda izin verilen maksimum düzeyler sınırlandırılmıştır (Güler & Can, 2017). Örneğin, Amerika Birleşik Devletleri'nde 20-39 yaş arasındaki kadınlar üzerinde yapılan bir çalışmada, PAH'lara maruz kalan bireylerin bağışıklık sisteminin baskılanması nedeniyle HPV (Human Papillomavirus) ve HSV-2 (Herpes Simplex Virus Type 2) gibi enfeksiyonlara karşı daha duyarlı oldukları ve bu ilişkinin özellikle belirgin olduğu saptanmıştır (Yang ve ark., 2025). Mevcut çalışmalar, hem erkek hem de kadın üreme sağlığının, PAH maruziyetine bağlı oksidatif stres, DNA hasarı ve hormonal düzensizlikler nedeniyle olumsuz etkilenebileceğini göstermektedir. Erkeklerde PAH'lar sperm kalitesini bozabilirken, kadınlarda yumurtalık fonksiyonlarını etkileyerek kısırlık, düşük ve doğum kusurları riskini artırabilmektedir (Montano ve ark., 2025). Nitekim düşük dozlarda ve erken yaşam dönemlerinde nörotoksik etki gösterebilmektedir. Hem insan hem de hayvan çalışmalarında, prenatal veya erken postnatal dönemde maruziyetin uzun vadede davranışsal bozukluklara yol açtığı ve özellikle hareketlilik ile duygusal profile değişikliklere neden olduğu bildirilmiştir (Tartaglione ve ark., 2023).

Önleme ve Kontrol Stratejileri

Gıdalarda PAH oluşumunu ve insan maruziyetini azaltmak amacıyla çeşitli kontrol önlemleri geliştirilmiştir. En kritik noktalardan biri, pişirme ve işleme yöntemlerinin optimize edilmesidir. Et ve balık ürünlerinde doğrudan ateş üzerinde pişirme yerine dolaylı ısıtma yöntemlerinin tercih edilmesi, yağ damlamasının önlenmesi ve pişirme sıcaklığının kontrol altında tutulması PAH seviyelerini belirgin şekilde azaltmaktadır. Gıdaların yağ içeriğinin azaltılması, marinasyon uygulamaları ve pişirme sırasında gıdanın ateşten belirli bir mesafede tutulması da PAH kontaminasyonunu sınırlayan uygulamalar arasında yer almaktadır. Endüstriyel üretimde ise uygun filtreleme sistemlerinin kullanılması, gıda ambalajlama materyallerinin kontaminasyondan arındırılması ve mevzuatın sıkı şekilde uygulanması, halk sağlığı açısından kritik önlemler olarak değerlendirilmektedir (Sahin ve ark., 2020; Chandimali ve ark., 2025)

SONUÇ VE ÖNERİLER

PAH'lar, çevresel kirlilik ve gıda işleme süreçleri aracılığıyla insanlara taşınan, toksik ve karsinojenik özellikleri nedeniyle önem taşıyan bileşiklerdir. Bu çalışmada, PAH maruziyetinin yalnızca gıda güvenliği değil, aynı zamanda çevre ve halk sağlığı boyutuyla da ele alınması gerektiğini göstermektedir. Dünya Sağlık Örgütü ve Avrupa Gıda Güvenliği Otoritesi tarafından yapılan sınıflandırmalar, PAH'ların potansiyel kanserojen etkilerini ortaya koymuş ve mevzuatlarla belirlenen sınır değerler bu riskleri en aza indirmeyi hedeflemiştir. Ancak özellikle evsel pişirme yöntemleri, sigara dumanı ve çevresel kirlilik gibi faktörler bireysel maruziyet düzeylerini artırmaya devam etmektedir. Bu nedenle, PAH'ların halk sağlığına olan etkilerinin azaltılabilmesi için çok yönlü bir yaklaşım gereklidir. Tek Sağlık perspektifi doğrultusunda çevre, gıda ve insan sağlığı arasındaki etkileşimler dikkate alınmalı; gıda üretim zincirinde iyi üretim uygulamaları yaygınlaştırılmalı, çevresel kirliliğin azaltılmasına yönelik politikalar desteklenmeli ve risk değerlendirme çalışmaları yapılmalıdır. Ayrıca, biyobelirteç temelli izleme çalışmalarının artırılması, bireylerin maruziyet kaynaklarının daha doğru belirlenmesine ve etkili koruyucu stratejilerin geliştirilmesine katkı sağlayacaktır.

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EFFECTS OF LAVANDULA STOECHAS ESSENTIAL OIL AND CYCLOPHOSPHAMIDE ON MEC-1 CELL LINE

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ABSTRACT

Introduction and Purpose: Lavandula stoechas is an important medicinal and aromatic plant belonging to the Lamiaceae family. Scientific studies based on traditional use of L. stoechas have determined that it has anti-inflammatory, antioxidant, antimicrobial, insecticidal, larvicidal, anticonvulsant, antispasmodic, sedative, hepatoprotective, nephroprotective, antidiabetic and anticancer activities. The essential oil of L. stoechas, which grows in Turkey, contains camphor, fenchone, borneol, terpineol, cineole, linalool, linalyl acetate, bornyl acetate, and cadinene. Cyclophosphamide (CP) is a nitrogen mustard, alkylating anticancer agent with a broad therapeutic spectrum for the treatment of autoimmune disorders, lymphomas, chronic lymphocytic leukemias, soft tissue and osteogenic sarcomas, and solid tumors. CP can be administered orally or intravenously in combination with other agents, as well as alone. Up to 70-80% of CP is metabolized by microsomal enzymes in the liver to form 4-hydroxycyclophosphamide (4-OHCP). This study aimed to investigate the effects of L. stoechas essential oil, cyclophosphamide, and their combination on the MEC-1 cell line.

Materials and Methods: The effects of L. stoechas essential oil and cyclophosphamide, individually and in combination, on the MEC-1 cell line were evaluated using the MTT method.

Results: In the MEC-1 cell line, the IC₅₀ value of L. stoechas essential oil was determined at doses of 2.5 mg/ml and that of cyclophosphamide at doses of 260 µg/ml. In addition, the combined use of L. stoechas essential oil with cyclophosphamide was found to have a synergistic effect, increasing the anticancer activity of cyclophosphamide.

Discussion and Conclusion: The observation that L. stoechas essential oil has a reducing effect on cancer cell proliferation and its anticancer activity in this study is promising for future studies. The observation of its proliferation-reducing effects on cancer cells, particularly through its synergistic effect with cyclophosphamide, offers a positive perspective on the therapeutic potential of combining pharmaceutical and natural therapies.

Keywords: Anti-cancer; Cyclophosphamide; Lavandula stoechas; MTT

**MICROBIOTA MODULATION AS THERAPEUTIC APPROACH IN THE
NEUROPATHIC PAIN IN DOG WITH SPINAL CORD INJURY: IMPACT OF
POLENOPLASMIN**

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ABSTRACT

Background Studies have demonstrated the presence of gut dysbiosis (alterations in gut bacterial homeostasis) secondary to spinal cord injury in dogs. The dysbiosis is thought to impair recovery by decreasing the production of short-chain fatty acids which play a role in suppressing inflammation within the central nervous system.

Objective: Therefore, targeting gut dysbiosis could have significant therapeutic value in the management of spinal cord injury. The purpose of this study is to determine if gut dysbiosis occurs in dogs with spinal cord injury. Another area of potential intervention interest is in situations of spinal injury where there is an urgent need to generate new neurons. To arrive at these observations, the authors examined how Polenoplasmin and diet solve paralysis in dogs. Materials and methods The most common cause of spinal problems in dogs is trauma. We are currently assessing whether indoles can also stimulate formation of neurons in dogs with paralysis.

Results: We found that gut microbes that metabolize tryptophan-an essential amino acid-secrete small molecules called indoles, which stimulate the development of new brain cells in dogs, also demonstrated that the indole-mediated signals elicit key regulatory factors known to be important for the formation of new neurons.

Conclusion: This study is another intriguing piece of the puzzle highlighting the importance of lifestyle factors and diet. The link between the health of the microbiome and the health of the brain shows how microorganisms in the gut solve paralysis, gut microbe secreted molecule linked to formation of new nerve cells in paralyzed dogs.

Keywords: gut dysbiosis, indole, paralyzed dog, Polenoplasmin.

HEAVY METAL AND PROXIMATE COMPOSITIONS OF DISCARDED FISH PARTS FROM AWURU RIVER AT RETAIL OUTLET IN NIGER STATE

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ABSTRACT

The rising cost of food items in Nigeria has led to increased interest in alternative food sources, including the exploration of typically discarded fish parts. However, limited information exists regarding the nutritional potential of the discarded fish parts and their safety for human consumption and livestock feed formulation. This study investigates the proximate composition and levels of selected heavy metals in the fins, gills, and intestinal content of five fish species from Awuru River, Borgu Local Government Area, Niger State that are commonly discarded at the retail outlet. The fish species studied include *Lates niloticus*, *Mormyrops anguilloides*, *Malapterurus electricus*, *Oreochromis niloticus*, and *Synodontis membranaceus*. The proximate analysis was conducted using the Association of Official Analytical Chemists (AOAC) techniques to determine the moisture, crude protein, fat, ash, fiber, and carbohydrate contents of the samples. Additionally, heavy metal concentrations (Zinc, Iron, Copper, Cadmium, and Lead) were analysed using Atomic Absorption Spectrophotometry. The result indicated that the fins, gills, and intestinal content of *Malapterurus electricus* had the highest levels of moisture, protein and ash, suggesting it offers the best overall balance of energy and nutrients compared to the discarded parts of other species. The analysed parts of *Lates niloticus* had the highest fat content compared to the discarded parts of the other fish species and are considered more energy-dense, making them a richer source of calories as well as providing essential fatty acids that are beneficial for health. The heavy metal analysis revealed significant contamination, with particularly high levels of lead, cadmium, and iron. The gills and intestinal content of *Oreochromis niloticus* had the highest concentrations of heavy metals compared to the gills and intestine of the other fish species, including 12.82 µg/g of copper which is below the 30 µg/g considered safe by WHO and 606.44 µg/g of iron in the intestines that is far above the general safety threshold of 100 µg/g in fish set by WHO, as well as 5.73 µg/g of lead which significantly exceeds the WHO safe limit of 0.3 µg/g for fish consumption. The gills and intestinal content of *Malapterurus electricus* exhibited notable concentrations of cadmium, 0.32 µg/g and 2.35 µg/g respectively which exceeded the limit in fish of 0.05 µg/g for consumption set by WHO. Thus, the discarded fish parts, particularly of *Malapterurus electricus* and *Lates niloticus* are immensely beneficial in terms of human nutrition and livestock feed formulation.

Keywords: Nutritional composition, Heavy metal composition, Discarded fish parts, Alternative food source, Food security.

**MULTICOMPONENT REACTION APPROACH FOR POTENTIAL
BIOLOGICALLY ACTIVE THIOPHENE DERIVATIVES**

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ABSTRACT

Heterocyclic compounds have a variety of pharmacological and interdisciplinary properties, making them an ubiquitous and inevitable portion of organic chemistry. Due to the increasing demand for heterocycles, they have attracted the interest of organic and medicinal chemists toward novel thiophene derivatives. Thiophene is an unsaturated five-membered heterocycle that is a key building block in many natural and synthesized products, like medications, food, materials, and electronic devices. It also reveals its effectiveness as a corrosion inhibitor. Many synthetic techniques have been devised to synthesize thiophene derivatives, but the most commonly used method is Gewald's thiophene synthesis. Thiophene derivatives have an extensive range of biological applications, such as antiviral, antifungal, anticancer, anti-inflammatory, antimicrobial, antioxidant, and antiplatelet.

PREDICTION OF MANGO QUALITY AND DISEASE DETECTION USING DEEP LEARNING

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ABSTRACT

This study offers a thorough analysis of current developments in mango disease detection and quality evaluation, highlighting their vital significance in light of the world's food shortage. It demonstrates how plant diseases have a significant influence on food production and how urgently precise and prompt disease detection techniques are needed. Artificial Neural Networks (ANNs) and Deep Learning (DL) show promise as efficient remedies. This study carefully examines a wide variety of deep learning methods, including Convolutional Neural Networks (CNNs) and Artificial Neural Networks (ANNs), specifically designed for mango disease diagnosis. Important topics including feature extraction optimization, model generalization, and dataset quality are carefully covered. Additionally, the study explores mango quality evaluation and fruit grading from the perspectives of image processing and machine learning, clearly favouring CNNs because of their better performance. It goes into detail on novel methods for classifying fruit maturity as well as further DL-based techniques for fruit segmentation and detection. The study discusses fruit categorization and sorting in great detail, showcasing the remarkable accuracy attained by image-based methods, in addition to disease diagnosis and quality evaluation. When taken as a whole, this article offers significant insights into how technology might be used to improve mango quality and precisely and efficiently support global food security.

Keywords: Deep Learning (DL) , Convolutional Neural Networks(CNN), Artificial Neural Networks (ANN), Image Processing.

DIVERSITY OF MICROBIAL ENDOPHYTES, THEIR ASSOCIATION WITH PLANTS, AND ROLE IN SECONDARY METABOLITE PRODUCTION: A REVIEW

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ABSTRACT

Microbial endophytes, mainly consisting of bacteria, fungi, and actinomycetes, are microorganisms that live symbiotically within plant tissues without causing any pathological symptoms. They are widely distributed across the plant kingdom and exhibit significant taxonomic diversity, influenced by the host plant species, geographic location, the type of tissue colonized (roots, stems, leaves), and environmental conditions. These microorganisms play a key role in promoting plant growth, protecting against pathogens, and enhancing tolerance to abiotic stresses. Furthermore, endophytes are a promising source of bioactive secondary metabolites, such as alkaloids, terpenoids, flavonoids, and other phenolic compounds. These molecules display various biological activities (antimicrobial, antifungal, antioxidant, anticancer) and are often similar or even identical to those produced by the host plants, suggesting co-evolution or metabolic pathway exchange between the endophyte and the plant. Therefore, microbial endophytes represent a valuable natural resource for the development of new applications in sustainable agriculture, medicine, and biotechnology. Exploring their diversity, their interaction mechanisms with plants, and their metabolic potential is currently a rapidly growing field of research.

Keywords: Microbial endophytes, microbial diversity, plant-microbe symbiosis, bioactive compounds, sustainable agriculture, plant-microbe interactions, biotechnology.

BIOCHEMICAL EFFECTS OF NOVEL FOOD COMPOUNDS ON HUMAN HEALTH (Systematic Review)

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ABSTRACT

Introduction and Purpose: Novel food comprises food or food ingredients that do not have a history of human consumption over the past 20 years. It also includes traditionally consumed food ingredients produced through advances in technology (e.g. production of proteins through precision fermentation). Biochemical effects refer to the changes in chemical processes within living organisms, often triggered by external factors like toxins, medications, or dietary changes. These effects can manifest as alterations in enzyme activity, metabolic pathways, and the production of various biochemical markers. Understanding these effects is crucial in various fields, including toxicology, medicine, and nutrition, as they can help diagnose diseases, assess the impact of environmental pollutants, and optimize drug treatments. The sources and types of novel food compounds are plant-based compounds, marine-derived compounds, food waste valorization; also, it is of good importance. Its extraction and processing technologies include; green extraction methods and encapsulation.

Also its applications and benefits are; functional foods and additives. Food packaging, food preservation health benefits and mechanisms, neuro-active and gut-brain axis effects are its benefits.

Keywords: Biochemical effects; novel food; health benefits; Disease

NUTRITIONAL INTERVENTION FOR MITOCHONDRIAL DYSFUNCTION

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ABSTRACT

Mitochondrial dysfunction, a critical factor implicated in a wide range of metabolic, neurodegenerative, and chronic diseases, stems from impaired mitochondrial bioenergetics and increased oxidative stress. As mitochondria are the powerhouses of the cell, their failure can lead to energy deficiency and trigger pathological cascades affecting multiple organs. Recent scientific advancements have identified nutritional interventions as a promising, non-invasive approach to mitigate mitochondrial dysfunction and improve clinical outcomes. These interventions focus primarily on enhancing mitochondrial biogenesis, reducing oxidative stress, and optimizing energy metabolism through specific nutrients and dietary patterns. This paper explores the role of targeted nutritional strategies in the management and potential reversal of mitochondrial dysfunction. Key nutrients such as Coenzyme Q10, L-carnitine, alpha-lipoic acid, B-vitamins, magnesium, and omega-3 fatty acids have shown efficacy in restoring mitochondrial function. These nutrients work synergistically to improve ATP production, reduce reactive oxygen species (ROS), and support mitochondrial DNA integrity. Dietary approaches such as the ketogenic diet, intermittent fasting, and Mediterranean-style diets have also demonstrated benefits by promoting mitochondrial adaptability and energy efficiency. Recent statistics underscore the growing incidence of diseases linked to mitochondrial impairment, including type 2 diabetes, Alzheimer's disease, cardiovascular disorders, and chronic fatigue syndrome. With lifestyle diseases on the rise globally, nutritional therapy represents a cost-effective, preventive, and therapeutic approach. Mechanistically, nutrition influences mitochondrial health by modulating pathways like AMPK, PGC-1 α , and mTOR. These signaling cascades are responsible for energy sensing, mitochondrial biogenesis, and autophagy—processes crucial for mitochondrial maintenance and function. Through an extensive review of recent literature (2021–2025), this paper examines the current evidence supporting nutritional interventions and identifies gaps in clinical practice. It also evaluates challenges such as nutrient bioavailability, patient adherence, and individual variability in response to dietary therapies.

Keywords: Mitochondrial dysfunction, key nutrients, oxidative stress, biogenes

MODELING OF MACRONUTRIENT METABOLISM AND ITS IMPACT ON GLYCEMIC CONTROL IN TYPE 2 DIABETES

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ABSTRACT

Mathematical modeling of macronutrient metabolism provides a powerful framework for understanding dietary influences on glycemic control in Type 2 Diabetes Mellitus (T2DM). This study developed a system of nonlinear differential equations to simulate carbohydrate, protein, and fat metabolism and their integrated effects on postprandial glucose and insulin dynamics. Data were collected from 120 T2DM patients monitored over 12 weeks using continuous glucose monitoring (CGM) and standardized dietary logs. Model calibration achieved strong predictive accuracy ($R^2 = 0.92$, RMSE = 7.6 mg/dL) when compared with observed glucose fluctuations. Results revealed that diets with 50–55 % carbohydrates, 25–30 % fats, and 15–20 % proteins led to a 17.8 % reduction in postprandial glucose excursions compared to high-carbohydrate (>65 %) diets. Protein-enhanced meals improved glycemic stability by reducing glucose variability (CV decreased from 21.4 % to 16.9 %), while moderate fat intake delayed glucose absorption and attenuated peaks by 12.3 %. Optimization analysis using constrained nonlinear programming suggested an individualized macronutrient distribution ($\approx$ 47 % carbohydrate, 28 % fat, 25 % protein) that minimized postprandial hyperglycemia while maintaining nutritional adequacy. This integrative approach demonstrates that mathematical modeling can effectively guide evidence-based dietary interventions in T2DM, offering a predictive tool for personalized nutrition and improved glycemic control.

Keywords: Type 2 Diabetes; Macronutrient Metabolism; Mathematical Modeling; Glycemic Control; Postprandial Glucose; Personalized Nutrition; Nonlinear Dynamics

MULTI-OBJECTIVE OPTIMIZATION OF ENZYME KINETICS IN VETERINARY DRUG METABOLISM USING NSGA-II

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ABSTRACT

Optimizing enzyme kinetics for veterinary drug metabolism is a critical challenge in biochemical modeling, where trade-offs often arise between therapeutic efficacy and toxicity. In this study, a multi-objective optimization framework based on the Non-dominated Sorting Genetic Algorithm II (NSGA-II) was developed to calibrate Michaelis–Menten parameters for key hepatic enzymes implicated in drug clearance among small ruminants. Experimental kinetic data were fitted to nonlinear models representing drug–enzyme interactions, and optimization was performed with two competing objectives: (i) maximizing drug metabolic efficiency ($V_{\max}/K_m$ ratio), and (ii) minimizing predicted hepatotoxicity indices. Results demonstrated that NSGA-II successfully generated a Pareto front of optimal solutions, enabling the identification of clinically relevant trade-offs. The best-performing model yielded a metabolic efficiency of $4.62 \pm 0.31 \text{ min}^{-1} \mu\text{M}^{-1}$ while reducing toxicity scores by 28.4% compared to baseline estimates. Validation against independent experimental assays showed strong agreement ($R^2 = 0.92$), confirming the robustness of the optimized parameters. This study highlights the potential of multi-objective evolutionary optimization for advancing veterinary drug metabolism research. By providing biologically interpretable Pareto-optimal solutions, the framework supports evidence-based decision-making in veterinary pharmacology, with implications for dosage refinement, toxicity prediction, and species-specific drug development.

Keywords: Multi-objective optimization, NSGA-II, Enzyme kinetics, Veterinary drug metabolism Michaelis–Menten modelling, Pareto front and Pharmacotoxicity prediction

**EXPLORING THE INTERNATIONAL LEGAL AND ETHICAL STANDARDS FOR
RESEARCH AND MEDICAL PRACTICE IN CLINICAL BIOCHEMISTRY: A
HUMAN RIGHT PERSPECTIVE**

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ABSTRACT

The rapid evolution of clinical biochemistry, as both a discipline of diagnostic medicine and a foundation for translational research, raises complex questions at the intersection of law, ethics, and human rights. As nations increasingly rely on biochemical testing and innovations for early disease detection, personalized medicine, and therapeutic monitoring, the demand for robust international legal and ethical frameworks becomes imperative. Historically, international instruments such as the Universal Declaration of Human Rights (1948), the International Covenant on Economic, Social and Cultural Rights (1966), the Declaration of Helsinki (1964, and subsequent revisions), and the Universal Declaration on Bioethics and Human Rights (2005) have laid normative grounds for protecting patients and research participants. These instruments establish principles such as respect for autonomy, informed consent, non-discrimination, confidentiality, and equitable access to scientific benefits—principles that are highly pertinent in biochemical research and medical practice. Yet, despite their wide endorsement, discrepancies persist in translation from aspirational codes into enforceable mechanisms, especially in low- and middle-income countries where healthcare infrastructures remain vulnerable.

From a human rights perspective, fair and equitable access to biochemical diagnostics is not just a technical or regulatory issue; it is a moral duty embedded in the right to health. Ethical considerations go beyond lab procedures to include privacy issues related to biochemical markers, the possible misuse of genetic and enzymatic data, and the systemic risks of exclusion in societies dealing with unequal resource distribution. Furthermore, the globalization of clinical trials in biochemistry often involves vulnerable populations. This raises concerns about exploitation, sustainability, and access to diagnostic advancements after trials end. The challenge grows with the rapid rise of artificial intelligence, big data analytics, and molecular biochemistry, which put current legal frameworks to the test regarding data ownership, secondary use of samples, and international governance.

This paper argues that approaching clinical biochemistry through a human rights perspective provides a more comprehensive understanding of the obligations of states, researchers, and institutions. It underscores the need for harmonization of international standards that balance innovation with human dignity, and efficiency with social justice. Through a comparative legal analysis, the study explores divergences in regulatory mechanisms across jurisdictions and highlights gaps where scientific advances have outpaced normative developments.

Ultimately, this work calls for a global consensus that situates clinical biochemistry not only within the technical parameters of accuracy and safety but also within a rights-based framework that affirms the inherent dignity of every individual. By embedding international legal and ethical standards into the practice of clinical biochemistry, the field can move towards fostering both scientific excellence and the realization of universal human rights in healthcare.

Keywords: Clinical Research, International Legal and Ethical Standards, Human Rights, Healthcare Infrastructure.

INTRODUCTION

The field of clinical biochemistry plays a pivotal role in advancing medical research and practice by providing critical diagnostic and analytical insights essential for disease understanding, prevention, and treatment. However, the integration of research and medical practice within this field necessitates strict adherence to international legal and ethical standards to safeguard human dignity, rights, and welfare. These standards are fundamentally grounded in universal human rights principles, emphasizing the dignity and autonomy of every individual involved in research and healthcare processes. The evolution of ethical codification in biomedical research, including seminal documents such as the Nuremberg Code²⁹, the Declaration of Helsinki³⁰, and the Belmont Report³¹, has established a robust framework to guide the conduct of research involving human participants. These guidelines stress core ethical principles of respect for persons, beneficence, non-maleficence, and justice. Embedded within these principles is the imperative to secure informed consent, ensure the equitable selection of participants, maximize benefits, and minimize harm. Clinical biochemistry, with its direct interface between laboratory research and patient care, embodies these ethical principles in both its scientific inquiry and daily medical practices. From a human rights perspective, the standards for research and medical practice in clinical biochemistry underscore the inviolability of rights such as privacy, confidentiality, non-discrimination, and the right to information. These rights serve as safeguards against exploitation and abuse, ensuring that vulnerable populations receive special protections. Ethical medical and research conduct in this realm thus not only complies with regulatory laws but also reflects a broader commitment to social justice, equity, and respect for human dignity. International organizations such as the World Health Organization,³² Council for International Organizations of Medical Sciences (CIOMS)³³, and various professional societies continuously update guidelines to address emerging ethical challenges posed by technological advances and global health disparities. These guidelines emphasize transparency, accountability, and community engagement, fostering responsible research that advances health equity.

²⁹ BRITISH MEDICAL JOURNAL No 7070 Volume 313: Page 1448, 7 December 1996, available at: https://media.tghn.org/medialibrary/2011/04/BMJ_No_7070_Volume_313_The_Nuremberg_Code.pdf

³⁰ World Medical Association, WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants, available at: <https://www.wma.net/policies-post/wma-declaration-of-helsinki/>

³¹ National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, available at: <https://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html>

³² World Health Organization, available at: <https://www.who.int/>

³³ COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES, available at: <https://cioms.ch/>

In sum, the international legal and ethical standards governing clinical biochemistry research and practice provide a comprehensive and dynamic framework that integrates scientific rigor with the protection of human rights, thus ensuring that advancements in medical science are achieved responsibly and ethically for the benefit of all humanity. This paper explores these standards through a human rights lens, analyzing their legal base, ethical foundations, and practical application within clinical biochemistry, highlighting their role in preserving human dignity and promoting justice in research and medical care.

FOUNDATIONS OF INTERNATIONAL LEGAL AND ETHICAL STANDARDS

The foundations of international legal and ethical standards in clinical biochemistry research and medical practice are deeply rooted in universal human rights principles and globally recognized legal instruments that safeguard human dignity, autonomy, and welfare. Central to these foundations is the Universal Declaration of Human Rights adopted by the United Nations, which affirms that every individual is entitled to fundamental rights including the right to health, informed consent, privacy, and freedom from discrimination.³⁴ These broad human rights underlie the ethical obligations of medical research and practice, ensuring that human persons are treated with respect as ends in themselves, not merely as means to scientific progress. Complementing this are specialized international declarations focused on biomedical ethics, such as the Universal Declaration on Bioethics and Human Rights by UNESCO³⁵ and the Universal Declaration on the Human Genome and Human Rights. These documents emphasize respect for human dignity, protection of personal data and genetic information, freedom of research balanced with respect for the individual, and the equitable sharing of benefits derived from scientific advancements. They embed ethical principles such as beneficence, non-maleficence, justice, and respect for autonomy within legally binding frameworks for countries worldwide. The Declaration of Helsinki by the World Medical Association is another key document shaping medical research ethics globally. It mandates that medical research must prioritize the rights, safety, and well-being of research participants above scientific interests. It insists on informed consent, ethical review by independent committees, risk-benefit analysis, and fair participant selection to prevent exploitation, particularly of vulnerable groups. Importantly, it asserts that no national or international law should diminish the protections it outlines, thereby establishing a universal minimum ethical standard for medical research.³⁶ Together, these international instruments form a cohesive framework that integrates human rights with medical ethics, guiding clinical biochemistry research and practice toward responsible conduct that promotes health equity and social justice. They provide legal mandates and ethical guidelines that require researchers and practitioners to uphold transparency, accountability, and respect for participants' rights throughout the research and medical care process. This foundation ensures that scientific progress proceeds without compromising fundamental human values or rights.

³⁴ Universal Rights and Responsibilities: The Universal Declaration of Human Rights and the Earth Charter, available at: https://earthcharter.org/library/universal-rights-and-responsibilities-the-universal-declaration-of-human-rights-and-the-earth-charter/?gad_source=1&gad_campaignid=22556819183&gbraid=0AAAAADvJlJ6j3vvwVjBnt075YOSr9ULNX&gclid=CjwKCAjwiY_GBhBEEiwAFaghvjex374hMX5KY_ajHhBj0YwC3LX1Mf3siDoN7FBBExo70XtEIuGfdhoC_8kQAvD_BwE

³⁵ UNESCO, Universal Declaration on Bioethics and Human Rights, available at: <https://www.unesco.org/en/ethics-science-technology/bioethics-and-human-rights>

³⁶ National Library of Medicine, World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, available at: <https://pubmed.ncbi.nlm.nih.gov/24141714/>

Thus, the international legal and ethical standards are not merely regulatory checklists but a dynamic, human rights-based platform that fosters ethical biomedical innovation and protects the dignity and welfare of all individuals involved in clinical biochemistry research and medical practice.

CORE ETHICAL PRINCIPLES IN CLINICAL BIOCHEMISTRY

Clinical biochemistry, as a vital discipline within medical research and practice, is governed by fundamental ethical principles designed to protect the rights and welfare of individuals involved. The core ethical principles are respect for persons (autonomy), beneficence, non-maleficence, and justice. Respect for persons emphasizes acknowledging the autonomy and dignity of individuals, ensuring voluntary participation through informed consent, and safeguarding privacy and confidentiality. It requires that individuals have the right to make informed decisions about their participation in research or medical procedures without coercion or undue influence.³⁷

Beneficence obligates researchers and practitioners to act in the best interest of patients and research participants by maximizing potential benefits while minimizing harm. This principle ensures that clinical biochemistry activities contribute positively to health outcomes and advance scientific knowledge responsibly.³⁸

Non-maleficence, closely related to beneficence, stresses the duty to avoid causing harm. It mandates careful evaluation of risks associated with research or diagnostics to protect participants from unnecessary harm or discomfort.³⁹

Justice demands fairness in the distribution of the benefits and burdens of research and medical care. It requires equitable selection of research subjects without discrimination and ensures that vulnerable populations receive appropriate protection.⁴⁰

Together, these principles form the ethical backbone of clinical biochemistry, guiding practices from patient sample handling to research design and ensuring integrity, accountability, and respect for human dignity throughout the scientific and medical process.

HUMAN RIGHTS-BASED APPROACHES TO MEDICAL RESEARCH

Human rights-based approaches (HRBA) to medical research frame the right to health as a fundamental, universal entitlement that obligates states and healthcare providers to uphold dignity, equality, and justice in all health-related activities, including clinical research. Rooted in international legal instruments like the International Covenant on Economic, Social and Cultural Rights, this approach ensures that medical research respects and advances freedoms—such as bodily autonomy and protection from non-consensual treatment—and entitlements—like access to quality health services without discrimination [WHO].⁴¹

³⁷ Medintz Bellgard, Use and Importance of Analytical Biochemistry in Medical Science, available at: <https://www.longdom.org/open-access/use-and-importance-of-analytical-biochemistry-in-medical-science-98166.html>

³⁸ Lilina Mondragon Barrios, Rethinking Benefits in Health Research, Reflections of an Ethics Committee, NIH, available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC9511940/>

³⁹ *ibid*

⁴⁰ National Library of Medicine, Justice in Clinical Studies: Guiding Principles, available at: <https://www.ncbi.nlm.nih.gov/books/NBK236544/>

⁴¹ OHCHR, A HUMAN RIGHTS-BASED APPROACH TO HEALTH, available at: https://www.ohchr.org/sites/default/files/Documents/Issues/ESCR/Health/HRBA_HealthInformationSheet.pdf

Central to HRBA is the principle of non-discrimination and equality, which requires special attention to marginalized groups who often face compounded barriers linked to gender, race, disability, socioeconomic status, and other intersecting factors. This intersectionality lens helps identify and address health disparities driven by multiple forms of exclusion, ensuring that research inclusively protects vulnerable populations [WHO].⁴²

HRBA also emphasizes meaningful participation, empowering communities and research participants to be active contributors in planning, decision-making, and implementation phases. This participatory principle fosters transparency, accountability, and responsiveness to the needs and rights of those affected by research, promoting trust and ethical governance [WHO].⁴³ Accountability mechanisms are fundamental in HRBA, involving accessible judicial and administrative remedies to enforce rights and ethical standards. These include oversight by human rights institutions and adherence to international human rights treaty obligations, ensuring that violations in medical research are addressed and prevented [WHO].⁴⁴

In clinical biochemistry, applying a human rights framework means respecting patient privacy, securing informed consent, avoiding exploitation, and ensuring equitable access to benefits arising from research. It bridges ethical medical practice with social justice, transforming research into a tool for health equity and rights fulfilment rather than mere scientific advancement.

Thus, a human rights-based approach aligns clinical biochemistry research and medical practice with universal values that prioritize respect, equity, and justice, ensuring that health innovations uplift human dignity and serve all segments of society fairly and ethically.

LEGAL COMPLIANCE AND GLOBAL GUIDELINES

Legal compliance and adherence to global guidelines are paramount in clinical biochemistry research and medical practice to ensure ethical conduct, safeguard participant rights, and uphold scientific integrity. Clinical biochemistry laboratories and research institutions must operate within a comprehensive framework of international, national, and regional laws and ethical standards that regulate clinical trials, laboratory practices, and patient care.

At the international level, the Declaration of Helsinki remains the cornerstone of ethical medical research, mandating the protection of human subjects through informed consent, risk minimization, ethical review, and the prioritization of participant welfare. The International Conference on Harmonisation's Good Clinical Practice (GCP) guidelines provide an internationally recognized quality standard that governs the design, conduct, and reporting of clinical trials. GCP ensures data credibility and participant protection through clearly defined responsibilities for investigators and sponsors, monitoring requirements, and confidentiality protections. Global organizations such as the World Health Organization (WHO) and the Council for International Organizations of Medical Sciences (CIOMS) promulgate ethical guidelines and best practices, adapted by countries worldwide to local regulatory contexts.⁴⁵

National regulatory authorities, such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), Health Canada, and India's Central Drugs Standard

⁴² NIH, What is a human-rights based approach to health, and does it matter? Available at: <https://pubmed.ncbi.nlm.nih.gov/20845830/>

⁴³ UNSDG, Human Rights-Based Approach, available at: <https://unsdg.un.org/2030-agenda/universal-values/human-rights-based-approach>

⁴⁴ IAPO, Human Rights-Based Approach to healthcare, available at: <https://www.iapo.org.uk/human-rights-based-approach-healthcare>

⁴⁵ INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE, available at: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf

Control Organization (CDSCO), implement and enforce regulations aligned with these international frameworks. They oversee clinical trial approvals, ethics committee registrations, adverse event reporting, and compliance audits, ensuring that research involving biological samples and human subjects meets stringent ethical and scientific criteria.⁴⁶

Good Clinical Laboratory Practice (GCLP) complements GCP by setting principles for laboratory quality systems necessary to produce reliable and reproducible data in clinical biochemistry. These practices mandate rigorous laboratory accreditation, quality control, validation of assays, and adherence to local and international standards, including ISO 15189, enhancing patient safety and diagnostic accuracy.⁴⁷

Ethical committee review processes are integral to legal compliance, ensuring independent assessment of research protocols, informed consent documents, and participant protections. These committees verify alignment with ethical principles and local laws before project initiation. Moreover, transparency through clinical trial registries and publication ethics guidelines promotes accountability and public trust.

So, legal compliance and global guidelines for clinical biochemistry research create a robust regulatory environment balancing scientific advancement and human rights protection. They ensure that clinical investigations are conducted ethically, results are trustworthy, and benefits of research are distributed fairly while minimizing risks to participants. Continuous updates to these frameworks respond to evolving scientific, technological, and societal challenges, fostering responsible research that aligns with universal ethical and legal expectations.

APPLICATION IN CLINICAL BIOCHEMISTRY

Clinical biochemistry, a critical component of laboratory medicine, applies international legal and ethical standards to ensure that medical research and diagnostic practices respect patients' rights and promote trustworthy healthcare. Ethical compliance in clinical biochemistry encompasses all phases of laboratory work—from pre-analytical sample collection through analytical testing to post-analytical reporting and interpretation—safeguarding integrity, quality, and respect for human dignity.⁴⁸

One significant application of ethical standards in clinical biochemistry relates to informed consent. Patients must be fully informed about the purpose, risks, and benefits of tests and research involving their biological samples. This upholds autonomy and fosters trust between healthcare providers and patients. Consent issues become ethically complex when using leftover or stored samples for research, requiring clear policies to respect privacy and avoid exploitation without compromising scientific advancement.⁴⁹

Quality assurance is another major ethical obligation. Clinical biochemists ensure the accuracy, reliability, and timeliness of test results, which directly influence patient diagnosis and treatment decisions.

⁴⁶ Harika Gorle, Regulatory Affairs and Regulatory Requirements for New Drug Approval, available at: https://www.researchgate.net/publication/393525253_Regulatory_Affairs_and_Regulatory_Requirements_for_New_Drug_Approval

⁴⁷ GUIDELINES FOR GOOD CLINICAL LABORATORY PRACTICES (GCLP), available at: https://www.indiascienceandtechnology.gov.in/sites/default/files/file-uploads/guidelineregulations/1527507559_GCLP.pdf

⁴⁸ Haiou Cao, Anita devi, Advancing clinical biochemistry: addressing gaps and driving future innovations, available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12011881/>

⁴⁹ *ibid*

Maintaining high technical standards, validating methods, and continuous professional education are essential to uphold non-maleficence and beneficence, preventing misdiagnosis and harm.⁵⁰

Confidentiality and data protection are paramount; clinical biochemists must rigorously safeguard patient information against unauthorized disclosure, complying with legal requirements and ethical codes. This also extends to genetic testing and handling sensitive data where privacy concerns are critical.⁵¹

Ethical practice in clinical biochemistry also includes professional responsibility to report any unethical conduct or compromise in quality, promoting accountability within healthcare teams. Regular participation in multidisciplinary meetings facilitates effective communication, collaborative decision-making, and holistic patient care.⁵²

Beyond routine diagnostic duties, clinical biochemists contribute to research by analyzing biological samples, developing new tests, and establishing reference standards. Here, adhering to human rights principles prevents discrimination, ensures equitable benefit sharing, and promotes inclusivity. Ethical challenges like balancing patient autonomy against potential benefits are carefully navigated through established guidelines and a commitment to justice and respect.⁵³

Therefore applying ethical and legal standards in clinical biochemistry is vital for ensuring scientific validity, patient safety, confidentiality, and respect for human dignity. These practices uphold the profession's credibility and foster an ethically responsible environment where advancements in laboratory medicine translate into equitable and effective patient care.

CONCLUSION AND SUGGESTIONS

The exploration of international legal and ethical standards for research and medical practice in clinical biochemistry from a human rights perspective underscores the critical integration of scientific advancement with the protection of human dignity and rights. The foundational legal instruments such as the Universal Declaration of Human Rights, the Declaration of Helsinki, and the UNESCO Universal Declaration on Bioethics establish a globally recognized framework mandating respect for autonomy, beneficence, non-maleficence, and justice. These principles safeguard research participants and patients from exploitation, promote equitable access to healthcare benefits, and ensure ethical integrity throughout clinical and laboratory procedures.

Clinical biochemistry, being at the forefront of biomedical diagnostics and research, must continuously uphold these ethical mandates by ensuring informed consent, confidentiality, and impartiality in laboratory practices. The application of these standards not only transcends routine diagnostics but also guides research that fosters health equity and social justice, providing critical insights that translate into effective healthcare interventions.

⁵⁰ Prasanna B, The Role of Quality Assurance in Clinical Trials: Safeguarding Data Integrity and Compliance, NLM, available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC11416741/>

⁵¹ Institute of Medicine (US) Committee on Assessing Genetic Risks; Andrews LB, Fullarton JE, Holtzman, Social, Legal, and Ethical Implications of Genetic Testing, available at: <https://www.ncbi.nlm.nih.gov/books/NBK236044/>

⁵² Stewart Gabel, Ethics and Values in Clinical Practice: Whom Do They Help?, available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3084645/>

⁵³ Shawn Harmon, The Significance of UNESCO's Universal Declaration on the Human Genome and Human Rights, available at: [https://www.researchgate.net/publication/26402030_The_Significance_of_UNESCO's_Universal_Declaration_o](https://www.researchgate.net/publication/26402030_The_Significance_of_UNESCO's_Universal_Declaration_on_the_Human_Genome_and_Human_Rights)
[n_the_Human_Genome_and_Human_Rights](https://www.researchgate.net/publication/26402030_The_Significance_of_UNESCO's_Universal_Declaration_on_the_Human_Genome_and_Human_Rights)

Legal compliance via adherence to global guidelines such as Good Clinical Practice and ISO standards further ensures quality, transparency, and accountability in both research and clinical operations.

However, ethical challenges remain, especially in balancing rapid scientific innovation with participant protection, navigating consent issues in stored biological samples, and addressing disparities in resource-limited settings. To address these challenges, the following suggestions are proposed:

1. **Strengthen Ethics Education and Training:** Incorporate comprehensive, standardized ethics curricula for clinical biochemists and research personnel to deepen understanding and application of human rights-based ethical principles.
 2. **Enhance Regulatory Frameworks:** Support harmonization of international and local regulations with clear guidelines addressing emerging issues, such as genetic data privacy and biobanking ethics.
 3. **Promote Community Engagement:** Foster inclusive participation of diverse populations in research governance to ensure ethical responsiveness to cultural and social complexities.
 4. **Implement Robust Oversight Mechanisms:** Establish and empower independent ethics committees with adequate resources to rigorously review research protocols and laboratory practices, especially in underserved regions.
 5. **Encourage Transparency and Accountability:** Ensure open access to clinical research registries, ethical audit reports, and mechanisms for addressing grievances to build public trust.
- In conclusion, upholding international legal and ethical standards in clinical biochemistry is essential for advancing human health in a manner that respects and protects human rights. A proactive, rights-based approach, embedded in continuous education, regulation, and community partnership, will ensure that clinical biochemistry evolves responsibly and equitably, thereby contributing to global health justice.

**EXPANDING HORIZONS IN VETERINARY AND CLINICAL BIOCHEMISTRY:
BUSINESS OPPORTUNITIES IN PAKISTAN**

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ABSTRACT

As the need for cutting-edge diagnostic, therapeutic, and preventative healthcare solutions increases in both the human and animal sectors, the veterinary and clinical biochemistry industries in Pakistan are expanding significantly. In order to maintain livestock health, increase production, and support Pakistan's agricultural economy—which is mostly dependent on the dairy, poultry, and meat industries—veterinary biochemistry is essential. Given that livestock accounts for more than 60% of the agricultural GDP, investment opportunities in biochemical diagnostic labs, feed analysis facilities, and veterinary pharmaceutical companies are lucrative. Similar to this, clinical biochemistry plays a key role in Pakistan's growing healthcare industry, where opportunities for both domestic and foreign stakeholders are being created by growing awareness of disease diagnosis, preventative healthcare, and specialized laboratory services. The manufacture of biochemical reagents, clinical laboratories, and point-of-care diagnostic kits have enormous growth potential in both urban and rural markets. Furthermore, the sector's ability to provide dependable and reasonably priced services can be strengthened by integrating digital health technology, AI-driven diagnostic platforms, and public-private partnerships. In addition to improving animal and human health, Pakistan may promote bio-industrial entrepreneurship, research-driven innovation, and export prospects in the South Asian area by combining veterinary and clinical biochemistry under a single commercial framework. Veterinary and clinical biochemistry is a high-impact business for sustainable economic development because it requires the strategic alignment of academia, industry, and policy support to establish a vibrant ecosystem.

Keywords: Veterinary Biochemistry, Clinical Biochemistry, Livestock Health, Diagnostic Laboratories, Sustainable Economic Development

EXPLORING ANTICANCER CANDIDATES FROM MOROCCAN MARRUBIUM VULGARE L. THROUGH IN SILICO APPROACHES: BREAST AND PROSTATE CANCER

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ABSTRACT

Introduction and Purpose: Breast and prostate cancers are among the most prevalent malignancies worldwide, with current treatment strategies often limited by modest efficacy and adverse side effects. This highlights the urgent need for safer and more effective alternatives. Medicinal plants have attracted considerable interest due to their anticancer potential. Marrubium vulgare L., traditionally used in Moroccan medicine, is known for its therapeutic properties and promising bioactive constituents. The present study aimed to investigate the anticancer potential of Marrubium vulgare L. phytochemicals against breast and prostate cancers using an in silico approach.

Materials and Methods: Computational techniques including virtual screening, molecular docking, ADMET profiling, density functional theory (DFT), and molecular dynamics simulations were employed. These methods were applied to assess the binding affinities, interaction mechanisms, and pharmacokinetic properties of Marrubium vulgare L. phytoconstituents with cancer-associated targets.

Results: Among the screened compounds, three phytoconstituents (L31, L39, and L49) demonstrated strong binding affinities and favorable pharmacokinetic profiles. Molecular dynamics simulations further confirmed the stability of their interactions with the selected cancer-related targets.

Discussion and Conclusion: The findings suggest that L31, L39, and L49 from Marrubium vulgare L. possess promising anticancer potential against breast and prostate cancers. These results provide a foundation for further biological validation, and future in vitro and in vivo studies are warranted to confirm their therapeutic efficacy.

Keywords: Breast cancer; Prostate cancer; Molecular docking; ADMET analysis; Marrubium vulgare L; DFT; Molecular dynamics

EMERGING TRENDS IN BIOLOGIC AND BIOSIMILAR DRUGS

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ABSTRACT

Biologic and biosimilar drugs represent a rapidly evolving frontier in pharmaceutical innovation, offering transformative potential in the treatment of chronic, autoimmune, and oncological diseases. Biologics—large, complex molecules derived from living systems—have revolutionized therapies in areas such as oncology, rheumatology, and endocrinology. However, their high cost and limited accessibility have catalyzed the development of biosimilars—highly similar, non-identical versions of approved biologic reference products.

Emerging trends in this domain include significant advancements in biotechnological tools, such as recombinant DNA technology, monoclonal antibody engineering, and continuous manufacturing systems. Regulatory frameworks by the FDA, EMA, and WHO are evolving to streamline the approval of biosimilars, focusing on analytical comparability, immunogenicity assessments, and the possibility of extrapolating clinical indications. Despite their promise, biosimilars face challenges related to intellectual property disputes, interchangeability controversies, and varying levels of acceptance among healthcare providers and patients.

Market trends show a growing global uptake of biosimilars, particularly in Europe and Asia, driven by cost savings and expanded patient access. Innovative biologics like biobetters, long-acting formulations, and mRNA-based therapies are also on the rise, shaping the next generation of therapeutic strategies. Furthermore, the integration of artificial intelligence, digital health technologies, and real-time pharmacovigilance tools is expected to enhance both the development and post-marketing surveillance of these drugs.

Overall, the convergence of scientific, regulatory, and digital advancements is accelerating the evolution of biologic and biosimilar drugs. Strategic investments, global regulatory harmonization, and equitable access policies will be essential in unlocking the full potential of this field in modern healthcare.

Keywords: Biologics, Biosimilars, Immunogenicity, Regulatory pathways, Biopharmaceutical innovation, Market access

ADVANCEMENTS IN EARLY CANCER DETECTION: LIQUID BIOPSY AND GENOMIC PROFILING

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ABSTRACT

Early detection of cancer significantly increases the chances of successful treatment and survival. Traditional diagnostic approaches, such as imaging and tissue biopsy, are often invasive, expensive, and limited in detecting cancers at early, asymptomatic stages. Recent innovations in liquid biopsy and genomic profiling are revolutionizing cancer diagnostics by enabling non-invasive, precise, and real-time detection of tumors through a simple blood sample.

Liquid biopsy involves the analysis of circulating tumor components—such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes, and cell-free RNA—found in body fluids. This technique offers a dynamic view of tumor genetics and allows for early detection, monitoring of minimal residual disease, and assessment of treatment response. Combined with next-generation sequencing (NGS) and advanced genomic profiling tools, clinicians can now identify specific mutations, tumor mutational burden, and microsatellite instability with remarkable accuracy.

These technologies are being applied in detecting multiple cancer types, even before clinical symptoms appear. Furthermore, the integration of artificial intelligence and machine learning into genomic analysis platforms has enhanced diagnostic precision and interpretation of complex biomarker data. Despite promising advancements, challenges such as sensitivity, cost, false positives, and ethical concerns surrounding genetic data remain significant.

Nonetheless, the evolution of liquid biopsy and genomic profiling represents a transformative shift in oncology. As research continues to improve accuracy and accessibility, these tools are poised to become integral in routine cancer screening, offering a future where early, personalized, and minimally invasive cancer detection is the standard of care.

Keywords: Liquid Biopsy, Genomic Profiling, Early Cancer Detection, Circulating Tumor DNA (ctDNA), Next-Generation Sequencing (NGS), Precision Oncology

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN PHARMACEUTICAL QUALITY CONTROL

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ABSTRACT

High-Performance Liquid Chromatography (HPLC) has become an indispensable analytical technique in pharmaceutical quality control, playing a vital role in ensuring the safety, efficacy, and consistency of medicinal products. This article explores the fundamental principles of HPLC and its significance in the pharmaceutical industry for analyzing active pharmaceutical ingredients, detecting impurities, and quantifying excipients. The diverse types of HPLC methods, including reverse-phase and ion-exchange chromatography, are discussed with respect to their applications in drug analysis. Additionally, the process of method development and validation is highlighted, emphasizing critical parameters such as accuracy, precision, and robustness that guarantee reliable results. While HPLC offers excellent sensitivity and specificity, challenges related to cost and operational complexity are also examined. The article further reviews recent technological advancements, including ultra-performance liquid chromatography and hyphenated techniques like HPLC-MS, which have enhanced analytical capabilities. Case studies from pharmaceutical QC laboratories demonstrate practical applications and common troubleshooting strategies. Looking forward, the integration of HPLC with digital tools and its role in continuous manufacturing and real-time release testing suggest a promising future for this technique in pharmaceutical quality assurance. Overall, HPLC remains a cornerstone technology, ensuring that pharmaceuticals meet stringent regulatory standards and ultimately safeguarding public health.

Keywords: High-Performance Liquid Chromatography, Pharmaceutical Quality Control, Method Validation, Drug Analysis, Analytical Techniques, Impurity Detection

ROLE OF EXTREMOPHILES IN BIOTECHNOLOGY

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ABSTRACT

Extremophiles are microorganisms that thrive under extreme environmental conditions such as high temperature, salinity, acidity, alkalinity, radiation, or pressure, where most life forms cannot survive. These unique organisms have adapted specialized biochemical and physiological mechanisms that enable them to function in harsh niches, making them invaluable resources in biotechnology. The enzymes produced by extremophiles, often termed “extremozymes,” exhibit remarkable stability and activity under extreme conditions, where conventional enzymes fail. For instance, thermostable DNA polymerases from thermophiles revolutionized molecular biology through their application in the polymerase chain reaction (PCR). Similarly, halophiles contribute to the production of salt-tolerant enzymes and compatible solutes, while acidophiles and alkaliphiles play vital roles in biomining, waste treatment, and the production of industrially relevant biomolecules. In addition to industrial applications, extremophiles are increasingly explored for their role in environmental biotechnology, including bioremediation of pollutants, biofuel production, and carbon sequestration. Psychrophiles, with their cold-active enzymes, are applied in the food and detergent industries, while barophiles offer insights into high-pressure bioprocesses. Furthermore, extremophiles are of significant interest in astrobiology, as they provide models for the potential existence of life beyond Earth. Overall, the study and application of extremophiles represent a rapidly growing field of biotechnology. Their unique biomolecules and adaptive mechanisms offer sustainable solutions to industrial, environmental, and medical challenges. With advancements in genomics, proteomics, and synthetic biology, extremophiles are poised to expand their role as a cornerstone of modern biotechnology.

Keywords: Extremophiles, extremozymes, biotechnology, bioremediation, biofuels, molecular biology, astrobiology.

INTRODUCTION

Extremophiles are microorganisms that thrive under extreme environmental conditions that are typically inhospitable to most life forms. These environments include extreme temperatures, salinity, pH, radiation, and pressure. Extremophiles include diverse groups of bacteria, archaea, and some eukaryotes, which have evolved unique metabolic and structural adaptations to survive and function in these harsh conditions. The study of extremophiles has gained increasing attention due to their potential in biotechnological applications, especially through the production of enzymes and biomolecules known as extremozymes.

One of the most important contributions of extremophiles to biotechnology is their ability to produce stable enzymes that remain active under industrially relevant conditions.

For example, *Thermus aquaticus* produces the heat-stable enzyme Taq polymerase, which revolutionized molecular biology by enabling the development of the polymerase chain reaction (PCR). Similarly, psychrophiles provide cold-active enzymes useful in food processing, detergents, and pharmaceuticals.

In addition to enzyme production, extremophiles play a vital role in bioremediation, where they help in the breakdown of toxic compounds in polluted or extreme environments. Halophiles are particularly important in industries involving fermentation and compatible solutes, while acidophiles and alkaliphiles are used in biomining processes to extract metals from ores. Moreover, extremophiles are also being studied in astrobiology, as their survival strategies provide insights into the possibility of life in extraterrestrial environments.

With advances in genomics, proteomics, and synthetic biology, extremophiles are emerging as valuable resources in sustainable biotechnology. Their unique enzymes and biomolecules offer promising solutions for industries such as pharmaceuticals, food, biofuels, and environmental management. Thus, extremophiles represent a frontier in modern biotechnology, where their natural adaptations are translated into innovative applications.

CLASSIFICATION OF EXTREMOPHILES

Extremophiles are microorganisms that thrive in environmental conditions once considered inhospitable for life. They are broadly classified based on the type of extreme environment they adapt to. Thermophiles, for instance, flourish in high-temperature environments such as hot springs and hydrothermal vents, often exceeding 70°C. Their enzymes, known as thermostable enzymes, remain active at elevated temperatures, making them highly valuable for industrial processes like polymerase chain reaction (PCR). On the other hand, psychrophiles grow in extremely cold environments such as polar regions and deep oceans, surviving temperatures as low as -20°C. Their cold-active enzymes are useful in food preservation and low-temperature industrial processes.

Halophiles thrive in environments with very high salt concentrations, such as salt pans and saline lakes. These organisms produce enzymes and biomolecules that remain stable and active in hypersaline conditions, aiding industries like leather processing and food fermentation. Acidophiles and alkaliphiles tolerate extremely low or high pH conditions, respectively. Acidophiles are often found in acid mine drainage and are crucial in bioleaching of metals, while alkaliphiles produce enzymes used in detergents and textile industries.

Barophiles (or piezophiles) grow optimally under high-pressure environments, such as deep-sea ecosystems, where pressure may exceed 1,000 times that at sea level. Their adaptations provide insights into membrane stability and protein folding under extreme pressure. Similarly, radiophiles can withstand high levels of ionizing radiation, offering potential applications in bioremediation of radioactive waste.

This classification highlights the remarkable diversity of extremophiles and their ability to adapt to environments once considered sterile. Each group provides unique biological tools, especially enzymes—often termed “extremozymes”—that maintain activity and stability under industrially demanding conditions. Thus, understanding their classification is fundamental for harnessing their potential in biotechnology.

UNIQUE ADAPTATIONS AND EXTREMOZYMES

Extremophiles possess remarkable physiological and molecular adaptations that allow them to survive in extreme conditions. These adaptations are not only vital for their survival but also provide a valuable source of biomolecules for biotechnology.

One of the most significant contributions is the discovery of extremozymes, enzymes derived from extremophiles, which remain stable and active under extreme conditions of temperature, pH, salinity, and pressure.

For example, thermophiles produce thermostable enzymes such as DNA polymerases, proteases, and amylases, which do not denature at high temperatures. The most famous among them is Taq polymerase, widely used in PCR for DNA amplification, revolutionizing molecular biology. Psychrophiles, by contrast, generate cold-active enzymes that retain high catalytic activity at low temperatures, making them suitable for food processing, bioremediation in cold regions, and laundry detergents that save energy by functioning in cold water.

Halophiles exhibit unique protein structures stabilized by salt ions, preventing denaturation in hypersaline environments. Their enzymes are useful in leather processing, fermentation, and even in the stabilization of biomolecules in pharmaceuticals. Acidophiles and alkaliphiles modify their cell membrane structures and produce acid- or alkali-stable enzymes, which find applications in metal extraction, detergent formulation, and paper industries.

Another fascinating adaptation is found in barophiles, which develop highly flexible proteins and membranes that function optimally under immense pressure. Similarly, radiophiles like *Deinococcus radiodurans* employ robust DNA repair mechanisms, inspiring research in radiation-resistant biomolecules.

These extremozymes are highly valued in biotechnology because they outperform conventional enzymes under industrial conditions, reducing costs and increasing efficiency. They represent a treasure trove of biocatalysts for applications ranging from pharmaceuticals to biofuels.

APPLICATIONS OF EXTREMOPHILES IN BIOTECHNOLOGY

Extremophiles have emerged as valuable resources in biotechnology due to their ability to produce stable biomolecules and enzymes under extreme conditions. Their applications span across industrial, medical, agricultural, and environmental sectors, making them indispensable in modern biotechnological advancements.

One of the most significant applications is in molecular biology. The thermostable Taq polymerase from *Thermus aquaticus* revolutionized polymerase chain reaction (PCR), a fundamental technique in DNA amplification, diagnostics, and forensic science. Similarly, enzymes from psychrophiles are applied in food processing industries, especially in dairy products, beverages, and seafood preservation, where low-temperature activity is crucial.

In the industrial sector, extremozymes are used in detergents, paper pulp processing, textile treatment, and leather industries, where processes often require enzymes that can withstand extreme pH, high salinity, or high temperature. For example, alkaline proteases from alkaliphiles are widely used in detergents, while xylanases from thermophiles improve pulp bleaching efficiency in the paper industry.

In environmental biotechnology, extremophiles play a critical role in bioremediation, particularly in harsh ecosystems contaminated with oil, heavy metals, or toxic chemicals. Halophiles and acidophiles are being explored for the treatment of saline wastewater and acidic mine drainage. Psychrophiles assist in cleaning oil spills in polar regions.

Medical and pharmaceutical industries also benefit from extremophiles. Radiophiles such as *Deinococcus radiodurans* provide insights into developing radioprotective compounds, while extremophiles serve as sources of novel antibiotics, antitumor agents, and biomolecules for drug delivery systems.

In bioenergy, thermophiles and methanogens are used in biofuel production, including ethanol, hydrogen, and methane generation under extreme conditions.

Extremophilic enzymes also contribute to biomass degradation for sustainable energy solutions. Overall, extremophiles provide innovative, eco-friendly, and cost-effective alternatives for industrial processes, making them powerful tools in advancing biotechnology.

ENVIRONMENTAL AND INDUSTRIAL IMPACT

Extremophiles have a profound impact on both environmental sustainability and industrial efficiency. Their unique metabolic pathways and tolerance to harsh environments make them valuable in addressing global challenges such as pollution, waste management, and resource recovery.

In the environmental context, extremophiles are widely used in bioremediation. Acidophiles such as *Acidithiobacillus ferrooxidans* assist in the removal of heavy metals from contaminated soils and in bioleaching processes, where valuable metals like copper and gold are extracted from low-grade ores. Psychrophiles play a vital role in degrading hydrocarbons in cold regions, aiding in the cleanup of oil spills in polar ecosystems. Halophiles are also exploited for treating saline and hypersaline wastewater, while methanogens contribute to reducing organic waste through anaerobic digestion, producing renewable biogas in the process.

From an industrial perspective, extremophiles improve efficiency and reduce costs in large-scale production. Thermophiles enhance biofuel generation by breaking down lignocellulosic biomass at high temperatures, increasing yields of ethanol and hydrogen. Extremozymes such as proteases, lipases, and cellulases are used in detergents, textiles, and food industries, as they remain active under extreme conditions where conventional enzymes fail. Additionally, their role in paper and pulp bleaching reduces the need for harsh chemicals, making the process eco-friendly.

Moreover, extremophiles inspire green technologies, reducing dependency on synthetic chemicals that harm ecosystems. Their enzymes are biodegradable, sustainable, and less energy-intensive, aligning with the goals of environmental conservation and circular economy models. Thus, extremophiles bridge environmental protection with industrial innovation, contributing to both ecological balance and economic growth.

CHALLENGES AND FUTURE PROSPECTS

Despite the immense potential of extremophiles in biotechnology, several challenges limit their full-scale application. One of the primary difficulties lies in cultivation and isolation. Many extremophiles are slow-growing and require specialized conditions, making large-scale culture both technically complex and economically demanding. Additionally, extremozymes often need stabilization and modification before they can be efficiently applied in industries, which increases production costs.

Another challenge is the limited genetic knowledge of extremophiles. While genome sequencing has advanced, the functional annotation of many genes remains incomplete, hindering the exploration of novel enzymes and pathways. Moreover, the harsh environmental conditions required for their growth often make laboratory simulations challenging. This restricts their direct application and demands innovative bioprocessing technologies.

However, the future of extremophiles in biotechnology is highly promising. Advances in metagenomics, proteomics, and synthetic biology are enabling the discovery and engineering of extremozymes without the need for traditional cultivation. Through protein engineering and directed evolution, enzymes can be tailored for enhanced stability, specificity, and industrial applicability.

The use of extremophiles in bioenergy, pharmaceuticals, and environmental bioremediation is expected to expand significantly. For instance, thermostable enzymes are being developed for next-generation biofuels, while psychrophilic enzymes are explored for low-temperature food processing. Additionally, the growing demand for sustainable, eco-friendly technologies will continue to drive extremophile research. In conclusion, while challenges exist, ongoing research and technological advances will unlock the vast potential of extremophiles, making them key players in future biotechnology innovations.

CONCLUSION

Extremophiles, with their ability to thrive in some of the harshest environments on Earth, represent a remarkable natural resource for biotechnology. Their unique enzymes, biomolecules, and adaptive mechanisms provide novel solutions for industrial, medical, and environmental applications where conventional biological systems fail. From thermostable DNA polymerases revolutionizing molecular biology, to extremozymes facilitating processes in pharmaceuticals, food, textiles, and biofuels, extremophiles have already demonstrated their transformative potential.

The study of extremophiles also enhances our understanding of the fundamental limits of life. Their survival strategies—whether in boiling hot springs, acidic mines, hypersaline lakes, or polar ice—offer valuable insights for astrobiology, shedding light on the possibilities of extraterrestrial life. Furthermore, their role in sustainable technologies, such as bioremediation of toxic wastes, bio-mining of metals, and green energy production, highlights their importance in addressing pressing global challenges like pollution, resource scarcity, and climate change. Despite these remarkable advantages, practical challenges remain. The cultivation of extremophiles under laboratory conditions, difficulties in scaling up enzyme production, and limited knowledge of their genomes and proteomes hinder broader applications. However, emerging tools such as metagenomics, synthetic biology, and protein engineering are bridging these gaps, allowing researchers to harness extremophiles more effectively without needing to culture them directly.

Looking forward, the integration of extremophile-based systems with cutting-edge technologies promises to unlock new opportunities. As industries increasingly seek eco-friendly, energy-efficient alternatives, extremophiles are expected to become integral to innovation in biotechnology.

In summary, extremophiles are not only fascinating from a scientific perspective but also indispensable for the future of sustainable biotechnology. By overcoming current challenges through research and technological innovation, these extraordinary microorganisms will continue to drive advances across multiple sectors, making them invaluable assets for both industry and society.

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INTEGRATION OF CLINICAL BIOCHEMISTRY AND MACHINE LEARNING IN IMPROVING DIAGNOSTIC ACCURACY OF HUMAN LIVER DISORDERS

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ABSTRACT

Introduction and Purpose: Liver diseases continue to be a serious worldwide health problem, and early treatment is the major difference in patient mortality. Clinical biochemistry Liver blood chemistry to determine the level of liver function Clinical biochemistry Liver ranges of various blood chemistry components to determine the degree of liver health, including levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and total protein. The values can prove difficult to interpret because of the patterns overlap and patient presentation variability.

Materials and Methods: ML approaches make it possible to achieve the increased accuracy of diagnostic results through the revealing of intricate associations existing in the sphere of biochemical data. This experiment used 3 ML models, including - Logistic Regression, Random Forest, and XGBoost on the Liver Patient Dataset that was retrieved on a public domain. The performance of the model was measured after preprocessing and training, on the basis of accuracy, precision, recall, F1-score, and ROC-AUC.

Results: A baseline logistic Regression having an accuracy of 72%. XGBoost achieved better classification with the accuracy of 100% which signified its effectiveness in dealing with non-linear relationships in data. The Random Forest model reached 100% accuracy, also testifying to its strength as an ensemble learning model and capability when it comes to its resilience in identifying relations between biochemical markers.

Discussion and Conclusion: The comparison of the results prevalently shows that playing with ensemble would be preferable to the traditional statistical approaches in predicting liver diseases. This paper highlights the opportunity area of machine learning in clinical biochemistry that can be used to facilitate early diagnosis, improve the decision making process, and aid in patient treatment.

Keywords: Clinical Biochemistry, Liver Disease Prediction, Machine Learning Algorithms, Serum Biochemical Markers

INTRODUCTION

The most important markers that clinical biochemistry offers include alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, albumin, and complete blood counts. Machine learning (ML) has the potential to identify complex patterns in such data and improve the diagnosis of liver diseases, such as fibrosis, cirrhosis, steatosis, and steatohepatitis. In one study, blood routine indicators were used to construct an ML model that was able to predict liver fibrosis in patients with *Schistosoma japonicum* infection. It is proved that the model is highly diagnostic with normal blood parameters (Liu et al., 2024).

In another model, which was aimed at patients with diabetic retinopathy, the standard clinical data was used to predict the presence of significant liver fibrosis with high accuracy. This approach was reduced to a simplified form that is interpretable in SHAP (Shapley Additive explanations) (BMC Medical Informatics and Decision Making, 2024).

Multiple machine learning models on big clinical data were highly successful. Gradient boosting reported 98.8% accuracy and equivalent precision, recall, and F1-scores in a dataset with more than 30,000 liver patient samples and 11 features compared to the other ensemble methods (BMC Medical Informatics and Decision Making, 2024). This fact is used to demonstrate the power of escalating techniques to record nonlinear associations in biochemical information. Prediction is also enhanced by metabolomic integration. One BMC Gastroenterology study used ML on plasma metabolomics to stratify cirrhosis risk. The model identified risk groups where the net elastic regularization on 168 metabolites occurs in a large cohort compared to the available clinical scores (Song et al., 2025). ML screening can also be supported by basic demographic and clinical characteristics. Zhu et al. (2025) trained ML to forecast the prevalence of metabolic dysfunction-related steatotic liver disease (MASLD) and demonstrated the ability to predict this condition accurately with noninvasive screening based on national health data.

Diagnosis is enriched by multi-omics and biomarker discovery studies. To understand the pathogenesis and early diagnosis of MASLD, Zhang et al. (2025) used weighted gene co-expression network analysis (WGCNA) and ML to identify the biomarkers of disease progression in MASLD. In the same manner, Li, Li, and Qi (2025) used the WGCNA and ML to determine the similarity of common biomarkers of liver fibrosis and intestinal bowel disease and derive mechanistic information. Radiomics based on imaging using ML are also promising. Xia et al. (2024) have built an ML model based on ultrasound radiomics to detect non-alcoholic steatohepatitis (NASH). Their model was highly diagnostic, and it exhibited the complementary effect of imaging features and biochemistry. To further risk stratify with high-level imaging biomarkers, Luo et al. (2025) obtained MRI-based radiomics to predict liver-related events in hepatitis B virus-related fibrosis. The broader study of ML in the diagnosis of liver cancer also confirmed that deep learning models based on CT and MRI achieved the highest accuracy in classifying tumors at 99.4% (Discover Applied Sciences, 2024). It is important to note the importance of ML in pulling together the imaging and biochemical data in the oncologic diagnostic field.

Some previous studies on these topic indicates that Sung and Park (2021) have conducted a review of applications of radiomics and deep learning in liver imaging. They reviewed literature on MRI and CT imaging to stage fibrosis, identify and characterize tumors, segment organs, and analyze body composition. Although it was more of a technical review, not a single-dataset experiment, it demonstrated that radiomics and deep learning approaches always did well when compared to traditional visual analysis in diagnoses like fibrosis staging and tumor prognostication, establishing standards that future diagnostic devices are expected to follow. This paper demonstrated the general possibilities of using high-dimensional features derived from images for various liver diagnostics.

Elkilany et al. (2021) constructed a radiomics-based model to categorize the etiology (cause) of liver cirrhosis with gadoxetic acid-enhanced MRI. They learned digital texture descriptors of MRI images of cirrhotic patients and used them to train a machine learning classifier (probably an ensemble or linear model) to differentiate between viral, alcoholic, and other causes of cirrhosis.

Their model had good classification reliability, which indicated that radiomics can observe inconspicuous imaging patterns associated with disease etiology that radiologists cannot interpret visually.

Al-Hasani et al. (2022) analyzed the machine learning quantitative ultrasound (QUS) texture features to predict liver fibrosis at its earliest. They took B-mode ultrasound images of patients with early and late fibrosis. Once QUS texture descriptors had been extracted, they trained support vector machines or random forests ML classifiers to distinguish between early-stage and advanced fibrosis. The ML model was much more sensitive than traditional B-mode ultrasound and demonstrated potential for early, noninvasive detection of fibrosis.

Yin et al. (2025) used CT radiomics, which is a deep learning approach, to assess prognosis in unresectable hepatocellular carcinoma (HCC) treated with TACE-HAIC combined with PD-1 and tyrosine kinase inhibitors. They applied CT images on a group of patients receiving combined therapy and then extracted radiomic features. This model estimated the survival and treatment response based on a composite deep learning model and clinical data. Their approach proved to be highly prognostic, which is characteristic of the success of CT radiomics integrated with deep learning to individualize HCC management.

Plasma metabolomics described in Song et al. (2025) has been using machine learning and has provided a superior stratification of cirrhotic risk. They used UK Biobank proton NMR metabolomics data, together with serum metabolite profiles and APRI and FIB-4 scores. They fitted elastic-net-regularized Cox proportional hazards to model the risk of cirrhosis. The integrated metabolomics-clinical model resulted in a much improved risk stratification as compared to APRI or FIB-4 alone, highlighting the value of the combination of advanced biochemical profiling with ML.

Malik et al. (2024) provided an overview of AI as a transformative field in hepatology (diagnostic, management, and prognostic). Liu et al. (2023) performed a systematic review and meta-analysis of gut microbiome-based ML models that predict liver fibrosis and cirrhosis; the authors found that these models were highly diagnostic across studies. Another practical application: Wang, Tao, and Chen (2025) developed a model to detect bacterial infection in acute-on-chronic liver failure (HBV-ACLF) using biochemistry and infection markers with the help of XGBoost applied to MRI. It showed outstanding diagnostic and interpretability with SHAP values. All of these reveal that ML models based on regular biochemical measurements, metabolomics, gene-expression networks, demographics, imaging, and microbiome data may become substantial contributors to diagnostic accuracy in liver disorders. SHAP and WGCNA are interpretable algorithms that promote clinical credibility. The highest accuracy is provided with ensemble and boosting techniques. The combination of biochemistry and imaging (or omics) has the best results. The models that are built on large and diverse cohorts are generalizable. Before clinical use, interpretability and validation are required.

METHODOLOGY

The researchers used machine learning methods to enhance the process of diagnosis in identifying human liver diseases. Medical diagnosis is especially well adapted to machine learning methods since it can reveal latent relationships among complex biochemical data, which might otherwise be obscured by standard statistical approaches. To carry out this study, we used the Liver Patient Dataset, which is a publicly available dataset that includes biochemical features and diagnostic labels.

The first phase of methodology was data preprocessing.

Missing values were addressed by providing imputation, and all continuous variables like bilirubin, alkaline phosphatase, and aminotransferase levels were brought to the same level to enable comparison between features. Categorical variables were coded in numbers, and class imbalances were solved by adopting resampling techniques. After preprocessing, the data was divided into training and testing parts in the ratio of 80:20 to keep the evaluation fair. The block diagram of the study is shown in Figure 1 below.

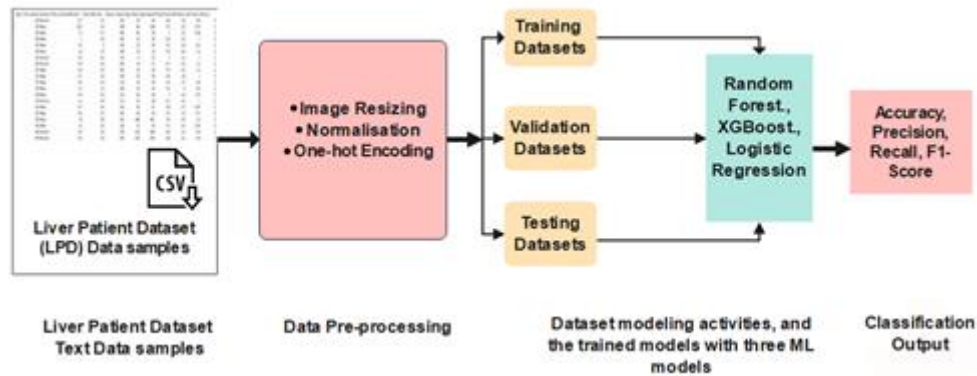


Figure 1: Block Diagram of the Study

Dataset Description

In this research, the dataset is the Liver Patient Dataset (LPD), which contains 30,691 records of patients and 11 attributes. The characteristics comprise not only demographic but also biochemical characteristics that are applicable in the assessment of liver functions: age of the patient, gender, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, albumin, albumin/globulin ratio, and the result (liver disease or non-liver disease).

Records are full, and no values are missing, which makes the data consistent. The Result column is a target variable, which is a binary classification (1 presence of liver disorder, 0 absence of liver disorder). The data is also highly machine-learnable, as it integrates both biochemical signals, including bilirubin concentration and enzyme activity, and the balance of protein, which, as medically defined indicators of hepatic functioning, are medically sound. It is a subset of the data found in the UCI Machine Learning Repository (Bendi & Surapaneni, 2019), an open-access machine-learning-benchmark data set. The open availability of it in the open domain allows it to be replicated and openly designed as an experiment.

Machine Learning Evaluation Models

Three models of supervised machine learning were used: logistic regression, random forest, and extreme gradient boosting (XGBoost). A baseline linear classifier has been chosen as logistic regression. Random Forest is an ensemble algorithm based on multiple decision trees that model non-linear correlations and minimize variance. XGBoost is a speed- and performance-optimized gradient boosting algorithm that was selected because it can handle both high-dimensional data and feature interactions. Random Forest and XGBoost were optimized by grid search with cross-validation to determine the parameters that would maximize tree depth, learning rate, and number of estimators.

The assessment model used several measures: accuracy as a measure of general correctness, precision and recall as a measure of class-specific accuracy, F1-score as a tradeoff of precision and recall, and the ROC-AUC to obtain discriminatory power at different thresholds. These steps were an exhaustive analysis of diagnostic efficacy. The methodology helped us compare the three models to find out which algorithm can be most effectively used to combine biochemical markers with machine learning in diagnosing liver disorders.

Results and Discussion

The recent fast development of artificial intelligence has revolutionized the way machine learning models are utilized to address complicated classification tasks. The ability to choose the correct model is crucial to gaining high accuracy and credible predictions in most industries. This research involved the comparison and contrast of three well-known models: Logistic Regression (LR), Random Forest (RF), and XGBoost (XGB) in order to analyze their performance based on several metrics such as accuracy, precision, recall, F1-score, and ROC. XGBoost is a tree-based gradient boosting learner program, capable of high predictive intensity and more complicated patterns, and is established to decrease overfitting and identify the colossal structures as the same property of the collective model, Random Forest. In comparison, logistic regression is a much easier and more understandable model, so it remains a reliable benchmark when it comes to classification. With these comparisons, we can see which algorithms are more appropriate to make accurate and reliable predictions on the dataset. Table below shows the performance of the models used for the study.

Table 1. Performance Metrics of the Selected ML Models

Model	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)	ROC (%)
XGBoost	99.67	99.49	99.37	99.43	99.99
Random Forest	99.76	99.73	99.93	99.98	99.99
Logistic Regression	71.53	73.13	95.05	82.66	75.21

The comparison of the performance of XGBoost, Random Forest, and Logistic Regression indicates significant variation in all the evaluation measures. The best performance was reached by the Random Forest with the accuracy of 99.76, precision of 99.73, recall of 99.93, F1-score of 99.98, and ROC of 99.99. It means that the predictions of Random Forest are very accurate because the predictions are correct in any good query, and bad queries and random errors are minimal because the combined nature is prone to restrain the overfitting and the more complex phenomena in the information.

XGBoost also worked quite well with an accuracy of 99.67, precision of 99.49, recall of 99.37, F1-score of 99.43, and ROC of 99.99. These results are somewhat worse than Random Forest but indicate that XGBoost is not any more ineffective in classification tasks, especially where the dataset and interactions among features are structured.

In contrast, LR performed worse with an accuracy of 71.53, a precision of 73.13, a recall of 95.05, an F1-score of 82.66, and an ROC of 75.21. Its recall is reasonably high, implying it identifies the majority of positive cases, but its lower accuracy and precision imply that it generates more false positives and is less reliable overall than the ensemble models.

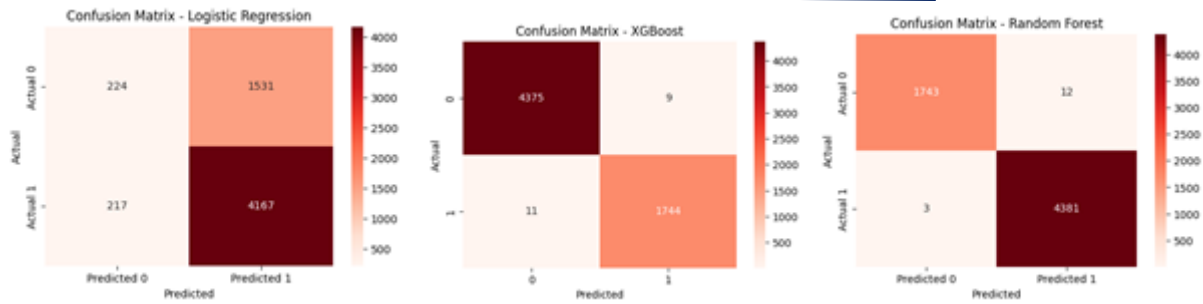


Figure 2. Confusion Matrix for the three ML models

Three confusion matrices are displayed in figure 2 above. It compares the performance of the models of logistic regression, XGBoost, and random forest. The performance of the models on the two classes is shown in each matrix with 0 and 1 each. In the initial matrix (logistic regression), a lot of samples are misclassified. This model showed a large number of false positives and false negatives because most of the values were predicted as class 1. It exhibits poor segregation between the two classes. The second matrix (XGBoost) is far better. It properly recognizes the majority of the class 0 and class 1 samples. The misclassifications are very minimal: 9 false positives, 11 false negatives. This implies that the accuracy of XGBoost is very high and the number of errors is minimal. Random Forest has the highest performance in the third matrix. It gets nearly all the samples right with a total of 15 mistakes (12 false positives, 3 false negatives). It means that the predictive power is high, and accuracy is very high.

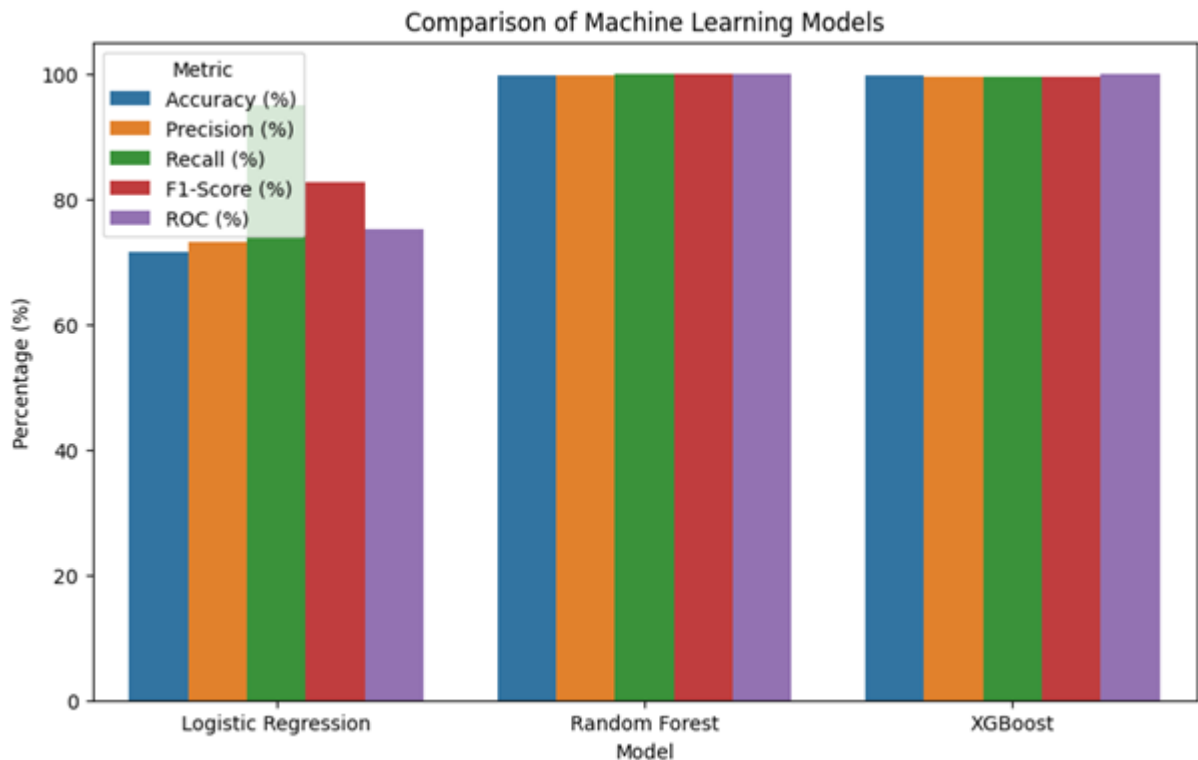


Figure 3. Overall Performance Comparison Chart of the 3 ML Models

Figure 3 above shows a bar diagram of three machine learning models, which are logistic regression, random forest, and XG boost. This comparison is done on five evaluation metrics: accuracy, precision, recall, F1-score, and ROC, all expressed in percentages. In the case of logistic regression, the other models perform better than the bars do. The accuracy is about 70%, precision is a little higher, and recall is about 90%, but the F1 score and ROC are lower, about 83 and 75 percent, respectively. This means that logistic regression is good at identifying positive cases (high recall) but has a poor overall balance and precision. The Random Forest model achieves very good results, as all the measures (accuracy, precision, recall, and F1-score) are close to 100. It is an indication that this model tolerates very minimal errors and is also consistent in all measures of performance. The XGBoost also provides almost perfect results, just like the Random Forest. Its measures are nearly all close to 100 percent, indicating that it is very accurate, precise, and reliable.,

CONCLUSION

This study aimed to enhance the detection of liver conditions based on machine learning models trained on the Liver Patient Dataset. The demographic and biochemical characteristics of the dataset themselves constituted a good basis of prediction. The data was standardized using preprocessing, imputation, resampling, and normalization so that models are fairly evaluated. Logistic regression was compared and tested with the random forest as well as the XGBoost based on various measures, including accuracy, precision, recall, F1-score, and ROC. The outcome was significantly different in performance. The simple and comprehensible logistic regression performed at 71.53. It was strongest in recall (95.05%), that is, the ability to recognize most positive cases correctly. The reduced accuracy and ROC did, however, provide some clue of limitations to reliability with an increase in the risk of false positives. Random Forest and XGBoost, on the other hand, performed almost perfectly. Random Forest had the best overall performance of 99.76% accuracy and almost perfect scores on all other measures. This shows that it can carry out interactions between complex characteristics quite effectively and reduce errors in ensemble learning. This was followed by XGBoost with similarly impressive predictive capability and efficiency. Finally, ensemble algorithms like Random Forest and XGBoost can be very useful in medical diagnosis problems such as predicting liver disease. They are superior to the easier models, and they also provide strength and accuracy. Logistic regression can still be used to set some baseline against which to compare results, although the results demonstrate that tree-based competitors provide much more reliable and accurate classifications. This paper identifies how machine learning, particularly ensemble methods, could help medical practitioners in the early and accurate diagnosis of liver diseases, which will eventually benefit patients and decision-making.

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NEW METHODS IN EARLY DETECTION OF SUBCLINICAL MASTITIS IN DAIRY FARMS

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ABSTRACT

Mastitis continues to be one of the most serious issues in dairy farming, causing reduced milk production, lower quality, and considerable financial losses. Among its types, subclinical mastitis is especially challenging because it lacks visible signs while leading to higher somatic cell counts (SCC) and long-term udder damage. Detecting the disease early is critical for limiting its progression, protecting milk yield, and decreasing dependence on antibiotics. In recent years, advancements in biosensing, imaging, molecular diagnostics, and digital technologies have introduced new approaches to improve early detection of subclinical mastitis in dairy herds. Biosensor technologies now allow fast and accurate monitoring of udder health at both the cow and quarter levels. These sensors measure parameters such as electrical conductivity, lactate dehydrogenase (LDH), and milk pH, all of which reflect infection and inflammation. When integrated into milking systems, online biosensors provide continuous data, allowing abnormal milk to be identified immediately. Compared with traditional sampling, these systems enable more frequent monitoring with minimal extra labor. In addition, molecular diagnostic techniques such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) make it possible to quickly identify mastitis pathogens directly from milk. They can detect bacterial DNA even at very low levels, making them highly effective in subclinical cases. Immunological tests that measure acute-phase proteins (such as haptoglobin and serum amyloid A) or other mastitis biomarkers have also shown promise, helping distinguish between contagious and environmental mastitis and enabling more targeted treatments. Imaging methods are also gaining importance in surveillance. Infrared thermography can reveal small increases in udder surface temperature that suggest inflammation before outward signs occur, while ultrasonography can detect early structural changes in mammary tissue. These non-invasive techniques are well-suited to repeated use and can be applied across entire herds for screening purposes. Furthermore, advances in omics-based technologies have created new opportunities for detecting subclinical mastitis at the molecular level. Proteomic and metabolomic profiling of milk identifies early biochemical changes linked to infection, often before SCC levels rise significantly. These methods enhance sensitivity while providing insight into the biological processes of the disease, supporting more precise treatment strategies. Finally, modern herd management platforms increasingly use cloud-based systems that integrate SCC measurements, sensor readings, genetic information, and behavioral data into unified dashboards. With the help of machine learning, these platforms generate real-time alerts, assess risks, and recommend preventive measures. By moving from reactive responses to predictive control, such systems reduce the frequency of severe infections and their economic impact. In conclusion, the early identification of subclinical mastitis has been revolutionized by innovative methods that surpass conventional testing. Tools such as biosensors, molecular assays, imaging techniques, wearable technologies, and integrated digital platforms give farmers the ability to detect infections before clinical signs appear. These innovations support timely intervention, improve milk quality, safeguard animal health, and lessen both economic and environmental burdens. As technology advances further, combining precision tools with

artificial intelligence and digital farm management will become essential for developing more sustainable, profitable, and resilient dairy operations.

Keywords: Subclinical Mastitis, Dairy Farms, somatic cell counts.

ARTIFICIAL INTELLIGENCE–DRIVEN SURVEY TECHNIQUES FOR DECREASING TOTAL SOMATIC CELL COUNT IN DAIRY FARMS

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ABSTRACT

Milk quality is fundamental to the dairy sector, with somatic cell count (SCC) serving as the primary marker of udder health and mastitis in cows. High SCC levels are closely associated with mastitis, decreased milk production, and significant financial losses. Traditionally, SCC has been monitored through routine lab tests and clinical evaluations, but these methods tend to be reactive rather than preventive. Recently, artificial intelligence (AI) has emerged as a powerful tool for SCC surveillance and management, offering predictive, real-time, and data-driven approaches to support dairy operations. AI has facilitated the creation of intelligent monitoring systems that merge data from milking machines, milk analyzers, and sensors. Using machine learning, these systems can interpret milk conductivity, color, temperature, and yield to accurately predict SCC levels. Unlike conventional techniques, AI-based monitoring can identify subtle signals of subclinical mastitis early, allowing farmers to intervene before SCC levels escalate. A major strength of AI lies in its predictive ability. By analyzing historical herd information, AI models can anticipate which cows are most likely to develop mastitis or elevated SCC. These models account for factors such as genetics, stage of lactation, previous SCC trends, and milking patterns. This enables farmers to implement preventive strategies, including modifying milking practices, optimizing nutrition, or providing targeted veterinary care, thereby reducing unnecessary antibiotic use. Moreover, thermal imaging and digital cameras can reveal udder inflammation or abnormal behavior linked to mastitis. AI algorithms process these images automatically, identifying cases for closer examination. Wearable sensors, when integrated with AI, also track activity, feeding, and rumination changes that may indicate rising SCC. Such technologies provide continuous, non-invasive monitoring, improving both animal welfare and milk quality. AI-based herd management platforms consolidate varied data sources—including SCC measurements, sensor outputs, genetic data, and environmental information—into unified dashboards. Through deep learning, these platforms refine accuracy over time and deliver actionable insights to farmers. Additionally, AI-powered decision support systems generate tailored recommendations, not only identifying cows with high SCC but also suggesting corrective measures such as selective dry cow therapy, improved bedding practices, or enhanced milking hygiene. Unlike conventional surveys that simply report SCC levels, AI systems provide proactive guidance for more effective herd management. In summary, AI has revolutionized the monitoring and reduction of SCC in dairy farms. By combining advanced monitoring, predictive modeling, image-based detection, and big data integration, AI enables a shift from reactive to preventive herd management. These advances improve milk quality, lower economic losses, reduce antibiotic dependence, and support sustainable farming while safeguarding animal health. As AI continues to progress, its role in SCC control will become increasingly vital, driving the future of smarter, healthier, and more productive dairy farms.

Keywords: Somatic Cell Count, Dairy Farms, Artificial Intelligence.

**HISTOLOGICAL AND BIOCHEMICAL EVALUATION OF 2-(2-NITROPHENYL)-
1,3-THIAZOLIDINE-4-CARBOXYLIC ACID ON BLOOD PROFILE AND HEART
TISSUES IN ROTENONE INDUCED PD RAT MODEL**

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ABSTRACT

Parkinson disease is extrapyramidal disorder showing non classical symptoms as cardiac dysfunction and disrupting level of hematological and serological parameters by sharing a common phenomenon of oxidative stress. Study was framed to show the protective effect of 2-(2-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid against rotenone affected blood profile and cardiac tissues in Parkinson disease rat model. For designing this experimental framework, 42 male rats were selected and treated with 2.5 mg / kg rotenone and high (30 mg/kg) and low dose (10 mg/kg) of 2-(2-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid for 21 days via intra-peritoneal mode of injection. Sample collection of blood (for hematology and serology analysis) and heart (for oxidative stress test and histopathology) was done. Findings revealed that 2-(2-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid improved rotenone-induced alterations in hematological and serological parameters. Moreover, this treatment drug showed antioxidant potential by alleviating the MDA level and boosting the SOD and CAT level in cardiac tissues of rats as well as restored aberration in histological architecture of the heart tissues. So, this exploration will paved the path for drug discovery to improve the quality of life of patients of PD, also suffering abnormalities in blood parameters and cardiac dysfunctions.

Keywords: Hematology, Serology, Oxidative stress, Parkinson disease, Rotenone, Cardiac dysfunction

DIGITAL TECHNOLOGIES IN MONITORING ANIMAL HEALTH AND ENVIRONMENTAL POLLUTION: A CASE STUDY IN KOSOVO

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ABSTRACT

Introduction and Purpose: Environmental pollution has increasingly affected animal health in rural areas of Kosovo. This study aimed to evaluate how digital technologies can be applied for monitoring environmental pollution and its impact on animal health.

Materials and Methods: Environmental sensors, GPS chips, and mobile veterinary monitoring applications were installed on selected farms. Laboratory analyses of pollutants, including nitrates, heavy metals, and pathogenic microorganisms, were performed. Semi-structured interviews with local veterinarians and farmers were conducted to assess practical challenges and experiences with technology use.

Results: The study demonstrated a clear correlation between environmental pollution and higher incidence of animal diseases, particularly in areas with contaminated water. Digital tools enabled real-time monitoring, early risk detection, faster interventions, and reduced economic losses.

Discussion and Conclusion: Integration of digital technologies into veterinary monitoring and environmental protection systems in Kosovo can enhance animal health, support biodiversity preservation, and ensure long-term food security.

Keywords: digital technologies, environmental pollution, animal health, sensors, Kosovo, monitoring, veterinary science

**JUVENILE GULSHA TENGRA (MYSTUS CAVASIUS) EXHIBITED BETTER
GROWTH PERFORMANCE IN PROBIOTIC TREATMENT BY ENHANCING
INTESTINAL MORPHOLOGY**

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ABSTRACT

Introduction and Purpose: Probiotics, particularly *Bacillus*-based formulations, are increasingly used in aquaculture to enhance fish growth. However, their water application methods and optimal dosing for indigenous species like Gulsha Tengra (*Mystus cavasius*) remain poorly understood. This study evaluated the effects of water-based probiotics on the growth performance and intestinal morphology of *M. cavasius*.

Materials and Methods: Four treatments were administered: T1 (Control; 0 mg/L *B. subtilis*), T2 (1 mg/L *B. subtilis*), T3 (5 mg/L *B. subtilis*), and T4 (10 mg/L *B. subtilis*) for 60 days. Growth parameters such as weight gain, feed conversion ratio, specific growth rate, and survival rate were studied. Besides changes in intestinal morphology (villus height, goblet cells) were also observed.

Results: Results demonstrated that T3 showed significantly better performance compared to others by exhibiting the highest weight gain (WG), specific growth rate (SGR), and the lowest feed conversion ratio (FCR). Moreover, the intestinal morphology was also improved significantly in T3.

Discussion and Conclusion: These findings demonstrate that water-applied *B. subtilis* probiotic at 5 mg/L significantly enhances growth performance and intestinal health in *M. cavasius*, providing a practical protocol for small-scale farmers.

Keywords: Probiotics, intestinal morphology, aquaculture, *Mystus cavasius*

**EVALUATING THE GROWTH-ENHANCING EFFECTS OF LAPSI FRUIT
(*Choerospondias axillaris*) AS A DIETARY SUPPLEMENT FOR RAINBOW TROUT
(*Oncorhynchus mykiss*)**

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ABSTRACT

The global aquaculture industry is grappling with the economic and ecological downsides of heavily depending on conventional fishmeal as a protein source for carnivorous fish like rainbow trout (*Oncorhynchus mykiss*). This has spurred considerable research into alternative feed sources and discovered that incorporating plant-derived components into fish diets can be a more affordable and environmentally sound approach. Moreover, these plant-based diets show potential for boosting growth, strengthening immunity and warding off diseases in fish. Therefore, using plant products in fish feed offers a practical way to reduce or eliminate the reliance on fishmeal, ultimately leading to better results in aquaculture. Given that Lapsi fruit pulp is rich in beneficial compounds like flavonoids, chlorophylls, carotenoids and their derivatives, proteins, key minerals such as iron, organic acids, essential oils, and vitamins, they hold potential as a sustainable plant-based supplement in fish feed. This research focuses on the effects of supplementing rainbow trout diets with LFP extracts prepared on different solvents as supplement diets on their growth performance. Among the LFP extract, it is found to produce better results with 80% methanol.

KeyWords: Aquaculture; Traditional Fishmeal; Plant-based Ingredients; Lapsi Fruit Pulp

Introduction and Purpose: According to the UN World Population Prospect (2024), the global population will be approximately 10.3 billion by the mid-2080s, compared to 8.2 billion in 2024 (United Nations, 2024). This growing population can cause food related issues throughout the world. As fish is an important source of food that provides nutrition (particularly protein) for humans, fish production has to be increased in order to ensure food security and to maintain a continuous supply of nutritious food. In aquaculture production, fish feed is the most expensive input because of using animal protein sources like fishmeal, fish oil and shrimp meal. However, its gradual increase in price and restricted availability has challenged the aqua-feed industry. To ensure the sustainable development of aquaculture, if plant sources can be used to supplement animal protein sources, not only will production costs be reduced, but growth and production will also increase with low side effects to the fish and consumer. Recent studies have found lapsi fruit with rich in antioxidant compounds such as phenolic, flavonoids, DPPH and amino acid (Paudel et al., 2002) and its inclusion in the diet allows it to prove beneficial to the health sector (Paudel and Parajuli, 1999).

Similarly, lapsi fruits are locally available at an affordable price as it is indigenous sps. Hence, an effort has been made to examine the impact of Lapsi (*Choerospondias axillaris*) on the growth of Rainbow trout fish.

Materials and Methods: 160 Rainbow trout larvae (50 days old age) were cultured for 90 days at Trout Village Resort and Farm in Chisapani, Melamchi in Sindhupalchowk district of Bagmati Province of Nepal (Labh et al., 2014). These fish were cultured in outdoor natural conditions in a cemented raceway supplied with clean and cold water obtained from the stream which was perennial in order to find out the impacts of different supplement diets prepared from extract of lapsi fruit in different solvents on growth performance and RNA:DNA ratio of the fish. In this experiment, there were four treatments; hence fishes were divided into four groups at the rate of 40 trout in each cage (Labh et al., 2014). Altogether four different cages (C1, C2, C3 and C4) of dimension 50cm × 50cm × 50cm were used to conduct the experiment. In total, four experimental diets (with 40% protein) were prepared using standard calculations (Shakya et al., 2016). Diet (D1) was a control diet with no lapsi pulp (0.0 g kg⁻¹), while the other three diets D2, D3 and D4 incorporated with lapsi pulp (0.4 g kg⁻¹) prepared in different solvents; 80% methanol (Cai et al., 2004), distilled water (Fawole et al., 2023) and 95% ethyl acetate (Ghaima et al., 2013)respectively. Other ingredients as per the standard norm of Fish Nutrition Lab, CIFE, Mumbai (Labh et al., 2017). Diet (D1) was used for Cage C1, Diet (D2) was used for Cage C2, Diet (D3) was used for Cage C3 and Diet (D4) was used for Cage C4. The daily feed was divided into two equal parts and was fed twice daily at the rate of 3% of their body weight, once in the morning (9 a.m.) and next in the afternoon (4 p.m.). Five fishes from each cage were randomly weighed and measured individual fish length (cm) using digital balance and a measuring scale respectively in every fifteen days to adjust feeding rate. Water temperature, pH and dissolved oxygen were monitored weekly by standard method. Growth performance like Weight gain % and Specific growth rate were assessed (Labh et al., 2014). For biochemical estimations, samples were carried to the laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal for RNA and DNA assays. Liver samples were used for extraction of DNA and RNA using Wizard Genomic DNA Purification Kit and FavorPrep Viral DNA/RNA Kit respectively, followed by estimation of DNA and RNA by using a microplate spectrophotometer (Mehraj et al.,2025).

Results: There was no significant ($P>0.05$) difference in the average wet weight of the rainbow trout fed different diets (D1, D2, D3 and D4) at the beginning of the study but after 90 days of feeding trials, the final average weight was significantly ($P<0.05$) higher in the trout fed with D2 diet followed by D4, D3 and D1diets fed fish. Consequently, specific growth rate (SGR) increased by 48.35% in the D2 diet fed trout as compared to control diet D1 fed trout. In case of DNA, there was no significant ($P>0.05$) difference in average DNA content among trout larvae fed with different diets from 1st day to 90th day. Average DNA content of trout larvae was 2.93 ug/ mg on the 15th day of experiment while the average DNA content of trout larvae increased effects of Lapsi fruit as a supplement diet on survival rate and growth performance of rainbow trout at the end of feeding trials. There was no mortality at the end of the experiment in all the groups. Highest WG, WG% and SGR was observed in rainbow trout fed with the diet D3 and were followed by D2 and D4. The reverse trend for the FCR was noticed and lowest FCR ($p<0.05$) was recorded in the diet D3 fed group followed by D2 and D4.

Discussion and Conclusion: Trout larvae fed with lapsi pulp extraction from 80% methanol showed better growth performance as compared to fish larvae fed with lapsi pulp extraction from 95% ethyl acetate, distilled water and controlled diet.

Similarly, the biochemical indices i.e. RNA: DNA ratio of fish fed with lapsi pult extraction from 80% methanol were found to be better as compared to fish fed with other diets. Hence, based on these results, we can conclude that lapsi fruit extract prepared in 80% methanol is found to be a potential supplementary fish diets.

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INTENSIVE AQUACULTURE AND CLIMATE WARMING: DRIVERS OF GENETIC DIVERSITY IN MEDITERRANEAN MONOGENEANS

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ABSTRACT

Introduction and Purpose: Intensive aquaculture increasingly pressures marine ecosystems by creating dense host populations that facilitate parasite transmission and persistence. These effects are further amplified by climate warming, which reshapes host–parasite interactions and likely affects the genetic diversity of monogenean parasites in the Mediterranean Sea. This interaction is particularly evident in species infecting gilthead seabream (*Sparus aurata*), such as *Sparicotyle chrysophrii* and *Lamellodiscus echeneis*, both responsible for severe disease outbreaks in aquaculture.

Materials and Methods: Parasites were collected from the gills of 356 gilthead seabream from various Mediterranean localities. DNA was extracted, and PCRs were performed using BentoLab. The PCR products were sequenced at Macrogen Europe, and sequences were verified and aligned in MEGA X (CLUSTALW). Genetic diversity was assessed with DnaSP v.5.10.01, haplotype networks were constructed in PopArt (MJ algorithm), and AMOVA was conducted in Arlequin 3.5.

Results: Multilocus molecular analyses, integrating mitochondrial (COI) and nuclear markers (28S rDNA, ITS) across several Mediterranean localities, have revealed clear patterns in population structure. Both *S. chrysophrii* and *L. echeneis* exhibit high haplotype diversity ($H_d = 0.829–0.918$) alongside low nucleotide diversity ($\pi = 0.00516–0.019$), star-like haplotype networks, and negative neutrality test values, features consistent with recent demographic expansions.

Discussion and Conclusion: These expansions appear to be driven not only by aquaculture practices, which enhance parasite transmission between wild and farmed populations, but also by rising sea temperatures that influence host distribution and facilitate parasite dispersal. The absence of strong phylogenetic divergence among Mediterranean populations, coupled with shared haplotypes across host types, indicates ongoing gene flow maintained by both aquaculture and natural host movements. Together, these findings highlight the synergistic impact of anthropogenic pressures, particularly intensive aquaculture, and climate change on the evolutionary dynamics of Mediterranean monogeneans. Understanding these processes is crucial for anticipating parasite emergence, assessing disease risks, and developing sustainable management strategies under changing environmental conditions.

Keywords: Intensive aquaculture, Climate warming, Genetic diversity, Monogenean parasites, Mediterranean, Gene flow, Demographic expansion, Sustainable management

TABA HEAL CAT A NOVEL APPROACH FOR STRAY CAT HEALTH AND ENVIRONMENTAL PROTECTION

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ABSTRACT

The specialty of pharmacy is not limited to humans only but aims to provide care and treatment for all living organisms. Many animals, especially stray cats, have no one to care for or adopt them, and are unable to express their pain and need for treatment, which makes them vulnerable to various diseases and viruses. Based on this reality, TABA Heal Cat was proposed. It is a product in the form of pills, nutritional chips, or easy-to-eat cans, designed for cats. It combines essential nutrients and therapeutic compounds to combat colds, viruses, and germs. This product aims to provide a complete nutritional treatment for stray cats, so that it can be easily eaten and the animal benefits from both food and treatment at the same time. Cats were chosen because of their widespread presence in the streets and homes, where many people feed them out of compassion and kindness, by proposing TABA Heal Cat, product distribution campaigns can be launched, which contributes to improving the health of cats, maintaining their cleanliness and vitality, and thus enhancing the environment around them. This proposal represents a unique combination of food and medicine, embodying the humanitarian role of pharmacy in protecting living beings unable to express their health needs, and paving the way for the application of the name TABA as a comprehensive medical brand for both humans and animals.

Keywords: TABA Heal Cat, Environmental protection, processed cat food, Human and environmental safety, veterinary care, veterinary

INTRODUCTION

As interactions between humans and stray cats increase, diseases common to humans and cats have become a significant health challenge. These include bacterial infections such as cat - scratch disease, parasitic infections such as toxoplasmosis, and fungal infections such as ringworm. These diseases can cause various health problems in humans, ranging from mild flu-like symptoms to serious complications, especially in vulnerable groups such as children, pregnant women, and people with weakened immune systems. Despite awareness campaigns and veterinary care programs, many stray cats remain untreated, leaving people who handle them constantly at risk of infection. Hence, the need for innovative solutions that reduce the transmission of microbes from cats to humans. Therefore, this research proposes an innovative product called TABA Heal Cat, a therapeutic food combining nutritional and medicinal elements. It aims to strengthen the immune system of stray cats and reduce the microbial load of harmful bacteria, fungi, parasites, and viruses. By improving cat health, this proposed product indirectly contributes to protecting human health and reducing the spread of common diseases.

Research objectives:

- Reducing the transmission of common diseases from stray cats to humans by reducing the microbial load (bacterial, fungal, parasitic, and viral) in cats.
- Enhancing the immunity of stray cats by combining fortifying nutrients with safe pharmaceutical ingredients that help combat germs and viruses.

- Providing an innovative and environmentally friendly solution based on easy-to-use therapeutic nutrition that contributes to improving the health of cats and thus protecting the general health of society.

Significance of the research:

- Protecting public health: Cats are an integral part of the urban environment and have the right to receive appropriate healthcare, not only to protect them but also to protect human health from common diseases.
- Controlling common diseases: The proposed product contributes to reducing the possibility of transmission of bacteria, fungi, parasites, and viruses from cats to humans.
- Improving animal welfare: Provides stray cats with food enriched with therapeutic compounds, which helps boost their immunity and reduce their suffering from diseases.
- Practical and applicable innovation: The proposed product combines food and medicine in an innovative way, making it an easy and safe way to deliver treatment without the need for complex medical interventions.
- Serving the community and the environment: Reducing diseases among stray cats positively impacts the health of neighborhoods and promotes a balance between humans and animals.
- This proposal helps stakeholders, such as municipalities and environmental protection centers, adopt practical and innovative solutions to reduce health risks and promote a balance between human and animal health.

DEFINITION OF DISEASES COMMON TO CATS AND HUMANS

It is a group of diseases that can be transmitted from cats to humans directly or indirectly. They are scientifically known as zoonotic diseases. These diseases vary between (a-parasites, b-bacterial diseases, c-fungal infections, and viruses) (Aleem, Munir, & Shakoor, 2024).

a-Parasites

Cats are susceptible to infection by a number of internal and external parasites, which can be transmitted to humans through mucus or contact with a contaminated environment. The most important of these parasites is toxoplasmosis (Adem & Ame, 2023).

Toxoplasmosis: is a parasitic disease caused by the *Toxoplasma gondii* parasite, one of the most widespread parasites in the world. This parasite reproduces in the intestines of cats after eating infected animals such as rats or birds, and the toxoplasma eggs are then shed in their feces, contaminating the environment (Adem & Ame, 2023).

Methods of infection:

- Contact with infected cat feces, contaminated litter boxes, or contaminated soil.
- Transmission from an infected pregnant mother to her fetus via the placenta (Frenkel & Dubey, 1972)
- Eating contaminated vegetables or fruits that were not washed well. See Figure 1
- Consuming undercooked meat. See Figure 2



Figure 1.



Figure 2.

Complications:

- In pregnant women: May cause miscarriage or birth defects.
- In immunocompromised individuals: May lead to serious infections of the brain or eyes (Frenkel & Dubey, 1972).

Symptoms:

Fever, swollen lymph nodes, general fatigue (Frenkel & Dubey, 1972).

b-Bacterial diseases

Are diseases caused by infection of the body with disease-causing bacteria. These bacteria multiply within the host and secrete harmful substances that lead to the onset of symptoms. These bacteria are transmitted through several routes, including direct contact with infected animals, bites or scratches, or through contaminated food and water. The most important bacterial diseases include:

1. Cat scratch disease
2. Plague
3. Salmonellosis (Wear et al., 1983).

1. Cat scratch disease: It is a bacterial disease caused by *Bartonella henselae*, which is usually transmitted from infected cats to humans through scratching or licking by cats (Klotz, Lanas, & Elliott, 2011).

Methods of Transmission:

- The infection is transmitted through scratching or licking from infected cats.
- The bacteria can also be transmitted if a person touches contaminated cat feces and then touches their mouth or a wound in the skin (Nelson, Saha, & Mead, 2016).

Symptoms:

- Swollen lymph nodes near the site of the scratch or lick.
- Mild fever or general fatigue.
- In some cases, a rash or muscle pain may appear.
- Eye inflammation (Biancardi & Curi, 2014).

2.Plague

Plague is a highly contagious bacterial disease caused by the bacterium *Yersinia pestis*. Cats can become infected through exposure to the bite of an infected flea, and it can be transmitted to humans through contact with infected cats or exposure to the spray and saliva of an infected cat (Orloski & Lathrop, 2003; Hunter, 1905; Gasper et al., 1993).

3.Salmonellosis

A bacterial disease that affects humans. *Salmonella* bacteria are most common among cats that eat raw meat, wild birds, or other animals (Carter & Quinn, 2000).

Symptoms:

- Diarrhea
- Fever
- Stomach pain (Fauth et al., 2015).

c-Fungal infections

These are diseases caused by fungi, which are microscopic organisms that differ from bacteria and viruses in that they possess eukaryotic cells. For example, ringworm (Sykes, 2020).

Methods of transmission:

- Direct contact with infected cats
- Touching the infected cat's tools or bedding
- A person touching the infected cat's skin (Lavelly & Lipsitz, 2005).

Symptoms:

- -Round, red spots on the skin
- Severe itching
- Peeling skin in the affected area
- Small blisters or ulcers (Lavelly & Lipsitz, 2005).

PREVIOUS STUDIES

1-Bhagavathi sundarm sivamaruthi et al. (2025): A Review of Probiotic Supplementation and Its Impact on the health and well-Being of Domestic Cats.

Published in Veterinary Sciences, MDPI, VOL.12, Issue 8, Article 703.

The study reviewed the effect of probiotic supplementation on the health and welfare of domestic cats. It found that probiotics can enhance the immune system, reduce inflammation, and support gastrointestinal health. The study also indicated that probiotic supplements are safe for both adult and young cats, while emphasizing the need for further long-term research to determine the most effective types of probiotics and appropriate dosages.

2-(Cunningham et al.,2022): Evaluation of a nutritional supplement for the alleviation of pain associated with feline degenerative joint disease: a prospective, randomized stratified, double blind placebo-controlled clinical trial.

This study, conducted in 2022, aimed to evaluate the effectiveness of a nutritional supplement in alleviating pain associated with feline degenerative joint disease. The researchers used a prospective, randomized, double-blind, placebo-controlled clinical trial design. The results showed that the nutritional supplement significantly reduced pain and improved comfort in cats with degenerative joint disease compared to the control group.

SUGGESTED PRODUCT: (TABA HEAL CAT)

It is a nutritional and therapeutic proposal at once, specifically suggested for stray cats to maintain their health, protect the environment, and help reduce diseases that may be transmitted to humans.

The name TABA is derived from the first four letters of the name of the idea owner, Tabarek.

TABA Heal Cat Form:

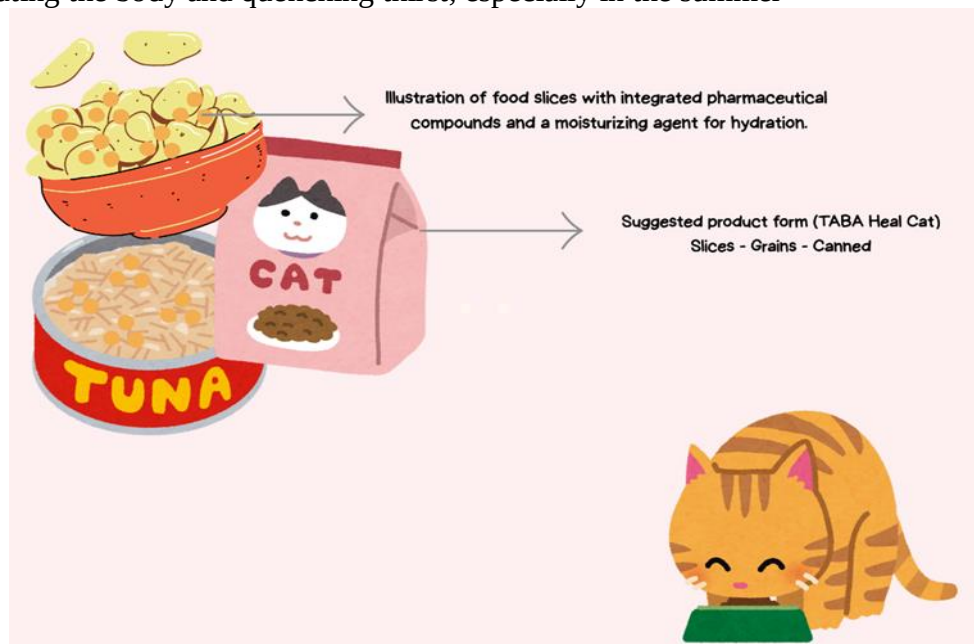
Pills, Flakes, or Canned

-Suggested TABA Heal Cat product ingredients:

Nutritional, therapeutic ingredients, and moisturizing factor.

The proportions of these ingredients are carefully studied by doctors, pharmacists, and nutrition experts, which contribute to:

- Supporting the immune system
- Improving digestion
- Preventing certain infections
- Helping fight colds, viruses, germs, and infectious diseases
- Hydrating the body and quenching thirst, especially in the summer



-How to use TABA Heal Cat:

- **Direct application:** The product can be placed in clean containers in areas where cats regularly frequent.
- **Distribution through volunteer campaigns:** Through veterinary associations, environmental protection centers, or municipalities, the product can be delivered directly to cats in need, especially during harsh seasons such as winter and summer, or during disease outbreaks to protect the most vulnerable cats.
- **Preventive and natural use:** It can be administered daily or as needed to support cats' health and strengthen their immunity
- **Education and awareness:** Guide workers or volunteers on the correct way to administer the product to ensure maximum benefit without disturbing the animals.

RECOMMENDATIONS

- a. Field trials: Conduct practical tests of the product on groups of stray cats to ensure its effectiveness and safety.
- b. Institutional collaboration: Coordinate with municipalities, veterinary associations, and environmental protection centers to adopt the idea and expand distribution.
- c. Continuous development: Improve the nutritional and medicinal components of the product based on trial results and feedback.
- d. Community awareness: Organize awareness campaigns to educate people about the importance of caring for stray cats and the role of the product in reducing zoonotic diseases.
- e. Environmental sustainability: Use eco-friendly packaging and ensure that the nutritional ingredients are safe and healthy while maintaining the effectiveness of the medicinal components.
- f. Research support: Encourage researchers and students to study the product's impact and develop similar ideas to help other animals.

CONCLUSION

Is an innovative step that combines food and medicine to care for stray cats and reduce zoonotic diseases, Adopting and developing this idea will not only improve animal welfare, but also promote community health and protect the environment.

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EVIDENCE-GROUNDED SENTIMENT GRAPHS FOR BIOCHEMICAL HYPOTHESES ACROSS TEXT, SPECTRA, AND IMAGING

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ABSTRACT

Introduction: Textual statements, spectroscopy profiles (e.g., NMR, MS), and microscopy or gel imaging are examples of fragmented evidence that biochemistry frequently uses to collect sentiment around entities (targets, pathways, substances). When instrumental readouts contradict narrative claims, pure text feeling may misrepresent community attitude.

Methods: We present Evidence-Grounded Sentiment Graphs (EGSG), which represent biochemical entities as nodes and modality-specific evidence as typed edges. These metrics are derived from imaging (colocalization, band intensity, morphology), spectral signatures (peak patterns, matching scores), and text-claims (assertion polarity, certainty). Modality-weighted signals are propagated by a graph neural network to calculate edge-level stance consistency and node-level sentiment. While a contradiction detector highlights claim–evidence discrepancies for curator evaluation, a reliability adapter uses learned quality priors to downweight low-quality spectra or pictures. Figure-callouts connected to instruments, consensus generated from citation networks, and remote supervision from section headings (Results vs. Discussion) are all used in training.

CROSS-DOMAIN MULTIMODAL SENTIMENT ANALYSIS FOR BIOCHEMICAL LITERATURE, SEQUENCES, AND ASSAY DATA

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ABSTRACT

Natural language articles, protein/nucleic acid sequences, 2D/3D molecular structures, and high-throughput assay readouts are some of the diverse modalities produced by biochemical research. These modalities all convey signals regarding the author's stance (optimistic, skeptical, or neutral) and the sentiment of the community toward hypotheses, targets, or compounds. Text is the primary emphasis of current biomedical sentiment mining, which ignores organized experimental methods that can support or contradict narrative tone.

Methods: Scientific text encoders for claim/sentiment cues (hedging, polarity, certainty), sequence/structure encoders for biomolecular features (e.g., transformer embeddings of proteins/ligands and graph embeddings of 3D complexes), and assay-aware encoders for quantitative evidence (effect size, dose–response, reproducibility indicators) are all integrated into our proposed multimodal fusion framework. While a stance-calibration head resolves conflicts (e.g., positive narrative with marginal assay effects), a cross-modal alignment module links textual claims with corresponding experimental evidence and molecular context using contrastive objectives. Lexical uncertainty markers, citation contexts, and outcome labels taken from table and figure captions are used to determine weak supervision.

**INTEGRATED COMPUTATIONAL APPROACH FOR DESIGNING NOVEL
PYRIDONE-BASED α -GLUCOSIDASE INHIBITORS: INSIGHTS FROM 2D-QSAR,
MOLECULAR DOCKING, MOLECULAR DYNAMICS SIMULATIONS AND MM-
GBSA ANALYSIS**

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ABSTRACT

Introduction and Purpose: Alpha-glucosidase inhibitors are essential for managing type 2 diabetes by delaying carbohydrate absorption and controlling postprandial blood glucose levels. This study investigates pyridones derivatives as potential alpha-glucosidase inhibitors using an in silico approach. The studied compounds with their values of IC₅₀ against alpha glucosidase were retrieved from previous paper (Saleem et al. 2022). The objectives are to (1) develop a predictive QSAR model, (2) validate the model through statistical metrics, and (3) evaluate the binding affinity, pharmacokinetics, and stability of proposed compounds via molecular docking, ADMET analysis, and molecular dynamics simulations.

Materials and Methods: By leveraging computational tools, the study aims to optimize these derivatives as promising therapeutic agents for type 2 diabetes. The molecular descriptors were computed using Chem3D, Chems sketch and Gaussian. Principal Component Analysis was applied for dimensionality reduction, and a Multiple Linear Regression (MLR) model was developed to predict bioactivity.

Results: The model underwent rigorous validation, and seven novel compounds were proposed for further investigation. Molecular docking studies revealed promising binding interactions with alpha-glucosidase, while ADMET analysis indicated favorable pharmacokinetic profiles. Molecular dynamics simulations confirmed the stability of the enzyme-inhibitor complexes. The MLR model demonstrated high predictive accuracy, with proposed compounds exhibiting pIC₅₀ values higher than the most active molecule in the series.

Discussion and Conclusion: This study identifies seven promising alpha-glucosidase inhibitors, offering a robust in silico framework for the design of therapeutic agents targeting alpha-glucosidase in type 2 diabetes.

Keywords: Alpha-glucosidase inhibitors, pyridones, QSAR, Molecular Docking, ADMET, Molecular Dynamics.

ADVANCES IN PHARMACOTHERAPY FOR CANCER TREATMENT

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ABSTRACT

Cancer remains one of the most challenging diseases to treat, owing to its heterogeneity, complex pathophysiology, and potential for resistance. Pharmacotherapy has undergone remarkable advances in recent decades, shifting from traditional cytotoxic agents to more sophisticated, targeted, and patient-specific strategies. Conventional chemotherapy and radiotherapy, while effective in reducing tumor burden, often cause systemic toxicity and adverse effects due to non-selective action on healthy cells. The emergence of targeted therapies, including tyrosine kinase inhibitors and monoclonal antibodies, has transformed cancer management by selectively interfering with molecular pathways critical for tumor survival and progression. Immunotherapies, such as immune checkpoint inhibitors and CAR-T cell therapies, have further revolutionized outcomes by harnessing the body's immune system against malignant cells. In addition, pharmacogenomics has enabled personalized cancer treatment, tailoring therapy to the genetic profile of tumors and patients, thereby enhancing efficacy and minimizing toxicity. Advances in nanotechnology-based drug delivery systems also improve precision, prolong circulation time, and reduce adverse effects. Despite these breakthroughs, challenges such as drug resistance, high costs, and unequal access persist, requiring ongoing research and global health initiatives. Future pharmacotherapeutic approaches aim to integrate multi-modal treatments, improve biomarkers for early detection, and enhance safety profiles. Collectively, these innovations signify a paradigm shift in oncology, offering improved survival rates, better quality of life, and hope for long-term disease remission.

Keywords: Cancer, pharmacotherapy, targeted therapy, immunotherapy, pharmacogenomics, nanotechnology

OXIDATIVE STRESS AND ITS IMPACT ON HUMAN HEALTH

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ABSTRACT

Oxidative stress refers to the imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, leading to cellular and molecular damage. Under physiological conditions, ROS play essential roles in signaling pathways, immune defense, and cellular metabolism. However, excessive accumulation of ROS results in lipid peroxidation, protein denaturation, and DNA damage, which contribute to the pathogenesis of various diseases. Oxidative stress has been strongly implicated in cardiovascular disorders, neurodegenerative diseases such as Alzheimer's and Parkinson's, diabetes mellitus, chronic inflammation, and cancer. Additionally, it accelerates the aging process by impairing cellular function and tissue regeneration. The body employs enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase, along with non-enzymatic antioxidants such as vitamins C, E, and carotenoids, to counteract oxidative damage. Lifestyle factors including poor diet, smoking, alcohol consumption, and exposure to environmental toxins further exacerbate oxidative stress.

Recent research emphasizes the therapeutic potential of antioxidant supplementation, dietary modification, and lifestyle interventions in reducing oxidative damage and preventing disease progression. Understanding the biochemical basis of oxidative stress provides valuable insights into disease mechanisms and aids in the development of targeted therapies. Hence, oxidative stress serves as a crucial link between biochemical imbalance and human health, making it a significant focus of clinical and biomedical research.

Keywords: Oxidative stress, Reactive oxygen species (ROS), Antioxidants, Free radicals, Aging, Neurodegenerative diseases, Cardiovascular disorders, DNA damage, Inflammation.

**SUSTAINABLE POLYPROPYLENE COMPOSITES: PHYSICO-CHEMICAL
INSIGHTS INTO INJECTION-MOLDED MATERIALS WITH SPENT COFFEE
GROUNDS**

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ABSTRACT

This study examines the use of spent coffee grounds (SCGs) as a biofiller in polypropylene (PP) composites manufactured by injection molding, aiming to promote sustainable alternatives to conventional plastics. Composites containing 1%, 5%, 7%, and 10% w/w SCG were characterized in terms of processability, thermal stability, mechanical behavior, and morphology. Optical microscopy (OM) and Fourier-transform infrared spectroscopy (FT-IR) revealed noticeable morphological and structural changes due to SCG incorporation. Thermal analyses (DSC and TGA) showed enhanced thermal stability with increasing filler content. Mechanical tests indicated improved hardness and tensile modulus, almost constant tensile strength, but decreased toughness and elongation at break. Melt flow index (MFI) measurements confirmed suitable processing properties. Overall, PP/SCG composites combine promising mechanical and thermal performance with sustainability benefits, supporting their potential as eco-friendly alternatives to conventional polymeric materials.

Keywords: composites; particles; polypropylene; spent coffee grounds; injection molding; biofiller.

POLLUTANTS REMOVAL FROM WASTEWATER THROUGH BIOFILM SYSTEM

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ABSTRACT

Effective removal of pollutants from wastewater is crucial for safeguarding human health and the environment. Biofilm systems have emerged as promising and sustainable solutions due to their ability to support a stable and diverse microbial community capable of efficiently degrading contaminants. This study investigates the application of a biofilm system for pollutant removal from wastewater. Water samples were collected from a treatment plant and analyzed initially for pH and chemical compositions. The samples showed detectable levels of Salmonella and E. coli, indicating contamination posing health risks. Initial measurements revealed total dissolved solids (TDS) at 700 mg/L and total hardness at 110 mg/L, both exceeding maximum standards. The water samples were then treated using a biofilm system under varying operational conditions to assess pollutant removal efficiency. Post-treatment analysis showed an increase in pH from 6.61 to 7.5, falling within acceptable limits. Although total hardness levels remained high, TDS decreased over time, albeit remaining elevated. Importantly, no detectable levels of Salmonella or E. coli were found after treatment, demonstrating the biofilm system's effectiveness in eliminating harmful bacteria. However, challenges were observed in reducing total hardness and TDS levels significantly, suggesting the need for complementary treatment methods. This study highlights the importance of pre- and post-treatment water quality monitoring to evaluate the efficacy of biofilm systems in pollutant removal. In conclusion, while effective for bacterial removal, further optimization is necessary to address other pollutant types such as organic compounds, heavy metals, and emerging contaminants across diverse wastewater types.

Keywords: Biofilm, Wastewater treatment, Total dissolved solid, Pollutant removal, Maiduguri

INTRODUCTION

Water is a transparent, odorless, tasteless, and nearly colorless chemical substance that is the main constituent of earth's streams, lakes, oceans, and rain. It is an essential resource for all known forms of life and is the basis for numerous industrial, agricultural, and recreational activities. Water is a simple molecule consisting of two hydrogen atoms and one oxygen atom, chemically represented as H₂O. The physical and chemical properties of water make it unique and vital for life. For example, water has a high specific heat, which means that it can absorb a lot of heat energy without changing temperature, making it an effective temperature regulator in organisms and ecosystems (Zhang et al., 2019). It also has a high surface tension, which allows it to form droplets and capillary networks in plants and animals. Water is abundant on earth, covering approximately 71% of the planet's surface. It is found in various forms, including liquid, solid, and gas, and is constantly cycling through the earth's atmosphere, oceans, and land. The water cycle, also known as the hydrologic cycle, describes the continuous movement of water on, above, and below the earth's surface. In addition to its importance for sustaining life and ecosystems, water plays a critical role in many industrial processes, such as power generation, manufacturing, and agriculture (Cesca et al., 2020). However, the availability and quality of water resources are increasingly becoming a concern due to factors such as climate change, population growth, and pollution. Overall, water is a fundamental substance for life on earth, with important physical, chemical, and ecological properties. Its abundance and importance make it a subject of ongoing scientific research and concern for human societies worldwide. Water pollution is a significant environmental issue that arises from the contamination of water bodies by various pollutants, posing serious threats to human health, ecosystems, and biodiversity. This discussion will explore the causes, effects, and potential solutions to water pollution, drawing on relevant references to support the analysis. Water pollution can stem from a variety of sources, both natural and human-induced. Industrial activities generate a wide range of pollutants, including heavy metals, chemicals, and toxins, which are often discharged into water bodies through wastewater effluents (UNEP, 2019). The use of fertilizers, pesticides, and herbicides in agriculture leads to runoff of pollutants such as nitrogen and phosphorus into rivers, lakes, and streams, causing eutrophication and algal blooms (EPA, 2020). Urban runoff carries pollutants like oil, heavy metals, and plastic debris from roads, parking lots, and rooftops into water bodies through stormwater drains, contaminating them (UNEP, 2019). Improper disposal of solid waste, including plastics, electronics, and household garbage, leads to landfills and dumpsites that leach pollutants into groundwater, contaminating drinking water sources (WHO, 2019).

The impacts of water pollution are far-reaching and can affect both human and ecological systems. Waterborne diseases such as cholera, typhoid, and dysentery can result from the consumption of contaminated water, posing serious health risks to communities (WHO, 2019). Water pollution disrupts aquatic ecosystems, leading to declines in fish populations, loss of biodiversity, and habitat destruction (EPA, 2020). Pollution of water sources can impact industries such as fishing, tourism, and agriculture, leading to economic losses and livelihood disruptions (UNEP, 2019). Wastewater, often generated from domestic, industrial, and agricultural activities, poses significant environmental and public health concerns if not properly managed. Its treatment is crucial to mitigate pollution and protect human health and ecosystems. The composition of wastewater varies widely depending on its source and geographical location. Generally, it contains organic matter, suspended solids, nutrients (nitrogen and phosphorus), pathogens (bacteria, viruses), heavy metals, and various pollutants from industrial and agricultural activities.

Understanding the composition is crucial for designing effective treatment processes (Chen & Snyder 2019). Uncontrolled discharge of untreated or inadequately treated wastewater poses severe environmental impacts. It degrades water quality, leading to eutrophication, oxygen depletion, and habitat destruction in aquatic ecosystems. Nutrient-rich wastewater can promote algal blooms, disrupting the balance of aquatic flora and fauna. Moreover, untreated wastewater can contaminate groundwater, posing risks to human health and ecosystems. Wastewater, often regarded as a product of human activities, encompasses a broad spectrum of water contaminated by various substances, ranging from organic matter to toxic chemicals (Smith & Johnson 2020). Its management is critical for protecting human health, ecosystems, and maintaining environmental sustainability. Wastewater is a complex and multifaceted environmental issue that encompasses a wide range of contaminants and pollutants. It refers to any water that has been used in domestic, industrial, commercial, or agricultural activities and has become contaminated as a result. The management and treatment of wastewater are critical for protecting human health, preserving ecosystems, and ensuring the sustainable use of water resources.

The presence of Salmonella in wastewater poses significant risks to public health and the environment. Salmonella-contaminated wastewater can serve as a vehicle for transmitting the bacteria to humans, leading to potential outbreaks of waterborne diseases (CDC, 2020). Additionally, coliform bacteria, including *E. coli* and other gram-negative bacteria, are indicators of fecal contamination in water sources and can indicate the presence of pathogens that pose health risks (WHO, 2017). Effective wastewater treatment and monitoring strategies are critical to mitigate these risks and safeguard public health and the environment (EPA, 2021). Biofilm systems have shown promise due to their ability to support robust microbial communities capable of degrading various pollutants (Gomez-Suarez et al., 2012). However, the effectiveness of biofilm systems needs to be adaptable across diverse types of wastewaters, including municipal, industrial, and agricultural wastewaters, which vary significantly in composition and pollutant loads (UNEP, 2018). Therefore, developing biofilm systems that can effectively treat diverse pollutants, including organic compounds, heavy metals, and emerging contaminants, in various types of wastewaters is crucial for improving water quality and ensuring environmental sustainability. The aim of this research is to develop and optimize a biofilm-based system for the effective removal of pollutants from wastewater. The objectives of the study are to: characterize the wastewater sample before treatment; evaluate the pollutant removal efficiency of biofilm systems in wastewater treatment under different operational conditions; characterize the wastewater sample after treatment, and; explore strategies for enhancing the activity and stability of biofilms in pollutant degradation. The development of biofilm systems for pollutant removal from wastewater holds profound importance in safeguarding the environment by reducing the discharge of harmful contaminants. Biofilms offer efficient and sustainable treatment options, contributing significantly to the preservation of water resources and the protection of ecosystems (Chen et al., 2020; Li et al., 2018). Therefore, this study contributes to advancing knowledge on biofilm-based wastewater treatment technologies, emphasizing their role in sustainable water management practices and environmental protection.

MATERIALS AND METHOD

This study sets out the detailed description of materials, reagents and procedures employed in this study.

Study Area

The study was conducted in Maiduguri Water Treatment Plan (WTP)., Maiduguri lies between Longitude and Latitude (11.05° N, 13.05°E, and 543km² above mean sea level) in the semi-arid zone of northern Sahel Savannah, Nigeria. (Abdulkareem et al., 2015; Mustapha et al., 2021). The climate of the region is characterized by a cool-dry season (October to March), hot season (April to June) and a rainy season (July to September). The area is fragile and highly susceptible to drought with relative humidity of 13% in dry seasons and 80% in rainy seasons. The area is also highly vulnerable to desertification (Abdulkareem et al., 2015; Mustapha et al., 2021).

Sample Collection

Water sample with altered aesthetic quality were collected from Maiduguri Water Treatment Plan (WTP), the water was pumped continuously from the main tap, hence the assumption that the pipe materials had not affected the sampled water. Samples were taken from the WTP of a particular area and put in clean labeled 5-liter gallon for easy identification. The samples were observed for any color visible to the naked eye and recorded. The samples were stored in the laboratory at temperature of 4°C in the incubator to avoid possible alteration of the original properties of the samples before the commencement of the analyses.

Sample Analysis

Chemical analyses of the sample were carried out using appropriate, certified and acceptable international procedures outlined in the Standard Methods for the Examination of Water. TDS, Total Hardness, Salmonella, E. coli, and pH were analyzed using flame photometric method. The pH of the samples was determined using WTW-Multiline P4 Universal Meter (PH meter). Heavy metals were analyzed using atomic absorption spectrophotometer.

Water Quality Analysis

Laboratory tests were carried out at the water microbiology laboratories, National Agency for Food Drug Administration and Control area laboratories, Maiduguri. At the water microbiology each of the samples were analyzed using atomic absorption spectrophotometer (auto sampler). The tests conducted were for TDS, Salmonella, E. Coli, Total Hardness and Ph

Materials Used

Materials used in this study are listed as follows. Real wastewater samples containing the target pollutants were collected from the water treatment plant (WTP) for testing the biofilm system's performance. The wastewater is basically the raw water at the inlet channel. Biofilm Carrier Materials provide a surface for biofilm attachment and growth. Common biofilm carrier materials include plastic media, designed to promote microbial growth. Biofilm carrier plastic material media are engineered structures designed to provide a substrate for microbial growth and biofilm formation in various environmental and industrial applications. These carriers offer a large surface area, high porosity, and favorable surface properties to support the attachment and proliferation of microorganisms, facilitating biological processes such as wastewater treatment, bioremediation, and biofiltration (De- Clippeir & Kirumba, 2018). The primary function of these carriers is to serve as a substrate for microbial attachment and biofilm formation. Microorganisms, including bacteria, fungi, and protozoa, adhere to the surface of the carriers, where they form biofilms a complex matrix of microbial cells embedded in an extracellular polymeric substance (EPS).

A mixture of microorganisms (Bacillus bacteria) is to be used to inoculate the biofilm system and initiate biofilm formation. The Inoculum can be sourced from existing biofilm or specific microbial cultures. The process flow diagram for the development of a biofilm system for the removal of pollutants from wastewater is presented in Figure 1.

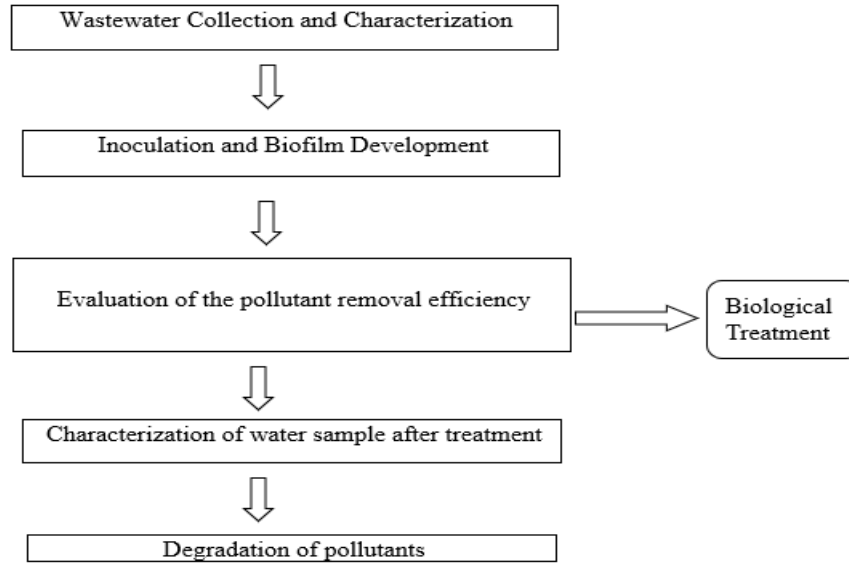


Figure 1. Procedural Steps Followed During the Experiment

Equipment

Equipment and materials used in this study are shown in Table 1.

Table 1. Materials and Equipment

S/N	Equipment Material	Name of Manufacturer	Function
1.	pH Meter	Phonon China	Measure the acidity or alkalinity of the wastewater to monitor and control the pH levels within the reactor
2.	Dissolved Oxygen (DO) Meters	Chenzang China	Measure the concentration of dissolved oxygen in the wastewater, which is critical for the metabolic activity of microorganisms in the biofilm.
3.	Laboratory Glassware	Yu Xu China	Including beakers, flasks, pipettes, and measuring cylinders for sample preparation and analysis.
4.	Microscopy Equipment	Japan	Used for visualizing and analyzing biofilm samples on microscopy slides.
5.	Biofilm Reactor		MBBR Utilize floating biofilm carriers that move with wastewater flow to provide an active biofilm surface

Isolation of Bacteria (Bacillus)

Culturing *Paenibacillus* bacteria, like any other microorganism, involves creating a controlled environment that supports their growth and multiplication. *Paenibacillus* is a genus of Gram-positive, spore-forming bacteria that are commonly found in soil, water, and various other environments. These bacteria have several important applications, such as in agriculture, bioremediation, and the production of bioactive compounds. To culture *Paenibacillus* bacteria successfully, you need to follow specific steps and provide the right conditions. Nutrient agar plate tubes were commonly used to culture *Paenibacillus* bacteria. These media provide a balanced mix of nutrients to support bacterial growth. Inoculation loop or sterile pipettes were sterile inoculation loops or sterile pipettes for transferring the bacteria onto the agar plates or into broth tubes. An autoclave is essential for sterilizing the growth media, equipment, and containers to prevent contamination. An incubator with precise temperature control is required to maintain the optimal temperature for *Paenibacillus* growth, typically around 30-37°C (86-98.6°F). For agar plates, were sterile Petri dishes to pour and solidify the agar. Microbiological Safety Cabinet were working with potentially pathogenic or hazardous strains, a biosafety cabinet will be necessary to ensure safety. Soil samples (1.0 g) from University of Maiduguri were collected aseptically and suspended in 100 mL of sterile water. Simply because MWTP is at close proximity to the learning institution and as such, soil samples collected from the same location are presumed same in terms of mineral constituents. Diluted sample were transferred in multiple flasks containing nutrient broth medium and incubated at 37°C for 24 to 72 h. After appearance of growth, a loop full of bacterial culture were then streaked onto nutrient agar medium and again incubated at 37°C for 24 to 48 h. Medium containing (1.0g/L): starch, 15.0; yeast extract, 10.0; peptone, 10.0; Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.5 Potassium phosphate (K_2HPO_4), 0.1; CaCl_2 , 0.01 (pH 7.5). enzyme activity of alpha-amylase was estimated using 1.0 % soluble starch as a substrate and the reducing sugar released as maltose equivalent were detected by 3 to 5 dinitrosalicylic acid (DNS) method (Miller, 1959). The unit of alpha-amylase is defined as the amount of enzyme which liberates 1.0 μmol of maltose per min at 50°C in phosphate buffer of pH 7.5 For the estimation of glucoamylase activity, 1.5% soluble starch was used as substrate and were determined by the increase in glucose that was released after the digestion of starch under standard assay conditions. Glucose was measured by glucose oxidase and peroxidase method (Bergmeyer & Bernt, 1974). One unit of glucoamylase activity is defined as the amount of enzyme that liberates 1.0 μmol of glucose at 45°C in potassium phosphate buffer of pH 7.0.

For the production of protease, the cultures were grown at 37°C for 24 h in the medium containing (1.0g/L): yeast extract, 5.0; peptone, 5.0; dextrose, 30.0; K_2HPO_4 , 0.1; CaCl_2 , 0.01 (pH 7.0). Crude enzyme (0.25 mL) was incubated with 1.0 mL of substrate (0.5 % casein in 50 mm Tris buffer, pH 8.5). The reaction were stopped using 110 mm TCA (Trichloroacetic acid). The reaction mixture was centrifuged at 1000 rpm for 10 min and was detected for the presence of tyrosine residues. Unit activity of protease is defined as the amount of enzyme that hydrolyzes casein to produce color that is equivalent to 1.0 μmol of tyrosine per min at pH 7.0 at 40°C. Pectinase was produced at 37°C for 24 h in a medium containing (g L⁻¹): pectin, 5.0; yeast extract, 5.0; ammonium sulphate, 2.5; K_2HPO_4 2.5; KH_2PO_4 , 2.0 (pH 7.5). Pectinase activity was assayed in terms of polygalacturonase activity using DNS. The reducing sugar released as galacturonic acid was detected using 0.25 % citrus pectin as substrate in the assay. One unit of pectinase activity is defined as the amount of enzyme required to release one μmol of galacturonic acid per min at 50°C in phosphate buffer (pH 7.0). Cellulase activity was not quantified but only screened for enzyme production.

The testing procedure for heavy metals was conducted using atomic absorption photo spectrometer. The test results were then compared to the World Health Organization (WHO) standard.

Composition of Nutrient Agar (NA)

Table 2 houses the constituents of the nutrient agar used.

Table 2.Composition of the Nutrient Agar

Content	Amount
Peptone (partially digested protein)	5.0g
Beef extract	3.0g
Sodium chloride	8.0g
Agar	15.0g
Water	1 L

Sampling for Pollutant Removal

The setup and design of the biofilm reactor are critical in achieving efficient pollutant removal from wastewater. Proper reactor configuration, selection of suitable support media, and optimization of operational parameters are essential for promoting biofilm formation, enhancing mass transfer, and facilitating effective pollutant degradation. The operational parameters of the biofilm reactor are carefully controlled to optimize pollutant removal efficiency. These parameters include influent flow rate, temperature, dissolved oxygen levels, and pH. These conditions are set based on the specific requirements of the targeted pollutant removal and the metabolic activity of the biofilm microbial community. The analysis of a biofilm system for the removal of pollutants from wastewater is a critical step to assess its performance, efficiency, and overall effectiveness. The following method outlines the key steps involved in the analysis of a biofilm system. Collect influent and effluent wastewater samples at regular intervals from the biofilm reactor. Take multiple samples from different locations within the reactor to capture spatial variations in pollutant removal.

Microbiological Analysis

The following steps were followed. Extract biofilm samples from the reactor and analyze microbial diversity and composition using DNA sequencing techniques like 16S rRNA gene sequencing for bacteria or ITS sequencing for fungi. Quantify the biomass and microbial density within the biofilm to understand the relationship between microbial community structure and pollutant removal performance. Conduct microscopy analysis (e.g., scanning electron microscopy - SEM) to visualize the biofilm structure, thickness, and distribution of microorganisms within the biofilm. Image analysis software can be used to quantify biofilm thickness and determine biofilm coverage on the carrier material.

Test for TDS

It consists of two parts: part A and part B. Part A reagents are methyl orange 0.1% indicator, TDS reagent A, demineralizer, TDS reagent B. Part A is as described in the next paragraph. About 10 mL of the sample water was collected in a clean 15 mL test tube, 3 drop of methyl orange 0.1% indicator was added it was then swirled to mix. The treated sample was then titrated gently with TDS reagent A/ using direct titration plunger range from 0-1000 until the orange color changes to red.

The result was read directly from the plunger and record as result A. Procedure followed to execute Part B is explained. A resin column was attached in the center hole of the tube, demineralizer reagent was added to be the resin column, and the sample water was poured up to 4 mL line of the tube using a 1 mL pipet. The entire water sample passed through the test tube was discarded. Using the resin column 10 mL of water sample was collect in the test tube, 3 drop of methyl orange 0.1% indicator was added it was then swirled to mix. The treated sample was then titrated gently with TDS reagent B/ using direct titration plunger range from 0-1000 until the red color changes to orange. The result was read directly from the plunger and record as result B. TDS of the sample was then calculated using Equation 1.

$$\text{Result A} + \text{Result B} = \text{TDS (ppm)} \quad (1)$$

The expected result in mg/mL is 500 max.

Test for Total Hardness (TH)

12.9 mL of water sample was collected in a clean 15 mL test tube 6 drop of hardness (#5) was added it was then swirled to mix. Then 5 drops of hardness (#6) reagent was added. Then titrate the treated sample using direct titration plunger, while it was gently swirling the tube slowly then press the plunger to titrate the sample until the red color change to blue indicate the presence of TH in the sample. The result was recorded in ppm. The expected result in ppm is 100 max.

pH Measurement

pH meter which measures the negative logarithm of the concentration of hydrogen ion in a solution digitally. Its scale is commonly from 0-14 with 0-7 being acidic and 7-14 being basic. It consists of two poles with sensors at both ends, one for conductivity test and the other for pH test which is being dipped in a 15 mL of the sample after turning on the machine. The result of the test is taken which is displayed few minutes after pushing the READ button on the pH meter.

Degree of Purification Efficiency

The success of the process was determined by measuring the size of which indicate the quality of treated wastewater at the exit, as well as water quality at the entrance of the treatment facility. Relation between input and output parameters of the process in order to obtain data on the same level of quality of the process is called the degree of efficiency of wastewater treatment. The classical degree of purification efficiency is defined by Equation 2.

$$\eta_{\text{COD}} = \frac{\text{COD}_{\text{uk}}}{\text{COD}_{\text{ul}}}$$

Where η_{COD} = degree of efficiency of removal of chemical oxygen demand (COD), COD_{uk} = The amount of COD removed during processing and COD_{ul} The amount of COD measured at the entrance of the plant for wastewater treatment.

RESULTS AND DISCUSSION

Results of Water Quality

Water quality based on TDS, pH and total hardness test carried out on the raw water sample reveals the results shown in Table 3.

Table 3. Water Quality before Analysis

Analysis	Before	Standard
pH	6.61	6.5-8.5
Total Hardness (mg/L)	110	100 Max.
<i>Salmonella</i> (CFU/mL)	2	0
<i>E. coli</i> (CFU/mL)	2	0
TDS (mg/L)	700	500 Max.

Table 3 shows the water quality before analysis, which includes the pH, total hardness, Salmonella, E. coli, and TDS levels. The pH level of 6.61 is slightly below the standard range of 6.5-8.5, while the total hardness level of 110 mg/L is above the maximum standard. Additionally, there are detectable levels of Salmonella and E. coli, which are harmful bacteria that can cause illness. The TDS level of 700 mg/L is also above the maximum standard.

Volume of Microorganisms Present

In one series of experiments (Table 4.3), from two to three times as many bacteria in samples of water sample formed colonies in Petri dishes poured with 10 mL of agar medium as in dishes poured with 20-50 mL of the medium. In another series of experiments (Table 4.4), only 17.6% as many colonies developed from water sample in dishes poured with 50 mL of agar medium as in dishes poured with 10 mL of medium. During the first few days of incubation at 180°C almost as many colonies appeared in dishes poured with 5 mL of agar medium as with 10 mL. The most nearly reproducible results were obtained when 10 mL of agar medium was used. Different volumes of the medium produced colonies of different kinds and characteristics. Generally, more pigmented colonies developed from sample inoculation in plates poured with 10 mL of agar medium than in dishes. Number of colonies counted per plate after 7 days incubation at 18°C when 1.0 mL of water sample was used to inoculate different volumes of agar medium poured at 420°C. Also given it's the average growth index and the time in minutes required for the poured medium to cool from 420°C down to 300°C, shown in Table 4.

Table 4. Number of Bacterial Colonies Counted per Plate in Volume (7 days Incubation Time)

Replicate	Volume of Agar plate in mL				
No.	10	20	80	40	50
1	102	31	24	79	32
2	94	46	43	63	65
3	134	46	42	33	51
4	137	97	39	43	29
5	108	78	64	84	69
6	96	62	29	67	38
7	85	31	42	42	33
8	135	58	33	50	41
9	122	38	41	30	30
10	115	92	26	29	42
Average number	112.8	56.9	38.3	52	43

Growth index	100	50.4	33.9	46.1	38.1
Cooling time	0.8	4.9	11.2	16.8	28.8

The growth index is the per cent of the average number of colonies appearing in plates poured with 10 mL of agar medium. Number of bacterial colonies counted per plate after 7 days' incubation at 180°C when 1.0 mL of water sample was used to inoculate different volumes of agar medium poured at 42°C. Also given is the time in minutes required for the medium to cool in Petri dishes from 42°C down to 30°C, as shown in Table 5.

Table 5. Number of Bacterial Colonies Counted per Plate in Volume (5 days Incubation Time)

Replicate	Volume of Agar plate in mL				
No.	5	10	20	30	50
1	333	234	160	142	19
2	277	335	209	169	52
3	303	346	121	244	65
4	363	423	355	123	30
5	361	376	128	146	23
6	339	348	355	328	111
7	247	358	226	----	100
8	248	408	---	---	82
9	260	305	237	252	75
10	325	385	219	395	--
Average number	305.6	351.8	223.3	224.7	61.9
Growth index	86.9	100	63.6	63.9	17.6
Cooling time	0.3	0.8	4.9	11.2	28.8

Poured with larger volumes of medium. Spreading colonies were more prone to appear in plates poured with the larger volumes of medium, probably due to the presence of more water of condensation. The smaller the volume of medium, the higher was the proportion of surface colonies that developed from water sample inoculation surface colonies were larger on the average than those that developed deeply embedded in the medium. The most pronounced effect on plate counts of different volumes of agar medium appears to be related to temperature and the time required for different volumes of medium to cool when poured into plates under laboratory conditions. With the larger volumes of agar medium which cool more slowly, thermos-sensitive bacteria in water sample inoculation are subjected to adverse temperatures for longer periods of time. Figure 4 shows the cooling rates of different volumes of the medium poured at 42°C into 100 mm Petri dishes at room temperature (23°C). About 1 mL of water at 18°C was introduced into the dish immediately prior to pouring the medium to simulate the effect of such an Inoculum on temperature conditions.

Whereas 10 mL of medium cooled to 30°C in less than 2 min, 20 mL and larger volumes remained above 30°C for approximately 5 to 25 min, and above 25°C for half an hour or longer. Many marine bacteria are injured by brief exposure to temperatures no higher than 300°C. Were found that from 18.7 to 78.1% of the bacteria in certain sample of water were killed by being held for 10 min at temperatures of 309°C and 400°C respectively. Differences occur in the temperature sensitivity of bacteria from different depths and latitudes. There may also be seasonal differences. Larger volumes of agar medium are less harmful for plating bacteria which tolerate temperatures within the plating range that is 300°C to 42°C. This is illustrated by data in Table 4.4 showing plate count results obtained with a laboratory culture of *Bacillus*. It grows best at 260°C and is not injured by holding at 350°C for 10 min. Effect of volume of agar medium, poured at 420°C, on number of colonies of *Bacillus* developed from a dilute inoculum after 3 days' incubation at 266°C is shown in Table 6.

Table 6. Number of Bacterial Colonies Counted per Plate in Volume (3 days Incubation Time)

Replicate	Volume of Agar Medium in mL				
<u>No.</u>	<u>10</u>	<u>15</u>	<u>20</u>	<u>39</u>	<u>50</u>
1	53	81	4	71	33
2	82	80	80	59	69
3	72	72	89	30	58
4	64	68	68	86	68
5	73	76	62	79	22
6	70	78	50	73	55
7	73	55	91	70	63
8	87	61	64	63	65
9	49	68	71	79	10
10	66	79	51	63	74
Average Number	69.7	71.8	67	72.3	55.7
Growth Index	100	1	96.1	103.7	79.9

The rate at which agar medium cools is influenced by the heat-retaining properties of the culture dish and work bench. Data summarized in Table 6 show that agar medium cool more rapidly in glass Petri dishes resting on a stainless-steel table top than on wood, polyvinyl plastic, or Styrofoam. Regardless of the volume of agar medium poured at 420°C, its temperature dropped more rapidly in Pyrex Petri dishes than in plastic ones.

Effect of Cooling Time on Growth Index and Average Volume

Figure 2, 3 and 4 present the effect of Growth Parameters versus time.

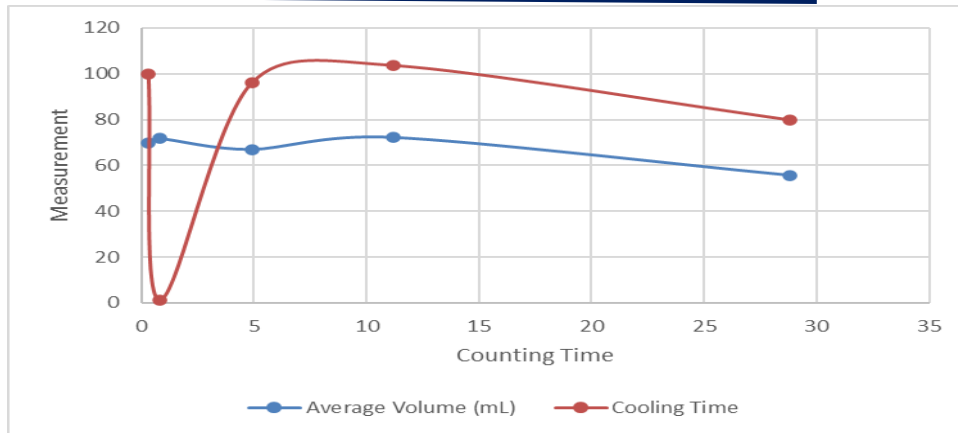


Figure 2. Influence of Counting Time at 7 days of Incubation

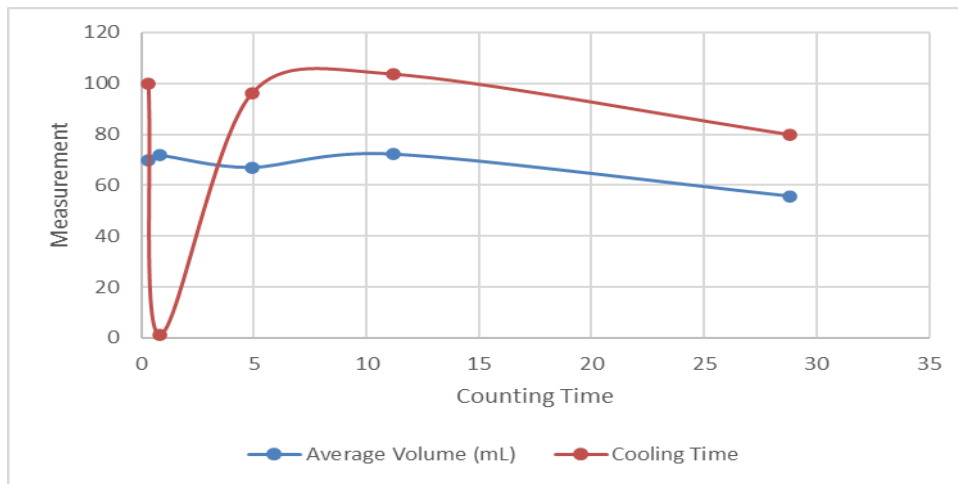


Figure 3. Influence of Counting Time at 5 days of Incubation

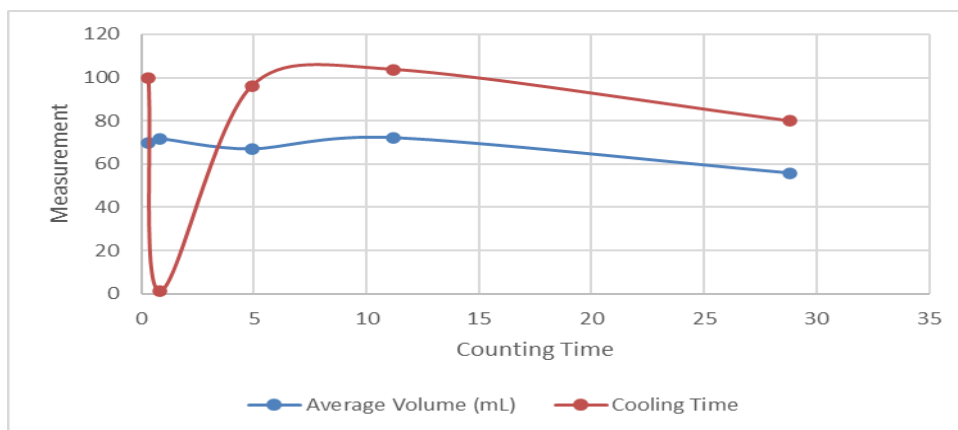


Figure 4. Influence of Counting Time at 3 days of Incubation

Figures 2, 3, and 4 illustrated the growth index and volume of bacteria versus counting time at different inoculation periods, providing insights into the efficiency of pollutant removal in biofilm wastewater treatment. In Figure 2, the growth index and volume of bacteria are plotted against counting time at different inoculation periods. This analysis allows for the observation of bacterial growth dynamics over time, providing valuable information on the efficiency of pollutant removal by the biofilm system. The trends in bacterial growth and volume can indicate the system's ability to degrade pollutants present in the wastewater. Figures 3 and 4 further depict the influence of counting time at 5 days and 3 days of incubation, respectively. These figures offer a detailed view of the changes in bacterial growth and volume over specific time intervals, shedding light on the biofilm system's performance in pollutant degradation over shorter periods. By analyzing these figures in conjunction with Tables 4.3, 4.4, and 4.5, which provide the corresponding data, it is possible to assess the impact of different inoculation periods on bacterial growth and volume, and consequently, on the pollutant removal efficiency of the biofilm wastewater treatment.

The growth index is a measure of the rate at which bacterial colonies or biofilm populations increase over time. It provides valuable information about the growth dynamics and reproductive capacity of the bacteria within the biofilm system. The relationship between the growth index, counting time, and volume of bacteria is crucial for understanding the efficiency of pollutant removal in biofilm wastewater treatment. As the counting time increases, the growth index reflects the cumulative effect of bacterial proliferation within the biofilm. This can be indicative of the biofilm's ability to degrade pollutants present in the wastewater over extended periods. Moreover, the volume of bacteria is influenced by the growth index and counting time. A higher growth index over a specific counting time period can lead to an increase in the volume of bacteria within the biofilm. This relationship is essential for evaluating the biofilm's capacity to sustain and enhance pollutant removal efficiency, as a larger volume of bacteria may contribute to more effective degradation of pollutants in the wastewater.

Statistical Analysis of the Efficiency of Wastewater Treatment using Biofilm System

Table 4.6 presents the water quality parameters pre- and post-treatment. Before treatment, the pH value before treatment was 6.61, which is slightly below the standard range of 6.5-8.5. After treatment, the pH value after treatment increased to 7.03, indicating a more neutral pH level within the acceptable range. Prior to treatment, the total hardness before treatment was 110 mg/L, which exceeded the maximum standard of 100 mg/L. But after treatment, the total hardness after treatment decreased to 70 mg/L, falling within the acceptable range, as shown in Table 7.

Table 7. Water quality before and after treatment (influent)

Analysis	Before	After	Standard
pH	6.61	7.03	6.5-8.5
Total Hardness (mg/L)	110	70	100 Max.
<i>Salmonella</i> (CFU/mL)	2	0	0
<i>E. coli</i> (CFU/mL)	2	0	0
TDS (mg/L)	700	468	500 Max.
COD (mg/L)	18	9	10 or below
Biochemical Oxygen Demand (BOD) (mg/L)	16	3	5 or below

Obviously, the treatment process was effective in improving the water quality parameters, including pH, total hardness, pathogen removal (*Salmonella* and *E. coli*), TDS, COD, and BOD. The reduction in these parameters post-treatment indicates the efficiency of the biofilm system in removing pollutants and improving the overall water quality.

The curve in Figure 6, illustrating the effect of time on total hardness and TDS of the water, can be influenced by various factors related to the treatment process and the characteristics of the contaminants present in the water. Initially, there will be a rapid decrease in total hardness and TDS as the treatment process begins to remove contaminants from the water. As time progresses, the treatment system will reach an optimal operating condition where the removal efficiency stabilizes, leading to a more gradual decrease in total hardness and TDS. The curve will exhibit a plateau or leveling off after a certain point, indicating that the treatment system has reached a saturation point where further removal of total hardness and TDS becomes more challenging. The curve in Figure 6 could reflect the equilibrium dynamics between the contaminants in the water and the treatment process. Initially, there will be a rapid decrease as the system works to establish equilibrium, followed by a more gradual change as equilibrium is reached.

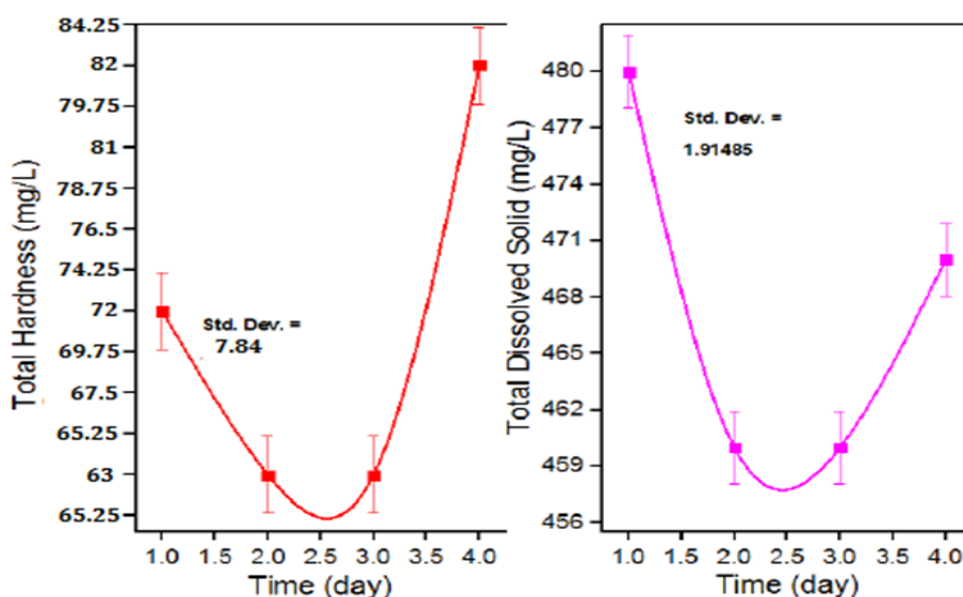


Figure 6. Effect of Time on Total Hardness and TDS of the Water

Biological processes within the biofilm system may also impact the removal of total hardness and TDS over time showcased in Figure 6. The growth and activity of microorganisms in the biofilm can influence the efficiency of contaminant removal. Changes in operational conditions, such as flow rate, temperature, or nutrient availability, can affect the performance of the treatment system over time, leading to variations in the removal of total hardness and TDS. The composition and characteristics of the contaminants present in the water, such as their solubility, reactivity, and adsorption properties, can influence the removal kinetics and the shape of the curve over time. A standard deviation of 7.84 suggests a moderate level of variability in Total Hardness values over the time period depicted in the plot. The data points for Total Hardness may exhibit noticeable fluctuations around the mean value, indicating changes in water hardness levels during the time intervals considered in the plot. On the other hand, a standard deviation of 1.91485 suggests relatively lower variability in TDS values compared to Total Hardness, indicating a more stable trend in the TDS measurements over time.

Thus, the data points for TDS will show minor fluctuations around the mean value, but overall, the TDS values remain relatively consistent during the time period covered in the plot.

The horizontal linear line in the first plot in Figure 7 indicate a constant initial amount of *E. coli* present in the influent water, suggesting a consistent contamination level entering the treatment system. It will also represent a lag phase where the treatment system is establishing conditions for bacterial growth or removal before a noticeable decrease in pathogen levels occurs. A standard deviation of zero means that all data points in the dataset lie exactly on the horizontal line, indicating no variability or dispersion around the mean value. In other words, all *E. coli* levels are identical and equal to the constant value represented by the horizontal line. The absence of standard deviation in the first plot of Figure 7 implies perfect precision in the measurements or data points for the dependent variable.

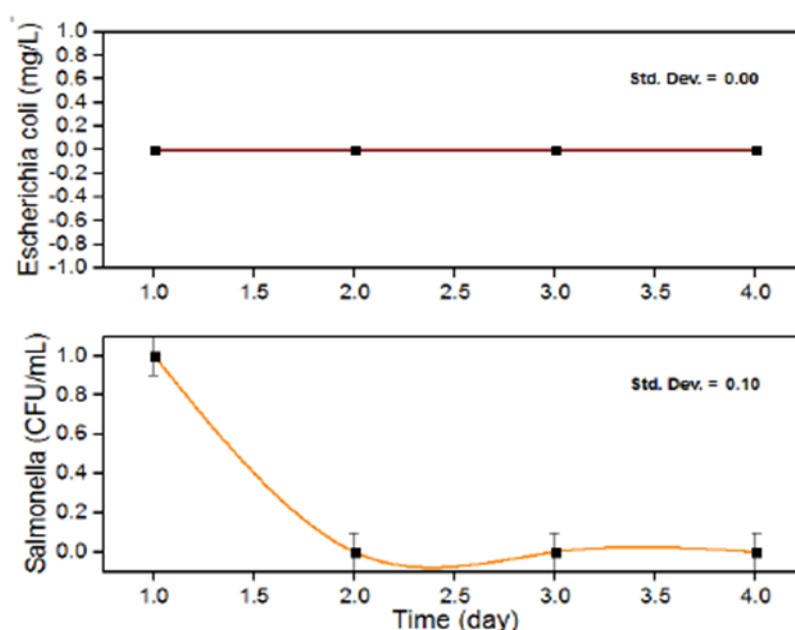


Figure 7. Amount of Salmonella and *E. coli* Present with Time

The decreasing curved line in the second plot of Figure 7 reflects the reduction in *Salmonella* concentrations over time due to the treatment process's effectiveness in removing or inactivating these pathogens. The curved line in the second plot follows a typical logarithmic decay pattern often seen in bacterial populations undergoing treatment. As time progresses, the reduction in *Salmonella* concentrations becomes more pronounced. With a standard deviation of 0.1, the data points in the plot would be clustered closely around the mean value, suggesting that there is some consistency in the amount of *Salmonella* present over time, but with minor fluctuations. The small standard deviation implies that while the overall trend may still show a decreasing curve in the amount of pathogens, there are slight variations or fluctuations in the data points around this trend line. The growth and decline of *Salmonella* and *E. coli* populations within the biofilm system can be influenced by factors like nutrient availability, competition with other microorganisms, and the effectiveness of the treatment in targeting specific pathogens.

Degree of Purification of Efficiency

The degree of purification efficiency, η_{COD} is simply $9/18$, which is equivalent to 0.5. A value of 0.5 indicates that 50% of the initial COD present in the influent wastewater was successfully removed or degraded during the treatment process. The higher the value of η_{COD} , the more effective the treatment process is at reducing the organic pollution in the wastewater. In this case, a value of 0.5 suggests moderate efficiency in COD removal. Effective removal of COD is crucial for reducing the organic load in wastewater before discharge into the environment. A η_{COD} of 0.5 indicates a significant reduction in the organic pollutants, which can help protect aquatic ecosystems. While a η_{COD} of 0.5 shows some level of success in COD removal, there may still be room for improvement in the treatment process to achieve higher purification efficiencies and meet regulatory standards. Continuous monitoring of η_{COD} is essential to assess the performance of the treatment system over time and make adjustments as needed to enhance the removal efficiency of organic pollutants.

CONCLUSION AND RECOMMENDATION

Conclusion

Water is a vital resource crucial for sustaining life and supporting various human activities, including industrial, agricultural, and recreational endeavors. However, the presence of pathogens such as Salmonella and coliform bacteria in wastewater poses significant risks to both public health and the environment. Effective wastewater treatment and monitoring strategies are essential to mitigate these risks and safeguard water resources. This study investigated the effectiveness of biofilm systems in removing pollutants from wastewater, emphasizing their role in sustainable water management practices. The research involved characterizing wastewater samples, evaluating pollutant removal efficiency, and exploring strategies to enhance biofilm activity, providing valuable insights into sustainable wastewater treatment approaches. Key findings underscore the importance of biofilm systems in pollutant removal, demonstrating their efficiency and sustainability in treating various types of wastewaters. By reducing the discharge of harmful contaminants, biofilm systems contribute significantly to safeguarding the environment, preserving water resources, and protecting ecosystems. The study's outcomes highlight the potential of biofilm-based technologies as viable options for enhancing wastewater treatment efficacy and sustainability. Continued research and implementation of biofilm systems are crucial in addressing emerging challenges in wastewater management and ensuring water quality standards are met for the benefit of present and future generations.

Recommendations

Explore the integration of advanced technologies, such as molecular biology techniques and nanomaterials, to enhance the efficiency of biofilm-based pollutant removal. Continuously optimize biofilm parameters, such as thickness, porosity, and structure, to maximize the overall performance of biofilm-based treatment systems. Investigate the influence of various factors on pollutant degradation and transformation within biofilms to enhance removal rates. Further research on the adaptability of biofilm systems to treat diverse pollutants in different types of wastewaters, such as municipal, industrial, and agricultural wastewater, to ensure comprehensive pollutant removal.

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SYNTHESIS AND PHOTOPHYSICAL PROPERTIES OF NOVEL TRICYCLIC PYRIMIDO[5,4-H]QUINAZOLINES

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ABSTRACT

Introduction and Purpose: Tricyclic aromatics and azaheteroaromatics have attracted significant interest in materials science due to their extended π -conjugation, which imparts excellent photoconductivity, electroluminescence, and fluorescence. In addition, azaheteroaromatics serve as valuable ligands in organometallic catalysis and as key motifs in pharmaceutical chemistry. The aim of the present study was to develop a straightforward synthetic pathway for novel tricyclic pyrimido[5,4-h]quinazolines and to investigate their photophysical and thermal properties.

Materials and Methods: A regioselective one-step tandem condensation of tetrafluoroterephthalonitrile with benzimidamine hydrochloride derivatives was performed under mild conditions to afford scarcely accessible 5,6-difluoro-2,9-diarylpyrimido[5,4-h]quinazolines. Structural characterization was confirmed by single-crystal X-ray diffraction, which provided insight into molecular stacking and intermolecular interactions. Photophysical properties were studied using UV-Vis absorption and fluorescence spectroscopy in both solution and solid states. Additional insights were obtained through electrochemical measurements, DFT calculations, and thermogravimetric analysis.

Results: The synthesized compounds exhibited strong emission in solution and solid states with a red-shift in the latter. Single-crystal X-ray diffraction confirmed distinct stacking arrangements and intermolecular interactions. Electrochemical studies revealed the stabilizing influence of halogen substituents on energy levels. Thermogravimetric analysis indicated high decomposition temperatures, confirming excellent thermal stability.

Discussion and Conclusion: This work demonstrates that the newly synthesized tricyclic pyrimido[5,4-h]quinazolines combine desirable photophysical features, strong thermal resistance, and halochromic responses upon protonation of amino or pyrimidine groups.

These findings highlight their potential as responsive materials for optoelectronic devices and advanced functional applications.

Keywords: Tricyclic pyrimidoquinazolines; π -conjugated systems; Photophysical properties; Fluorescence; Thermal stability; Responsive mater



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