

INTERNATIONAL
A E G E A N
CONFERENCES
on Natural & Medical Sciences~V
February 25-26, 2022 / Izmir, Turkey

Proceedings Book

Editors

*Assoc. Prof. Dr. Tamilla ALIYEVA
Dr. Mehmet Emin KALGI*

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INTERNATIONAL AEGEAN CONFERENCES
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PROCEEDINGS BOOK

EDITORS

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CONFERENCES TITLE

- **INTERNATIONAL AEGEAN CONFERENCES**
 - **on Natural & Medical Sciences**

DATE AND PLACE

- February 25-26, 2022 / Izmir, Turkey

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- ATLAS INTERNATIONAL JOURNAL ON SOCIAL SCIENCES
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 DATE • 25.02.2022	 TIME • 10 ⁰⁰ -12 ³⁰	 SESSION • HALL-2 • SESSION-1
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HEAD OF SESSION: Assoc. Prof. Dr. Dilek ÇAVUŞOĞLU

NAJIM ABDULLA YASSIN EKRAM LUGMAN ISMAEL IMAN HAMEED MIKHA	<i>College of Medicine</i> <i>General Shekhan Hospital</i> <i>War-child UK Organization</i>	PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIAL AGENTS OF URINARY TRACT INFECTION AND GENITAL TRACT AMONG WOMEN IN SHEKHAN DISTRICT, IRAQ
Hilda. A. Emmanuel-Akerele	<i>Anchor University</i>	MICROBIAL AND PHYSICO-CHEMICAL ASSESSMENT OF SOIL AND WATER AROUND WASTE DUMP SITES IN LAGOS
KHALID Ibtissame MEZIANE Mustapha ELAYACHI Moussa	<i>Mohamed first university</i> <i>Mohamed first university</i> <i>Mohamed first university</i>	CLASSICAL IDENTIFICATION OF THE GENERA OF LACTIC ACID BACTERIA ISOLATED FROM MATURE HUMAN MILK
Mohamed Kouighat Hafida Hanine Mohamed El Fechtali Abdelghani Nabloussi	<i>Institut National de la Recherche</i> <i>Agronomique</i> <i>Université Sultan Moulay</i> <i>Slimane</i>	ASSESSMENT OF SOME SESAME MUTANTS UNDER DROUGHT CONDITIONS
Assist. Prof. Dr. K.R.Padma K.R.Don	<i>Sri Padmaвати Mahila</i> <i>Visvaavidyalayam (Women's)</i> <i>University</i> <i>Sree Balaji Dental College and</i> <i>Hospital</i>	THE ROLE OF GUT MICROBIOTA ON ENDOCRINE SYSTEM
Dr. Ranjana Bengani B.R Subba K.P Limbu	<i>Veer Narmad South Gujarat</i> <i>University</i> <i>Post Graduate Campus</i> <i>Post Graduate Campus</i>	RESTORATION OF DEAD LAKE - A CASE STUDY OF CHIMDI LAKE, SUNSARI, NEPAL
Assoc. Prof. Dr. Dilek ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU	<i>Isparta University</i> <i>Isparta University</i> <i>Giresun University</i>	THE PROTECTIVE ROLE OF LYCOPENE AGAINST POTASSIUM BROMATE GENOTOXICITY: THE ALLIUM TEST
Assoc. Prof. Dr. Dilek ÇAVUŞOĞLU Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN	<i>Isparta University</i> <i>Giresun University</i> <i>Isparta University</i>	PROTECTIVE ROLE OF ROYAL JELLY AGAINST PHENOXYETHANOL- INDUCED TOXICITY IN ALLIUM CEPA L.: GENETIC APPROACH
Assoc. Prof. Dr. Faik GÖKALP	<i>Kırıkkale University</i>	A THEORETICAL RESEARCH ON THE INHIBITORY EFFECTS FOR THE ACTIVE COMPONENTS OF OPUNTIA FICUS- INDICA L. ON CANCER AND SARS-COV- 2 VIRUS RECEPTORS

 DATE • 25.02.2022	 TIME • 10 ⁰⁰ -12 ³⁰	 SESSION • HALL-3 • SESSION-1
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HEAD OF SESSION: Prof. Dr. Osman SAĞDIÇ




Melissa URUÇAY Lect. Beyza MENDEŞ	<i>Bezmi Alem University</i>	THE ROLE OF ANTHOCYANINS IN NUTRITION AND HEALTH
Kübra EKMEKÇİ Büşra ÇALIK Nesibe Nur YALÇIN Assist. Prof. Dr. İsmail Hakkı TEKİNER	<i>İstanbul Sabahattin Zaim University</i>	EVALUATION OF EFFECT OF DEUTERIUM OXIDE ON UNSATURATED FATTY ACIDS EXPOSED TO GAMMA, UV, AND HEAT STRESS FACTORS
Assoc. Prof. Dr. Erdem SEVEN	<i>Batman University</i>	DISTRIBUTION AND BIOLOGY OF LEUCOMA WILTSHIREI COLLENETTE, 1938 (LEPIDOPTERA, EREBIDAE, LYMANTRIINAE) IN SOUTH-EASTERN TURKEY
Lect. Ayşen ARSLAN Dr. Zeynep Hazal TEKİN ÇAKMAK Assoc. Prof. Dr. Salih KARASU Prof. Dr. Osman SAĞDIÇ	<i>Istinye University Yıldız Technical University</i>	THE EFFECT OF THE USE OF SALEP POWDER OBTAINED FROM DIFFERENT WILD ORCHID SPECIES IN TURKEY ON THE RHEOLOGICAL PROPERTIES OF ICE CREAM
Fatma Hacet Assoc. Prof. Dr. Eda Becer Prof. Dr. Hafize Seda Vatansever Prof. Dr. Sevinç Yücecan	<i>Near East University Celal Bayar University Lokman Hekim University</i>	EFFECT OF ALLYL ISOTHIOCYANATE AND SULFORAPHANE ON CELL VIABILITY IN AN IN VITRO MODEL OF ALZHEIMER'S
Nesli Nur MERCAN Dr. Leila MEHDIZADEHTAPEH Assist. Prof. Dr. Hüseyin ABDİK Assist. Prof. Dr. İsmail Hakkı TEKİNER	<i>İstanbul Sabahattin Zaim University Eryiğit Medical Devices and Biotechnology Inc.</i>	INVESTIGATION OF INTERACTIONS AMONG BETA-CAROTENE, BIOTIN, AND CROHN DISEASE USING IN-SILICO, IN VITRO AND GENE EXPRESSION METHODS

 DATE • 25.02.2022	 TIME • 10 ⁰⁰ -12 ³⁰	 SESSION • HALL-4 • SESSION-1
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HEAD OF SESSION: Assoc. Prof. Dr. Ali ACAR




Asst. Prof. Dr. Kübra Gülnur TOPSAKAL Ebru YURDAKURBAN Şule GÖKMEN Gökhan Serhat DURAN Serkan GÖRGÜLÜ	<i>University of Health Sciences</i>	COMPARISON OF MAXILLARY CENTRAL INCISORS CROWN-ROOT ANGLE IN SKELETAL CLASS 1 AND CLASS 3 MALOCCLUSION INDIVIDUALS
Dr. Samir GÖYÜŞOV Dr. Osman Özkan DOĞAN Dr. Süleyman Emre MEŞELİ	<i>Istanbul Aydın University</i>	ASSESSMENT OF DENTAL ANXIETY STATUS AMONG PATIENTS WITH DIFFERENT EDUCATIONAL LEVELS
Assist. Prof. Dr. Kübra Gülnur TOPSAKAL Şule GÖKMEN Ebru YURDAKURBAN Assoc. Prof. Dr. Gökhan Serhat DURAN Prof. Dr. Serkan GÖRGÜLÜ	<i>University of Health Sciences</i>	ARTIFICIAL INTELLIGENCE (AI) APPLICATIONS IN ORTHODONTICS
Res. Ast. Dr. Tuba UNVER Assoc. Prof. Ayse Sebnem ERENLER	<i>Inonu University Malatya Turgut Ozal University</i>	DEFENSE MECHANISM METABOLITES IN THE FEMALE REPRODUCTIVE SYSTEM
Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	INVESTIGATION OF THE GENOTOXIC EFFECTS OF FENPYROXIMATE WITH THE COMET ASSAY
Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	DETERMINATION OF DNA DAMAGE INDUCED BY AFLATOXIN B2 WITH COMET ASSAY

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 DATE	• 25.02.2022	 TIME	• 13⁰⁰–15³⁰	 SESSION	• HALL-2 • SESSION-2
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HEAD OF SESSION: Dr. Kave Koorehpaz

Assoc. Prof. Dr. Elzbieta PATKOWSKA	<i>University of Life Sciences in Lublin</i>	THE HEALTH STATUS OF PEA (PISUM SATIVUM L.) PLANTS AFTER APPLYING OF BIOSTIMULANTS
Tanveer Alam Murtaza Gani Rukhsana Rahman Khalid ul Islam Rather	<i>HNB Garhwal University Srinagar Shere Kashmir University High End Instrumentation Lab High End Instrumentation Lab</i>	HPLC QUANTIFICATION OF THE CHEMICAL CONSTITUENTS FROM INDIGENOUS FRUITS AND VEGETABLES OF INDIAN HIMALAYAN REGION
B. Akhila	<i>International School of Technology and Science</i>	REVIEW ON FARMING AGRICULTURE ROBOTS
Akil A. Khan	<i>Gandhi Faiz-e-Aam College</i>	PHYTOPLASMA - A SERIOUS THREAT TO VARIOUS PLANT SPECIES IN INDIA
Shoeb Ahmad	<i>Gandhi Faiz-e-Aam College</i>	PHYTOPLASMA OCCURRING VARIOUS SYMPTOMS IN DIFFERENT PLANT SPECIES
Ibrahim-Olesin, Sikiru Igberi, Christiana Ogonna	<i>Alex Ekwueme Federal University</i>	INCOME DISTRIBUTION OF THE ADOPTERS AND NON ADOPTERS OF CROP ROTATION AND DIVERSITY PRACTICES
Dr. Kave Koorehpaz	<i>Urmia University</i>	FELINE LEUKEMIA VIRUS INFECTION AND RELATED DISEASES IN CATS
M. Hamayoon Mohammad	<i>Khazar University</i>	MAJOR DIRECTION TO INCREASE THE COMPETITIVENESS OF AGRICULTURE ENTERPRISES OF AFGHANISTAN
Sanchita Chandra Subham Sarkar Paramita Mandal	<i>The University of Burdwan</i>	UNRAVELING THE DIFFERENTIAL MOLECULAR PROFILES OF CERVICAL CANCER BASED ON TISSUE TYPE
Assist. Prof. Dr. Ə.G.Cəlilov A.R.Əliyev S.Ş.Süleymanov	<i>Institute of Zoology NASA</i>	HYDROFAUNA OF THE MIDDLE CASPIAN AZERBAIJANI AQUATORIUM

 DATE	• 25.02.2022	 TIME	• 13⁰⁰–15³⁰	 SESSION	• HALL-3 • SESSION-2
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HEAD OF SESSION: Dr. Ahmed Siddique Ammar

Major Giurgiu Gheorghe Prof. Dr. Cojocaru Manole	<i>Deniplant-Aide Sante Medical Center Titu Maiorescu University</i>	THE THERAPEUTIC EFFECTS OF DENIPLANT NATURAL MODULATOR ON THE GUT MICROBIOME IN PATIENTS WITH PSORIASIS
Dr. Alessio ZANZA Dr. Rodolfo REDA Dr. Gabriele Miccoli Dr. Dario DI NARDO Prof. Dr. Luca TESTARELLI	<i>Sapienza University</i>	ROLE OF THE CRYSTALLOGRAPHIC PHASE OF NITI ROTARY INSTRUMENTS: WHAT CHANGES IN DIFFERENT BENDING CONDITIONS
Sabina Farhadova	<i>Azerbaijan National Academy of Science University of Montpellier, France</i>	DISSECTING THE MULTI-LEVEL CONTROL OF IMPRINTING AT THE Dlk1-Dio3 And Igf2-H19 DOMAINS IN DEVELOPMENT AND DISEASE
Raghad Adel Omer Mohammed Yawuz Jamal	<i>University of Baghdad</i>	SELF-REPORTED SLEEP DISORDER, ANXIETY AND DEPRESSION IN POST MYOCARDIAL INFRACTION PATIENTS IN IRAQ
Anar Abdullayev Zemfira Nadirli	<i>Azerbaijan Medical University</i>	WORMIAN BONE OF THE SAGITTAL SUTURE ON A METOPIC FEMALE SKULL OF THE MIDDLE AGES WITH OCCIPITAL DEFORMATION
Manisekaran Hemagirri Sreenivasan Sasidharan	<i>Universiti Sains Malaysia</i>	ANTIAGING ACTIVITY OF POLYALTHIA LONGIFOLIA LEAF IN SACCHAROMYCES CEREVISIAE BY611 YEAST MODEL VIA MICROSCOPIC APPROACHES
Ruta MINELGAITE Vilija MALINAUSKIENE	<i>Lithuanian University of Health Sciences</i>	PECULIARITIES OF ANXIETY IN ADOLESCENTS
Gerald Oladipo Okparah Vilija Malinauskiene	<i>Lithuanian Sports University</i>	BURNOUT, PSYCHOSOCIAL FACTORS AT WORK AND NUTRITION AMONG MEDICAL DOCTORS: AN OBSERVATIONAL STUDY REPORT
Dr. Saba Zubair Prof. Dr. Huma Ali	<i>Jinnah Sindh Medical University</i>	PREVALENCE AND CATEGORIES OF FREQUENTLY OCCURRING DRUG INTERACTIONS IN ICU SETTINGS. AN OBSERVATIONAL PROSPECTIVE STUDY FROM KARACHI PAKISTAN
Prof. Dr. Huma Ali Dr. Saba Zubair	<i>Jinnah Sindh Medical University</i>	A CROSS SECTIONAL STUDY ON HEPATITIS PREVALENCE FROM KARACHI PAKISTAN. CONTRIBUTING FACTORS AND RELATED CO-MORBIDITIES ASSESSMENTS
Dr. Ahmed Siddique Ammar	<i>Consultant General & Laparoscopic Surgeon</i>	ESWEP SCORE, A NEW STATISTICALLY VALID SCORING CRITERIA FOR DECISION BETWEEN REPAIR OR ILEOSTOMY IN PATIENTS WITH PERITONITIS DUE TO ENTERIC PERFORATION
Angelina Kirkova-Bogdanova Daniela Taneva Maria Becheva	<i>Medical University</i>	TRAINING HEALTHCARE FACULTY IN A LEARNING CONTENT MANAGEMENT SYSTEM (LCMS)

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HEAD OF SESSION: Prof. Dr. Emine YALÇIN

Şadiye DEMİR ATMACA Tülay TOPRAK Sedat YALÇINYİĞİT Prof. Dr. Rüveyde TUNÇTÜRK Prof. Dr. Mehmet ÜLKER Prof. Dr. Murat TUNÇTÜRK Assoc. Prof. Dr. Erol ORAL	<i>Van Yüzüncü Yıl University</i>	THE EFFECTS OF GLOBAL WARMING ON AGRICULTURE
Dr. Ayşegül AKPINAR	<i>Bilecik Seyh Edebali University</i>	NITROGEN ASSIMILATION CAPACITY OF CHENOPODIUM ALBUM L. LOCATED IN POLLUTED AREAS
Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	CYTOTOXIC AND GENOTOXIC EFFECTS OF FUMONISIN B2 ASSISTED WITH MOLECULAR DOCKING STUDIES
Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	ANTIMUTAGENIC EFFECTS OF TRACHYSTEMON ORIENTALIS FLOWER EXTRACT
Hüseyin YILMAZ Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	INVESTIGATION OF THE PROTECTIVE ROLE OF GRAPE SEED AGAINST BENZYL BENZOATE GENOTOXICITY IN ALLIUM CEPA L.
Hüseyin YILMAZ Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	INVESTIGATION OF THE PROTECTIVE ROLE OF GINKGO BILOBA LEAF EXTRACT AGAINST CHROME GENOTOXICITY WITH ALLIUM TEST
Assoc. Prof. Dr. Deniz KURT Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	PROTECTIVE ROLE OF GREEN TEA AGAINST DIETHYL PHTHALATE TOXICITY IN ALLIUM CEPA L.: A BIOCHEMICAL APPROACH
Assoc. Prof. Dr. Deniz KURT Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	PROMETHRIN TOXICITY IN ALLIUM CEPA L.: GENETIC APPROACH
Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	PROTECTIVE ROLE OF SALVIA OFFICINALIS AGAINST PARAQUAT TOXICITY IN SWISS ALBINO MICE: A GENETIC APPROACH
Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN Assoc. Prof. Dr. Ali ACAR	<i>Giresun University</i>	THE PROTECTIVE ROLE OF LYCOPENE AGAINST CYPERMETHRIN TOXICITY IN SWISS ALBINO MICE: A BIOCHEMICAL APPROACH

 DATE	• 25.02.2022	 TIME	• 16⁰⁰-18³⁰	 SESSION	• HALL-3 • SESSION-3
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HEAD OF SESSION: Assoc. Prof. Dr. Tuğçe KALEFETOĞLU MACAR

Dr. Oksal MACAR Assoc. Prof. Dr. Tuğçe KALEFETOĞLU MACAR Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU	<i>Giresun University</i>	IRON (FE) - INDUCED MICRONUCLEUS (MN) FORMATION IN DIFFERENT CELL TYPES OF ALBINO MICE
Dr. Oksal MACAR Assoc. Prof. Dr. Tuğçe KALEFETOĞLU MACAR Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN	<i>Giresun University</i>	PROTECTIVE ROLE OF GINGER AGAINST GENOTOXICITY CAUSED BY TETRACONAZOLE FUNGICIDE IN ALLIUM CEPA L.
Assoc. Prof. Dr. Tuğçe KALEFETOĞLU MACAR Dr. Oksal MACAR Prof. Dr. Emine YALÇIN Prof. Dr. Kültiğın ÇAVUŞOĞLU	<i>Giresun University</i>	PROTECTIVE ROLE OF POMEGRANATE SEED EXTRACT AGAINST BIOCHEMICAL TOXICITY INDUCED BY ABAMECTIN PESTICIDE IN ALLIUM CEPA L.
Assoc. Prof. Dr. Tuğçe KALEFETOĞLU MACAR Dr. Oksal MACAR Prof. Dr. Kültiğın ÇAVUŞOĞLU Prof. Dr. Emine YALÇIN	<i>Giresun University</i>	MANGANASE GENOTOXICITY IN ALLIUM CEPA L.
Prof. Dr. Selami CANDAN Assoc. Prof. Dr. Nurcan ÖZYURT KOÇAKOĞLU	<i>Gazi Universtiy</i> <i>Gazi Universtiy</i>	THE ANATOMY AND HISTOLOGY OF THE DIGESTIVE SYSTEM OF PIMELIA SUBGLOBOSA (PALLAS, 1781) (COLEOPTERA: TENEBRIONIDAE)
Dr. Çağlar ADIGÜZEL Dr. Hatice KARABODUK Assoc. Prof. Dr. Fatma Gökçe APAYDIN Prof. Dr. Yusuf KALENDER	<i>Gazi Universtiy</i>	LEAD NITRATE AND CADMIUM CHLORIDE INDUCED RATS THYROID PATHOLOGY AND EFFECTS OF SESAMOL
Buket KOYUKAN Yiğit Osman AKYILDIZ Mediha GÜMÜŞ Özge ÇAĞLAR Elif ÖZYILMAZ	<i>Selçuk University</i>	EFFECT OF DANDELION ON HEPATOCELLULAR CARCINOMA
Derya ÇİÇEK POLAT Muhammed Mesud HÜRKUL	<i>Ankara University</i>	EVALUATION OF COTINUS COGGYGRIA SCOP. FRUITS IN TERMS OF TOTAL PHENOL COMPOUND AMOUNT AND ANTIOXIDANT ACTIVITY
Şeyda YAYLA Muhammed Mesud HÜRKUL	<i>Ankara University</i>	TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF VITIS LABRUSCA L. FRUITS AND LEAVES

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HEAD OF SESSION: Dalal Adnan Amer Maturi

Osarumwense Peter Osarodion	<i>Ondo State University of Sciences and Technology</i>	SYNTHESIS, ANTI-INFLAMMARTORY ACTIVITY OF 3-AMINO 5-METHOXYL-2-METHYL QUINAZOLIN-4(3H)-ONE AND AMINO-6-METHOXYL-2-METHYL OF 4H-BENZO[D] [1,3]-OXAZINE-4-ONE
Benchettou oumaïma Bentbib Abdeslam hafid Bouhamidi abderrahman	<i>University Cadi Ayyad - 93000 University Littoral</i>	AN ACCELERATED TENSORIAL DOUBLE PROXIMAL GRADIENT METHOD FOR TOTAL VARIATION REGULARIZATION PROBLEM
Mahrouk abdelkader Mosbah asma Noreddine Kacem Chaouche	<i>University Frère Mentouri Constantine 1</i>	SCREENING FOR POTENTIAL NOVEL PROBIOTICS WITH ANTIOXIDANT, ANTIMICROBIAL ACTIVITIES
Cindy Moyna CLARA Sutopo HADI Yandri YANDRI	<i>Universitas Lampung</i>	SYNTHESIS, CHARACTERIZATION, AND BIOACTIVITY TEST OF DIPHENYLTIN(IV) DI-2 CHLOROBENZOATE AND TRIPHENYLTIN(IV) 2-CHLOROBENZOATE COMPOUNDS AS DISINFECTANT
Aisyah Larasaty SUSANGKA Sutopo HADI Noviany NOVIANY	<i>Universitas Lampung</i>	SYNTHESIS, CHARACTERIZATION, AND DISINFECTANT BIOACTIVITY TEST OF SOME TRIPHENYLTIN(IV) COMPOUNDS
Cornelia Nichita	<i>University of Bucharest</i>	BIOACTIVE AND PROTECTIVE EFFECTS OF PHENOLIC COMPOUNDS FROM CURCUMA LONGA L. RHIZOME EXTRACTS AGAINST OXIDATIVE STRESS
Dalal Adnan Amer Maturi	<i>King Abdulaziz University</i>	THE SERIES SOLUTION METHOD SOLVING VOLTERRA INTEGRAL EQUATIONS OF THE FIRST KIND USING MAPLE
Dossan Aray	<i>Abay University</i>	METHODS OF FORMATION OF COGNITIVE ACTIVITY IN THE PROCESS OF TEACHING BIOLOGY IN THE SUBJECT INFORMATION AND EDUCATIONAL ENVIRONMENT
Saba Ajaz Baloch Huma Ali Farya Zafar Saba Zubair	<i>Jinnah Sindh Medical university University of Karachi</i>	DRUG UTILIZATION PATTERN OF ANTIBACTERIAL INCLUDING PIPERICILLIN+TAZOBACTUM IN TERTIARY CARE HOSPITALS: ASSESSMENT OF THERAPEUTIC OUTCOMES AND BENEFITS
Dr. Yasemin Tümer	<i>Karabuk University</i>	SYNTHESES OF TRISPIROCYCLOTTRIPHOSHAZENE COMPOUNDS

 DATE • 26.02.2022	 TIME • 10 ⁰⁰ –12 ³⁰	 SESSION • HALL-3 • SESSION-1
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HEAD OF SESSION: Prof. Dr. Ramazan ERENLER

Volkan TAŞDEMİR	<i>Van Yüzcüncü Yil University</i>	METAL-CATALYZED SYNTHESIS OF FURAN USING YINON DERIVATIVES
Bahar Meryemoglu Berna Nis Burcak Kaya Ozel	<i>Cukurova University Bursa Technical University Bursa Technical University</i>	LC-TOF/MS ANALYSIS OF ORGANIC ACIDS WITH DIFFERENT COLUMNS
Ahsen KARADAĞ Prof. Dr. Sibel DEMİREL	<i>Kocaeli University Kocaeli University</i>	INVESTIGATION OF INHIBITION EFFECT OF 4-AMINO-N-(1,3) - THIAZOLE-2-YL BENZENE SULFONAMIDE ON CORROSION OF COPPER IN ACIDIC SOLUTION
Assist. Prof. Dr. Ercan SARUHAN	<i>Muğla Sıtkı Koçman University</i>	EVALUATION OF MEASUREMENT UNCERTAINTY FOR THYROID FUNCTION TESTS
Prof. Dr. Ramazan ERENLER	<i>Tokat Gaziosmanpaşa University</i>	BIOSYNTHESIS OF SILVER NANOPARTICLES USING ETHYL ACETATE EXTRACT OF ORIGANUM ONITES: CHARACTERISATION AND ANTIOXIDANT ACTIVITY
Prof. Dr. Ramazan ERENLER	<i>Tokat Gaziosmanpaşa University</i>	CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL OF ECHIUUM VULGARE
Dr. Mahira Firudin AMİROVA	<i>Azerbaijan Medical University</i>	THE ROLE OF CELL RECEPTORS AND OXIDATIVE STRESS IN THE SARS-COV INFECTION SPREAD
Dr. Mahira Firudin AMİROVA	<i>Azerbaijan Medical University</i>	BASIC PRINCIPLES OF SYNTHESIS OF ANTIMICROBIAL PEPTIDES
Assist. Prof. Dr. Ümit YILDIKO	<i>Kafkas University</i>	N,N-BIS(4-AMINOPHENYL)PYRIDINE-4-AMIN SYNTHESIS AND CHARACTERIZATION: DFT STUDIES AND MOLECULAR DOCKING ANALYSIS
Dr. Mehmet DEMİRALAY Dr. Cansu ALTUNTAŞ	<i>Artvin Çoruh University</i>	EFFECTS OF EXOGENOUS SOME POLYAMINE BIOSYNTHESIS INHIBITORS ON SOME PHOTOSYNTHETIC PARAMETERS UNDER DROUGHT STRESS

 DATE • 26.02.2022	 TIME • 13 ⁰⁰ -15 ³⁰	 SESSION • HALL-2 • SESSION-2
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HEAD OF SESSION: DR. MUHAMMAD FAISAL




DR. MUHAMMAD FAISAL	<i>Sindh Madressatul Islam University</i>	VERSATILE SLIDING MODE CONTROL OF PARTICULAR SELF-RECONFIGURABLE SPACE APPARATUS WITH TIME DEFER ASSESSMENT BY AI TECHNOLOGY
DR. MUHAMMAD FAISAL	<i>Sindh Madressatul Islam University</i>	PAKISTANI NAVAL AND AERONAUTICAL DISPOSAL ASSETS CAN BE UTILIZED FOR THE MARINE AND SPACE ADVENTURES BY ROBOTICS
Muhammad Aliff Khars Fathanah Kahar Lisa Maere Hildegarda Angkangon Jasper Jilo Jude Bayad Billy Alexander	<i>Keningau Vocational College</i>	IMPROVING THE INFRASTRUCTURE OF A COMMUNITY PLAYGROUND THROUGH CONSTRUCTION WORK
Dr. Hanane Ait Hmeid Pr. Mustapha Akodad Pr. Mourad Baghour Pr. Abdelmajid Moumen Pr. Ali Skalli Pr. Ghizlane Azizi	<i>Mohamed First University</i>	PRELIMINARY CHARACTERIZATION AND EVALUATION OF THE POTENTIAL USE OF CLAY MATERIALS FROM NORTH-EAST MOROCCO IN THE CERAMIC INDUSTRY
Hanane Ait Hmeid Mustapha Akodad Mourad Baghour Abdelmajid Moumen Ali Skalli Ghizlane Azizi Hicham Gueddari Yassine El Yousfi Lahcen Daoudi	<i>Mohamed First University</i> <i>Abdelmalek Essaadi University</i> <i>Cadi Ayyad University</i>	MINERALOGY AND GEOCHEMISTRY OF RHYOLITIC DOMES AND PERLITES ROCKS IN THE TIDINIET MASSIF, (NADOR, NORTHERN MOROCCO)
Maryam	<i>Kohat University of Science and Technology</i>	GAUSS HYPERGEOMETRIC GAMMA AND BETA FUNCTIONS WITH PROPERTIES CONNECTING TO INTEGRAL TRANSFORMS
Hayat Ullauh	<i>Kohat University</i>	A MODIFIED BIPOLAR SOFT SETS BASED MODEL FOR SOLVING DECISION-MAKING PROBLEMS

 DATE • 26.02.2022	 TIME • 13 ⁰⁰ –15 ³⁰	 SESSION • HALL-3 • SESSION-2
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HEAD OF SESSION: Anisha CHAUHAN

Aditi Chauhan	<i>Banasthali Vidyapith University</i>	XENOTRANSPLANTATION
Anshima SINGH	<i>Banasthali Vidyapith University</i>	BIOTECHNOLOGY
Anisha CHAUHAN	<i>Banasthali Vidyapith University</i>	MEDICAL ETHICS AND MEDICAL EDUCATION: A NEW CHALLENGE IN OUTGOING PANDEMIC
Anisha CHAUHAN	<i>Banasthali Vidyapith University</i>	BIOTECHNOLOGY AND ITS APPLICATIONS: A REVIEW
Vishnu PRIYA	<i>Banasthali Vidyapith University</i>	AN ANALYTICAL STUDY OF HEALTH TOURISM INDUSTRY - A STUDY ON POTENTIALITY OF INDIAN HEALTH TOURISM PERSPECTIVE.
Akanksha kumari	<i>Banasthali Vidyapith University</i>	GENE THERAPY AND IT'S FUTURE
Kaberi Pramanik	<i>Banasthali Vidyapith University</i>	EVOLUTIONARY BIOLOGY
Ms. Aishwarya K A	<i>Banasthali Vidyapith University</i>	GENETIC SCREENING
Shailja Sharma	<i>Banasthali Vidyapith University</i>	TOPIC- ON-OFF SWITCH FOR INHIBITING BACTERIAL GROWTH
Bhavika Bisht	<i>Banasthali Vidyapith University</i>	BIOTECHNOLOGY ABSTRACT
Shrishty gupta	<i>Banasthali Vidyapith University</i>	COVID-19 PANDEMIC AND LIFE THROUGH IT
Vidha Pandey	<i>Bansthali Vidyapeeth University</i>	GENE-EDITING TECHNOLOGY




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HEAD OF SESSION: Assoc. Prof. Dr. Nilgun ULUTASDEMIR

Assoc. Prof. Dr. Nilgun ULUTASDEMIR	<i>Gümüşhane University</i>	RISK MANAGEMENT IN DISASTERS
Assoc. Prof. Dr. Nilgun ULUTASDEMIR	<i>Gümüşhane University</i>	PREPAREDNESS FOR DISASTERS
Assist. Prof. Dr. Servet AŞKIN	<i>Iğdir University</i>	DISSOLVED RATE OF ARSENIC- MOLYBDENUM IN MOUNT ARARAT VOLCANIC ROCKS IN ULTRAPURE WATERS
Assist. Prof. Dr. Servet AŞKIN	<i>Iğdir University</i>	COMPARISON IRON, COBALT, AND NICKEL IN VOLCANIC ROCKS AND SANDSTONES CONCENTRATION MEASUREMENTS OF WITH ICP-MS
Assist. Prof. Dr. Hüseyin ASLAN	<i>Sakarya University</i>	FREQUENCY OF LABORATORY TESTING IN THYROIDECTOMY PATIENTS
Assoc. Prof. Dr. Buket BORA SEMİZ Assist. Prof. Dr. Tarık SEMİZ	<i>Bilecik Şeyh Edebali University</i> <i>Bilecik Şeyh Edebali University</i>	DIGITAL MARKETING IN HEALTH TOURISM: A RESEARCH FOR PRIVATE HEALTH INSTITUTIONS
Dr. CEFEROVA Peri Dr. AHUNDOV Perviz Dr. İBRAHİMOVA Almaz	<i>National Institute of Sports and Medical Rehabilitation</i>	SOME RESULTS OF THE MEDICAL REHABILITATION PROGRAM OF KARABAKH WAR VETERANS

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HEAD OF SESSION: Prof. Huseynova Gulgiz Agahasan

Assist. Prof. Dr. Oya GÜVEN Dr. Bedriye Feyza KURT	<i>Kırklareli University</i> <i>Kırklareli Training and Research</i> <i>Hospital</i>	RETROSPECTIVE ANALYSIS OF PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH POISONING AFTER ORAL DRUG INGESTION
P.H.Najafgulyeva	<i>II Department of Surgical</i> <i>Diseases of AMU</i>	MODERN ASPECTS OF THE TREATMENT AND PREVENTION OF GASTRODUODENAL HEMORRHAGE IN POSTKOVID PERIOD
Dr. Muhammed Raşit ÖZER Dr. Kader Zeybek AYDOĞAN Dr. Ali AVCI Dr. İsmail BALOĞLU	<i>Karaman Training and Research</i> <i>Hospital</i> <i>Niğde Training and Research</i> <i>Hospital</i>	FACTORS PREDICTIONING TO INTENSIVE CARE HOSPITALIZATION IN PARENTS APPLYING TO THE EMERGENCY DEPARTMENT DUE TO COVID-19
Res. Ast. Sema KONATEKE Prof. Dr. Şükriye İlkay GÜNER	<i>Gaziantep University</i>	MALNUTRITION RISK AND EVALUATION OF NUTRITIONAL SCREENING TOOLS IN SURGERY PATIENTS
Prof. Dr. Şükriye İlkay GÜNER Res. Ast. Sema KONATEKE	<i>Gaziantep University</i>	PATIENT SAFETY PROBLEM IN THE OPERATING ROOM: RETAINED SURGICAL ITEM
Prof. Huseynova Gulgiz Agahasan Mammadova Arifa Jumail	<i>Azerbaijan Medical University</i>	ABOUT THE GLANDS GENERAL EXCRETORY DUCTS OF THE HUMAN URINARY BLADDER IN NORM
Anar Abdullayev Nigar Allahverdiyeva	<i>Azerbaijan Medical University</i>	RELATIONSHIP OF THE METOPISM WITH FEATURES OF THE FRONTAL AND SPHENOID SINUSES

Kaydediliyor... Görünüm

OBSERVER H-4...

H4-Süleyman Emre

OBSERVER H-4 ÜLKÜ EKŞİN

H-4, Osman Özkan DOĞAN

H-4, Ali Acar

H-4, ŞULE GÖKMEN - EBRU YURDAKURBAN

H44-Tuba UNVER

Sesi aç Videoyu Başlat Katılımcılar 6 Sohbet Ekran Paylaşımı Kaydı Duraklat/Durdur Ara Odaları Tepkiler Odadan Çık

Kaydediliyor... H-4, Osman Özkan DOĞAN ekranını görüntüleyorsunuz Görüntü Seçenekleri Konuşan: H-4, Osman Özkan D...

1 2 3 4 5 6 7 8 9 10 11 12

DENTAL ANKSİYETE

Yaş Age

Eğitim Düzeyi Educational Level

Cinsiyet Gender

Olumsuz Deneyimler Unfavorable Experiences

Tedavi Kaygısı Worry in Treatment

Ağrı Pain

Dental Tedavi Sıklığı Dental Visit Frequency

Kişilik Yapısı Personality

□ Muğlalı M, Kömenik N. Ağrı cerrahisi ve anksiyete. CÜ Diş Hek Fak Derg 2005; 8(2):88- 88.
□ Kumar S, Bharzav P, Patel A et al. Does dental anxiety influence oral health-related quality of life? Observations from a cross-sectional study among adults in Usdaipur district, India. J Oral Sci 2009; 51(2): 245-254

Not eklemek için tıklayın

Slayt 3 / 18 Ofis Teması

Sesi aç Videoyu Başlat Katılımcılar 6 Sohbet Ekran Paylaşımı Kaydı Duraklat/Durdur Ara Odaları Tepkiler Odadan Çık

Kaydediliyor... H-4, ŞULE GÖKMEN - EBRU YURDAK... ekranını görüntüyorsunuz Görüntü Seçenekleri Konuşan: H-4, ŞULE GÖKMEN ...

YAPAY ZEKA - ARTIFICIAL INTELLIGENCE

- Bilgisayar sisteminin insan benzeri öğrenme ve sorun çözme işlevlerini taklit edebilme becerisi
- İlk defa 1956 yılında ortaya atılmıştır.
- *The ability of the computer system to emulate human-like learning and problem-solving functions.*
- *It was first introduced in 1956.*

Artificial Intelligence (AI)

Machine Learning (ML)

Deep Learning (DL)

Convolutional Neural Networks (CNN)

<http://sitn.hms.harvard.edu/flash/2017/history-artificial-intelligence>

Yapay zeka, bilgisayar sisteminin öğrenme ve sorun çözme gibi insan benzeri bilişsel işlevleri taklit edebilme becerisini tanımlayan genel bir terimdir. İlk yapay zeka programı 1956 yılında bir sunulmuştur. Yapay zeka algoritmalarının mimarisinde insan beyninin biyolojik sınırlarından esinlenilmiştir. Çeşitli yapay nöronlar, katmanlar halinde düzenlenmiş bir ağ oluşturarak birbirlerine bağlanır ve sayıları yapay zekanın eğitileceği görevin karmaşıklığına bağlı olarak değişir. Tıpta ve dış hekimliğinde yapay zekanın farklı özelliklerdeki alt kümelerinden yararlanır.

Sesi aç Videoyu Başlat Katılımcılar 7 Sohbet Ekran Paylaşımı Kaydı Duraklat/Durdur Ara Odaları Tepkiler Odadan Çık

Kaydediliyor... Görünüm

H3-Ahisen KARADAĞ

OBSERVER-H-3 ÖLKÜ EKŞİN

Hall-3-Ramazan Erenler

H3-3 Assoc. Prof. Dr. Mahira Amirova

Hall-3, Cansu Altuntaş

H3-Umit VILDJKO

H3-Volkan TAŞ... H3-Volkan TAŞDEMİR

H3-Ercan Saruhan

H3-Berna Niş

Sesi aç Videoyu Başlat Katılımcılar 9 Sohbet Ekran Paylaşımı Kaydı Duraklat/Durdur Ara Odaları Tepkiler Uygulamalar Odadan Çık

Kaydediliyor... Görünüm

H-3, Kaberi Pramanik;session-2

OBSERVER H-3 ÖLKÜ EKŞİN

H-3, Aditi Chauhan

H-3, anisha Chauhan

H-3 Shailja Sharma

H-3 Aishwarya K.A

H3-Vidha pandey

6 atanmamış katılımcı

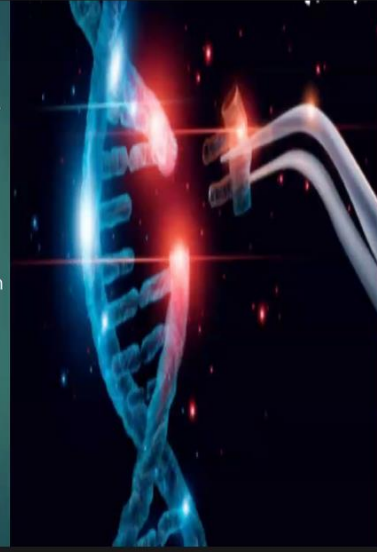
H3-Vidha pandey

Sesi aç Videoyu Başlat Katılımcılar Sohbet Ekran Paylaşımı Kaydı Duraklat/Durdur Ara Odalar Tepkiler Uygulamalar Odadan Çık

Kaydediliyor...

- ▶ Biotechnology is the technology that utilizes biological systems, living organisms or parts of this to develop or create different products.
- ▶ Brewing and baking bread are examples of processes that fall within the concept of biotechnology to produce the desired product.
- ▶ Biotechnology deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.

- ▶ Biotechnology is the technology that utilizes biological systems, living organisms or parts of this to develop or create different products.
- ▶ Brewing and baking bread are examples of processes that fall within the concept of biotechnology to produce the desired product.
- ▶ Biotechnology deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.



KURAKLIK STRESİ



- Kuraklık stresi dünya çapında birçok bölgede verim ve ekonomik kayıplara neden olan en önemli streslerden biri olarak kabul edilmektedir.
- Ortalama verimi %50 veya daha fazla azaltarak tahıl kalitesini ve tahıl eldesini ciddi şekilde etkileyebilir (Wang vd., 2003).
- Kurak ve yarı kurak iklimlerde, toprak su kavnağındaki azalma veya bitkilerde yüksek terleme oranı yüzünden kuraklık stresi meydana gelebilir (Somerville vd., 2001).
- Kuraklık stresi, hücre içinde meydana gelen bir dizi metabolik reaksiyonu olumsuz yönden etkilemesinin yanı sıra (Ashraf ve Foolad, 2007), fotosentez, kuraklıktan büyük ölçüde etkilenen en önemli metabolik süreçlerden biridir (Kalaji vd., 2016).
- Sonucunda, bitkilerde stoma iletkenliğinin (gs) azalması ve stomaların kapanması durumu meydana gelir (Ali ve Ashraf, 2011).
- Hem stomaların kapanması hem de metabolik bozuklukların meydana gelmesi fotosentez için önemli bir sınırlama olduğu bilinmektedir.





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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIAL AGENTS OF URINARY TRACT INFECTION AND GENITAL TRACT AMONG WOMEN IN SHEKHAN DISTRICT, IRAQ

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ABSTRACT

Background: Urinary tract and urogenital tract is linked and interconnected so invasion by resistant microbial pathogens can extends and complicate women health.

Objective: to know bacterial causes and efficacy of antibiotics of urinary and genital tracts infection of Iraqi women for better understanding of antibiotic treatment strategy.

Method: During January 2018 till September 2020, 224 women (from inside and outside of Shekhan in campuses) that clinically suspected with urinary and urogenital tract infections were referred to Gynecologist clinic in Shekhan. Midstream urine (78) and HVS (146) samples were collected in the laboratory and inoculated on blood, Chocolate, MacConkey agar media in duplicate aseptically and incubated aerobically at 37°C. Colony forming units were counted 24 hours later, and if count $\geq 100,000/\text{ml}$, antimicrobial sensitivity studies were then performed and read after a further 24 hours. Microorganism identification and characterization using routine morphological and biochemical methods were applied. Antibiotic-susceptibility test was performed using disk-diffusion method.

Results: Exactly 107 (73.2%) and 57 (73%) revealed positive growth for HVS and urine respectively. Single infections were predominant. *Escherichia coli* revealed the highest prevalence 18 (32%) in urinary tract, while *non-hemlytic Streptococcuss* was highest in genital tract 32(30%). *Staphylococcus aureus* showed the next highest rates 21(20%) and 8(14%) in HVS and urine respectively. *Candida albicans* detected 19% and 9 % in HVS and urine respective. Amikacin expressed efficacy 87% and 71% for urine and HVS isolates, respectively followed by nitrofurantoin 77%, 63%, gentamicin 64%, 68% for urine and HVS isolates, respectively. The highest resistance rates and low efficacy were recorded to ampicillin, amoxicillin, cefixime and nalidixic acid. There was no statistical significant variation in drug susceptibility between isolate from urine and HVS

Conclusion: various microbial causes could interconnect to infect urinary tract and vaginal tissues. Moreover, incorrect use and high dose of antibiotics may leads to selective pressure and emergence of multiple resistant isolates. These results are worrisome and essential care should be taken in maternity unit and drug prescribing policy should be monitored and updated.

Keywords: Urinary tract infection, Urogenital tract, *Escherichia coli*, *Candida albicans*
Comparative study

1. Background

Almost urinary tract infection (UTI), cystitis, vaginitis and pyelonephritis are the most common cause and concerns of urogenital infections among female and women in all age groups (Onuh, 2005; Nwadike, 2015) That is why, more female gender in all-purpose seeks to visit hospitals and gynecology clinic within her routine checkup regardless of any signs of infections. The risks of incidence of asymptomatic infections were in increasing among ante-natal by hypertension, still birth, abortion, preterm labor and thrombosis (Anyadoh, 2010). There are various microbial causes behind UTI (frequency, urgency, nocturia, urge incontinence) and genital tract in women. Several studies have shown that in approximately half of the patients, the symptoms are due to bacterial cystitis caused by well-known urinary pathogens such as *Escherichia coli* and other bacteria species and *Candida* (Scaldazza, and Morosetti, 2006). Moreover, recurrent UTI and reproductive tract infections can further complicate the situation and create symptoms of sexually transmitted diseases. However, it is noticeable that very often women in our locality do not report to healthcare facilities due to various impediments such as their low status in society, illiteracy, ignorance, and rigid social norms (Ray *et al*, 2009).

In our locality, complication of UTI and urogenital infections that specifically caused by multidrug resistant microbes can cause serious problems for women. This serious problem is more concern in developing countries where drug prescribing policy is not controlled. Over and above, random antibiotic treatment will develop multidrug resistant bacteria (MDR) making infection treatment more difficult task that threats to women and fetus in case of pregnancy (Nwadike, 2015). This situation here will reduce rate of using safe antibiotic and hence treatment successes hard to achieve and infection impossible to eliminate (Neu, 1992). In each study setting, specifically in underdeveloped countries, where prescribing antibiotics is not controlled reporting of rates of gram negative and gram positive multiple-drug resistant (MDR) suppose to be performed and its urgent indication for a better drug surveillance control system (Momoh *et al*, 2007). Therefore better surveillance of microbial causes and drug policy is required for rescheduling of empirical therapy (Onuh, 2006). Most studies in our area concentrated on prevalence of UTI with less concern of genital tract infection. The purpose behind this study is to find out prevalence of microbial causes and their drug resistant patterns

among woman suspected with UTI and genital tract that stay inside and outside(in campuses) Shekhan district, Duhok city, Iraq.

2. Material and Methods

2.1 Study setting and Population

The study population enrolled 224 women staying inside and outside Shekhan district (in Campuses). Those women that staying inside Shekhan district they were directly referred as outpatients to visit Gynecologist clinic (Dr Ekram Lugman) that located in center of Shekhan district. For those women in campuses, the (War child Uk organization) had coordinated, encouraged, facilitated and taken responsibility transferring then referring them to visit that Gynecologist clinic or further consultation and doing laboratory investigations. Formal oral consent was taken from each subject before participating in the study.

2.2 Inclusion Criteria

Patients not treated with any antibiotics and antifungal treatment within 3 days ago.

2.3 Exclusion Criteria

Patients already treated with any antibiotics and antifungal treatment during 3 days of their visit to Gynecologist clinic and those who refused formal oral consent.

2.4 Sample Collection, Processing and Identification

Overall 224 women, 146 and 78 were clinically suspected with urogenital tract infection and UTI respectively. As routine laboratory examination work, clean-catch MSU (Mid-Stream Urine) in wide open mouth sterile container and HVS (high vaginal Swab) samples were collected form suspected women as described by (White, 2011). Patients were instructed to wash and clean urethro-genital organs and to pass the first void of urine then to collect midstream sample into sterile container. Samples were labeled and analyzed within 30 minutes of collections. Urine and HVS samples were examined microscopically according to (Lane and Takhar, 2011). Those showed 10 white blood cells/ mm³ were regarded as pyuric and urogenital infection (Tantry and Rahiman, 2012). Samples were cultured on blood, chocolate, MacConkey agars and Sabouraud dextrose agar (Difco) in duplicate aseptically and incubated aerobically at 37°C. All isolated bacteria were identified and characterized by Gram stain followed by microscopic examination, motility test and biochemical tests as described by (AAFP, 2004). The characterization of *Candida albicans* was done by the germ tube test and morphological examination. Bacterial colony forming units were counted 24 hours later, and if number is $\geq 100,000$ colony/ml of urine, antimicrobial sensitivity studies were then performed and read after a further 24 hours.

2.5 Antibiotic Susceptibility Assay

Antibiotic susceptibility assay for all isolates were performed according to Kirby-Bauer disc diffusion technique (Bauer *et al.*, 1966) as per the recommendation of Clinical and Laboratory Standards Institute (CLSI) (Weinstein *et al.*, 2017). Antibiotics panels were; amikacin(10 µg/ml), ampicillin(20 µg/ml), amoxicillin(25 µg/ml) , cefixime(30 µg/ml), cephalothin(10 µg/ml), cefotaxime (30 µg/ml), amoxiclav (25/20 µg/ml), nitrofurantoin(20 µg/ml), nalidixic acid(10 µg/ml), norfloxacin(30 µg/ml), rifampicin(10 µg/ml), ciprofloxacin (10 µg/ml), gentamicin (10 µg/ml), trimethprime-sulphamethoxazol (10/50 µg/ml),

3. Results

A total of 224 women clinically suspected with UTI and urogenital tract infection that were referred to the laboratory for urine and HVS culture , 164 of them revealed microbial growth giving prevalence rate of 57(73%) and 107(73.2%) for urine and HVS sample, respectively. The rates were close with little of HVS higher than urinary tract infection. The prevalence and the rate of positive cultures revealed predominant of single isolates that were (74.6%) and (66.3%) for urine and HVS respectively. *E. coli* was the predominant organism with prevalence rate of 18(32 %) in urine samples while *non-hemolytic Streptococcus* showed the highest prevalence rate 32(30%) for HVS samples as in Table 2.1

Table 3.1: Rates of Microbial profile of UTI and genital tract infection

Microbial causes	UTI No. (%)	HIV No. (%)
<i>E coli</i>	18 (32)	16 (15)
<i>Klebsiella pneumoniae</i>	7 (12)	9 (8)
<i>Enterobacter</i>	1 (1.7)	2 (1.8)
<i>Proteus</i>	1 (1.7)	1 (0.9)
<i>Acenitobacter</i>	1 (1.7)	1 (0.9)
<i>Pseudomonas aeruginosa</i>	----	1 (0.9)
<i>Staphylococcus aureus</i>	8 (14)	21 (20)
<i>Non-hemolytic Streptococcus</i>	13 (23)	32 (30)
<i>Strep pyogenes</i>	2 (3.4)	-----
<i>Strep agalactiae</i>	-----	2 (1.8)
<i>Strep fecalis</i>	-----	1 (0.9)
<i>Listeria monocytogenis</i>	1 (1.7)	1 (0.9)
<i>Candida albicans</i>	5 (9)	20 (19)
Total of positive growth	57 (73)	107 (73.2)
No growth	21 (27)	39 (26.8)

Table 3.2 shows results of antibiotic susceptibility test that amikacin was expressed efficacy 87% and 71% for urine and HVS isolates, respectively followed by nitrofurantoin 77%, 63%, gentamicin 64%, 68% for urine and HVS isolates, respectively. The highest resistance rates and low efficacy were recorded to ampicillin, amoxicillin, cefixime and nalidixic acid. There was no statistical significant variation in drug susceptibility between isolate from urine and HVS. These results are worrisome and essential care should be taken for pregnant women and efficient drug prescribing policy should be explored.

Table 3.2: Antibiotic susceptibility rates of Microbial profile of UTI and genital tract infection

Antibiotics	UTI susceptibility rate %	HIV susceptibility rate %
Amikacin	87	71
Nitrofurantoin	77	63
Gentamicin	64	68
Ciprofloxacin	64	59
Cefotaxime	52	43
Rifampicin	48	47
Amoxicillin-clavulinic acid	48	48
Norfloxacin	37	36
Cephalothin	37	30
Sulfa-methoxazol	27	25
Nalidixic acid	15	13
Cefixime	12	6
Amoxicillin	8	3
Ampicillin	2	1

Table 3.3 shows the number and rate of samples with mixed growth culture (polymicrobial) that was 7.5% and 7.0% for HVS and urine samples, respectively. Most of them occurred with *Candida+ non-hemolytic Streptococcus* and among HVS isolates.

Table 3.3: Rates and patterns of mixed cultures (polymicrobial)

Co infection profile	UTI No.	HIV No.
<i>Candida+ non-hemolytic Streptococcus</i>	3	5
<i>Candida+ Klebsiella pneumoniae</i>	1	2
<i>Candida+ Staph aureus</i>	---	1
Total (12)	4 (7%)	8 (7.5%)

4. Discussion

Knowledge of bacterial causes and patterns of their antibiotics susceptibility patterns of urine and HVS routinely is could be mandatory for initiation of empirical therapy. The overall prevalence of bacterial isolates of HIVS and urine was (73.3%) and (73.0%) respectively, found in this study. However, prevalence rate is not as high as but rather close to each other and may suggesting an interlinked female urogenital microbiota that is not only limited to pathogens but is also characteristic of health-associated commensals. Although most of the vaginal bacteria are not pathogenic unless they have chance to overgrown in numbers or in case of an abrasion they will induce infection (Whiteside *et al*, 2015). Our data was in accordance with a study in Jordan found close results of urine and HVS samples as (51.3%) and 31(43.7%), respectively (Battikhi, 2018). Similar data also reported by (Nwadike, 2015). Other studies disagree with our data found high prevalence of urine isolates over HVS sample and concluded that could be related to the site of collection (Sylvia *et al*, 2015; Stacey *et al*, 2003). This is quite understandable due to different geographical location and social and economical status of women. Current study stated that *E. coli* was the prevalent organism followed by *non-hemolytic Streptococcus* in urine samples however; *non-hemolytic Streptococcus* was the prevalent bacterial isolate from genital tract followed by *E. coli* as in Table 3.1. Dissimilar to our results, others observed *E. coli* prevalent organism followed by *S.aureus* in urine samples and *S. aureus* prevalent isolate followed by *E. coli* from genital tract (Sylvia *et al*, 2015; Battikhi, 2018). During women cycle any hormonal changes may provide the ideal environment for UTI-causing bacteria and increase risk of infections. (Homeier, 2004). *Candida albicans* showed higher incident rate in samples isolated from HVS 20(19 %) than urine 5(9%) in this study. This data agree with (Sylvia *et al*, 2015; Battikhi, 2018) who they found *Candida albican* more in HVS than urine. Women vagina work as suitable niche condition supporting for growth of different types of aerobic and anaerobic bacteria as well as *C. albicans* due to moisture habitat and rich glycogen content (Homeier, 2004; Stacey *et al*, 2003). Nevertheless, some samples in current study showed no bacterial growth culture that could be due to sphincter spasms or anatomical variations of the genitalia (Kaplan *et al*, 1980) or non-cultivable microorganisms (Browne *et al*, 2016).

In present study the polymicrobial infections was appeared in 7.5% and 7.0% for HVS and urine samples, respectively. Co-infection with *Candida + non-hemolytic Streptococcus* isolates occurred more among HVS and urine samples. This is already mentioned by (Sylvia *et al*, 2015; Battikhi, 2018) but correlation was more with *Candida + S. aureus*. The association of the existence of polymicrobial cultures is that bacterial synergism and antibiotic resistance make the selection of an optimal antibiotic regimen challenging (Melven, 1090; Sylvia *et al*, 2015), particularly in the developing countries including Iraq, where poor health services, insufficient drug supplies and unsure drug quality all favors the emergence of microbial resistance.

Regarding antibiotics susceptibility pattern, current study demonstrated that amikacin was most effective drug 87% and 71 % over urine and HVS samples. Bittichi, 2015 found ciprofloxacin was most efficient 89%, 86% among urine and HVS (Battikhi, 2018). Other study also concluded ciprofloxacin most effective antibiotic 88.6% and 87.8% in urine and HVS samples (Sylvia *et al*, 2015). Ciprofloxacin efficacy was somewhat consistent between the specimens 64 % and 59 % in current study and was the fourth in rank of efficacy regimen. This is due to fluoroquinolones are newer drugs with mode of action central on inhibition of the DNA replication (Knobler, 2003; Kaplowitz, 2005) but are relatively not expensive in our locality therefore they are more likely available for abuse. Present study showed nitrofurantoin and gentamicin expressed good efficacy more than ciprofloxacin and nalidixic acid 77 %, 66% and 64 %, 68% over urine and HVS respectively. While, other researchers observed moderate efficacy (Sylvia *et al*, 2015; Battikhi, 2018). This indicate rational use of gentamicin in UTI and genital infection treatment namely, in Iraqi pregnant women as first line of treatment for the fact that gentamicin is safe during pregnancy (Olukoya *et al*, 1995). Additionally, nitrofurantoin may have some medical implication efficacious more in UTIs little quite efficacious in vaginal infections (Sylvia *et al*, 2015). In present study, no significant statistical variation in drugs susceptibility of urine and HVS isolates seen by amoxiclav that showed equal efficacy 48% respectively. This data is in agreement with (Nwadike, 2015). In current study, moxicillin, ampicillin and cefixime showed very low efficacy (<10%) this is not surprising result since these drugs used widely for treatment of various type of infections caused development of drug resistant strains. This agree with (AAFP, 2004; Onuh, 2006; Sylvia *et al*, 2015). Therefore, it is not recommended to use those drugs for therapy of UTI and vaginal infection in our setting.

Our study concludes that various microbial causes were behind UTI and urogenital tract infections and they could interconnect and linked. Incorrect use and high dose of antibiotics may leads to selective pressure and emergence of multiple resistant isolates. Our recommendation is to carry out routine HVS and urine culture for all symptomatic women in any population to emphasize correct empirical therapy to ensure good health for future especially during pregnancy and fetus regardless sign of infections.

5. Acknowledgments and Declaration

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**MICROBIAL AND PHYSICO-CHEMICAL ASSESSMENT OF SOIL AND WATER
AROUND WASTE DUMP SITES IN LAGOS**

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ABSTRACT

In Nigeria, the reliance on sanitary landfills is a common phenomenon in the disposal of waste materials. The aim of the study was to ascertain the physicochemical and microbiological effect landfill has on its surrounding soil and water. Four water samples and five soil samples were collected each from Ile-Epo and Legacy dumpsites and the adjoining areas. Physicochemical parameters determined were temperature, pH, total dissolved solids (TDS), total hardness (TH), and electrical conductivity. Most of these parameters indicated pollution but were below the World Health Organization (WHO) limits for consumption in the water. Microbiological analysis was carried out using standard microbiological procedures. The mean bacteria count and fungal count for water and soil samples are 26.41 CFU/mL and 10.00 CFU/mL; and 26.30 and 14.50 (CFU/G) respectively. The antibiotic susceptibility pattern of the bacterial isolates against conventional antibiotics displayed varying degree of susceptibility and resistance; the bacterial contaminants were susceptible to Augmentin, Gentamycin and Chloramphenicol and resistant to Pefloxacin, Amoxicilin, Tarivid, Streptomycin, Sparfloxacin and Ciprofloxacin. The results obtained in this study showed that the leachate generated from the landfill site has a minimal impact on the groundwater and soil quality in the locality.

Keywords: Antibiotics, Dumpsite, Landfill, Microbiological, Physicochemical.

**CLASSICAL IDENTIFICATION OF THE GENERA OF LACTIC ACID BACTERIA
ISOLATED FROM MATURE HUMAN MILK**

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ABSTRACT

Breast milk is one of the vectors of transmission of the microbiota to the baby. The microbiota is influenced by various factors such as: the stage of lactation, genetics, mode of delivery, the health status of the mother, nutrition, etc...

In this study, we took more than ten milk samples from healthy mothers, naturally delivered and lactating infant aged from 540 to 700 days postpartum. We found that the estimated lactic flora (catalase negative and gram positive) at this stage is lower compared to non-lactic flora (already catalase positive or gram negative).

We identified lactic strains at the genus level using morphological methods (size, color and diameter of colonies, gram stain, bacillus or coccus) and some biochemical methods (catalase test, growth at 10°C for 10 days, growth at 45°C for 48 h, production of CO₂ from glucose, growth in the presence of 6.5% NaCl...etc).

From the results of these tests, we concluded the presence of a dominant strain which is the enterococci. The latter are characterized by their resistance to hostile conditions (like pH 9...).

Enterococci are used as human probiotics, to treat diarrheal diseases caused by some pathogens. They play also a beneficial role in the flavor development of certain fermented food products such as cheeses.

Keywords: Breast milk, lactic acid bacteria, enterococci.

ASSESSMENT OF SOME SESAME MUTANTS UNDER DROUGHT CONDITIONS

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ABSTRACT

In the context of climate change and water scarcity, it is necessary to develop and use drought-resistant crops. Sesame is an oleaginous and aromatic plant with high nutritional value. It is a tropical crop but is also grown in arid and semi-arid environments such as Morocco. Our objective is to evaluate (in two separate experiments) the response of 11 M2 sesame mutants and two wild types to drought stress at the germination and flowering stages. Severe water stress was simulated at the germination stage by applying (in Petri dishes) polyethylene glycol (PEG) at an osmotic potential of -1.2 MPa to sesame seeds. The measured/calculated parameters were germination percentage (GP), mean germination time (MGT), root to shoot ratio (RSR), and seedling vigor index (SVI). At the flowering stage, stress was simulated (in pots) by suspending and reducing irrigation to 50% of the control from the beginning of flowering until the appearance of the first boll. Drought tolerance was estimated by analyzing their morphological, physiological, and agronomic responses. The results showed a significant effect of genotype, drought, and interaction on all parameters studied in two stages. Interestingly, the mutants 'ML2-5', 'ML2-10' and 'ML2-37' were identified as the most tolerant to severe drought at the germination stage. These mutants also confirmed their drought tolerance at the flowering stage by showing high productivity compared to the other genotypes. These mutants could be considered valuable and relevant genetic material for developing drought-tolerant cultivars.

Keywords: Moroccan sesame, mutant, drought stress, flowering stage, germination stage, drought tolerance.

THE ROLE OF GUT MICROBIOTA ON ENDOCRINE SYSTEM

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ABSTRACT

Dissemination of the gut microbiota exerts several influences on the intestinal regional but specific effects on endocrine organs. In women's lifetime course, microbiota plays a key role as they are associated with the reproductive endocrine system influencing hormones such as estrogen, androgens, insulin, and other hormones. Any disparity on gut microbiota can lead to imbalance in microgenderome process and further aggravates other disease conditions, such as gastrointestinal (GI) diseases, pregnancy complications, polycystic ovary syndrome (PCOS), endometriosis, and cancer. However, trillions of microbes live inside human beings but research on their mechanisms is limited. Our article prime focus is to explore the mechanism of microbiota-hormone-mediated maladies due to imbalance in environmental factors. Further, we have depicted the computer-based approach on metabonomics, proteomics, DNA sequencing in order to afford novel therapeutic as well as preventive strategies. Therefore, our article provides broad perspective on host-microbe interaction and paves way for understanding the role of hormones on gut microbiota.

Keywords: Microbiota, Reproductive endocrine system, Polycystic ovary syndrome, Metabonomics

**RESTORATION OF DEAD LAKE - A CASE STUDY OF CHIMDI LAKE, SUNSARI,
NEPAL**

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ABSTRACT

The information regarding the diversity during rehabilitation stage in any lake area is very few. The study has attempted to know the present condition of the lake in order to have an outcome of restoration of the lake again. This natural lake was totally occupied by the local people and was made farmland. Chimdi Lake, locally known as 'Birju Tal' is the study area located at Chimdi village development committee (VDC) in Sunsari district, Nepal. The total area of the lake is approximately 101.6 hectare. The study carried out for 15 months concluded rich biodiversity of avifauna, herpetofauna, fishes and molluscs after restoring the natural abiotic condition of the lake. The study also showed rich diversity of Zooplanktons. By the effort of local authorities and non-government organization, it was being brought to rehabilitation stage. During the restoration period local villagers especially women contributed their time and effort for conservation of the lake. Abiotic and biotic components of any system are closely interrelated to each other. A change in abiotic component, suitable for aquatic life has increased the diversity of the lake. This is how the study revealed that if the lake is restored again it can provide habitat for many important species of biota. However the continuous management, conservation, planning and infrastructure development would increase the biodiversity of the lake which will be a permanent condition.

Keywords: Rehabilitation, species, biodiversity, villagers, local women

**THE PROTECTIVE ROLE OF LYCOPENE AGAINST POTASSIUM BROMATE
GENOTOXICITY: THE *ALLIUM* TEST**

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ABSTRACT

Potassium bromate (KBrO₃) is used in the production of bakery products, especially in fermented and fermented products such as bread, to make the product whiter in color and appear fluffier. Another purpose of use is to extend the shelf life of bakery products. However, some studies carried out in recent years have revealed that overdose of Potassium bromate is carcinogenic. Lycopene is a natural carotenoid that gives fruits and vegetables their red color. It is found in fruits and vegetables such as tomatoes, watermelon, grapefruit and red peppers, and in unprocessed products such as tomato juice, tomato paste and ketchup. Lycopene is a very powerful antioxidant. In this study, genotoxicity induced by Potassium bromate and the protective role of lycopene against this genotoxicity were investigated by using *A. cepa* (n=16) as an indicator. Mitotic (MI) index, micronucleus (MN) and chromosomal aberration (CA) numbers were used as indicators of genotoxicity. *A. cepa* bulbs were divided into six (6) groups as one (1) control and five (5) treatments. The bulbs in the control group were germinated with tap water and the bulbs in the application group were germinated with 100 mg/L dose of Potassium bromate and two different doses of lycopene (215 and 430 mg/L). The germination process was continued uninterruptedly for 72 hours. At the end of the period, the germinated root tips were washed, cut to a certain size and made ready for microscopic examinations using the crush preparation technique. As a result, the highest MI and lowest MN and CA numbers were observed in the control group (group I), 215 and 430 mg/L lycopene treated groups. No statistically significant difference was observed between the measured genotoxicity values in these groups (p>0.05). A significant (p<0.05) decrease in MI and a significant (p<0.05) increase in MN and CA numbers were detected in Group IV exposed to 100 mg/L dose of Potassium bromate. Potassium bromate exposure promoted CAs in root tip meristem cells in the form of fragment > sticky chromosome > vagrant chromosome > unequal distribution of chromatin > reverse polarization > nucleus bud > irregular mitosis. Administration of 215 and 430 mg/L doses of lycopene together with Potassium bromate decreased the genotoxicity of Potassium

bromate in Groups V and VI. In addition, it was determined that the 430 mg/L dose of lycopene was more effective in reducing genotoxicity than the 215 mg/L dose. As a result, it was determined that Potassium bromate causes genotoxicity at a certain dose level, while lycopene has a toxicity limiting effect in reducing this toxicity due to its strong antioxidant properties.

Keywords: *Allium cepa*, Genotoxicity, Lycopene, Potassium bromate

INTRODUCTION

Potassium bromate (KBrO_3) is a white, crystalline and widely used food additive. It acts as a ripening agent. It is a well known flour improver. It is generally used as a dough softener to increase bread volume and elasticity. It is also used in the brewing, cheese production, pharmaceutical and cosmetics industries. Potassium bromate can also be formed as a pollutant in drinking water, in the process of converting naturally occurring bromide in water to bromate by ozone. Some studies carried out in recent years have shown that Potassium bromate causes redness and pain in the eyes and skin, as well as kidney failure and the development of cancer. It has been stated by the International Agency for Research on Cancer that there is sufficient evidence that Potassium bromate may be a carcinogen. Therefore, it is classified as a 2b carcinogen. On the other hand, it has been reported that Potassium bromate causes oxidative damage to basic cellular macromolecules by promoting free radical production [1, 2].

In recent years, studies to reduce the toxicity caused by chemicals have focused on the use of herbal products such as *Ginkgo biloba*, sage, green tea, ginger, grape seed, which have antioxidant properties. One of these herbal products is lycopene. Lycopene is a non-cyclic carotenoid pigment that cannot be synthesized in the human body. It is especially found in tomato and tomato-based products (tomato paste and ketchup). It is the pigment that gives the red and orange color of fruits and vegetables such as watermelon, orange, grapefruit, rosehip, pepper. Its main function is to protect plants against photosensitization by absorbing light during photosynthesis. Lycopene is the most potent antioxidant measured in a food. It is one of the most important deactivators of reactive oxygen species (ROS). It can scavenge oxygen radicals ten times more than beta-carotene and alpha-tocopherol. Some studies conducted in recent years have revealed that frequent lycopene consumption reduces the risk of cardiovascular disease, cancer (prostate, esophageal, colorectal and oral cancer), diabetes, osteoporosis and infertility [3, 4].

The aim of this study is to investigate the genotoxic effects of Potassium bromate, which has been used as a food additive for about 90 years, with the help of the *Allium* test and to reveal the potential protective role of lycopene against these effects.

MATERIAL AND METHOD

Product and Chemicals

Lycopene extract (90 capsules x 430 mg) was purchased from SepeNatural, *Allium cepa* L. (n=16) was purchased from a commercial market in Giresun province, and Potassium bromate (CAS number: 7758-01-2) was purchased from Sigma Aldrich.

Test Material and Experimental Groups

A. cepa bulbs of approximately equal size were divided into 6 groups, each group containing 10 bulbs.

- Group I: Control
- Group II: 215 mg/L lycopene
- Group III: 430 mg/L lycopene
- Group IV: 100 mg/L Potasyum bromat
- Group V: 100 mg/L Potassium bromate + 215 mg/L lycopene
- Group VI: 100 mg/L Potassium bromate + 430 mg/L lycopene

The bulbs were placed in pre-sterilized glass beakers. The control group was germinated at 24 °C for 72 hours with tap water. The application groups were germinated with two different doses (215 and 430 mg/L) of lycopene and Potassium bromate at a dose of 100 mg/L for 72 hours at 24 °C. The beakers were checked every 24 hours and the decreasing solution was added. After 72 hours of application, the bulbs were washed with distilled water [5].

Genotoxicity Tests (MI, CAs, MN)

A. cepa root tips were cut about 1 cm long, fixed in Clarke solution for 2 hours, washed in 96% ethanol for 15 minutes, hydrolyzed in 1 N HCl for 17 minutes at 60 °C, kept in 45% glacial acetic acid for 30 minutes and stained overnight in acetocarmine. After staining, the root tips were placed on a slide and lightly crushed with the help of a coverslip and examined under the Irmeco IM-450 TI model research microscope. Detected MN and CAs photographed at x400 magnification [6].

Three criteria suggested by Fenech et al. [7] were taken as basis for the detection of MN.

1. MN should be approximately 1/3 of the nucleus in diameter,
2. MN should be round or oval in shape,
3. If the MN and the nuclear membranes come into contact, they should be clearly distinguishable from each other.

MI, which shows the proportion of cells undergoing mitosis in the total cell, was calculated using Equation (1).

$$MI (\%) = \text{number of cells in mitosis} / \text{total number of cells} \times 100 \quad (1)$$

RESULTS

Genotoxicity induced by potassium bromate exposure in *A. cepa* meristem cells is shown in Table 1 and Figure 1. The highest MI value and the least number of MN and CAs were observed in the control group and Group II and Group III, which were administered two different doses (215 and 430 mg/L) of lycopene. There was no statistically significant difference between the genotoxicity values obtained in these groups ($p > 0.05$). Potassium bromate exposure at a dose of 100 mg/L decreased MI significantly ($p < 0.05$) in Group IV, and increased the number of MN and CAs significantly ($p < 0.05$). Compared to the control group, MI was reduced by approximately 3.59% in Group IV. In addition, MN formation was determined at a rate of 54.6 ± 5.21 in this group. Potassium bromate exposure induced CAs in root tip meristem cells in the form of fragments, sticky chromosome, vagrant chromosome, unequal distribution of chromatin, reverse polarization, nucleus bud and irregular mitosis. The greatest effect of potassium bromate on chromosomes was fragmentation. Fragment damage was observed at a rate of 57.3 ± 5.48 in Group IV, which was administered Potassium bromate at a dose of 100 mg/L. Administration of lycopene together with potassium bromate decreased the toxicity of potassium bromate and caused an improvement in the genetic parameter values examined. It was determined that this improvement was directly related to the applied lycopene dose. Compared to Group IV, in Group VI administered lycopene at a dose of 430 mg/L, MI increased by approximately 1.35%, number of MN decreased approximately 1.73 times, and fragmentation, which was the most observed type of CAs, decreased approximately 1.41 times. It was determined that this increase and decrease was statistically significant ($p < 0.05$).

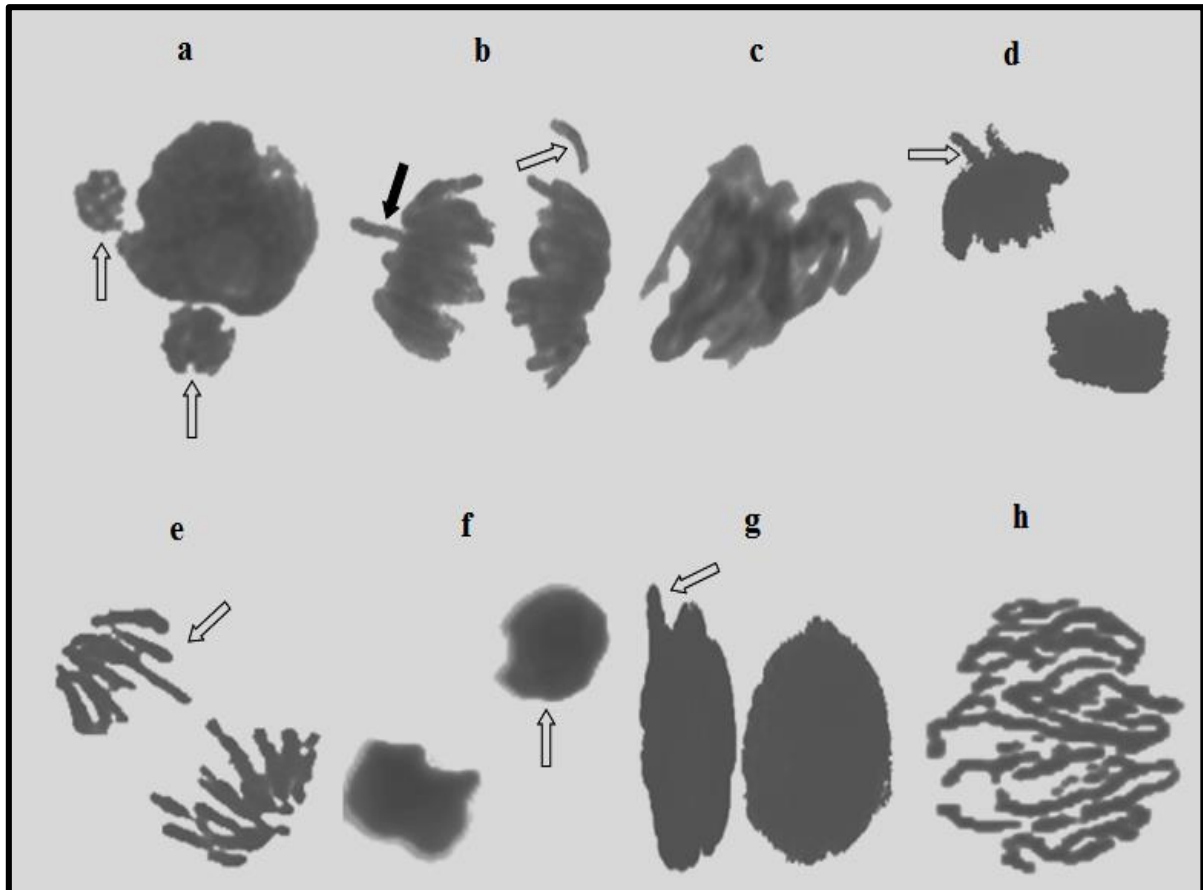


Figure 1 Chromosomal Abnormalities Caused by $KBrO_3$ in Root Tip Meristematic Cells. MN (a), fragment-*white arrow*, vagrant chromosome-*black arrow* (b), sticky chromosome (c), vagrant chromosome (d), unequal distribution of chromatin (e), reverse polarization (f), nucleus bud (g), irregular mitosis (f)

Table 1 The Protective Role of Lycopene Against Genotoxicity Induced by KBrO₃ in Root Tip Meristematic Cells

Damages	Group I	Group II	Group III	Group IV	Group V	Group VI
MI (%)	875±36.7 ^a (8.75)	884±36.9 ^a (8.84)	869±35.4 ^a (8.69)	516±22.8 ^d (5.16)	577±25.6 ^c (5.77)	651±27.5 ^b (6.51)
MN	0.24±0.29 ^d	0.15±0.21 ^d	0.18±0.22 ^d	54.6±5.21 ^a	44.8±4.73 ^b	31.5±3.16 ^c
FRG	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	57.3±5.48 ^a	50.9±4.98 ^b	40.7±4.38 ^c
SC	0.16±0.23 ^d	0.24±0.30 ^d	0.11±0.18 ^d	48.9±4.74 ^a	41.6±4.42 ^b	34.8±3.35 ^c
VC	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	35.7±3.40 ^a	27.5±2.80 ^b	21.3±2.44 ^c
UDC	0.12±0.16 ^d	0.07±0.12 ^d	0.00±0.00 ^d	26.4±2.75 ^a	20.7±2.32 ^b	12.6±1.66 ^c
RP	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	16.8±1.95 ^a	12.1±1.50 ^b	8.50±0.91 ^c
NB	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	12.6±1.62 ^a	8.40±0.88 ^b	5.70±0.63 ^c
IM	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	7.50±0.71 ^a	4.20±0.58 ^b	1.90±0.36 ^c

*Group I: Control, Group II: 215 mg/L lycopene, Group III: 430 mg/L lycopene, Group IV: 100 mg/L KBrO₃, Group V: 100 mg/L KBrO₃+ 215 mg/L lycopene, Group VI: 100 mg/L KBrO₃+ 430 mg/L lycopene. Data are shown as mean ± SD (n=10). MN and CAs were calculated by analyzing 1.000 cells in each group, and MI by analyzing 10.000 cells in each group. The averages shown with different letters^(a-d) in the same line are significant at p<0.05. MI: mitotic index, MN: mikronucleus, FRG: fragment, SC: sticky chromosome, VC: vagrant chromosome, UDC: unequal distribution of chromatin, RP: reverse polarization, NB: nucleus bud, IM: irregular mitosis

DISCUSSION AND CONCLUSION

Potassium bromate application at a dose of 100 mg/L caused genotoxicity by reducing MI and promoting the formation of MN and chromosomal damage in *A. cepa* root tip meristem cells. In the literature, there are very limited studies investigating genotoxicity caused by Potassium bromate in plants. For example, Öztürk et al. [8] investigated the genotoxicity induced by potassium bromate at 25, 50 and 100 mg/L doses in *A. cepa* root meristem cells. They used MI, MN and CAs as indicators of genotoxicity. In conclusion, they reported that potassium bromate decreased MI in root meristem cells and increased the numbers of MN and CAs, depending on the dose. Hoda et al. [9] showed that the application of Potassium bromate at doses of 3, 5, 7 and 9 g/L caused genotoxicity in *Allium sativum* (garlic) root tip cells by significantly reducing the DNA content at all concentrations in a dose-dependent manner. Romnick and Somera [10] reported that exposure to Potassium bromate at doses of 10, 25, 50, 75 and 100 ppm caused a dose-dependent increase in the number of MN in *A. cepa* var. *aggregatum* root tip meristem cells. In this study, the decrease in MI and the increase in MN and CAs numbers as a result of exposure to Potassium bromate may be due to the direct interaction of Potassium bromate with spindle fibers and chromosomes or its indirect effects by creating reactive oxygen species (ROS). Because in some studies, it has been reported that Potassium bromate promotes CAS by causing the production of reactive oxygen radicals. [11].

In recent years, studies to reduce the toxicity caused by chemical agents have focused on the use of different plant extracts with antioxidant properties. In this study, lycopene extract was used against genotoxicity induced by Potassium bromate. By reducing the toxicity of lycopene Potassium bromate, it caused an improvement in MI, MN and CAs parameter values. It was determined that this improvement was directly related to the applied lycopene dose. The potent antioxidant nature of lycopene is thought to play a role in reducing the genotoxic effects of Potassium bromate. Because there are some studies in the literature that lycopene provides effective protection by reducing the toxicity caused by chemical and radioactive agents, thanks to its antioxidant property. For example, Çavuşoğlu and Yalçın [12] observed that lycopene reduced the genotoxicity caused by gamma radiation in human lymphocytes. Kurt and Çavuşoğlu [13] showed that lycopene decreased 1,4 dioxane genotoxicity in *A. cepa* root tip meristem cells. Kalefetoğlu Macar et al. [14] reported that lycopene also reduced the genotoxicity caused by cobalt in *A. cepa* root tip meristem cells.

In conclusion, exposure to Potassium bromate at a dose of 100 mg/L caused genotoxicity in *A. cepa* root tip cells. Administration of lycopene at doses of 215 and 430 mg/L decreased the toxic effects of Potassium bromate and resulted in dose-dependent improvements in the genetic parameter values studied. For this reason, foods containing lycopene should be the foods that must be included in the daily diet in order to reduce the toxic effects of chemical agents such as Potassium bromate.

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**PROTECTIVE ROLE OF ROYAL JELLY AGAINST PHENOXYETHANOL
INDUCED TOXICITY IN *ALLIUM CEPA* L.: GENETIC APPROACH**

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ABSTRACT

Phenoxyethanol is widely used in the manufacture of personal care products such as foundation, make-up products, skin care products, bath foam and detergents, which are frequently used in daily life, due to its antibacterial and antifungal properties. Royal jelly, on the other hand, is a nutrient consisting of flower pollen, honey extract and enzymes produced in the bees' upper jaw glands. It is especially used for feeding baby bees, but it is the basic food for the queen bee in the hive. In addition to B vitamins, it contains minerals such as Fe, Ca, Cu, P and K potassium. In this study, genotoxicity induced by Phenoxyethanol was investigated by using *A. cepa* (n=16) as an indicator. In addition, the protective role of royal jelly against this genotoxicity was tested. Mitotic (MI) index, micronucleus (MN) and chromosomal aberration (CA) numbers were used as the main indicators of genotoxicity. *A. cepa* bulbs were divided into 6 groups as 1 control and 5 applications. Control group bulbs were germinated with tap water for 72 hours. The bulbs in the application group were germinated with 10 mM dose of Phenoxyethanol and two different doses (250 and 500 mg/L) of royal jelly for 72 hours. At the end of the period, the root tips were washed, cut into approximately 1 cm length and made ready for microscopic examination with the help of the crush preparation technique. As a result, the highest MI and lowest MN and CA numbers were observed in the control group (group I), groups II and III, treated with 250 and 500 mg/L of royal jelly, respectively. No statistically significant difference was observed between the genotoxicity values determined in these groups ($p>0.05$). A significant ($p<0.05$) decrease in MI and a significant ($p<0.05$) increase in MN and CA numbers were detected in Group IV exposed to 10 mM dose of Phenoxyethanol. Phenoxyethanol exposure induced CAs in *A. cepa* root tip meristem cells in the form of fragment, vagrant chromosome, bridge, unequal distribution of chromatin, irregular mitosis, nucleus bud, reverse polarization and spindle abnormality. The application of royal jelly at doses of 250 and 500 mg/L together with Phenoxyethanol decreased the genotoxicity of Phenoxyethanol in Groups V and VI. A significant increase in MI ($p<0.05$), and a significant decrease ($p<0.05$) in the MN and CAs frequency were also observed. 500 mg/L dose of royal jelly was more effective in reducing

genotoxicity than 250 mg/L dose. As a result, royal jelly can be used as a very useful nutritional supplement in reducing the toxic effects of chemicals such as Phenoxyethanol or in protecting from their effects.

Keywords: *Allium cepa*, Genotoxicity, Phenoxyethanol, Royal Jelly

INTRODUCTION

Phenoxyethanol is an aromatic glycol ether found naturally in green tea and can also be produced in the laboratory due to its commercial importance. It has a molecular weight of 138.17, a melting point of 14 °C and a boiling point of 237-245.2 °C. Due to its pleasant smell and colorless appearance, Phenoxyethanol is used in the manufacture of products that we frequently use in daily life, such as moisturizing creams, soaps, disinfectants, mascara, sunscreens, shaving creams, shampoos and perfumes. On the other hand, it is also used in the production of insecticides, antiseptics, solvents, ultrasound gels, anesthetics, paint and ink. Phenoxyethanol has a broad spectrum of antimicrobial activity. For this reason, it is especially used for the disinfection of gram negative bacteria such as *Pseudomonas aeruginosa* and Gram + bacteria such as *Staphylococcus aureus* and yeasts such as *Candida albicans*. It exhibits antimicrobial activity by separating oxidative phosphorylation from respiration and competitively inhibiting malate dehydrogenase. It also functions as a bactericidal agent by increasing the permeability of the cell membrane to K ions. It also has a direct inhibitory effect on microbial DNA and RNA synthesis. However, taken in high concentrations via ingestion, inhalation and skin it can cause toxic effects. These effects include skin irritation, liver, lung and kidney damage, and nervous system disorders. It is also used as an insecticide due to its lethal effect on invertebrates and especially insects at low concentrations. Therefore, many non-target organisms in agricultural applications may also be exposed to Phenoxyethanol. Plants are among the organisms most affected by Phenoxyethanol toxicity [1, 2].

Studies carried out in recent years have focused on the use of biological products to reduce the toxicity caused by chemicals. One of these biological products is royal jelly. Royal jelly is a creamy-white and gelatinous product secreted from the hypopharyngeal and mandibular glands of worker bees. It is used to feed the bee larvae in the hive. In addition, the nutrition of the queen bee is provided with this milk. Royal jelly consists of 60-70% water, 11-23% carbohydrates, 9-18% protein and 4%-8% fatty acids, lipids, vitamins and minerals. In recent years, studies focusing on reproduction, neurological diseases, aging and cancer have shown that royal jelly has antioxidant, antibacterial, anti-inflammatory and antitumor activities. These activities depend on substances such as royalicin, major royal jelly proteins, biotin, niacin, thiamine, pyridoxine, riboflavin, inositol, gamma globulin, folic acid, pantothenic acid and 10-hydroxy-2-decenoic acid in the structure of royal jelly. In addition, royal jelly contains plenty of minerals such as Ca, Cu, Fe, S and P [3, 4].

The aim of this study is to investigate the genotoxic effects of Phenoxyethanol, which is in the structure of cosmetic and cleaning products that are frequently used in daily life, with the help of *Allium* test and to reveal the potential protective role of royal jelly against these effects.

MATERIAL AND METHOD

Product and Chemicals

Royal jelly (500 mg x 10 ampoule) was purchased from SepeNatural, *Allium cepa* (n=16) was purchased from a market in Giresun, and Phenoxyethanol (CAS number: 0000122996) was purchased from Sigma Aldrich.

Test Material and Experimental Groups

A. cepa bulbs were divided into six (6) groups, with ten (10) bulbs in each group.

Group I: Control

Group II: 250 mg/L royal jelly

Group III: 500 mg/L royal jelly

Group IV: 10 mM Phenoxyethanol

Group V: 10 mM Phenoxyethanol + 250 mg/L royal jelly

Group VI: 10 mM Phenoxyethanol + 500 mg/L royal jelly

Bulbs were placed in sterile glass beakers. Control group bulbs were germinated at 24 °C for 72 hours with tap water. The bulbs in the application group were germinated at 24 °C for 72 hours with 10 mM doses of Phenoxyethanol and 250 and 500 mg/L doses of royal jelly. The beakers were checked daily and the decreasing solution was added. At the end of the period, the bulbs were washed with distilled water [5].

Mitotic Index (MI), Chromosomal Abnormalities (CAs) and Micronucleus (MN) Tests

To determine the genotoxicity, the germinated root tips of *A. cepa* were cut about 1 cm long, fixed in Clarke solution for 2 hours, washed in 96% ethanol for 15 minutes, hydrolyzed in 1 N HCl for 17 minutes at 60 °C, kept in 45% glacial acetic acid and stained in acetocarmine for 24 hours. The stained root tips were placed on a slide and crushed with the help of a coverslip and examined under the Irmeco IM-450 TI model microscope. Displayed MN and chromosomal abnormalities were photographed at x400 magnification [6].

MN determination was based on the criteria determined by Fenech et al. [7] These:

4. The diameter of the MN should be approximately 1/3 of the nucleus,
5. MN should be round or oval,
6. MN and nuclear membranes should be distinguishable from each other in contact.

MI, which shows the proportion of cells undergoing mitosis in the total cell, was calculated with the help of the Equation (1) below.

$$MI (\%) = \text{number of cells undergoing mitosis} / \text{total number of cells} \times 100 \quad (1)$$

RESULTS

The genotoxicity induced by phenoxyethanol application in root tip meristem cells of *A. cepa* is shown in Table 1 and Figure 1. The highest MI value and the least MN formation were observed in the control group (Group I) and Group II and Group III, where two different doses of royal jelly were applied. There was no statistically significant difference between the MI and MN values counted in these groups ($p > 0.05$). In addition, no CAs were detected in these three groups. Phenoxyethanol exposure at a dose of 10 mM caused a decrease in MI and an increase in the number of MN and CAs in Group IV. These decreases and increases were found to be statistically significant ($p < 0.05$). Compared to the control group, MI was reduced by approximately 2.51% in Group IV. In addition, MN formation was determined at a rate of 50.7 ± 3.64 in Group IV. Phenoxyethanol administration promoted CAs in the form of fragment, vagrant chromosome, bridge, unequal distribution of chromatin, irregular mitosis, nucleus bud, reverse polarization, and spindle fiber abnormality. The greatest effect of phenoxyethanol on chromosomes was fragmentation. In Group IV exposed to 10 mM dose of Phenoxyethanol, 55.4 ± 3.70 fragment formation was observed. The application of royal jelly together with Phenoxyethanol (at doses of 250 and 500 mg/L) reduced the toxicity of Phenoxyethanol and caused an improvement in the genetic parameter values examined. It has been determined that this improvement depends on the dose of royal jelly applied. Compared to Group IV, MI increased approximately 1.15%, MN number decreased approximately 1.65 times, and fragment formation, which is the most observed CA, decreased approximately 1.51 times in Group VI, where 500 mg/L dose of royal jelly was administered. These observed increases and decreases were found to be statistically significant ($p < 0.05$).

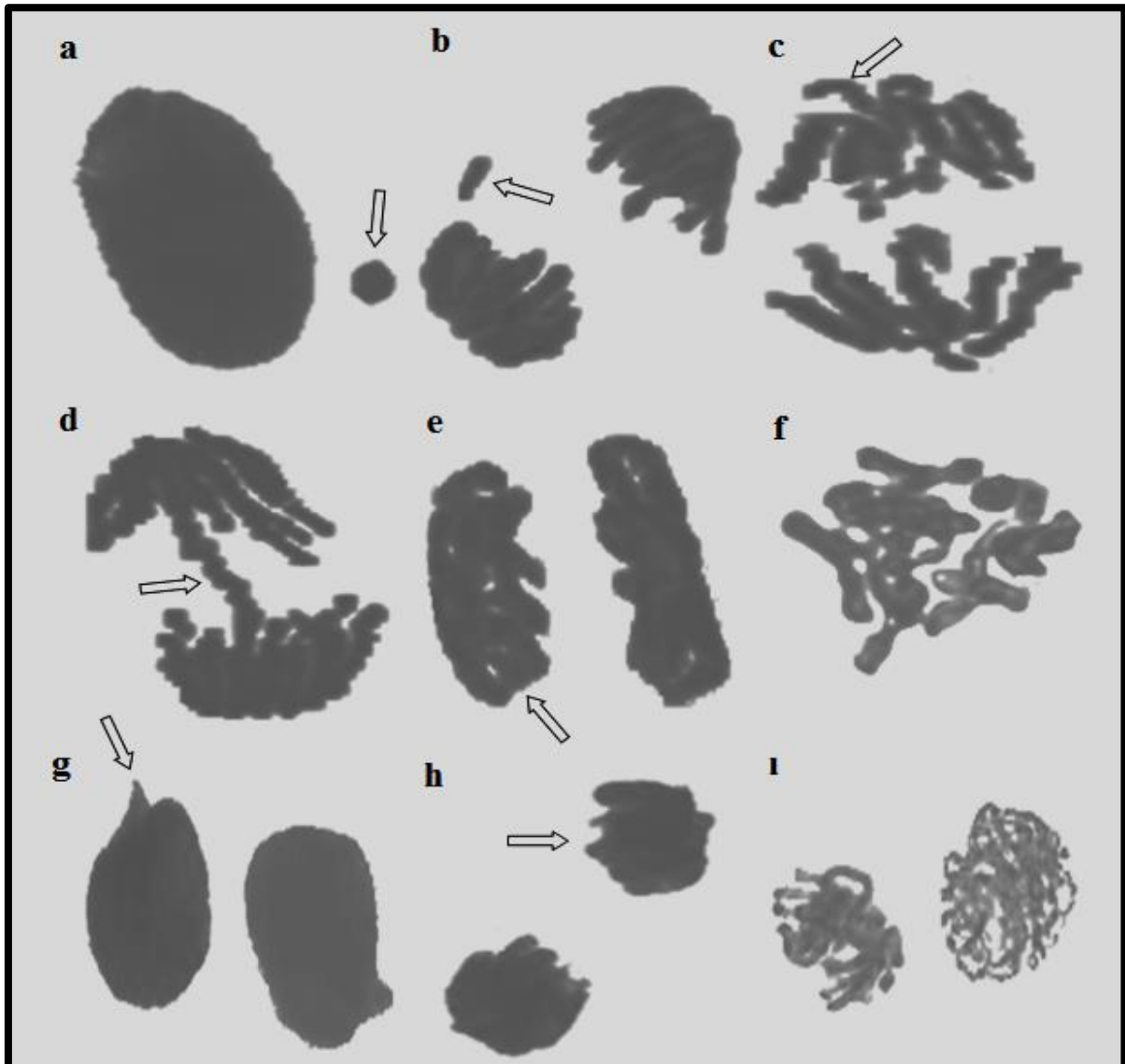


Figure 1 Chromosomal Abnormalities Induced by Phenoxyethanol in Root Tip Meristem Cells. MN (a), fragment (b), vagrant chromosome (c), bridge (d), unequal distribution of chromatin (e), irregular mitosis (f), nucleus bud (g), reverse polarization (h), spindle fiber abnormality (i)

Table 1 Protective Role of Royal Jelly Against Genotoxicity Induced by Phenoxyethanol in Root Tip Meristem Cells

Abnormalities	Group I	Group II	Group III	Group IV	Group V	Group VI
MI (%)	851±40.9 ^a (8.51)	845±38.2 ^a (8.45)	854±41.3 ^a (8.54)	600±30.4 ^d (6.00)	658±32.7 ^c (6.58)	715±35.8 ^b (7.15)
MN	0.18±0.25 ^d	0.21±0.26 ^d	0.12±0.18 ^d	50.7±3.64 ^a	41.2±3.15 ^b	30.7±2.84 ^c
FRG	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	55.4±3.70 ^a	47.1±3.62 ^b	36.8±2.91 ^c
VC	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	40.6±3.10 ^a	32.7±2.88 ^b	25.1±2.62 ^c
B	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	35.8±2.85 ^a	29.4±2.71 ^b	20.6±2.48 ^c
UDC	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	22.5±2.54 ^a	17.6±2.23 ^b	11.5±1.86 ^c
IM	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	15.3±1.90 ^a	11.9±1.54 ^b	7.70±1.15 ^c
NB	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	11.5±1.47 ^a	8.00±0.96 ^b	4.80±0.62 ^c
RP	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	8.70±0.98 ^a	5.10±0.64 ^b	3.00±0.48 ^c
SFA	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	7.20±0.85 ^a	4.30±0.54 ^b	1.80±0.32 ^c

*Grup I: Control, Grup II: 250 mg/L royal jelly, Grup III: 500 mg/L royal jelly, Grup IV: 10 mM Phenoxyethanol, Grup V: 10 mM Phenoxyethanol + 250 mg/L royal jelly, Grup VI: 10 mM Phenoxyethanol + 500 mg/L royal jelly. Data are shown as mean ± SD (n=10). MN and CAs were calculated by analyzing 1.000 cells in each group, and MI by analyzing 10.000 cells in each group. The averages shown with different letters^(a-d) in the same line are significant at p<0.05. MI: mitotic index, MN: mikronucleus, FRG: fragment, VC: vagrant chromosome, B: bridge, UDC: unequal distribution of chromatin, IM: irregular mitosis, NB: nucleus bud, RP: reverse polarization, SFA: spindle fiber abnormality

DISCUSSION AND CONCLUSION

10 mM Phenoxyethanol application caused genotoxicity by reducing MI and promoting the formation of MN and chromosomal damage in root tip meristem cells of *A. cepa*. There are very limited studies in the literature investigating the genotoxicity caused by phenoxyethanol in plants. Therefore, the results obtained were compared with the results of studies on the genotoxic effects of other chemicals. Akgündüz et al. [2] investigated the genotoxic effects of 2.5 mM, 5.0 mM and 10.0 mM doses of Phenoxyethanol in *A. cepa*. MI, MN, and CAs were used as indicators of genotoxicity. In conclusion, it has been reported that exposure to Phenoxyethanol reduces MI in root meristem cells and causes an increase in the number of MN and CAs. In addition, it was observed that these decreases and increases were more pronounced at the 10 mM dose of Phenoxyethanol. Kurt et al. [8] determined that exposure to 1,4 dioxane at a dose of 100 mg/L caused genotoxicity by decreasing MI and increasing MN and CAs in *A. cepa* root tip cells. In addition, 1,4 dioxane promoted CAs such as fragments, sticky chromosomes, unequal distribution of chromatin, bridge and vacuole nucleus in root tip cells. Acar et al. [9] reported that exposure to benzyl benzoate at 10,000, 25,000 and 50,000 mg/L doses caused a dose-dependent decrease in MI and an increase in the number of MN and CAs in *A. cepa* root tip meristem cells. In addition, benzyl benzoate promoted chromosomal damage in the form of fragment, sticky chromosome, bridge, unequal distribution of chromatin and c-mitosis. In this study, the decrease in MI and the increase in MN and CA numbers as a result of Phenoxyethanol administration may be due to the fact that Phenoxyethanol interacts directly with chromosomes or acts indirectly through the free radicals it creates, causing damage and inhibition of spindle fiber synthesis. Because some studies have reported that phenoxyethanol may have a direct inhibitory effect on DNA biosynthesis [10].

In recent years, it has been seen that biological products such as grape seed, *Ginkgo biloba*, green tea, sage, green coffee, ginger and pomegranate have been used in studies to reduce the toxicity caused by chemical agents. In this study, royal jelly was used against genotoxicity induced by Phenoxyethanol. Royal jelly reduced Phenoxyethanol toxicity and caused improvement in MI, MN and CAs values, although not as much as the control group (Group I). It has been determined that this improvement depends on the dose of royal jelly applied. It is thought that antioxidant substances and minerals such as royalicin, biotin, niacin, thiamine, pyridoxine, riboflavin, inositol, gamma globulin, folic acid, pantothenic acid, Ca, Cu, Fe, S and P in the content of royal jelly play a role in reducing the genotoxic effects of Phenoxyethanol. Because there are some studies in the literature that royal jelly provides protection by reducing the toxicity caused by chemical agents, thanks to its antioxidant property. For example, Türkmen et al. [11] reported that royal jelly reduced the genotoxicity caused by petroleum wastewater in *A. cepa* root tip cells. Çavuşoğlu et al. [12] showed that royal jelly decreased cadmium genotoxicity in albino mice. Acar et al. [13] observed that royal jelly reduced genotoxicity caused by lambda cyhalothrin in Swiss albino mice.

As a result, 10 mM dose of Phenoxyethanol application caused genotoxicity in root tip cells of *A. cepa*. The application of royal jelly at doses of 250 and 500 mg/L decreased the toxic effects of Phenoxyethanol and caused a dose-dependent improvement in the values of the genetic parameters examined. Therefore, royal jelly can be used as a very useful nutritional supplement in reducing the toxic effects of chemical agents such as Phenoxyethanol.

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A THEORETICAL RESEARCH ON THE INHIBITORY EFFECTS FOR THE ACTIVE COMPONENTS OF OPUNTIA FICUS-INDICA L. ON CANCER AND SARS-COV-2 VIRUS RECEPTORS

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ABSTRACT

Opuntia ficus-indica L., known as prickly fig or prickly fig, has many species, but it is very important in terms of the active ingredients it contains and their effects on diseases have been investigated recently. In this study, the inhibition effects of sinapic acid, ferulic acid and quercetin, which are important phytochemicals in the fruit of this plant, on lung cancer and SARS-CoV-2 virus receptors were compared with the chemical calculation method. It is important to give the effect of important phytochemicals in this plant fruit by comparing it with the chemical calculation method docking in this epidemic period in cancer patients and especially in individuals with lung cancer, in terms of giving direction to experimental and clinical studies and preventing time and material loss.

Keywords: Opuntia ficus-indica L., sinapic acid, ferulic acid, quercetin, docking

INTRODUCTION

Indian Fig, concentrated in rural areas, contains protein, oil, vitamins (1) and many important active substances to meet the nutritional and health needs of people from ancient times to modern times. Bioactive compounds in *O. ficus-indica* extracts have a chemopreventive role in human cancer cells (2). *Opuntia ficus-indica* is also stated to have anti-COVID-19 utility as a source of potential antiviral drugs (3).

Sinapic acid also binds to the COVID-19 active site via hydrogen bond interaction with His163A, Ser144A and Glu166A (4). Sinapic acid (SA) has been found to show promising selective in vitro antiviral potential (5). Ferulic acid and its derivatives sodium ferulate alone or in combination with other compounds are important compounds in the fight against such emerging diseases like COVID-19 (6).

Quercetin has been reported to be effective in reducing COVID-19 (7). Sinapic acid (SA) is a potent free radical scavenging agent that can inhibit lipid peroxidation and restore endogenous antioxidants (8). Ferulic acid (FA) and its derivatives were found to have positive anti-cancer activities (9). Quercetin, a widely distributed bioflavonoid, is well known to induce growth inhibition in a variety of human cancer cells (10).

MATERIALS AND METHOD

In this study, the inhibition effect of active compounds in prickly figs on Covid-19 and lung cancer receptors was investigated by using docking, one of the chemical calculation methods, by comparing them with docking energy scores (11).

RESULTS AND DISCUSSIONS

The interaction docking scores of phytochemicals (sinapic acid, ferulic acid and quercetin) in prickly fig (*Opuntia ficus-indica* L.) with Covid-19 and lung cancer receptors (6LU7,1mox) are given in Table 1.

Table1 . The interaction docking scores of phytochemicals (sinapic acid, ferulic acid and quercetin) in prickly fig (*Opuntia ficus-indica* L.) with Covid-19 and lung cancer receptors

Receptörs / Ligands	sinapic acid	ferulic acid	quercetin
6LU7(COVID-19)	-311.99	-331.03	-404.58
1mox (LUNG)	-96.93	-69.34	-87.41

(6LU7,1mox)

As shown in Table 1: When the inhibition effects of the main active ingredients (sinapic acid, ferulic acid and quercetin) in *Opuntia ficus-indica* L. on the Covid-19 receptor are listed from largest to smallest;

Quercetin > Ferulic acid > Sinapic acid

As shown in Table 1: When we list the inhibition effects of the active ingredients (sinapic acid, ferulic acid and quercetin) in *Opuntia ficus-indica* L. on the 1mox (LUNG) receptor from largest to smallest;

Sinapic acid > Quercetin > Ferulic acid

Here, as shown in Table 1 receptor-ligand interactions can be mentioned, and based on the literature (12,13), it has been concluded that they form hydrogen bonds, hydrophobic, polar bonds and the other interactions.

CONCLUSION

The hydrogen bonds, hydrophobic, polar bonds and the other interactions between 6LU7(COVID-19) and 1mox (LUNG) receptors and the natural active substances (sinapic acid, ferulic acid and quercetin) show that the inhibitory effect is quite high.

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ANTOSİYANİNLERİN BESLENME VE SAĞLIKTAKİ ROLÜ
THE ROLE OF ANTHOCYANINS IN NUTRITION AND HEALTH

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ÖZET

Antosiyaninler suda çözünen bitki pigmentleri olup doğada 600'den fazla sayıda bulunduğu bilinmektedir. Böğürtlen, yaban mersini gibi birçok bitki dokusunun mavi, mor ve kırmızı rengini verir. Meyve ve meyve suyunun tek bir porsiyonunda ortalama 400-500 mg antosiyanin bulunmaktadır. Antioksidan içerikleri zengin olmasından dolayı sıklıkla bu etkileri ve faydaları ile literatürde adını sıklıkla duyurmaktadır. Antioksidanlar, vücutta oksijenli solunum sonucu ortaya çıkan reaktif oksijen türlerine (ROS) karşı savaşarak vücut savunmasında aktif rol oynamaktadır. Epidemiyolojik ve biyolojik kanıtlar, polifenol tüketimi ile kardiyovasküler hastalık (KVH) riski arasında ters bir ilişki olduğunu tespit etmiştir. Birçok fitokimyasal gibi antosiyaninler, adipogenezini azaltıp lipolizi uyararak yağ asidi β -oksidasyonunu artırma gibi süreçler ile antropometrik değerleri düzenler. Antosiyanin bakımından zengin gıdaların tüketimi de daha düşük tip 2 diyabet riski ile ilişkilendirilirken, diğer flavonoid alt sınıfları için anlamlı bir korelasyon bulunmamaktadır. Aynı zamanda antosiyanin açısından zenginleştirilmiş besinler ile insan kolon kanseri hücresi arasında hücredeki büyümeyi baskılayıcı etkisi gözlenmiştir. Antosiyanin ve bağırsak ilişkisi üzerine yakın zamanlı yapılan üç in vitro, iki hayvan ve bir insan çalışmalarında da sağlıklı bakterilerin artışı ve patojen mikroorganizmaların baskılanması kanıtlanmıştır. Aynı zamanda antosiyanin alımı ile bağırsak geçirgenliğinde azalma, bağırsak uzantılarında iyileşme, bakteri dengesi gibi bağırsak sağlığı ile ilişkili etkenler üzerine olumlu sonuçlar tespit edilmiştir. Antosiyaninlerin uzun zamanlı sağlık üzerine olan etkilerinin anlaşılması için daha fazla çalışmaya ihtiyaç vardır. Bu çalışmada antosiyaninlerin sağlık üzerine olan etkileri ve beslenme ile ilişkisi incelenmiştir.

Anahtar Kelimeler: Antosiyanin, antioksidan, polifenol, sağlık, beslenme

ABSTRACT

Anthocyanins are water-soluble plant pigments, and it is known that there are over 600 of them in nature. It gives the blue, purple and red color of many plant tissues such as blackberries and

blueberries. A single serving of fruit and fruit juice contains about 400-500 mg of anthocyanin. It is commonly mentioned in the literature with these effects and advantages due to its high antioxidant concentration. Antioxidants play an active role in body defense by fighting against reactive oxygen species (ROS) that occur as a result of aerobic respiration in the body. According to epidemiological and biological studies, the intake of polyphenols is negatively related to the risk of cardiovascular disease (CVD). Anthocyanins, like many phytochemicals, modulate anthropometric parameters by increasing fatty acid oxidation while decreasing adipogenesis and increasing lipolysis. Anthocyanin-rich foods were also associated with a lower incidence of type 2 diabetes, while other flavonoid subclasses has not shown any significant correlations. At the same time, a cell growth suppressive effect was observed between anthocyanin-enriched foods and human colon cancer cells. In recent studies three in vitro, two animal, and one human research on the interaction between anthocyanin and gut, the rise of beneficial bacteria and reduction of pathogenic microorganisms were proven. Moreover, positive results were found in factors related to gut health, such as decreased intestinal permeability, healing of intestinal appendages, and bacterial balance of anthocyanin intake. More research is needed to fully comprehend anthocyanins' long-term health impacts. This review was written to examine the properties of anthocyanins, their effects on health and their relationship with nutrition.

Keywords: Anthocyanin, antioxidant, polyphenol, health, nutrition

**GAMA, UV VE ISI STRES FAKTÖRLERİ ALTINDA DÖTERYUM OKSİTİN
DOYMAMIŞ YAĞ ASİTLERİ ÜZERİNE ETKİSİNİN ARAŞTIRILMASI**
EVALUATION OF EFFECT OF DEUTERIUM OXIDE ON UNSATURATED FATTY
ACIDS EXPOSED TO GAMMA, UV, AND HEAT STRESS FACTORS

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ÖZET

Döteryum oksit (D₂O) doğada eser miktarda (%0.015) bulunan, nükleer reaktörlerde reaksiyon hızını ve ısı salımını kontrol etmek için kullanılan normal suyun bir izotopudur. D₂O, biyoloji, tıp, farmasötik, veterinerlik ve biyoteknoloji alanlarında değerlendirilmek için araştırılmaya başlanmıştır. Ancak, gıda ve beslenme konularında çalışmaya rastlanmamaktadır. Bu çalışmada, D₂O'nun, gamma (γ), ultraviyole (UV) ve ısı (Δ), streslerine maruz bırakılan pastörize yumurta sarısı (PYS) bileşimindeki doymamış yağ asitleri üzerine etkisini araştırmak amaçlanmıştır. Bu bağlamda dört adet cam şişede örnekler hazırlanmıştır. Birinci şişeye (kontrol) 40 ml PYS, ikinci (γ ışımaya), üçüncü (UV ışımaya) ve dördüncü (Δ ısı) şişelere ise 40 ml PYS ve 6 ml D₂O pipetlenmiştir. Şişeler 20 rpm/250 s karışmaya bırakılmıştır. γ ışınması ort. 10 kGy/180 dk (min. 5 kGy, maks. 15 kGy), UV ışınması 120 dk (Osram Vital 300W E, 280-400 nm) ve Δ ısı işlem uygulaması ise etüvde 61.5°C/24 saat gerçekleştirilmiştir. Stres uygulamaları sonrası örneklerin doymamış yağ asitleri (linolenik, linoleik, oleik, araşidonik ve DHA) kompozisyonu gaz kromatografi-kütle spektrometri (GC-MS) yöntemi ile yarı-kantitatif analiz edilmiştir. Elde edilen bulgular, D₂O'nun, doymamış yağ asitleri kompozisyonunu, Δ ısı işlemine karşı %98.9, γ maruziyetine karşı %97.5 ve UV maruziyetine karşı %96.0 oranında koruduğunu göstermiştir. Özetle, D₂O ve biyomoleküllerin olumsuz şartlarda stabilite

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davranışlarının gıda ve beslenme açısından sunabileceği olası potansiyel fırsatların daha ileri testler ile araştırılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Döteryum oksit, Doymamış yağ asitleri, Gamma, UV, Heat

ABSTRACT

Deuterium Oxide (D₂O) is an isotope of the regular water present at trace amounts (0.015%) in nature that is used to control the reaction kinetics and heat released in the nuclear reactors. The potential utilization of D₂O has been investigated in biological, medical, pharmaceutical, veterinary, and biotechnological fields. However, it remains unclear in the food and nutritional area. This study aims to evaluate the effect of D₂O on the unsaturated fatty acids composition of the pasteurized egg yolk (PEY) exposed to gamma (γ), ultraviolet (UV), and heat (Δ) stress factors. For that, four samples of PEY were prepared in glass bottles. The first bottle (control) was filled with 40 ml of PEY, and the second (γ radiation), third (UV radiation), and fourth (Δ heat) ones contained 40 ml of PEY and 6 ml of D₂O. Following that, the bottles were initially allowed for shaking at 20 rpm/250 s and subsequently subjected to γ radiation (Ave. 10 kGy/180 min; min. 5 kGy and max. 15 kGy), UV radiation for 120 min by the UV light source (Osram Vital 300W E, 280-400 nm), and Δ heating at 61.5°C for 24 h. After the stress applications, the samples' unsaturated fatty acid composition (linolenic, linoleic, oleic, arachidonic, and DHA) was determined semi-quantitatively by gas chromatography-mass spectrometry (GC-MS). The findings revealed that D₂O protected 98.9% of the unsaturated fatty acids composition against Δ heat stress, 97.5% against γ exposure, and 96.0% against UV radiation. In summary, we concluded that we should investigate the potential opportunities of D₂O to stabilize the behaviors of some biomolecules from food and nutritional perspective.

Keywords: Deuterium oxide, Unsaturated fatty acids, Gamma, UV, Heat

**DISTRIBUTION AND BIOLOGY OF *LEUCOMA WILTSHIREI* COLLENETTE, 1938
(LEPIDOPTERA, EREBIDAE, LYMANTRIINAE) IN SOUTH-EASTERN TURKEY**

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ABSTRACT

The current study deals with probing the distribution and biology of *Leucoma wiltshirei* Collenette, 1938. The situation of the species in Turkey, which is known as a pest on oak in Iran, is discussed and mentioned. Pictures and data of the larva, parasitized larva, pupa, newly discovered parasitoids, and adult are presented and illustrated.

Keywords: Pest, *Leucoma wiltshirei*, Lymantriidae, Lepidoptera, Turkey

INTRODUCTION

The lymantriid genus *Leucoma* was established by Hübner in 1822, based on the type species *Phalaena salicis* Linnaeus, 1758 (Syst. Nat. (Edn. 10) 1: 502). *Leucoma salicis* is widely distributed in Europe as the only species in the genus, as well as *L. wiltshirei* Collenette, 1938 also spreads in Turkey. *L. wiltshirei* was named in Collenette (1938) on the one male holotype and one male paratype specimen, collected by E.P. Wiltshire on 30-31 July 1935 from Northern Iraq. The male of the species was also reported by Wiltshire (1957) in Iraq. The female was caught and described by Mirzayans and Abai (1974) from north-western Iran. Abai (1980, 1981) reported detailed studies on *L. wiltshirei* from the systematics, distribution, morphology, biology, population dynamics, and control measures standpoints. *L. wiltshirei* has a limited distribution from northern Iraq to west and northwest Iran, and only a few southeast provinces in Turkey (Abai, 1980; Koçak and Kemal, 2018). *L. wiltshirei* is known to be a pest in the oak forest in Iran (Mirzayans and Abai, 1974; Abai, 1981). The larvae of the species were found in high numbers in the study area, southeast Turkey.

MATERIAL AND METHODS

The larvae were collected from Siirt Prov., southeast of Turkey in the oak forest during 2018 and 2020. The samples were taken to the laboratory in a transparent box with the food plant for rearing. Specimens were fed and their development was observed, daily. A total of 10 larvae were collected to investigate from 4 locations (Table 1). Photographs of development stages were taken with Fujifilm Finepix HS30EXR camera. Parasitoids are photographed under the SMZ1000 stereomicroscope. For terminology, Collenette (1938) and Abai (1980, 1981) were

followed. The collection is preserved in the Entomology Laboratory of Batman University (BTU), Batman, Turkey.

RESULTS

Leucoma wiltshirei Collenette, 1938 (Figure 1. e-f)

Original reference: Ann. Mag. Nat. Hist., (11) 2 (20): 369. Holotype: 1 Male, Rowanduz, northern Iraq.

Material examined. Siirt: 1♂ Larva, Şirvan, centre, 1050 m, 21.05.2018; 3♀♂ Larvae, Akyamaç, 650 m, 03.05.2019; 4♀♂ Larvae, Botan Valley, Gökçebağ, 850 m, 31.05.2020, 2♀♂ Larvae, Tillo, 1100 m, 01.06.2020; leg. E. Seven, in coll. BTU.

Range. Iran (Kermanshah, Kordestan, Khuzestan, Fars) (Mirzayans and Abai, 1974; Abai, 1980, 1981; Mehrdad et. al., 1997), Iraq (Rowanduz, Diana: Current name Soran) (Collenette, 1938; Wiltshire, 1957) and Turkey (Kemal et. al., 2013; Koçak and Kemal, 2018).

Distribution in Turkey. Diyarbakır, Hakkari, Siirt (Kemal et. al., 2013; Koçak and Kemal, 2018), Batman (Seven, 2019), Elazığ (Seven and Çakır, 2019).

Biology. The larvae colour is green to brown or red and each larva has 5-6 larval stages and takes about 40-50 days (Mehrdad et. al, 1997) and during this stage, they feed on oak leaves. The pupa colour is green with a yellow band. The pupal period takes about 6-8 days. *L. wiltshirei* has 3 generations in the Baghmalek area (Iran) and its hibernation takes place in the 2nd and 3d larval stages within cracks of the bark of the trunk of oak trees. The over-winter period takes about 8-9 months (Mehrdad et. al, 1997).

Diagnosis. Adult of *L. salicis* is noticeably bigger than *L. wiltshirei* and, legs of *L. salicis* have black rings, while in *L. wiltshirei* are completely white.

Table 1. The developmental stages and sites of some collected larvae of *L. wiltshirei*

Larva	Pupa	Emerged	Parasitized	Died	Location
1 sample 21.05.2018	06.06.2018	Male, 13.06.2018	-	-	Şirvan
3 samples 09.05.2019	2 sample, 20.05.2019, 22.05.2019	2 males, 27.05.2019, 28.05.2019	-	1 sample, at larval stage	Akyamaç
4 samples 31.05.2020	1 sample, 11.06.2020	Female 18.06.2020	1 sample, (Reared: 1 dipter, 1 braconid, 16.06.2020)	2 sample, at larval stage	Gökçebağ
2 samples 01.06.2020	-	-	-	2 sample, at larval stage	Tillo

DISCUSSION

The known parasitoid species of *L. wiltshirei* contains mostly Hymenoptera group. However, *L. wiltshirei* is also known to be parasitized or hunted by Diptera and Heteroptera species (Table 2). In this research, one larva parasitized from the collected samples, and a possibly distinct hymenopter (braconid) and a different dipter species reared (Table 2, Figure 1. g-h).

Table 2. Predators and parasitoids of *L. wiltshirei*

Parasitoid	Family, Ordo	Host	Resource
<i>Dibrachys cavus</i>	Pteromalidae, Hym.	Stage not specified	Lotfalizadeh & Gharali, 2008
<i>Mesopolobus amaenus</i>	Pteromalidae, Hym.	Stage not specified	Lotfalizadeh & Gharali, 2008
<i>Ooencyrtus</i> sp.	Encyrtidae, Hym.	Egg parasitoid	Mehrdad et. al., 1997
<i>Apanteles</i> sp.	Braconidae, Hym.	Larvae parasitoid	Mehrdad et. al., 1997
<i>Compsilura concinnata</i>	Tachinidae, Dipt.	Larvae parasitoid	Mehrdad et. al., 1997; Abai, 1981
<i>Torymus triangularis</i>	Torymidae, Hym.	Pupal parasitoid	Mehrdad et. al., 1997
<i>Lioterphus</i> sp.	Torymidae, Hym.	Pupal parasitoid	Mehrdad et. al., 1997
<i>Tryphon</i> sp.	Ichneumonidae, Hym.	Pupal parasitoid	Mehrdad et. al., 1997
<i>Brachymeria intermedia</i>	Chalcididae, Hym.	Pupal parasitoid	Mehrdad et. al., 1997; Abai, 1981
<i>Tetrastichus evonymellae</i>	Eulophidae, Hym.	Hyper parasitoid of <i>B. intermedia</i>	Mehrdad et. al., 1997
<i>Tetrastichus defimbriatus</i>	Eulophidae, Hym.	Hyper parasitoid of <i>B. intermedia</i>	Mehrdad et. al., 1997
<i>Nagusta simonii</i>	Reduviidae, Het.	Predator	Abai, 1981
<i>Nagusta goedeli</i>	Reduviidae, Het.	Predator	Abai, 1981
<i>Telenomus</i> sp. (?)	Scelionidae, Hym.	Egg parasitoid	Abai, 1981
<i>Gryon</i> sp. (?)	Scelionidae, Hym.	Egg parasitoid	Abai, 1981
<i>Apanteles lacteicolor</i>	Braconidae, Hym.	Larvae parasitoid	Abai, 1981
<i>Apanteles</i> sp.	Braconidae, Hym.	Larvae parasitoid	Abai, 1981
<i>Exorista sorbillans</i>	Tachinidae, Dipt.	Larvae parasitoid	Abai, 1981
X sp.	Family (?), Dipt.	Larvae parasitoid	Current discovery
<i>Apanteles</i> sp. (?)	Braconidae, Hym.	Larvae parasitoid	Current discovery

L. salicis was reported a serious pest by numerous caterpillars on the foliage of *Populus* woodland in the Nemrut Caldera, Bitlis, east of Turkey (Kemal and Koçak, 2015). *L. wiltshirei* was given as a pest on *Quercus* trees in Iran (Mirzayans and Abai, 1974; Abai, 1981). So yet, there has been no such pest report for *L. wiltshirei* in Turkey. As the larval population in the studies sites is high in numbers especially at Tillo and Gökçebağ locations, and harm to the leaves of many oak trees, it is thought that *L. wiltshirei* may cause damage to the oak forest in southeast Anatolia, Turkey.

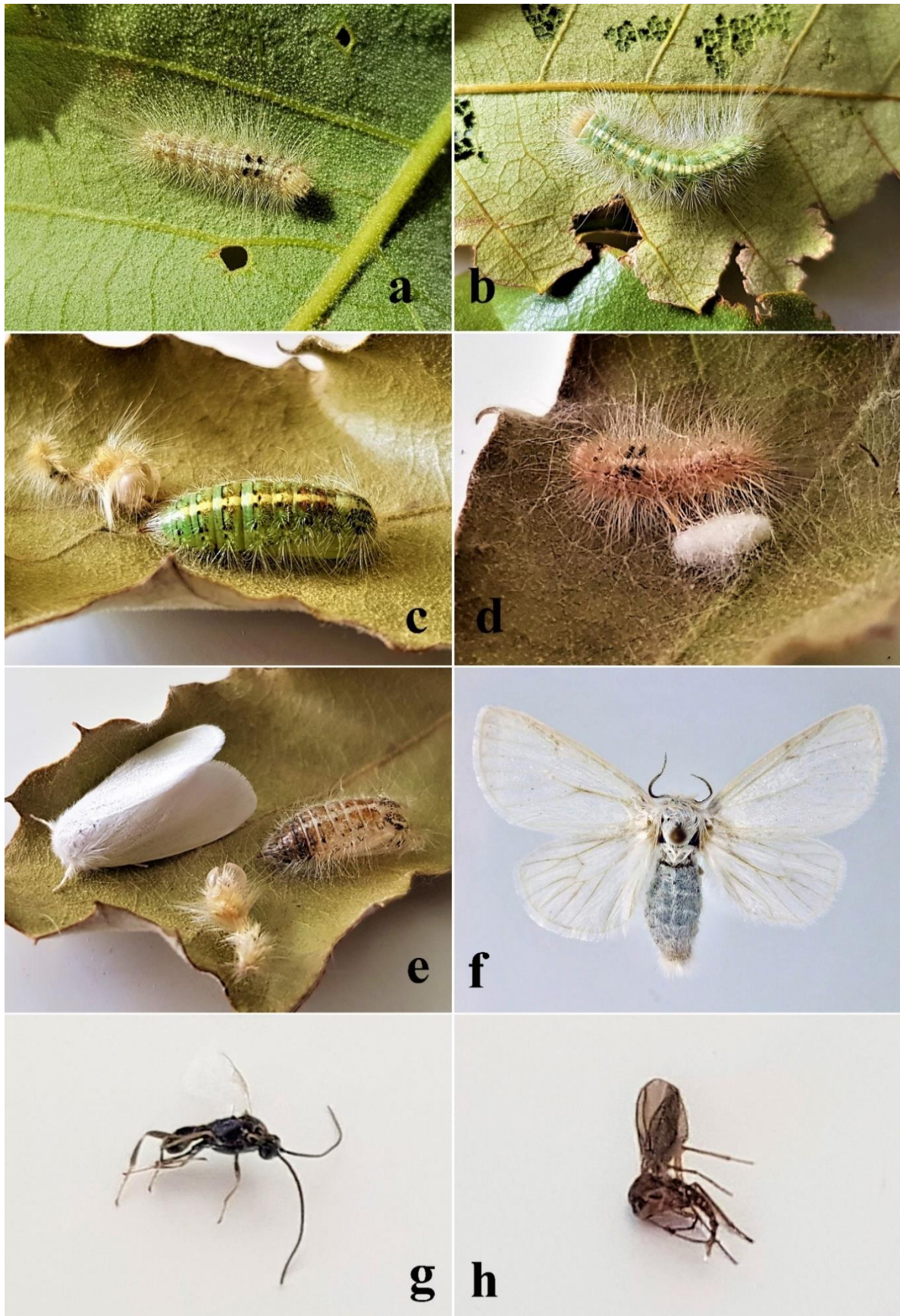


Figure 1. *L. wiltshirei*: a-b. Larvae, c. Pupa, d. Parasitized larva, e-f. Adult, g-h. Parasitoids

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**TÜRKİYE’DE YETİŞTİRİLEN FARKLI YABANI ORKİDE TÜRLERİNDEN ELDE
EDİLEN SALEBİN DONDURMADA KULLANIMI İLE REOLOJİK**

ÖZELLİKLERİNİN DEĞERLENDİRİLMESİ

**THE EFFECT OF THE USE OF SALEP POWDER OBTAINED FROM DIFFERENT
WILD ORCHID SPECIES IN TURKEY ON THE RHEOLOGICAL PROPERTIES OF ICE
CREAM**

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ÖZET

Salep *Orchidaceae* familyasına ait, bilimsel adı ‘tubera salep’ olan yabancı orkide türlerinin yumrularının kurutulup öğütülmesiyle elde edilmektedir. Salep yapısında bulunan stabilizatör ajanlar sebebiyle stabilizatör madde olarak gıda endüstrisinde özellikle dondurma üretiminde kullanılmaktadır. Dondurma karışımının yapısında bulunan süt proteinleri, şeker, yağ globülleri, buz kristalleri, hava kabarcıkları ve diğer maddelerin istenilen tekstür ve fiziksel kalitede olması için stabilizatör maddeler karışıma mutlaka eklenmelidir. Salep dondurmanın vizkozitenin artmasına ve ürünün reolojik özelliklerinin gelişmesine katkıda bulunmaktadır.

Çalışmanın amacı Türkiye'nin farklı bölgelerinde yetişen salep türlerinin dondurmanın reolojik özelliklerine etkisini araştırmaktır. Bu amaçla Türkiye'nin sahip olduğu doğal mikroflorada bulunan farklı bölgelerde yetişen (Mersin, Yozgat, Muğla, Kahramanmaraş) 10 farklı tür salep toplanmış ayrıca kontrol olarak bir adet ticari salep örneği temin edilmiştir. Saleplerin sulu çözeltilerinin reolojik özellikleri incelenmiş ve değerlendirme sonucunda dondurma üretiminde en uygun formülizasyonu oluşturacak miks karışımı için salep örnekleri aynı konsantrasyonlarda (%2) dondurma miksine eklenmiştir. Stabilizatör olarak eklenen farklı salep türlerinin dondurmada farklı reolojik akış davranışları sergilediği ortaya konulmuştur. Dondurma mikserlerinin akış davranış özellikleri, frekans taraması ve tiksotropik özellikleri çalışılmıştır. Bir dondurma karışımı için beklenen akış davranışı olan kayma incelenmesi akış özellikleri tüm dondurma karışımlarında görülmüştür. Dondurma karışımları için K ve n değerleri sırasıyla 0.03–35.08 Pasn ve 0.33–0.80 olarak belirlendi. Tüm dondurma örnekleri yeterli zeta potansiyel değerine sahiptir ve zeta potansiyel değerleri -25.87 mV ile -33.95 mV arasındadır. Dinamik reolojik parametreler de power-law modeli kullanılarak hesaplanmıştır. Üçüncü aralıktaki tüm dondurma örnekleri tiksotropik davranış sergilemiştir. Çalışma sonucundaki veriler doğrultusunda nesli tükenmekte olan salep orkideleri içinde dondurma üretimine en uygun türü bulup o türe ait salep orkidesinin yetiştirilmesini destekleyecek, stabilizatör olarak dondurma üretiminde en uygun salep türünün kullanım olanakları değerlendirilmiş olmaktadır.

Anahtar Kelimeler: Salep, stabilizatör, dondurma, reoloji.

ABSTRACT

Salep belongs to the *Orchidaceae* family and is obtained by drying and grinding the tubers of wild orchid species whose scientific name is 'tubera salep'. Salep is used as a stabilizer agent in the food industry, especially in the production of ice cream. Ice cream mixture composed of milk proteins, sugar, fat globules, ice crystals, air bubbles, and other substances. Stabilizer substances must be added to the mixture because of having the desirable texture and physical quality. Salep contributes to the increase in the viscosity of ice cream and the improvement of the rheological properties of the product.

The aim of this study is to investigate the effect of salep species grown in different regions of Turkey on the rheological properties of ice cream. For this purpose, 10 different types of salep grown in different regions (Mersin, Yozgat, Muğla, Kahramanmaraş) in the natural microflora of Turkey were collected and a commercial salep sample was obtained as a control. The rheological properties of the aqueous solutions of salep were examined and it was determined that the proper consistency was 2% aqueous solution. 2% salep is added for the most suitable formulation in ice cream production. It has been revealed that different types of salep added as stabilizers exhibit different rheological flow behaviors in ice cream. Flow behavior, frequency

sweep, and thixotropic properties of ice cream mixes were studied. Shear-thinning flow characteristics, which is the expected flow behavior for an ice cream mix, were observed in all ice cream mixes. The K and n values for the ice cream mixes were determined as 0.03–35.08 Pasⁿ and 0.33–0.80, respectively. All ice cream samples have sufficient zeta potential values, with zeta potential values between -25.87 mV and -33.95 mV. Dynamic rheological parameters were also calculated using the power-law model. All ice cream samples in the third range exhibited thixotropic behavior. In conclusion, the most suitable species for ice cream production among the wild salep orchids will be found and the possibilities of using the most suitable salep species as a stabilizer in the production of ice cream will be evaluated.

Keywords: Salep, stabilizer, ice cream, rheology.

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**İN VİTRO ALZHEİMER MODELİNDE ALLİL İZOTİYOSİYONAT VE
SULFORAFANIN HÜCRE CANLILIĞI ÜZERİNE ETKİSİ**
EFFECT OF ALLYL ISOTHIOCYANATE AND SULFORAPHANE ON CELL
VIABILITY IN AN IN VITRO MODEL OF ALZHEIMER'S

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ÖZET

Alzheimer hastalığı, ilerleyici nörodejeneratif bir hastalıktır ve gün geçtikçe yaygınlaşan bu hastalığın 2050'li yıllarda dünya genelinde yaklaşık 100 milyon kişiyi etkileyeceği düşünülmektedir. Bu yüzden de bir halk sağlığı problemi ve sosyoekonomik bir yük olarak ifade edilmektedir. Son yıllarda ise; beslenmenin Alzheimer hastalığı (AH) üzerine olan etkileri dikkat çekmiş ve bu konu araştırılmaya başlanmıştır. Yapılan çalışmalar organosülfür bileşiklerinden allil izotiyosiyonat ve sulforafanın Alzheimer hastalığının önlenmesi ve tedavisinde sahip oldukları güçlü antioksidan ve anti inflamatuvar özelliklerinden dolayı potansiyel etki gösterebilecekleri düşünülmektedir. Çalışmada *in vitro* Alzheimer modeli oluşturularak, allil izotiyosiyonat ve sulforafanın bu hücreler üzerindeki hücre canlılığı etkilerinin incelenmesi amaçlanmıştır. *In vitro* AH modeli insan nöroblastoma hücrelerine (SK-N-AS) 24 ve 48 saat boyunca $A\beta_{25-35}$ peptit uygulaması ile gerçekleştirilmiştir. Model kontrolü Thioflavin T (ThT) boyası ile yapılmıştır. *In vitro* AH model hücrelerinde farklı dozlarda (1, 5, 15, 25 and 50 μ M) allil izotiyosiyonat ve sulforafan uygulanır iken, kontrol grubu hücrelere sadece kültür vasatı eklenmiştir. Hücre canlılığı 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazoliumbromide (MTT) analizi ile değerlendirilmiştir. Model için $A\beta_{25-35}$ uygulaması sonrasında, zamana bağlı olarak amyloid beta oluşumunun arttığı gözlemlenmiş ve 48 saat 1 μM $A\beta_{25-35}$ uygulamasının uygun olduğu sağlanmıştır. MTT analiz sonuçları değerlendirildiğinde, 50 μM allil izotiyosiyonat ve 15 μM sulforafanın 48 saatlik uygulama sonrasında uygulamaya yapılmayan *in vitro* AH model hücrelerine göre hücre canlılığını artırdığı saptanmıştır. Yapılan deneylerin sonucunda hem allil izotiyosiyonat hem de sulforafan uygulamasının *in vitro* AH model hücreleri üzerinde hücre canlılığını artırıcı etkiye sahip oldukları ve bu iki bileşenin Alzheimer tedavisinde nöroprotektif etki yaratabileceği düşünülmektedir.

Anahtar Kelimeler: Allil izotiyosiyonat, Sulforafan, *In vitro* Alzheimer modeli, $A\beta_{25-35}$

SUMMARY

Alzheimer's disease (AD) is a progressive neurodegenerative disease, and it is thought that this disease, which is becoming more common day by day, will affect approximately 100 million people worldwide in the 2050s. In recent years; The effects of nutrition on AD have attracted attention and this issue has begun to be investigated. Studies suggest that allyl isothiocyanate (AITC) and sulforaphane (SFN), which are organosulfur compounds, may have potential effects in the prevention and treatment of AD due to their strong antioxidant and anti-inflammatory properties. In this study, it was aimed to examine the cell viability effects of AITC and SFN on these cells by creating an *in vitro* AD model. The *in vitro* model of AD was performed by administering $A\beta_{25-35}$ peptide to human neuroblastoma cells (SK-N-AS) for 24 and 48 hours. Model control was performed with Thioflavin T (ThT) dye. While different doses (1, 5, 15, 25 and 50 μM) of AITC and SFN were applied to *in vitro* AD model cells, only culture medium was added to control cells. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) analysis. After $A\beta_{25-35}$ application for the model, it was observed that amyloid beta formation increased depending on time, and it was ensured that 1 μM $A\beta_{25-35}$ application for 48 hours was appropriate. When MTT analysis results were evaluated, it was determined that 50 μM AITC and 15 μM SFN increased cell viability compared to *in vitro* AD model cells that were not applied after 48 hours of application. As a result of the experiments, it is thought that both AITC and SFN application have a cell viability-enhancing effect on *in vitro* AD model cells and these two components may have a neuroprotective effect in the treatment of AD

Keywords: Allyl isothiocyanate, Sulforaphane, *In vitro* Alzheimer's model, $A\beta_{25-35}$

**BETA-KAROTEN, BİOTİN VE CROHN HASTALIĞI ARASINDAKİ
ETKİLEŞİMLERİN *İN SİLİKO*, *İN VİTRO* VE GEN EKSPRESYONU YÖNTEMLERİ
İLE ARAŞTIRILMASI**

INVESTIGATION OF INTERACTIONS AMONG BETA-CAROTENE, BIOTIN, AND
CROHN DISEASE USING *İN-SİLİKO*, *İN VİTRO* AND GENE EXPRESSION METHODS

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ÖZET

Crohn hastalığı (CD) immünite temelli kronik bir inflamatuvar bağırsak hastalığıdır. Gıdaların ve beslenme alışkanlıklarının dünyada ve ülkemizde görülme sıklığı artış gösteren CD üzerinde etkilerini belirlemeye dönük incelemelere gerek duyulmaktadır. Bu çalışmanın amacı, beta-karoten ve biotin ile CD arasındaki etkileşimlerin *in siliko*, *in vitro* ve gen ekspresyonu yöntemleri ile araştırmaktır. Bu bağlamda, Nimbal ve Imppat veri tabanlarından CD'ye antiinflamatuvar yanıt verdiği bildirilen toplam 25 adet gıda kaynaklı biyoaktif bileşik seçilmiştir. Seçilen bileşiklerin Aril Hidrokarbon Reseptörü (AhR) ile etkileşim seviyeleri *in siliko* moleküler kenetlenme tekniği ile analiz edilmiş ve aralarından en yüksek ve en düşük kimyasal afinite değeri veren beta-karoten ve biotin ileri analizler için seçilmiştir. İki bileşimin farklı zaman (24. - 48.saat) ve dozlarda (25 µM - 400µM) tek başlarına ve birlikte HCT-116 hücre hattı üzerindeki etkileri *in vitro* MTS testi ile ölçülmüştür. MTS testi hücre canlılık sonuçlarına göre seçilen zaman ve dozlarda iki bileşimin uygulandığı hücrelerden RNA izole edilmiş, izolatlar cDNA'ya dönüştürülmüş ve dört hedef genin (CYP1A1, CYP1B1, IDO1 ve IDO2) ekspresyon seviyesini belirlemek real-time PCR yöntemi ile analiz edilmiştir. *In siliko* hesaplamalı bulgulara göre, AhR ile en yüksek bağlanma enerjisi beta-karoten (-8.4 kkal/mol)

¹ Sorumlu Yazar

ve en düşük bağlanma enerjisi biotin (-5.3 kkal/mol) için belirlenmiştir. *In vitro* MTS bulgularına göre, beta-karoten uygulamalarının, 50-200 µM 24. saatte ve 100-200 µM 48. saatinde hücre canlılığını kontrol grubuna kıyasla kayda değer arttırdığı görülmüştür. Biotinin ise, 24. ve 48. saatlerde farklı doz uygulamaları sonucunda, hücre canlılığını kontrol grubuna göre anlamlı şekilde etkilemediği kaydedilmiştir. Ayrıca iki bileşiğin en yüksek dozunun (400 µM) hücrelere tek tek ve birlikte uygulanmasının 24. ve 48. saatlerde hücre canlılığını kontrol grubuna göre anlamlı şekilde etkilemediği kaydedilmiştir. Gen ekspresyonu analizine göre, beta-karoten; CYP1A1 ve CYP1B1 genlerinin ekspresyonlarını arttırırken, IDO1 ve IDO2 genlerin ekspresyon düzeylerini azaltmıştır. Biotin ise yalnızca IDO1 genin ekspresyonu seviyesini düşürmüştür. İki bileşiğin birlikte dört hedef genin ekspresyon düzeylerini yükselttiği görülmüştür. Özetle, CD ile gıda ve beslenme arasındaki ilişkiyi daha iyi anlamak açısından klinik bilimleri ile birlikte *in-vivo* incelemelere gerek olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Crohn Hastalığı, AHR, HCT-116, Betakaroten, Biotin, *in siliko*, *in vitro*, Gen ekspresyonu

ABSTRACT

Crohn's disease (CD) is a chronic, autoimmune and inflammatory bowel disorder. The studies on the effects of food and nutritional habits on CD with its increasing frequency in the world and our country remains unclear. This study aims to investigate the interactions among beta-carotene, biotin, and CD using *in silico*, *in vitro*, and gene expression methods. Twenty-five bioactive compounds with antiinflammatory responses to CD were selected from Nimbal and Imppat databases. The interactions levels of the selected compounds with Aryl Hydrocarbon Receptor (AHR) were assessed by *in silico* molecular docking technique, and the beta-carotene with the highest chemical affinity value and biotin with the lowest chemical affinity value Among them were considered for further analyses. Following that, the effects of these two compounds on the cell line HCT-116 to different durations (24th – 48th hour) and different doses (25 µM - 400µM) were measured by *in vitro* MTS techniques. RNA was isolated from the cells according to the duration and dose results based on the cell viability data of the MTS test, the isolates were converted to cDNA, and the expression levels of the four target genes (CYP1A1, CYP1B1, IDO1 ve IDO2) were subjected to real-time PCR application. The *in-silico* results revealed that the highest binding energy level with AHR belonged to beta-carotene (-8.4 kcal/mole)), while the lowest binding energy level was to biotin (-5.3 kcal/mole). The *in vitro* MTS results indicated that the beta-carotene significantly increased the cell viability at 50-200 µM/24th h and 100-200 µM/48th has compared to the control group. However, the different doses of biotin at 24th and 48th h did not significantly impact the cell viability. In addition to that, the alone and combined application of the highest quantities of the two compounds (400 µM) 24th and 48th h did not significantly affect cell viability concerning the control group. The

gene expression analysis provided that beta-carotene upregulated the expression levels of both CYP1A1 ve CYP1B1 while downregulated IDO1 ve IDO2. On the other hand, the biotin downregulated the expression level of IDO1 only. The combined application of both compounds upregulated the expression levels of all target genes. Overall, we concluded that in vitro studies should be conducted together with the clinical sciences to understand better the correlation between CD, food, and nutrition.

Keywords: Crohn Disease, AHR, HCT-116, Beta-carotene, Biotin, *in silico*, *in vitro*, Gene ekspresion

**İSKELETSEL SINIF 1 VE SINIF 3 MALOKLÜZYONLU BİREYLERDEKİ
MAKSİLLER SANTRAL KESİCİ DİŞLERİN KRON KÖK AÇILARININ
KARŞILAŞTIRILMASI**

**COMPARISON OF MAXILLARY CENTRAL INCISORS CROWN-ROOT ANGLE IN
SKELETAL CLASS 1 AND CLASS 3 MALOCCLUSION INDIVIDUALS**

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ÖZET

Bu çalışmanın amacı iskeletsel Sınıf I ve Sınıf III maloklüzyonlu bireylerin lateral sefalometrik radyografilerinde maksiller kesici dişlerindeki kron kök açılarını karşılaştırmaktır.

Bu retrospektif çalışma 120 lateral sefalometrik radyografi üzerinde gerçekleştirildi. Çalışmaya maksiller santral dişlerinde sayı eksikliği, yapısal bozukluğu, belirgin morfolojik değişikliği, travma geçmişi ve restorasyonu olmayan bireylere ait lateral sefalometrik filmler dâhil edildi. Seçilen örnekler ANB açısı ve Witts değerleri ile Angle klasifikasyonuna göre İskeletsel Sınıf III maloklüzyonlu çalışma grubu ve İskeletsel Sınıf I maloklüzyonlu kontrol grubu oluşturmak üzere iki gruba ayrıldı. Kök ve kronun uzun eksenleri arasındaki açı kollum açısı olarak ölçülmüştür. Collum açısının ölçümü Delivanis ve Kuftinec tarafından geliştirilen “Collum açısı ölçümü yöntemi” ile yapıldı. 1-NA (mm) ve Wits değerlerine sahip örneklerin SNA, SNB, ANB, 1/NA°, SN-GoGn, Y eksenini, kollum açılarının tanımlayıcı istatistikleri minimum, maksimum, ortalama ve standart sapma olarak değerlendirildi. Gruplar arası karşılaştırma için Mann Whitney-U testi yapıldı.

Çalışma ve kontrol grubu SNA açı ortalamaları yakın değerlerde iken, SNB açı ortalamaları arasında 4,37°, ortalama Witts değerleri arasında ise 6,77mm’lik fark olduğu gözlenmiştir. Mann Whitney U testi sonucuna göre çalışma grubundaki collum açıları kontrol grubuna göre istatistiksel olarak anlamlı ve yüksekti. İki grubun ortalama 1/NA° ve 1-NA değerleri arasındaki farklar sırasıyla 5,1° ve 0.92 mm olarak belirlendi. Ortalama SN-GoGn açısı değerleri benzerdi. Grupların ortalama Y eksenini açı değerleri arasında 2°’den az fark vardı.

Çalışmamız, iskeletsel sınıf 3 maloklüzyonlu bireylerdeki maksiller santral kesici diş collum açılarının iskeletsel sınıf 1 maloklüzyonlu bireylerinkinden anlamlı düzeyde daha yüksek olduğunu göstermiştir.

Anahtar Kelimeler: Collum Açısı, Kron Kök Açısı, Sınıf III Maloklüzyon.

ABSTRACT

The aim of this study was to compare the crown root angle of the maxillary incisors on lateral cephalometric radiographs of individuals with skeletal Class I and Class III malocclusions.

This retrospective study was performed on 120 lateral cephalometric radiographs. Lateral cephalometric films of individuals without missing teeth, structural disorder, significant morphological changes, trauma history and restoration in their maxillary central teeth were included in the study. Selected samples were divided into two groups according to ANB angle and Witts values and Angle classification, as the study group with Skeletal Class III malocclusion and the control group with Skeletal Class I malocclusion. The angle between the long axes of the root and crown was measured as the collum angle. Descriptive statistics of

SNA°, SNB°, ANB°, I/NA°, SN-GoGn°, Y-axis°, collum angles of samples with I-NA(mm) and Wits(mm) values were evaluated as minimum, maximum, mean and standard deviation. Mann Whitney-U test was used for comparison between groups.

While the mean SNA angles of the study and control groups were close, it was observed that there was a difference of 4.37° between the mean SNB angles and 6.77mm between the mean Wits values. According to the results of Mann Whitney U test, the collum angles in the study group were statistically higher than in the control group. The differences between the mean I/NA° and I-NA values of the two groups were determined as 5.1° and 0.92 mm, respectively. Mean SN-GoGn angle values were similar. There was less than 2° difference between the mean Y-axis angle values of the groups.

Our study showed that the maxillary central incisor collum angles in individuals with skeletal class 3 malocclusion were significantly higher than those of individuals with skeletal class 1 malocclusion.

Keywords: Collum Angle, Crown-Root Angle, Class III Malocclusion.

FARKLI EĞİTİM DÜZEYİNE SAHİP HASTALARIN DENTAL ANKSİYETE DURUMLARININ BELİRLENMESİ
ASSESSMENT OF DENTAL ANXIETY STATUS AMONG PATIENTS WITH DIFFERENT EDUCATIONAL LEVELS

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ÖZET

Amaç: Dental anksiyete kişilerin diş tedavilerine karşı geliştirdiği hoşnutsuzluk ve kaygı durumunu tanımlayan klinik bir tablodur. Dental anksiyetenin objektif olarak değerlendirilmesinde kullanılan çeşitli anketler ve psikometrik ölçekler mevcuttur. Bu çalışmanın amacı dental tedaviye başvuran farklı eğitim düzeyine sahip hastaların dental anksiyete durumlarının karşılaştırılması olarak değerlendirilmesidir.

Gereç ve Yöntem: Çalışmaya, İstanbul Aydın Üniversitesi Diş Hekimliği Fakültesi Hastanesine başvuran, yaşları 18-70 arasında değişen, kadın/erkek=72/89 olan toplam 161 katılımcı dahil edildi. Katılımcılar eğitim düzeylerine göre ilkökul, ortaokul, lise, önlisans/lisans, lisansüstü ve üniversite öğrencisi olmak üzere 6 gruba ayrıldı. Hastaneye yaptıkları ilk başvuruyu takiben katılımcılara 7 gün sonraya diş yüzey temizliği için randevu verildi. Planlı randevusuna gelen hastalar tedavi öncesinde, diş hekimi ve dental tedavi anksiyetesini değerlendiren, Modifiye Dental Tedavi Anksiyete Skalası (MDAS) doldurdu. MDAS, her soruya 1-5 arasında puan verilen 5 sorudan oluşan ve skor aralığı 5-25 aralığında değişen bir ölçektir. Ek olarak, katılımcıların diş hekimini ziyaret sıklığı ve diş fırçalama sıklıkları ile ilgili veriler de kayıt altına alındı. Çalışmada istatistiksel anlamlılık $p \leq 0.05$ düzeyinde değerlendirildi.

Bulgular: Çalışma popülasyonunun %60'ını gençler ve genç erişkinler; ve yaklaşık %70'ini en az üniversite mezunu olanlar oluşturmaktaydı. MDAS skor ortalaması kadınlarda anlamlı

olarak daha yüksekti ($p=0.001$). Eğitim düzeyi ve diş hekimi ziyaret sıklıklarına göre katılımcıların MDAS skor ortalamaları karşılaştırıldığında gruplar arası fark bulunamadı ($p>0.05$). Katılımcıların diş fırçalama sıklığına göre MDAS ortalamaları kıyaslandığında, günde 1 kere fırçalayanların MDAS ortalaması, günde 1 kereden daha fazla fırçalayanlara kıyasla istatistiksel olarak anlamlı derecede yüksek bulundu ($p=0.02$)

Sonuç: Bu çalışmanın sınırları dahilinde sonuçlarımız, katılımcıların eğitim düzeyi ve diş hekimi ziyaret sıklığının dental anksiyeteleri düzeylerinde fark oluşturmadığını göstermektedir.

Anahtar Kelimeler: Ölçek, dental anksiyete, modifiye dental anksiyete skalası

ABSTRACT

Objective: Dental anxiety is a clinical condition which is the feeling of discomfortable and anxiety that one suffers against to dental treatments. A variety of psychometric scales are introduced to objectively determine dental anxiety. This study aimed to assess the dental anxiety status among patients with different educational attainment levels.

Materials and Methods: This study enrolled a total of 161 participants (F/M=72/89), aged 18-70 years, admitted to the Istanbul Aydın University Faculty of Dentistry Hospital. Based on their education level, participants were categorized into six groups: primary school, secondary school, high school, associate/bachelor's degree, master's/PhD degree, and students still enrolled in a university. Following their first application to the hospital, participants were scheduled for a dental scaling session on 7th day. Before the treatment, patients who attended their scheduled appointments completed the Modified Dental Treatment Anxiety Scale (MDAS), which assessed their dental anxiety status. The MDAS is a five-question scale in which each question is assigned a score between 1 and 5, total score ranging from 5 to 25 points. Participants were also asked how often they visit the dentist and brush their teeth. The statistically significant level was set at $p\leq 0.05$.

Results: Over 60% of the participants in the study were adolescents and young adults, and approximately 70% had at least had undergraduates. The mean MDAS score was significantly higher in women ($p = 0.001$). There was no difference between groups in terms of educational level and frequency of dental visit ($p>0.05$). The mean MDAS score of those who brushed their teeth once a day was significantly higher ($p=0.02$) than those who brushed their teeth more than once a day.

Conclusion: Within the limits of this study, our results demonstrate that dental anxiety level is not influenced by educational level or frequency of dental visits.

Keywords: Educational level, dental anxiety, questionnaire, modified dental anxiety scale

ORTODONTİDE YAPAY ZEKA UYGULAMALARI
ARTIFICIAL INTELLIGENCE (AI) APPLICATIONS IN ORTHODONTICS

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ÖZET

Son yıllarda dijitalleşmenin hızlanması ve tıbbi cihaz teknolojisindeki gelişmelerle birlikte klinik veri miktarı artmakta ve bu verilerin analizinde desteğe ihtiyaç duyulmaktadır. Hız kazanarak gelişmeye devam eden yapay zekâ çalışmaları, tıp ve yaşam bilimleri gibi pek çok

disiplinde olduğu gibi, diş hekimliğinin de yükselen trendi olmaya adaydır. Yapay zekâ, bilgisayar sisteminin öğrenme ve sorun çözme gibi insan benzeri bilişsel işlevleri taklit edebilme becerisini tanımlayan genel bir terimdir. Bu bildirinin amacı yeni ve hızla gelişmekte olan yapay zeka teknolojisinin ortodonti alanındaki uygulamalarını anlatmaktır.

Ortodonti; dişler ve çenelerdeki düzensizliklerin (maloklüzyon) teşhisi, önlenmesi ve tedavisiyle ilgilenen diş hekimliği dalıdır. Bu düzensizliklerin tanımlanmasında 1920'lerden beri, standart sefalometrik radyografiler kullanılarak çeşitli açısal ve doğrusal veriler oluşturulmaktadır. Bu ölçülebilir veriler ile ortodontistler, önceden tanımlanmış standartları kullanarak bireydeki mevcut maloklüzyonun tanımlamasını sağlar. Bu bilgi, tedavi planını oluşturmada ve prognozun değerlendirilmesinde önemli bir role sahiptir. Yapay zekanın bu alandaki yazılımlarda kullanılmasıyla sefalometrik filmler üzerinde, dişleri ve çeneleri ilgilendiren analizler otomatik olarak kısa süre içinde hekime sunulmaktadır. 2010 yılında yapılan bir araştırmada ortodontik tedavi planının oluşturulmasında yapay zekanın doğruluğu değerlendirilmiştir. Buna göre, maloklüzyonun düzeltiminde diş çekimine gerek olup olmadığına karar vermek üzere kurulan bir nöral network ağı ile yapay zekanın %80 doğruluğa ulaştığı belirtilmiştir. Yapay zekanın ortodontideki kullanım alanlarından biri de, servikal vertebra maturasyon aşamaları üzerinden büyüme ve gelişim periodunun tespitidir. Farklı yapay zeka algoritmalarının verdikleri kararlar ile ortodontistlerinkinin karşılaştırıldığı bir araştırmada, yapay nöral ağların (ANN) stabil sonuçlar sağladığı ve bu alanda kullanılabileceği belirtilmiştir.

Sonuç olarak, ortodonti alanında gelişmekte olan yapay zekâ uygulamalarının, hekimleri kısıtlı bir süreç içerisinde belirli klinik kararları vermede doğru şekilde yönlendirildiği görülmektedir.

Anahtar Kelimeler: Yapay Zekâ, Ortodonti, Sefalometri.

ABSTRACT

In recent years, with the acceleration of digitalization and developments in medical device technology, the amount of clinical data has increased and support is needed in the analysis of these data. Artificial intelligence studies, which continue to develop by gaining speed, are a candidate to be the rising trend of dentistry, as in many disciplines such as medicine and life sciences. Artificial intelligence is a general term that describes the ability of a computer system to mimic human-like cognitive functions such as learning and problem solving.

The purpose of this paper is to explain the applications of artificial intelligence, which is a new and rapidly developing technology, in the field of orthodontics. Orthodontics; It is the branch of dentistry that deals with the diagnosis, prevention and treatment of teeth and facial irregularities (malocclusion). Since the 1920s, orthodontists have generated small measurable data using standard cephalography. These data are then collected and processed, enabling

orthodontists to identify the malocclusion using predefined standards. The use of AI in orthodontics has increased significantly in recent years to make the diagnostic process more accurate and efficient in the orthodontic treatment process. In a study conducted in 2010, they reached 80% accuracy with a neural network established to decide whether tooth extraction is needed in orthodontic treatment. One of the uses of artificial intelligence in orthodontics is to determine the growth and development period by using the cervical vertebra stages. In a study comparing the decisions made by different artificial intelligence algorithms with those of orthodontists, it was stated that artificial neural networks (ANN) provide stable results and can be used in this field.

As a result, artificial intelligence applications can guide physicians to make better decisions while making certain clinical decisions within a limited time.

Keywords: Artificial Intelligence, Orthodontics, Cephalometry.

**DEFENSE MECHANISM METABOLITES IN THE FEMALE REPRODUCTIVE
SYSTEM**

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ABSTRACT

Lactobacillus species, which are dominant in the microflora here, have a significant role in the health and functioning of the female urogenital system. *Lactobacillus* species play critical protective roles in vaginal health through diverse mechanisms. *Lactobacillus* microorganisms suppress the growth of other microorganisms in the vagina by producing metabolites such as lactic acid, hydrogen peroxide, and bacteriocins. Production of organic acids by lactobacilli decreases vaginal pH. At the same time, the production of hydrogen peroxide and bacteriocins by lactobacilli suppresses endogenous pathogenic bacteria to maintain a healthy vaginal ecosystem. Maintaining a healthy vaginal ecosystem is essential for both the vaginal and general health of the patient. The release of various metabolites into the vaginal environment and the acidic pH formed by lactobacilli is critical for protecting the vaginal tract. Disruption of this balance in the vaginal environment is interpreted as a preliminary diagnosis of various infections. The production and concentration of lactic acid, directly proportional to lactobacilli, ensures the exclusion of pathogenic microorganisms and is seen as an important biomarker of vaginal health. The decrease in vaginal pH causes pathogenic microorganisms to consume more energy, protein denaturation, loss of vitality, and deterioration of their DNA. In addition, *Lactobacillus* species produce bacteriocins. Bacteriocins cause the death of pathogenic microorganisms by inhibiting cell wall biosynthesis and disrupting the structure of the membrane through pore formation. At the same time, most *Lactobacillus* species produce hydrogen peroxide with their specific enzymes in the presence of oxygen. Hydrogen peroxide inhibits the glucose transport system, hexokinase activity, and glyceraldehyde-3-phosphate dehydrogenase enzymes of pathogenic microorganisms.

Keywords: Vaginal defense metabolites, *Lactobacillus* sp, Lactic acid, Bacteriocin, Hydrogen peroxide

INTRODUCTION

The health and functioning of the female urogenital system depend on the species that make up the microflora here. This protection is mainly attributed to *Lactobacillus* species which are dominant in the vaginal flora of healthy women (Beamer et al, 2017; Ventolini, 2015; Cribby et al, 2008; Larsen and Monif, 2001). *Lactobacillus* species play critical protective roles through various mechanisms. *Lactobacillus* microorganisms suppress the growth of other endogenous bacteria in the vagina by producing metabolites such as lactic acid, hydrogen peroxide, and bacteriocins. The production of organic acids by lactobacilli keeps the vaginal pH between 3.8 and 4.5, thus creating an unsuitable environment for the growth of most endogenous pathogenic bacteria (Beamer et al, 2017; Mendling, 2016). Along with lactic acid, the presence of hydrogen peroxide and bacteriocins further suppresses endogenous pathogenic bacteria to maintain a healthy vaginal ecosystem (Figure 1).

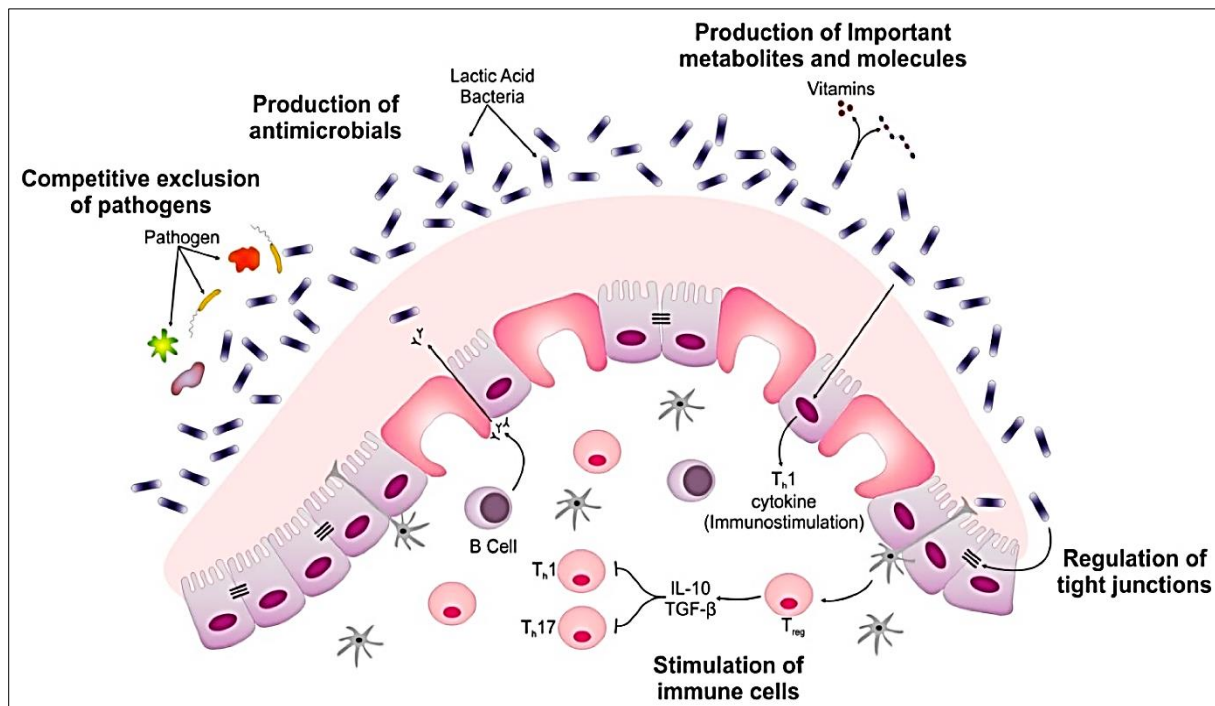


Figure 1: Protective roles of *Lactobacillus* on epithelial surface (Ayivi et al., 2020)

Importance of Lactic Acid Production (Vaginal pH)

In healthy reproductive-aged women, the vaginal microbiome generally shows the dominance of the genus *Lactobacillus* (Ravel et al., 2011). The condition in which *Lactobacillus* species that make up the vaginal microbiota is dominant is called vaginal eubiosis, while the condition is characterized by the overgrowth of multiple anaerobes such as bacterial vaginosis is called vaginal dysbiosis. *Lactobacillus* species found in the vagina naturally or as probiotics produce lactic acid by fermentation, lowering the vaginal pH and increasing the environment's acidity (Figure 2). The antimicrobial effects of lactic acids are provided by their undissociated structure and their pH-reducing properties (Erkmen and Bozoğlu, 2008a, 2008b). Therefore, in the case

of vaginal dysbiosis, it creates eubiosis by killing pathogenic microorganisms (Tachedjian et al., 2017). In the case of vaginal dysbiosis, the presence of polymicrobial populations in the vagina with a moderate *Lactobacillus* load (middle microbiota) or without *Lactobacilli* (bacterial vaginosis, BV) is observed (Ravel et al., 2011; Ling et al., 2010; Nugent et al., 1991).

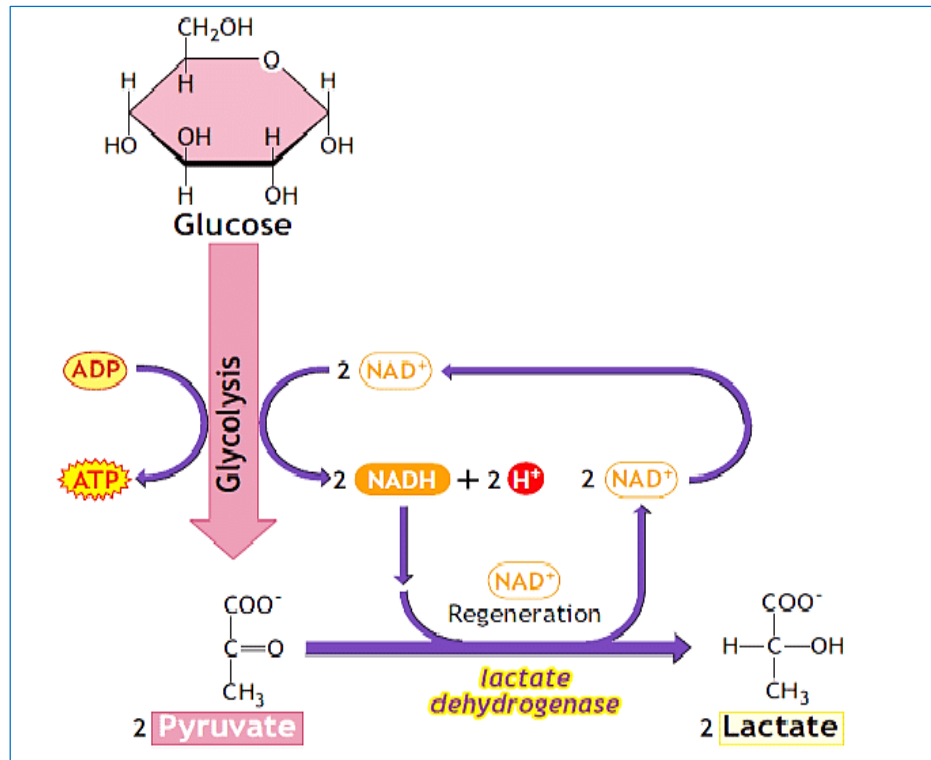


Figure 2: Graphical representation of lactic acid production by glucose fermentation (Shopstore, 2022)

The production of lactic acid, which is directly proportional to the presence of lactobacilli, is considered the beneficial activity of the vaginal microflora. The presence and concentration of lactic acid, respectively, ensure the exclusion of pathogenic microorganisms and are seen as an important biomarker of vaginal health. Vaginal pH is widely used to diagnose genital infections and is thus another indicator of vaginal health. The decrease in vaginal pH causes pathogenic microorganisms to consume more energy, protein denaturation, loss of vitality, and deterioration of their DNA. Therefore, the decrease in pH affects cellular components, structures, and intracellular functions. While vaginal pH increases in bacterial vaginosis and trichomonas vaginitis diseases, it decreases in vaginal candidiasis (Nenadic and Pavlovic 2015; Ryu and Min 2006). In some studies, it has been reported that lactic acid can inactivate a wide variety of reproductive tract pathogens such as *C. trachomatis* and HIV-1 (Gong, 2014; Aldunate et al., 2013).

Bacteriocins

It is known that *Lactobacillus* species that dominate the vaginal flora can produce antimicrobial metabolites that can control the vaginal epithelial surface. Bacteriocins are antimicrobial metabolites of bioactive bacterial peptides or proteins with antimicrobial activity against other bacteria (Gaspar et al., 2018). They are molecules with bactericidal or bacteriostatic activity against closely related species (narrow spectrum) or genera (broad-spectrum) (Cotter et al., 2005; Klaenhammer, 1993). Bacteriocins cause cell death by inhibiting cell wall biosynthesis and/or disrupting the membrane structure through pore formation (Mills et al., 2011). Both Gram-positive and Gram-negative bacteria produce them. Mainly lactic acid bacteria produce these antimicrobial peptides and proteins in ribosomes (Muriana and Klaenhammer, 1991).

The term bacteriocin has been defined as a toxic protein or peptide that is antimicrobial active on related bacteria but does not harm the producer cell. Bacteriocins are named according to the type of microorganism from which they are produced. For example, the first identified bacteriocin produced by *E. coli* was named colisine. The bacteriocin produced from the bacterium formerly known as *Pseudomonas pyocyanea*, now known as *Pseudomonas aeruginosa*, is called pyocin. The bacteriocin produced from *Enterobacter cloacae* is called cloacin, while the bacteriocin produced from *Klebsiella* is called klebicin. Bacteriocins produced from *Lactococcus* are called lactococin, and staphylococin bacteriocins are produced from *Staphylococcus* (Karpiński and Szkaradkiewicz, 2016; Lagos, 2013).

Bacteriocins stabilize the flora of the healthy vagina through 3 mechanisms, as shown in Figure 3. Bacteriocins can act as colonizing peptides, facilitating the competition of a probiotic with the resident microbiota. They can function to kill pathogens by directly eliminating them. They can also serve as signaling peptides to the immune system (Dobson et al., 2012).

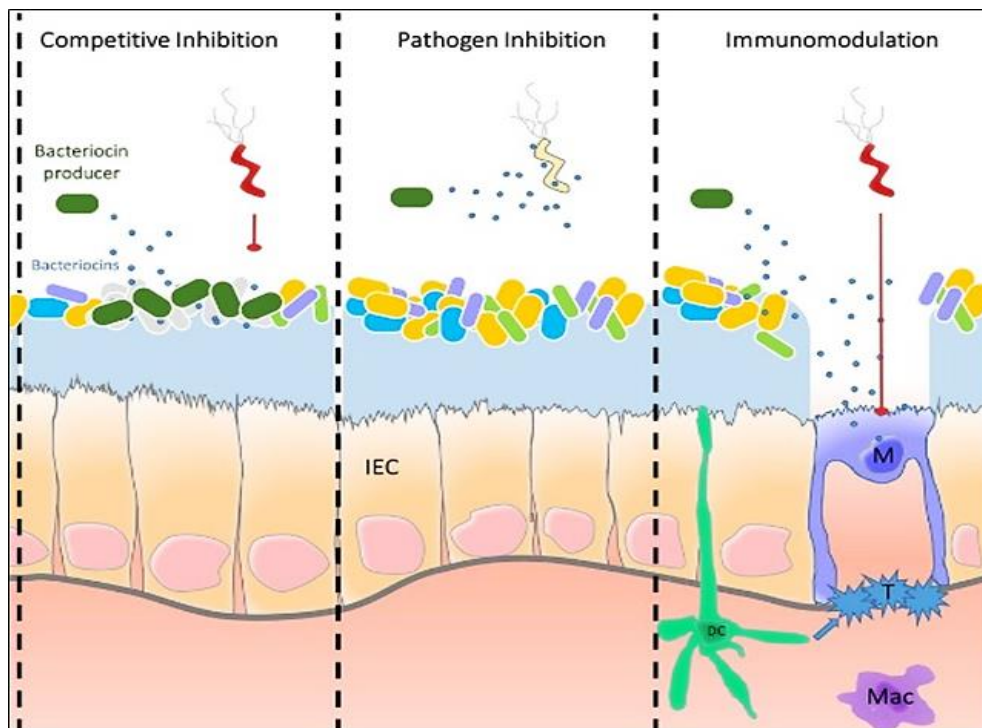


Figure 3: Graphical illustration of the bacteriocins mechanism (Naver 2018).

The largest and best-known group of bacteriocins are the bacteriocins produced by lactic acid bacteria. Because of their antimicrobial activities, bacteriocins are used in medicine and pharmacy to treat infections caused by pathogenic multidrug-resistant strains. In more than 40 countries, it is used as a food preservative in dairy products, cheeses, meat, fish, salads, and canned goods (Karpiński and Szkaradkiewicz, 2016).

Hydrogen peroxide (H₂O₂)

Hydrogen peroxide (H₂O₂) is mainly produced by *Lactobacillus* species to varying degrees and is an essential indicator of vaginal health. Therefore, H₂O₂ is an important antimicrobial compound that provides protection against genital tract pathogens and is often the primary mechanism to prevent the entry of pathogens. (Zheng et al, 2019; Aldunate et al, 2015; O'Hanlon et al, 2013). Most *Lactobacillus* species produce H₂O₂ with their flavoprotein oxidase enzyme in the presence of oxygen (Yang, 2000; Erkmen and Bozoğlu, 2008b). However, *Lactobacillus* species produce H₂O₂ with nicotinamide adenine hydroxy dinucleotide (NADH) peroxidase enzymes (Yang, 2000). Since *Lactobacillus* species do not have catalase enzymes, H₂O₂ accumulates in the environment. H₂O₂ inhibits the glucose transport system, hexokinase activity and glyceraldehyde-3-phosphate dehydrogenase enzymes of the pathogenic microorganism (Akpınar and Kılıç, 2012). H₂O₂ is effective on bacteria, yeast, molds, and viruses (Erkmen and Bozoğlu, 2008b). Studies have reported the inhibitory effect of H₂O₂ against *C. albicans* (Larsen and White, 1995; Lenander-Lumikari, 1992).

CONCLUSION

Lactobacillus species, which make up the vaginal microflora to a large extent, form a defense mechanism against pathogenic microorganisms in the vaginal microenvironment with the lactic acid they form due to fermentation and the metabolites they produce at the same time. While the lactic acid produced maintains the pH balance of the vaginal microenvironment, H₂O₂ and bacteriocins inhibit endogenous pathogenic bacteria of the vaginal microflora. Thus, maintaining the dominance of *Lactobacillus* also against opportunistic pathogens creates a healthy vaginal microenvironment.

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INVESTIGATION OF THE GENOTOXIC EFFECTS OF FENPYROXIMATE WITH THE COMET ASSAY

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ABSTRACT

Fenpyroximate acaricide, which is used to combat mites in agricultural production, has a molecular weight of 421.50 g/mol and a closed formula of $C_{24}H_{27}N_3O_4$. The genetic effects of fenpyroximate were investigated in this study using *Allium cepa* L. test material. As test materials, *A. cepa* L. (2n=16) bulbs obtained from a commercial market in Giresun province were used. *A. cepa* L. bulbs were divided into four groups, one control group and three treatment groups. Tap water was applied to the control group for 72 hours. The application groups were treated with 25 mg/L, 50 mg/L and 100 mg/L doses of fenpyroximate for 72 hours. At the end of the period, the root tips were cut 1 cm long and prepared for the alkaline comet assay. Fluorescence microscopy was used to analyze the prepared slides after they had been stained with ethidium bromide. DNA breakage measurement and DNA damage quantification were performed using Comet Assay software version 1.2.3b (CaspLab). As a result, the lowest values of tail DNA percentage, tail moment and olive tail moment were observed in the control group treated with tap water, while the highest values were observed in the group treated with 100 mg/L fenpyroximate. It was determined that tail DNA increased and head DNA decreased depending on the fenpyroximate application dose. The increased percentage of tail DNA, tail moment (TM) and olive tail moment (OTM) showed statistically significant differences ($p<0.05$) relative to the control group with DNA damage caused by increasing doses of fenpyroximate. The use of this acaricide, which is used to protect against the harms of mites in agricultural production, should be abandoned considering the DNA damage it may cause in the environment and in living things other than the target organism, especially humans and animals.

Keywords: *Allium cepa*, Fenpyroximate, DNA damage, comet assay.

INTRODUCTION

Chemical fertilizers and pesticides are increasingly being used to fulfill the growing need for agricultural produce as the global population grows. Chemical residues by fertilizers and pesticides create environmental pollution by contaminating the soil, agricultural irrigation streams, and rivers surrounding agricultural regions, as well as exposing non-target creatures to these chemicals (Huber et al. 2000). Pesticides are classified in different ways according to their physical structures, formulations and the harmful groups they affect. The most widely used is

to classify them as herbicides, insecticides and acaricides according to the pest groups they are effective in (Öncüer, 1995). One of the acaricides used to combat mites in agricultural production is fenpyroximate. Fenpyroximate has a molecular weight of 421.50 g/mol and empirical formula of $C_{24}H_{27}N_3O_4$. Fenpyroximate is a powerful acaricide that is both acid and alkaline-stable. In Japan, China, and Switzerland, it was initially approved and used to control mites in 1991. It was licensed and began to be used in 28 other countries, including Turkey, after this date. Fenpyroximate is an acaricide that is effective against red spiders (*Tetranychusurtica*), which harm vineyards, citrus fruits, cotton, and vegetables, as well as whitefly, thrips, lepidopter larvae, aphids, leaf psyllid, rust beetle, and potato beetle. It works by preventing oxidative phosphorylation, or the creation of ATP (Doğan et al., 2013). By inhibiting mitochondrial NADH-ubiquinone oxidoreductase enzyme activity, it inhibits electron transport and ATP production in the redox respiratory chain (Motoba et al., 2000).

Because of its sensitivity and strong correlation with mammalian test methods, the *Allium cepa* L. was characterized as an effective test material commonly used to investigate the genotoxic potential of substances in the environment (Grant, 1982; Chauhan et al., 1999). The *A. cepa* L. test method is a quick and effective test that is used to determine toxicity in a short time. It gives important information on the toxicity parameters of chemical agents and environmental pollutants at cytological and genetic levels. (Haq et al. 2017; Roa et al. 2012).

The comet assay was used in this research to analyze DNA damage induced by fenpyroximate at 25 mg/L, 50 mg/L, and 100 mg/L concentrations in root tip cells of *Allium cepa* L. bulbs ($2n=16$).

MATERIAL AND METHOD

Test Material

Fenpyroximate was administered as a chemical agent, and *A. cepa* L. bulbs ($2n=16$) purchased from a Giresun commercial market were used as test materials.

Experimental Design

A total of four groups, one control and three applications, each with ten bulbs, were created.

- Group I: Control
- Group II: 25 mg/L Fenpyroximate
- Group III: 50 mg/L Fenpyroximate
- Group IV: 100 mg/L Fenpyroximate

The control group bulbs were germinated with tap water and the bulbs in the application group were treated with fenpyroximate at room temperature for 72 hours. After the application, the root tips were cut around 1 cm length and prepared for the comet assay.

Comet Assay

The alkaline comet assay was performed following the procedure developed by Tice et al. (2000) to assess DNA damage in *A. cepa* root tip meristem cells subjected to three different doses of fenpyroximate. Fluorescence microscopy was used to analyze the prepared slides that had been stained with ethidium bromide. For each group, 100 cells were computed.

The Comet Assay software version 1.2.3b (CaspLab) (Końca et al., 2003) was used to assess DNA breakage, calculate the amount of DNA damage. The results were represented as a percentage of DNA in the comet's tail.

Statistical Analysis

The analyses were carried out using the "IBM SPSS Statistics 22" package program, and the results were presented as mean \pm standard error of the mean (SEM). Duncan's test and One-way ANOVA were used to establish statistical significance between means, and p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The comet assay was used to assess the genotoxicity of fenpyroximate doses of 25 mg/L, 50 mg/L, and 100 mg/L in nuclei isolated from *A. cepa* root tip cells (Figure 1). Table 1 shows the statistically evaluated data obtained with the Comet Assay (CASP) program. In Group I (control group), tail DNA percentage was calculated as 1.06, tail moment 0.18 and olive tail moment 1.15. In Group II, which applied 25 mg/L fenpyroximate, the tail DNA percentage increased sharply to 36.73. In addition, tail moment and olive tail moment parameters increased in this group and were calculated as 24.32 and 17.35, respectively. Tail DNA percentage was calculated as 53.26, tail moment 39.68 and olive tail moment 28.74 in Group III where 50 mg/L fenpyroximate was applied. In the study, the highest tail DNA percentage, tail moment and olive tail moment values were determined in Group IV, where 100 mg/L fenpyroximate was administered, which was the highest application dose, and these values were 79.13%, 59.10 and 41.17, respectively. Increased fenpyroximate doses resulted in an increase in the tail DNA percentage, tail moment (TM) and olive tail moment (OTM), as well as significant ($p < 0.05$) DNA damage compared to the control group. Further, the DNA damage in the fenpyroximate applied groups increased according to the application dose, and the difference between the groups was found to be statistically significant ($p < 0.05$).

There are researches undertaken by other researchers that validate the findings have obtained. Graillot et al. (2012) reported that the administration of fenpyroximate showed genotoxic activity in human T-cell leukemia cell line and SH-SY5Y cells even at low concentration and that fenpyroximate could cause DNA damage in human cells by causing oxidative stress. Seven

et al. (2009) investigated the effects of fenpyroximate exposure in albino mice, and reported that fenpyroximate exposure had a dose-dependent genotoxic effect. Macar et al. (2021) reported that fenpyroximate application causes micronucleus formation and chromosomal aberrations in root tip cells of *A. cepa*.

Table 1. Fenpyroximate induced DNA damages in nuclei isolated from *A. cepa* roots

Groups	Head DNA (%)	Tail DNA (%)	Tail Moment	Olive Tail Moment
Group I	98.94±0.20 ^a	1.06±0.20 ^d	0.18±0.03 ^d	1.15±0.11 ^d
Group II	63.27±1.94 ^b	36.73±1.94 ^c	24.32±2.49 ^c	17.35±2.05 ^c
Group III	46.74±1.88 ^c	53.26±1.88 ^b	39.68±1.93 ^b	28.74±2.02 ^b
Group IV	20.87±2.23 ^d	79.13±2.23 ^a	59.10±2.06 ^a	41.17±1.98 ^a

* Group I: control, Group II: 25 mg/L Fenpyroximate, Group III: 50 mg/L Fenpyroximate, Group IV: 100 mg/L Fenpyroximate. Data are presented as mean ± SEM. The averages in the same column with different letters are statistically significant ($p < 0.05$).

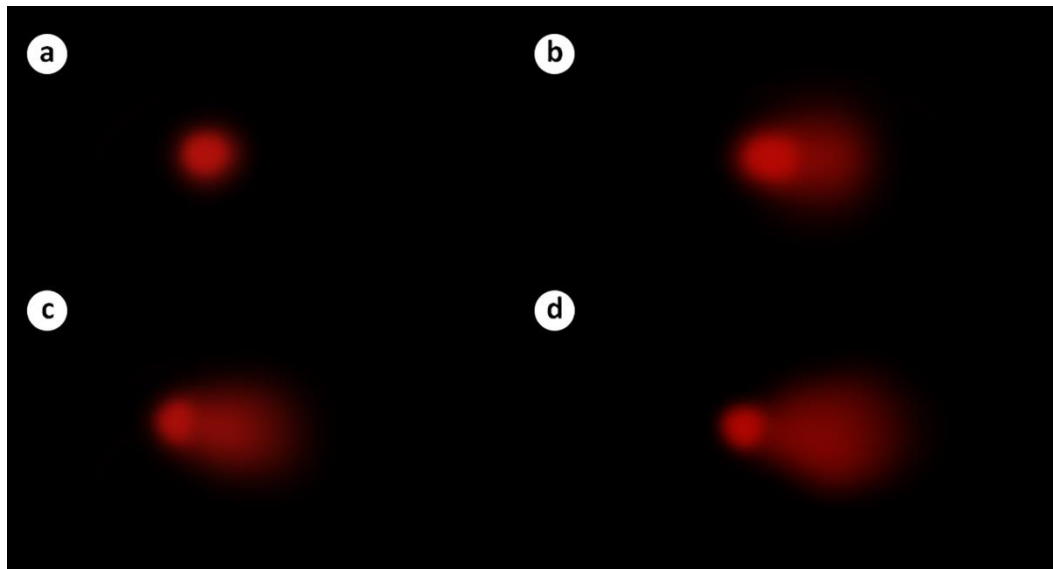


Figure 1. The effects of fenpyroximate application on DNA in *A. cepa* root tip cell nuclei (a: control, b: 25 mg/L fenpyroximate, c: 50 mg/L fenpyroximate, d: 100 mg/L Fenpyroximate).

CONCLUSION

The comet assay with nuclei obtained from *A. cepa* root tip cells demonstrated that the administration of fenpyroximate caused DNA damage dose-dependent manner. The use of this acaricide, which is used to protect against the harms of mites in agricultural production, should be abandoned considering the DNA damage it may cause in the environment and in living things other than the target organism, especially humans and animals.

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**DETERMINATION OF DNA DAMAGE INDUCED BY AFLATOXIN B2 WITH
COMET ASSAY**

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ABSTRACT

Aflatoxins are occurs naturally mycotoxins that can contaminate food and hence have an impact on human health. *Aspergillus* species multiply fast and create aflatoxins in feeds, grains, and dry foods due to high humidity and warmth. They are typically generated by *Aspergillus* species such as *A. bombycis*, *A. Australis*, *A. ochraceoroseus* and *A. flavus*. There are eighteen distinct types of aflatoxin species discovered, with the primary species being aflatoxins B1, B2, G1, G2, and M1. In this study, genetic effects of aflatoxin B2 were investigated by comet assay using *Allium cepa* L. test material. *A. cepa* L. (2n=16) bulbs acquired from a local market in Giresun province were used as test materials. The bulbs were separated into four groups: one control and three application groups. Tap water was applied to the bulbs in the control group and 40 µg/L, 80 µg/L and 120 µg/L aflatoxin B2 doses were administered to the treatment groups during 72 hours. The root tips were cut 1 cm long at the end of the period and prepared for the alkaline comet assay. The prepared slides were stained with ethidium bromide and examined by fluorescence microscopy. DNA breakage measurement and DNA damage quantification were performed using Comet Assay software version 1.2.3b (CaspLab). As a result of the study, the tail DNA increased due to aflatoxin B2 application, while the head DNA decreased. Increased tail moment (TM) and olive tail moment (OTM) rises were found to be significant when compared to the control group ($p < 0.05$). It was found that depending on the aflatoxin B2 application dose, tail DNA increased and head DNA decreased. With increasing doses of aflatoxin B2, the increased percentage of tail DNA, tail moment (TM), and olive tail moment (OTM) exhibited a statistically significant difference ($p < 0.05$) compared to the control group. In conclusion, in this study, it was determined that exposure to aflatoxin B2 causes dose-related DNA damage. Considering the genetic damage that aflatoxins may cause in humans and other organisms, which may occur due to adverse conditions that may occur in the food storage and supply chain, it is necessary to be more sensitive about the presence of aflatoxins in the foods offered for consumption.

Keywords: *Allium cepa*, aflatoxin B2, DNA damage, comet assay.

INTRODUCTION

Aflatoxins are occurs naturally mycotoxins that can contaminate food and hence have an impact on human health (Milićević et al., 2010). *Aspergillus* species multiply fast and create aflatoxins in feeds, grains, and dry foods due to high humidity and warmth. They are typically generated by *Aspergillus* species such as *A. bombycis*, *A. Australis*, *A. ochraceoroseus* and *A. flavus*. There are eighteen distinct types of aflatoxin species discovered, with the primary species being aflatoxins B1, B2, G1, G2, and M1 (Bennett and Klich, 2003; Maragos, 2001). Colorless to pale yellow crystals of aflatoxins glow when exposed to ultraviolet (UV) light. Group B aflatoxins shine blue under UV light, while Group G aflatoxins glow green; hence, they are referred to as B and G, respectively. Aflatoxins are very slightly soluble in water, but readily soluble in moderately polar organic solvents such as methanol, chloroform, and dimethyl sulfoxide. They are entirely insoluble in non-polar solvents. Aflatoxins are unstable when exposed to UV radiation, when exposed to O₂, when exposed to high pH (3, >10), and when exposed to oxidizing chemicals. Aflatoxins are extremely sensitive to a wide range of chemical agents and can breakdown in the presence of strong acids, alkalis, and ammonia (IARC, 2012).

Aflatoxins may be found in a wide range of food and feed commodities across the world, most notably cereals, animal fodder, oilseeds, spices, nuts, and dried fruits. Furthermore, aflatoxin and its derivatives have been discovered in the meat, milk, and eggs of animals given aflatoxin-contaminated feeds (Ismail et al., 2018). Aflatoxins are linked to a variety of disorders, including aflatoxicosis, in both people and animals across the world, and are thought to be mostly harmful to health due to their immunosuppressive, carcinogenic, mutagenic and teratogenic effects (Lanyasunya et al., 2005)

In this study, the comet assay was used to assess DNA damage caused by aflatoxin B2 at concentrations of 40 µg/L, 80µg/L and 120 µg/L in root tip cells of *Allium cepa* L. bulbs (2n=16).

MATERIAL AND METHOD

Test Material

Aflatoxin B2 was utilized as a hazardous agent, while *A. cepa* L. bulbs (2n=16) acquired from a commercial market in Giresun were used as test materials.

Experimental Design

A total of 4 groups, 1 control and 3 applications, were created with 10 bulbs in each group.

Group I: Control

Group II: 40 µg/L Aflatoxin B2

Group III: 80 µg/L Aflatoxin B2

Group IV: 120 µg/L Aflatoxin B2

The bulbs in the control group were treated with tap water, whereas the bulbs in the application group were treated with aflatoxin b2 for 72 hours at room temperature. At the completion of the application, the root tips were trimmed to around 1 cm length and prepared for the comet assay.

Comet Assay

The alkaline comet assay was performed in accordance with the procedure developed by Tice et al. (2000) to assess DNA damage in *A. cepa* root tip meristem cells subjected to three different doses of aflatoxin B2. Fluorescence microscopy was used to analyze the prepared slides that had been stained with ethidium bromide. For each group, 100 cells were computed.

The Comet Assay software version 1.2.3b (CaspLab) (Końca et al., 2003) was used to assess DNA breakage, calculate the amount of DNA damage. The results were represented as a percentage of DNA in the comet's tail.

Statistical Analysis

The analyses were carried out using the "IBM SPSS Statistics 22" package program, and the results were presented as mean \pm standard error of the mean (SEM). Duncan's test and One-way ANOVA were used to establish statistical significance between means, and p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Figure 1 and Table 1 show the effects of different dosages of aflatoxin B2 on nuclei extracted from *A. cepa*. According to the study performed using Comet Assay software version 1.2.3b, the head DNA in the control group was 98.46 percent, whereas it ranged from 56.82 to 28.71 percent in aflatoxin B2 treated groups. While the tail DNA in the control group was just 1.54 percent, it varied from 43.18 percent to 71.29 percent in the administration groups. When aflatoxin B2 doses were increased, tail DNA percentage, tail moment (TM), and olive tail moment (OTM) rose, but head DNA percentage decreased, indicating that DNA damage was increased significantly ($p < 0.05$) compared to the control group and the application dose.

Other researchers have conducted experiments that confirm our findings and indicate that aflatoxins cause DNA damage. Zhang et al. (2015) reported that aflatoxin B1 and aflatoxin M1 cause genetic damage in Caco-2 cells in a time- and dose-dependent manner. Gursoy-Yuzgullu et al. (2011) reported that genotoxic doses of aflatoxin B1 can cause an incomplete and inefficient checkpoint response in human cells, which may contribute to the mutagenic and carcinogenic effects of aflatoxins. Gündüz et al. (2021) reported that exposure to aflatoxin B2 triggered MN formation in buccal epithelium, erythrocyte and leukocyte cells and CAs formation in bone marrow cells in albino mice.

Table 1. DNA damages induced by aflatoxin B2 in nuclei isolated from *A. cepa* roots

Groups	Head DNA (%)	Tail DNA (%)	Tail Moment	Olive Tail Moment
Group I	98.46±0.24 ^a	1.54±0.24 ^d	0.21±0.02 ^d	1.04±0.10 ^d
Group II	56.82±1.96 ^b	43.18±1.96 ^c	37.74±1.85 ^c	28.16±1.34 ^c
Group III	41.38±2.05 ^c	58.62±2.05 ^b	41.25±2.34 ^b	33.22±2.11 ^b
Group IV	28.71±1.81 ^d	71.29±1.81 ^a	60.37±2.19 ^a	46.75±2.36 ^a

* Group I: control, Group II: 40 µg/L Aflatoxin B2, Group III: 80 µg/L Aflatoxin B2, Group IV: 120 µg/L Aflatoxin B2. Data are presented as mean ± SEM. The averages in the same column with different letters are statistically significant (p<0.05).

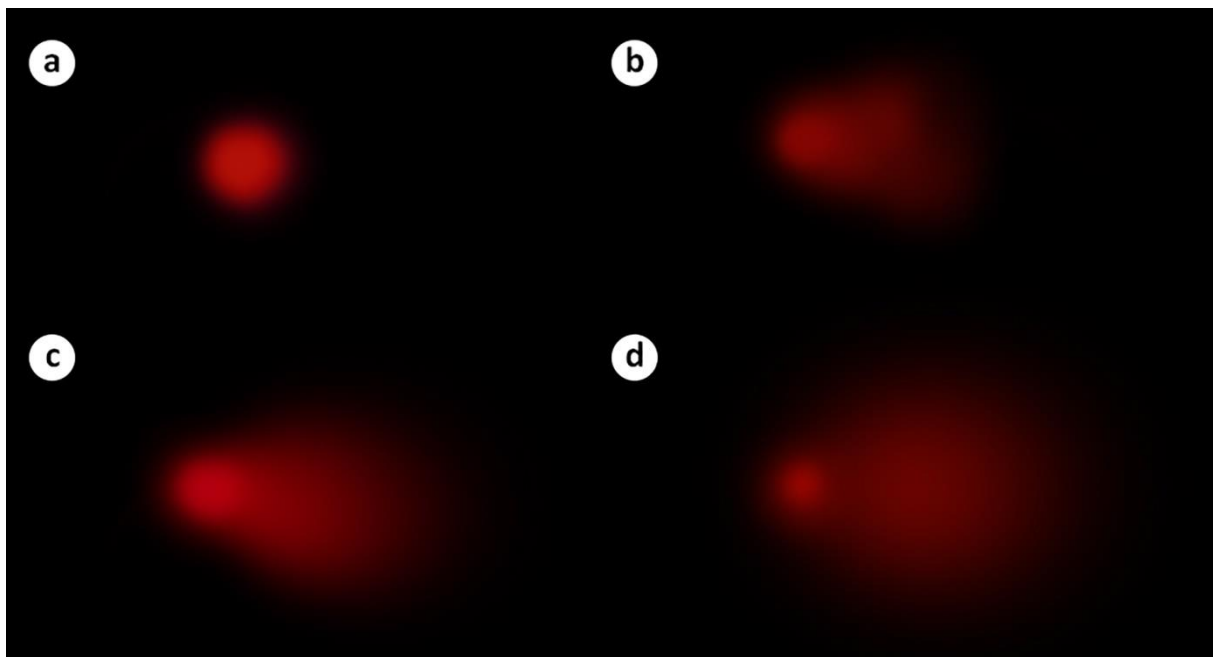


Figure 1. Effects of aflatoxin B2 application on DNA in *A. cepa* root tip cells nuclei (a: control, b: 40 µg/L Aflatoxin B2, c: 80 µg/L Aflatoxin B2, d: 120 µg/L Aflatoxin B2).

CONCLUSION

Aflatoxin exposure of *A. cepa* L. root tip meristem cells caused an increase in tail DNA percentage, tail moment and olive tail moment parameters depending on the application dose. Obtained findings have shown that exposure to aflatoxin causes genetic damage by causing DNA fragmentation. Considering the genetic damage that aflatoxins may cause in humans and other organisms, which may occur due to adverse conditions that may occur in the food storage and supply chain, it is necessary to be more sensitive about the presence of aflatoxins in the foods offered for consumption.

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**THE HEALTH STATUS OF PEA (*PISUM SATIVUM* L.) PLANTS
AFTER APPLYING OF BIOSTIMULANTS**

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ABSTRACT

The aim of the study was to determine the effect of biostimulants Timorex Gold 24 EC (based on tea tree oil) and Trianum P (spores of *Trichoderma harzianum* Rifai T-22) on the health status of *Pisum sativum* L. and on the bacteria and fungi populations in the rhizosphere of this plant.

Field studies were conducted in the Experimental Station in Felin belonging to the University of Life Sciences in Lublin, Poland. Before the sowing, the seeds of pea were dressed with Trianum P, Timorex Gold 24 EC and Miedzian 50 WP fungicide (50% oxychloride of copper). The number of the grown plants and their health status was established four weeks after seed sowing and at anthesis. Plants with the symptoms of necrosis were taken for a laboratory mycological analysis. A microbiological analysis of the rhizosphere soil of pea was conducted in the laboratory. The obtained isolates of bacteria *Pseudomonas* spp., *Bacillus* spp. and fungi *Myrothecium* spp., *Clonostachys* spp., *Penicillium* spp., *Trichoderma* spp. were used to determine their antagonistic effect towards the following fungi pathogenic towards pea plants: *Peyronellaea pinodes*, *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium culmorum*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*.

The number of plants grown from the seeds dressed with Trianum P exceeded the number of plants obtained after the application of Timorex Gold 24 EC and Miedzian 50 WP fungicide. The following fungi were isolated from the infected plants and rhizosphere soil: *Peyronellaea pinodes*, *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Antagonistic microorganisms dominated in the rhizosphere of plants grown from the seeds dressed with Trianum P.

Biostimulants Trianum P and Timorex Gold 24 EC can be effective in biocontrol of *Pisum sativum* from soil plant pathogens. The tested biostimulants stimulate the development of antagonistic bacteria and fungi, at the same time limiting the occurrence of pathogenic soil-borne fungi.

Keywords: *Pisum sativum* L., Biocontrol, Healthiness of Plants, Biostimulants

**HPLC QUANTIFICATION OF THE CHEMICAL CONSTITUENTS FROM
INDIGENOUS FRUITS AND VEGETABLES OF INDIAN HIMALAYAN REGION**

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ABSTRACT

The purpose of the present work was to determine the phytochemical profiles by HPLC of the indigenous fruits and vegetables. The phenolic contents showed diverse variation in the selected fruits and vegetables. Development of genuine and dependable analytical methods with profile marker phytoconstituents in an extract containing a mixture of several components is a challenging task. A simple, rapid, precise and reliable HPLC method was developed for the quantification of phytochemicals from the extracts of selected minor fruits and vegetables. The *Taraxacum officinale* genus comprised a mixture of different bioactive compounds belonging to different chemical types, such as flavonoids, sesquiterpenes, triterpenes, phenolic acids, sterols. *Malva neglecta* contains different compounds including several phenolic acids, flavonoids and some non-phenolic compounds. Caffeoylquinic acids (3-, 4-, and 5-O-caffeoylquinic acids and 3,5-dicaffeoylquinic acid) are mainly present in *Cydonia oblonga* pulps. Three different hydroxycinnamic acid derivatives (neochlorogenic acid, p-coumaroylquinic acid and chlorogenic acid) were detected and quantified in *Prunus avium*.

Keywords: Analysis; Crops; Gradient; HPLC; Minor; Phytochemicals.

REVIEW ON FARMING AGRICULTURE ROBOTS

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ABSTRACT

Robotics is playing a significant role in agriculture production and management there is a need for autonomous and time saving technology in agriculture to have efficient farm management. The agricultural robots have been researched and developed principally for harvesting, chemical spraying, picking fruits, and monitoring of crops. Robots like these are perfect substitute for manpower to a great extent as they deploy unmanned, sensing and a machinery system. The prime benefits, of development of autonomous, and intelligent agricultural robots are to improve repeatable precision, efficacy compaction and drudgery. Major operations in open arable farming includes tilling, soil analysis, seeding, transplanting, crop scouting, pest control, weed removal and harvesting where robots can assist in performing all of the tasks. Each specific operation, requires, axillary devices and sensors with specific functions. While robots are becoming the inseparable parts of the modern farms, results showed that the robots travelled with a maximum lateral error of 47 minutes and sprayed with a productivity of 16-20 plants/minute. Also, weeding action was taken in time. It can work 20 hours/day. so far, the robots are 61% accuracy in picking ripe fruits. The designed robot has adaptability, versatility because the different operations can be archived on the same platform. Our conclusion is that it is not realistic to expect an entirely automated farming system in the future.

Keywords: Precision farming, Agricultural robots, task-based agricultural robots, soil analysis, seeding, weed detection, harvesting, productivity, adaptability, versatility.

PHYTOPLASMA – A SERIOUS THREAT TO VARIOUS PLANT SPECIES IN INDIA

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ABSTRACT

In India recent evidences showed that phytoplasma associated with plants including crops, fruits, trees, ornamental, sugarcane, grasses and weeds increasing at alarming rate. Phytoplasma cause diseases in several plant species and resulted in serious threat as a source of alternative natural host for the spread of phytoplasma pathogen to other economically important plants and thereby chances of causing severe losses. In earlier days very few phytoplasma diseases were identified in India merely on the basis of bright field, fluorescence, electron microscope observations, tetracycline treatment and to a lesser extent by serological assays. Among these microscopic methods do not attain pathogen identification, and all of them are not always sufficiently sensitive to detect phytoplasma infections in low titer hosts. Today detection of phytoplasma based on molecular genetic methods including PCR assays are efficiently carried out in India and on that basis several plant species are reported to have phytoplasma infections. So far more than 39 plant species have been reported to be associated with phytoplasma infections from all over India. The “Candidatus phytoplasma asteris”, “Candidatus phytoplasma aurantifolia”, “Candidatus phytoplasma trifolii” and “Candidatus phytoplasma cynodontis” belong to 16SrI, 16SrII, 16SrVI and 16SrXIV group of phytoplasmas are the major groups associated with different species reported to be infected with phytoplasma throughout India. Here we have discussed overall progress on phytoplasma disease on plant species in India in terms of taxonomy, symptomatology, economic significance, transmission and characterization.

Keywords: Phytoplasma, 16Sr- RNA sequences, genetic diversity, India.

PHYTOPLASMA OCCURRING VARIOUS SYMPTOMS IN DIFFERENT PLANT SPECIES

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ABSTRACT

Phytoplasmas are associated with diseases in several hundreds of cultivated herbaceous and woody plants. Their impact in agriculture and the periodical outbreak of worrying epidemics make very important, besides precise laboratory-based diagnosis, the direct in field recognition of phytoplasma disease symptoms. Even if some symptoms are typical of this kind of pathogens, in field diagnosis requires the knowledge of the host plant, strong field experience, and awareness of the symptom variability of the various organs of the plant during different seasons and under various environmental conditions. It is therefore very important to be familiar with factors like environmental conditions, agronomical features, and disease progression that influence symptom expression. Therefore, a satisfactory diagnosis should be based on repeated and complete observations scored over the entire plant and across different times of the year. A more suitable diagnosis is possible if the observer is able to recognize and distinguish the symptoms of other biotic or abiotic diseases. A general rule is to observe three different symptoms, at least, and to seek input from the grower about the initial development, frequency, diffusion, and particular characteristics of the disease. After a short introduction the following symptoms are presented: the most common and representative symptoms caused by phytoplasmas; the most common symptoms of phytoplasma diseases occurring in particular plant organs, with some references to specific diseases; phytoplasma symptoms on the model plant periwinkle (*Catharanthus roseus*); the main factors influencing phytoplasma symptoms expression; and several practical procedures that should be followed for suitable diagnosis.

Keywords: Chlorosis, Decline, Plant diseases, Virescence, Witches' broom

**INCOME DISTRIBUTION OF THE ADOPTERS AND NON ADOPTERS OF CROP
ROTATION AND DIVERSITY PRACTICES**

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ABSTRACT

This study determined the income distribution of the adopters and non adopters of crop rotation and diversity practices among rural farmers in Ebonyi State, Nigeria, in a bid to know whether the adoption of crop rotation cropping system has financial benefit over non adoption. Multistage sampling technique was used to select 180 respondents, and data collection was done with the use of structured questionnaires and interview schedules. The results of the study revealed that the majority (87.78%) of the farmers were not aware of crop rotation practices, but were rather involved in other cropping systems. The mean annual income of the farmers was N455,055.60, while the majority of the farmers earn between N300,000.00 to N499,000.00. The highest income earners were the adopters of crop rotation, with 81.8% of them, representing 10% of the entire population of the respondent, earning between N700,000.00 to N899,000.00, while 18.2% of them, representing 2.2% of the entire population of the respondents, earn N900,000.00 and above. The Chi-square test static revealed that at $P=0.000$, income is a significant determinant of the adoption of crop rotation cropping system, while the major identified constraints were lack of the knowledge of the cropping system (85.55%), lack of planning skills (73.33%), lack of extension service delivery (72.22%), and Inexistence demonstration plots (71.11%). It concludes that the adoption of crop rotation increases farmers' income, and the study recommends proper extension services, demonstration plots and outreaches to make it known and practiced by the farmers for increased income.

Keywords: Farmers, Rural people, Cropping systems, Adoption, and Farming practices

FELINE LEUKEMIA VIRUS INFECTION AND RELATED DISEASES IN CATS

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ABSTRACT

Feline leukemia virus (FeLV) is one of the most common infectious agents in cats worldwide. There are different courses of FeLV infection: progressive, regressive, abortive, and focal atypical infection, that have been characterized experimentally. Progressive FeLV infection causes various tumors in cats, most commonly lymphoma and less commonly leukemia and other hematopoietic tumors as well as some unusual tumors, such as neurolymphomatosis, osteochondromas, olfactory neuroblastoma, uterine adenocarcinoma, and cutaneous horns. Regressive infections and their implications especially are still poorly understood, and their impact on the health of cats is likely underestimated. The outcome of FeLV infection is determined by a battle between the cat's immune system and the virus; this is true particularly in the early phase of infection, usually over the first 12 weeks after exposure, when the course the infection will take is determined. In a few cats, the balance between virus and immune system will be unstable lifelong. FeLV contains a protein core with single-stranded RNA protected by an envelope. It replicates within many tissues, including bone marrow, salivary glands, and respiratory epithelium. If the cat's immune response does not intervene after initial infection, FeLV spreads to the bone marrow and infects hematopoietic precursor cells. The ability of the virus to become part of the host's own DNA is crucial for the lifelong persistence of the virus after bone marrow infection. FeLV is contagious and spreads through close contact between virus-shedding cats and susceptible cats. Transmission of FeLV occurs primarily via saliva. Progressively infected cats shed millions of virus particles in saliva, and shedding through saliva occurs relatively consistently throughout the life of these cats. FeLV is passed effectively horizontally among communal cats that have close contact. Fleas have been considered a potential route of transmission because FeLV RNA has been detected in fleas and flea feces, but flea transmission does not seem to play an important role in nature. Studies report a prevalence of progressive FeLV infection of 2.3% to 3.3% in the United States, 0.7% to 15.6% in Europe, 3.0% to 28.4% in South America, and 0.5% to 24.5% in Asia and Australia/New Zealand. The 3 best and longest known FeLV subgroups are FeLV-A, FeLV-B, and FeLV-C. FeLV-A is contagious and passed horizontally from cat to cat in nature. Pathogenicity of FeLV-B and FeLV-C is higher than that of FeLV-A alone. FeLV-B is commonly associated with malignancies; it is particularly frequently associated with mediastinal lymphoma, but also occurs in multicentric lymphoma. Bone marrow suppression, especially anemia, is the most common clinical syndrome associated with FeLV infection and results from infection of both

hematopoietic stem cells and bone marrow stromal cells that constitute the supporting environment for blood precursor cells. Nevertheless, Unfortunately no curative treatment currently exists to eradicate retroviral infection.

Keywords: FeLV, Retrovirus, Infection, Cats.

**MAJOR DIRECTION TO INCREASE THE COMPETITIVENESS OF
AGRICULTURE ENTERPRISES OF AFGHANISTAN**

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ABSTRACT

This paper aims to set forth the fundamental collapse of the Afghanistan government and its drawback on the private sectors in general and on the agriculture enterprises in special, challenges, security concerns, unemployment, and living conditions of the people, during and after the government collapsed. To that end, studies on Afghanistan's affected economic situations, 20 years of war, instabilities in the country were reviewed and data collected. Firstly, the reports, articles in the newspapers and journals, economists' perspectives about the effects of the collapse on agriculture enterprises are analyzed. In the research contents, concepts, and terms like the competitiveness of agriculture enterprises in the local markets, the competitiveness of Afghanistan's agricultural products in the regional and international markets, were searched in the search engines. However, there has been enough related literature on Agricultural products and enterprises of Afghanistan to review, many books or theses based on agriproducts being researched, covering the characteristics of the agricultural products as well the agriculture enterprises' challenges, risks, concepts, strategies, and constant developments were found out. Recently some articles published in journals partially estimating the effects of the government collapse and withdrawal of the international forces from Afghanistan reflects some facts and figures but in no circumstances, it shows the real damages and impacts government collapse has on the agricultural enterprises. Data for agriculture enterprises research were obtained through quantitative and qualitative methods. The researchers carried out their studies by categorizing the concerned period of these studies dated August 15, 2021, to December 31, 2021, according to their subjects, objectives, methods, findings, and results. This study was qualitatively carried out and the data were analyzed through content analysis. According to the results of the research, it was determined that the political collapse has fundamentally affected the private sectors, agribusinesses, household incomes, and socio-economical lives of the people.

Keywords: Private sector risks, impacts of politics on agribusiness, the competitiveness of enterprises.

**UNRAVELING THE DIFFERENTIAL MOLECULAR PROFILES OF CERVICAL
CANCER BASED ON TISSUE TYPE**

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ABSTRACT

Cervical cancer is one of the leading causes of women's death worldwide. The prevalence of adenocarcinoma (AC) and squamous cell carcinoma (SCC) has been increasing gradually but the genetic basis underlying tissue-specific cervical carcinogenesis is largely unknown. The study aimed to find differential gene mutations, DNA methylation, and mRNA expression profiles among different categories of tissue-specific cervical cancer samples.

The gene mutations, promoter methylation, and gene expression data of patients with cervical cancer from The Cancer Genome Atlas (TCGA) Database of Genomic Data Commons (GDC) portal were used in this study. The association of the significant gene mutations with the patient's survival among different tissue types was analyzed. The association of CpG island methylation with the gene expression was also determined by negative correlation analysis.

This study identified the ERBB signaling pathway and proteoglycans pathway which were significantly associated with AC cases compared to SCC cases. In these pathways, missense mutation of S310F in ERBB2 gene and G12D and A146T in KRAS gene was significantly associated with AC cases compared to SCC cases. Additionally, promoter methylation study revealed hypermethylation in the CpG island of the protocadherin gene cluster and PIWIL1 was associated with downregulation of the genes among SCC cases compared to AC cases.

Cervical squamous cell carcinoma and adenocarcinoma have distinctive molecular profiles. This result provides valuable insight into the differential molecular markers among the categories of cervical cancer which helps our ability to classify these cancers and for targeted therapy.

Keywords: Cervical cancer, squamous cell carcinoma, adenocarcinoma, mutation, CpG methylation, RNA, expression, CNV

HYDROFAUNA OF THE MIDDLE CASPIAN AZERBAIJANI AQUATORIUM

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ABSTRACT

Against the background of the impact of anthropogenic factors and species on the hydrofauna of the coastal waters of the Caspian Sea, as well as the coastal waters of the Azerbaijani sector of the Middle Caspian (*Mnemiopsis leidyi*, etc.), the study of conservation and protection of Caspian biodiversity is a topical issue. *Mnemiopsis combs* have been used by zooplankton to compete with kilka fish, leading to a sharp decline in their stocks. Changes in the level of the Caspian Sea are also one of the most important factors affecting the distribution of hydrobionts. In addition to oil extraction and other industrial pollutants, the influx of species into the Caspian Sea from various parts of the globe has become even more relevant in modern times. As a result of anthropogenic impacts, significant changes have taken place in the Caspian ecosystem, including fish biodiversity, population structure and reserves. Detection of these changes allows us to determine the direction in which they are going, to predict what problems may arise in the future. Therefore, the scientific and practical significance of such research is great. Taxonomic and ecological diversity of zooplankton and ichthyofauna in the coastal waters of the Azerbaijani sector of the Middle Caspian in 2017-2020 was studied; A general conclusion was reached on the number, development dynamics, distribution, population structure, as well as their unity of zooplankton and fish. Mesoplankton samples and ichthyological materials were collected and processed according to the general rules adopted in hydrobiology. 100 mesoplankton samples were taken from Nabran, Khudat, Khachmaz, Siyazan, Zarat, Gilazi, Sitalchay, Yashma and Haji Zeynalabdin waters of the Middle Caspian.

In 2017-2020, the hydrofauna of the Azerbaijani waters of the Middle Caspian was monitored by seasons. Mesoplankton has 31 species belonging to 3 groups (Rotatoria, Cladocera, Copepoda) and larvae of other invertebrates belonging to 3 groups. The Rotatoria group was represented by 9 species, the Cladocera group by 17 species and the Copepoda group by 5 species. Invasive species make up 22.73% of the mesoplankton fauna. In terms of biomass, the Copepoda group dominates. From the ecological point of view, neritic species predominate in the mesoplankton community (90.91%). Shortcorn species predominate in the taxocene

(42.11%). The share of freshwater species is 31.58%, and the share of marine species is 26.31%. The biodiversity of the ichthyofauna of the Azerbaijani sector of the Middle Caspian was also studied and the current situation was assessed. 13 species and semi-species of sea fish belonging to 17 taxa, 4 species and semi-species of transient and semi-transient fish belonging to 17 taxa were registered in the Azerbaijani waters of the Middle Caspian. In the Azerbaijani waters of the Middle Caspian, adult fish are found in all seasons at a depth of 15-25 m, and juvenile fish at a depth of 1-5 m.

Keywords: Middle Caspian, Mesoplankton, Caspian Sea, hydrofauna

**THE THERAPEUTIC EFFECTS OF DENIPLANT NATURAL MODULATOR
ON THE GUT MICROBIOME IN PATIENTS WITH PSORIASIS**

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ABSTRACT

Background A growing body of evidence highlights that intestinal dysbiosis is associated with the development of psoriasis. The gut–skin axis is the novel concept of the interaction between skin diseases and microbiome through inflammatory mediators, metabolites and the intestinal barrier. The gut microbiome affects skin homeostasis through its influence on the signaling pathways that coordinate epidermal differentiation.

The objective of this study was to synthesize current data on the Deniplant natural modulator of the gut microbiome in patients with psoriasis.

Materials and methods All studies confirmed the association of psoriasis and gut microbiota dysbiosis. We describe the recent advances regarding the interplay between gut microbiota and the skin. Thus, the microbiome can be considered an effective therapeutical target for treating this disorder.

Results This presentation provides a detailed and comprehensive systematic study regarding gut microbiome in patients with psoriasis. These results are supported by clinical observations based on a case serie showing improvement in psoriatic skin lesions after Deniplant natural modulator. It is still not clear whether psoriasis is an effect or a cause of the observed disbalance between beneficial and pathogenic microbes. In this context, the study provides very interesting results, showing significantly greater changes in the gut microbiome of patients with psoriasis treated Deniplant natural modulator

Conclusion There is a significant association between alterations in gut microbial composition and psoriasis. Intestinal dysbiosis is a state of imbalanced gut microbiome that eventually has a negative impact on skin function and integrity. Deniplant natural modulator is a potential therapeutic strategy in patients with psoriasis

Keywords: Dysbiosis, Microbiome, Psoriasis, Gut-Skin Axis, Gut Barrier, Deniplant Natural Modulator

ROLE OF THE CRYSTALLOGRAPHIC PHASE OF NITI ROTARY INSTRUMENTS: WHAT CHANGES IN DIFFERENT BENDING CONDITIONS?

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ABSTRACT

The scope of this study was to assess the role of the crystallographic phase of Nickel titanium (NiTi) rotary instruments in determining their torsional resistance during different bending conditions, such as different degrees and angles of curvature. 200 F-One 20.04 instruments (Fanta) Dental, Shanghai, China) were used, 100 austenitic instruments and 100 martensitic instruments. Each group was divided in 5 subgroups according to the different bending conditions (straight canal, 90 or 60 of curvature degrees and 3 mm or 5 mm of radius of curvature). The static torsional test was performed by using a device composed of an electric motor capable of recording torque values (Ncm); a vice used to secure the instruments at 3 mm from the tip; and artificial canals, which allow instruments to remain flexed during test. Each instrument was rotated at 500 rpm with a torque limit set to 5.5 Ncm until its fracture. Torque at Fracture (TtF) was registered. A scanning electron microscopy (SEM) observation was conducted. The collected data confirm that an increase in the angle of curvature and a decrease in the radius of curvature of the artificial canals lead to an increase of TtF values with a statistically significant difference ($p < 0.05$), both in the austenitic and martensitic groups. Regarding the comparison between austenitic and martensitic groups in the same bending condition, a statistically significant difference was found only when the torsional test was

performed in the canals with the degrees of curvature of 90 and the radius of curvature of 3 mm and 5 mm, with the austenitic instruments showing a higher TtF than the martensitic ones. In conclusion, it can be stated that the crystallographic phase influences the maximum torque at fracture when the instruments are subjected to severe bending and that the radius of curvature significantly influences their torsional resistance.

Keywords: Crystallographic Phase; Endodontics; Nickel-Titanium; Root Canal Treatment; Torsional Stress

DISSECTING THE MULTI-LEVEL CONTROL OF IMPRINTING AT THE *Dlk1-Dio3* And *Igf2-H19* DOMAINS IN DEVELOPMENT AND DISEASE

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ABSTRACT

Genomic imprinting is an epigenetic marking process that causes genes to be expressed or repressed depending on their parental origin. It is essential for normal mammalian development and its perturbation can lead to complex congenital diseases in humans. Most imprinted genes are organized in clusters, which generally contain both protein-coding and non-coding RNA genes. In our laboratory, we have explored the evolutionarily conserved paternally imprinted domains *Dlk1-Dio3* and *Igf2-H19*. The *Dlk1-Dio3* imprinted gene cluster expresses several protein-coding genes from the paternal chromosome, whereas the maternal chromosome expresses multiple non-coding RNAs, from a single polycistron whose expression is driven by the *Meg3* lncRNA promoter. Previously, we reported that *Meg3* expression controls the *Dlk1* gene in cis, by preventing its transcriptional up-regulation on the maternal chromosome during development (Sanli et al. Cell reports 2018). In order to further dissect the *Meg3* polycistron's role -particularly that of the lncRNA *Meg3* itself- we generated hybrid mouse embryonic stem cells (ESCs) that express truncated forms of *Meg3*, by inserting a poly-adenylation signal (PAS) at different positions. In ESCs with PAS insertion in intron-1 of *Meg3*, the insertion gave strongly reduced expression of all the ncRNAs of the polycistron, and affected the expression of *Dlk1*. In parallel, we assess the mechanistic consequences of specific epi-mutations (i.e., loss of DNA methylation) that are commonly associated with the imprinting disorders Silver-Russell Syndrome (loss of methylation at *Igf2-H19* domain) and Temple Syndrome (loss of methylation at *Dlk1-Dio3* domain). For that, we apply a novel CRISPR-dCas9 based technology to recruit multiple copies of the catalytic domain of TET1, to induce loss of methylation at the *Meg3* promoter and at the *Igf2-H19* imprinting control region. In the obtained hybrid ESCs, we unravel the precise transcriptional, chromatin-structural and phenotypic consequences, both in ESCs and in derived differentiated cells.

Keyword: genomic imprinting, CRISPR-dCas9 based technology, imprinting disorders

SELF-REPORTED SLEEP DISORDER, ANXIETY AND DEPRESSION IN POST MYOCARDIAL INFRACTION PATIENTS IN IRAQ

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ABSTRACT

Myocardial infarction (MI), characterized by the myocardial cell necrosis due to significant and sustained ischemia, is the main manifestation of CHD and has been a significant burden of both high-income countries and low-income countries. Post myocardial infarction sequels may be psychosocial. The common psychosocial sequels of myocardial infarction include anxiety, fear of impending death, social isolation, sleep disturbance, and depression. The aim of this study is to assess the outcomes of sleep quality, depressive symptoms and anxiety among post myocardial infarction patients. A descriptive cross-sectional design was used to collect data from patients (n =94) who were with in first months post myocardial infarction diagnosis. The Arabic version of the Pittsburgh Sleep Quality Index, Patient Health Questionnaire-9and generalized anxiety disorder 7 (GAD7) were used to assess sleep quality, depressive and anxiety symptoms respectively. The sample mean age was 60.35 ± 8.35 years. Poor sleep quality affected 69.1 % (65) of the participants. The significant predictors of poor sleep quality were the intervention and low income which associated with higher rate of sleep difficulty. The most impacted domains of sleep quality were sleep latency, Sleep duration, and sleep disturbances. The prevalence of depression in patient with MI approximately half participants have mild depression (51.1%) according to PHQ 9 scale and The prevalence of anxiety in in patient with MI were most of them have moderate anxiety (40%) according to GAD7 scale.

The sleep quality of post myocardial infarction patients was poor and the prevalence of depression and anxiety was high. So this findings show the importance of regular assessment to early diagnosed and manage sleep problems, anxiety and depressive symptoms in post myocardial infarction patient

Keywords: Myocardial Infarction, Sleep Quality, Anxiety And Depressive.

**WORMIAN BONE OF THE SAGITTAL SUTURE ON A METOPIC FEMALE
SKULL OF THE MIDDLE AGES WITH OCCIPITAL DEFORMATION**

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ABSTRACT

Artificially deformed skulls are of great historical and anthropological interest. All kinds of combinations of various anatomical modifications can be found on these skulls, including a metopic suture with Wormian bones. There are many indications of the localization of the Wormian bones, mainly in the lambdoid suture; however, the combination of the Wormian bone of sagittal suture with the metopic suture is quite rare. The occurrence of this combination on artificially deformed skulls dating back to the Middle Ages has not yet been described.

The study aimed to investigate an artificially deformed female's metopic skull dating back to the Middle Ages.

The material for the study was an artificially deformed skull taken from the craniological collection of the educational museum of the Department of Human Anatomy and Medical Terminology of the Azerbaijan Medical University. The deformation belonged to the occipital type. The study used cranioscopic, craniometric, and computed tomography methods.

The results of the study showed that the length of the metopic suture was 79.01 mm. The suture can be divided into 3 segments. The length of the first segment is 35.47 mm. The intermediate segment, 7.35 mm long, passed into the third segment, 36.19 mm long. The dentation of the lower segment was weakly expressed, the upper segment of the metopic suture had a rather pronounced dentation. A Wormian bone was located in the sagittal suture. The anteroposterior dimension of this bone was 14.59 mm; the width was 8.28 mm.

Artificial cranial deformation, apparently affecting the normal growth of the bones of the skull, causes such modifications as the metopic suture and Wormian bones. Being a natural model of bone growth transformation, this phenomenon is subject to deep study in anatomical terms.

Keywords: Artificial cranial deformation, Metopic suture, Wormian bone, Sagittal suture.

**ANTIAGING ACTIVITY OF *POLYALTHIA LONGIFOLIA* LEAF IN
SACCHAROMYCES CEREVISIAE BY611 YEAST MODEL VIA MICROSCOPIC
APPROACHES¹**

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ABSTRACT

Plants produce variety of bioactive compounds. High phytochemical concentrations in medicinal plants, is critical in cell rejuvenation and anti-aging effects. Based on the medicinal plant *Polyalthia longifolia*'s prominent antioxidative properties, this could contribute towards their antiaging benefits. Hence, the present study was undertaken using *Saccharomyces cerevisiae* BY611 strain yeast model to appraise the antiaging property of *P. longifolia* leaf methanol extracts (PLME) on the basis of morphology via various microscopic approaches. Prior to the evaluation, the BY611 yeast cells were treated with 1.00 mg/mL PLME extract. The antiaging activity was then evaluated by assessing the replicative and total lifespan, vacuole morphology, extra-morphology by Scanning (SEM) and Transmission (TEM) Electron Microscopy for intra-morphology. As yeast cells were treated with PLME, their replicative and total lifespans were significantly boosted when compared to untreated cells, as evidenced from replicative and total lifespan experiments. Furthermore, light microscopy studies demonstrated that PLME administration might delay the formation of large apoptotic-like type 3 yeast cell vacuoles, which is consistent with PLME's favourable effect on yeast cell lifespan. Besides, we discovered notable age-related changes in phenotypes for the untreated yeast cells compared to the PLME-treated yeast cells using the SEM and TEM. SEM study of untreated yeast cells exhibited prominent aging extracellular morphology such as shrinkage, regional invaginations and wrinkled cell surfaces. Meanwhile, TEM analysis of untreated yeast cells displayed quintessential aging intracellular morphology such as swollen, wrinkled, or damaged vacuole, circular endoplasmic reticulum formation, ruptured nuclear membrane, nucleus fragmentation, and fully damaged cytoplasm. However, in the PLME-treated yeast cells, such major morphological alterations were delayed further validating the vacuole morphology study in

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establishing their protective effect. Conclusively, using three microscopic approaches – SEM, TEM, and bright-field light microscope – the results show that *P. longifolia* leaf extract has an antiaging effect in the *S. cerevisiae* BY611 yeast model, which helps to increase yeast cell proliferation and decrease their propensity to undergo apoptotic cell death.

Keywords: Scanning electron microscopy, transmission electron microscopy, light microscope, antiaging, *P. longifolia*, yeast

PECULIARITIES OF ANXIETY IN ADOLESCENTS

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ABSTRACT

There is a lack of studies in gender differences of anxiety among adolescents. Therefore, this leads to a problem question: what are the levels of anxiety in males and females during adolescence? The purpose of the study is to evaluate anxiety in adolescents. The hypothesis was formulated that females, compared to males, would report significantly higher levels of anxiety.

The Beck Anxiety Inventory (BAI) a 21-item self-report multiple-choice questionnaire, was used to assess the severity of symptoms related to anxiety. Each answer in BAI had a score on a scale value of 0–3. The range of total scores was from 0 to 63. The cut off points for classification are as follows: 0–7 minimal anxiety, 8–15 mild anxiety, 16–25 moderate anxiety, and 26–63 severe anxiety. We selected 315 adolescents from Kaunas region in our sample. The average age of males was $M = 16.73$ years ($SD = 2.38$) and females $M = 16.34$, years ($SD = 2.62$) years. One-hundred-sixty-seven female and 148 male adolescents took part in the survey.

Use of Student's t-test revealed that statistically significant differences were found between the two study groups in levels of anxiety: females reported higher levels of anxiety ($10,17 \pm 5,39$) than males ($8,97 \pm 5,28$): $t(71) = 1.99$; $p < .05$. The hypothesis, which expected that females, compared to males, would report significantly higher levels of anxiety was supported. It should be noted that some researchers (for instance, Beck and Steer) found significant differences in anxiety according to gender. They reported that women with anxiety disorders tend to score, on average, four points higher than men with anxiety disorders using the BAI. Conclusion: The results of the present study revealed that females reported higher concern than males regarding the symptoms of anxiety.

Keywords: anxiety, adolescents, gender differences.

**BURNOUT, PSYCHOSOCIAL FACTORS AT WORK AND NUTRITION AMONG
MEDICAL DOCTORS: AN OBSERVATIONAL STUDY REPORT**

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ABSTRACT

Job burnout is a special type of work-related stress, either in a state of physical or emotional exhaustion, which in turn can initiate a sense of reduced accomplishment and loss of personal identity. "Burnout" is not a regular medical diagnosis but has been considered by experts to happen because of depression. Stating also that individual factors, such as personality traits and family life, can influence who experiences job burnout. Burn-out is included in the 11th Revision of the International Classification of Diseases (ICD-11) as an occupational phenomenon. It is not classified as a medical condition. The prevalence of burnout is taking an increase in self-reported data, of which the numbers are showing a more frequent risk emergence among women than men. Overall, the data are difficult to compare, as they build on different definitions. This is becoming a growing problem as many hospital administrators are trying to address the current issue, as more clinicians are starting to leave their jobs. For many health care workers, the stress of last year's pandemic situation does not seem to be going away anytime soon.

The aim of this study was to investigate the associations between burnout, physical activity and emotional eating among medical practitioners in Lithuania and Nigeria, with the use of a burn out online anonymous questionnaire as data source. Data information was acquired using a shared anonymous voluntary sampling google online questionnaire. Numerical values derived were analyzed using the Microsoft Excel spreadsheet SPSS. Statistical analysis was done according to age, gender, psychosocial factors at work, lifestyle habits, nutrition, emotional eating, and the life orientation of the gotten responses. Correlation analysis was the final step employed to understand the concomitant associations between psychosocial factors at work and emotional eating, while also considering the possible influence from lifestyle, and life orientation.

A total of 117 responses were received spreading ranging different fields of front-line health workers which included Doctors (Pediatricians, Gynecologists, Family Physicians), Surgeons, Dentists, Optometrists, Nurses, Anesthesiologists and Laboratory Scientists. The priority field was Medical Doctors with a frequency of 45.3% response from these professionals and a mean age of 42 (SD 8) years consisting of both men and women. The analysis from Spearman's correlations were calculated between percentiles of burnout scores, Emotional eating behavior, Exhaustion, Depersonalization, Personal achievement, Demands, Control, and social support.

We tested statistical differences in eating behaviors, do you often exercise (play sports, run, etc.), how many hours per night they sleep, night work, compared to a year ago how they rate their health, how they access their health in general, if they drink alcohol, several types of foods they consume.

Strong correlations were observed between emotional eating and the effect it had on their health a year ago, level of alcohol intake and coffee intake. Suffering from exhaustion was seen to be mostly because of total sleeping hours and night shift which could be traced to be a cause for depersonalization and how they judged their health performance from the previous year, with their health in general being influenced by several values of control and social support. At the baseline, eating behavior showed several correlations between several unhealthy foods and the emotions to which they are taken by these individuals. The highest correlations ranged from soft drinks, energy drinks, coffee and light beverages, influencing their different reactions to burnout occurrences.

The findings of these studies theoretically seem plausible because several physiological and psychological mechanisms underlying burnout with the hospital working place can be identified and investigating the relationship between physical activity, burnout and the corresponding psycho-social effects can help reduce burnout of these professionals within the hospital workplace. The healthcare industry is one of the most hazardous environments to work in, with employees in this industry being constantly exposed to a complex variety of health and safety hazards in the course of their work. Burnout is becoming a rapid problem with this industry and this research can be used to give advice on ways to which most of these situations can be reduced.

Keywords: Burnout, Job Burnout, Emotional Eating Behavior, Nutrition among Medical Doctors, Psychosocial Factors at Work.

PREVALENCE AND CATEGORIES OF FREQUENTLY OCCURRING DRUG INTERACTIONS IN ICU SETTINGS. AN OBSERVATIONAL PROSPECTIVE STUDY FROM KARACHI PAKISTAN

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ABSTRACT

Background:

Drug Drug Interactions are major cause of extensive mortality ratios and prolonged hospitalization with associated increase of healthcare cost. These Interactions may range from mild to severe in term of intensity of may be of multiple nature. Healthcare professionals are often required to change of modify prescribe medications. They poses greater consideration in low socio-economic region where affordable medical facilities are highly challenging.

Methodology:

The aim of this study was to evaluate the Drug-drug Interactions incidence, prevalence and related facts in ICU settings of Karachi, Pakistan. In this study we have estimated the prevalence of DDIs in tertiary care settings in ICU cohort. The study design was prospective, observational and conducted in cross sectional manner and various drug interactions were also calculated using a well designed questionnaire. Moreover impact of these interactions were correlated with length of hospitalization and associated increase in cost.

Results:

Results were calculated with spss software version 20 and p values were considered significant with less than 0.05. Delayed and rapid onset of effects were 52% and 35% respectively. Association of gender was found insignificant while age was determined as significant parameter for higher values of DDIs.

Conclusion:

Prescriptions contain higher number of drugs and handling of prescriptions are also major causes of such aspects. Trained professionals with better understanding of drug related issues are likely to be appointed in such settings including pharmacist and applications of drug management software's is recommended to avoid the risk of mortality and associated morbidity.

Keywords: drug interactions, ICU, geriatric, incidence, facts

**A CROSS SECTIONAL STUDY ON HEPATITIS PREVALENCE FROM KARACHI
PAKISTAN. CONTRIBUTING FACTORS AND RELATED CO-MORBIDITIES
ASSESSMENTS**

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ABSTRACT

Background

Hepatitis is considered as threatening and widespread disease in low socio-economic region where hygiene and health care services are under targeted. Public health perception and lifestyle management are key attributes for progressive disease burden.

Methodology

In this observational and cross sectional study we investigated the various mode of viral transmission, associated risk factors, prevalence of different categories of hepatitis, co-morbid status of patients cohort and available options of treatment and compliance. A structured questionnaire was developed to collect the related information of study respondents (N=200). This investigation was carried out to screen the health status of local population. Medical records of patients were also reviewed to gather the required information.

Results

An inclined rate of hepatitis C and B were found in respective order of 58% and 24%. Rare cases were seen of Hepatitis D. Hepatitis A rates were comparatively lower in targeted population up to 15%. Results were analyzed statistically with SPSS 20. Though a structure and systematic vaccination campaigns are now being made over the couple of years which could be considered as a small seed towards better quality of life. But poorly treated water and blood borne infections transmissions are prevalent in society.

Conclusion

While hepatitis B and C stay serious, it very well may be overseen and treated. Yet, individuals with hepatitis B or C face social distraction and separation, deterring them from looking for clinical assistance that could forestall conversion of their sickness to more serious issues like liver malignancy and growth. A mass screening of population for this infection must be established at district and union council levels. Public should be taken in various counselling and education activities regarding safe practice during infection period for self and care givers.

Moreover a treatment support programme may be initiated for needy population and property advertising and contact details may be provided for better compliance of medication utilization.

Keywords: Hepatitis, lifestyle management, Risk factors, perception, Prevalence, transmission.

**ESWEP SCORE, A NEW STATISTICALLY VALID SCORING CRITERIA FOR
DECISION BETWEEN REPAIR OR ILEOSTOMY IN PATIENTS WITH
PERITONITIS DUE TO ENTERIC PERFORATION**

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ABSTARCT

Enteric fever leading to enteric perforation is very common surgical emergency in the developing nations. The two surgical solutions used worldwide are to repair or exteriorize the perforation as ileostomy. The aim of this study was to setup and validate a statistically reliable scoring system for decision between repair and ileostomy in patients with peritonitis due to enteric perforation. It was an observational cross-sectional study done at EAST Surgical Ward of MAYO Hospital Lahore, Pakistan. 256 patients were selected by consecutive non probability sampling after ethical approval. The duration of study was 2 years from 1st August to 2018 to 30th July 2020. Patients with age more than 13 years presented in emergency department with diagnosis of peritonitis due to enteric perforation were included. A preformed scoring criterion named as East Surgical Ward Enteric Perforation (ESWEP) Score was set by giving each variable a score of 1 to 3. Cutoff value between repair and ileostomy was observed. In this study, 142 (55.4%) patients were male and 114 (44.5%) were female. Male to female ratio was 1: 1.24. The average age of patients is about 37 years with SD of ± 9.67 years. ROC curve showed cutoff of pre-operative ESWEP Score of 4 (Sensitivity 78%, Specificity of 88%), per operative score of 7 (Sensitivity 96 %, Specificity of 85%) and total ESWEP Score of 11 (Sensitivity 94%, Specificity of 88%).

This study concluded that ESWEP Score is a new score which helps in standardization of operative procedure for the patients with peritonitis due to enteric perforation.

Keywords: - Enteric, ileostomy, Peritonitis, Repair, Score.

**TRAINING HEALTHCARE FACULTY IN A LEARNING CONTENT
MANAGEMENT SYSTEM (LCMS)**

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ABSTRACT

The COVID-19 pandemic was stressful for educational systems and especially for healthcare education, where practical skills are mastered in the laboratories and at the bed of the patient. Academic staff of health educational institutions need support and training as they adapt to the new situation and face the instructional challenges brought by the pandemic. This presentation deals with the development, the provision and the evaluation of a course “E-learning. Moodle” for healthcare faculty.

The course was developed following the instructional design principles and it was launched at the beginning of the 2020/2021 academic year, which was also marked by long periods of lockdown.

The training was developed as a course in Moodle – the formal learning content management system at the Medical University of Plovdiv. Thus, faculty has the opportunity to experience being an e-student, which will help to gain a better understanding of both the difficulties that students would face and their expectations. The training combines competencies from the relevant subject area of each participant with pedagogical and technological knowledge and skills.

The objective was for the participants in the course to acquire basic knowledge and skills for creating e-learning content and working with the learning content management system Moodle. To meet the objective, seven topics were developed:

1. Introduction to e-learning;
2. Introduction to instructional design;
3. Tips how to create engaging and interactive resources;
4. Working with Moodle;

5. Separate sections devoted to the test module and the lesson module;
6. Content protection.

The evaluation of the training was carried out through a self-reported online anonymous survey among academic staff training healthcare majors at the Medical University of Plovdiv. A total of 109 teachers were enrolled, of them, 92 took part in the training. The number of colleagues, who completed the questionnaire was 69.

The training of the academic staff in the learning content management system in which they teach enhances the direct transfer of knowledge and skills in teaching and provides a better notion of students' experience. Confidence in computer skills and pedagogical qualification are strong predictors of the success of such training – both in terms of evaluation and achievement of educational goals, and in terms of satisfaction and increasing motivation to develop e-learning. Training aimed at the teaching staff is beneficial when its design allows independent asynchronous learning, but there is also a synchronous component. Training should be following the concept of micro-learning – to consist of short videos demonstrating particular skills directly related to the topic objectives.

Keywords: E-Learning, COVID-19, Healthcare, Faculty

KÜRESEL ISINMANIN TARIM ÜZERİNE ETKİLERİ
THE EFFECTS OF GLOBAL WARMING ON AGRICULTURE

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ÖZET

Küresel ısınma en başta atmosfer olmak üzere okyanus ve kara kütleleri üzerinde sıcaklık artışı olarak tanımlanır. Bu kavramın günümüzde en popüler karşılığı “**küresel iklim değişikliği**”dir. Bu duruma fosil yakıtlar başta olmak üzere sanayileşme, ormansızlaştırma, arazi kullanım

değişikliği ve diğer beşerî faaliyetler neden olmaktadır. Atmosfere salınan sera gazlarının birikmesi ve sonrasında yerküredeki sıcaklık artışına paralel olarak iklimde değişiklikler ortaya çıkmıştır. Bu değişim etki ve sonuçları itibari ile yaşantımızı çok büyük oranda etkilemiştir. Özellikle kuraklık, erozyon ve sonrasında çölleşme, iklim kuşaklarında yer değiştirmeler, ani ve şiddetli hava olayları, buzul erimeleri sonucunda deniz seviyesinde yükselmeler, salgın hastalıklar, doğal dengenin bozulması sonucunda birçok canlı türünün nesli ya tükenmiş ya da tehlike altına girmiştir. Bitkisel üretimde küresel ısınmaya bağlı olarak artan sıcaklık değerleri fotosentez aktivitesinin azaltarak verim ve kalite parametrelerini olumsuz etkilemiştir. Bitki büyüme ve gelişmesi, çoğalma ve dölleme yetisi çok azalmıştır. Araştırmacıların üzerinde çalıştığı bazı iklim projeksiyonlarında tropik ve sub tropik bölgelerde tarımsal üretim azalırken, kuzey bölgeleri başta tahıllar olmak üzere birçok ürün için elverişli hale geleceği tahmin edilmektedir. Bu bakımdan Ülkemiz küresel ısınmanın olumsuz etkileri açısından en riskli ve şansız bir konuma sahiptir. Son yıllardaki su kaynaklarında azalma, orman yangınları çölleşme ve tarımsal üretimdeki rekolte kayıpları bunun bir göstergesi olarak ortaya çıkmıştır. Uzayda söz sahibi ülkelerin ülkemize ait resim ve görsellerin paylaşımı ve bazı ödüllere konu olmasındaki temel nedenin küresel iklim değişiminin **en net** görüldüğü bir coğrafyada yer bulunmamızdır. İnsanlığın geleceği adına tüm ülkelerin bir araya gelerek küresel iklim değişikliğine karşı bir eylem planı hazırlaması ve uygulaması kaçınılmazdır. Aksi bir durumda tarihte olduğu gibi bitkiler, hayvanlar ve insanların daha kuzeye veya dağlık alanlara doğru kitlesel göç hareketlerine şahit olabiliriz. Küresel ısınmanın tarımsal üretimi sekteye uğratması, gıda arzındaki gerileme Dünya üzerinde sosyo-ekonomik krizi tetikleyerek yeni bir kaosu neden olacaktır.

Anahtar Kelimeler: İklim, tarım, küresel ısınma, kuraklık.

ABSTRACT

Global warming is defined as an increase in temperature over the ocean and land masses, especially in the atmosphere. The most popular equivalent of this concept today is “global climate change”. This situation is caused by fossil fuels, industrialization, deforestation, land use change and other human activities. In parallel with the accumulation of greenhouse gases released into the atmosphere and the subsequent increase in temperature in the world, changes in the climate have occurred. This change has greatly affected our lives in terms of its effects and results. Many species have become extinct or endangered, especially as a result of drought, erosion and subsequent desertification, displacements in climatic zones, sudden and severe weather events, rise in sea level as a result of glacial melting, epidemics, and deterioration of natural balance. Increasing temperature values due to global warming in plant production decreased photosynthesis activity and negatively affected yield and quality parameters. Plant growth and development, reproduction and fertilization ability are greatly reduced. In some

climate projections that researchers are working on, it is predicted that while agricultural production will decrease in tropical and sub-tropical regions, northern regions will become suitable for many products, especially grains. In this respect, our country has the most risky and unfortunate position in terms of the negative effects of global warming. The decrease in water resources, forest fires, desertification and yield losses in agricultural production in recent years have emerged as an indicator of this. The main reason why countries that have a say in space share pictures and images of our country and are the subject of some awards is that we are located in a geography where global climate change is most clearly seen. On behalf of the future of humanity, it is inevitable for all countries to come together and prepare and implement an action plan against global climate change. Otherwise, we may witness mass migration movements of plants, animals and people to the north or to mountainous areas as in history. The disruption of agricultural production by global warming and the decrease in food supply will trigger a socio-economic crisis on the world and cause a new chaos.

Keywords: Climate, agriculture, global warming, drought.

GİRİŞ

Avrupada ilk olarak 18 ve 19. yüzyıllarda başlayan başlayan sanayi devrimi ile birçok alanda önemli gelişmeler görülmüştür. Bu refah ve zenginleşme süreci kendisi ile birlikte bir takım çevresel problemlerinde ortaya çıkmasına neden olmuştur((Akalin, 2014). Özellikle son yüzyıl içerisinde artan sera gazı emisyonları nedeniyle yeryüzü 0.5-1°C sıcaklık artışı meydana gelmiştir(Stern, 2007). Artan sanayileşme ve benzeri beşeri faaliyetler bu artışın daha tehlikeli bir boyut kazanacağını göstermektedir. İngiliz bilim insanı Stren (2007) tarafından yapılan iklim modellemelerinde herhangi bir önlem alınmadığı takdirde gelecek yıllarda yeryüzünün 1.4- 5.8 °C arasında ısınacağı belirtilmiştir. Bu ısınma atmosferik ve hidrolojik denegeyi bozarak yağış ve su rejimlerinde dalgalanmalara neden olacağı belirtilmiştir. Bu bozulma kendisi ile birlikte çok ekstrem iklim olaylarının (Kasırğa, fırtına, hortum, sel baskınları, yağışın miktarı ve şekli, buzullarda erime vb) görünme sıklığı ve şiddetinin artmasına neden olacaktır. İklimlerde meydana gelen bu değişimler makro ve mikro ölçekte bazı etkilerinin olması kaçınılmazdır. Bu değişimler tarımsal üretim, bitki ve orman örtüsü, biyolojik çeşitlilik, enerji kaynakları, su kaynakları, deniz suyu seviyesi ve çevre-insan sağlığı üzerine menfi etkilerinin olacağı bir gerçektir (Akalin, 2014). Tüm bu etkilerin günümüzdeki en popüler karşılığı “küresel ısınma” olarak ifade edilmektedir.

Dünyadaki sıcaklığın sistematik olarak artması “küresel ısınma” olarak tanımlanmıştır. Sıcaklık artıka su kaynaklarındaki buharlaşma yükselerek yağış ve hava hareketleri değişir. Küresel iklim değişikliği olarak bilinen bu durumun ortaya çıkmasında 10-15 yıllık bir zaman dilimine ihtiyaç duyulmaktadır. Kısa süreli hava olayları ile bu durumun karıştırılmaması gerekmektedir. Küresel iklim değişikliğinin etkilerinin tespiti amacıyla 1983 yılından itibaren

çok ciddi ölçümler yapılmıştır. Bu çalışmalardan elde edilen sonuçlar en sıcak ve en kurak son 20 yılda yaşandığı tespit edilmiştir. Bu değişimlerin en net ölçüldüğü yerler hiç şüphesiz kutuplar olmuştur. Örneğin, güney kutbundan şimdiye kadar görülmemiş büyüklükte buzul parçalarının koparak ayrılmıştır. İzlanda, Himalaya ve Alplerdeki buzullarında son 30 yılın en büyük erime olayı görülmüştür. Uzayda ve yeryüzünde yürütülen bazı çalışmalarda sera gazları olarak bilinen; karbondioksit, kloroflorkarbon gazları (CFC-11, HCFC-22, CF4, vb.), metan, azotoksitleri, ozon ve su buharı olarak nitelenen bazı gazların atmosferdeki miktarlarının arttığı ve yeryüzüne yakın atmosfer tabakaları ile sıcaklığının yapay olarak yükseldiği görülmüştür (Anonim, 2022). Bu gazlar atmosferi kapladıkları gibi güneş ışınları ile karşılaşınca ısı açığa çıkmaktadır. Bu gazlar ısı enerjisi dalgalarının yeryüzünden atmosfere doğru yükselmesine; başka bir ifadeyle, karasal ışıma(radyasyon) olayı ile atmosferin yüksek katmanlarına ulaşmasına engelledikleri bilinmektedir. Bu gazlar yükselen ısının bir kısmını absorbe ederek yeniden yeryüzüne yansıtır. Gazların neden olduğu bu olaya “küresel ısınma” denir.

Küresel ısınma ve bunun etkileri sosyo-ekonomik hayatı olumsuz yönde etkilemiştir (Doğan ve Tüzer, 2011). Nüfus artış hızına paralel olarak küresel iklim değişikliği tarımsal üretim ve gıda güvenliğini tehdit etmeye başlamıştır. Dünya nüfusunun %30-35'nin tarım ve tarıma dayalı sektörlerden geçimini sağladığı düşünüldüğünde etkileri çok can yakıcı olmuştur. İklim değişikliğinin en fazla etkilediği tarım sektörü tüm aktörleri ile bunu tartışmalıdır. Küresel çapta kurulacak ortak bir platformda konunun önemini tartışması ve gerekli tedbirleri ortaya koyması gerekmektedir. Günümüzde küresel ısınmaya bağlı olarak ortaya çıkan gıda güvenliğini ve arzını riske sokacak tehditlerin bertaraf edilmesinde küresel bir eylem planına ihtiyaç duyulmaktadır. Küresel ısınma ve bunun tarım üzerindeki etkilerini hafifletecek uzun ve kısa vadede pratik çözümler üretilmelidir(Doğan ve Tüzer, 2011). Aksi durumda tarımsal üretimde yaşanacak bir darboğaz sosyo-ekonomik ve siyasal alanda büyük bir kaosa neden olma potansiyeli göz ardı edilmemelidir.

Sonuç olarak küresel ısınmanın tarımsal üretim üzerindeki etkilerinin tam tespiti kısmen mümkün olabilmektedir. Özellikle bu konuda yürütülecek çalışmalardan elde edilen verileri ve tahminleri kıymetli kılacaktır. Bu çalışmada Dünyada ve Ülkemizde olası iklim projeksiyonları ve senaryolarının tarımsal üretim üzerindeki olumsuz etkileri ve buna karşın alınabilecek tedbirlerin belirlenmesi amaçlanmıştır.

ARAŞTIRMA VE BULGULAR

Küresel İklim Değişikliğinin Tarım Üzerine Etkileri

Küresel iklim değişikliğinin olumsuz etkileri başta tarım olmak üzere birçok sektörü etkilemektedir. Türkiye coğrafi konumundan dolayı yarı-kurak bir iklim kuşağında bulunması ve topografik yapısı gereği düzensiz yağış rejimine sahip olması nedeniyle sürekli kuraklık riski taşıyan bir ülkedir. Bu nedenle, Türkiye küresel iklim değişikliğinden ve özellikle de

kuraklıktan etkilenebilecek ülkelerden biridir. Ülkemizin 100 yıl sonra Kuzey Afrika'ya dönüşme olasılığı çok uzak bir ihtimal değildir. Elde edilen verilere göre son 70 yılda 100'e yakın istasyonda kaydedilen sıcaklık verilerine göre durum çok ciddi bir hale dönüşmüştür. Akdeniz ve Güneydoğu Anadolu bölgeleri artarak ısınmakta ve ısınma oranları, her 10 yılda 0.07- 0.34 derece arasında olduğu bildirilmiştir. Dünya Yaban Hayatı Koruma Fonu (WWF) nın raporuna göre Akdeniz havzasında bulunan Türkiye'de 40 dereceye yakın sıcaklıklar mevsim normali olarak kabul edilecektir. Geliştirilen iklim projeksiyonları ve modeller tarım alanlarının ise yüzde 40'ını kaybetme riski ile karşı karşıya olduğumuz belirtilmiştir (Anonim, 2022).

Gerçekte bu sorun tüm ekolojik sistemi alt üst eden büyük bir probleme dönüşmüştür. Mevsimlerin değiştiği bir birine karıştığı bir girdaba dönüşmeye başlamıştır. Bahar ayını görmeden yaz ayını sonbaharı yaşamadan kışı görmeye başladığımız gerçeği herkesin malumudur. Ancak bu etkilenme olayı denize kıyısı olan ve olmayan bölgelerde farklı etkilerle kendini göstermektedir. (DPT, 2000). Kuraklık; uzun bir zaman içerisinde yağışların belirgin bir şekilde normal değerlerin altına düşmesi olarak tanımlanan meteorolojik kuraklık; meteorolojik kuraklığın uzaması durumunda yeraltı suları, akar sular ve göllerin seviyesinde ani bir düşüşe neden olan hidrolojik kuraklık; toprak muhtevasında bitkinin ihtiyacı olan suyun bulunmaması olarak tanımlanan tarımsal kuraklık olarak üç çeşittir (Anonim,2007a).



Anonim 2022(<https://www.google.com/search?q=VANG>).

Kuraklık öncelikle tarım alanlarında ve suya dayalı sektörler üzerinde etkisini göstermektedir. Çünkü bitkiler için yıl boyunca yağın toplam yağıştan ziyade, büyüme dönemlerinde bitki kök bölgesinde barındırdığı su çok daha önemlidir. Dolayısı ile bitkilerin çıkış ve gelişme döneminde ihtiyaç duyabilecekleri suyun toprakta bulunmaması tarımsal kuraklık anlamına gelmekte olup, tarımsal verimliliği yakından etkilemektedir. Tarımsal kuraklığın izlenmesinde en önemli unsur sıcaklık, yağış, yağışın dağılımı, toprak profil derinliği, toprak nemi, toprak yapısı ve bünyesidir. (Kapluhan, 2013).

Ürün kalitesini ciddi oranda etkileyen tarımsal kuraklık, ekonomisi tarıma dayalı olan bölgeler için en önemli sorunların başında gelmektedir. Kuraklığın yaşandığı bölgelerde gözlenen diğer sorunlar arasında, düzensiz yağış rejimleri, yağışların beklenen düzeyinde altında kalması, su kaynaklarının nitelik ve nicelik olarak azalması, artan nüfusla beraber su kaynaklarının kontrolsüz ve plansız kullanımından dolayı beklenen verimi sağlamaması gibi sorunlar yer almaktadır (TAKEP, 2008; Başoğlu ve Telatar, 2013). Bunun doğal sonucu olarak tarımsal üretimde yıllar itibari ile büyük dalgalanmalar meydana gelmektedir (Tablo 1, Tablo 2).

Tablo:1 Türkiyede tarım alanlarının yıllar itibari ile dağılımı

TÜRKİYE'DE TARIM ALANLARI

Tarım Alanı	1990		2002		2017		2018		2019		2020	
	(Bin ha)	%	(Bin ha)	%	(Bin ha)	%	(Bin ha)	%	(Bin ha)	%	(Bin ha)	%
Tarla Bitkileri	18.868	67,7	17.935	67,5	15.532	66,4	15.421	66,5	15.387	66,6	15.615	67,5
Nadas	5.324	19,1	5.040	19,0	3.697	15,8	3.513	15,2	3.387	14,7	3.173	13,7
Sebze	635	2,3	930	3,5	798	3,4	784	3,4	790	3,4	779	3,4
Meyve, İçecek ve Baharat	3.029	10,9	2.674	10,1	3.343	14,3	3.457	14,9	3.525	15,3	3.564	15,4
Süs Bitkileri	-	-	-	-	5	0,02	5,1	0,02	5,2	0,02	5	0,02
TOPLAM	27.856	100	26.579	100	23.375	100	23.180	100	23.094	100	23.136	100

Kaynak:TÜİK

Tablo-2: Türkiyede üretilen bazı ürünlerin yıllar itibari ile ekim ve üretim miktarları

Ürünler	Ekim Alanı (1000 ha)								Üretim (1000 ton)							
	2012	2014	2015	2016	2017	2018	2019	2020	2012	2014	2015	2016	2017	2018	2019	2020
Buğday	7.529	7.919	7.867	7.672	7.669	7.299	6.846	6.922	20.100	19.000	22.600	20.600	21.500	20.000	19.000	20.500
Arpa	2.749	2.787	2.784	2.740	2.424	2.468	2.869	3.097	7.100	6.300	8.000	6.700	7.100	7.000	7.600	8.300
Çavdar	143	115	112	115	101	111	112	104	370	300	330	300	320	320	310	296
Yulaf	89	94	103	99	113	106	110	113	210	210	250	225	250	260	265	315
Çeltik	120	111	116	116	110	120	126	125	880	830	920	920	900	940	1.000	980
Aspir	16	44	43	40	27	25	16	15	20	62	70	58	50	35	22	21
Ayçiçeği	605	657	685	720	780	734	753	729	1.370	1.638	1.681	1.671	1.964	1.949	2.100	2.067
Dane Mısır	623	659	688	680	639	592	639	692	4.600	5.950	6.400	6.400	5.900	5.700	6.000	6.500
Kolza	30	32	35	35	17	38	53	35	110	110	120	125	60	125	180	122
Kuru Fasulye	93	91	94	90	90	85	89	103	200	215	235	235	239	220	225	280
Kütü	488	468	434	416	502	519	478	359	2.320	2.350	2.050	2.100	2.450	2.570	2.200	1.774
Pamuk	237	250	224	252	293	277	282	248	438	345	360	365	430	353	354	371
Mercimek	416	389	359	360	395	514	521	512	518	450	460	455	470	630	630	630
Nohut	174	130	154	145	143	136	141	148	4.795	4.166	4.760	4.750	4.800	4.550	4.980	5.200
Soya	32	34	37	38	32	33	35	35	122	150	161	165	140	140	150	155
Şeker Pancarı	281	289	274	322	339	292	314	338	15.000	16.743	16.023	19.593	21.149	17.436	18.086	21.000
Tütün	108	99	92	93	95	93	81	75	73	75	68	70	80	80	70	77
Soğan(kuru)	73	60	58	60	58	53	61	68	1.736	1.790	1.879	2.121	2.132	1.931	2.200	2.280
Çay	76	76	76	76	82	78	78	83	1.250	1.266	1.328	1.350	1.300	1.481	1.407	1.418

Kaynak: TÜİK

Türkiye'nin 643 mm olan uzun yıllar yağış ortalaması küresel ısınmanın etkisiyle giderek azalmaktadır. Bu yağış azalışları tarımsal üretim üzerinde, olumsuz bir etki yaratacaktır. Olabilecek bir küresel iklim değişikliği sonucunda, su kaynaklarının azalması, ekolojik dengelerin bozulması ve buna bağlı olarak, orman yangınları, kuraklık ve çölleşme gibi

faktörlere maruz kalacak risk grubundaki ülkeler arasına girecek olan ülkemizin de daha sıcak ve kurak bir iklim kuşağına dahil olabileceği tahmin ediliyor. Özellikle sıcaklık artışından en fazla çölleşme tehdidi altında bulunan Güney Doğu ve İç Anadolu gibi kurak ve yarı kurak bölgelerle, yeterli suya sahip olmayan yarı nemli Ege ve Akdeniz Bölgeleri etkilenecektir (Öztürk, 2002).

Sıcaklık ve yağış ülkemizde tarımı etkileyen en önemli iklim elemanlarıdır. Bu doğal koşullara bağlı olarak da tarımsal üretim, iklim, toprak koşulları ve uygulanan tarım tekniklerine göre artmakta veya azalmaktadır. Kurak bölgelerde aşırı sıcaklar verim düşüklüğüne neden olmaktadır. Sulu tarım yapılan bölgelerde ise bitkiler sıcaklık stresine girerek, yeterli sulama yapılırsa dahi verimlerinde düşme söz konusu olmaktadır. Sulu tarım yapılan bölgelerde sıcaklık artıktı, sulama sayısı da artmakta böylece yeraltı ve yerüstü su kaynaklarının aşırı kullanılmasına neden olmakta ve var olan doğal denge de bozulmaktadır. Bu durum tarım yapılan alanlarda ve havzalarda ekosistemde yaşayan canlı bitki ve hayvan tür ve popülasyonunu da etkilemektedir. Kurak iklim şartlarına uyum sağlayamayan birçok bitki ve hayvan türünün yok olması söz konusu olabilmektedir (Celik ve Karakayacı 2007).

Doğal nedenlere bağlı olarak yıllarca yavaş yavaş değişim gösteren iklim elemanları, günümüzde önemli derecede hissedilebilecek hızlı bir değişim sürecine girmiştir. Yağış miktarlarının azalması ve sıcaklıkların yukarı yönlü değişmesi ile birlikte oluşan kuraklık, tarım alanları ve su kaynaklarının etkilenmesine, bitkilere yeteri kadar suyun toprakta bulunmamasına ve su dengesinde bozulmalara sebep olmaktadır. Yağış ve sıcaklıklarda ki değişimler küresel ısınmanın etkisiyle, bölgelerin sahip olduğu mevcut su potansiyellerinde de önemli azalmalar meydana gelmektedir. (McCarthy ve ark., 2001; Christensen ve ark., 2007; Fıstıkoğlu ve Biberoglu, 2008). Örneğin belirli bir aralığın üstüne çıkan sıcaklık değerleri, bitkilerde tohum gelişimini bozmakta ve verimin azalmasına neden olmaktadır. Ayrıca yüksek sıcaklıklar bitkilerin su kullanımına etki etmektedir (Doğan Demir ve Demir, 2016). (Karabulut, 2012).

Kuraklığın bir diğer olumsuz etkisi de tuzluluk olacaktır. Küresel ısınmadan dolayı su istekleri artan bitkilere verilen su ile toprağa tuz ilavesi yapılmakta ve bu arada toprağın derinliklerinde bulunan tuzlarda kapillar yükselme ile toprak yüzeyine çıkarak birikmektedir. Toprakta biriken tuzlar bitkide fizyolojik kuraklığa sebep olur ve bitki strese girerek verimde önemli düşüşler meydana gelir.

Kuraklık, bitki hastalıklarına, böcek istilasına, ürünlerde kayıba, ürün kalitesinde düşüklüğe, orman yangınlarına, orman ürünlerinde kayıba, ağaç hastalıklarına ve çiftçi gelirlerinde kayıplara neden olabilir (Öztürk 2002).

İklimsel değişim bitkilerde olumlu etki gösterdiği kadar olumsuz etkilerde ortaya çıkarmaktadır. Sıcaklık ve CO₂ miktarı bitki gelişim dönemleri için önemli birer faktördürler. Sıcaklık ve CO₂ konsantrasyonundaki artış, bitki gelişim hızını arttırır, gelişim periyodu kısalmır

ve potansiyel büyüme daha az bir şekilde sonuçlanabilir. Tahıllar günümüzde insan beslenmesi, hayvan beslenmesi ve sanayi sektörü de dahil çoğu alanda kullanılan insanoğlunun temel ihtiyaçlarından en önemli olanıdır. Olası bir küresel ısınma durumunda tahıllar kapladıkları ekim alanı bakımından etkilenme olasılığı en yüksek olan bitkilerin başında gelmektedir. Özellikle sıcaklık artışlarının tahılların dane doldurma dönemlerini hızlandırdığı görülmektedir. Olgunlaşma süresi ve dane doldurma süresi toplam verim üzerine etkili olduğundan dolayı artan sıcaklık sebebi ile kısalan dane doldurma süresi birçok üründe verim ve kalitenin azalmasına neden olmaktadır (Bongaart, 1994).

Sıcaklıkların artmasıyla su kaynaklarının azalması, ortadan kalkması, sulu şartlarda yetişen çeltik, gelişim periyodu sırasında ve verimliliği suya bağlı olarak artan mısır gibi diğer tahılların verim ve kalite parametrelerinde önemli azalışlar meydana gelecektir. Kuraklık nedeni ile bitki deseninde önemli farklılıkların meydana geleceği düşünülmektedir. Kuraklık görülen alanlarda özellikle; buğday, pamuk, mısır, mercimek gibi bitkiler ön plana çıkmaktadır. Dünyada en çok üretilen C4 bitkileri olan mısır ve çeltik küresel ısınmanın oluşturduğu kuraklığın etkisiyle ve çoğu tahıllarda yaşanacak üretimde meydana gelen kayıplar, gıda ve yem bitkilerinde ciddi açıklar oluşmasına neden olacaktır. Başta buğday, arpa gibi bitkiler olmak üzere kuraklığa adaptasyonu iyi olan türlerin yetiştiriciliği yaygınlaşacak, diğer yandan artan erozyon, sel ve taşkın gibi doğa olayları sonucu üretim ve verim üzerinde ciddi tehdit unsurları oluşacaktır.

Ülkemizde bazı bölgelerde yağışın az olması, yağış rejiminin düzensiz olması, kaynakların yetersiz olması, bazen de tersi durumların yani aşırı yağışların meydana gelmesi sonucu birçok bölgede sel olaylarının yaşanması tarımsal üretiminin kısıtlanmasına neden olmaktadır. Yaşanan kuraklık ilk olarak İç Anadolu Bölgesinde görülmekte daha sonra ise Akdeniz, Ege, Marmara ve Karadeniz bölgelerine yayıldığı görülmektedir. İlk olarak belirlenen meteorolojik kuraklık, daha sonra tarımsal kuraklığa dönüşmüş, bunun bir sonucu olarak çiftçilerimiz pek çok üründe ciddi zararlarla karşı karşıya kalmışlardır.

Türkiye’de 2007 yılında çok şiddetli ve geniş bir yayılış gösteren kuraklık, 2007 yılı kış, ilkbahar ve yaz aylarında yeni bir meteorolojik kuraklık yaşanmasına ve böylece tarımsal, hidrolojik kuraklık sonucu sosyoekonomik çöküntüye neden olacağı belirtilmiştir (Türkeş, 2007). Ayrıca buğday üretimi 2007 yılında bir önceki yıla göre %13,3 oranında azalarak 20 milyon ton dan 17,3 milyon tona gerilemiş, arpa üretimi ise %22,4 azalışla 7,4 milyon tona gerilemiştir. Bu miktarların azalmasında diğer birçok üründe olduğu gibi yaşanan kuraklık etkili olmaktadır. Bu konuda Hindistan özelinde yapılan bir modellemede olası bir küresel ısınma etkisiyle eğer sıcaklık 4 °C derece artacak olursa, tahıl üretimi %25-40, çeltik üretimi %15-25, buğday üretimi %30-35 oranında azalacağı varsayılmıştır (Mendelsohn, 2000).

Şimdiye kadar iklim ve iklim faktörleri ile ilgili yapılan çalışmalarda son dönemlerde sıcaklıkların arttığı, yağışların ise azalmakta olduğu görülmektedir. Türkeş ve ark., (1996)

tarafından yapılan bir çalışmada, günümüzde iklim değışikliklerinin etkilerinin zamanla arttığı, bunun sonucunda ise sıcaklık ve yağış değerlerinde değışkenlik meydana geldiği belirlenmiştir.

Kuraklık ve Etkileri

Tarım sektörünün gelişmiş olduğu ülkelerde bile, tarımsal üretimin en önemli sorunlarından bir tanesi de kuraklıktır. Kuraklığın ilk etkileri; tarımsal üretim yapılan alanlarında ve suya ihtiyaç duyulan sektörlerde görülmektedir. Tarım sektöründe kuraklıktan kaynaklı oluşan etkilerin diğer sektörlerde oluşan etkilerden farklı olduğu bilinmektedir (Kapluhan, 2013). Farklı şiddetlerde meydana gelen ve genotipe bağlı olan kuraklıktan etkilenme derecesi, o genotipin stres altında oluşturduğu metabolik farklılıklara bağlıdır (Kayabaşı, 2011). Bitkiler üzerinde meydana gelen kuraklık stresi; kuruma ve su noksanlığı olmak üzere ikiye ayrılmaktadır (Smirnoff, 1993). Su noksanlığı; gaz değışiminde kısıtlamaya ve stomalarda kapanmaya sebep olan orta düzeydeki su kaybıdır. Su noksanlığı ile karşı karşıya kalan bitkilerde stomaların kapanmasının bir sonucu olarak karbondioksit alımı azalmaktadır. Kuruma ise metabolizma ve hücre yapısının tamamen bozulmasına ve enzimle katalizlenen reaksiyonların durmasına sebep olan fazla miktardaki su kaybı olarak ifade edilmektedir (Smirnoff, 1993; Kalefetoğlu ve Ekmekçi, 2005). Kuruma olayında solma çok ileri bir evreye gelmişse, bitkinin artık eski haline dönmesi mümkün değildir (Kaçar, 2015). Kurumaya duyarlı vasküler bitkilerin çoğu vejetatif doku, % 30'un altındaki oransal su kapsamına ulaştıktan sonra iyileşme sürecine artık girememekte ve bitki ölmektedir (Kalefetoğlu ve Ekmekçi, 2005).

Bitkilerin, büyüme ve gelişme dönemlerinde gereksinim duydukları suyun yeterli miktarda toprakta bulunmaması durumunda tarımsal verimlilikte azalmalar görülmektedir. Buna tarımsal kuraklık adı verilmektedir. Tarımsal kuraklık, tarımsal üretimi ve verimliliği önemli derecede etkilemektedir (Kapluhan, 2013). Ekonomik olarak tarıma bağlı olan bölgeler için tarımsal kuraklık, başlıca sorunlardandır. Kuraklığın meydana geldiği diğer bölgelerde gözlemlenen sorunlar arasında; yağışların istenen düzeyde olmaması, düzensiz yağış rejimlerinin görülmesi, nüfusun hızla artmasına bağlı olarak su kaynaklarının kontrolsüz ve plansız kullanılması bulunmaktadır (TAKEP, 2008; Başoğlu ve Telatar, 2013). Verimliliğe etki eden diğer faktörler arasında; mevsime bağlı olarak meydana gelen kuraklıklar, yaz aylarında özellikle buharlaşma oranının yüksek seviyede olduğu dönemlerde sulama faaliyetlerinin az olması ve beklenmedik epizodik kuraklıklar görülmektedir (Partigoç ve Soğancı., 2019). Tarımsal kuraklığın uzun süre görülmesi bölgede felaketin oluşmasına neden olmaktadır. Kuraklığın uzun süre devam etmesi bitki ölümlerine neden olmakta ve bunun sonucunda bitkiden verim alınamamaktadır. Yaşanılan bu olay kuraklık felaketi olarak tanımlanmaktadır. Kuraklık felaketinin görüldüğü bölgelerde dışarıdan ürün alındığından maliyet yükselmektedir (Wang ve ark., 2003; Kaçar ve ark., 2006; Özen ve Onay., 2007; Mengü ve ark., 2011).

Türkiye yarı kurak iklim kuşağında bulunan bir ülkedir. Bu nedenle yağışların alana ve zamana dağılımı oldukça düzensizdir. Hızla artan nüfusun ve sanayi ihtiyaçlarını, mevcut su kaynakları

karşulamakta yetersiz kalmakta, yüzey sulama sistemleri ile tarımsal üretimde suyun büyük bir bölümü bilinçsiz bir şekilde kullanılmakta; suyumuzun kalitesi giderek azalmaktadır (Wang ve ark., 2003; Kaçar ve ark., 2006; Özen ve Onay., 2007; Mengü ve ark., 2011).



(Anonim, 2022 a. Kuraklık stresine ait bir görüntü. <https://images.app.goo.gl/EpKgXYVEMzj5FQgx5>).

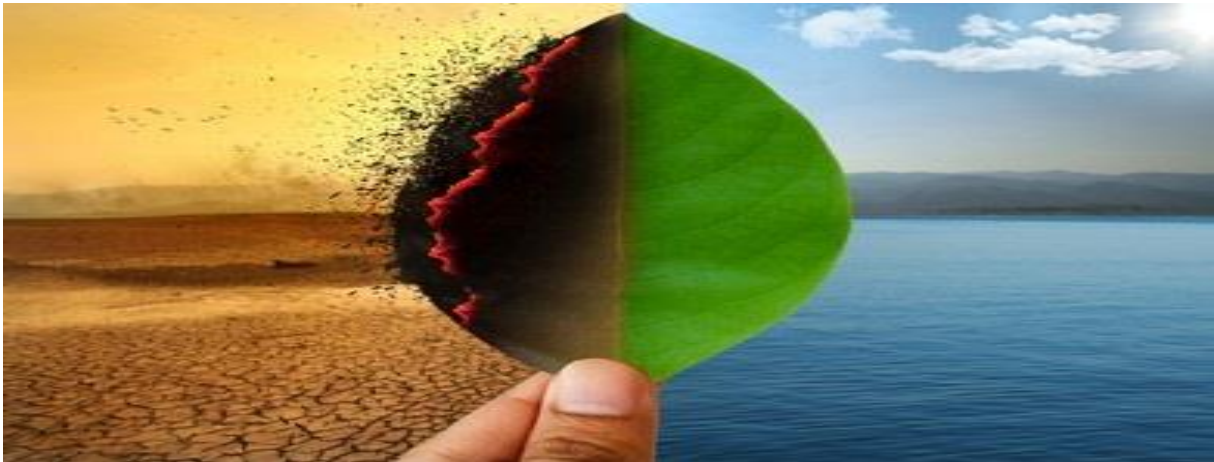
Bitkilerde su eksikliği; toprakta bitkinin ihtiyacı olan su miktarının normalden az olması, atmosferik koşulların etkisiyle evaporasyon ve transpirasyon sonucu su kaybının devam etmesine bağlı olarak meydana gelir. Suyun yeterli miktarda olmaması durumunda floem ve ksilemdeki madde iletimi olumsuz etkilenmektedir. Bunun sonucunda meyveler küçük kalmakta, tahıllarda ise danelerin yeteri kadar dolmamasına ve ürün kalitesinin düşmesine neden olduğu görülmektedir (Kaçar ve ark., 2006; Özen ve Onay., 2007; Kavar ve ark., 2008). Tarımsal üretimde kullanılan sulama sistemleri ile suyun daha az kullanılması sağlanabilmektedir. Tarımsal üretimde damlama veya yağmurlama sulama sistemi gibi modern sulama sistemlerinin kullanılması ile salma sulamaya göre; su kullanım miktarının % 70 oranında azaltırken, ürün verimini ise % 90 oranında arttırmaktadır (Kapluhan, 2013).

Bitkiler gereksinim duydukları su ihtiyacına göre veya buldukları çevrede suyun miktarına bağlı olarak; mezofitler, hidrofiter ve kserofitler olmak üzere 3 gruba ayrılmaktadırlar. Mezofitler, toprak neminin uygun olduğu alanlarda büyüyen, hidrofiter, bir kısmı veya tamamı suyun içerisinde yaşayan, kserofitler ise kurak alanlarda büyüyen bitkiler olarak tanımlanmaktadır (Öztürk ve Seçmen, 1992).

Kuraklık stresi altında bulunan bitkilerin kök bölgesinde farklılıklar görülmektedir. Bu farklılıklar sonucunda bitkinin kök bölgesi mantara benzeyen kalın bir doku tabakası ile örtülmektedir. Bu tabaka, altta bulunan canlı hücreleri, sıcak ve kurak olan toprak etkisinden koruyabilmektedir. Kuraklık oluşması durumunda, toprak üstü organlardan köklere doğru çözünebilir karbonhidratlar taşınmaktadır. Bunun sonucunda köklerdeki ozmotik basınç yükselerek suyu emme güçleri artmaktadır (Çırak ve Esenal., 2003). Kurak koşullar altında;

yapraklarda oluşan morfolojik farklılıklar, genellikle transpirasyon sonucu kaybedilen su miktarını azaltmak; köklerde oluşan morfolojik farklılıklar sonucu ise topraktaki suyu yüksek bir kuvvetle absorbe etmeyi amaçlamaktadır. Kuraklık stresi altında kökün gövdeye oranı artmakta ve kökün gelişimi hızlanmaktadır (Öztürk ve Seçmen 1992).

Kuraklık anında, bitkinin toprak üstü kısımlarından köklere doğru çözünebilir karbonhidratlar taşınmaktadır. Bunun sonucu olarak, köklerin ozmotik basınçları artarak, su emme güçlerinin yükseldiği görülmektedir. Bitkilerde yaprak yüzey alanının genişliği arttıkça bunun bir sonucu olarak su kaybı da artmaktadır (Göksoy ve Turan, 1991). Bitkiler su kaybını en aza indirmek için toplam yaprak alanlarını küçültmeye çalışırlar. Bu durum genellikle çöl bitkilerinde çok sık görülmektedir. Yaprak büyümesi, kuraklık stresine karşı oldukça hassastır. Bu durum fotosentezin azalmasına bağlıdır. Ayrıca, kuraklık stresine bir tepki olarak bitkiler yaprak yüzeylerini sık tüylerle kaplamaktadır. (Göksoy ve Turan, 1991).



(Anonim, 2022 b. Kuraklık stresine ait bir görüntü. <https://www.shutterstock.com/tr/image-photo/climate-change-global-warming-concept-burning-1092120809>)

Son dönemde ülkemizde yaşanan kuraklık, kış aylarında oluşan yağış miktarının büyük oranda azalması ile meteorolojik kuraklıktan, hidrolojik ve tarımsal kuraklığa doğru değiştiği görülmektedir. Yaşanılan iklim değişiklikleri ile yakın zamanda meydana gelen kuraklığın, uzun dönemde tekrarlayan bir doğa olayı olmaktan çıkarak gündelik yaşamımızın bir parçası haline dönüşmeye başladığı görülmektedir. Bunun bir sonucu olarak tarımsal alanda su kullanımının, kuraklık şartlarına uygun hale getirilmesi amaçlanmalıdır (Levent, 2014).

Stres koşullarına karşı öncelikli olarak yürütülen çalışmalar; bitki ıslah metodları ile kuraklığa karşı dayanıklı çeşitlerin bulunup, geliştirilmesi ve aşılı fidelerin yaygınlaştırılması gerekmektedir. Ayrıca nemin toprakta tutulmasını arttıran bazı toprak düzenleyicilerin kullanılması, toprakta bulunan neminin doğru ve düzenli bir şekilde izleyen sensörlerin sulama uygulamalarında kullanılması, bazı topoğrafik önlemlerin alınması ve kısmi kök kuruluğu gibi

birbirinden farklı sulama tekniklerinin kullanılması gibi çeşitli çalışmalar son dönemlerde yoğun bir şekilde yapılmaktadır. Suyun az veya yetersiz olduğu alanlarda hayatta kalabilen bitkilerin mekanizmaları incelenerek, strese dayanıklı genotipler belirlenmeye çalışılmıştır. Kuraklığa dayanıklı çeşitlerin geliştirilmesi önümüzdeki yıllar için gıda ihtiyacının sağlanmasında büyük önem taşımaktadır (Örs ve Ekinci, 2015).

SONUÇ ve ÖNERİLER

Küresel iklim değişikliği tarımsal üretim üzerine etkilerine yönelik makro ve mikro ölçekte alınabilecek bazı tedbirler aşağıda belirtilmiştir.

- 1-Vangölü havzasında mevcut su kaynaklarına ait tam bir envanterinin çıkartılması,
- 2-Bir komisyon marifeti ile İlimizdeki nüfus ve ekonomik artış hızına bağlı olarak artan su talebi ve tüketiminin mevcut kaynaklar üzerindeki baskılarının nasıl azaltılacağı ve alınacak tedbirler belirlenmeli,
- 3- Bölgemizde tüm paydaşların bir araya gelerek iklim değişikliğinin su kaynakları üzerindeki olumsuz etkilerini en aza indirecek bir acil eylem planının hazırlanması,
- 4-Evlerde tüketilen günlük içme ve kullanma suyu için kayıp/kaçak oranlarının azaltılması, denetim ve cezaların artırılması,
- 5-Su israfının azaltılmasına yönelik radyo-televizyon programları ile tanıtım materyalinin hazırlanarak dağıtımı ve bu konuda bir farkındalığın oluşturulması,
- 6-Yağmur ve kar sularının hasadını sağlayacak suni göl ve göletlerin yapılması,
- 7-Tarla ve bahçe sulamasında kullanılan kanal ve kanaletlerin tamiri ve bakımının yapılması,
- 8-Buharlaştırma yoluyla oluşacak su kaybını azaltacak kapalı sistem kanal ve kanalet yapımına öncelik verilmesi,
- 9-Sera gazı salınımını artıran ağır toprak işleme metotları yerine minimum toprak işleme yöntemlerinin geçilmesi,
- 10- Küresel ısınmaya bağlı ortaya çıkan kuraklık tehlikesine karşı suyu daha ekonomik kullanan strese dayanıklı bitki tür ve çeşitlerinin belirlenmesi(Ürün güncellemesi)
- 11-Salma ve vahşi sulama yöntemleri yerine damlama, yağmurlama, toprakaltı sulama gibi basınçlı sulama sistemlerine geçilmesi,
- 12-Basınçlı sulama sistemlerinin teşvikine yönelik hibe-fon kaynaklarının çeşitlendirilmesi,
- 13- İçme ve kullanım suyunun tarla ve bahçe sulamada kullanılmasına yönelik caydırıcı cezaların artırılması,

- 14-Sanayii ve evsel su tüketiminde daha ekonomik ekipman kullanımı (Araba ve halı yıkama işletmeleri)
- 15- Tarımda ve evsel kullanımdaki atık suların yeniden kullanılmasına olanak sağlayacak sistemlerin geliştirilmesi,
- 16-İyi ve organik tarım uygulamalarına ağırlık verilmesi (teşvik, destek),
- 17- Tarımda ilaç ve gübre kullanımını sınırlandıracak organik gübre, biyolojik ve kombine mücadele yöntemlerine geçilmesi,
- 18- Küresel ısınma ve kuraklık ile mücadele kapsamında çiftçilere yönelik eğitim faaliyetlerinin artırılması,
- 19- Su kullanım etkinliği ve yönetiminde paydaşların tamamına temsil yetkisi verilmeli,
- 20-Ağaçlandırma faaliyetlerine hız verilmeli,
- 21-Kuraklık ve sonuçlarının değerlendirildiği bilimsel toplantı ve çalıştay sayısının artırılması

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**NITROGEN ASSIMILATION CAPACITY OF *CHENOPODIUM ALBUM L.*
LOCATED IN POLLUTED AREAS**

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ABSTRACT

Phytoremediation offers a sustainable treatment option for polluted sites. It is ecological, low-cost, and cost-effective, leading to the establishment of long-term green cover in the contaminated area. Fast-growing plant species characterized by high biomass production is used in phytoremediation.

Nitrogen metabolism is one of the main physiological processes of plants and is directly related to growth. The nitrogen assimilation (N) by plants requires the uptake of nitrate (NO_3^-) from the soil and reduction to nitrite (NO_2^-), and then, the conversion of NO_2^- to ammonium (NH_4^+). And it is followed by the incorporation of NH_4^+ into organic compounds. The activity of nitrate reductase, which is the key enzyme in nitrogen assimilation, is considered an indicator of nitrogen metabolism.

This paper aims to set forth the changes in nitrogen assimilation capacity of *Chenopodium album L.* which were naturally established on the sewage sludge lagoons. The present study is determined the nitrate reductase activity (NRA) and biomass parameters in *Chenopodium album L.* NRA of samples was determined according to the *in vivo* test. This spectrophotometric method is based on the determination of the absorbance of nitrite (NO_2^-) formed with the reduction of nitrate in incubation medium. Our results were shown that the rapid growth and high nitrogen assimilation ability of *Chenopodium album L.* allow it to settle naturally in degraded areas. It is important to reveal the physiological characteristics of the plant species that are frequently used in phytoremediation, in order to learn the functioning of the plant.

Keywords: Nitrogen assimilation, nitrate reductase activity (NRA), biomass, phytoremediation, polluted areas.

**CYTOTOXIC AND GENOTOXIC EFFECTS OF FUMONISIN B₂ ASSISTED WITH
MOLECULAR DOCKING STUDIES**

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ABSTRACT

Mycotoxins are secondary metabolites produced by fungi and exhibit toxic effects. Fumonisin are among the most common members of the mycotoxin family, produced by *Fusarium* species occurring in maize, wheat and other grains. There are different types of fumonisin and the toxic effects of fumonisin B₁ have been studied in detail. Although there are many studies on the mechanism of action and toxicity of fumonisin B₁, studies on other fumonisin species are at a low level. Fumonisin B₂ is produced by *Fusarium verticillioides* and *Aspergillus niger*. Fumonisin B₂, a structural analogue of fumonisin B₃, is more cytotoxic than fumonisin B₁. In this study, the cytotoxic and genotoxic effects of fumonisin B₂ were determined in vivo by the *Allium* test and the mechanism of toxicity was elucidated by molecular docking studies. In this context, mitotic index and chromosomal aberration analyzes were performed in the slides prepared using *Allium* bulbs applied with fumonisin. Biovia Discovery Studio 2020 Client was used for molecular docking. As a result, it was determined that the application of fumonisin B₂ decreased the mitotic index rate by 40.5% compared to the control group. The mitotic index is an indicator of the proliferation of a cell. The proliferation-reducing effect of fumonisin B₂ has been associated with its interaction with tubulin proteins. As a result of molecular docking studies, it was determined that there are hydrogen and hydrophobic bonds between fumonisin B₂ and tubulin proteins. In the chromosomal aberration analysis, anomalies such as sticky chromosome, unequal distribution of chromatin and vagrant chromosome were detected after fumonisin B₂ application. These abnormalities can be explained by the disruptions in genome integrity, and this result was confirmed by the histone-fumonisin B₂ interactions obtained after molecular docking analysis. As a result, fumonisin B₂ exhibited cytotoxic and genotoxic effects by causing a decrease in mitotic index rates and a high rate of chromosomal aberration. The cytotoxic effect was associated with fumonisin-tubulin, and the genotoxic effect was associated with fumonisin-histone interactions.

Key words: Fumonisin B₂, Chromosomal abnormality, Mitotic index, Molecular docking

INTRODUCTION

Fungi, known as strong sources of antibiotics, also produce various metabolites that are toxic to humans and animals. Secondary metabolites produced by fungi and exhibiting toxic effects are called mycotoxins. The term mycotoxin was first used in the UK in 1961 after a crisis in which thousands of animals died, and it was determined that the disease was caused by ingestion of a peanut contaminated with a toxin produced by the filamentous fungus *Aspergillus flavus* [1,2]. In general, mycotoxins are low molecular weight compounds synthesized by filamentous fungi during secondary metabolism. Their chemical structures can vary from simple C₄ compounds to complex substances. Mycotoxins are natural pollutants in raw materials, foods and feeds. Mycotoxin-producing fungi species can grow on a wide variety of substrates under different environmental conditions, and this feature threatens agricultural crops around the world. Mycotoxins, even at low concentrations, are toxic to vertebrates and other animal groups and exhibit allergenic, teratogenic, carcinogenic and mutagenic properties. Mycotoxins, unlike bacterial toxins with antigenic properties and protein structure, have different biological activities and a high variety of chemical structures. In agriculture and food industry, mycotoxins are divided into five main classes: aflatoxins, ochratoxins, fumonisins, zearalenone and patulins [2,3]. Fumonisins are important toxins produced by *Fusarium* species. Different types of fumonisins such as fumonisins B₁, B₂, and B₃ are the main forms found in foods. *F. verticillioides* and *F. proliferatum* are the two most important fumonisin producing species. Fumonisin B₂ (FB₂) is a fumonisin produced by *F. verticillioides* and *A. niger*. It is a structural analogue of fumonisin B₃ and differs from fumonisin B₁ in that it does not contain a hydroxyl group [4,5].

FB₂ is more cytotoxic than fumonisin B₁ and has a toxic effect by inhibiting sphingosine acyltransferase. While FB₂ and other fumonisins frequently contaminate maize and other crops, it has recently been found to cause contamination of coffee beans as well [5,6]. Studies on mycotoxins are mostly related to aflatoxins. Although the studies on fumosins are less, these studies mostly focused on fumonisin B₁. Therefore, studies investigating FB₂ toxicity have a high specific value. In this study, the cytotoxic and genotoxic effects of FB₂ were determined using the *Allium* test. Information on the toxicity mechanism was provided by molecular docking studies. Mitotic index was used to determine cytotoxic effects, and micronucleus and chromosomal abnormalities were examined to determine genotoxic effects.

MATERIAL AND METHODS

The *Allium* test, which is an important indicator test for determining FB₂ toxicity was used. *Allium cepa* bulbs of equal size and appearance were used as test material. In order to determine the dose-related toxic effect, four different experimental groups were formed. The control group was coded as Group I and the bulbs of this group were germinated with tap water. Group II, Group III and Group IV were germinated with 10 µg/mL, 20 µg/mL and 40 µg/mL FB₂,

respectively. 10 bulbs were used for each group and germination was carried out at 24 °C for 72 hours. At the end of the period, cytotoxic and genotoxic effects were investigated in root tip cells.

Cytotoxic Effects

In order to determine the cytotoxic effects of FB₂, the mitotic index (MI) ratios of each group were determined. For this purpose, root tips of 1-2 cm in length were collected from the root tip cells of each group at the end of the germination period. Root tips were washed with distilled water to remove surface residues. The samples, which were kept in Clarke fixator for 2 hours, were kept in 96% ethanol for 15 minutes. At the end of the time, the samples were kept in 1N HCl at 60 °C for hydrolysis. The samples were stained in carmine (aceto-carmin) solution prepared in 45% acetic acid. At the end of the period, the samples were examined under a microscope [7,8]. To determine the effect of FB₂ on MI, 10000 cells were counted in each group, and MI (%) was calculated by taking the ratio of dividing cells to total cells.

Genotoxic Effects

In order to determine the genotoxic effects of FB₂, the micronucleus (MN) and chromosomal abnormality (CA) frequencies of each group were determined. For this purpose, root tips of 1-2 cm in length were collected from the root tip cells of each group at the end of the germination period. Root tips were subjected to fixation, hydrolysis and staining processes. A total of 1000 cells were counted in each treatment group for the frequency of micronucleus and chromosomal abnormalities, and abnormalities were determined under the microscope and photographed at X500 magnification [8,9].

Molecular Docking

Molecular docking studies with macromolecules have been carried out to elucidate the mechanism of the toxic effect of FB₂. In particular, its interaction with histone proteins and tubulin proteins was investigated in order to determine its cytotoxic and genotoxic mechanism of action. The 3D crystal structure of the proteins was obtained from the protein database. Biovia Discovery Studio 2020 Client was used for molecular docking. For proteins, energy minimization with Gromos 43B1 using Swiss-Pdb Viewer20 (v.4.1.0) software, and ligand energy minimization with uff-force field using Open Babel v.2.4.0 software 21 [10].

Statistical Analysis

Statistical analyzes were performed using the “IBM SPSS Statistics 22” package program. Data are shown as mean ± SD (standard deviation). Statistical significance between the means was determined by One-way ANOVA and Duncan's test, and a p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Cytotoxic Effects

In order to determine the cytotoxic effects of FB₂, MI rates were examined and the results are given in Figure 1. MI of the control group was found to be 8.7%. It has been determined that FB₂ reduces MI in a dose-dependent manner. It was determined that 10 µg/mL FB₂ decreased the mitotic index, but this decrease was not statistically significant ($p > 0.05$). 20 µg/mL and 40 µg/mL FB₂ applications significantly decreased MI rates compared to the control group, and this decrease was found to be statistically significant ($p < 0.05$). MI is an indicator of cell proliferation. In normal cells, the cell cycle continues without any interruption or pause. As a result of damage to cells, cytotoxic or genotoxic effects, the cell cycle stops at certain cycles and cell division is delayed. In damaged cells, this abnormality is eliminated by repair mechanisms and completes the cell cycle. In the presence of irreparable damage, the cell tends to controlled death [11,12]. Delays and pauses in cell division generally indicate a cytotoxic effect. The fact that FB₂ reduces MI rates at the root tip of *Allium cepa* indicates a cytotoxic effect.

To understand the mechanism of formation of this effect, FB₂-tubulin interactions were investigated (Figure 2, Table 1). Tubulin proteins polymerize to form microtubules. Microtubules are the main components of mitotic spindles used to separate eukaryotic chromosomes from each other, and abnormalities in these proteins directly cause delays in mitosis. FB₂ interacts with tubulin proteins, causing deterioration in their three-dimensional structure, and since microtubule polymerization does not occur, the movement of chromosomes to the poles is restricted, and this causes both disruptions in mitotic stages and formation of CA.

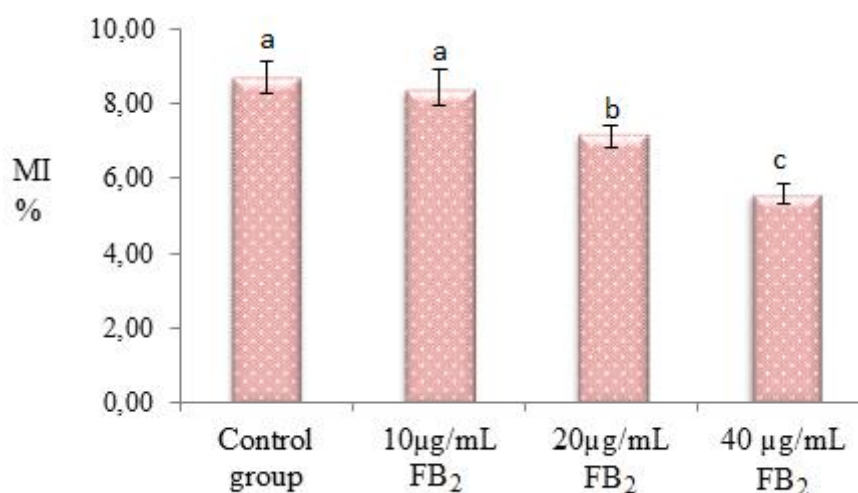


Figure 1 Effects of FB₂ on Mitotic Index

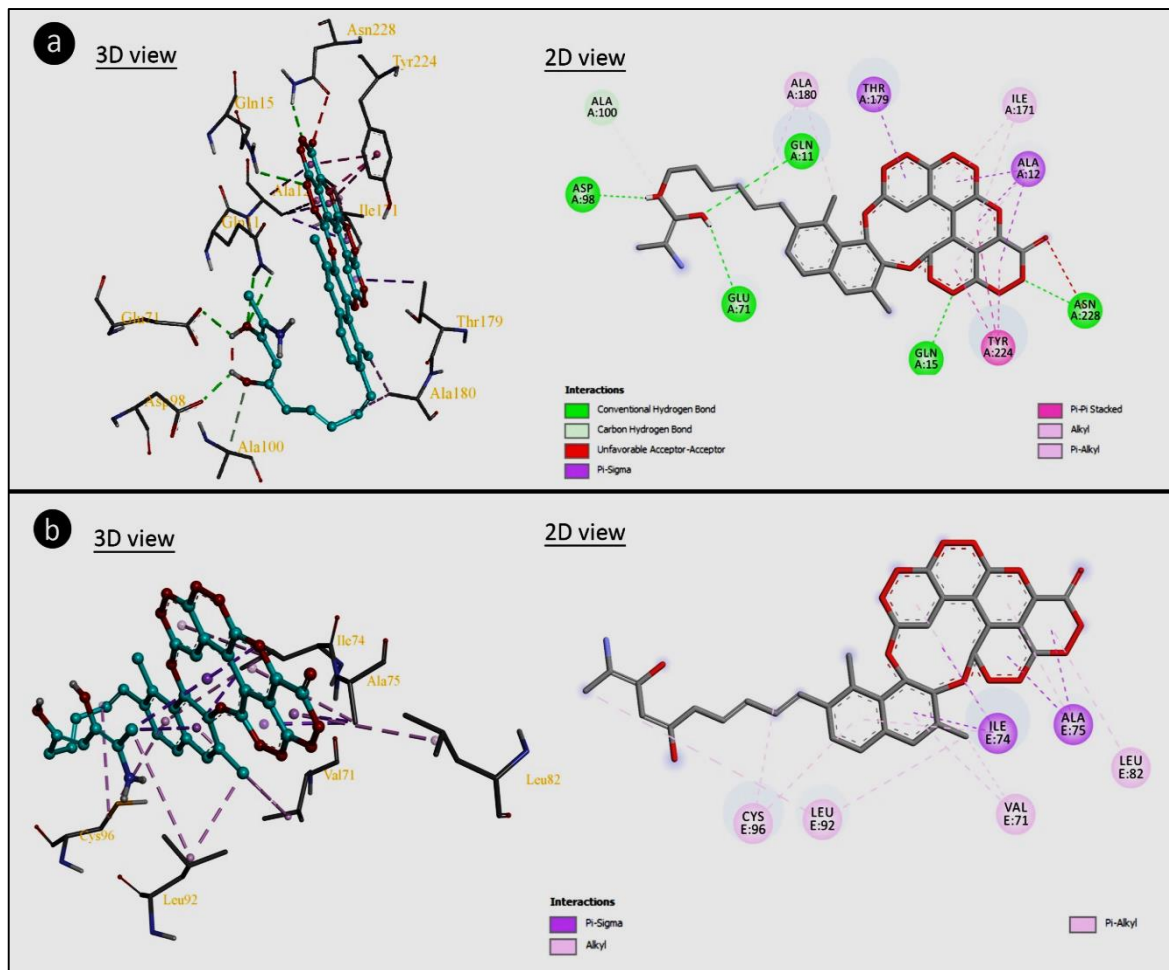


Figure 2 The Molecular Interactions of FB₂ with Tubulin and Histone ProteinS (**a:** tubulin alpha 1B chain, **b:** histone H2A).

Table 1 Binding Affinities and Interactions of FB₂ with Tubulin and Histone Proteins

Macromolecule	Free energy of binding (kcal/mol)	Inhibition constant (Ki)	Hydrogen bond interactions	Hydrophobic interactions
Tubulin alpha-1B chain	-8.17	1.04 uM	GLN11 (x2) GLN15 ASN228 GLU71 ASP98 ALA100	ALA12 (x4), THR179 TYR224 (x3) ALA180 (x2) ILE171 (x2)
Histone H2A	-6.59	14.85 uM	-	ILE74 (x4) ALA75 (x3) CYS96 (x2) VAL71 (x2) LEU92 (x2) LEU82

Genotoxic Effects

In order to determine the genotoxic effects of FB₂, the frequencies of MN and CA were examined and the results are given in Figure 3. While no MN formations were observed in the control group, significant MN formations were observed in the FB₂ applied groups. It was determined that the frequency of MN increased with the increase in the dose of FB₂ application. The highest MN frequency was determined as 23.3 ± 2.1 in the group administered 40 $\mu\text{g/mL}$ FB₂. The frequencies of CA induced by FB₂ are given in Table 2. No abnormality was observed in the control group, except for a few broken and sticky chromosome formations, and these abnormalities were found to be statistically insignificant. It has been determined that FB₂ causes abnormalities in the form of breaks, fragments, sticky chromosomes, vacuolated nuclei, binuclear cells and reverse polarization at an increasing level depending on the dose. The highest frequency of abnormalities was detected in the group treated with 40 $\mu\text{g/mL}$ FB₂, and breaks occurred with the highest frequency among chromosomal abnormalities. There are many studies in the literature reporting the genotoxic effects of mycotoxins. Gündüz et al. reported that AFB₂ causes CA formation and this effect is associated with oxidative stress [13]. Fetaih et al. stated that AFB₁ application causes macro-DNA damages such as gap, break, deletion, dicentric chromosome, sticky chromosome, hypopolyploidy, centromeric rearrangements [14]. Various interactions between FB₂ and histone proteins through various amino acid residues were determined by molecular docking studies (Figure 2, Table 1). These interactions between FB₂ and histone proteins weaken the DNA-histone binding and cause degradation/dissolution of DNA and chromosome structure. This situation causes both a decrease in the rate of MI and an increase in the frequency of MN and CA.

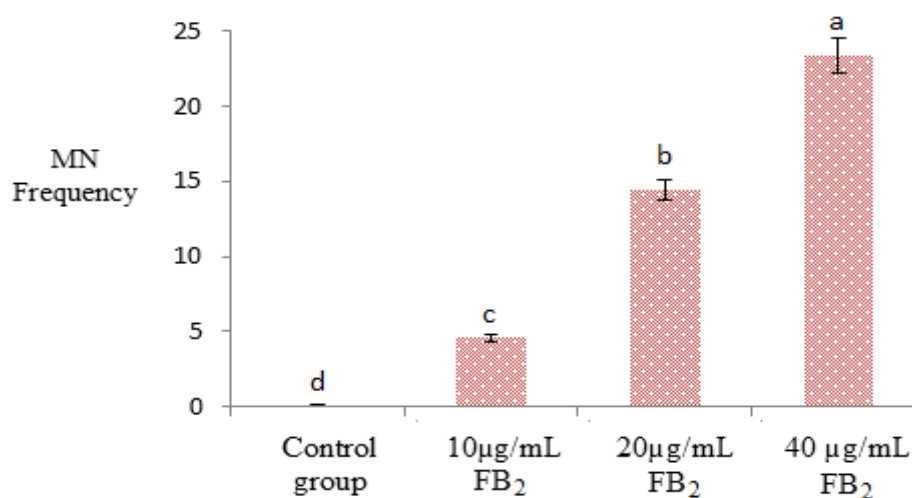


Figure 3 The effects of FB₂ on Frequency of Micronucleus

Table 2 Frequency of Chromosomal Abnormalities Induced by FB₂

	Group I	Group II	Group III	Group IV
Break	0.1±0.0d	15.7±1.2c	21.2±1.2b	35.4±2.7a
Fragment	0.0±0.0d	11.8±1.7c	19.8±1.9b	23.6±1.6a
Vacuolized nucleus	0.0±0.0d	10.8±1.1c	17.3±0.8b	22.6±1.9a
Sticky chromosome	0.2±0.0d	9.7±0.9c	14.6±1.1b	19.2±0.8a
Binucleated cell	0.0±0.0d	7.6±0.5c	9.1±0.2b	15.9±0.7a
Reverse polarization	0.0±0.0d	5.4±0.3c	9.9±0.5b	11.7±0.6a

*A total of 1,000 cells were analyzed in each group for MN and chromosomal damage counts. The averages shown with different letters (a-d) in the same line are significant at p<0.05.

CONCLUSION

In this study, the cytotoxic and genotoxic effects of FB₂, an important mycotoxin species, were investigated. These effects were investigated using the *Allium* test and the mechanism of action was clarified by Molecular docking. As a result, FB₂ interacts with tubulin proteins in *Allium cepa* root tip cells, reducing the MI rate and exhibiting a cytotoxic effect. FB₂ interacts with histone proteins through various bonds and exhibits a genotoxic effect by causing MN and CA formations.

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**ANTIMUTAGENIC EFFECTS OF *TRACHYSTEMON ORIENTALIS*
FLOWER EXTRACT**

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ABSTRACT

T. orientalis is a hermaphrodite perennial plant with rhizomes that can stay green all seasons, with hard and hairy leaves and blue-red flowers. It spreads in Northern Anatolia and Black Sea region in Turkey. The height of the flower can vary between 15 and 30 cm, and the diameter of the petals of the plant can reach about 1 cm. Especially its leafy stems are consumed as a vegetable along with its flowers, and its antipyretic, blood purifying, diuretic, antiviral and antioxidant activity effects are known. The color of the flowers suggests that the secondary metabolites that provide this color formation have important activities. In this study, the antimutagenic activity of *T. orientalis* flowers was investigated and the first data entry was provided to the literature on this subject. Flower tissues of *T. orientalis* collected from Giresun were dried and extracted under sterile conditions in laboratory environment. The antimutagenic activity of the obtained extract was examined by the *Allium* test. The group treated with sodium azide (NaN_3), a potent mutagen, was accepted as the positive control and the antimutagenic effect was calculated based on the NaN_3 +extract treated group. No significant differences were observed in the control group and the extract treated group. This indicates that the extract does not cause any mutagenic effect or chromosomal abnormality formation. Serious mutagenic effects were detected in the positive control group. Abnormalities such as breaks, sticky chromosome, bridges and unequal distribution of chromatin were detected with a high frequency in the NaN_3 treated group. It was determined that the frequency of these abnormalities was decreased in the group administered with NaN_3 +extract. As a result, *T.orientalis* flowers have a strong protective property against induced mutagenity and the frequency of NaN_3 -induced chromosomal aberrations decreased with the application of *T. orientalis* flower extract.

Key words: *Trachystemon orientalis*, *Allium* test, Chromosomal abnormality, NaN_3

INTRODUCTION

The mutagenic effect is the hereditary base changes that occur spontaneously in the genome or is formed by chemical substances taken from the outside of the body. Many studies have shown that mutations that occur as a result of the effect of chemical substances are associated with cancer. It is also accepted that many compounds that become active by biotransformation and cause cancer, either directly or indirectly, also have mutagenic effects. This situation was investigated with test systems in which approximately 300 chemicals were used, and it was determined that 157 of 175 carcinogens were mutagenic and 94 of 108 non-carcinogenic substances were not mutagenic [1,2]. It has been determined that 90% of the chemical agents with carcinogenic effects are mutagenic, and 87% of the chemicals that do not have a carcinogenic effect do not have a mutagenic effect. Although every substance that causes mutation does not cause cancer, the parallelism between mutagenicity and cancer formation makes the research of antimutagenic compounds pioneering studies. The antimutagenic effect is the effect of reducing/eliminating the effect of toxic agents with mutagenic effects or inactivating/preventing the mutation in the genome. Antimutagens are classified into two groups according to their mechanism of action as desmutagens and bioantimutagens [2,3]. As a result of antimutagenicity studies, antimutagenic activities of natural and herbal components with different structures were determined. Some of these compounds are vitamins, flavonoids, phenolics, carotenoids, icoumarin, anthraquinone, tannin, terpenoid, saponin and other secondary compounds produced by plants [4,5].

In this study, the antimutagenic effect of *Trachystemon orientalis* L. flower extract was investigated. *T. orientalis* is a hermaphrodite perennial herbaceous plant with rhizomes that can stay green all seasons, with hard and hairy leaves and blue-red flowers. It spreads in Northern Anatolia and Black Sea region in Turkey. The height of the flower can vary between 15 and 30 cm, and the diameter of the petals of the plant can reach about 1 cm. The low light intensity of the growing regions reduces the seed forming feature and accordingly the development of rhizome root structures occurs [6,7]. Its distinctive features are the spirally curved corolla lobes and the long hairy filaments. *T. orientalis* tissues contain resin, nitrate salts, saponin, tannin, mucilage and essential oil [6-8]. In addition, the presence of catechic tannins, choline and β -sitosterol has also been demonstrated by similar studies. These components detected in *T. orientalis* are used in various treatments in alternative medicine. Especially its leafy stems are consumed as a vegetable and its antipyretic, blood-purifying and urinary-increasing effects are known. It is also reported that some tissue extracts exhibit antiviral and antioxidant activity [9,10]. From this point of view, in this study, the antimutagenic effect of *T.orientalis* flower extract, which has a colorful appearance, was investigated.

MATERIAL AND METHOD

Extraction

In this study, the antimutagenic effect of *Trachystemon orientalis* flower extract was investigated and the flower extract was first prepared for this purpose. *T. orientalis* plants collected by the local public market in Giresun were defined in the Biology Department of Giresun University. The flower tissues of the plants were separated and dried in sterile conditions. After drying, it was extracted in methanol for 12 hours. Centrifugation was made at 10000 rpm in order to remove the solid particles and the extract obtained by evaporation of the supernatant in the evaporator was used in antimutagenic studies [11,12]. *Trachystemon orientalis* flower extract obtained in this way was used by coding as Toex throughout the study.

Experimental Groups

The *Allium* test was used to determine the antimutagenic activity of Toex. In this test, *Allium cepa* bulbs were germinated and root tips with frequently divided meristem tissue were used for analysis. For the determination of antimutagenic activity, firstly, four different groups containing 10 *Allium* bulbs were formed. Control group germinated with tap water and was coded as Group I. The bulbs of Group II were germinated using only 10 mg/mL Toex and it was investigated whether Toex alone was mutagenic in this group. The bulbs of Group III were germinated with 10 mg/mL sodium azide with known mutagenic activity. The bulbs of Group IV were treated with 10 mg/mL Toex+10 mg/mL sodium azide, and the protective role of Toex against induced genotoxicity was investigated in this group and the antimutagenic activity was calculated based on the data of this group.

Antimutagenic Activity

Root tips of each group were collected at the end of the germination period to determine the antimutagenic activity. Root tips were washed with distilled water to remove surface residues and fixed in Clarke solution for 2 hours. Preparations was examined under the microscope after hydrolysis (1N HCl for 17 min at 60 °C) and staining (aceto-carmin) procedures [13,14]. Antimutagenic activity (%) was calculated based on the data of the group treated with sodium azide and 10 mg/mL Toex+10 mg/mL sodium azide.

RESULTS AND DISCUSSION

In this study, the antimutagenic activity of Toex was investigated with the *Allium* test and the results are given in Figure 1 and Figure 2. Within the scope of the study, both mutagenic and antimutagenic activities of Toex were investigated. For this purpose, four different groups were formed and chromosomal abnormalities at root tips of bulbs belonging to each group were examined. The values of Group I in which tap water was applied and Group II in which only Toex was applied were found to be at similar levels. Negligible abnormalities were found in both groups. While a few break and sticky chromosome were found in the control group, only

negligible sticky chromosomes were observed in the Toex-treated group. Similar results were obtained in both groups in terms of break and sticky chromosome frequencies. This result shows that Toex alone did not induce the formation of these abnormalities. It was determined that 67.3 frequency of break and 85.2 frequency of sticky chromosome occurred in Group III, which was applied only sodium azide. This result confirms that sodium azide is a potent mutagen. It was determined that break and sticky chromosome abnormalities regressed in Group IV, which was used as a basis for the determination of antimutagenic activity.

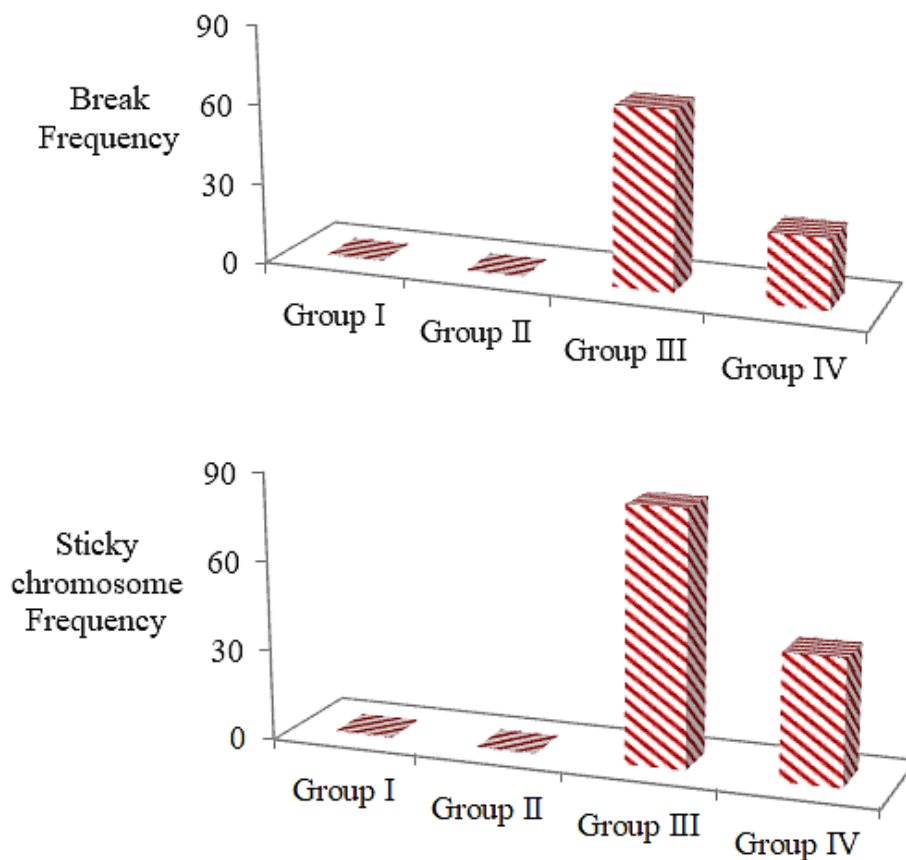


Figure 1 Break and Sticky Chromosome Frequencies in Experimental Groups

The frequency of break and sticky chromosomes induced by sodium azide was decreased by 61.19% and 50.58%, respectively, after Toex application. This decrease shows that Toex exhibits a protective feature against break and sticky chromosome aberrations. Other types of chromosomal abnormalities detected in the study are bridge and unequal distribution of chromatine (Figure 2). Except for a few bridges in the control group, these abnormalities were not found in Group I and only Toex-applied Group II. Similar results were obtained in both groups in terms of bridge and unequal distribution of chromatine frequencies, indicating that

Toex alone did not induce bridge and unequal distribution of chromatine formation. In Group III, in which only sodium azide was applied, it was determined that unequal distribution of chromatine occurred with a frequency of 33.4 and bridge at a frequency of 34.9. Sodium azide induced chromosomal breaks and sticky chromosome formations along with breaks and unequal distribution of chromatine. This result confirms that sodium azide is a potent mutagen that causes many chromosomal abnormalities. Bridge and unequal distribution of chromatine abnormalities were found to regress in Group IV where Toex and sodium azide were administered together. Among these abnormalities induced by sodium azide, a decrease of 70.58% in bridge formation and 48.4% in the frequency of unequal distribution of chromatine were observed, respectively, after Toex application (Figure 3). These results show that Toex exhibits a protective feature against bridge and unequal distribution of chromatine abnormalities.

The anti-mutagenic activity of Toex against chromosomal abnormalities is related to the active ingredients it contains. Considering that the flowers of *T. orientalis* are blue-red, it is thought to contain many pigments. While pigments such as carotene, lycopene and betalain are responsible for red color formation in plant tissues, anthocyanins are responsible for blue color formation. In short, all these pigments in the content of *T. orientalis* flowers have antioxidant properties and exhibit antimutagenic activity due to these properties [15,16]. There are many studies in the literature reporting these pigments to be protective against mutagenic effects. Acar et al. reported that beta carotene is protective against ammonium sulfate toxicity [17]. Çavuşoğlu et al. reported that lycopene reduces chromosomal abnormalities induced in *A. cepa* [18]. Macar et al. reported that anthocyanin-rich bilberry extract decreased the chromosomal abnormalities importantly [19].

As a cumulative result of the biological roles of the active compounds in the Toex content, the extract exhibits significant biological activity. In this study, Toex was found to be particularly antimutagenic. This feature is closely related to the antioxidant role of the components in the high content of Toex.

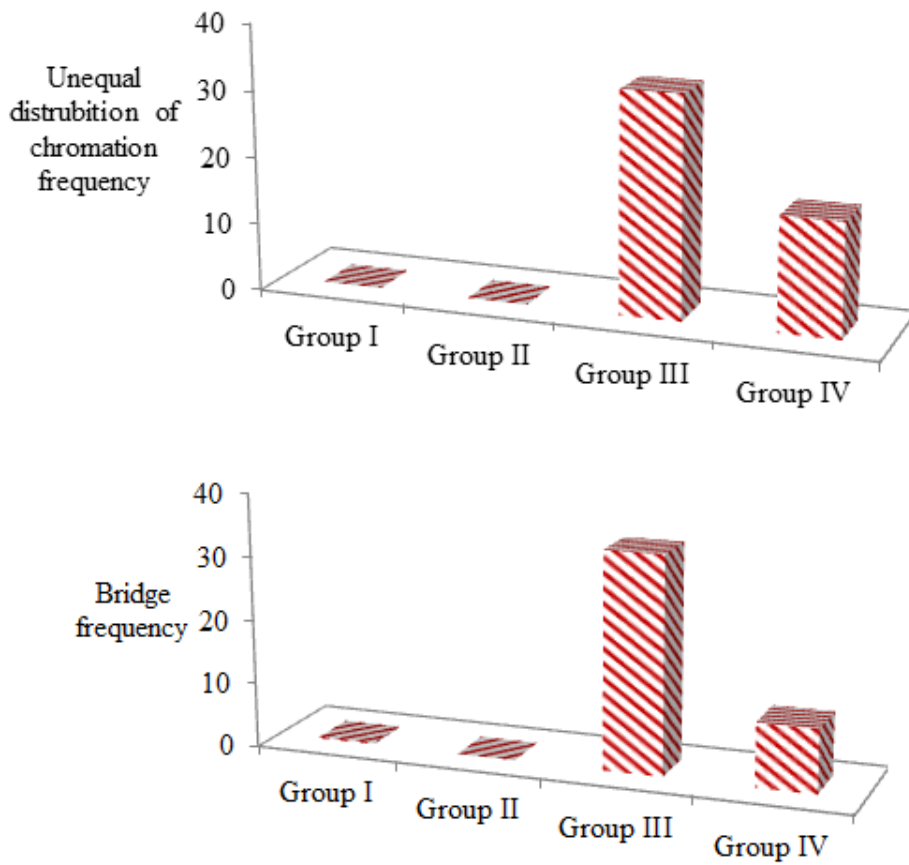


Figure 2 Unequal Distribution of Chromatin and Bridge Frequencies in Experimental Groups

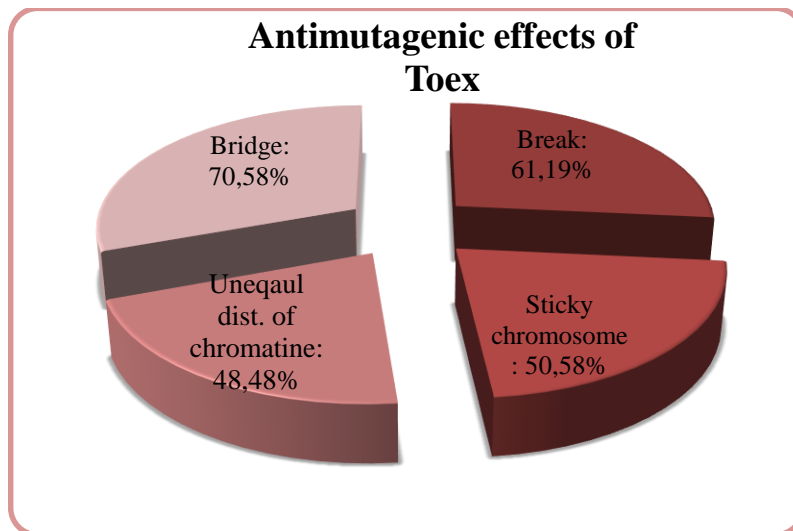


Figure 3 Antimutagenic Effects of Toex Against Chromosomal Abnormalities

CONCLUSION

With the increase in technology and industrialization, increasing diseases and each chemical formulation developed for treatment against these diseases cause side effects and new diseases. This situation increases the tendency towards natural, side-effect-free and protective ingredients. For this purpose, the antimutagenic effects of natural and plant tissues should be investigated more and these studies should be a guide against orientation to natural resources.

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**INVESTIGATION OF THE PROTECTIVE ROLE OF GRAPE SEED AGAINST
BENZYL BENZOATE GENOTOXICITY IN *ALLIUM CEPA* L.**

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ABSTRACT

Benzyl benzoate ($C_{14}H_{12}O_2$) is a chemical compound and acaricide used in the treatment of lice and scabies infections. Grape seed is an industrial derivative obtained as a result of crushing black grape seeds. Thanks to the high vitamin E and procyanidins in its content, it helps to prevent the toxic effects of free radicals on the cell. In this study, genotoxicity induced by 50.000 mg/L dose of Benzyl benzoate was investigated in *A. cepa* (n=16) bulbs. In addition, the protective role of grape seed extract (GSE) against this toxicity was determined. Mitotic (MI) index, micronucleus (MN) frequency and chromosomal aberration (CA) numbers were used as indicators of genotoxicity. *A. cepa* bulbs were divided into a total of six (6) groups (*one control and five treatments*). Tap water was applied to the control group for 72 hours. The application groups were treated with 50.000 mg/L dose of Benzyl benzoate and 465 mg/L and 930 mg/L doses of GSE for 72 hours. At the end of the period, germinated root tips were obtained and made ready for genotoxic investigations with the help of crushing technique. As a result, the highest MI and lowest MN frequency and CA numbers were observed in the control group (group I) and groups II and III, which were exposed to two different doses of GSE. No statistically significant difference was observed between the genotoxicity values observed in these groups ($p > 0.05$). Compared to the control group, MI decreased the frequency of MN and the number of CAs increased in Group IV, where Benzyl benzoate was administered at a dose of 50.000 mg/L. These decreases and increases were found to be statistically significant ($p < 0.05$). Benzyl benzoate application promoted CAs in root meristem cells in the form of fragment>sticky chromosome>bridge>unequal distribution of chromatin>irregular mitosis. GSE application with Benzyl benzoate at doses of 465 mg/L and 930 mg/L decreased Benzyl benzoate genotoxicity in Groups V and VI, and a statistically significant ($p < 0.05$) increase in

MI again, and A statistically significant decrease in the number of MN and CAs were observed. In addition, 930 mg/L dose of GSE was determined to be more effective in reducing genotoxicity. As a result, GSE can be used as a useful nutritional supplement in reducing or protecting from the toxic effects of environmental pollutants such as Benzyl benzoate.

Keywords: *Allium cepa*, Benzyl benzoate, Genotoxicity, Grape seed

INTRODUCTION

The empirical formula for Benzyl benzoate is $C_{14}H_{12}O_2$, and its molecular weight is 212.2. It exists in the form of leaflets with a melting point of $21^{\circ}C$ or as an oily liquid with a boiling temperature of $323-324^{\circ}C$ and a specific gravity of 1.1210 at $16.5^{\circ}C$. It has a light, pleasant scent and a strong, searing flavor. In the presence of alkali, it hydrolyzes to benzoic acid and benzyl alcohol. In addition to being synthesized, benzyl benzoate occurs naturally in Peruvian and Tolu balsams, as well as some essential oils. It is used on humans as a repellent for chiggers, ticks, and mosquitoes in liquid form (300 mg/L). It is used on humans as an acaricide in the form of emulsions or lotions (200-350 mg/L). Benzyl benzoate is used as a scabicide and pediculicide in veterinary medicine. Other nonpesticide applications for Benzyl benzoate include solvent, camphor replacement, and perfume fixative; it is also used in the flavoring of some confectionery and chewing gum (Hayes and Laws, 1991). In cosmetic formulations, benzyl alcohol, benzoic acid and its salts, and Benzyl benzoate have been identified as aroma components, insecticides, pH adjusters, preservatives, solvents, and/or viscosity reduction agents (Gottschalck and Bailey, 2010).

In recent years, there has been an increase in the use of plant extracts against the toxicity caused by chemical agents. One of them is GSE. GSE is a natural extract derived from the *Vitisvinifera* seed. It is a rich source of proanthocyanidins oligomers, one of the most beneficial classes of plant flavonoids. These flavonoids have a variety of health-promoting properties, including the ability to scavenge oxidants and free radicals (Ozer et al., 2011). Grapes and grape derivatives are high in dietary flavonoids, which are potent antioxidants. Grape seed extract is a complex polyphenol combination that includes dimers, trimers, and various oligomers (procyanidins) of catechin and epicatechin (Ahmet et al., 2007; Raina et al., 2007). Grape seed extract is a potent antioxidant with high quantities of bioflavonoids, vitamin C, and vitamin E (Sachs, 1997). It purifies the human body from harmful components. It has a positive effect on circulation. It reduces atherosclerosis and is therefore very beneficial for heart health. It is known that grape seed is good for skin problems, eczema, calcification, sinusitis and urinary tract infections (Gupta et al., 2020).

The aim of this study is to investigate the protective effect of GSE against genotoxicity induced by Benzyl benzoate with the help of *Allium* test.

MATERIAL AND METHOD

Product and Chemicals

GSE (200 capsules x 930 mg) was purchased from SepeNatural, *Allium cepa* L. (n=16) was purchased from a commercial market in Giresun province, and Benzyl benzoate (CAS number: 120-51-4) was purchased from Sigma Aldrich.

Test Material and Experimental Groups

A. cepa bulbs of approximately equal size were divided into 6 groups, each group containing 10 bulbs.

Group I: Control

Group II: 465mg/L GSE

Group III: 930 mg/L GSE

Group IV: 50.000 mg/L Benzyl benzoate

Group V: 50.000 mg/L Benzyl benzoate+ 465 mg/L GSE

Group V: 50.000 mg/L Benzyl benzoate+ 930 mg/L GSE

The bulbs were placed in pre-sterilized glass beakers. The control group was germinated at 24 °C for 72 hours with tap water. The application groups were germinated with two different doses (465 and 930 mg/L) of GSE and Benzyl benzoate at a dose of 50.000 mg/L for 72 hours at 24 °C. The beakers were checked every 24 hours and the decreasing solution was added. After 72 hours of application, the bulbs were washed with distilled water (Qian, 2004).

Genotoxicity Tests (MI, CAs, MN)

A. cepa root tips were cut about 1 cm long, fixed in Clarke solution for 2 hours, washed in 96% ethanol for 15 minutes, hydrolyzed in 1 N HCl for 17 minutes at 60 °C, kept in 45% glacial acetic acid for 30 minutes and stained overnight in acetocarmine. After staining, the root tips were placed on a slide and lightly crushed with the help of a coverslip and examined under the Irmeco IM-450 TI model research microscope. Detected MN and CAs photographed at x400 magnification (Staykova et al., 2005).

Three criteria suggested by Fenech et al. (2003) were taken as basis for the detection of MN.

7. MN should be approximately 1/3 of the nucleus in diameter,
8. MN should be round or oval in shape,
9. If the MN and the nuclear membranes come into contact, they should be clearly distinguishable from each other.

MI, which shows the proportion of cells undergoing mitosis in the total cell, was calculated using Equation (1).

$$MI(\%) = \text{number of cells in mitosis} / \text{total number of cells} \times 100 \quad (1)$$

RESULTS

Genotoxicity induced by Benzyl benzoate application in *A. cepa* meristem cells is given in Table 1 and Figure 1. The control group, as well as Groups II and III, which were given two different dosages of GSE (465 mg/L and 930 mg/L), had the greatest MI value and the fewest MN and CAs. The genotoxicity results reported in these groups did not differ statistically significantly ($p > 0.05$). Benzyl benzoate treatment at a dose of 50.000 mg/L substantially lowered MI ($p < 0.05$) in Group IV while considerably increasing the number of MN and CAs ($p < 0.05$). In Group IV, the MI rate fell by almost 4.20% as compared to the control group. Furthermore, MN formation was shown to occur at a rate of 45.2 ± 4.58 in this group. Benzyl benzoate application induced CAs in root tip meristem cells in the form of fragment, sticky chromosome, bridge, unequal distribution of chromatin and irregular mitosis. The most significant effect of Benzyl benzoate on chromosomes was fragment formation. Fragment formation was observed at a rate of 58.9 ± 5.12 in Group IV, which was administered Benzyl benzoate at a dose of 50.000 mg/L. The administration of GSE with Benzyl benzoate reduced Benzyl benzoate toxicity and improved the genetic parameter values evaluated. It was determined that this improvement was directly related to the applied GSE dose. Compared to Group IV, in Group V administered GSE at a dose of 465 mg/L in addition to 50.000 mg/L Benzyl benzoate, MI increased by approximately 0.90%, number of MN decreased approximately 20.13%, and fragment formation, which was the most observed type of CAs, decreased approximately 19.52%. In Group VI, where 930 mg/L GSE was administered in addition to 50.000 mg/L Benzyl benzoate, MI increased by approximately 1.80%, MN frequency decreased approximately 36.95%, and fragment formation decreased approximately 38.20%, compared to Group IV. It was determined that these increases and decreases were statistically significant ($p < 0.05$). In addition, when compared to Group IV, it was determined that all CAs reduced occurred in the groups in which GSE was applied in addition to 50.000 mg/L Benzyl benzoate, depending on the application dose.

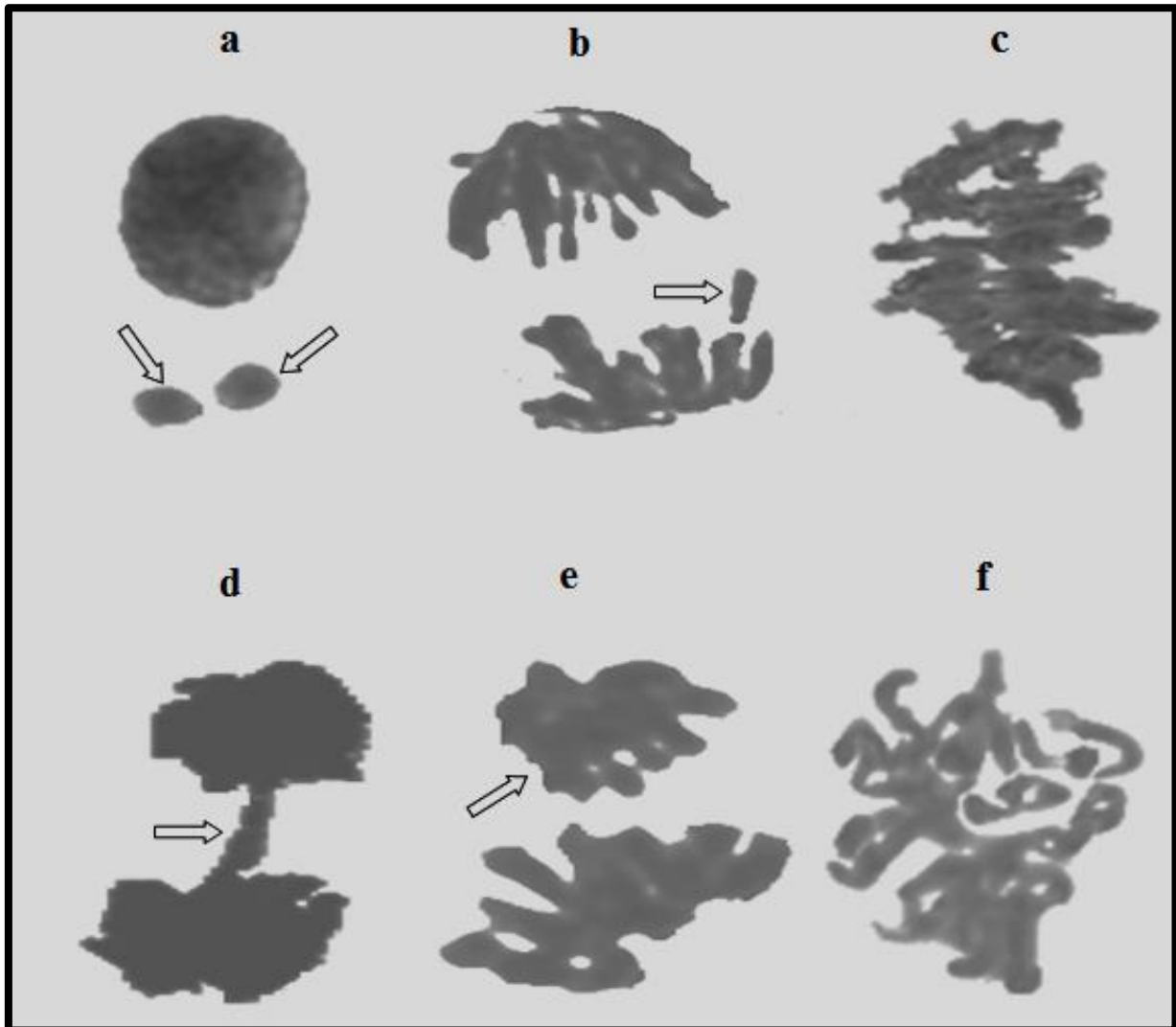


Figure 1 Chromosomal Abnormalities Induced by Benzyl Benzoate in Root Tip Meristematic Cells. MN (a), fragment(b), sticky chromosome (c), bridge (d), unequal distribution of chromatin (e), irregular mitosis (f).

Table 1 Protective Role of GSE against Genotoxicity Induced by Benzyl Benzoate

Damages	Group I	Group II	Group III	Group IV	Group V	Group VI
MI	920±34.52 ^a	912±33.96 ^a	931±34.88 ^a	500±15.74 ^d	596±17.79 ^c	685±20.46 ^b
(%)	(%9.2)	(%9.1)	(%9.3)	(%5.0)	(%5.9)	(%6.8)
MN	0.24±0.30 ^d	0.18±0.23 ^d	0.13±0.19 ^d	45.2±4.58 ^a	36.1±3.77 ^b	28.5±2.93 ^c
FRG	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	58.9±5.12 ^a	47.4±4.65 ^b	36.4±3.78 ^c
SC	0.12±0.17 ^d	0.14±0.20 ^d	0.06±0.10 ^d	39.7±3.81 ^a	30.5±2.97 ^b	20.3±2.54 ^c
B	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	36.5±3.80 ^a	28.6±2.95 ^b	19.2±2.47 ^c
UDC	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	32.3±3.14 ^a	25.8±2.52 ^b	16.7±1.96 ^c
IM	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	18.6±1.98 ^a	11.7±1.36 ^b	6.80±0.83 ^c

*Group I: Control, Group II: 465 mg/L GSE, Group III: 930 mg/L GSE, Group IV: 50.000 mg/L Benzyl benzoate, Group V: 50.000 mg/L Benzyl benzoate + 465 mg/L GSE, Group VI: 50.000 mg/L Benzyl benzoate + 930 mg/L GSE. Data are shown as mean ± SD (n=10). MN and CAs were calculated by analyzing 1.000 cells in each group, and MI by analyzing 10.000 cells in each group. The averages shown with different letters^(a-d) in the same line are significant at p<0.05. MI: mitotic index, MN: micronucleus, FRG: fragment, SC: sticky chromosome, B: bridge, UDC:unequal distribution of chromatin, IM: irregular mitosis.

DISCUSSION AND CONCLUSION

50.000 mg/L Benzyl benzoate treatment-induced genotoxicity in *A. cepa* L. root tip meristem cells by decreasing MI, increasing MN formation, and causing CAs. Studies investigating the genotoxicity caused by Benzyl benzoate in plant test materials are very limited. Acar et al. (2020) investigated the effect of different doses of Benzyl benzoate with *A. cepa* L. test material, and reported that benzyl benzoate caused a decrease in MI and an increase in MN and CAs formations, resulting in genotoxicity depending on the application dose. Demir et al. (2010) investigated the genotoxic effects of benzyl derivatives with the alkali comet test on peripheral lymphocyte cells, and reported that the application caused DNA damage by causing an increase in tail moment and tail DNA percentage parameters. In this study, it is thought that the decrease in MI and the increase in MN and CAs as a result of Benzyl benzoate application are due to the formation of free radicals in the cell by Benzyl benzoate. These free radicals formed by Benzyl benzoate can not only prevent the formation of spindle fibers in the cell, but also attack DNA and cause damage. In some studies, it has been reported that benzene and benzoate derivatives stimulate reactive oxygen species or free radical production in the cell (Piper, 2018; Acar et al., 2020)

In recent years, attempts to minimize the toxicity produced by chemical agents have concentrated on the use of various plant extracts having antioxidant characteristics. Some of these plant extracts can be listed as *Ginkgo biloba* leaf extract, ginger extract, green tea leaf extract, sage leaf extract and green coffee extract. GSE was employed in this investigation to prevent genotoxicity caused by Benzyl benzoate. GSE reduced the toxicity of Benzyl benzoate and improved the MI, MN and CAs parameter values dose-dependently. The powerful antioxidant activity of GSE is considered to have a role in mitigating the genotoxic effects of Benzyl benzoate. This antioxidant feature is due to the proanthocyanins that make up approximately 90% of the GSE. Because of its antioxidant activity, GSE has been shown in several studies to provide effective protection by lowering the toxicity caused by chemical agents. For example, Macar et al. (2021) reported that GSE has a protective role against cobalt (II) nitrate genotoxicity. Yalçın et al. (2010) determined that GSE is a highly effective antioxidant in protection from doxorubicin-induced genotoxicity and oxidative stress in albino mice.

As a result, it has been demonstrated by the *A. cepa* L. test material that exposure to Benzyl benzoate at a dose of 50.000 mg/L causes genotoxicity. Application of GSE at doses of 465 mg/L and 930 mg/L decreased the toxic effects of Benzyl benzoate and resulted in dose-related improvements in the genetic parameter values studied. For this reason, inclusion of GSE in the daily diet can be very beneficial in order to protect from the toxic effects of chemical agents such as Benzyl benzoate.

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INVESTIGATION OF THE PROTECTIVE ROLE OF *GINKGO BILOBA* LEAF EXTRACT AGAINST CHROME GENOTOXICITY WITH *ALLIUM* TEST

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ABSTRACT

Chromium (Cr) is a trace element in the group of heavy metals with a specific gravity greater than 4. Cr is an essential element that must be present in small amounts in the body. Cr contributes to the functionality of insulin. It also has important effects on carbohydrate, protein and fat metabolism. On the other hand, Cr is widely used in metallurgy, chemistry, casting, automotive, metal-steel industry and kitchen-bathroom industries. Excessive amounts of Cr are highly toxic to living things. *Ginkgo biloba* is one of the oldest living tree species that has survived from 2,600 BC to the present day. In particular, its leaves have been used in Chinese alternative medicine for about 500 years. Its leaves are rich in terpenes, flavonoids, biflavonoids, organic acids, phenolics compounds. In this study, genotoxicity induced by Cr in *A. cepa* (n=16) bulbs and the protective role of *G. biloba* leaf extract against this genotoxicity were investigated. Mitotic (MI) index, micronucleus (MN) and chromosomal aberration (CA) numbers were chosen as the main indicators of genotoxicity. *A. cepa* bulbs were divided into 6 groups as 1 control and 5 applications. The bulbs in the control group were treated with tap water, and the bulbs in the application group were treated with 12.5 mg/L doses of Cr and 200 mg/L and 400 mg/L doses of *G. biloba* leaf extract. The application was continued uninterruptedly for 72 hours. At the end of the period, the germinated root tips were collected and made ready for genotoxic studies using the crush preparation technique. In conclusion, the highest MI and lowest MN and CA numbers were observed in the control group (group I) and groups II and III exposed to 200 mg/L and 400 mg/L doses of *G. biloba* leaf extract. There was no statistically significant difference between the genotoxicity values observed in these groups ($p>0.05$). Cr exposure at a dose of 12.5 mg/L decreased MI and increased MN and CA numbers in Group IV. These decreases and increases were found to be statistically significant ($p<0.05$). Cr exposure promoted CAs in root tip meristem cells in the form of fragment>sticky

chromosome>bridge>unequal distribution of chromatin>irregular mitosis>reverse polarization. The application of *G. biloba* leaf extract in two different doses (200 mg/L and 400 mg/L) together with Cr caused an improvement in the genotoxic effects of Cr in Groups V and VI, increased MI ($p<0.05$), decreased MN and CAs frequency ($p<0.05$). It was also determined that 400 mg/L dose of *G. biloba* leaf extract was more effective against genotoxicity. As a result, *G. biloba* leaf extract can be used as an effective nutritional supplement to protect and reduce the toxic effects of heavy metals such as Cr.

Keywords: *Allium cepa*, Chromium, Genotoxicity, *Ginkgo biloba*

INTRODUCTION

Cr is a trace element in the group of heavy metals with a specific gravity greater than 4. It is a transition metal in group VI B of the periodic table and is classified as heavy metal. Cr is an essential element that must be present in small amounts in the body. Cr contributes to the functionality of insulin. It also has important effects on carbohydrate, protein and fat metabolism. It is the seventh most plentiful element in the world, with around 107 tons generated each year (Han et al., 2004; Hua et al., 2012). While there are natural sources of Cr in the environment, notably Cr (III), the great bulk of Cr (VI) comes from industrial operations. The mining of chromite, mainly ferrous chromite, is the first step in the industrial usage of Cr (Kimbrough et al., 1999). Cr and its compounds have multifarious industrial uses. They are extensively employed in leather processing and finishing (Nriagu, 1988), in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals. Hexavalent Cr compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and, until recently, wood preservation. The complicated electronic chemistry of Cr has posed a significant barrier to understanding its toxicity mechanism in plants. The effect of Cr contamination on plant physiology is determined by metal speciation, which is responsible for metal mobilization, subsequent absorption, and toxicity in the plant system. Cr toxicity in plants is reported at a variety of levels, ranging from decreased yields to impacts on leaf and root development, as well as suppression of enzyme activity and mutagenesis (Shankar et al., 2005).

In recent years, the use of plant extracts has increased to reduce the toxicity induced by heavy metals. One of these extracts is *G. biloba* leaf extract. *G. biloba* L. is thought to be the oldest tree species in the world, with a history extending back over 200 million years. Some *Ginkgo* trees have been documented to survive for 1,000 years or more. *Ginkgo* products are standardized to include 24 percent of the leaf bioflavonoids, as well as ginkgolides and bilobilides, a complex collection of substances found solely in the *Ginkgo* tree. *G. biloba* leaf extract is the most widely used phytomedicine in Europe, where it is used to treat early-stage Alzheimer's disease, chronic cerebral insufficiency, vertigo, peripheral claudication, heart disease, eye diseases, vascular dementia, dementia, brain trauma and vascular tinnitus (Tenney,

1996; Sierpina et al., 2003). *G. biloba* extract has been found to have an active function in the stability of cell membrane structure and the elimination of free radicals in studies. *G. biloba* extract has also been shown to modulate vascular flexibility and prevent metabolic and neurological problems caused by oxidative stress (Chang et al., 2011; Guidetti et al., 2001).

In this study, genotoxicity caused by Cr in *A. cepa* L. test material and the protective role of *G. biloba* leaf extract against this genotoxicity were investigated.

MATERIAL AND METHOD

Product and Chemicals

G. biloba leaf extract (60 capsules x 400 mg) was purchased from SepeNatural. *Allium cepa* L. (n=16) was purchased from a commercial market in Giresun province, and potassium dichromate ($K_2Cr_2O_7$) (CAS number: 7778-50-9) was purchased from Sigma Aldrich.

Test Material and Experimental Groups

A. cepa L. bulbs of approximately equal size were divided into 6 groups, each group containing 10 bulbs.

Group I: Control

Group II: 200 mg/L *G. biloba* leaf extract

Group III: 400 mg/L *G. biloba* leaf extract

Group IV: 12.5 mg/L $K_2Cr_2O_7$

Group V: 12.5 mg/L $K_2Cr_2O_7$ + 200 mg/L *G. biloba* leaf extract

Group V: 12.5 mg/L $K_2Cr_2O_7$ + 400 mg/L *G. biloba* leaf extract

The bulbs were placed in pre-sterilized glass beakers. The control group was germinated at 24 °C for 72 hours with tap water. The application groups were germinated with two different doses (200 mg/L and 400 mg/L) of *G. biloba* leaf extract and $K_2Cr_2O_7$ at a dose of 12.5 mg/L for 72 hours at 24 °C. The beakers were checked every 24 hours and the decreasing solution was added. After 72 hours of application, the bulbs were washed with distilled water (Qian, 2004).

Genotoxicity Tests

A. cepa root tips were cut about 1 cm long, fixed in Clarke solution for 2 hours, washed in 96% ethanol for 15 minutes, hydrolyzed in 1 N HCl for 17 minutes at 60 °C, kept in 45% glacial acetic acid for 30 minutes and stained overnight in acetocarmine. After staining, the root tips were placed on a slide and lightly crushed with the help of a coverslip and examined under the Irmeco IM-450 TI model research microscope. Detected MN and CAs photographed at x400 magnification (Staykova et al., 2005).

Three criteria suggested by Fenech et al. (2003) were taken as basis for the detection of MN.

10. MN should be approximately 1/3 of the nucleus in diameter,

11. MN should be round or oval in shape,

12. If the MN and the nuclear membranes come into contact, they should be clearly distinguishable from each other.

MI, which shows the proportion of cells undergoing mitosis in the total cell, was calculated using Equation (1).

$$MI(\%) = \text{number of cells in mitosis} / \text{total number of cells} \times 100 \quad (1)$$

RESULTS

The MN formation and CAs in *A. cepa* L. root tip cells caused by Cr treatment indicate in Table 1 and Figure 1. The highest MI value and the least frequency of MN and CAs were determined in the Control group and Groups II and III, which were given two different doses of *G. biloba* leaf extract (200 mg/L and 400 mg/L). The genotoxicity data reported in these groups did not differ statistically substantially ($p > 0.05$). Cr treatment at a dose of 12.5 mg/L substantially reduced MI ($p < 0.05$) while increasing the number of MN and CAs ($p < 0.05$). When compared to the control group, the MI rate in Group IV reduced by about 3.90%. MN formation was also determined to occur at a rate of 57.3 ± 5.63 in this group. Cr treatment-induced CAs in root tip meristem cells in the form of fragment, sticky chromosome, bridge, unequal distribution of chromatin, irregular mitosis and reverse polarization. The formation of fragment was the most important effect of Cr on chromosomes. In Group IV, which administered Cr at a dose of 12.5 mg/L, fragment formation occurred at a rate of 67.4 ± 6.25 . Application of *G. biloba* leaf extract in addition to Cr reduced the resulting toxicity in dose-dependent manner and resulted in improvement in the evaluated genetic parameters. Compared to Group IV, when *G. biloba* leaf extract was administered at a dose of 200 mg/L in addition to 12.5 mg/L Cr in Group V, MI increased from 4.4% to 5.1%, MN frequency was decreased by about 17.63%, the most observed CAs type of fragment formation, on the other hand, decreased by approximately 20.47%. In Group VI, where 400 mg/L *G. biloba* leaf extract was administered in addition to 12.5 mg/L Cr, MI increased from 4.4% to 6.3%, MN frequency decreased approximately 37.52%, and fragment formation decreased approximately 42.28%, compared to Group IV. These increases and decreases were found to be statistically significant ($p < 0.05$). Furthermore, as compared to Group IV, all CAs were reduced in the groups that applied *G. biloba* leaf extract in addition to the application of 12.5 mg/L Cr, depending on the administration dose, and these changes were statistically significant ($p < 0.05$).

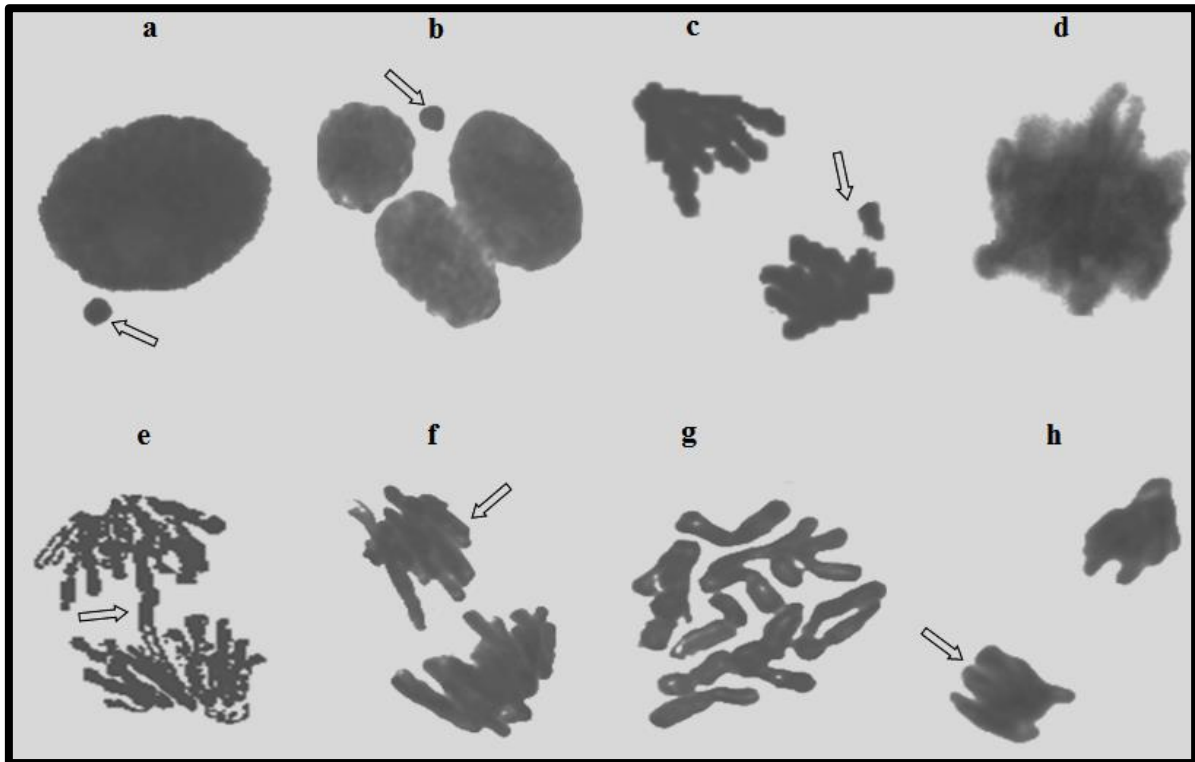


Figure 1 Chromosomal Aberrations Caused by Cr Exposure. MN (a,b), fragment(c), sticky chromosome (d), bridge (e), unequal distribution of chromatin (f), irregular mitosis (g), reverse polarization (h).

Table 1 Protective Role of *G. biloba* Leaf Extract against Genotoxicity Caused by Cr

Damages	Group I	Group II	Group III	Group IV	Group V	Group VI
MI (%)	850±30.75 ^a (%8.5)	844±29.98 ^a (%8.4)	837±27.84 ^a (%8.3)	445±13.92 ^d (%4.4)	510±16.77 ^c (%5.1)	634±19.23 ^b (%6.3)
MN	0.15±0.22 ^d	0.08±0.14 ^d	0.00±0.00 ^d	57.3±5.63 ^a	47.2±4.36 ^b	35.8±3.74 ^c
FRG	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	67.4±6.25 ^a	53.6±4.78 ^b	38.9±3.90 ^c
SC	0.23±0.28 ^d	0.26±0.32 ^d	0.18±0.22 ^d	50.6±4.52 ^a	41.8±3.96 ^b	33.7±3.65 ^c
B	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	40.2±3.85 ^a	30.4±3.14 ^b	22.6±2.58 ^c
UDC	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	27.1±2.83 ^a	19.5±2.30 ^b	11.5±1.53 ^c
IM	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	21.5±2.44 ^a	15.7±1.67 ^b	10.4±1.46 ^c
RP	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	10.8±1.47 ^a	6.30±0.98 ^b	3.70±0.56 ^c

*Group I: Control, Group II: 200 mg/L *G. biloba* leaf extract, Group III: 400 mg/L *G. biloba* leaf extract, Group IV: 12.5 mg/LK₂Cr₂O₇, Group V: 12.5 mg/LK₂Cr₂O₇+ 200 mg/L *G. biloba* leaf extract, Group VI: 12.5 mg/LK₂Cr₂O₇+ 400 mg/L *G. biloba* leaf extract. Data are shown as mean ± SD (n=10). MN and CAs were calculated by analyzing 1.000 cells in each group, and MI by analyzing 10.000 cells in each group. The averages shown with different letters^(a-d) in the same line are significant at p<0.05. MI: mitotic index, MN: mikronucleus, FRG: fragment, SC: sticky chromosome, B: bridge, UDC:unequal distribution of chromatin, IM: irregular mitosis, RP: reverse polarization.

DISCUSSION AND CONCLUSION

The treatment of *A. cepa* L. root tip meristem cells with 12.5 mg/L Cr caused genotoxicity by lowering MI, increasing MN formation, and inducing CAs. Similar results regarding Cr toxicity have been reported by other researchers in the literature. Gürel et al. (2018) reported that Cr application caused a dose-dependent decrease in MI rate, an increase in MN frequency and CAs formation in *A. cepa* L. root tip cells. Liu et al. (1992) reported that both trivalent and hexavalent Cr application inhibited root growth in *A. cepa* L. and caused mitotic irregularities including c-mitosis, anaphase bridges, chromosome stickiness, and chromosome fragmentation and lagging. Similarly, Hemechandra and Pathiratne(2014) reported that Cr exposure caused MI depression and nuclear abnormalities in *A. cepa* L. In this study, the toxicity induced by Cr can be explained by the direct interaction of Cr with chromosomes, namely DNA, or its indirect interaction with the free radicals it generates. In both cases, the synthesis of spindle fibers can be inhibited and CAs can be promoted. In the literature, it has been reported that heavy metal ions directly or indirectly through free radicals interact with microtubules and DNA, reducing the MI and promoting CAs (Liu et al., 2009; Matos, 2017).

Various plant extracts such as sage, green tea, green coffee, turmeric, ginger and pomegranate have been used against heavy metal toxicity in scientific studies carried out in recent years. In this study, *G. biloba* leaf extract was used against genotoxicity induced by Cr. *G. biloba* leaf extract reduced the Cr-induced toxicity and enhanced the MI, MN and CAs parameter values dose-dependent manner. According to Pereira et al. (2013), *G. biloba* has high antioxidant activity. This antioxidant activity is due to various components such as terpenoids, flavonoids, biflavonoids, organic acids and phenolics. Therefore, this antioxidant activity is assumed to be the reason that *G. biloba* leaf extract reduces genotoxic effects. Due to its antioxidant activity, it has been shown in various studies that *G. biloba* extract provides effective protection by reducing the toxicity caused by heavy metals and chemical agents. Çavuşoğlu et al. (2009) reported that *G. biloba* leaf extract treatment showed dose-dependent amelioration of uranium-induced genotoxicity and oxidative stress in Swiss albino mice. Silva et al. (2019) reported that *G. biloba* leaf extract showed a protective effect against oxidative DNA damage induced by paraquat.

As a result, the *A. cepa* test material has indicated that Cr exposure at a level of 12.5 mg/L produces genotoxicity. *G. biloba* leaf extract at doses of 200 mg/L and 400 mg/L reduced Cr toxicity and resulted in dose-related improvements in the genetic parameter values investigated. Therefore, it has been understood that *G. biloba* leaf extract can be used as an effective nutritional supplement to protect and reduce the toxic effects of heavy metals such as Cr.

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**PROTECTIVE ROLE OF GREEN TEA EXTRACT AGAINST DIETHYL
PHTHALATE TOXICITY IN *ALLIUM CEPA* L.: A BIOCHEMICAL APPROACH**

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ABSTRACT

Diethyl phthalate (C₁₂H₁₄O₄) is a member of the group of phthalic acid esters. It is a clear and colorless liquid. The most common usage area is the cosmetics industry. It is used in the manufacture of personal care products such as perfume and nail polish in the cosmetics industry. In addition, thanks to its plasticizing feature, it is used in the production of automotive parts, toothbrushes, food packaging and plastic toys. In addition to these, Diethyl phthalate is also used in epoxy resins, pharmaceutical applications, and the manufacture of sports equipment. Green tea is a type of tea obtained by drying the leaves of the *Camellia sinensis* plant. It is a strong source of antioxidants thanks to the catechin substance it contains. In this study, biochemical toxicity induced by 4.4 µM dose of Diethyl phthalate in root tips of *A. cepa* was investigated. In addition, the protective role of green tea extract against this biochemical toxicity was tested. Malondialdehyde (MDA) levels, which are the most important indicators of lipid peroxidation, and superoxide dismutase (SOD) and catalase (CAT) activities, which are antioxidant enzymes, were used as the main indicators of toxicity. *A. cepa* bulbs were divided into 6 groups (1 control and 5 treatments), with 10 bulbs in each group. The bulbs in the control group were germinated with tap water for 72 hours. The bulbs in the application groups were germinated with 4.4 µM Diethyl phthalate and green tea extract at 140 mg/L and 280 mg/L doses for 72 hours. At the end of the period, the root tips were washed with distilled water, cut and made ready for biochemical analysis. As a result, the lowest MDA level and the lowest SOD and CAT enzyme activity were measured in the control group (group I) and groups II and III, which were exposed to two different doses of green tea extract. There was no statistically significant difference between the measured biochemical parameter values in these groups

($p > 0.05$). Compared to the control group, MDA levels, SOD and CAT enzyme activities increased significantly ($p < 0.05$) in Group IV exposed to Diethyl phthalate at a dose of 4.4 μM . The administration of green tea extract at doses of 140 mg/L and 280 mg/L together with Diethyl phthalate reduced Diethyl phthalate toxicity in Groups V and VI, and again caused significant ($p < 0.05$) decreases in MDA levels, SOD and CAT enzyme activities. In addition, it was observed that the improvement in biochemical parameter values was more pronounced at the dose of 280 mg/L of green tea extract. As a result, it has been shown that Diethyl phthalate, which is in the structure of the products we frequently use in daily life, can cause genotoxicity when it reaches a certain dose level, and green tea extract can be used as a very useful nutritional supplement in reducing this toxicity.

Keywords: *Allium cepa*, Diethyl phthalate, Genotoxicity.

INTRODUCTION

Developing technology and increasing population growth in our daily lives have caused us to interact directly or indirectly with many chemicals. Exposure to chemicals causes adverse effects on the environment and the living population. Plastics are the most exposed chemicals. Although coal, oil and natural gas are the raw materials of plastics, properties such as durability, flexibility and strength can be increased with some additives during the production stages. Phthalates are one of these additives and are used to make plastic materials more flexible. Over time, it started to be used for different purposes in many sectors and started to be used in areas such as cosmetics and perfume industries with its softness, solvent and binder properties. The negative effects of the contamination of phthalates to living things reveal the necessity of investigating the toxic effects of phthalates on living things in detail [1,2]. Phthalates, which are frequently contaminated with the environment as a result of intensive use of plastic, have various toxic effects on living things. It is known that phthalates have hormone-disrupting effects on living things. Since the immune system is not fully developed, it is much more dangerous for babies and children than adults. Phthalates can cause deterioration in reproductive cells, infertility, and developmental disorders in the genitals in men. Phthalates are carcinogenic chemicals. In addition, phthalates may have a triggering effect in many diseases, as they have a detrimental effect on the balance of hormones [3,4]. The toxic effects of phthalates, which threaten many agricultural areas in the environment, on plants have not been adequately studied.

In this study, the toxic effect of diethylphthalate on *Allium cepa* and the protective properties of green tea against this effect was investigated. The tea plant (*Camellia sinensis*) has been grown in Asia for thousands of years. Today, more than two-thirds of the world's population consumes this popular beverage. However, most of the tea consumed in the world (78%) is black tea, while green tea consumption makes up only 20%. There are chemical compounds in

very different structures and properties in the structure of green tea. The main ones are: enzymes, polyphenols, alkaloids, nitrogenous compounds, carbohydrates, pigments, vitamins, organic acids, minerals [5,6]. 20-40% by weight of green tea consists of polyphenols, of which 60-80% are catechins. Epigallocatechin gallate (EGCG) is the most abundant and most researched catechin in tea. Polyphenols such as theaflavins, thearubigins, and especially compounds such as catechins are believed to be responsible for the antioxidant effects. Studies have shown that green tea has various pharmacological effects such as antioxidative, anti-aging, anti-inflammatory and antimutagenic [5-8].

MATERIAL AND METHODS

In this study, the toxic effect of diethyl phthalate (DfP) and the protective properties of green tea extract (GTex) against this effect were investigated with the *Allium* test. For this purpose, *Allium cepa* bulbs of equal size were divided into 6 groups, and the applications in each group are given in Figure 1.

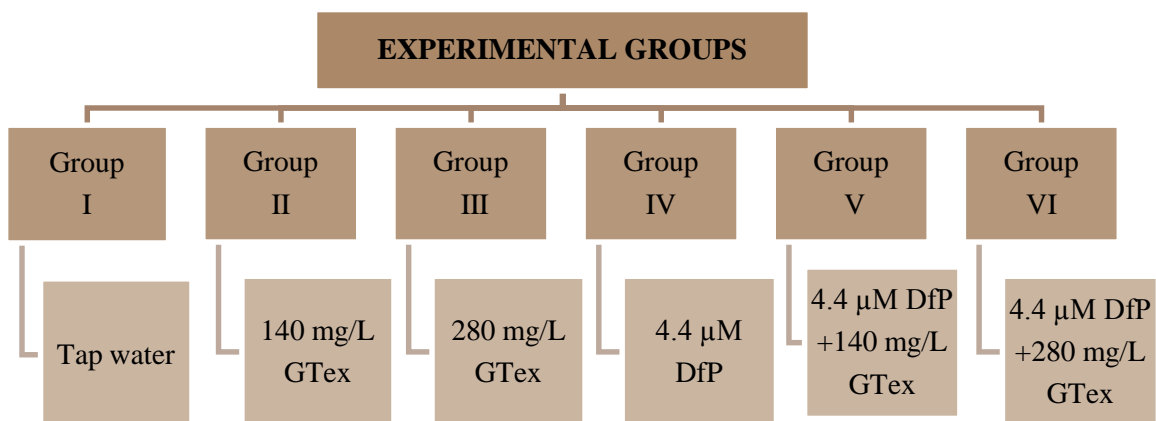


Figure 1 Experimental Groups

Group I was treated with tap water only as the control group. Groups II and III were germinated with different doses of GTex and the effect of GTex alone was determined in these groups. The effect of DfP was determined in Group IV, the protective property of GTex against DfP toxicity was determined in Groups V and VI. The bulbs in all groups were germinated for 72 hours and analyzes were performed on the root tip cells at the end of the period.

Extraction

Root tissues were extracted before SOD, CAT and MDA analyses. For this purpose, root tip samples were collected from each group. The samples were homogenized in phosphate buffer and the supernatant obtained after centrifugation was used for analysis [9].

SOD and CAT Activity

For SOD activity, a procedure was applied proposed by Demiraş et al.[10]. CAT activity was measured by the method proposed by Çavuşoğlu et al [11].

MDA Analysis

Approximately 0.5 g samples taken from root tips were homogenized in trichloroacetic acid. The homogenates were centrifuged at 12.000 rpm for 15 minutes, the obtained supernatant and thiobarbituric acid were mixed in equal volumes and boiled at 96 °C for 25 minutes. After the time, the mixture was cooled and centrifuged at 10,000 rpm for 5 minutes. The absorbance of the obtained supernatant was measured at 532nm [12].

RESULTS AND DISCUSSION

MDA is an important indicator of lipid peroxidation in cells. The effects of DfP and GTeX on MDA level in *Allium cepa* root tip cells are given in Figure 2. MDA levels were found to be similar in Groups I, II and III. This indicates that GTeX administered alone does not have an MDA-increasing or lipid peroxidation-inducing effects. Application of 4.4 μ M DfP increased the MDA level 2.6 times compared to the control group. This result shows that DfP application alone significantly increases lipid peroxidation. Similarly, Demirtaş et al. reported that DfP application increased MDA level by inducing lipid peroxidation [10]. In Groups V and VI, in which GTeX was applied with DfP, a regression was detected in MDA levels. The most significant regression was observed in Group VI administered 280 mg/L GTeX. This result indicates that GTeX prevents lipid peroxidation induced by DfP. In the literature, the protective property of GTeX against lipid peroxidation has been reported in many studies. Yalçın et al. determined that green tea has a protective feature against oxidative damages induced by formaldehyde [7].

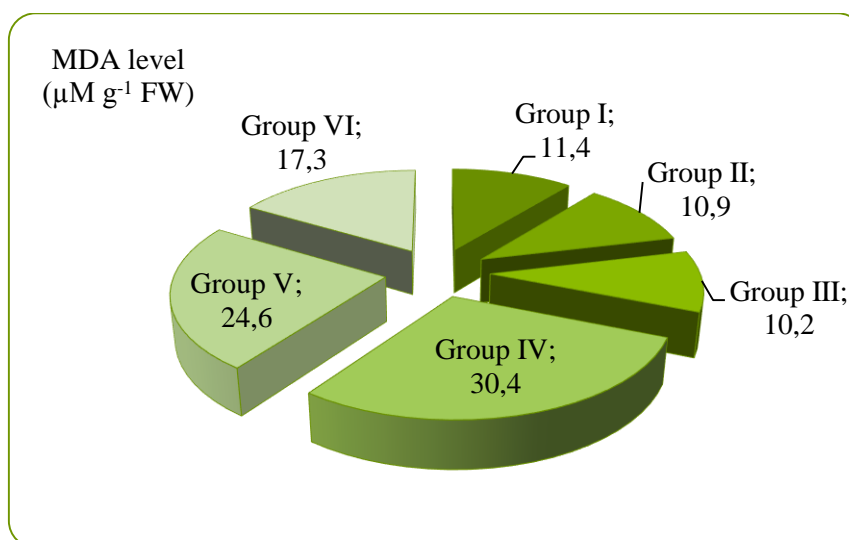


Figure 2 The Effects of DfP and GTeX on MDA Level

SOD is an important intracellular antioxidant enzyme and its activity changes in the presence of oxidative stress in the cell. The effects of DfP and GTeX on SOD level in *Allium* root tip cells are given in Figure 3. SOD levels were found to be similar in Groups I, II and III. This result indicates that GTeX treatment alone does not have any effect on SOD activity. Application of 4.4 μ M DfP increased SOD activity 2.21 times compared to control. SOD is an enzyme that neutralizes the superoxide radical, and the induction of SOD activity indicates the presence of oxidative stress in the cell [13]. In Groups V and VI, in which GTeX was applied together with DfP, a decrease was observed in the increased SOD level. This result shows that oxidative stress in the cell begins to decrease. The most significant decrease in SOD activity was detected in Group VI, which was administered 280 mg/L Gtex with DfP.

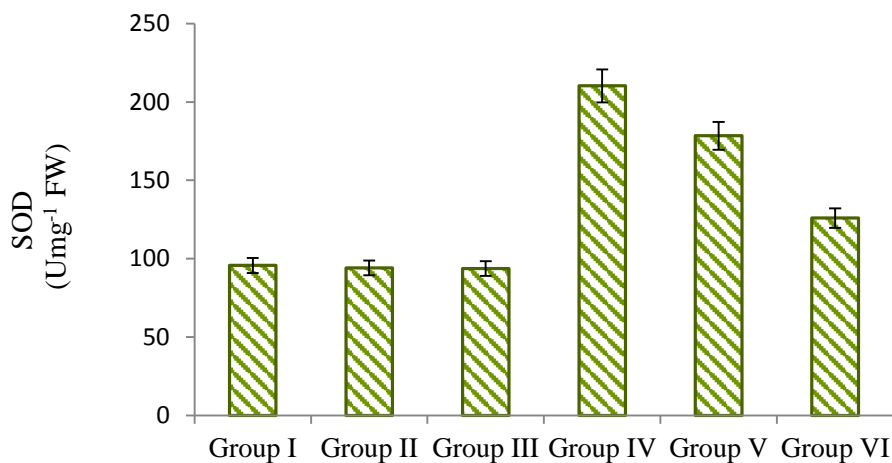


Figure 3 The Effects of DfP and GTeX on SOD Acitivity

Like SOD, CAT is an important intracellular antioxidant enzyme and functions in the removal of hydrogen proxide from the cell. The effects of DfP and GTeX on CAT level in *Allium* root tip cells are given in Figure 4. CAT levels were found to be similar in Groups I, II and III. This indicates that GTeX administered alone does not have a modifying effect on CAT activity. Application of 4.4 μ M DfP increased CAT activity 2.64 times compared to control. This increase in CAT activity indicates an increase in hydrogen peroxide-induced oxidative stress in the cell. In Groups V and VI, in which GTeX was applied with DfP, a regression was observed in the increased CAT level. This result shows that oxidative stress in the cell begins to decrease. The most significant decrease in CAT activity was detected in Group VI administered 280 mg/L Gtex with DfP.

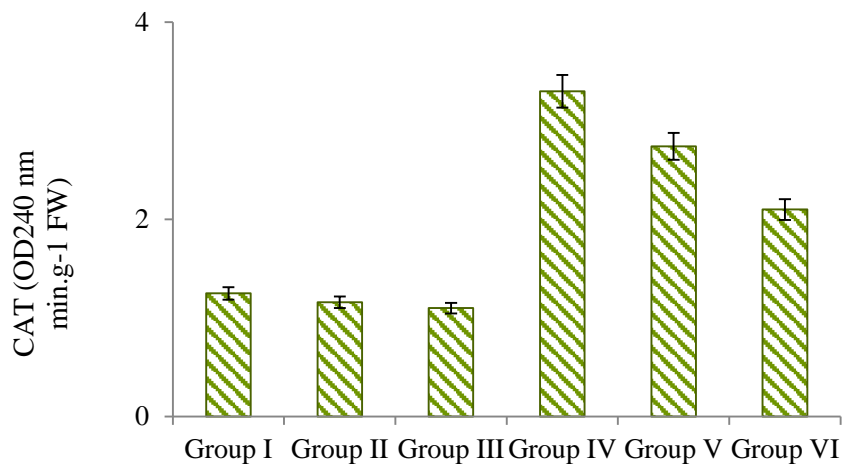


Figure 4 The effects of DfP and GTeX on CAT level

CONCLUSION

In this study, it was determined that DfP, which is widely used in the production of plastic products and is an important environmental contaminant, causes a deterioration in the antioxidant/oxidant balance in *Allium cepa* root tip cells. MDA level, SOD and CAT activity were used as indicators in the disruption of this balance. It was determined that GTeX application caused an improvement in the deteriorated balance, and this improvement increased depending on the dose. This protective property of GTeX is associated with its antioxidant activity.

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PROMETRYN TOXICITY IN *ALLIUM CEPA* L.: GENETIC APPROACH

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ABSTRACT

Prometryn is a herbicide of the methylthiothiazine group. It is a selective herbicide used to control perennial grasses (such as meadow grass, goose grass) and broadleaf weeds (such as nightshade, bird grass) in a variety of crops such as cotton, celery, potatoes, sunflowers, carrots, peanuts, peas and dill. It is known to inhibit photosynthesis in susceptible species. In this study, genotoxicity induced by three different doses of Prometryn in root tip meristem cells was investigated by using *Allium cepa* L. (n=16) as a biological indicator. Mitotic (MI) index, micronucleus (MN) and chromosomal aberration (CA) numbers were used as the main indicators of genotoxicity. *A. cepa* bulbs were divided into four groups: Group I: control, Group II: 2 g/L Prometryn, Group III: 4 g/L Prometryn, Group IV: 6 g/L Prometryn. The bulbs in the control group were germinated with tap water, and the bulbs in the treatment groups were germinated with three different doses of Prometryn. The germination process was continued uninterruptedly for 72 hours at 24°C. At the end of the period, the root tips were washed with distilled water, cut into 1 cm lengths and made ready for microscopic examination using the crush preparation technique. As a result, the highest MI and lowest MN and CA numbers were observed in the control group (group I). Prometryn exposure significantly decreased MI ($p<0.05$) and increased MN and CA numbers significantly ($p<0.05$), depending on the dose increase in groups II, III and IV. It was observed that 6 g/L dose of Prometryn was more effective in these increases and decreases. Prometryn exposure induced CAs in *A. cepa* root tip meristem cells in the form of fragments, sticky chromosomes, vagrant chromosomes, bridges, unequal distribution of chromatin, nucleus with vacuoles, and reverse polarization. As a result, it has been determined that Prometryn herbicide can cause genotoxicity when it reaches a certain dose level.

Keywords: *Allium cepa*, Genotoxicity, Prometryn.

INTRODUCTION

Pesticides are mixtures used to prevent harmful organisms, to control them or to keep their harm to a minimum. Pesticides can be a chemical substance or a biological agent such as a virus or bacteria. Pesticides are used especially against insects, plant pathogens, weeds, molluscs, birds, mammals, fish, worms and microbes that harm food sources. Pesticides are classified in different ways according to the organisms they affect. Herbicides, a subgroup of pesticides, are substances used to control wild plants. Herbicides act by creating toxicity in plants [1,2]. Prometryn is a herbicide belonging to the triazines chemical class. Prometryn is one of the most widely applied herbicides worldwide in the second half of the twentieth century due to its low toxicity and relatively low environmental persistence. However, significant evidence has been obtained that S-triazine pesticides, including prometryn, have various toxic properties (endocrine disrupting, genotoxic and immunotoxic) and are groundwater pollutants. This evidence led to the limited use of many triazine pesticides and eventually to their complete ban in many countries. Prometryn, which is still produced in large quantities, was first used against wild plants in cotton and celery cultivation. Later, its use became widespread and it became frequently used in sunflower, beans, carrots, celery, fennel, lentils, leeks, parsley, peas, potatoes, peanuts cultivation. The toxic effects of pesticides on target organisms are clear, but their effects on non-target organisms are not clear. In this respect, the effects of each pesticide on non-target organisms should be investigated [3,4].

In this study, the toxic effects of prometryn herbicide were investigated with the *Allium* test. In this way, genotoxic effects of prometryn were detected in the non-target organism *Allium cepa*. *Allium cepa* test is widely used by many researchers as a bioindicator of environmental pollution. It is a sensitive test used to evaluate the genotoxic potential or toxicity-reducing properties of medicinal plants as well as environmental pollution. With the *Allium* test, substances that cause damage to DNA in eukaryotes can be detected and the results obtained can be used as a preliminary assessment in all animal and plant biodiversity [5,6]. In this study, the frequencies of MI, MN and CA in *A. cepa* treated with prometryn were examined and the cytotoxic and genotoxic effects were clarified.

MATERIAL AND METHOD

Allium test was used to determine prometryn toxicity. *Allium cepa* bulbs of equal size and appearance were used as test material. In order to determine the dose-related toxic effect, 4 different groups were formed. The control group was coded as Group I and the bulbs of this group were germinated with tap water. Group II, Group III and Group IV were germinated with 2g/L, 4g/L and 6g/L prometryn, respectively. 10 bulbs were used for each group and germination was carried out at 24°C for 72 hours. At the end of the period, cytotoxic and genotoxic effects were investigated in root tip cells.

Cytotoxic Effects

In order to determine cytotoxic effects, the mitotic index (MI) ratios of each group were determined. For this purpose, root tips of 1-2 cm in length were collected from the root tip cells of each group at the end of the germination period. Root tips were washed with distilled water to remove surface residues. Routine preparations for MI analysis were prepared according to the method suggested by Çavuşoğlu [7]. To determine the effect of prometryn on MI, 10000 cells were counted in each group, and MI (%) was calculated by taking the ratio of dividing cells to total cells.

Genotoxic Effects

In order to determine the genotoxic effects, the micronucleus (MN) and chromosomal abnormality (CA) frequencies of each group were determined. For this purpose, root tips of 1-2 cm in length were collected from the root tip cells of each group at the end of the germination period. Root tips were subjected to fixation, hydrolysis and dyeing processes. A total of 1000 cells were counted in each treatment group for the frequency of micronucleus and chromosomal abnormalities, and abnormalities were determined under the microscope and photographed at X500 magnification [8].

RESULTS AND DISCUSSION

In order to determine the effect of prometryn application on proliferation potential, the number of dividing cells was calculated and MI rates were examined (Figure 1). 10000 cells were counted for each group. While the number of dividing cells was 790 in the control group, it was determined that prometryn application decreased the number of dividing cells. The most significant reduction was detected in the 6g/L prometryn group. the number of dividing cells in this group decreased by 1.35 times compared to control. It was determined that the number of dividing cells decreased as the dose of prometryn administration increased. Pesticides cause a decrease in MI rates by various mechanisms. Disruptions in the cytoskeleton and spindle fiber, delays in the cell cycle are some of these mechanisms [9,10].

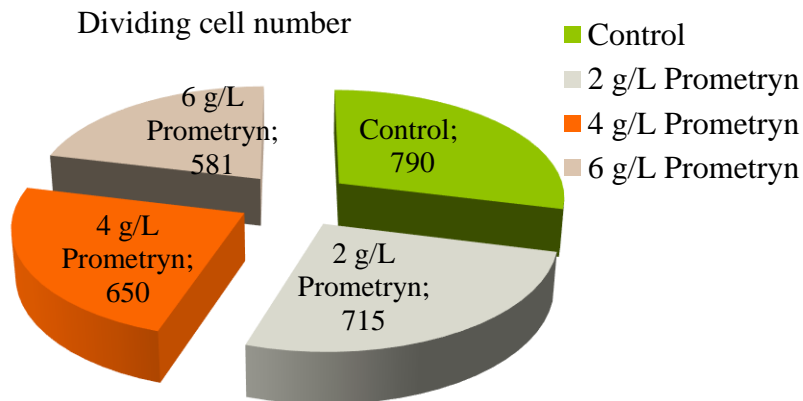


Figure 1 Prometryn Effects on Cell Proliferation

The effect of prometryn application on the frequency of MN, which indicates a genotoxic effect, is given in Figure 2. While a few MN formations that were not statistically significant were found in the control group ($p>0.05$), an increased frequency of MN was detected in the groups treated with prometryn, depending on the dose. The MN frequencies for Group II, Group III and Group IV were found to be 15.5 ± 1.14 , 29.6 ± 2.10 and 66.9 ± 4.38 , respectively. MN formation in a cell indicates genotoxic effect. MN formations can occur from the remaining chromosomes or chromosome breaks in a cell [11].

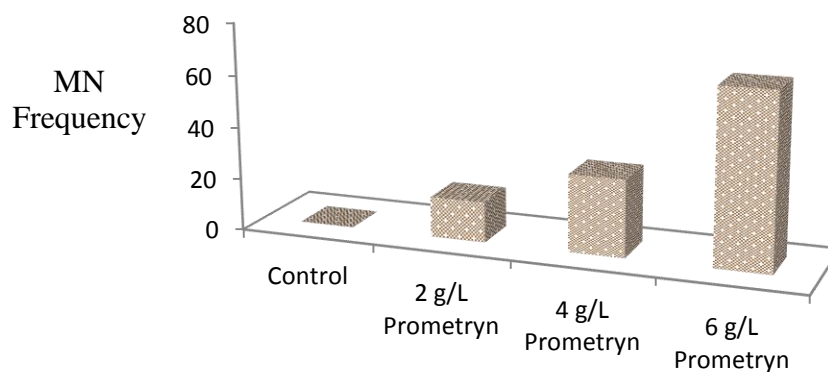


Figure 2 Prometryn Effects on MN Frequency

The effect of prometryn application on CA formations is given in Table 1, Figure 3. No abnormalities were found in the control group, except for a few adherent chromosomes. Different types of CA formations were determined in the groups treated with prometryn. The highest CA formations were detected in Group IV. The types of CA detected include fragment, sticky chromosome, vagrant chromosome, bridge, unequal distribution of chromatin, nucleus

with vacuoles and reverse polarization. The highest rate of fragment was observed among CA. This result indicates the genotoxic effect of prometryn. Pesticides induce CA formations by different mechanisms. The most important mechanism occurs through the formation of oxidative stress. Oxidative stress causes chromosome breaks and fragments. Other CA abnormalities occur as a result of rearrangement of chromosome breaks [12,13].

Table 1 Prometryn Effects on CA Frequency

	Group I	Group II	Group III	Group IV
FRG	0.00±0.00 ^d	13.7±1.05 ^c	25.4±1.83 ^b	52.7±3.96 ^a
SC	0.15±0.22 ^d	10.4±0.94 ^c	19.7±1.55 ^b	44.3±3.37 ^a
VC	0.00±0.00 ^d	7.50±0.72 ^c	15.8±1.27 ^b	35.6±2.98 ^a
B	0.00±0.00 ^d	6.10±0.68 ^c	13.5±0.98 ^b	29.4±2.12 ^a
UDC	0.00±0.00 ^d	5.30±0.54 ^c	10.3±0.90 ^b	24.2±1.80 ^a
NC	0.00±0.00 ^d	4.20±0.47 ^c	9.10±0.88 ^b	19.5±1.52 ^a
RP	0.00±0.00 ^d	3.60±0.42 ^c	7.50±0.74 ^b	15.8±1.29 ^a

* The averages shown with different letters (a-d) in the same line are significant at $p < 0.05$. FRG: fragment, SC: sticky chromosome, VC: vagrant chromosome, B: bridges, UDC: unequal distribution of chromatin, NC: nucleus with vacuoles, RP: reverse polarization

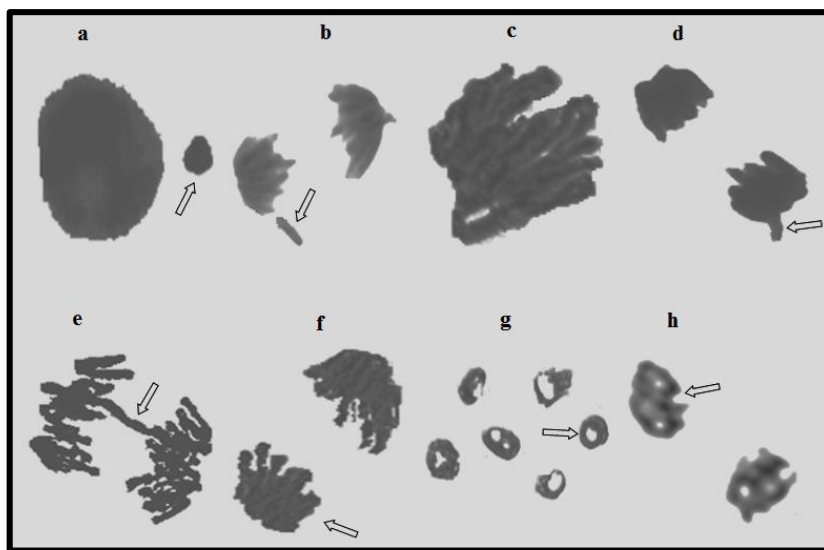


Figure 3 Prometryn Induced CA and MN appearance. MN (a), fragment (b), sticky chromosome (c), vagrant chromosome (d), bridge (e), unequal distribution of chromatin (f), nucleus with vacuoles (g), reverse polarization (h)

CONCLUSION

Herbicides, a subgroup of pesticides, are widely used in the control of wild plants. Prometryn is a widely used herbicide especially in cotton cultivation. Prometryn causes toxic effects against wild plants and its toxic effects on the target organism can also occur in non-target

organisms. In this study, cytotoxic and genotoxic effects in *A. cepa*, which is a non-target for prometryn, were investigated. As a result, prometryn showed a cytotoxic effect by causing a decrease in MI rates in *A. cepa* root tip cells. It also showed a genotoxic effect by causing an increase in the frequencies of MN and KA. It has been determined that all these effects increase depending on dose. Based on these results, the use of prometryn should be limited, and in cases where its use is inevitable, low doses should be preferred.

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**PROTECTIVE ROLE OF *SALVIA OFFICINALIS* AGAINST PARAQUAT
TOXICITY IN SWISS ALBINO MICE: A GENETIC APPROACH**

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ABSTRACT

Although Paraquat is banned in some countries, it is still one of the most widely used herbicides in the world. It is used to control weeds in agricultural lands and wetlands. Paraquat is a highly toxic herbicide. Some studies carried out in recent years have revealed that Paraquat plays a role in carcinogenesis. Sage (*Salvia officinalis* L.) is a plant from the Lamiaceae family that stays green all seasons and blooms purple, blue and pink flowers in summer. Sage contains more than 160 polyphenols (*such as rosmarinic, caffeic, chlorogenic, ellagic acid*) that strengthen the immune system. It is also rich in vitamins such as K, B3, B6, B9, C, E and A and flavonoids such as apigenin, luteolin and diosmetin. It also contains the minerals potassium, calcium, iron, manganese, zinc, magnesium and copper. On the other hand, it contains small amounts of protein, fat and fiber. In this study, micronucleus (MN) formation induced by Paraquat in buccal mucosa epithelium, erythrocyte and leukocyte cells and chromosomal aberrations (CAs) in bone marrow cells were investigated by using *Mus musculus* var. albinos species as a biological indicator. And also the protective effects of sage against Paraquat toxicity were determined. For this aim, albino mice were divided into 6 groups, with 6 mice in each group. Mice in the control group were fed with tap water and pellet food for 28 consecutive days. On the other hand, the mice in the application group were fed with pellet food orally with 200 mg/kg b.w dose of Paraquat and two different doses of sage (190 and 380 mg/kg b.w) for 28 consecutive days. At the end of the period, the mice were anesthetized under halothane anesthesia, sacrificed, and bone marrow, blood and buccal mucosal epithelial tissues were collected. The collected tissue samples were subjected to routine preparation and made ready for microscopic examinations. The criteria determined by Fenench et al. (2003) for the detection of MN and by Savage (1976) for the detection of CAs were taken as basis. As a result, the lowest MN and CA numbers were observed in the control group (group I) and in groups II and III, where 190 and 380 mg/kg b.w doses of sage extract were administered. There was no statistically significant difference between MN and CA numbers in these groups ($p>0.05$). In Group IV exposed to Paraquat at a dose of 200 mg/kg b.w, significant ($p<0.05$) increases were

detected in the numbers of MN and CAs in all cell types examined. The most MN formation was observed in leukocyte>erythrocyte>buccal mucosa epithelial cells, respectively. Paraquat also promoted break, fragment, acentric, dicentric, gap and ring chromosomal damage in the bone marrow cells. The application of sage leaf extract at doses of 190 and 380 mg/kg b.w with Paraquat reduced the toxic effects of Paraquat in Groups V and VI, and again caused a significant ($p<0.05$) decrease in the number of MN and CAs. It was observed that these decreases were more pronounced at the dose of 380 mg/kg b.w of sage leaf extract. As a result, exposure to Paraquat promoted genotoxicity in albino mice, while administration of sage showed a reducing effect on genotoxicity. For this reason, sage can be used as a “toxicity limiting” nutritional supplement to prevent or reduce the toxic effects of environmental pollutants such as Paraquat.

Keywords: Albino mouse, Chromosomal aberrations, Micronucleus, Paraquat.

INTRODUCTION

Paraquat is also known as 1,1-dimethyl-4,4-bipyridylium chloride. It is a non-selective herbicide discovered in 1955. It is especially used to protect crops in agriculture and wetlands against weed invasion. Paraquat is also used as an active ingredient in the structure of many herbicides. Despite being banned in some countries, it is still one of the most widely used herbicides in the world. Paraquat is rapidly absorbed by green plants. It causes disorder in photosynthesis. It easily reaches all tissues and organs of the body by being taken in different ways in animal organisms. Therefore, it causes toxicity in humans and animals. Paraquat cannot be metabolized. Instead, it is reduced to an unstable free radical that is reoxidized to form a cation and a superoxide anion. Thus, it promotes lipid peroxidation, protein inactivation and DNA damage within the cell. The liver, kidney, heart, adrenal glands and central nervous system are the organs and systems most affected by Paraquat toxicity [1, 2].

In recent studies, plant extracts such as *Ginkgo biloba*, green tea, grape seed, lycopene and ginger have been used to reduce the toxicity caused by pesticides. In this study, sage (*Salvia officinalis*) leaf extract was used against Paraquat genotoxicity. The genus *Salvia* is the largest member of the Lamiaceae family, which includes more than 900 species worldwide. *Salvia officinalis* (sage) means "to heal" in Latin. It is an aromatic, perennial and antioxidant herb. For this reason, it is used as a pharmacological plant in alternative medicine and as a beverage in daily life. It can also be used as a flavoring spice. It is used in alternative medicine in the treatment of digestive and circulatory disorders, heart, cough, bronchitis, asthma, spasm, memory problems, inflammation, depression, skin diseases and excessive sweating. Sage contains abundant flavonoids, terpenoids and essential oils. These substances play a role in the anticancer, antimicrobial, antioxidant and free radical scavenging activities of sage. Sage is one of the herbs with the highest amount of essential oil. These are 1,8-cineole, camphor, borneol, bornyl acetate, camphene, α - and β -thujone, linalool, α - and β - caryophyllene, α -humulene, α -

and β -pinene, viridifluorool, pimaradien, salvianolic acid, rosmarinic acid, carnosolic acid, ursolic acid. Sage is also a natural source of polyphenolic compounds (carnosic acid, rosmarinic acid and caffeic acid) that have powerful antioxidant, radical scavenging and antibacterial activities. Another component of sage, ursolic acid, has powerful anti-inflammatory properties [3, 4].

The aim of this study was to investigate the protective role of sage against Paraquat-induced genotoxicity in Swiss albino mice.

MATERIAL AND METHOD

Product and Chemical

Paraquat herbicide (CAS number: 50635) was purchased from Merck and Sage leaf extract (90 capsules x 380 mg) was purchased from SepeNatural (İzmir).

Experimental Animal and Animal Care

36 male Swiss albino mice (*Mus musculus* var. *albinos*) were used as experimental animals. Mice were maintained in a laboratory environment in steel cages, at a cycle of 12 hours of light and 12 hours of dark, at 22 ± 3 °C and $55\pm 5\%$ relative humidity. All methods and techniques applied to mice were carried out in accordance with the criteria and permissions obtained by the World Health Organization (WHO) and Giresun University Animal Experiments Local Ethics Committee.

Group Formation Principles and Experimental Protocol

Albino mice were divided into 6 groups, with 6 animals in each group.

- Group I: (Control), tap water,
- Group II: 190 mg/kg b.w sage,
- Group III: 380 mg/kg b.w sage,
- Group IV: 200 mg/kg b.w Paraquat,
- Group V: 200 mg/kg b.w Paraquat + 190 mg/kg b.w sage,
- Group VI: 200 mg/kg b.w Paraquat + 380 mg/kg b.w sage,

Mice were brought to the laboratory 7 days before the application and their adaptation to the environment was ensured. The control group was fed *orally* with tap water and pellet food for 28 consecutive days. The application groups were fed orally with 200 mg/kg b.w Paraquat, two different doses of sage (190 and 380 mg/kg b.w) and pellet feed for 28 consecutive days. The cages were checked daily and the depleted feed and solution were added. At the end of the 28th day, the mice were stunned under Halothane anesthesia, sacrificed, and blood and tissue samples were collected.

Buccal Mucosa Epithelium MN Test

The mouths of mice stunned with halothane were rinsed with distilled water. Mucosa epithelial cells on the left and right buccals of the inside of the mouth were collected by scanning with a blunt and moist toothpick. Collected cells were placed on a sterile slide and the slides were left to dry at room temperature for 15 minutes. At the end of the period, the cells were fixed in a mixture of methanol (3 volumes) and acetic acid (1 volume) for 10 minutes, stained with feulgen and fast green, and examined under the Irmeco IM-450 TI model research microscope [5].

Erythrocyte MN Test

Blood samples were collected from the tail veins of mice stunned with Halothane with the help of a fine-tipped syringe. Approximately 5 μ L of blood sample was mixed with 3% EDTA and spread on a sterile slide. Cells were fixed in 70% ethanol for 2 minutes and left to dry at room temperature. At the end of the period, the slides were stained with giemsa (5%) for 15 minutes and examined under the Irmeco IM-450 TI model research microscope [6].

Leukocyte MN Test

Mice were stunned under Halothane anesthesia, blood samples were collected and centrifuged at 5.000 rpm for 10 minutes. At the end of the centrifugation process, 0.075 M 5 mL of KCl was added to the residue and left at room temperature for 20 minutes. At the end of the period, the tubes were centrifuged at 5.000 rpm for 10 minutes. After centrifugation, 5 mL of a washing solution consisting of methanol (3 volumes) and glacial acetic acid (1 volume) was added to the residue remaining after centrifugation, and it was kept at -20 °C for 30 minutes. At the end of the period, cells were spread on sterile slides, stained with giemsa (5%) and examined under an Irmeco IM-450 TI model research microscope [7].

Detection of MN in all three investigated cell types was made according to three criteria determined by Fenech et al. [8].

1. MN should be about 1/3 of the cell nucleus,
2. MN and cell nucleus should be the same color,
3. In case of contact between the MN and the membranes of the cell nucleus, the boundary between them should be clearly visible.

Determination of Chromosomal Aberrations

Mice were given 0.025% colchicine intraperitoneally 2 hours before sacrifice. At the end of the period, the mice were sacrificed under Halothane anesthesia. Bone marrow cells were aspirated from the femurs. Cells were washed in physiological saline, kept in 0.075 M KCl, fixed in Carnoy, stained overnight with giemsa (5%) and examined under an Irmeco IM-450 TI model research microscope [9]. CAs were detected and classified according to the criteria determined by Savage [10].

RESULTS

Genotoxicity caused by paraquat administration in Swiss albino mice is shown in Figure 1 and Table 1. In the control group (Group I) and the groups (Group II and Group III) treated with two different doses of sage (190 and 380 mg/kg b.w), MN formation was observed only in leukocyte cells, which was not statistically significant ($p>0.05$). No MN formation was observed in the buccal mucosa epithelium and erythrocyte cells of these groups. In addition, CAs were not detected in the bone marrow cells of these groups. In Group IV, where Paraquat was administered at a dose of 200 mg/kg b.w, MN formation was observed in all three cell types and chromosomal damage was observed in bone marrow cells. The MN counts observed in Group IV were leukocyte>erythrocyte>buccal mucosa epithelium. Paraquat application induced break, fragment, acentric, dicentric, gap and ring chromosome damage in bone marrow cells. The greatest effect of Paraquat on chromosomes is break formation with a rate of 57.4 ± 4.26 . The application of sage together with paraquat resulted in an improvement in genotoxicity and again a significant ($p<0.05$) decrease in the number of MN and CAs. It was also determined that these decreases were inversely proportional to the dose of sage. Compared to Group IV, MN count decreased 2.26 fold in buccal mucosal epithelial cells, 1.49 fold in erythrocyte cells and 1.50 fold in leukocyte cells in Group VI exposed to 380 mg/kg b.w of sage. A similar decrease was 1.38 fold for break, which is the most common type of CAs.

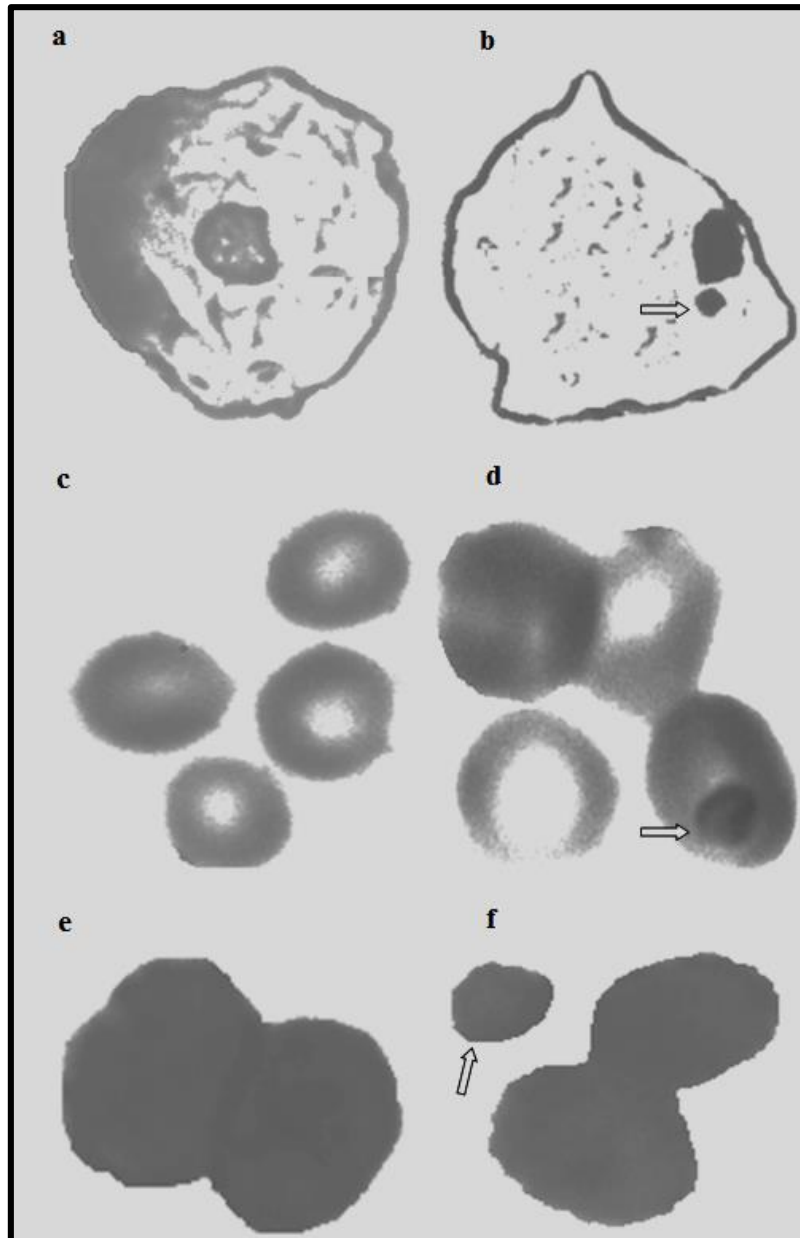


Figure 1 MN Formation Caused by Paraquat Herbicide in Different Cell Types. Buccal mucosal epithelium normal appearance (a), buccal mucosal epithelium with MN (b), erythrocyte cell normal appearance (c), erythrocyte cell with MN (d), leukocyte cell normal appearance-*lymphocyte* (e), leukocyte cell with MN-*lymphocyte* (f)

Table 1 Protective Role of Sage Against Genotoxicity Induced by Paraquat in Different Cell Types

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
BME-MN	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	45.7±3.64 ^a	33.8±3.12 ^b	20.2±2.36 ^c
Erythrocyte-MN	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	75.6±5.82 ^a	61.7±4.77 ^b	50.6±4.15 ^c
Leukocyte-MN	0.24±0.32 ^d	0.16±0.23 ^d	0.11±0.17 ^d	105.8±9.67 ^a	87.1±5.95 ^b	70.3±5.62 ^c
Break	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	57.4±4.26 ^a	49.3±3.98 ^b	41.5±3.37 ^c
Fragment	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	40.7±3.30 ^a	32.6±2.75 ^b	25.8±2.14 ^c
Acentric	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	29.3±2.61 ^a	23.5±1.98 ^b	19.8±1.55 ^c
Dicentric	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	23.6±1.94 ^a	18.4±1.46 ^b	12.5±1.10 ^c
Gap	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	13.9±1.18 ^a	9.90±0.95 ^b	5.30±0.68 ^c
Ring	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	11.7±1.02 ^a	8.80±0.92 ^b	4.70±0.56 ^c

*Grup I: Control, Grup II: 190 mg/kg b.w sage, Grup III: 380 mg/kg b.w sage, Grup IV: 200 mg/kg b.w Paraquat, Grup V: 200 mg/kg b.w Paraquat + 190 mg/kg b.w sage, Grup VI: 200 mg/kg b.w Paraquat + 380 mg/kg b.w sage. BME: buccal mucosal epithelium, MN: micronucleus. Values are shown as mean ± SD (n=6). 1.000 cells in each group were analyzed for MN frequency and 600 cells were analyzed for CAs. The averages shown with different letters^(a-d) on the same line are statistically significant (p<0.05).

DISCUSSION AND CONCLUSION

Paraquat administration induced MN and CAs formation in buccal mucosa epithelium, erythrocyte, leukocyte and bone marrow cells of Swiss albino mice. These results are consistent with the results of other limited studies on genotoxicity induced by Paraquat in albino mice and rats. For example, Tanaka and Amano [11] observed that the administration of Paraquat promoted the formation of gaps and breaks in the chromosomes of Chinese hamster lung cells in culture. Bakr et al. [12] reported that administration of Paraquat at a dose of 20 mg/kg bw for 4 weeks caused breakage, centromeric fusion and stickiness in chromosomes. Hafez [13] observed that administration of Paraquat at a dose of 10 mg/kg b.w in albino rats caused MN formation in polychromatic erythrocytes. Paraquat also promoted CAs in bone marrow cells in the form of centric fusion, stickiness, fragmentation, deletion and sister chromatid exchange. In Our study, it is thought that the increases observed in the number of MN and CAs as a result of exposure to Paraquat are due to the fact that Paraquat encourages the formation of free radicals in the cell and that these free radicals interact with cellular molecules such as DNA, chromosomes and spindle fibers (microtubules), and triggers their damages. Because, in some studies, it has been reported that Paraquat causes damage to various macromolecules, especially DNA, by promoting the formation of reactive oxygen species (ROS) in the cell [14]. In addition, the higher rate of MN observed in leukocyte cells compared to erythrocyte and buccal mucosa epithelial cells suggests that the mice swallowed Paraquat without keeping it in their mouths too long, and the erythrocyte cells lost their nuclei before entering the blood circulation. These two processes may have caused a decrease in MN counts in erythrocyte and buccal mucosal epithelial cells.

In recent years, an increase in the use of plant extracts has been observed in reducing the toxicity caused by pesticides. In this study, sage leaf extract was used against the genotoxicity induced by Paraquat in Swiss albino mice. Sage caused an improvement in the values of genetic parameters (MN and CAs) studied, reducing the toxicity induced by Paraquat. A remarkable decrease was observed in the number of MN and CAs depending on the applied sage dose. The decrease observed in these parameters is thought to be due to the antioxidant nature of flavonoids, terpenoids, essential oils and polyphenolic compounds in the content of sage. Because the results of some studies carried out on the protective effect of sage confirm this idea. For example, Ahmed et al. [15] reported that sage protected against Cyclophosphamide-induced cytogenetic, biochemical, and histopathological toxicity in albino mice. Alshubaily and Jambi [16] determined that sage extract had a protective effect against testicular and heart tissue damage caused by hypercholesterolemic diet in rats. Mourad et al. [17] observed that sage provided protection against tamoxifen-induced hepatotoxicity and nephrotoxicity in rats.

In conclusion, Paraquat administration caused genotoxicity by promoting MN and CAs formation in Swiss albino mice. The application of sage leaf extract, on the other hand, caused

an improvement in genotoxicity and promoted a decrease in the genetic parameter values studied. For this reason, it should be a food supplement that must be included in the daily diet in order to reduce the effects of toxic agents such as sage Paraquat or to protect them from their effects.

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**THE PROTECTIVE ROLE OF LYCOPENE AGAINST CYPERMETHRIN
TOXICITY IN SWISS ALBINO MICE: A BIOCHEMICAL APPROACH**

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ABSTRACT

Cypermethrin is a synthetic pyrethroid insecticide that is widely used in agricultural practices and insect control in living areas. It acts as a very fast acting neurotoxin in insects. It also has an anti-nutritional effect. Cypermethrin is used for the control of Coleoptera, Diptera and Hemiptera species, especially Lepidoptera species, in fruits and vegetables, cereals (such as corn, soybean, rice), vines, cotton, potatoes, cucurbits, lettuce, red peppers and tomatoes. It is also used for the control of insect species such as flies and cockroaches in homes. Lycopene is a bright red carotenoid pigment. It gives red color to tomatoes, watermelon and grapefruit. It is the most common carotenoid found in the human body. Lycopene is the highest antioxidant measured in a food. It is not only a color pigment, but also a powerful antioxidant. In this study, the biochemical toxicity induced by Cypermethrin and the protective role of lycopene against this toxicity were investigated by using *Mus musculus* var. *albinos* as a biological indicator. Malondialdehyde (MDA), glutathione (GSH), blood urea nitrogen (BUN) and creatinine levels; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities were used as indicators of biochemical toxicity. Albino mice were divided into 6 groups, with 6 mice in each group. Mice in the control group were fed with tap water and pellet food for 28 consecutive days. The mice in the application group were fed with pellet food together with two different doses of lycopene (215 and 430 mg/kg b.w) and Cypermethrin at a dose of 250 mg/kg b.w *orally* for 28 consecutive days. At the end of the period, the mice were anesthetized under halothane anesthesia, sacrificed, and the blood tissue, liver and kidneys were collected. Collected samples were subjected to routine preparation and made ready for biochemical analysis. As a result, the lowest MDA, BUN, creatinine levels and AST and ALT enzyme activities were measured in the control group (group I), groups II and III, in which lycopene doses of 215 and 430 mg/kg b.w were administered. In addition, the highest GSH levels were also measured in these groups. There was no statistically significant difference between the measured biochemical parameter values in these groups ($p>0.05$). Significant ($p<0.05$) increases in MDA, BUN, creatinine, AST and ALT values and significant ($p<0.05$) decreases

in GSH values were detected in Group IV exposed to Cypermethrin at a dose of 250 mg/kg b.w. Administration of lycopene at 215 and 430 mg/kg b.w doses together with Cypermethrin reduced the toxic effects of Cypermethrin in Groups V and VI. A significant ($p < 0.05$) decrease in MDA, BUN, creatinine, AST and ALT values, and a significant increase in GSH values ($p < 0.05$) were observed in these groups. These improvements observed in biochemical parameter values were more pronounced at a dose of 430 mg/kg b.w of lycopene. As a result, Cypermethrin exposure caused biochemical toxicity in Swiss albino mice, while lycopene administration showed toxicity reducing effect. Therefore, lycopene can be used as a “toxicity limiting” nutritional supplement to protect or reduce the toxic effects of environmental pollutants such as Cypermethrin.

Keywords: Albino mice, Antioxidant enzymes, Cypermethrin, Lipid peroxidation.

INTRODUCTION

Cypermethrin is a light yellow insecticide with a molecular weight of 416.30 g/mol and a distinctive smell. Cypermethrin is a broad spectrum pyrethroid insecticide with contact and stomach action. It is a fast and highly effective neurotoxin. Cypermethrin is used in agriculture and animal husbandry, especially for the control of harmful insects. In residential areas, it is widely used in the fight against flies, fleas and ticks. Due to its lipophilic structure, it accumulates in adipose tissue, skin, liver, kidney, adrenal glands, ovaries and brain. Nervous and muscular systems are the most affected organs. Cypermethrin may cause skin irritation, itching, tingling and burning sensation on the skin and eyes. It can also cause loss of bladder control, incoordination, seizures, and death. Cypermethrin has been detected in the milk of nursing mothers in South Africa. The short-term nephrotoxicity caused by Cypermethrin is primarily mediated by overstimulation of the central nervous system. Cypermethrin promotes neurotoxicity by modulating gamma-amino butyric acid (GABA) level. It also contributes to toxicity by promoting the formation of free radicals [1, 2].

In scientific studies carried out in recent years, plant extracts such as *Ginkgo biloba*, grape seed, green tea, green coffee, sage and ginger are used to reduce the toxicity caused by pesticides. In this study, lycopene extract was used against biochemical toxicity induced by Cypermethrin. Lycopene is a carotenoid pigment found naturally in tomatoes and tomato products (*such as ketchup, tomato paste, tomato juice*). It is also found in watermelon, orange, grapefruit, dried apricots and rose hips. It is the predominant carotenoid in human plasma. Lycopene is a powerful antioxidant. It is the highest antioxidant measured in a food. It has a very high free radical scavenging capacity. So that; It is the most effective antioxidant and radical scavenger among about 700 carotenoids. It reduces lipid peroxidation in the cell. It increases antioxidant levels and protects proteins, lipids and DNA from oxidation. It plays a supportive role in the antioxidant activities of vitamins C and E. Lycopene circulates in the blood and is stored in the fatty tissues and organs of the body, especially in the liver, lungs, colon, prostate and testicles.

Epidemiological studies have shown that dietary lycopene intake reduces the risk of gastric cancer and has antiproliferative effects on prostate and breast cancer cell lines [3, 4].

The aim of this study was to investigate the protective role of lycopene against the biochemical toxicity induced by Cypermethrin in Swiss albino mice.

MATERIAL AND METHOD

Product and Chemical

Cypermethrin (CAS number 52315-07-8) was purchased from Sigma Aldrich and lycopene extract (90 capsules x 430 mg) was purchased from SepeNatural (İzmir).

Care of Experimental Animals

In this study, 36 healthy male Swiss albino mice (*Mus musculus* var. *albinos*) were used. Mice were housed in steel cages in the laboratory, with a 12-hour light and 12-hour dark cycle, at 22±3 °C and 55±5% relative humidity. Experimental methods and techniques applied to mice were carried out according to the criteria and permissions determined by the World Health Organization (WHO) and Giresun University Animal Experiments Local Ethics Committee.

Group Formation Principles and Experimental Protocol

Male albino mice were divided into 6 groups, with 6 animals in each group.

- Group I: (Control), tap water,
- Group II: 215 mg/kg b.w lycopene,
- Group III: 430 mg/kg b.w lycopene,
- Group IV: 250 mg/kg b.w Cypermethrin,
- Group V: 250 mg/kg b.w Cypermethrin + 215 mg/kg b.w lycopene,
- Group VI: 250 mg/kg b.w Cypermethrin + 430 mg/kg b.w lycopene,

One week before the experimental application, the mice were brought to the laboratory and their adaptation to the environment was ensured. Control group mice were fed *orally* with tap water and pellet food. The mice in the application group were fed orally with Cypermethrin at a dose of 250 mg/kg b.w and lycopene extract at two different doses (215 and 430 mg/kg b.w) and pellet food. Feeding was continued for 28 days without interruption. The cages were checked daily and the necessary feed and solution additions were made. At the end of the 28th day, the mice were stunned and sacrificed under Halothane anesthesia, and blood and tissue samples were collected.

Blood Serum Analysis

Mice were stunned under Halothane anesthesia and whole blood samples were taken intracardiac into vacutainer blood tubes. Blood samples were centrifuged at 1.200 g for 10 minutes and stored at -20 °C until analysis. AST and ALT enzyme activities and BUN and

creatinine levels were measured in an autoanalyzer (Medispec 99 M) using commercial kits (Teco Diagnostics, USA) [5].

Tissue MDA and Glutathione Measurements

Mice were sacrificed by heart exsanguination method under Halotene anesthesia. The liver and kidneys of each mouse were harvested, washed in distilled water, dried and prepared for biochemical analysis. Liver and kidney tissues were homogenized in 0.15 M cold KCl at 16.000 rpm for 3 minutes (Homogenizer, Ultraturrax Type T25-B, IKA Labortechnik, Germany). The homogenates were centrifuged at 5.000 g for 1 hour at 4 °C and the supernatant was taken and stored at -40 °C until analysis [6]. Tissue MDA and GSH levels were measured in UV-spectrophotometer (UVmini-1240, Shimadzu, Japan) according to the colorimetric method proposed by Yoshiko et al. [7] and Beutler [8].

RESULTS

The biochemical toxicity induced by cypermethrin exposure in Swiss albino mice is shown in Table 1. The lowest AST and ALT enzyme activity and BUN, creatinine and MDA levels were measured in the control group (Group I) and Group II and Group III, which were administered two different doses of lycopene (215 and 430 mg/kg b.w). There was no statistically significant difference between the measured parameter values in these groups ($p>0.05$). In addition, the highest GSH levels were also measured in these groups. Cypermethrin administration at a dose of 250 mg/kg b.w caused statistically significant ($p<0.05$) increases in AST, ALT, BUN, creatinine and MDA activities/levels and significant ($p<0.05$) decreases in GSH levels in Group IV. Compared with the control group, AST increased approximately 1.54 fold, ALT approximately 1.81 fold, BUN approximately 2.15 fold and creatinine approximately 3.75 fold in Group IV. Liver MDA level increased approximately 1.58 and kidney MDA level increased approximately 1.39 fold. In addition, liver GSH level decreased approximately 2.1 fold and kidney GSH level decreased 1.97 fold. Application of lycopene extract together with Cypermethrin resulted in improvement in toxicity and significant ($p<0.05$) improvements in the biochemical parameter values examined. It was also determined that these improvements were directly related to the lycopene dose. When compared to Group IV, AST decreased approximately 1.32 fold, ALT approximately 1.42 fold, BUN approximately 1.31 fold, and creatinine approximately 2.0 fold in Group VI treated with 430 mg/kg b.w dose of lycopene extract. Similarly, liver MDA level decreased approximately 1.26 fold and kidney MDA level decreased approximately 1.15 fold. In addition, liver GSH level increased approximately 1.43 fold and kidney GSH level increased approximately 1.37 fold.

Table 1. Protective Role of Lycopene Against Biochemical Toxicity Caused by Cypermethrin

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (U/L)	78.0±4.96 ^d	79.0±4.85 ^d	76.0±4.90 ^d	120±7.85 ^a	107±7.12 ^b	91.0±5.86 ^c
ALT (U/L)	47.0±2.95 ^d	45.0±2.87 ^d	43.0±2.79 ^d	85.0±5.10 ^a	74.0±4.83 ^b	60.0±4.15 ^c
BUN (mg/L)	105±6.13 ^d	101±5.94 ^d	107±6.24 ^d	226±10.8 ^a	202±9.67 ^b	172±7.48 ^c
Creatinine (mg/L)	4.00±1.16 ^d	4.14±1.18 ^d	3.92±1.12 ^d	15.0±2.76 ^a	11.6±2.38 ^b	7.39±1.52 ^c
MDA _{Liver} (nmol/g)	0.250±0.12 ^d	0.264±0.13 ^d	0.241±0.10 ^d	0.396±0.42 ^a	0.352±0.38 ^b	0.315±0.32 ^c
MDA _{Kidney} (nmol/g)	0.196±0.08 ^d	0.191±0.06 ^d	0.207±0.09 ^d	0.273±0.31 ^a	0.255±0.29 ^b	0.238±0.25 ^c
GSH _{Liver} (mg/g)	0.315±0.44 ^a	0.308±0.41 ^a	0.321±0.46 ^a	0.150±0.25 ^d	0.177±0.27 ^c	0.215±0.30 ^b
GSH _{Kidney} (mg/g)	0.278±0.39 ^a	0.285±0.40 ^a	0.294±0.42 ^a	0.141±0.22 ^d	0.168±0.25 ^c	0.193±0.27 ^b

*Group I: Control, Group II: 215 mg/kg b.w lycopene, Group III: 430 mg/kg b.w lycopene, Group IV: 250 mg/kg b.w Cypermethrin, Group V: 250 mg/kg b.w Cypermethrin + 215 mg/kg b.w lycopene, Group VI: 250 mg/kg b.w Cypermethrin + 430 mg/kg b.w lycopene. AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, MDA: malondialdehyde, GSH: glutathione. Values are shown as mean±SD (n=6). The means indicated by different letters^(a-d) on the same line are statistically significant (p<0.05).

DISCUSSION AND CONCLUSION

Cypermethrin administration induced biochemical toxicity in Swiss albino mice. These results are largely in line with the results of the limited number of studies on the biochemical toxicity induced by Cypermethrin in animals. For example, Ateşşahin et al. [9] determined that oral exposure to 50 mg/kg b.w of Cypermethrin in rats caused an increase in tissue MDA and GSH-Px levels and a decrease in catalase enzyme activity. Mossa et al. [10] observed that exposure to 13.80 mg/kg b.w of Cypermethrin caused an increase in serum AST, ALT and ALP enzyme activities and tissue MDA levels and a decrease in tissue GSH levels in male albino mice. Eraslan et al. [11] reported that 125 mg/kg b.w Cypermethrin exposure caused an increase in tissue MDA levels and ALP activities, and a decrease in AST, ALT and glutathione peroxidase (GSH-Px) activities in female Wistar rats. AST is a transaminase enzyme produced by liver cells. Transaminases catalyze the transfer of the amino group of an amino acid to a keto acid. AST is also found in heart, muscle, kidney, brain and red blood cells. In cases of damage such as liver tissue degeneration or necrosis, a large amount of AST passes into the blood. ALT is also known as serum glutamic pyruvic transaminase. ALT is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite oxaloacetate. It is found in high concentration in liver cells. It is also found in kidneys and, in very small amounts, in heart and skeletal muscle cells. It is especially abundant in the hepatocyte cytoplasm. It functions in the conversion of nutrients that reach the liver after being taken into the body and digested into energy. Any damage to liver cells by viral and toxic agents increases serum ALT levels [12]. BUN and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite from dietary protein and tissue protein turnover. Ammonia occurs when the protein taken through food is broken down into amino acids by the digestive system. Ammonia reaching the liver through the blood is converted to urea here and given back to the blood and transported to the kidneys. The kidneys separate the urea nitrogen, which is toxic to the body, from the blood and throw it out through the urine. In case of kidney damage, the amount of urea in the blood rises. Creatinine is the product of muscle creatine catabolism. Creatinine is the breakdown product of a molecule called creatine, which is stored as energy in the muscles. Used creatine is converted to creatinine and excreted by the kidneys. High blood creatinine indicates kidney damage [13]. In this study, it is thought that the increase in AST and ALT enzyme activities and BUN and creatinine levels as a result of exposure to Cypermethrin is due to the damage of Cypermethrin to liver and kidney tissues. Because, in some studies, it has been shown that Cypermethrin promotes damage in the form of necrosis in the liver and kidney tissues of experimental animals [14].

MDA is a small and reactive organic molecule with two aldehyde groups. MDA can be induced non-enzymatically by reactive oxygen species (ROS) and enzymatically by lipoxygenase activity. MDA is the most important indicator of oxidative damage of lipids in cell membranes

[15]. GSH is a simple compound containing the thiol group found in many organisms and consisting of the amino acids cysteine, glycine and glutamine. GSH is produced naturally in the body. It is the strongest antioxidant that protects cells against the toxic effects of ROS such as free radicals and peroxides. It captures free radicals and transports them to the liver to be neutralized. For this reason, it is also called the mother of antioxidants [16]. In this study, it is thought that the increase in tissue MDA levels and the decrease in GSH levels as a result of exposure to Cypermethrin are due to the fact that Cypermethrin promotes the formation of free radicals and that these free radicals cause damage to the liver and kidney tissues. In some studies, it has been reported that exposure to Cypermethrin promotes high ROS formation in mice and rats [17].

In scientific researches carried out in recent years, plant extracts are used to reduce the toxicity caused by pesticides. In this study, lycopene extract was used against the biochemical toxicity induced by Cypermethrin in Swiss albino mice. Lycopene decreased the toxicity of Cypermethrin and caused an improvement in the biochemical parameter values examined. It was determined that this improvement was related to the lycopene dose. The improvement observed in biochemical parameter values is thought to be due to the antioxidant nature of lycopene. Because the results of the studies on the protective role of lycopene against the toxicity promoted by various agents in different cell types confirm this idea. For example, Cavusoglu and Yalcin [18] observed that lycopene provides protection against genotoxicity caused by gamma radiation in human lymphocytes. Cavusoglu et al. [19] reported that lycopene has a protective role against mercury (Hg) cytotoxicity in albino mice. Koksoy [20] determined that lycopene reduced oxidative damage in liver, heart and kidney tissues of mice exposed to cobalt (Co).

In conclusion, Cypermethrin administration induced biochemical toxicity in Swiss albino mice. Application of lycopene extract caused improvement in toxicity depending on the dose and improved the biochemical parameter values examined. For this reason, lycopene extract or foods containing lycopene are antioxidant products that must be taken in the daily diet in order to reduce the effects of toxic agents such as Cypermethrin or to protect them from their effects.

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**IRON (FE) - INDUCED MICRONUCLEUS (MN) FORMATION IN
DIFFERENT CELL TYPES OF ALBINO MICE**

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ABSTRACT

Iron (Fe) is a heavy metal that is essential in trace amounts for human, plant and animal cells and has vital importance in the transport of oxygen by taking place in the structure of the hemoglobin protein. However, excessive doses of Fe intake can cause toxicity in the organisms. The hematological system is one of the cellular structures most affected by Fe toxicity. In this study, micronucleus (MN) formation promoted by FeCl₂ metal in buccal mucosa epithelial, erythrocyte and leukocyte cells of *Mus musculus* var. *albinos* species as a biological indicator was investigated. Albino mice were placed in steel cages and divided into 4 experimental groups with 6 mice in each. Mice in the control group were fed with tap water and pellet food for 14 consecutive days. On the other hand, the mice in the treatment groups were fed with orally 50, 100 and 200 mg/kg b.w Iron (II) Chloride (FeCl₂) solutions together with pellet food for 14 days. At the end of the experimental period, blood samples of the mice anesthetized using halothane were collected by an injector, while buccal mucosal epithelial cells were taken using a sterile toothpick. Blood and epithelial tissue samples were prepared for microscopic examination of MN analysis using routine preparation procedures. Detection of MN was performed according to the criteria determined by Fenech et al. (2003). It was determined that Fe exposure induced MN formation in all three cell types depending on the application dose. The most frequent MN formation was observed in leukocyte>erythrocyte>buccal mucosa epithelial cells, respectively. For all three cell types, the maximum MN formation was determined in the group exposed to 200 mg/kg b.w Fe. As a result, although Fe is an essential element in trace amounts for organisms to maintain their metabolic activities, it has been

determined that excessive exposure to Fe may cause toxicity at a level that can affect the genetic structure.

Keywords: Albino Mice, Fe, micronucleus.

INTRODUCTION

Heavy metals, which are inorganic elements with a density above 5 g/cm³, are classified as essential and non-essential heavy metals [1]. As a necessary heavy metal, iron (Fe) participates in the structure of many enzymes such as aconitase, aldehyde oxidase, catalase, cytochrome, hemoglobin, myoglobin, succinate dehydrogenase, peroxidases, tryptophan 2,3-dioxygenase and play indispensable role in in the transport of oxygen [2-3]. Heavy metal pollution in water and soil has increased in the last few decades because of human activities. The fact that heavy metals are non-biodegradable pollutants raises the concern about this problem.

Although Fe is of vital importance for all organisms, it can become toxic, genotoxic, mutagenic, and carcinogenic if it is found at levels above the threshold levels [4,5]. Excessive iron concentrations can cause DNA strand breaks, molecular, cellular and tissue damage, and oxidative stress, ultimately resulting in cell death [6,7]. Fe accumulation resulting from excessive exposure to Fe causes many health problems such as hematological system problems, cancer, diabetes, liver and heart problems, neurodegenerative disorders, hormonal problems, and immune system disorders [8]. On the other hand, since the genotoxic effect of Iron is still a poorly understood issue, more studies are needed to clarify the potential toxicity of this metal.

Since it is both difficult and ethically problematic to study the dangerous effects of genotoxic compounds on humans, different model organisms and techniques are used to investigate this issue. MN are fragments of extranuclear chromatin that can form as a result of DNA double helix rupture or malfunctioning of the mitotic spindle apparatus [9]. Since MN are rarely formed during the mitosis of healthy cells, they are widely used to measure genotoxic damage caused by hazardous substances.

The goal of present study is to understand the genotoxicity of Fe, which is known to have dangerous toxic effects. For this purpose, the increase in the formation of MN originating from FeCl₂ in the buccal mucosa epithelial, erythrocyte and leukocyte cells of *Mus musculus* var. *albinos* species was investigated as a biological indicator.

MATERIAL AND METHOD

Test animals

This study was performed on a total of 24 six-week-old male mice (*Mus musculus* var. *albino*). Mice obtained from Refik Saydam Public Health Institute Animal Research Center (Ankara, Turkey) were kept in metal cages at 22°C (±3°C). The 12-hour light/dark cycle was maintained

throughout the experiment and the mice were fed a standard pellet and tap water diet. All experiments were carried out in accordance with the rules of Kırıkkale University Faculty of Veterinary Animal Experiments Ethics Committee and Kırıkkale University Faculty of Veterinary Animal Experiments Ethics Committee (protocol number: B.30.2.KKÜ.0.06.00/230 - 29.06.2007).

Materials

Experimental solutions were prepared using Iron (II) Chloride (FeCl_2) (Sigma-Aldrich; CAS No: 7758-94-3)

Experimental Plan

Four experimental groups of 6 mice each were formed. Mice in the control group were pellet food and fed with tap water for 14 consecutive days. On the other hand, the mice in the treatment groups were fed with orally 50, 100 and 200 mg/kg b.w Iron (II) Chloride (FeCl_2) aqueous solutions together with pellet food for 14 days.

Table 1 Experimental Plan

Groups	Application
Group I (Control)	Pellet food + tap water
Group II	Pellet food + 50 mg/kg b.w FeCl_2
Group II	Pellet food + 100 mg/kg b.w FeCl_2
Group II	Pellet food + 200 mg/kg b.w FeCl_2

Determination of MN on Buccal Mucosa Epithelial Cells

In order to detect MN formation, after cleaning the mouths of anesthetized mice (Halothane) with distilled water, epithelial cells were collected by gently scraping the epithelial mucosa on the right and left sides of their mouths with a moist toothpick. The collected epithelial cells were transferred to the slides before the slides were dried for 15 minutes. Epithelial cells on dried slides were fixed in a solution of methanol / acetic acid (3/1) for 10 minutes. Fixated cells were stained using Feulgen and Fast Green dyes. Examination preparations were prepared using Entellan and coverslips with stained cells [10].

Determination of MN on Polychromatic Erythrocyte Cells

5 μL of blood taken from the tail veins of mice anesthetized with Halothane using a syringe was spread on slides with 3% EDTA solution. The cells on the slide were fixed using ethanol (70%) for 2 minutes and then left to dry at room temperature for 24 hours. After the fixed slides were stained with Giemsa dye (5%) for 15 minutes, they were closed with a coverslip with the help of Entellan and prepared for examination [11].

Determination of MN on Leukocyte Cells

Blood samples of mice under halothane anesthesia were centrifuged at 5.000 rpm for 10 minutes, then 5 ml of KCl (0.075 M) solution was added to the residue taken and stored at room

temperature for 20 minutes. At the end of 20 minutes, the mixture was centrifuged again at 5.000 rpm for 10 minutes, and 5 ml of a washing solution (3 methanol / acetic acid 1) was added to the remaining residue, then it was kept at -20°C for 30 minutes. After 20 minutes the mixture was centrifuged again at 5.000 rpm for 10 minutes and 5 ml of washing solution (3 methanol / 1 acetic acid) was added to the remaining residue and then at -20°C for 30 minutes. Recovered leukocyte cells were spread on slides and stained with Giemsa (5%) [12].

The presence of MN in all three cell types was determined by screening a total of 1.000 cells in each group according to the criteria proposed by Fenech et al. [13]

Statistical Analysis

Results from the MN tests were analyzed using the ANOVA test ($p < 0.05$) and Duncan's multiple range tests using SPSS analytical software.

RESULTS AND DISCUSSION

The in vivo rodent MN test has been the common and most reliable test to determine the induction of chromosomal aberrations for the genotoxic risk assessment of hazardous substances [14]. Genotoxic effects of Fe on albino mice in terms of MN formation on showed in Table 2 and Figure 1. It was determined that exposure to increasing doses of FeCl_2 caused a statistically significant ($p < 0.05$) increase in the frequency of MN formation in all three cell types [buccal mucosal epithelial (Figure 1b), erythrocyte (Figure 1d), and leukocytes (Figure 1f)]. The highest increase in MN frequency was observed in leukocyte cells in group IV (68.7 ± 5.92). In addition, the higher rate of MN observed in leukocyte cells compared to erythrocyte and buccal mucosa epithelial cells suggests that the mice swallowed Fe without keeping it in their mouths too long, and the erythrocyte cells lost their nuclei before entering the blood circulation. These two processes may have caused a decrease in MN counts in erythrocyte and buccal mucosal epithelial cells.

Table 2. MN formation promoted by FeCl_2 exposure

Cell type	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)
Buccal mucosa epithelial	0.00 \pm 0.00 ^d	10.8 \pm 1.16 ^c	21.5 \pm 1.75 ^b	35.9 \pm 2.78 ^a
Erythrocyte	0.12 \pm 0.19 ^d	17.5 \pm 1.64 ^c	38.9 \pm 2.94 ^b	54.3 \pm 4.16 ^a
Leukocyte	0.18 \pm 0.24 ^d	24.6 \pm 1.83 ^c	41.7 \pm 3.26 ^b	68.7 \pm 5.92 ^a

*Group I: Control, Group II: 50 mg/kg FeCl_2 , Group III: 100 mg/kg FeCl_2 , Group IV: 200 mg/kg FeCl_2 . Values shown as mean \pm SD. Means indicated with different superscripts on the same line are statistically significant ($p < 0.05$).

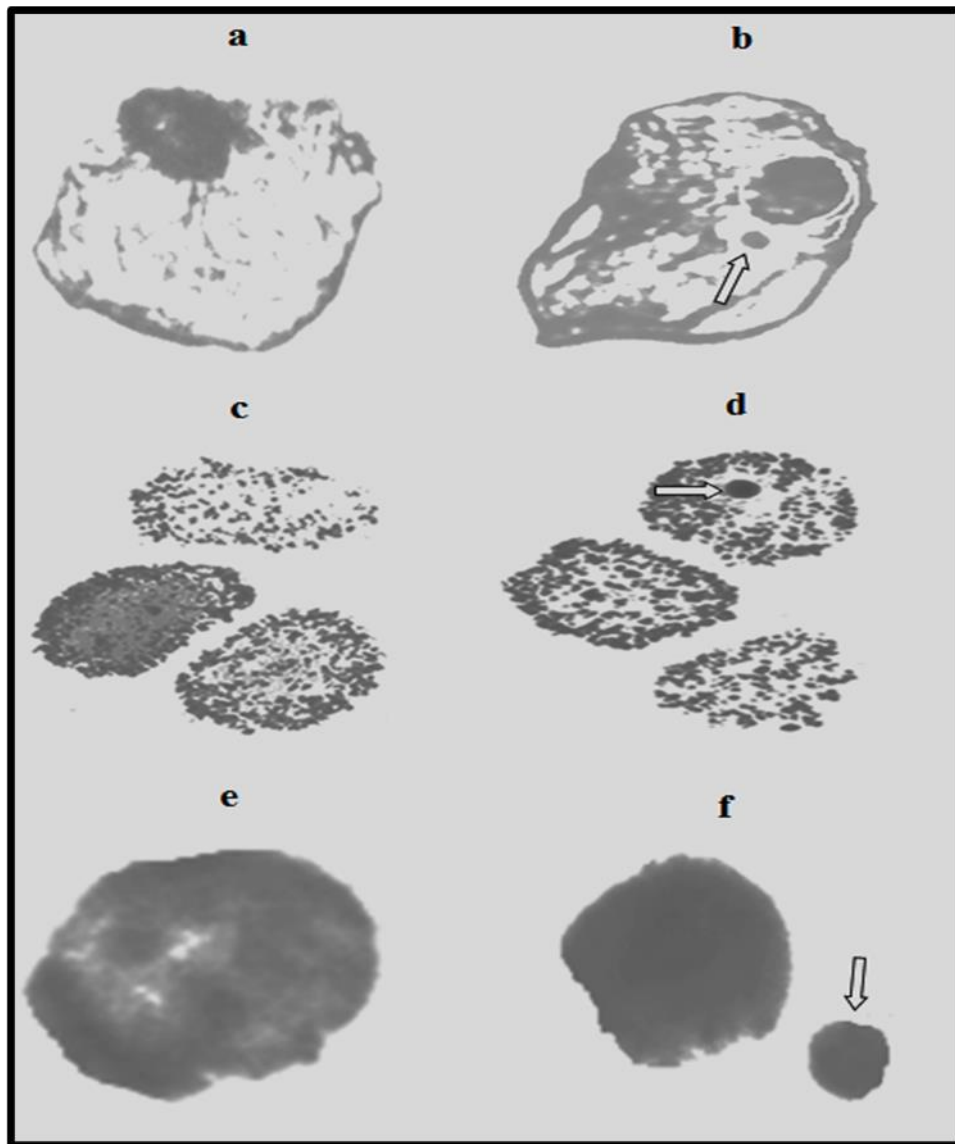


Figure 1 MN Formation Promoted by Fe in Different Cell Types. Normal appearance of buccal mucosa epithelial cell (a), buccal mucosa epithelial cell with MN (b), Normal appearance of erythrocyte cell (c), erythrocyte cell with MN (d), normal appearance leukocyte (e), leukocyte cell with MN (f)

Excessive accumulation of heavy metals triggers the formation of reactive nitrogen and oxygen species in organisms [3]. As a transition metal, Fe increases the formation of hydroxyl radicals through Fenton and Haber-Weiss reactions. In addition, Fe can react with DNA and proteins, causing their functional degradation [15]. Studies in in vivo model systems have shown that oxidative damage plays an important role in Fe toxicity [16]. In addition, it has been reported that excessive dietary Fe consumption increases the risk for various types of cancer [16].

Our results were in agreement with previous studies that mentioned the genotoxic effects of Fe on mammalian cell cultures [17-19] and mice [20-21], mainly due to oxidative stress resulting from extensive Fe accumulation in cells.

CONCLUSION

Although Fe is an essential heavy metal for the regular functioning of organisms, excessive doses have toxic effects. The results of the present study demonstrated the genotoxic effects of Fe, using the formation of MN resulting from FeCl₂ administration in the buccal mucosa epithelial, erythrocyte and leukocyte cells of *M. musculus*. It was also observed that the toxic and genotoxic effects of Fe were dose-dependent and the most damage was observed in leukocyte cells.

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**PROTECTIVE ROLE OF GINGER AGAINST GENOTOXICITY CAUSED BY
TETRACONAZOLE FUNGICIDE IN *ALLIUM CEPA* L.**

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ABSTRACT

Tetraconazole is a triazole systemic fungicide. Triazoles function by inhibiting cell growth and especially cell wall synthesis as well as by preventing ergosterol biosynthesis in cell membranes. Tetraconazole is widely used in the agriculture to combat fungal diseases. Ginger (*Zingiber officinale*) is a perennial herb, which can grow up to one meter in length. Its leaves are thin and long, it blossoms yellow and red flowers, and its tubers are used as a spice and medicinal drug. In the present study, genotoxicity induced by Tetraconazole and the mitigating effect of ginger extract (GE) against this genotoxicity were determined by using *Allium cepa* as a biological indicator. Mitotic index (MI) value, micronucleus (MN) and chromosomal aberration (CAs) frequencies were selected as genotoxicity bio-indicators. Six groups were formed as one control group and five treatment groups. Bulbs of *A. cepa* in the control group were soaked in water, while bulbs in the other groups were soaked in 240 mg/L GE, 480 mg/L GE, 10 mg/L Tetraconazole dose, 10 mg/L Tetraconazole dose + 240 mg/L GE and 10 mg/L Tetraconazole dose + 480 mg/L GE solutions, respectively, for 72 hours. At the end of the treatments, root tips were washed, cut and prepared for genotoxicity analyses by utilizing squash preparation technique. Group I (Control) and two different doses of GE applied Groups II and III exhibited the highest MI and the lowest MN and CAs levels. The differences between the values of genotoxicity results obtained from these first three groups were insignificant ($p>0.05$). On the other hand, MI reduced significantly ($p>0.05$) and MN and CAs incidences increased significantly ($p<0.05$) in Group IV (10 mg/L Tetraconazole). Tetraconazole administration triggered CAs, including fragment, sticky chromosome, vagrant chromosome, unequal distribution of chromatin, bridge, vacuolated nucleus and reverse polarization in the

meristematic cells of root tips. Treatment of Tetraconazole together with GE at doses of 240 mg/L and 480 mg/L in Groups V and VI mitigated the Tetraconazole-related genotoxicity. In Group V and Group VI, significant decreases in the levels of MN and CAs ($p < 0.05$) and a significant increase in MI were observed. 480 mg/L ginger concentration was found to be more effective in reducing the genotoxicity. As a result, ginger can be used as a highly effective nutritional supplement in reducing and protecting the toxic effects of pesticides such as Tetraconazole.

Keywords: *Allium cepa*, Genotoxicity, Ginger, Tetraconazole.

INTRODUCTION

In recent years, the use of fungicides to control diseases in agricultural areas has increased significantly [1]. Tetraconazole, a systemic triazole, is a broad-spectrum fungicide utilized to combat various plant diseases such as powdery mildew and rust [2]. As a demethylation inhibitor (DMIs), Tetraconazole interrupts cytochrome P450 14- α sterol demethylase and inhibits ergosterol biosynthesis [3] and blocks the mycelial growth. Due to its high stability and low mobility, Tetraconazole accumulates in agricultural soils and also pollutes surface waters [4]. Triazoles, including Tetraconazole, may cause endocrine diseases by adversely affecting steroid hormone biosynthesis in humans and animals. It is also known that triazoles can have an impact on photosynthesis, hormone balance, enzymatic activities and yield of plants [5, 6].

Ginger (*Zingiber officinale*), from Zingiberaceae family, is a perennial herb that can grow up to one meter tall. It has thin and long leaves as well as yellow and red flowers [7]. The rhizome of ginger is a well-known spice as well as very valuable in traditional medicine. The rhizomes of ginger have been used to cure many illnesses such as asthma, arthritis, belching, bloating, constipation, cold, cramps, diabetes, dementia, diarrhea, hypertension, fever, infectious diseases, gingivitis, rheumatism, migraine, sprains, pains, nervous diseases, stroke, dyspepsia, gastritis, gastric ulcerations, indigestion, and nausea [8]. There are also studies showing the antioxidant, anti-inflammatory, antiulcer and antitumor capacity of ginger [9].

Plants are widely used in bioanalysis examining the effects of pesticides and they often give successful results. Among these plants, *A. cepa* L. ($2n=16$) is the most preferred bio-model in showing chromosomal injuries and disorders in the mitotic cycle, thanks to its large and few chromosomes [10].

Main goal of this study was to understand genotoxicity induced by Tetraconazole and the mitigating effect of GE against this genotoxicity using *A. cepa* as a model plant. MI, MN and CAs frequencies were used as signs of genotoxicity.

MATERIAL AND METHOD

Materials

Bulbs of *A. cepa* were obtained from a grocery in Giresun-Turkey. Tetraconazole was purchased from ISAGRO S.P.A (Milan, Italy). Commercially available Ginger extract (Sepe Natural Organic Products İzmir, Turkey).

Experimental Plan

The brown scales of the bulbs were peeled before the bulbs were washed with tap water. Equal sized bulbs of *A. cepa* were selected to form six treatment groups as one control group and five treatment groups. Bulbs of *A. cepa* in the control group were soaked in tap water, while bulbs in the other groups were soaked with 240 mg/L GE, 480 mg/L GE, 10 mg/L Tetraconazole dose, 10 mg/L Tetraconazole dose + 240 mg/L GE and 10 mg/L Tetraconazole dose + 480 mg/L GE, respectively, for 72 hours at 23±2 °C. Application dose of Tetraconazole determined according to Macar [11].

Determination of Genotoxicity

After the germination period, the harvested roots were washed toughly with distilled water and the one-centimeter part from the tip was taken to be investigated. Roots were hydrolyzed with 1 N HCl at 60 °C for 12 min in hot water bath. After hydrolysis root tips were stained with solution of acetocarmine (1%) for 24 hr. The root tips of 2 mm, cut from the stained root tips, are squashed to prepare slides [12]. To determine the MI values and CAs and MN frequencies, ten slides randomly selected slides from each group were examined and photographed using a research microscope (500x). MN and CAs ratios were determined by evaluating 1.000 cells from each group. On the other hand, MI values were determined by evaluating 10.000 cells from each group. The occurrences of MNs were evaluated using the criteria proposed by Fenech et al. [13]. Values of MI were obtained as follows:

MI = Number of cells in mitosis/total number of cells [14].

Statistical Data Analysis

Data obtained from the study were analyzed using ANOVA test ($p < 0.05$) using SPSS 23 software. Duncan's multiple range tests was used to calculate the significant differences between treatment groups.

RESULTS AND DISCUSSION

The results obtained from the genotoxicity analyzes were presented in Figure 1 and Table 1. Ginger application did not cause a statistically significant change ($p > 0.05$) in genotoxicity parameters in the 240 mg/L GE and 480 mg/L GE groups when compared to control group. Genotoxicity results of the GE treatment groups, which clearly showed that they had no

genotoxic effect, are compatible with the literature [15-17]. On the other hand, administration of 10 mg/L Tetraconazole dose caused very sharp alterations ($p < 0.05$) in genotoxicity parameters. In 10 mg/L Tetraconazole group, MI was reduced to 612 ± 19.45 and MN frequency was raised to 70.3 ± 8.14 . In addition, Tetraconazole application also triggered an acceleration in formation of CAs including fragment, sticky chromosome, vagrant chromosome, unequal distribution of chromatins, bridge, vacuolated nucleus as well as reverse polarization. Dubey et al. [18] and Schwarzbacherová et al. [19] already mentioned genotoxic role of triazoles. Aragão et al. [20] associated the reduction in MI with the disrupting effect of triazoles on the disrupted G2 phase of the cell cycle or their suppressive effect on DNA. In addition, our results were accordance with Macar [11] who reported genotoxic effect of Tetraconazole on *A. cepa*.

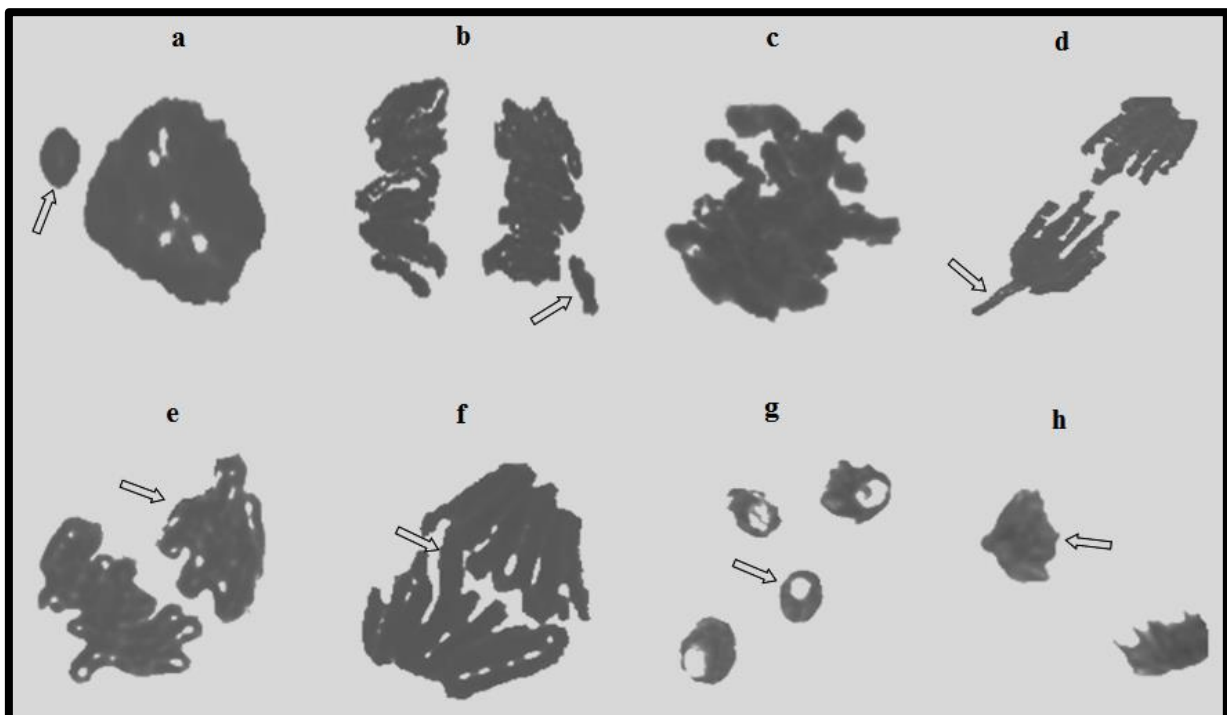


Figure 1 CAs Triggered by Tetraconazole. Micronucleus (a), fragment (b), sticky chromosome (c), vagrant chromosome (d), Unequal distribution of chromatins (e), bridge (f), vacuolated nucleus (g), reverse polarization (h).

GE administration along with Tetraconazole resulted in significant restoration of Tetraconazole-induced genotoxicity parameters in *A. cepa* in a concentration-dependent manner. Compared to the 10 mg/L Tetraconazole group, the most successful restoration was observed in the 10 mg/L Tetraconazole + 480 mg/L GE group, with an increase in MI (736 ± 25.73) and a reduction in MN (49.1 ± 4.18) and CAs. Although the genotoxicity mitigating effect of GE was very clear, none of the parameters examined reached the level of the control group. The anti-genotoxic role of GE due to its strong antioxidant power has been previously

reported by different researchers [15, 21-23]. In addition, Çavuşoğlu et al. [17] stated that the antioxidant power of GE is an important factor in reducing the genotoxic effect of Tetraconazole pesticide on onions. This superior antioxidant feature of ginger; it is due to bioactive components such as 6-shogaol and zingerone it contains [24].

Table 1 Mitigative Role of Ginger Against Tetraconazole-Induced Genotoxicity

Damages	Control	240 mg/L	480 mg/L	10 mg/L	10 mg/L	10 mg/L
	(Tap water)	GE	GE	Tetraconazole	Tetraconazole + 240 mg/L GE	Tetraconazole + 480 mg/L GE
MI	850±29.84 ^a	844±28.62 ^a	858±30.85 ^a	612±19.45 ^d	675±22.38 ^c	736±25.73 ^b
MN	0.28±0.35 ^d	0.21±0.27 ^d	0.12±0.16 ^d	70.3±8.14 ^a	58.5±5.46 ^b	49.1±4.18 ^c
FRG	N.A. ^d	N.A. ^d	N.A. ^d	65.6±7.78 ^a	52.2±5.14 ^b	45.3±3.86 ^c
SC	0.16±0.25 ^d	0.21±0.28 ^d	0.12±0.19 ^d	55.9±5.32 ^a	46.8±3.88 ^b	38.7±3.40 ^c
VC	N.A. ^d	N.A. ^d	N.A. ^d	50.4±4.14 ^a	41.7±3.72 ^b	34.6±3.12 ^c
UDC	N.A. ^d	N.A. ^d	N.A. ^d	44.2±3.80 ^a	38.6±3.36 ^b	30.9±2.85 ^c
B	N.A. ^d	N.A. ^d	N.A. ^d	41.5±3.70 ^a	33.4±2.98 ^b	25.1±2.38 ^c
VN	N.A. ^d	N.A. ^d	N.A. ^d	30.8±2.80 ^a	23.3±2.16 ^b	17.5±1.76 ^c
RP	N.A. ^d	N.A. ^d	N.A. ^d	23.7±2.11 ^a	15.8±1.54 ^b	8.8±0.84 ^c

* Means with different letters^(a-d) in the same line indicate significant differences at $p < 0.05$.

N.A.: Not available. MI: mitotic index, MN: micronucleus, FRG: fragment, SC: sticky chromosome, VC: vagrant chromosome, UDC: unequal distribution of chromatins, B: bridge, VN: vacuolated nucleus, RP: reverse polarization.

CONCLUSION

The results of this study revealed the genotoxic effects of Tetraconazole and GE on root tip cells of *A. cepa*. Although the genotoxic effect of exposure to Tetraconazole on living things are known; the protective and restorative effects of GE against these risks were demonstrated for the first time in vivo. GE application decreased the genotoxic effects of Tetraconazole in all parameters examined and increased this protective effect depending on the applied GE dose. The outstanding antioxidant capacity of GE is likely to play an important role in its anti-genotoxic effect. The fact that the doses of ginger administered alone did not cause any genotoxic effects underlines the potential of using ginger as a food.

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**PROTECTIVE ROLE OF POMEGRANATE SEED EXTRACT AGAINST
BIOCHEMICAL TOXICITY INDUCED BY ABAMECTIN PESTICIDE
IN *ALLIUM CEPA* L.**

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ABSTRACT

Abamectin, a pesticide widely used to combat species such as *Tetranychus* sp. (red spider), *Tetranychus urticae* (two-spotted scarlet spider) and *Panonychus ulmi* (European red spider) is found in many agricultural products such as pear, pepper, strawberry, tomato, apple, cucumber and eggplant. It is also known as red spider pesticide. Pomegranate (*Punica granatum* L.), which is also cultivated especially in the Southeastern Anatolia, Mediterranean and Aegean regions of Turkey, is a tree that grows in temperate climates and has been cultivated for centuries. Since pomegranate seeds are rich in polyphenol compounds, they contain plenty of anthocyanins and tannins, and in this respect, they exhibit a very strong antioxidant property. In this study, biochemical toxicity induced by 0.100 mL/L dose of Abamectin pesticide in root tips of *Allium cepa* L. (n=16) as well as the protective role of pomegranate seed extract against this biochemical toxicity were investigated. Malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD) and catalase (CAT) were utilized as toxicity indicators. *A. cepa* bulbs were divided into six (6) groups as one control and five treatments. Bulbs in the control group were germinated in tap water, while bulbs in the other groups were treated with 265 mg/L pomegranate seed extract, 530 mg/L pomegranate seed extract, 0.100 mL/L Abamectin dose, 0.100 mL/L Abamectin dose + 265 mg/L pomegranate seed extract and 0.100 mL/L Abamectin dose + 530 mg/L pomegranate seed extract, respectively, for 72 hours. At the end of the experimental period, root tips were washed, cut and prepared for biochemical analyses. The lowest MDA level and the least SOD and CAT activities were determined in control group (group I), 265 mg/L pomegranate seed extract-exposed group II and 530 mg/L pomegranate

seed extracted-exposed group III. There was no statistical difference between the values of biochemical parameters in these groups ($p > 0.05$). MDA accumulation and the activities of SOD and CAT enzymes were increased significantly ($p < 0.05$) in 0.100 mL/L Abamectin-exposed group IV, when compared to the control group. Administration of pomegranate seed extract at doses of 265 mg/L and 530 mg/L along with Abamectin in groups V and VI attenuated the toxicity of Abamectin as seen by the statistically reduced MDA level and the activities of SOD and CAT enzymes ($p < 0.05$). In addition, it was observed that the reduction of toxicity and the improvement in the values of biochemical parameters were more apparent at the dose of 530 mg/L of pomegranate seed extract. In conclusion, pomegranate seed extract can be used as a highly effective nutritional supplement to reduce the toxic effects of pesticides such as Abamectin, which can be frequently exposed in daily life, or to protect from their effects.

Keywords: Abamectin, *Allium cepa* L., Antioxidant Enzymes, Oxidative Stress, Pomegranate

INTRODUCTION

Pesticides are chemical compounds commonly used in agricultural areas to repel pests that cause vector-borne diseases and crop losses [1]. Despite the advantages of pesticides such as increasing product yield and minimizing post-harvest losses, when used for a long time and in high amounts, they accumulate in the environment and cause toxicity in non-target organisms [2]. The most probable risks in pesticide usage are the circulation of these substances in environmental systems, migration via food chain or accumulation by various living beings [3]. The health-threatening dangers related to the pesticides range from chronic to acute. Acute impacts (such as *dizziness, headache, nausea, vomiting, fatigue, stomachache, respiratory disorders and nerve-skin-eye irritations*) may occur immediately after exposure or over a 24-hour period, while chronic effects (such as *carcinogenic-mutagenic-teretogenic-oncogenic-allergic effects, sterility, hormone disorders and neurotoxicity including Parkinson and Alzheimer diseases*) can not be noticed within 24 hours following the exposure [4, 5].

Abamectin (avermectin B1a) is a pesticide produced from the fermentation of *Streptomyces avermitilis* and exhibits a marvellous insecticidal, acaricidal and antihelmintic action. In addition to 80% avermectin B1a content, 20% of Abamectin is avermectin B1b [6]. It belongs to the family of “avermectins” and the class of “macrocyclic lactones” [7, 8]. Abamectin, which is very effective against *Panonychus ulmi* and *Tetranychus urticae*, is also known as a red spider pesticide [9]. As a neurotoxic agent, the target site of Abamectin for paralyzing insects and nematodes is the glutamate-gated chloride channel which controls the nervous system [10]. Despite being used since the 1990s against the parasites in agricultural fields, it has been declared as highly toxic to bees and fish and extremely toxic to aquatic invertebrates and mammals by US Environmental Protection Agency Pesticide Fact Sheet [8, 11]. Another

classification for Abamectin based on its toxicity by ingestion or inhalation was declared as “T+, R48/25 Toxic” [12].

Pomegranate (*Punica granatum* L.), one of the predominant members of the *Punicaceae* family, is a mystical plant widely grown all over the world, especially in the Mediterranean Region. It is estimated that more than 300.000 hectares of agricultural land are allocated in the world for pomegranate production by global pomegranate producers, including Turkey [13]. It has been considered as “jewel of winter” and consumed for numerous nutritional and therapeutic functions since ancient times [14, 15]. In addition to fresh consumption, interest in processed food products made from pomegranate such as wine, juices, extracts, dehydrated seeds, jams, jellies, dried rinds and nutritional fiber has been increasing recently [16]. Due to its admirable dietary characteristics and protective phytochemicals, pomegranate fruit is accepted as a “Super Fruit” [17]. The reputation of the fruit in preventing and treating several diseases is undoubtedly due to its biologically active chemicals [18]. The Latin name of the fruit, granatum, means “seedy” [19] and the seeds are phenol-rich by-products that remain after the fruit has been juiced. Besides phenolic compounds, pomegranate seeds contain plenty of antioxidants, anti-inflammatory substances, sterols, fatty acids and vitamin E [20].

Although there are various papers evaluating the curative capacity of pomegranate limiting the detrimental effects of various compounds, the literature lacks a study showing the protective potential of pomegranate seed extract against Abamectin pesticide. The object of the current study was to investigate the protective role of pomegranate seed extract in a model organism, *A. cepa* L., against oxidative stress generated from Abamectin administration.

MATERIAL AND METHOD

Preparation of the Experimental Workflow

A. cepa bulbs bought from the grocery store were brought to the laboratory. Standard sized bulbs were separated from the others and washed thoroughly to eliminate dust. Then, old adventitious roots and thin leaves surrounding the bulbs were removed. 0.100 mL/L of Abamectin solution was prepared using the stock chemical “Torpedo EC-18 g/L Abamectin”, produced by Hektaş Ticaret T.A.Ş.–Kocaeli-Turkey. Abamectin dose applied in the study was determined according to the previous study of Kalefetoğlu Macar [21]. Pomegranate seed extract (90 capsules X 530 mg) (Sepe Natural) was utilized to prepare 265 mg/L and 530 mg/L doses of seed extracts. Six groups, each consisting of ten bulbs, were formed. Each group was kept in contact with the relevant solution for 72 hours in the dark. The solutions in which the groups were treated were as follows:

- Tap water (control)
- 265 mg/L pomegranate seed extract (PSE1)
- 530 mg/L pomegranate seed extract (PSE2)

- 0.100 mL/L Abamectin (ABM)
- 0.100 mL/L Abamectin + 265 mg/L pomegranate seed extract (APSE1)
- 0.100 mL/L Abamectin + 530 mg/L pomegranate seed extract (APSE2)

Analysis of Biochemical Parameters

Lipid Peroxidation

MDA analysis, which is a frequently used indicator of lipid peroxidation, was performed to comprehend the effects of abamectin pesticide and pomegranate seed extract applications on cellular membranes. MDA levels of the samples were estimated through the assay developed by Heath and Packer [22]. Root segments cut from the bulbs was centrifuged in 5% trichloroacetic acid (12000 rpm:15 minutes) and boiled in 0.5% thiobarbituric acid-20% TCA mixture. In order to interrupt the reactions, homogenates were cooled suddenly. Following a centrifuge step (10000 rpm:5 minutes), the absorbance of the supernatants was read at wavelength of 532 and 600 nm to calculate MDA amount. Each step of analyzes was repeated ten times.

Total Activities of Antioxidant Ezymes

The catalytic activities of two antioxidant enzymes, superoxide dismutase [SOD-E.C 1.15.1.1] and catalase [CAT-E.C 1.11.1.6] were detected in order to conceive the oxidative stress level of the groups. While the method developed by Zhang and Zhai [23] was used to determine SOD activity, CAT activity was analyzed according to Zhang et al. [24]. Both of the enzymes were extracted by using the same procedure based on centrifugation of a medium prepared with root samples and sodium phosphate buffer [25]. SOD containing reaction medium was illuminated using a fluorescent lamp (215 W) to initiate the catalysis, while CAT activity was monitored through the consumption of the substrate, hydrogen peroxide. The absorbances corresponding to the wavelengths of 560 nm (for SOD activity) and 240 nm (for CAT activity) were read. Each step of analyzes was repeated ten times.

Statistical Analysis

Data were analyzed statistically through SPSS (version 23) software and presented as mean \pm standard deviation. One-way ANOVA and Duncan tests were administered to the data to assess the significance between the mean values. In the table, superscripted letters near the mean values point the statistical difference between the means ($p < 0.05$).

RESULT AND DISCUSSION

Biochemical changes stemming from Abamectin and pomegranate seed extract treatments were investigated through oxidative stress state of the root cells. To achieve this goal, MDA level and the total catalytic activities of SOD and CAT enzymes in each group were analyzed (Table

1). Regardless of the dose, pomegranate seed extracts applied to PSE1 and PSE2 groups did not induce a significant change in MDA accumulation or enzyme activities compared to the control. This meant that the applied concentrations of pomegranate seed extract did not cause any disruption in the usual internal balance of the cells. Plants are equipped with a variety of enzymatic and non-enzymatic defense responses to overcome the biotic and abiotic stress factors they may encounter [26]. In case oxygen molecules cannot be completely reduced in cells, reactive oxygen radicals accumulate. The uncontrolled invasion of these radicals gives rise to a devastating process called oxidative stress, which may mediate a disruption of cellular membrane integrity [27]. SOD and CAT are among the first-line antioxidants to suppress the uprising of radicals [28]. Abamectin exposure in ABM group prompted a rise in SOD and CAT activities as well as MDA. MDA level of ABM was approximately 3.5 times that of the control group. In addition, the catalysis performances of SOD and CAT enzymes were nearly 1.6 times and 3.1 times those of the control, respectively. SOD, a metalloenzyme, reduces the amount of superoxide radical by converting this radical to hydrogen peroxide and oxygen [29]. Hydrogen peroxide is such an aggressive molecule that it destructively attacks cellular structures. CAT activity greatly contributes to the elimination of hydrogen peroxide by converting this molecule into water and oxygen [30]. Here, in this study, we declare that exposure to Abamectin pesticide triggered a burst of oxidative stress in *A. cepa* root cells. Although the activities of SOD and CAT enzymes increased significantly, collective effort of these enzymes was not sufficient to prevent membrane damage caused by the pesticide. Thus, in Abamectin administered group, reactive oxygen species that could not be destroyed by enzyme activities may have reacted with the lipid bilayer to produce a toxic compound, MDA. Our results confirmed the previous report of Kalefetoğlu Macar [21], who reported an increment in oxidative stress provoked by Abamectin in *A. cepa* roots. There are also many reports showing oxidative damage associated with Abamectin in experimental animals [31-33].

Co-administration of pomegranate seed extract with Abamectin reduced pesticide-related oxidative stress in APSE1 and APSE2 groups (Table 1). The protective potential of pomegranate seed extract tended to increase with increasing concentrations of the extract. Although pomegranate seed extract addition significantly decreased the severity of oxidative stress in these groups compared to the group treated with only abamectin (ABM), the MDA and enzyme activity results of APSE1 and APSE2 did not reach the control level. According to Karimi et al. [34] antioxidant nature of pomegranate is substantially associated with its polyphenol content, including catechins, anthocyanins and tannins. Anthocyanins, ellagic acid and punicalagin isomers dispersed in various parts of the pomegranate fruit are renowned for their virtues in scavenging reactive radicals and limiting lipid peroxidation [20]. Strong antioxidant effectiveness of fruit seeds prevents the DNA damages and the risk of cancer [35, 36] and the abundant oil content of the seeds contains 64–83% punicic acid, which attenuates several health problems mainly based on oxidative imbalance [37]. Our results were in a very

good agreement with Elhosary et al. [38] who reported the inhibitory effects of pomegranate seed extracts against agricultural fertilizer-induced abnormalities, including MDA increment and enzyme activity alterations.

Table 1 Protective role of Pomegranate Seed Extract Against the Biochemical Toxicity Promoted by Abamectin Treatment

Groups	MDA (n=10) ($\mu\text{M g}^{-1}$ FW)	SOD activity (n=10) (U mg^{-1} FW)	CAT activity (n=10) (OD _{240 nm} min.g^{-1} FW)
Control	6.50±1.94 ^d	65.00±5.66 ^d	0.50±0.10 ^d
PSE1	6.10±1.92 ^d	63.90±5.28 ^d	0.52±0.12 ^d
PSE2	5.80±1.85 ^d	64.70±5.54 ^d	0.48±0.08 ^d
ABM	22.60±3.95 ^a	125.80±9.52 ^a	1.56±0.34 ^a
APSE1	17.30±3.48 ^b	112.30±8.24 ^b	1.20±0.25 ^b
APSE2	11.40±2.64 ^c	98.60±7.11 ^c	0.98±0.18 ^c

*PSE1: 265 mg/L pomegranate seed extract, PSE2: 530 mg/L pomegranate seed extract, ABM: 0.100 mL/L Abamectin, APSE1: 0.100 mL/L Abamectin + 265 mg/L pomegranate seed extract, APSE2: 0.100 mL/L + Abamectin 530 mg/L pomegranate seed extract (n=10).

CONCLUSION

In brief, despite its widespread use, Abamectin pesticide clearly causes an oxidative stress in non-target organisms. *A. cepa* is a suitable organism for demonstrating the harmful effects of such pesticides. Pomegranate seed, which is a waste product of fruit juice industries, offers a remarkable protective potential against biochemical toxicity triggered by Abamectin with its unique antioxidant richness. From now on, the therapeutic action mechanisms of pomegranate seed extract need to be addressed through different chemicals that threaten the health of non-target biota.

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MANGANESE GENOTOXICITY IN *ALLIUM CEPA* L.

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ABSTRACT

Manganese (Mn), a heavy metal, is an essential trace element which acts as a cofactor for various enzymes and different metabolic processes including photosynthesis in plants. However, excessive Mn doses are highly toxic to plants. Mn can cause oxidative stress and phytotoxicity in plants as well as a decrease in fresh weight and photosynthesis rate. In this study, genotoxicity caused by different doses of Mn in root tip cells of *Allium cepa* L. (n=16), a biological indicator, was investigated. Mitotic index (MI), micronucleus (MN) and chromosomal aberration (CA) scores were screened as genotoxicity indicators. *A. cepa* bulbs were separated into four groups as GR I: control (tap water), GR II: 250 µM Mn, GR III: 500 µM Mn and GR IV: 1000 µM Mn. The bulbs in the control group were germinated using tap water, while the bulbs in the treatment groups were kept at corresponding Mn doses for 72 hours. At the end of the treatments, emerged root tips were washed with distilled water and cut approximately 1 cm long. Samples were prepared through squash preparation method for microscopic analyses. The highest MI value and the lowest CAs values were observed in the control group (GR I). On the other hand, MI decreased significantly ($p<0.05$), whereas MN and CAs increased significantly ($p<0.05$) in Mn-exposed Groups II, III and IV depending on the increase in Mn-application dose. It was evident that these alterations in the values of genotoxicity parameters were most apparent in GR IV, where 1000 µM-Mn was applied. Mn exposure resulted in CAs, including fragment, sticky chromosome, vagrant chromosome, unequal distribution of chromatin and bridge in the meristematic cells of *A. cepa* root tips. As a conclusion, although Mn as a trace element is essential for the metabolic activities of plants, its excessive exposure may cause toxicity affecting the genetic structure of plants.

Keywords: *Allium cepa*, Genotoxicity, Manganese

INTRODUCTION

Heavy metal exposure is not only an intriguing environmental issue, but also a health risk for all flora and fauna [1]. Accumulation of these pollutants has become prevalent due to the repeated practice of these materials in technological, agricultural, industrial, domestic and medical fields [2]. All undesirable effects that disrupt cell cycle pattern and genomic structure, including DNA, DNA repair apparatus, DNA polymerases, and spindle strands are defined as genotoxicity, and heavy metals are well-known genotoxins when exposed to excessive doses [3].

Manganese (Mn) performs two main tasks in almost all organisms, one being a cofactor or activator for hundreds of enzymes (such as oxalate oxidase located in the apoplast and the enzymatic antioxidant Mn-superoxide dismutase located in mitochondria and peroxisomes) and the other being a metal with catalytic activity in biological processes [4-6]. As a notable micronutrient, Mn is needed to sustain various metabolic roles in subcellular compartments of plant cells. The major functions of Mn in life cycle of plants include aerobic respiration, photosynthesis, scavenging of oxygen radicals, hormone signaling and the struggle against pathogen attacks [7]. Mn in the leaf tissues of plants was first demonstrated in vegetable ashes over 200 years ago. Despite this relatively early discovery, the place and importance of Mn in plant metabolism and development were only understood in the 1900s [5]. Noticing that both plants and algae cannot release O₂ under growth conditions without Mn, Pirson et al. [8] made history as the first scientists to discover the possible role of Mn in photosynthesis. Then, Spector and Winget [9] discovered that Mn is an integral component of oxygen evolving complex (or water-splitting complex) in PSII, the first stage of photosynthesis, of plants. During the process, the tetra-Mn cluster (Mn₄O₅Ca) achieves splitting 2 molecules of water into 4 protons, 4 electrons and molecular O₂ [10].

Mn toxicity due to over-accumulation is a well-known phenomenon in plants. Excessive amounts of Mn could reduce the rate of photosynthesis, block the biosynthesis of auxin hormone and chlorophyll pigments, increase the oxidized phenolics or prevent the absorption and distribution of various essential elements such as Mg, Ca, P and Fe [7, 11]. Yellowing and shrinking of leaves with brown spots are also visible symptoms of Mn toxicity [12]. In addition, it is known that Mn incites oxidative stress and damages in genetic material of the cells [13]. In some cases, degeneration of structural components of the cells such as proteins, nucleic acids, lipids and carbohydrates resulting from Mn toxicity ends with cell death [14]. Some studies report that overexposure to Mn leads to genetic instability at the chromosomal or DNA level [15].

A. cepa is an excellent instrument for biomonitoring the genotoxic effects of environmental contaminants due to its fast-growing adventitious roots [16]. Rapid growth also means rapid cell division, which provides a spectacular opportunity to screen the failures that can occur in

mitosis in the meristematic tissues of *Allium* roots [17]. The *Allium* test, which also shows a strong correlation with known mammalian tests, lays the groundwork for microscopic examination of the cellular effects of harmful chemicals due to the small number of large chromosomes ($2n=16$) of the onion. Herein, we designed the current study to demonstrate the genotoxic effects of different doses of Mn in *A. cepa* root meristem cells.

MATERIAL AND METHOD

Design of the Experimental Workflow

The loose scales and of *A. cepa* bulbs (about the same weight) bought from a grocery store were removed. Then, the old roots and root residues emerging from the disc stem were thoroughly scraped. Mn with CAS No. 7439-96-5 purchased from Merck was used to prepare the Mn solutions. While one beaker was filled with tap water, three different doses of Mn solution (50 μ M Mn, 500 μ M Mn and 1000 μ M Mn) were added to the other beakers. Four groups of onions, each consisting of 10 bulbs, were placed in beakers with their discs in contact with the relevant solutions. The beakers were kept in the dark for 72 hours to allow for the emergence of new roots. The entire testing process was carried out at room temperature.

Determination of Genotoxicity Parameters

The roots of the bulbs were harvested to complete the application period. Root tips were fixed in Clarke's fixator consisting of glacial acetic acid and ethanol (3:1 ratio) for 2 hours and transferred into ethanol (70%). Hydrochloric acid solution (1N) at 60 °C was used to hydrolyze the root materials. The roots were then left to stain in 25% acetic acid containing 1% acetocarmine for 24 hours. To screen the genotoxicity parameters, the root tips, on which 45% acetic acid was dripped, were crushed with the thumb. The Irmeco IM-450 TI model microscope was utilized to count CAs and the cells in mitosis as well as the amount of MN [18]. The rules of Fenech et al. [19] were taken into account to determine whether a nucleus-like package could be accepted as MN. MI assessment was done by analyzing 1.000 cells for 10 root tips in each group (a total of 10.000 cells for MI). On the other hand, MN and the other CAs scores were reached by analyzing 100 cells for 10 root tips in each group (a total of 1.000 cells for Mn and CAs).

Statistical Analysis

The statistical difference between the means ($p < 0.05$) was established according to one-way ANOVA and Duncan's test (SPSS 23 Software).

RESULT AND DISCUSSION

It is of great importance to elucidate the genotoxic effects caused by heavy metals by using different bio-indicator organisms. Genotoxicity incited by Mn applications is summarized in Figure 1 and Table 1. Damage to root tip cells of *A. cepa* definitely increased with the

application dose of Mn. The highest MI value was observed in the control (GR 1) (Table 1) and Mn administration reduced MI in a dose-dependent manner. Hence, as the applied Mn concentration increased, the mean MI level in the groups decreased significantly. Mercado and Caleño [20] stated that MI values below the control level are indicative of a halt or disruption in normal mitosis. Therefore, the applied concentrations of Mn were mitodepressive for *A. cepa* meristems. Owolarafe et al. [21] interpreted that the reason for the dose dependency in MI impairment may be retardation in cell proliferation or alteration of mitotic cycle processes. In addition, excessive Mn reduces cell division in meristematic tissues via suppressing auxin biosynthesis [22]. According to Dutta et al. [23], MI reflects the presence of a cytotoxin, while CAs are markers of genotoxicity generated from a contaminant. Our results were in line with data of Francisco et al. [24] who reported a Mn-related reduction in MI in *A. cepa* seeds.

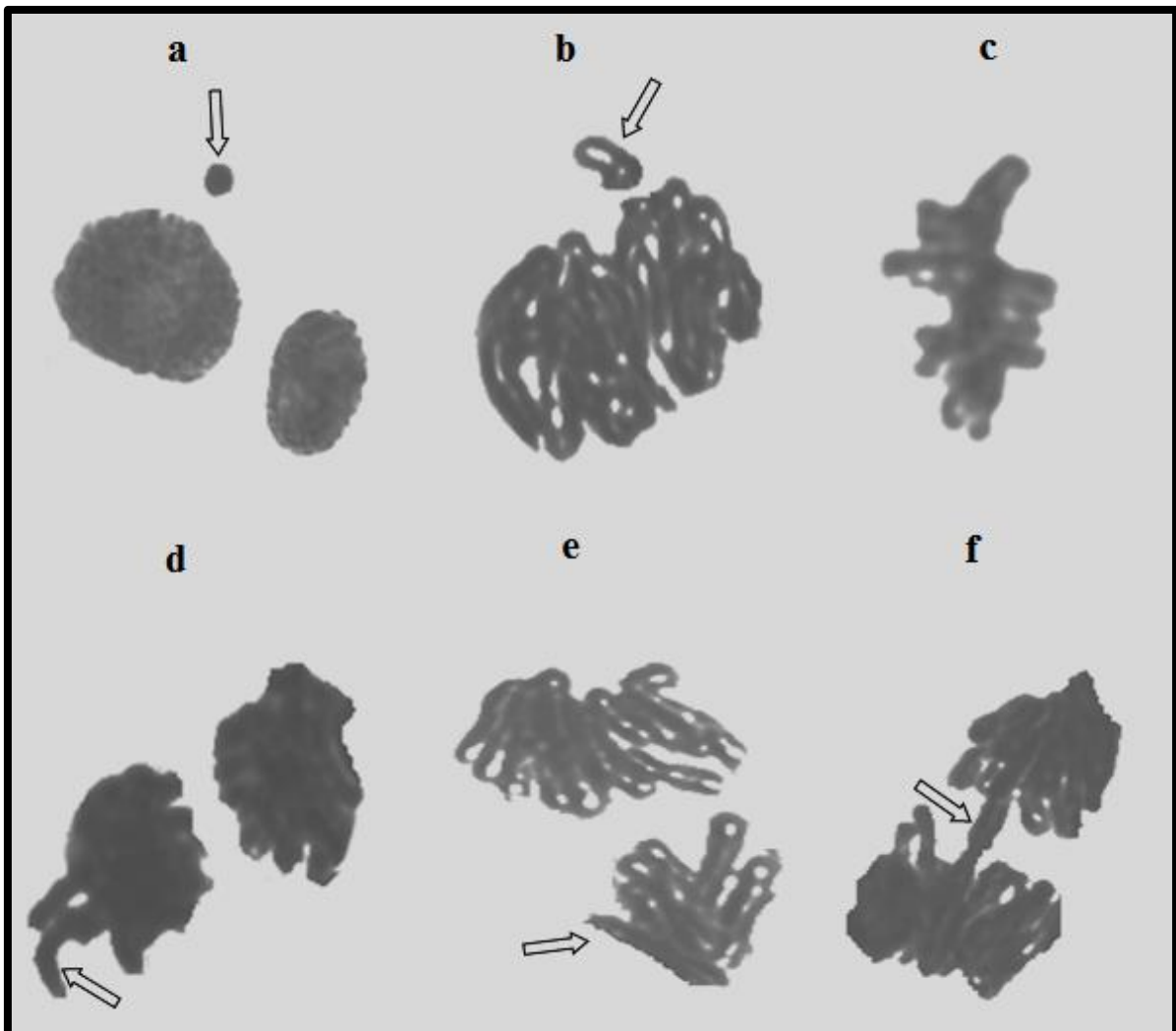


Figure 1 Chromosomal Disorders Induced by Manganese. MN (a), Fragment (FR) (b), Sticky Chromosome (STC) (c), Vagrant Chromosome (VGC) (d), Unequal Distribution of Chromatin (UDCH) (e), Bridge (BR) (f)

MN is a tiny nucleus-like body consists of lagging chromosomes or chromosomal fragments failed to involve in the main nucleus following the completion of mitotic division [25]. Mn administration led to a significant accumulation of MN in *Allium* root cells (Table 1, Figure 1a). MN values in all groups were statistically different from each other. Similar to the reduction in MI, the dose-related behavior in the MN increase was also striking in the Mn-treated groups. Since MN abundance is a sign of genetic instability [26], the current study clearly showed that Mn is an agent that induces genotoxic stress. Confirming our results, the MN promoting effect of Mn has also been demonstrated in different organisms [27-29].

Table 1 Genotoxicity Induced by Manganese

Damages	GR I	GR II	GR III	GR IV
MI	880±31.67 ^a	824±28.72 ^b	753±25.84 ^c	676±21.57 ^d
MN	0.14±0.19 ^d	17.3±1.28 ^c	29.4±2.96 ^b	55.8±5.16 ^a
FR	0.00±0.00 ^d	14.6±1.18 ^c	25.8±2.66 ^b	48.4±4.79 ^a
STC	0.18±0.24 ^d	9.70±0.98 ^c	18.6±1.36 ^b	39.5±3.80 ^a
VGC	0.00±0.00 ^d	7.20±0.84 ^c	15.3±1.20 ^b	30.6±3.12 ^a
UDCH	0.11±0.18 ^d	5.90±0.76 ^c	10.5±1.00 ^b	23.7±2.50 ^a
BR	0.00±0.00 ^d	4.50±0.58 ^c	7.70±0.85 ^b	15.3±1.16 ^a

*GR I: Control, GR II: 250 µM Mn, GR III: 500 µM Mn, GR IV: 1000 µM Mn. Values were given as mean ± standard deviation (n=10). MI: mitotic index, MN: micronucleus, FR: fragment, STC: sticky chromosome, VGC: vagrant chromosome, UDCH: unequal distribution of chromatin, BR: bridge. Different letters^(a-d) in the same line reflect statistical significance between the mean values (p<0.05).

Fragment was the second most common CAs after MN in Mn-applied groups (GR II: 14.6±1.18, GR III: 25.8±2.66 and GR IV: 48.4±4.79) (Table 1, Figure 1b). On the other hand, the control group treated with tap water did not indicate any fragment formation. The difference between fragment formation results of all groups was statistically significant. Unrepaired breaks are DNA double strand give rise to production of acentric fragments [25]. According to Debnath et al. [30], chromosomal fragmentations and bridges are attributed to sticky chromosomes, which are then followed by setback of separation and movement in anaphase. Stickiness was the third most frequent CAs in the groups treated with Mn solutions (Table 1, Figure 1c). GR IV administered with the highest Mn dose showed the most abundant sticky chromosome. Stickiness is an irreversible disorder that typically results in cessation of the cells [23]. Mn-induced vagrant formation (Table 1, Figure 1d) is a prominent aneugenic effect together with MN accumulation [31]. Rank [32] suggested that the main reason for vagrant appearance is spindle poisoning. The other CAs recorded in the groups treated with Mn

solutions was unequal distribution of chromatin (Table 1, Figure 1e) and bridge (Table 1, Figure 1f). Unequally distributed chromatins are generated from failures in the disjunction of chromatids in anaphase, causing an enhancement in vagrant accumulation [23]. Mishra [33] reported that two noticeable indicators of a mutagenic material are chromosomal bridges and stickiness. The mechanism underlying bridge formation is simultaneous pulling of a chromosome region to both poles of the spindle fiber during the segregation of chromosomes [34]. We are aware of some papers pronouncing aberrant chromosomes induced by excessive Mn exposure [35-37], confirming our results.

CONCLUSION

Based on the data of the study, we inferred that overdose of Mn, an essential element for plants, was a genotoxin for *Allium cepa* and the level of detrimental effect was strictly dose dependent. Arrested mitosis, MN accumulation and increment in CAs formation were obvious symptoms of the genotoxic effect of Mn administration on cells.

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***PIMELIA SUBGLOBOSA* (PALLAS, 1781) (COLEOPTERA: TENEBRIONIDAE)'NİN
SİNDİRİM SİSTEMİNİN ANATOMİSİ VE HİSTOLOJİSİ
THE ANATOMY AND HISTOLOGY OF THE DIGESTIVE SYSTEM OF *PIMELIA
SUBGLOBOSA* (PALLAS, 1781) (COLEOPTERA: TENEBRIONIDAE)**

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ÖZET

Bu çalışmada, *Pimelia subglobosa*'nın sindirim kanalı, tükürük bezleri, Malpighi tüplerinin anatomisi ve histolojisi ayrı ayrı prosedürler uygulanarak ışık ve taramalı elektron mikroskobu ile tanımlanmıştır. *P. subglobosa*'da sindirim kanalı; ön, orta ve arka bağırsak olmak üzere 3 kısımdan oluşmakta olup, tükürük bezleri ve Malpighi tüpleri de sindirim kanalıyla bağlantılı yapılardır. Başın hemen altında yer alan tükürük bezleri tek tabakalı silindirik epitelle çevrelenmiş ve hücreleri bazofilik çekirdeklidir. Ön bağırsak; farinks, özofagus, kursak ve proventrikulus olmak üzere 4 kısımdan oluşmaktadır. Ağızın devamında farinks yapısı özofagusa bağlanmaktadır. Özofagusu takip eden kursak dıştan enine kaslarla ve trake ağıyla çevrelenmiştir. Kursak duvarı intima, tek tabakalı kübik epitel ve kalın kas tabakası ile çevrelenmiştir. İntimanın iç yüzeyinde gruplar halinde kısa dikenler vardır. Kursak proventrikulusa bağlanmaktadır. Proventrikulusun intima tabakasının iç yüzeyinde çok sayıda, küçük, sivri diken şeklinde çıkıntılar almaktadır. Orta bağırsak anatomik olarak dıştan boyuna kaslarla ve trakelerle çevrelenmiştir. Orta bağırsak yüzeyinin genişlemesini sağlayan parmak şeklinde çok sayıda krypt vardır. Orta bağırsak histolojik olarak yuvarlak çekirdeklere sahip tek tabakalı silindirik epitelle çevrelenmiştir. Hücrelerin apikalinde emilimden sorumlu mikrovilluslar görülmektedir. Malpighi tüpleri orta bağırsak ile arka bağırsak arasında bağlanmaktadır. Histolojik incelemelerde Malpighi hücrelerinin tek tabakalı kübik epitelle çevrelendiği görülmektedir. Sindirim kanalının son kısmı olan arka bağırsak ileum, kolon ve rektum olmak üzere 3 kısımdan oluşmuştur. İleum yüzeyi içten enine kaslarla dıştan boyuna kaslarla çevrelenmiştir. İleumu çevreleyen tek tabakalı epitel hücresi kübik ve çekirdekleri yuvarlaktır. Intima tabakasının lümene bakan yüzeyinde çok sayıda kutikular çıkıntılar görülmektedir. Arka bağırsağın ikinci kısmı olan kolon duvarı intima ve epitel hücreleri ile çevrelenmiştir. Lümende çok sayıda yuvarlak şekilli bakterilere rastlanmıştır. Sindirim kanalının son kısmı rektum oldukça kalın ve enine kas tabakasıyla çevrelenmiştir. Sonuç olarak

bu çalışmada, *P. subglobosa* dahil Tenebrionidae'nin beslenme kanalı biyolojisine, anatomik ve sistematik çalışmalara katkı sağlanması amaçlanmıştır.

Anahtar Kelimeler: Sindirim sistemi, boşaltım sistemi, ışık mikroskobu, elektron mikroskobu

ABSTRACT

In this study, the anatomy and histology of the digestive tract, salivary glands, and Malpighian tubules of *Pimelia subglobosa* were described by light and scanning electron microscopy using separate procedures. The digestive tract in *P. subglobosa*, it consists of 3 parts: foregut, midgut and hindgut. In addition, salivary glands and Malpighian tubules are structures associated with the digestive tract. The salivary glands located just below the head are surrounded by a monolayer cylindrical epithelium and their cells are basophilic nuclei. Foregut consists of 4 parts: pharynx, esophagus, crop and proventriculus. In the continuation of the mouth, the pharynx structure is connected to the esophagus. The crop following the esophagus is surrounded externally by transverse muscles and a tracheal network. The crop wall is surrounded by the intima, a monolayer of cuboidal epithelium, and a thick muscle layer. There are groups of short spines on the inner surface of the intima. The crop is attached to the proventriculus. Numerous small, pointed spines are located on the inner surface of the intima layer of the proventriculus. The midgut is anatomically surrounded by longitudinal muscles and trachea. There are many finger-shaped crypts that allow the expansion of the midgut surface. Histologically, midgut is surrounded by a monolayer cylindrical epithelium with round nuclei. Microvilli responsible for absorption are seen at the apical part of the cells. The Malpighian tubules are connected between the midgut and hindgut. Histological examinations show that Malpighian tubules are surrounded by a monolayer cubical epithelium. The hindgut, which is the last part of the digestive tract, consists of 3 parts: ileum, colon and rectum. The surface of the ileum is surrounded internally by transverse muscles and externally by longitudinal muscles. The monolayer epithelial cell surrounding the ileum is cubic and the nuclei are round. Numerous cuticular protrusions are seen on the lumen-facing surface of the intima layer. The colon wall which is the second part of the hindgut, is surrounded by intima and epithelial cells. Numerous round shaped bacteria were found in the lumen. The last part of the digestive system, the rectum, is surrounded by a very thick and transverse muscle layer. In conclusion, in this study, it is aimed to contribute to the digestive system biology, anatomic and systematic studies of Tenebrionidae including *P. subglobosa*.

Keywords: Digestive system, excretory system, light microscope, electron microscope

INTRODUCTION

Tenebrionidae has 2,274 extant genera and approximately 30,000 species, distributed in 11 subfamilies (Bouchard et al., 2021). The Tenebrionidae fauna of Turkey includes approximately 543 species (Canpolat & Hasbenli 2012; Nabozhenko & Keskin 2014; Chigray et al. 2015a, 2015b). Tenebrionidae mostly live in soil, under logs and rocks, and in leaf litter. The family Tenebrionidae is abundant in semi-arid or arid regions all over the world and is considered as biological indicators of these environments (Borror et al. 1989; Wiggins et al. 2007; Ghahari et al. 2010; Thakare et al. 2012). Tenebrionids (both adults and larvae) are primarily saprophyte, feeding on a variety of dead plant and animal matter, including humus, leaf litter, rotting trees, carrion, and manure. However, some tenebrionids are predators or semi-predators that feed on other insects, while a number of geophyll larvae feed on live plant roots, stems or seedlings (Lawrence & Spilman 1991; Fattorini 2000). *Pimelia subglobosa* is the apterous tenebrionid beetle distributed in the arid area - the suitable object (model species) for study in the adaptation and evolutionary tendency.

In insects, digestive process varies greatly due to differences in the consumption of nutrients (Slansky, 1982). Intestinal length is often related to diet. Insects that usually consume a higher protein diet have a shorter gut (Pradhan, 1939). The digestive system distinctly consists of the foregut, midgut and hindgut, based on the differential structures and cell types (Beauregard 1890; Everly 1936; Mattingly 1938; Senarat et al. 2014; Özyurt Koçakoğlu et al., 2021a, b). The foregut begins at the mouth, includes pharynx, esophagus, and proventriculus. The midgut is the principal source of the digestive enzymes and also one of the main sites for the absorption of digested material from the gut-contents. The hindgut has ileum, colon, and rectum and terminates with the anus (Terra and Ferreira, 2009; Özyurt Koçakoğlu et al., 2021a, b). The Malpighian tubules in Coleoptera have cryptonephric condition. The distal ends of the tubules are closely applied to the posterior hindgut, zigzagging over its surface (Crowson 1981; Chapman 2013; Özyurt Koçakoğlu et al., 2021a-c).

The studies on different parts of the digestive system in the Tenebrionidae family is very few (Midler, 1931; McAllister et al., 1995; Sarwade and Bhawane, 2013). Miller (1931) described the histological structure of *Meracantha contracta* (Tenebrionidae) with drawings. Sarwade and Bhawane (2013) also examined the histology of the digestive tract of *Platynotus belli* (Tenebrionidae) under the light microscope. McAllister (1995) illustrated the histology of *Alphitobius diaperinus* (Tenebrionidae). Therefore, in this study, we studied in detail the anatomy and histology of the digestive tract, salivary glands, and Malpighian tubules of *P. subglobosa*. In conclusion, this study will contribute to the digestive system anatomical and histological studies on the Tenebrionidae family and other Coleoptera families.

MATERIALS AND METHODS

Insects

Adult 25 males and females of *P. subglobosa* were collected in Babadağ, Fethiye, Muğla, Turkey in April 2019. They were kept in the laboratory at Gazi University at 25 °C and perforated plastic jars with plants in their environment.

Preparation of Gut

For light microscopy studies, the samples were anesthetized with ethyl acetate evaporation, and their dissected guts in sodium phosphate buffer were photographed in stereomicroscope. They were transferred in Bouin's fixative, washed 70% alcohol, dehydrated in increasing ethanol series, cleared with xylol and embedded in pure paraffin. The obtained sections (5-7µm thickness) with microtome were stained with haematoxylin and eosin. These sections were photographed under a light microscope (Olympus BX51).

Scanning Electron Microscopy (SEM)

For SEM examinations, the dissected guts were fixed with 2.5% glutaraldehyde (pH 7.2, sodium phosphate buffered), washed three times with phosphate buffer (pH 7.2) for 15 minutes, and then dehydrated in ethanol series (70-100% for 15 min each). Then tissues were transferred Hexamethyldisilazane (HMDS) and they were dried in air. They were mounted by double-sided tape on SEM stubs, and then coated with gold for 2 minutes with a Polaron SC 502 sputter coater. The samples were examined with a JEOL (JSM 6060 LV) SEM operated at 10 kV, and photographed in an electron microscope laboratory in the faculty of science at Gazi University.

RESULTS

In *P. subglobosa*, the digestive system consists of 3 parts: foregut, midgut and hindgut. In addition, salivary glands and Malpighian tubules are structures associated with the digestive tract. The digestive system starts from the mouth. The salivary glands are in pairs around the esophagus just below the head and are white in color. Histologically, the salivary glands are surrounded by a monolayer cylindrical epithelium with small, rounded, basophilic nuclei. The lumen is narrow, surrounded by a thick cuticular layer (Figs. 1a, b). The foregut consists of 4 parts: pharynx, esophagus, crop and proventriculus. In the continuation of the mouth, the pharynx structure is connected to the esophagus. The esophagus continues with the crop (Fig. 1c). The crop is surrounded externally by transverse muscles and trachea network (Figs. 1d, 2a, 2b). Histologically, in the crop; intima, monolayer cubic epithelium and thick muscle layer are distinguished (Fig. 2c). There are groups of short spines on the inner surface of the intima (Fig. 2c). The lumen is quite large and filled with nutrients (Fig. 2c). It attaches to the esophagus proventriculus. (Fig. 3a) The proventriculus appears to have bifurcated deep folds in the transversely fractured SEM photographs (Fig. 3b). On the inner surface of the intima layer of

the proventriculus, nutrient particles were distinguished in some places among the numerous, small, triangular pointed spines (Figs. 3c, d). The proventriculus, the last part of the foregut, attaches to the second part of the digestive tract, the midgut (Figs. 4a, b). The midgut is anatomically surrounded by longitudinal muscles and trachea (Fig. 4b). On the outer surface of the midgut, there are numerous finger-shaped crypts that allow the midgut surface to expand (Figs. 4c, d). The midgut is histologically surrounded by a monolayer cylindrical epithelium with round nuclei (Figs. 4e-h). Microvilli responsible for absorption are distinguished at the apical part of the cells (Fig. 4h). The lumen is quite wide. It contains food particles (Fig. 4e). A white, thin, long pair of Malpighian tubules are distinguished, connecting between the midgut and the hindgut (Fig. 5a). Histologically, the Malpighian tubules have a monolayered cuboidal epithelium with oval nuclei located towards the base of the cell. There are wide spaces between the cells. A brush border is seen at the apical part of the cell (Fig. 5b). Hindgut of *P. subglobosa* which is the last part of the digestive canal, consists of 3 parts: ileum, colon and rectum. The ileum is a wavy, tubular structure that lies between the midgut and the colon. Anatomically, its surface is surrounded by transverse muscles from the inside and longitudinal muscles from the outside. Deep folds of intima and epithelium surrounding the ileum towards the lumen were distinguished in light microscope cross-sections and broken SEM photographs (Figs. 6a-d). The epithelial cells are monolayered and cubical, with rounded nuclei (Fig. 6c). The muscle layer surrounding the epithelial cells is thick (Figs. 6a-d). Numerous cuticular protrusions are distinguished on the lumen-facing surface of the intima layer (Fig. 6d). The colon, the second part of the hindgut, has a flat surface (Figs. 7a, b). Histologically and anatomically, the colon wall is surrounded by intima and epithelial cells. Malpighian tubules are distinguished between the colon wall and the sheath (Figs. 7c-e). Numerous round-looking bacteria were found in the lumen (Fig. 7f). Rectum is the last part of the digestive tract. In light microscopy and SEM examinations, rectum is surrounded by a very thick and transverse muscle layer (Figs. 8a, b). The muscle layer is clearly striated, with many nuclei present. The intima layer and epithelium have deep recesses towards the lumen (Figs. 8c, d). The epithelial cell surrounding the rectum is smaller than the cells surrounding the other parts of the hindgut (Figs. 6c, 7e, 8c).

DISCUSSION AND CONCLUSION

The morphology of the digestive tract of beetles is correlated to feeding habits (Halffter and Matthews, 1966; Umeya 1960). In Tenebrionidae including *P. subglobosa*, generally the digestive canal consists of three main divisions, the foregut, the midgut and the hindgut (Miller, 1931; McAllister, 1995; Sarwade and Bhawane, 2013; Özyurt Koçakoğlu 2021a, b). The foregut of the some tenebrionids including *P. subglobosa*, consists of a pharynx, an oesophagus, and a proventriculus (McAllister, 1995). The foregut of *A. diaperinus* (Tenebrionidae) includes the pharynx, maxillary glands and esophagus with no well-defined crop or proventriculus (Miller, 1931). In the gut in *Platynotus belli* (Tenebrionidae) consists of three regions, preoral

cavity, pharynx and oesophagus (Sarwade and Bhawane, 2013). The foregut of *P. subglobosa* with well-defined circular muscles along its length is similar to that of most beetles (Crowson, 1981; Mc Allister, 1995; Özyurt Koçakoğlu 2021a, b).

The shape of the epithelial cell varies according to the parts that make up the digestive system and species. As like in *M. contracta* (Tenebrionidae) and *A. diaperinus* (Tenebrionidae), the foregut and hindgut epithelium of *P. subglobosa* consists of cuboidal cells with oval nuclei (Miller, 1931; Mc Allister, 1995). But, in *C. populi*, the foregut has monolayer squamous epithelium (Özyurt Koçakoğlu et al., 2021d). In *A. bipunctata* (Coccinellidae), the epithelium of the foregut varied from squamous to simple cuboidal and columnar (Borges et al. 2015). As well as, the midgut epithelial cells of *P. subglobosa*, *M. contracta* (Tenebrionidae), *A. diaperinus* (Tenebrionidae) and *C. herbacea* (Chrysomelidae) are columnar and have a striated border (Miller, 1931; Mc Allister, 1995; Özyurt Koçakoğlu and Candan, 2021). In *P. subglobosa*, the foregut arises irregular, small punctures or invaginations in the intima, as like in *M. contracta* (Tenebrionidae) and *A. diaperinus* (Tenebrionidae) (Miller, 1931; Mc Allister, 1995). But intima of hindgut in *P. subglobosa* has irregular and large folds, as like in *A. diaperinus* (Tenebrionidae), *Platynotus belli* Fairmere (Tenebrionidae), *E. malachiticus* (Curculionidae) and *C. sycophanta* (Carabidae) (Mc Allister, 1995; Sarwade and Bhawane, 2013; Candan et al., 2019, 2021). As like in *P. subglobosa*, the intima of various parts of the foregut have spines in other species (Miller, 1931; McAllister, 1995; Díaz et al., 2003; Herve & Heat, 2011; Ingerson-Mahar 2014; Özyurt Koçakoğlu and Candan, 2021; Özyurt Koçakoğlu 2021a-d). The sclerotized spines of the foregut intima appear to play no significant part in the mechanical breakdown of food (Hochuli et al. 1992). The proventriculus weakly developed in *P. subglobosa*, as in like *M. picta decastigma* (Buprestidae), *Aporhina australis* (Heller) (Brentidae), *Mecysolobus bubo* (Fabricius) (Curculionidae), *Tillus elongatus* (Linnaeus) (Cleridae) and *Onychotillus vittatus* Chapin (Cleridae) (Calder, 1989; Opitz, 2014). But, in *Tanymecus dilaticollis* Gyllenhal, 1834 (Curculionidae) have a well-developed proventriculus (Candan et al., 2020).

On the outer surface of the midgut of *P. subglobosa*, there are numerous finger-shaped crypts. The midgut caeca or regenerative crypts were observed in *Tenebrio confusum*, *Tenebrio castaneum*, *Bolitotherus cornutus* (Panzer), *Tribolium* Macleay (Tenebrionidae), *Oryzaephilus* Ganglbauer (Cucujidae), *A. diaperinus* (Tenebrionidae) (Panzer) and *C. sycophanta* (Carabidae) (Auten, 1933; Sinha, 1958; Mc Allister, 1995; Candan et al., 2021). But, there are no regenerative crypts on the midgut of in *Meracantha contracta* Beauv. (Tenebrionidae), *A. diaperinus* (Tenebrionidae) and *C. sycophanta* (Miller, 1931; Mc Allister, 1995; Candan et al., 2021).

As like in *Meracantha contracta*, in *P. subglobosa*, Malpighian tubules originate at the union place of the mid-gut and hind-gut. As other most insect species, in *C. herbacea*, they are

composed of large cells with large, oval nuclei (Sinha, 1958; McAllister et al., 1995; Díaz et al., 2000, 2003; Rubio et al., 2008; Bu & Chen, 2009; Aldigail et al., 2013; Singh & Prasad, 2013; Candan et al., 2019; Candan et al., 2021; Koçakoğlu et al., 2020). They finally becoming attached to the wall of the hind-gut at the point where the colon commences (Miller, 1931; Crowson, 1981; Mc Allister, 1995).

As like in *M. contracta* (Tenebrionidae), in *P. subglobosa* the small intestine or ileum, the large intestine or colon, and the rectum (Miller, 1931; Candan et al., 2021). *Carabus violaceus*, the hindgut consists of only two parts as colon and rectum (Ali, 1964). In *P. subglobosa*, there are bacteria in colon lumen. As well as, bacteria were seen in the colon and rectum lumen of *C. sycophanta* (Candan et al., 2021). But, in *E. malachiticus* (Curculionidae) and *P. belli* (Tenebrionidae), the ileum lumen has a lot of bacteria clumps (Candan et al. 2019; Sarwade and Bhawane 2013). As like in *A. diaperinus* (Tenebrionidae), the rectum in *P. subglobosa* has six rectal pads with cavities and connective tissue at the base of each pad. Malpighian tubules are close association with the rectal pads.

It is thought that this study will make contributions to the knowledge of the digestive system of Tenebrionidae. Furthermore, it will be helpful to reveal the characters that may be useful for future studies; and it will be beneficial in obtaining the key information for future research into in the systematic, ecology and biological control agents of Coleoptera.



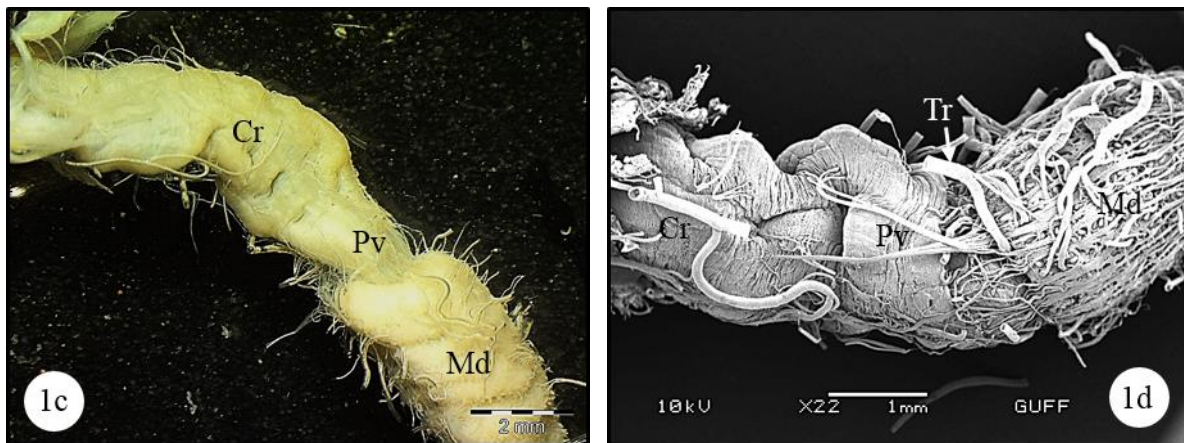


Fig. 1. a, b. The longitudinal section of salivary gland (x400, x1000) (LM). **c, d.** SM and SEM photograph of crop, proventriculus and midgut. Cr-crop, Ep-epithelium, In-intima, Lu-lumen, Md-midgut, Nu-nucleus, Pv-proventriculus, Tr-trachea, LM-Light microscope, SM-Stereo microscope, SEM-Scanning Electron Microscope.

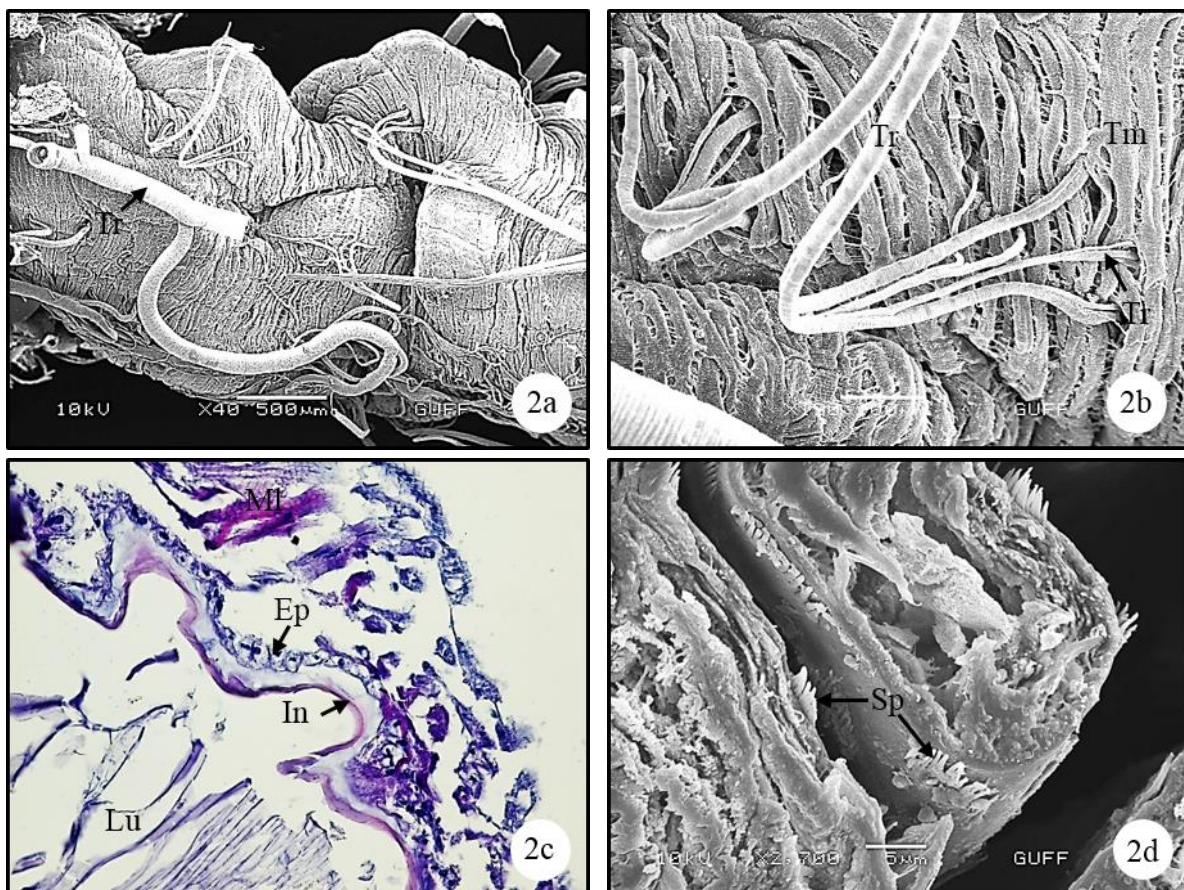


Fig. 2. a, b. The trachea and transverse muscles on surface the crop (SEM). **c.** The cross section of the crop wall (x400) (LM). **d.** SEM photograph of the spines in the crop intima. Ep-epithelium, In-intima, Lu-lumen, MI-muscle layer, Sp-Spine, Tm-transverse muscles, Tr-trachea, LM-Light microscope, SEM-Scanning Electron Microscope.

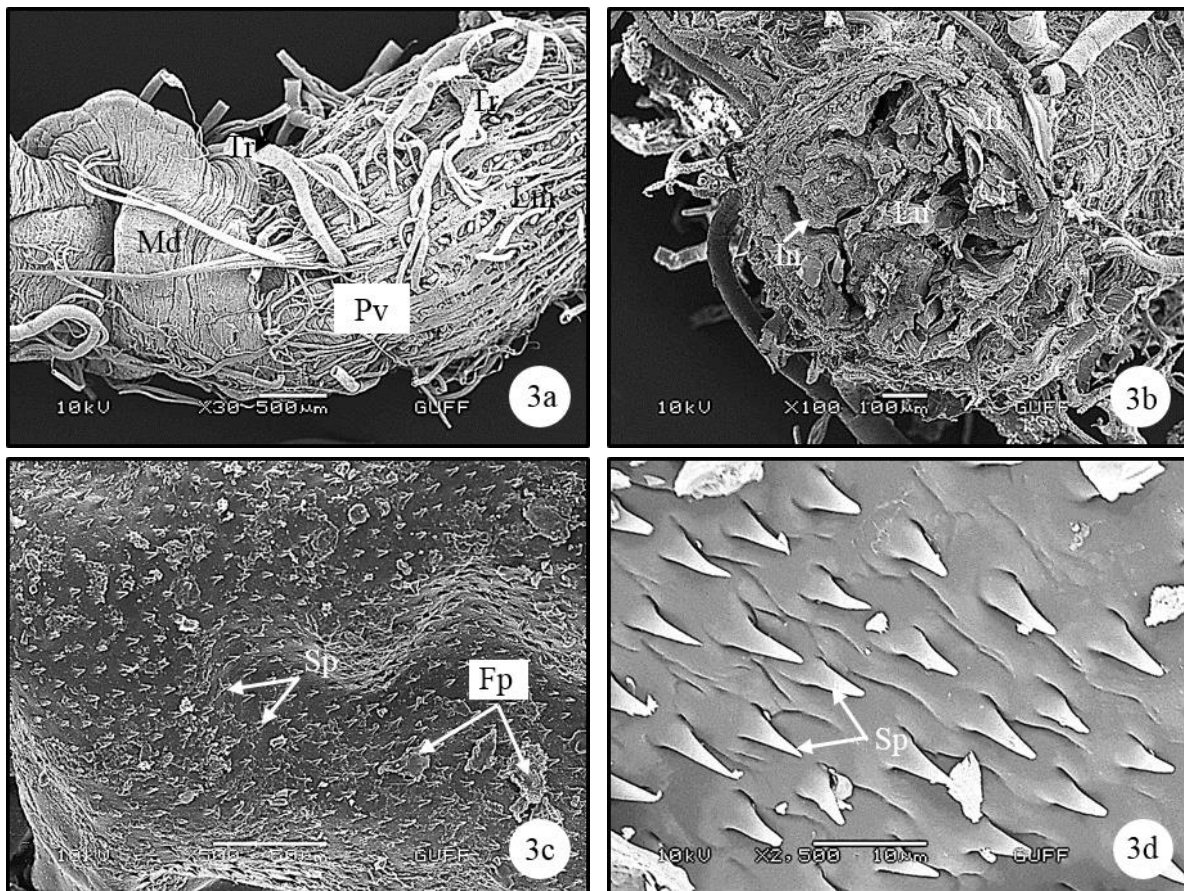
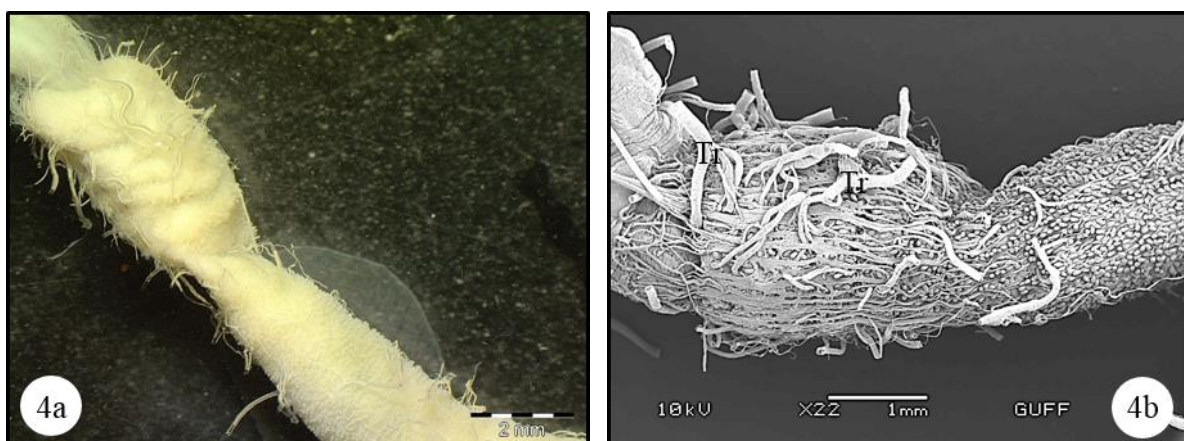


Fig. 3. a. Transition from proventriculus which is the last part of the foregut, to the midgut (SEM). b. SEM photograph of the proventriculus cross section. c, d. The spines in proventriculus intima (SEM). In-intima, Lm-longitudinal muscles, Lu-lumen, Md-midgut, MI-muscle layer, Pv-proventriculus, Tr-trachea, SEM-Scanning Electron Microscope.



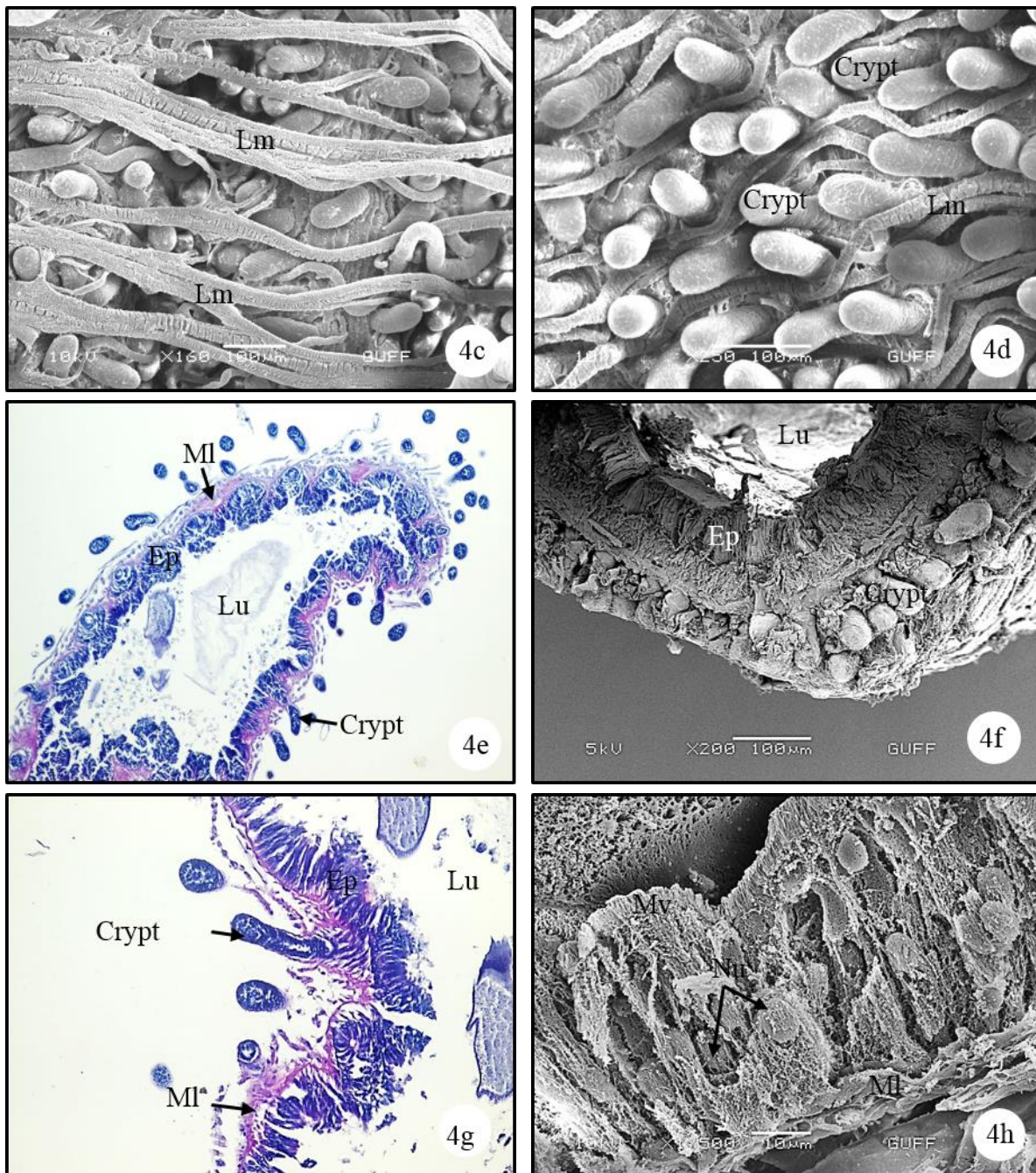


Fig. 4. a, b. SM and SEM photographs of the midgut surface. **c, d.** The longitudinal muscles on crypts surrounding midgut surface (SEM). **e.** The longitudinal section of midgut wall (X100) (LM). **f.** SEM photograph of the crypts surrounding midgut wall. **g.** The muscle layer and epithelium surrounding the midgut wall (X200) (LM). **h.** Microvilli extending from the apical of monolayer epithelial cells with rounded nuclei surrounding the midgut (SEM). Ep-epithelium, In-intima, Lm-longitudinal muscles, Lu-lumen, MI-muscle layer, Nu-nucleus, Tr-trachea, SM-Stereo microscope, LM-Light microscope, SEM-Scanning Electron Microscope.

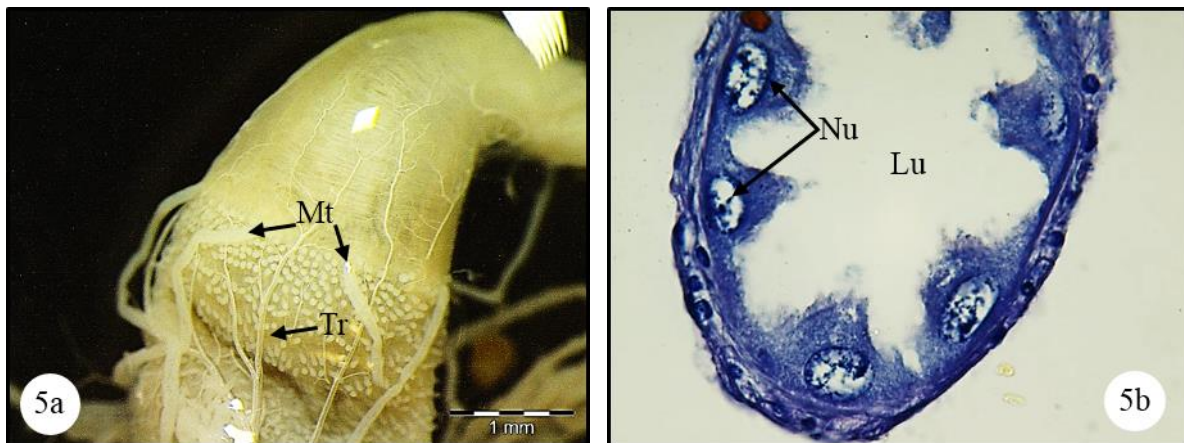


Fig. 5. a. The attachment of Malpighian tubules between midgut and hindgut (SM). **b.** Monolayer cubic epithelium with rounded nuclei surrounding the wall of the Malpighian tubules (X1000) (LM). Lu-lumen, Mt-Malphan tubule, Nu-nucleus, Tr-trachea, SM-Stereo microscope, LM-Light microscope.

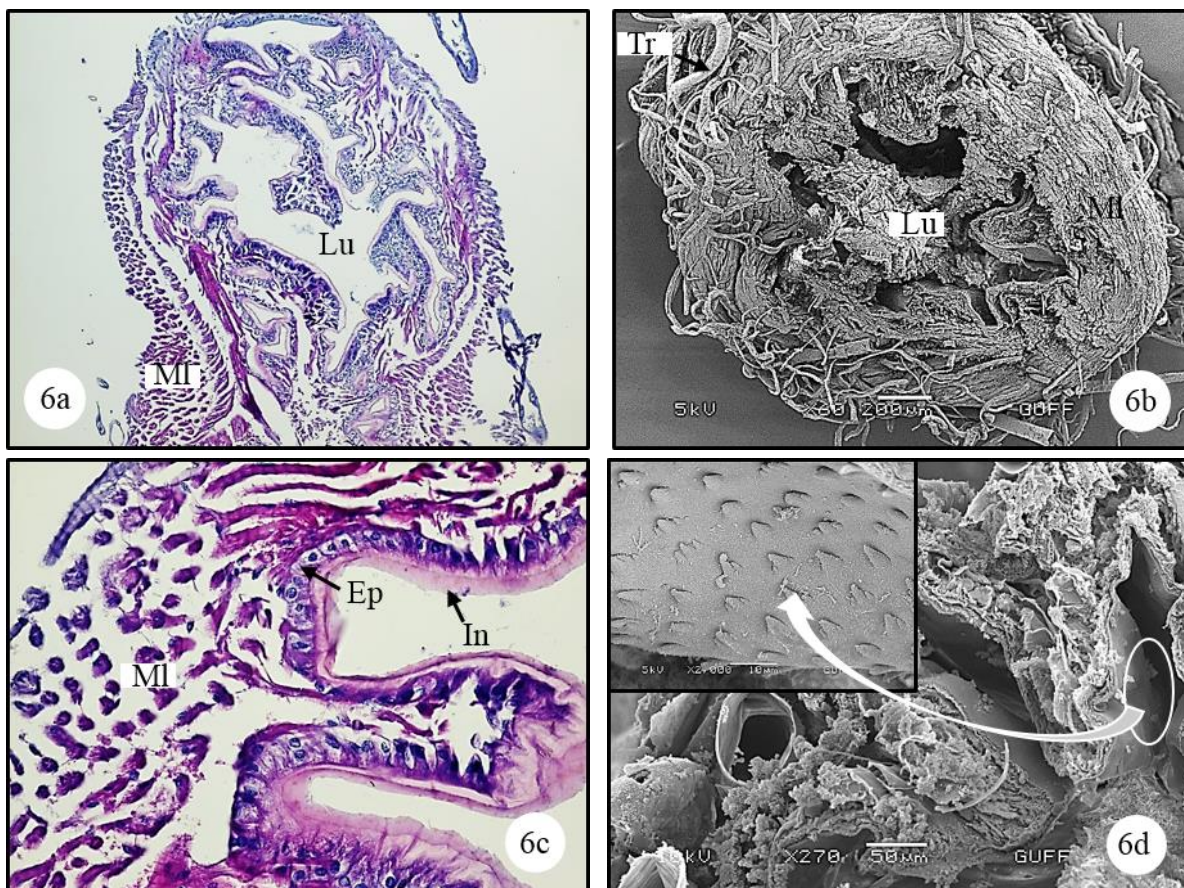


Fig. 6a, b. LM and SEM photographs of a transverse section of the ileum which is the first part of the hindgut. **c.** The muscle layer, epithelium and intima surrounding ileum wall (X400) (LM). **d.** The spines on the inner surface of the ileum intima (SEM). Ep-epithelium, In-intima, Lu-

lumen, MI-muscle layer, Tr-trachea, LM-Light microscope, SEM-Scanning Electron Microscope.

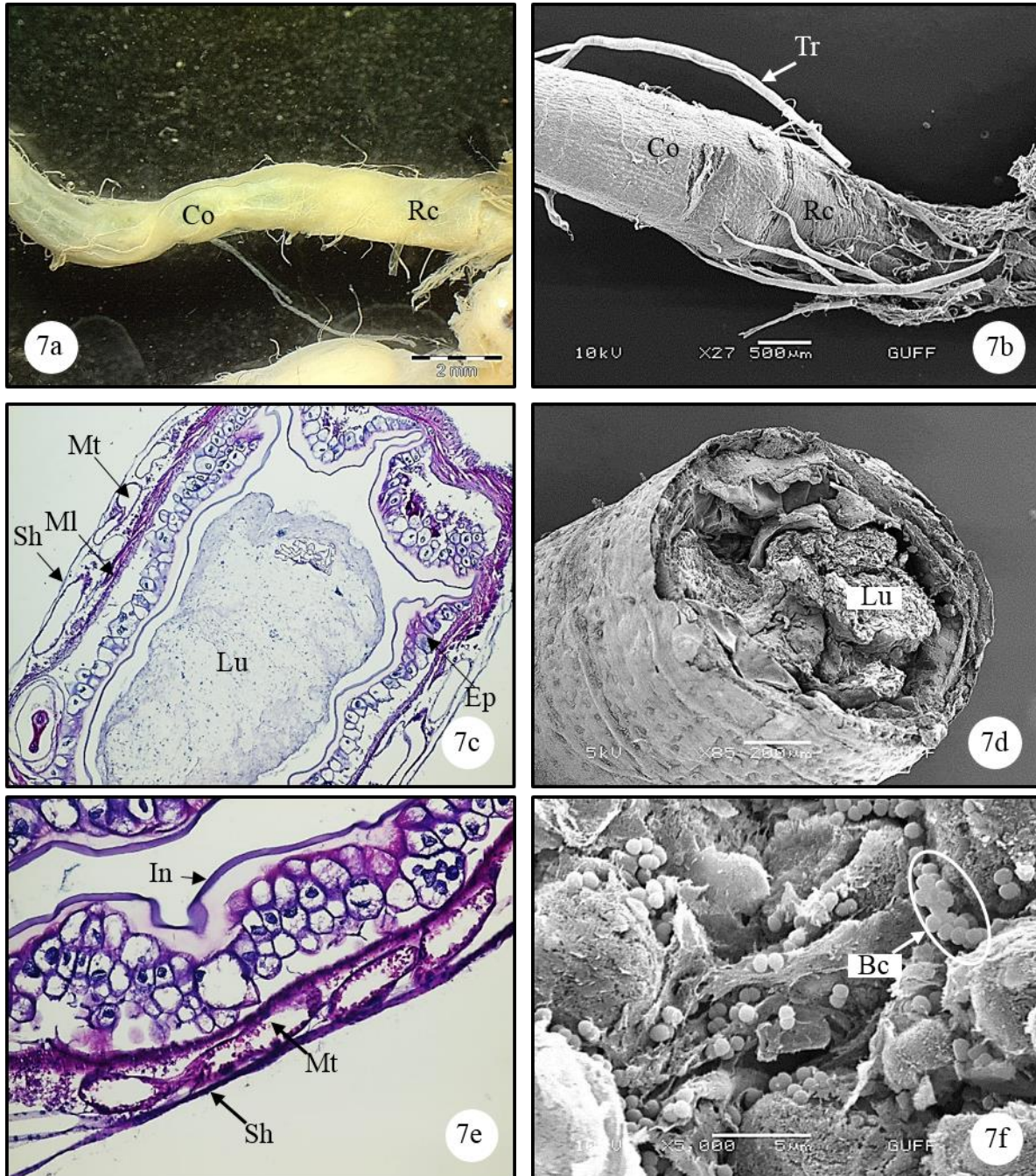


Fig. 7. a, b. SM and SEM photographs colon and rectum general views. **c, d.** LM and SEM photographs of Malpighian tubules surrounding the colon wall (X200). **e.** Malpighian tubules, epithelium, intima surrounding the colon wall (X400) (LM). **f.** SEM photograph of the bacteria in colon lumen. Bc-bacteria, Co-Colon, Ep-epithelium, In-intima, Lu-lumen, MI-muscle layer, Mt-Malphan tubule, Rc-rectum, Sh-sheath, SM-Stereo microscope, LM-Light microscope, SEM-Scanning Electron Microscope.

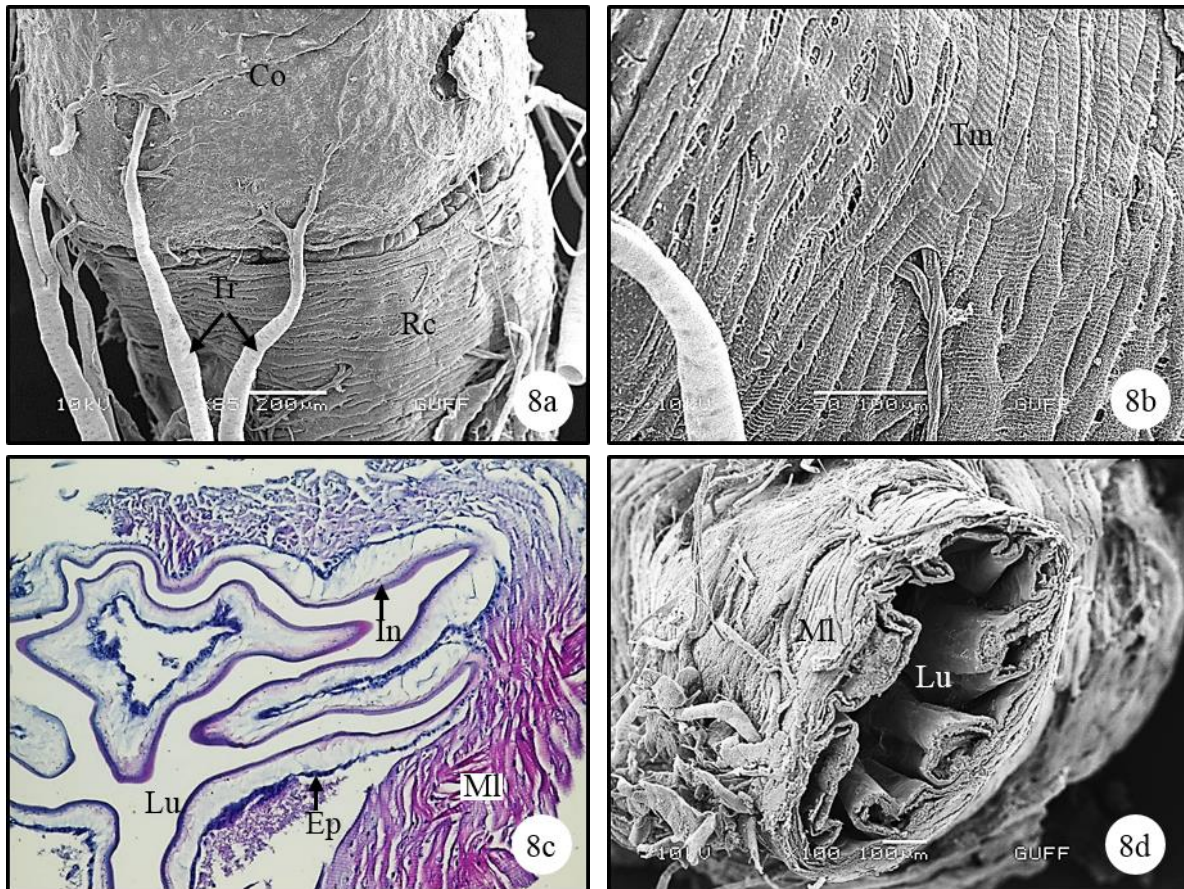


Fig. 8. a, b. SEM photographs of the trachea and transverse muscles surrounding rectum. **c.** The histological sections of muscle layer, epithelium, intima surrounding rectum wall (X400) (LM). **d.** SEM photograph of the rectum wall. Co-Colon, Ep-epithelium, In-intima, Lu-lumen, Ml-muscle layer, Rc-rectum, Tm-transverse muscles, Tr-trachea, LM-Light microscope, SEM-Scanning Electron Microscope.

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**LEAD NITRATE AND CADMIUM CHLORIDE INDUCED RATS THYROID
PATHOLOGY AND EFFECTS OF SESAMOL**
RATLARDA KURŞUN VE KADMIYUMUN NEDEN OLDUĞU TİROİD PATOLOJİSİ
VE SESAMOLUN ETKİSİ

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ABSTRACT

Lead and cadmium are heavy metals that are frequently used in daily life. It poses serious threats to both public health and environmental health. Sesamol is a powerful antioxidant with antibacterial and anti-inflammatory properties. The thyroid gland is a multivascular endocrine gland composed of follicle cells and colloid that is involved in the secretion of metabolism-regulating hormones such as triiodothyronine (T3) and tetraiodothyronine (T4). In this study, the protective role of sesamol against the toxicity of lead nitrate and cadmium chloride in rat thyroid tissue was investigated. 48 male Wistar rats were used in the study. Eight groups were formed as control, sesamol, lead nitrate, cadmium chloride, lead nitrate + cadmium chloride, lead nitrate + sesamol, cadmium chloride + sesamol and lead nitrate + cadmium chloride + sesamol. After 4 weeks of substance administration, the thyroid tissue of the rats was examined histologically. The thyroid tissues of the rats in the control group and sesamol group were histologically and morphologically normal and similar. The thyroid tissues of the rats in the groups treated with lead nitrate, cadmium chloride and lead nitrate + cadmium chloride were histologically different, and severe damage was detected in the follicle cells and colloid structures. A moderate improvement was detected in the groups treated with lead nitrate + sesamol and cadmium chloride + sesamol compared to the groups treated with lead nitrate and cadmium chloride. However, no improvement was observed in the lead nitrate + cadmium chloride + sesamol group.

Keywords: Heavy metal, antioxidant, histopathology, toxicity

ÖZET

Kurşun ve kadmiyum günlük hayatta sıklıkla kullanılan ağır metallerdir. Hem halk sağlığı hem de çevre sağlığı için ciddi tehditler oluşturmaktadır. Sesamol, antibakteriyel ve antiinflamatuvar özelliklere sahip güçlü bir antioksidandır. Tiroid bezi, triiyodotironin (T3) ve tetraiyodotironin (T4) gibi metabolizmayı düzenleyen hormonların salgılanmasında rol oynayan folikül hücreleri ve kolloidden oluşan çok damarlı bir endokrin bezdir. Bu çalışmada, sıçan tiroid dokusunda kurşun nitrat ve kadmiyum klorürün toksisitesine karşı sesamolün koruyucu rolü araştırıldı. Çalışmada 48 adet erkek Wistar sıçan kullanıldı. Kontrol, sesamol, kurşun nitrat, kadmiyum klorür, kurşun nitrat + kadmiyum klorür, kurşun nitrat + sesamol, kadmiyum klorür + sesamol ve kurşun nitrat + kadmiyum klorür + sesamol olmak üzere sekiz grup oluşturulmuştur. 4 haftalık madde uygulamasından sonra sıçanların tiroid dokusu histolojik olarak incelendi. Kontrol grubu ve sesamol grubundaki sıçanların tiroid dokuları histolojik ve morfolojik olarak normal ve benzerdi. Kurşun nitrat, kadmiyum klorür ve kurşun nitrat + kadmiyum klorür uygulanan gruplardaki sıçanların tiroid dokuları histolojik olarak farklıydı ve folikül hücrelerinde ve kolloid yapılarında ciddi hasar tespit edildi. Kurşun nitrat + sesamol ve kadmiyum klorür + sesamol uygulanan gruplarda, kurşun nitrat ve kadmiyum klorür uygulanan gruplara göre ılımlı bir iyileşme tespit edildi. Ancak kurşun nitrat + kadmiyum klorür + sesamol grubunda herhangi bir iyileşme gözlenmedi.

Anahtar Kelimeler: Ağır metal, antioksidan, histopatoloji, toksisite

INTRODUCTION

Because heavy metals are not biodegradable, their presence in the environment is a concern. Humans are in constant interaction with these metals in daily life, so even the lowest doses of exposure cause various harms. Due to many factors, toxic metals enter the nutrient chain. Consumption of these metals leads to disorders in the cardiovascular, reproductive and endocrine systems in humans (Sarwar et al., 2017; Sundseth et al., 2017). It has been stated that cadmium and lead are toxic to humans and cause devastating effects when exposed to sufficient levels through ingestion or inhalation (Yousif and Ahmed, 2009). Cadmium is one of the most important environmental toxicants, and it has been stated that in long-term exposures, it accumulates in tissues such as liver and kidney, testes and lungs, and causes damage to the digestive and respiratory systems (Shwartz and Reiz, 2000). Lead is very common in nature and is frequently used in many areas in industry. In case of exposure, it has been stated that when taken into the body by different means, it accumulates in organs and tissues (Elwood et al., 1984; Lyn Patrick, 2006). It has been stated that when exposed to lead for a long time, it causes hematological, neurological and pathological biochemical changes in the body, and liver and kidney function disorders (Lavicoli et al., 2003). Cadmium and lead are toxic metals that

can cause many serious illnesses when exposed to adequate inhalation or ingestion (Yousif and Ahmed, 2009). It has been observed that heavy metals such as cadmium and lead increase reactive oxygen species, cause oxidative stress, and cause organ and tissue damage (Mendez-Armenta et al., 2011; Apaydin et al., 2021). The thyroid gland is the place where metabolism regulator hormones such as triiodothyronine (T3), tetraiodothyronine (T4) and calcitonin (CT) are secreted (Zoeller et al., 2002). It has been stated that changes in serum concentration of these hormones indicate thyroidal disorders in their synthesis or secretion (Kelly, 2000). Studies have reported that cadmium gradually accumulates in the body and affects thyroid gland functions, which play an important role in energy use, oxygen consumption by cells, growth process and whole body metabolism (Badiei et al., 2009). Likewise, exposure to lead has been shown to adversely affect the endocrine glands (Zacharewski, 1998). It is known that heavy metals cause toxicity in the body and reduce the activity of antioxidant enzymes (Ognjanovic et al., 2010). It has been stated that antioxidants are used to prevent or reduce the chain damage that may occur with free radicals against these destructive effects of heavy metals (Lakshmi et al., 2013). It has been stated that antioxidant activities in natural products are frequently used in the adjuvant treatment of many diseases, preventing oxidation reactions caused by free radicals and delaying aging (Joshi et al., 2005; Zhou et al., 2021). One of these natural products is sesamol, which has a very high antioxidant activity in sesame seeds (Joshi et al., 2005). Sesamol has been shown to have anti-inflammatory, antibacterial and antifungal properties as well as antioxidant properties (Srisayam et al., 2014). Studies have shown that sesamol is a metabolism regulator and prevents the formation of lipid peroxidation (Kanu et al., 2010). In another study, it was observed that sesamol scavenges hydroxyl radicals and helps heal liver-related injuries (Chang et al., 2010; Joshi et al., 2005).

Considering this information, in this study, we aimed to investigate the protective role of sesamol against histopathological damage caused by lead nitrate and cadmium chloride on thyroid tissue in rats.

MATERIAL AND METHOD

In order to carry out this study, firstly, the approval of Gazi University Animal Experiments Local Ethics Committee was obtained. In the study, 48 Wistar males, weighing 200-250 g, obtained from Gazi University Laboratory Animal Breeding and Experimental Research Center, were used. 8 groups were formed with 6 rats in each cage. They were fed with a special laboratory diet and free water and were also subjected to a photoperiod of 12 hours light and 12 hours dark. Rats were kept at room temperature of 18-22 °C.

Group 1: Control group received normal food and free water

Group 2: Sesamol treated group (50 mg/kg bw per day)

Group 3: Lead nitrate treated group (90 mg/kg bw per day, 1/25 LD₅₀)

Group 4: Cadmium chloride treated group (3 mg/kg bw per day; 1/25 LD₅₀)

Group 5: Lead nitrate+Cadmium chloride treated group (90 mg/kg bw per day+3 mg/kg bw per day)

Group 6: Lead nitrate+sesamol treated group (90 mg/kg bw per day+50 mg/kg bw per day)

Group 7: Cadmium chloride+sesamol treated group (3 mg/kg bw per day+50 mg/kg bw per day)

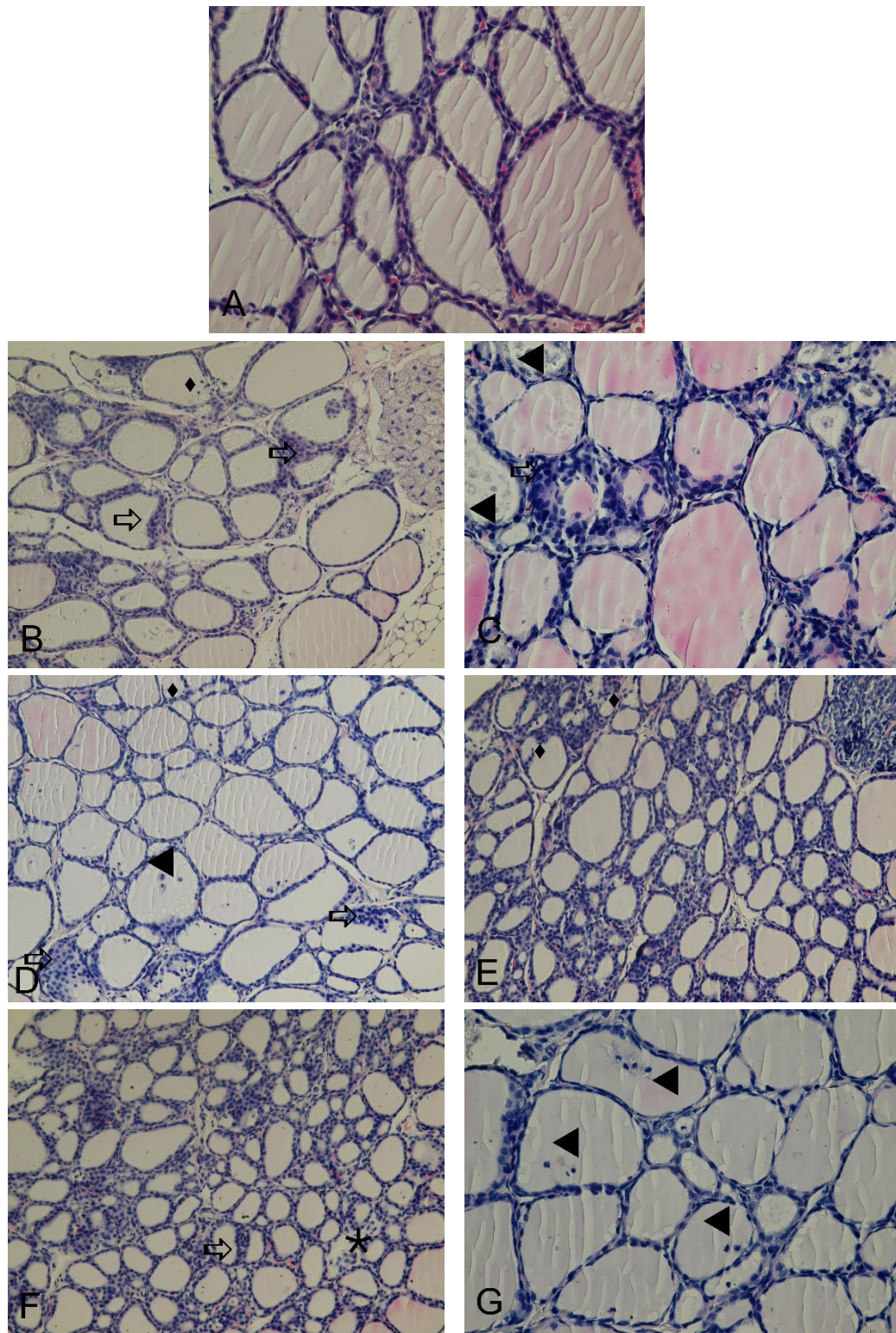
Group 8: Lead nitrate+Cadmium chloride+sesamol treated group (90 mg/kg bw per day+3 mg/kg bw per day+50 mg/kg bw per day)

Doses to be administered to animals are Plastunov and Zub (2008) for lead nitrate (90 mg, LD₅₀ 1/25), El-Demerdash et al. (2004) for cadmium chloride (3 mg, LD₅₀ 1/25), and for sesamol (50 mg) was selected based on Hemalatha et al. (2013).

In our experiment, which we created as 8 groups, the solutions were administered to the rats by gavage, and at the end of 28 days of application, they were stunned and sacrificed with a combination of ketamine and xylazine. The thyroid tissue was removed and the fixative solution was placed in formaldehyde, after which the tissues were washed and passed through alcohol series and embedded in paraffin blocks. Sections of 6-7 µm thickness were taken from the blocks, and stained with hematoxylin and eosin (H&E) after xylene and various alcohol series. Thyroid tissues were examined with a light microscope (Olympus BX51, Tokyo, Japan) and thyroid tissues were photographed by a camera (Olympus E-330, Olympus Optical, Japan) placed in the microscope.

RESULTS

No histopathological conditions were observed in the thyroid gland sections of the control group rats in light microscopy examinations, and the follicle cells were small and variable in size, covered with simple cuboidal epithelium, and lined up along a line. It was observed that the follicle cells were filled with acidophilic colloid and had a normal structure. The thyroid tissue sections of the sesamol group rats were similar to the control group (Figure A). Follicle degeneration and follicles desquamated were observed in the thyroid gland in the lead nitrate group (Figure B). In the group in which cadmium chloride was applied, desquamated was detected in the follicle cells (Figure C). Follicle degeneration, follicles desquamated and follicular cell hyperplasia were observed in the lead nitrate and cadmium chloride group (Figure D). Follicular degeneration was observed in the lead nitrate and sesamol group (Figure E), follicular cell hyperplasia and intercellular degenerations were observed in the cadmium chloride and sesamol group (Figure F). In the group in which lead nitrate, cadmium chloride and sesamol were administered together, follicles desquamated was detected (Figure G).



Figures. **A:** Photomicrographs of thyroids of the control group. **B:** Thyroid sections of lead nitrate treated rats, ◆: degenerations of follicles, ⇨: follicular cell hyperplasia. **C:** Thyroid sections of cadmium chloride treated rats, ▲: showing desquamated follicles, ⇨: follicular cell hyperplasia. **D:** Thyroid sections of lead nitrate and cadmium chloride treated rats, ◆: degenerations of follicles, ▲: showing desquamated follicles, ⇨: follicular cell

hyperplasia. **E:** Thyroid sections of lead nitrate and sesamol treated rats, **◆:** degenerations of follicles **F:** Thyroid sections of cadmium chloride and sesamol treated rats, **⇔:** follicular cell hyperplasia, ***:** intercellular degenerations. **G:** Thyroid sections of lead nitrate and cadmium chloride and sesamol treated rats, **▲:** showing desquamated follicles.

DISCUSSION

Increasing population growth in recent years has led to intense water, soil and air pollution, and this pollution has peaked with the development of the industry. Heavy metals have an important place in environmental pollution, industry, medicine and similar activities are the factors that increase heavy metal pollution (Türk and Osma, 2020). It has been stated that lead is both toxic for public health and a very strong pollutant for the environment. It has been stated that changes in function or damage in many tissues and organs are observed in lead exposure (Adıgüzel and Kalender, 2015). Cadmium, like lead, is a toxic metal, both occupational and environmental, and it has been shown that histopathological conditions occur in many tissues and organs when exposed to this metal (Çilenk et al., 2016; Ji et al., 2011; Kim and Soh, 2009). The occurrence or changes of histopathological conditions in tissues and organs are important for toxicology studies (Apaydın et al., 2019). In previous studies, it has been determined that it causes pathological conditions in the small intestine of rats, changes in physiological and biochemical parameters, degenerations in liver tissue, neuronal disorders and oxidative stress (Adıgüzel and Kalender, 2015; Baş and Kalender, 2016; Pagliara et al., 2003). In studies with cadmium, it has been stated that it causes free radical formation in case of exposure and causes lipid peroxidation in cell membrane structures (Al-Derawi, 2018). In another study, it was stated that cadmium administered to rats caused toxicity in the liver tissue and led to hepatocyte degeneration (Kim et al., 1998). In our study, we found that the damage to the thyroid tissue caused by the doses of lead and cadmium we applied to the rats was similar to the previous studies. It has been shown that antioxidants can have preventive or reducing effects against the formation of reactive oxygen species caused by heavy metals and the toxicity caused by these structures (Uzun and Kalender, 2013). Sesamol is a compound resistant to oxidative deterioration, effective in preventing the formation of lipid peroxidation with its chain breaking feature, and has strong antioxidant properties (Kuhad and Chopra, 2008). Studies have shown that sesamol is a hydroxyl radical inhibitor and increases antioxidant activities (Baş et al., 2021; Nayak et al., 2013). After subacute administration of lead nitrate and cadmium chloride, pathological changes were observed in the liver and kidneys of rats, while a significant decrease in antioxidant enzyme activity was detected. A curative effect was observed in the sesamol administered groups (Baş et al., 2021). As a result of the data we obtained in our study, it was determined that lead nitrate and cadmium chloride caused histopathological changes by causing severe damage to the thyroid tissue of rats. It has been observed that the application of sesamol has a positive effect, but does not provide complete protection.

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HEPATOSELLÜLER KARSİNOMA KARAHİNDİBA ETKİSİ
EFFECT OF DANDELION ON HEPATOCELLULAR CARCINOMA

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ÖZET

Hepatosellüler karsinom (karaciğer kanseri, HCC), dünya çapında en yaygın görülen beşinci kanser türüdür, kansere bağlı ölüm sıralamasında ise ikinci sırada yer almaktadır. HCC, genellikle sirozun ileri aşamalarında görülsede tamamen siroza bağlı gelişen bir hastalık değildir. Yapılan araştırmalarda büyük ölçüde metastaz ve cerrahi müdahale sonucunda ortaya çıktığı görülmektedir ve hastaların 5 yıllık sağ kalım oranı zayıftır. Hastalığın tedavisinde cerrahi rezeksiyon, kemoterapi, karaciğer nakli gibi yöntemler uygulanmaktadır. Damar yapılarında incelmeye de görülebildiğinden dolayı kemoterapi ve karaciğer nakli her zaman mümkün olmayabilir. Bu nedenle tedaviye yardımcı olabilecek yeni yaklaşımlara ihtiyaç duyulmaktadır.

Taraxacum officinale (Karahindiba, Dandelion) Kuzey Yarımküre'ye özgü bir Asteraceae familyası bitkisidir. İdrar söktürücü, antioksidan, anti kanserojen, analjezik, antihiperglisemik ve antikoagülan gibi özellikleri ile uzun zamandır tıbbi bir bitki olarak kullanılan önemli bir bitkidir. Son yıllarda bu bitkinin yaprak ve kökleri üzerinde yapılan incelemelerde, bu bitkinin yaprak ve köklerinin sindirim rahatsızlıklarının yanı sıra gastrointestinal problemler, akciğer kanseri, meme kanseri ve bağırsak kanseri tedavilerinde de etkisi olduğu gözlemlenmiştir.

Bu derlemede *Taraxacum officinale*'nin sadece ülkemizde değil tüm dünyada yaygın görülen bir hastalık olan HCC üzerindeki etkisinin anlatılması amaçlanmıştır.

Anahtar kelimeler: Hepatosellüler karsinom, Karaciğer kanseri, Karahindiba.

ABSTRACT

Hepatocellular carcinoma (HCC) is the world's fifth most prevalent cancer form and the second leading cause of cancer death. HCC is most commonly seen in the late stages of cirrhosis, however, it is not a condition that arises solely as a result of cirrhosis. In research is observed that it mostly emerges as a result of metastasis and surgical intervention, and the patients' five-year survival rate is low. Surgical resection, chemotherapy, liver transplant, and similar methods are applied in the treatment of the disease. Due to thinning of the vascular structures, chemotherapy and liver transplant is not always possible. Therefore, new approaches that may assist the treatment are required.

Taraxacum officinale (Dandelion, Dandelion) is an Asteraceae family plant native to the Northern Hemisphere. It is a significant plant that has long been used as a medical herb, with qualities such as diuretic, anti-oxidant, anti-carcinogenic, analgesic, anti-hyperglycemic, and anti-coagulant. In recent years, in examinations on the leaves and roots of this plant, it has been observed that the leaves and roots of this plant have an effect on gastrointestinal problems, lung cancer, breast cancer, and intestinal cancer treatments, in addition to digestive ailments.

This review aims to describe the effect of *Taraxacum officinale* on HCC, a disease that is prevalent not only in our nation but also around the world.

Keywords: Hepatocellular carcinoma, cancer, Dandelion.

***COTINUS COGGYGRIA* SCOP. MEYVELERİNİN TOTAL FENOL BİLEŞİK
MİKTARI VE ANTİOKSİDAN AKTİVİTESİ AÇISINDAN DEĞERLENDİRİLMESİ
EVALUATION OF *COTINUS COGGYGRIA* SCOP. FRUITS IN TERMS OF TOTAL
PHENOL COMPOUND AMOUNT AND ANTIOXIDANT ACTIVITY**

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ÖZET

Cotinus Mill. cinsi, Anacardiaceae familyasına ait olup 8 tür içermektedir. *Cotinus coggygria* Scop. (syn.: *Rhus cotinus* L.) türü ülkemizde doğal olarak yetişmesinin yanısıra güzel görünümü nedeniyle kültürü de yapılmaktadır. *C. coggygria* kışın yaprak döken, 5-7 m.'ye kadar büyüeyebilen, çalı formunda bir bitkidir. Özellikle yaprak ve dallarının, boğaz ağrısı, diş ağrısı, kabızlık ve deri lezyonları gibi durumlarda geleneksel kullanımı mevcuttur. Bu nedenle bu kısımlarla ilgili çalışmalar mevcuttur. Ancak meyveleri üzerinde çok fazla çalışmaya rastlanmamıştır. Bu çalışmada Çankaya/Ankara (Türkiye)'den *C. coggygria* meyveleri toplanmış ve gölgede kurutulmuştur. Toz haline getirilen meyveler ayrı ayrı metanol ve aseton çözücülerini ile maserasyon yöntemiyle ekstre edilmiştir. Böylece iki farklı polaritede ekstratlar elde edilmiştir. Bu ekstratlar üzerinden Folin-Ciocalteu yöntemi ile total fenol miktar tayini yapılmıştır; DPPH ve ABTS yöntemleriyle antioksidan aktiviteleri değerlendirilmiştir. Elde edilen sonuçlara göre en yüksek fenolik içerik metanol (163.56±2.99 mg GAE/g ekstre) ekstresinde tespit edildi. Ayrıca, DPPH ve ABTS testlerinde en güçlü radikal süpürücü kapasiteyi metanol ekstresi (IC₅₀: 0.020±3.87 mg/ml ve 0.027±6.36 mg/ml, sırasıyla) göstermiştir.

Anahtar Kelimeler: *Cotinus coggygria*, DPPH, ABTS, Toplam fenolik içerik

ABSTRACT

Cotinus Mill. genus belongs to the Anacardiaceae family and includes 8 species. *Cotinus coggygria* Scop. (syn.: *Rhus cotinus* L.) species is grown naturally in our country as well as cultivated due to its beautiful appearance. *C. coggygria* is a deciduous plant in winter and in

the form of a shrub that can grow up to 5-7 m. Especially the leaves and branches have traditional uses some illness such as sore throat, toothache, constipation, and skin lesions. Therefore, studies are usually related to leaves and branches. However, not many studies have been found on its fruits. In this study, *C. coggygia* fruits were collected from Çankaya/Ankara (Turkey) and dried in the shade. The powdered fruits were extracted separately with methanol and acetone solvents by maceration method. Thus, two different polarity extracts were obtained. Total phenol amount was determined on these extracts by Folin-Ciocalteu method; antioxidant activities were evaluated by DPPH and ABTS methods. According to the results obtained, the highest phenolic content was detected in the methanol (163.56±2.99 mg GAE/g extract) extract. In addition, methanol extract (IC₅₀: 0.020±3.87 mg/ml and 0.027±6.36 mg/ml, respectively) showed the strongest radical scavenging capacity in DPPH and ABTS tests.

Keywords: *Cotinus coggygia*, DPPH, ABTS, Total phenolic content

GİRİŞ

Anacardiaceae familyasından *Cotinus* Mill. cinsi, 8 tür ile temsil edilmektedir (POWO, 2022). Bu türlerden sadece *Cotinus coggygia* Scop. (syn.: *Rhus cotinus* L.) ülkemizde doğal olarak yetişmektedir. Çalı formundaki bu tür, 5-7 m.'ye kadar büyüeyebilen, kışın yaprak döken bir bitkidir. Bitkinin yaprakları oval veya obovat şekilli, pinnat damarlanmalı, alternat dizilişli olup, petalleri beyazımsı yeşil renklidir. Meyveleri 4-5 x 3-4 mm. boyutunda, buruşuk şekilli bir drupadır (Davis, 1967; Matić vd., 2016). Doğal olarak yetişmesinin yanında güzel görünümünden dolayı kültürü de yapılmaktadır. Ülkemizde halk arasında tetra, boyacı sumacı, sarıboya ağacı, sarıcan, sarı yaprak, pamuklu sumak veya duman ağacı olarak adlandırılmaktadır (Demirci vd., 2003).

C. coggygia yaprak ve dallarının geleneksel kullanımı mevcuttur. Ülkemizde yaprakları dekoksasyonu antiseptik, kabızlık, kalp hastalıkları, hipertansiyon, ateşdüşürücü ve kabızlık giderici olarak kullanılmaktadır (Baytop, 1999; Kültür, 2007; Bahadırılı, 2020). Diğer ülkelerde de *C. coggygia* yaprak ve ağaç kabuklarının dekoksasyon ve infüzyonları antiseptik, yara iyi edici, antienflamatuvar ve antimikrobiyal özellikleri nedeniyle boğaz ağrısı, diş ağrısı, diş eti iltihabı, hemoroid, gastrite, deri ve mukoza lezyonlarında karşı kullanılmaktadır. Sırbistan'da kanser tedavisi için kabukları kaynatılarak kullanıldığı belirtilmektedir (Antal vd., 2010; Matić vd., 2016). Bulgaristan'da parfümeride kullanılması için yapraklarından ve genç dallarından uçucu yağ elde edilmektedir (Bahadırılı, 2020).

Geleneksel kullanıma bağlı olarak yapılan çalışmalarla antimikrobiyal, antioksidan, antienflamatuvar, hepatoprotektif, antiülserojenik, immünoestimulan, yara iyi edici ve antikanser etkileri desteklenmiştir (Deniz vd., 2020; Gospodinova vd., 2021).

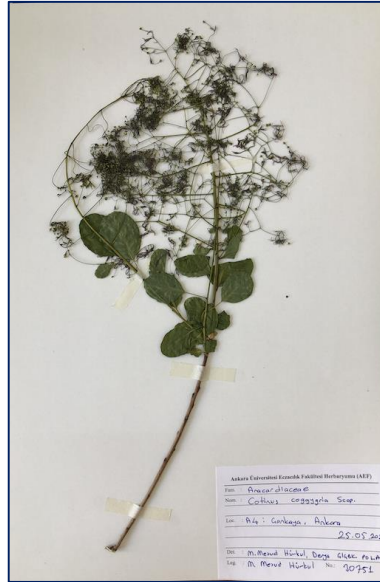
Bitkiler içerdikleri sekonder metabolitlerden kaynaklı olarak önemli biyolojik aktivitelere sahiptirler. Bugüne kadar *C. coggygia*'nın farklı kısımlarından flavonoidler (fisetin, fustin, kersetin, apigenin, mirisetin, taksifolin), auronlar (sülfüretin, disülfüretin, sülfüreïn), kalkonlar (butein, isoliquiritigenin), antosiyaninler (delphinidin-3-galaktoz, siyanidin-3-galaktoz, petunidin-3-glukozit), kateşinler ve diğler fenolikler bileşiler (gallik asit ve metil gallat) izole edilmiştir. Elde edilen uçucu yağlarda da limonen, β -osimen, β -karyofilen, α -pinen, karyofilen oksit ve α -mirsen majör bileşikler olarak tespit edilmiştir (Demirci vd., 2003; Deniz vd., 2020; Gospodnova vd., 2021; Shaboyan vd., 2021).

Bu çalışmada ülkemizde hem doğal olarak yetişen hem de kültürü yapılan *C. coggygia* türünün meyvelerinin farklı polaritelerdeki ekstralarının hem total fenol miktarı hem de antioksidan aktiviteleri açısından değerlendirilmesi yapılmıştır.

YÖNTEM

Bitki Materyali

Meyve örnekleri Ankara/Çankaya bölgesinden toplanmıştır. Meyvenin toplandığı bitkiden alınan herbaryum örneği Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu'nda (AEF30751) saklanmaktadır (Şekil 1).



Şekil 1. *C. coggygia* herbaryum örneği

Ekstrelerin Hazırlanması

Gölgede kurutulan meyveler öğütücü yardımıyla toz edildi. Toz edilen örnekler metanol ve aseton ile ayrı ayrı hareketli maserasyona tabi tutuldu (8 saat X 3 gün). Çözücüler rotavapor

yardımla düşük basınç altında uçuruldu. Elde edilen ekstrelerin verimleri % olarak kuru bitki üzerinden hesaplandı.

Toplam Fenolik Madde Miktarının Belirlenmesi

Ekstrelerin toplam fenolik madde miktarının belirlenmesi için Folin-Ciocalteu reaktifi kullanılarak, spektrofotometrik olarak yapılan, renk oluşumuna dayanan yöntem kullanılmıştır (Slinkard ve Singleton, 1977). Buna göre; 20 µl numune, 1580 µl distile su ile dilüe edilip, üzerine 10 µl Folin-Ciocalteu reaktifi eklenmiştir. 30 µl %20'lik Na₂CO₃ çözeltisi bu karışımın üzerine eklenip, karışım 2 saat süresince karanlık ortamda, oda sıcaklığında bekletilmiştir. Ölçümler 765 nm de spektrofotometrik olarak yapılmıştır. Sonuçlar ortalama mg gallik asit eşdeğeri (GAE)/g kuru ekstre olarak ifade edilmiştir.

DPPH Radikal Süpürücü Kapasite Tayini

DPPH testinde, ekstrelerin DPPH (2,2-difenil-1-pikrilhidrazil) radikalinin menekşe-mor rengini giderme yeteneği bitki ekstresinin antioksidan kapasitesini belirtmektedir. Bu test yöntemi ekstrelerin DPPH ile oluşturdukları rengin 517 nm'de spektrofotometrik olarak ölçülmesi ve standart madde ile kıyaslanması temeline dayanmaktadır (Blois, 1958). DPPH'in 100 µM etanol çözeltisi ve farklı derişimlerde bitki ekstrelerinden oluşan karışım oda sıcaklığında ve karanlıkta 30 dakika boyunca bekletilip, 517 nm'de absorbans değerleri ölçülmüştür. Deneyle 3 tekrarlı yapılmıştır. Pozitif kontrol olarak Gallik asit (GA) kullanılmıştır.

ABTS Radikal Süpürücü Kapasite Tayini

Ekstreler tarafından, radikal rengini giderme kapasitesine dayanan ABTS (2,2'-azino-bis-(3-etilbenzotiazolin-6-sülfonik asit)) radikal süpürücü kapasite testinde, 7 mM ABTS sulu çözeltisi, 2.45 mM potasyum persülfat ile reaksiyona sokularak ABTS radikal katyonunun oluşması sağlanır. Test edilecek numune 12-16 saat boyunca karanlıkta ve oda sıcaklığında bekletilen ve sonrasında etanol ile dilüe edilen bu karışım ile (100x) seyreltilir. 6 dakika sonra bu karışımın absorbans değeri spektrofotometrik olarak 734 nm'de ölçülüp, inhibisyon yüzdesi hesaplanır (Re vd., 1999). Deneyle 3 tekrarlı olarak yürütülmüştür. Pozitif kontrol olarak troloks kullanılmıştır.

BULGULAR

Elde edilen sonuçlara göre, *C. coggyria*'nın kuru meyvelerinden elde edilen ekstre verimleri Tablo 1'de gösterilmiştir.

Tablo 1. *C. coggyria* ekstrelerine ait % verimler

Ekstre tipi	% verim
Metanol	7.17
Aseton	7.47

Elde edilen sonuçlara göre en yüksek fenolik içerik metanol (163.56 ± 2.99 mg GAE/g ekstre) ekstresinde tespit edildi (Tablo 2). Ayrıca, DPPH ve ABTS testlerinde en güçlü radikal süpürücü kapasiteyi metanol ekstresi (IC_{50} : 0.020 ± 3.87 mg/ml ve 0.027 ± 6.36 mg/ml, sırasıyla) göstermiştir (Tablo3-4).

Tablo 2. *C. coggygia* ekstrelerine ait toplam fenolik madde içeriği

Ekstre tipi	Toplam fenolik madde içeriği (mg GAE/g kuru ekstre) \pm SS
Metanol	163.56 ± 2.99
Aseton	71.89 ± 3.69

Tablo 3. *C. coggygia* ekstrelerine ait DPPH radikal süpürücü kapasite sonuçları

Ekstre tipi	IC_{50} (mg/ml) \pm SS
Metanol	0.020 ± 3.87
Aseton	0.029 ± 4.67
Gallik asit	0.015 ± 4.33

Tablo 4. *C. coggygia* ekstrelerine ait kantitatif ABTS radikal süpürücü kapasite sonuçları

Ekstre tipi	IC_{50} (mg/ml) \pm SS
Metanol	0.027 ± 6.36
Aseton	0.036 ± 8.12
Trolox	0.021 ± 6.70

TARTIŞMA VE SONUÇ

Literatür verileri bu çalışmanın *C. coggygia* meyveleri üzerinde yapılan ilk toplam fenolik madde içeriği ve radikal süpürücü kapasite çalışması olduğunu göstermiştir. Çalışma verileri toplam fenolik madde içeriği bakımından en zengin ekstrenin, metanol ekstresi (163.56 ± 2.99 mg GAE/g ekstre) olduğunu göstermiştir. Bununla uyumlu olarak yine metanol ekstresinin (IC_{50} : 0.020 ± 3.87 mg/ml) DPPH radikal süpürücü kapasitesi, gallik asite (IC_{50} : 0.015 ± 4.33 mg/ml) oranla yüksek çıkmıştır. ABTS testinde Trolox'a (IC_{50} : 0.021 ± 6.70 mg/ml) kıyasla metanol ekstresi (IC_{50} : 0.027 ± 6.36 mg/ml) yüksek radikal süpürücü kapasite göstermiştir.

Yapılan diğer çalışmalara bakıldığında *C. coggygia* yapraklı dallarının metanol ekstrelerinde total fenolik bileşikler açısından daha zengin olduğu görülmüştür. Buna bağlı olarakta antioksidan aktivite açısından meyvelerinden daha aktif olduğu saptanmıştır (Nićiforović vd., 2010; Sukhikh vd., 2021).

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**VITIS LABRUSCA L. MEYVE VE YAPRAKLARININ TOPLAM FENOLİK İÇERİĞİ
VE ANTIOKSİDAN AKTİVİTESİ**

**TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF VITIS LABRUSCA
L. FRUITS AND LEAVES**

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ÖZET

Vitis labrusca L. (Vitaceae) Kanada'nın güneydoğusu ve ABD'nin doğu bölgelerinde doğal yayılış gösterir. Bitki Türkiye'nin Karadeniz bölgesinde yaygın olarak yetiştirilmektedir.

Bu çalışmada, *V. labrusca*'nın meyve ve yapraklarının toplam fenolik içerikleri ve antioksidan kapasitesi incelenmiştir. Toplanan bitki materyalleri gölgede kurutuldu. Parçalanmış bitki materyalleri metanol, etanol, diklorometan, etil asetat ve aseton ile maserasyon metodu uygulanarak ekstre edildi. Ekstrelerin toplam polifenol içeriği, standart olarak kullanılan fenol bileşiği olan gallik asidin kalibrasyon eğrisine atıfta bulunarak Folin-Ciocalteu yöntemi ile belirlendi. Sonuçlar, ortalama mg gallik asit eşdeğeri (GAE)/g kuru ekstre olarak ifade edildi. Ekstrelerin serbest radikal temizleme aktiviteleri DPPH ve ABTS yöntemleri kullanılarak test edildi. Sonuçlar inhibisyon yüzdesi olarak ifade edildi ve numunelerin yarı maksimum inhibitör konsantrasyonları (IC₅₀) lineer regresyon analizi ile hesaplandı.

Elde edilen sonuçlara göre en yüksek fenolik içerik meyve metanol (181.19±3.23 mg GAE/g ekstre) ve etil asetat (270.31±8.01 mg GAE/g ekstre) ekstralarında tespit edildi. Ayrıca hem kalitatif hem de kantitatif DPPH testinde en güçlü radikal süpürücü kapasiteyi meyve etil asetat ekstresi (IC₅₀: 0.019±2.12 mg/ml), ABTS testinde ise meyve metanol (IC₅₀: 0.027±3.70 mg/ml) ve etil asetat (IC₅₀: 0.024±4.14 mg/ml) ekstraları gösterdi.

Anahtar Kelimeler: *Vitis labrusca*, Toplam fenolik içerik, DPPH, ABTS

ABSTRACT

The native field of *Vitis labrusca* L. (Vitaceae) is southeastern Canada and eastern USA. The plant is widely cultivated in the Black Sea region of Turkey.

In this study, the total phenolic contents and antioxidant capacity of the fruits and leaves of *V. labrusca* were investigated. The collected plant materials were dried in the shade. The plant materials were extracted by using the maceration method with methanol, ethanol, dichloromethane, ethyl acetate and acetone. The total polyphenol content of extracts was determined by Folin-Ciocalteu method, referring to the calibration curve of gallic acid, phenol compound used as a standard. The results were expressed as mean mg gallic acid equivalent (GAE)/g dry extract. The free radical scavenging activities of the extracts were tested using DPPH and ABTS methods. The Results were expressed as inhibition percentage and the half-maximal inhibitory concentrations (IC₅₀) of the samples were calculated by linear regression analysis.

According to the results, the highest phenolic content was determined in fruit methanol (181.19±3.23 mg GAE/g extract) and ethyl acetate (270.31±8.01 mg GAE/g extract) extracts. In addition, fruit ethyl acetate extract (IC₅₀: 0.019±2.12 mg/ml) showed the strongest radical scavenging capacity in both qualitative and quantitative DPPH test, and fruit methanol (IC₅₀: 0.027±3.70 mg/ml) and ethyl acetate (IC₅₀: 0.024±4.14 mg/ml) extracts in ABTS test.

Keywords: *Vitis labrusca*, Total phenolic content, DPPH, ABTS

GİRİŞ

Vitaceae familyası içerdiği flavonoidler, antosiyaninler, flavonoller ve fenolik asitlerden kaynaklanan düşük yoğunluklu lipoprotein, ateroskleroz ve koroner kalp rahatsızlıklarına karşı koruma ile ilgili güçlü antioksidan özelliğe sahiptir (Evans, 2002). Familya 17 kabul edilmiş cins içerir ve bu cinsler içinden *Vitis* L. tıbbi ve ekonomik değeri yüksek olan en önemli cinstir (Evans, 2002; POWO, 2022). *Vitis* cinsi ılıman kuzey yarım küreden tropik dağlara kadar doğal bir yayılış alanına sahiptir (POWO, 2022).

Dünya genelinde kültürü yapılan *Vitis* cinsine ait türlerden biri olan *Vitis labrusca* L. “izabela” (Şekil 1) Türkiye'nin Karadeniz bölgesinde yaygın olarak kültüre alınmaktadır (Köse, 2014). *V. labrusca* gallik, vanilik, sirinjik, protokateşik, elajik, kafeik, *p*-kumarik, ferulik, klorojenik asitler, (+)-kateşin, (-)-epikateşin, mirsetin, kemferol, kersetin, rutin, narinjin, trans-resveratrol, tirozol gibi bitkisel kökenli antioksidan maddeleri içerdiği bilinmektedir (Burin vd., 2014; Dani vd., 2010; Lago-Vanzela vd., 2011; Ribeiro vd., 2015; Rockenbach vd., 2011; Santos vd., 2011).

YÖNTEM

Bitki Materyali

Bitki materyali Ankara/Çankaya bölgesinden toplanmıştır. Bir herbaryum örneği Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu'na (AEF 30929) kaydedilmiştir (Şekil 1).



Şekil 1. *V. labrusca*

Ekstrelerin Hazırlanması

Gölgede kurutulan bitki materyali bir öğütücü yardımıyla toz edildi. Toz edilen örnekler metanol, etanol, diklorometan, etil asetat ve aseton ile ayrı ayrı hareketli maserasyona tabi tutuldu (8 saat X 3 gün). Çözücüler rotavapor yardımıyla düşük basınç altında uçuruldu. Elde edilen ekstrelerin verimleri % olarak kuru bitki üzerinden hesaplandı.

Toplam Fenolik Madde Miktarının Belirlenmesi

Ekstrelerin toplam fenolik madde miktarının belirlenmesi için Folin-Ciocalteu reaktifi kullanılarak, spektrofotometrik olarak yapılan, renk oluşumuna dayanan yöntem kullanılmıştır (Slinkard ve Singleton, 1977). Buna göre; 20 µl numune, 1580 µl distile su ile dilüe edilip, üzerine 10 µl Folin-Ciocalteu reaktifi eklenmiştir. 30 µl %20'lik Na₂CO₃ çözeltisi bu karışımın üzerine eklenip, karışım 2 saat süresince karanlık ortamda, oda sıcaklığında bekletilmiştir. Ölçümler 765 nm de spektrofotometrik olarak yapılmıştır. Sonuçlar ortalama mg gallik asit eşdeğeri (GAE)/g kuru ekstre olarak ifade edilmiştir.

DPPH Radikal Süpürücü Kapasite Tayini

Kantitatif DPPH testinde, ekstrelerin DPPH (2,2-difenil-1-pikrilhidrazil) radikalinin menekşemor rengini giderme yeteneği bitki ekstresinin antioksidan kapasitesini belirtmektedir. Bu test yöntemi ekstrelerin DPPH ile oluşturdukları rengin 517 nm'de spektrofotometrik olarak ölçülmesi ve standart madde ile kıyaslanması temeline dayanmaktadır (Blois, 1958). DPPH'in

100 µM etanol çözeltisi ve farklı derişimlerde bitki ekstrlerinden oluşan karışım oda sıcaklığında ve karanlıkta 30 dakika boyunca bekletilip, 517 nm'de absorbans değerleri ölçülmüştür. Deneyler 3 tekrarlı yapılmıştır. Pozitif kontrol olarak Gallik asit kullanılmıştır.

Kalitatif DPPH testinde, mor renkli serbest radikal olan DPPH indirgendiğinde sarı renkli difenil pikril hidrazini verir. Bir İTK plağına püskürtüldüğünde, herhangi bir antioksidan bileşik, mor bir arka plan üzerinde sarı bir bölge olarak görülür. Etanol içinde %0.2 DPPH çözeltisi püskürtülen, 20°C'de bırakılan ve püskürtmeden 30 dakika sonra incelenen İTK plakalarına 2 µl test edilen ekstre çözeltisi uygulanmış ve renk değişimi gözlemlenmiştir. Referans bileşik olarak gallik asit kullanılmıştır.

ABTS Radikal Süpürücü Kapasite Tayini

Ekstreler tarafından, radikal rengini giderme kapasitesine dayanan ABTS (2,2'-azino-bis-(3-etilbenzotiazolin-6-sülfonik asit)) radikal süpürücü kapasite testinde, 7 mM ABTS sulu çözeltisi, 2.45 mM potasyum persülfat ile reaksiyona sokularak ABTS radikal katyonunun oluşması sağlanır. Test edilecek numune 12-16 saat boyunca karanlıkta ve oda sıcaklığında bekletilen ve sonrasında etanol ile dilüe edilen bu karışım ile (100x) seyreltilir. 6 dakika sonra bu karışımın absorbans değeri spektrofotometrik olarak 734 nm'de ölçülüp, inhibisyon yüzdesi hesaplanır (Re vd., 1999). Deneyler 3 tekrarlı olarak yürütülmüştür. Pozitif kontrol olarak troloks kullanılmıştır.

BULGULAR

Elde edilen sonuçlara göre, *V. labrusca*'nın meyve ve yapraklarından elde edilen ekstre verimleri Tablo 1'de gösterilmiştir.

Tablo 1. *V. labrusca* ekstrlerine ait % verimler

Ekstre	Meyve	Yaprak
Metanol	7.12	6.17
Etanol	6.42	5.04
Diklorometan	4.50	4.36
Etil asetat	5.59	4.51
Aseton	6.91	3.54

Elde edilen sonuçlara göre en yüksek fenolik içerik meyve ekstrlerinde etil asetat ekstresinde (270.31±8.01 mg GAE/g ekstre), yaprak ekstrlerinde ise metanol ekstresinde (88.04±2.91 mg GAE/g ekstre) tespit edilmiştir (Tablo 2).

Kantitatif DPPH testinde en güçlü radikal süpürücü kapasiteyi, gallik asite (IC₅₀: 0.015±1.05 mg/ml) kıyasla, meyve etil asetat ekstresi (IC₅₀: 0.019±2.12 mg/ml) göstermiştir (Tablo 3). Kalitatif DPPH testi, gallik asite kıyasla, meyve ekstrlerinde metanol, etanol ve etil asetat

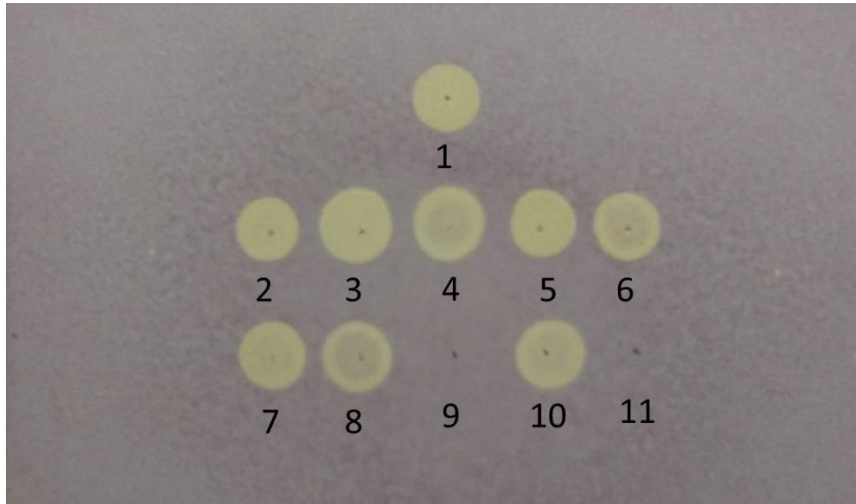
ekstrelerinin, yaprak ekstralarında ise metanol ve etil asetat ekstralarının daha güçlü radikal süpürücü kapasiteye sahip olduğunu göstermiştir (Şekil 2).

Tablo 2. *V. labrusca* ekstralarına ait toplam fenolik madde içeriği

Ekstre	Toplam fenolik madde içeriği (mg GAE/g kuru ekstre) ± SS	
	Meyve	Yaprak
Metanol	181.19±3.23	88.04±2.91
Etanol	148.01±4.11	75.91±5.51
Diklorometan	97.32±6.51	63.17±3.74
Etil asetat	270.31±8.01	84.02±3.41
Aseton	94.15±1.03	54.10±1.01

Tablo 3. *V. labrusca* ekstralarına ait kantitatif DPPH radikal süpürücü kapasite sonuçları

Ekstre/Standart	IC ₅₀ (mg/ml) ± SS	
	Meyve	Yaprak
Metanol	0.023±1.04	0.037±1.41
Etanol	0.027±2.31	0.041±7.01
Diklorometan	0.037±4.10	0.044±1.13
Etil asetat	0.019±2.12	0.035±5.10
Aseton	0.035±6.81	0.046±5.65
Gallik asit	0.015±1.05	



Şekil 2. *V. labrusca* ekstralarına ait kalitatif DPPH radikal süpürücü kapasite sonuçları (1: Gallik asit, 2: Meyve-Metanol, 3: Meyve-Etanol, 4: Meyve-Diklorometan; 5: Meyve-Etil asetat, 6: Meyve-Aseton, 7: Yaprak-Metanol, 8: Yaprak-Etanol, 9: Yaprak-Diklorometan; 10: Yaprak-Etil asetat, 11: Yaprak-Aseton)

ABTS testinde, Trolox'a (IC_{50} : 0.020 ± 1.31 mg/ml) kıyasla meyve ekstrelerinden etil asetat (IC_{50} : 0.024 ± 4.14 mg/ml) ve metanol (IC_{50} : 0.027 ± 3.70 mg/ml) ekstreleri, yaprak ekstrelerinden ise etil asetat (IC_{50} : 0.041 ± 1.04 mg/ml) ve etanol (IC_{50} : 0.041 ± 8.01 mg/ml) ekstreleri güçlü radikal süpürücü kapasite göstermiştir (Tablo 4).

Tablo 4. *V. labrusca* ekstrelerine ait ABTS radikal süpürücü kapasite sonuçları

Ekstre/Standart	IC_{50} (mg/ml) \pm SS	
	Meyve	Yaprak
Metanol	0.027 ± 3.70	0.043 ± 4.21
Etanol	0.029 ± 1.11	0.041 ± 8.01
Diklorometan	0.047 ± 7.41	0.047 ± 3.24
Etil asetat	0.024 ± 4.14	0.041 ± 1.04
Aseton	0.043 ± 3.61	0.049 ± 2.53
Trolox	0.020 ± 1.31	

TARTIŞMA VE SONUÇ

Elde edilen sonuçlara göre en yüksek fenolik içerik meyve ekstrelerinde etil asetat ekstresinde (270.31 ± 8.01 mg GAE/g ekstre), yaprak ekstrelerinde ise metanol ekstresinde (88.04 ± 2.91 mg GAE/g ekstre) tespit edilmiştir. Kantitatif DPPH testinde en güçlü radikal süpürücü kapasiteyi, gallik asite (IC_{50} : 0.015 ± 1.05 mg/ml) kıyasla, meyve etil asetat ekstresi (IC_{50} : 0.019 ± 2.12 mg/ml) göstermiştir. ABTS testinde, Trolox'a (IC_{50} : 0.020 ± 1.31 mg/ml) kıyasla meyve ekstrelerinden etil asetat (IC_{50} : 0.024 ± 4.14 mg/ml) ekstresi, yaprak ekstrelerinden ise etil asetat (IC_{50} : 0.041 ± 1.04 mg/ml) ve etanol (IC_{50} : 0.041 ± 8.01 mg/ml) ekstreleri güçlü radikal süpürücü kapasite göstermiştir.

Daha önce yapılan çalışmalarda meyveye ait toplam fenolik içerik 100 g taze bitkide 56.6 ± 6.10 mg GAE olarak tespit edilmiştir, ayrıca DPPH ve ABTS testinde taze meyvelerin radikal süpürücü kapasiteleri sırasıyla 104.3 ± 2.6 ve 208.6 ± 1.9 μ mol olarak hesaplanmıştır (Burin vd., 2014). Başka bir çalışma ise bitkinin yapraklarındaki toplam fenolik madde miktarının $\%3 \pm 0.1$ oranında olduğunu göstermiştir (Dresch vd., 2014).

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SYNTHESIS, ANTI-INFLAMMATORY ACTIVITY OF 3-AMINO 5-METHOXYL-2-METHYL QUINAZOLIN-4(3H)-ONE AND AMINO-6-METHOXYL-2-METHYL OF 4H-BENZO[D] [1,3]-OXAZINE-4-ONE

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ABSTRACT

Introduction: Quinazolinone derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity. They are widely used in pharmaceuticals and agrochemicals.

Methods: The condensation of 2-amino-methyl-5-dimethoxybenzoate with acetic anhydride yielded the cyclic compound 2-methyl-5-substituted-1,3-benzo-oxazine-4-one which further produced a novel 2,3-disubstituted quinazolin-4-ones via the reaction with hydrazine hydrate. The compounds synthesized were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance (^1H and ^{13}C), Gas chromatography-mass spectrophotometer and elemental analysis. The synthesized compounds were screened and evaluated pharmacologically for their in-vivo anti-inflammatory activity and the paw volume of each rat was measured before 1 h and after 3 h of carrageenan treatment with the help of a plethysmometer.

Results: Compound 1 had anti-inflammatory activity of 89.03% and 88.03% at 20 mg/kg and 10 mg/kg respectively, while compound 2 had anti-inflammatory activity of 94.79% and 90.30% at 20 mg/kg and 10 mg/kg respectively.

Discussion: Compound 1 displayed a singlet signal at: δ 3.78 attributed to methoxy group and singlet at δ 3.68 which was due to methyl group. Also, ^1H NMR spectrum of compound 2 showed a characteristic signal at δ 2.56 (singlet) corresponding to methyl group and duplet at: δ 3.90 for methoxy group. For the IR spectra, Compound 1 was characterized by absence of ν NH_2 and presence of ν C-O stretch in 1101cm^{-1} region of the compound. Compound 2 showed the highest anti-inflammatory activity at 20 mg.kg of 94.79% compared to compound 1 and indomethacin. These compounds synthesized had a higher anti-inflammatory activity than indomethacin which is a standard anti-inflammatory drug.

Conclusion: Compound 2 had a higher anti-inflammatory activity than Compound 1. The compounds synthesized had a higher anti-inflammatory activity than Indomethacin, a standard anti-inflammatory drug.

Keywords: Anti-inflammatory activity, Quinazolin-4(3H)-One, 6-methoxyl 2-methyl 4H-benzo[d] [1,3]-oxazine-4-One, Nucleophile, Synthesis, 3-Amino 6-methoxyl -2-Methyl.

**AN ACCELERATED TENSORIAL DOUBLE PROXIMAL GRADIENT METHOD
FOR TOTAL VARIATION REGULARIZATION PROBLEM**

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ABSTRACT

We consider the constraint tensorial total variation minimization problem for regularizing ill-posed multidimensional problems arising in many fields, such as image and video processing, multidimensional data completion, etc. The non-linearity and the non-differentiability of the total variation minimization problem make the resolution directly more complex. The aim of the present paper is to bring together the resolution of this problem using an iterative tensorial double proximal gradient algorithm and the acceleration of the convergence rate by updating some efficient extrapolation techniques in the tensor form. The general structure of the proposed method will expand its to many fields of application. We will restrict our numerical application to the multidimensional data completion which illustrates the effectiveness of the proposed method.

Keywords: Tensorial total variation regularization, proximal gradient method, polynomial extrapolation, t-product, tensorinpainting.

**SCREENING FOR POTENTIAL NOVEL PROBIOTICS WITH ANTIOXIDANT,
ANTIMICROBIAL ACTIVITIES**

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ABSTRACT

Probiotics are living microorganisms which, when administered in adequate amounts, confer a health benefit on the host, they are now accepted as treatments for several pathologies.

Comprehensive systematic screening studies for the isolation of probiotic bacteria from new sources are needed for the selection of new candidates applicable in the health and food industries.

To meet this need, we proceeded to the isolation of microbial strains from different ecosystems, among 54 isolates, 10 were preselected and identified as Gram-positive, catalase negative, non-spore forming and exhibited good survival at low pH (2.0, 3.0, 4.0, 5.0 and 6.4) and high bile salt concentration (0.3 and 1.0%). Also, their ability to tolerate a High salinity concentration NaCl (1%... to 8 %), and phenol (0.04% and 0.05%) They also demonstrated hydrophobicity activity, auto and co-aggregation activity, Agar well diffusion method has been used to determine the antimicrobial activities (against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella Typhimurium*, *Bacillus cereus*), antibiotic sensitivity, and anti-oxidative potential. The safety of these isolates was evaluated by their hemolytic activities.

Based on these results, the isolates with the best probiotic attributes were presented in this study. Exhibit a high tolerance at low pH with a survival rate varying between 50% to 94% proved their resistance to gastric conditions. They have also demonstrated hydrophobic activity, showing DPPH Free Radical scavenging activity 9.85 -72.34 %, auto and co-aggregation ranging from 49 % to 80 %, represented the adhesion properties of these isolates. and

antibacterial capacity against pathogenic strains. These properties can help bacteria colonize the intestine.

The results proved that strains exhibited promising probiotic properties and seem favorable for use in food industry as fermented foods and in the pharmaceutical industry as therapeutic compliments.

Keywords: Probiotics, Lactic bacteria, Antagonist activity, DPPH Free Radical scavenging.

**SYNTHESIS, CHARACTERIZATION, AND BIOACTIVITY TEST OF
DIPHENYLTIN(IV) DI-2-CHLOROBENZOATE AND TRIPHENYLTIN(IV) 2-
CHLOROBENZOATE COMPOUNDS AS DISINFECTANT**

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ABSTRACT

This research was conducted to study bioactivity of two derivative of organotin(IV) carboxylate compounds as disinfectant; diphenyltin(IV) di-2-chlorobenzoate (1) and triphenyltin(IV) 2-chlorobenzoate (2), against pathogenic bacteria. Compounds (1) and (2) was successfully synthesize with total yields respectively 87.71 % and 88.73 %. Several spectroscopy characterization such as UV, IR, ¹H-NMR, ¹³C-NMR, and microelemental analysis data also supported structures elucidation. The optical density test both of compounds at 600 nm shows a good bioactivity against *Salmonella sp.* and *S. aureus* bacteria. Based on this research result, compound (2) has greater bioactivity than compound (1) or local liquid disinfectant (2.5 % pine oil). Bioactivity of compound (2) optimum at 5×10^{-4} M and contact times with bacteria 15 minutes, this data corresponding with decrease of absorbance from 0.6640 to 0.0955 for *Salmonella sp.*, and 0.6565 to 0.1295 for *S. aureus*. Compound (1) optimum at 5×10^{-4} M and contact time with bacteria 15 minutes, this data corresponding with decrease of absorbance from 0.6640 to 0.1305 for *Salmonella sp.*, and 0.6565 to 0.196 for *S. aureus*. Decrease of absorbance imply, both of compounds potential as disinfectant to kills pathogenic bacteria.

Keywords: disinfectant, organotin(IV) carboxylate, optical density, *Salmonella sp.*, *S. aureus*

**SYNTHESIS, CHARACTERIZATION, AND DISINFECTANT BIOACTIVITY TEST
OF SOME TRIPHENYLTIN (IV) COMPOUNDS**

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ABSTRACT

The derivatives of organotin(IV) carboxylates are continue to attract interest due to their strong biological activity. This paper aims to report the synthesis of the derivative of organotin(IV) carboxylate of triphenyltin(IV) 4-aminobenzoate (**2**) and triphenyltin(IV) 4-nitrobenzoate (**3**) and tested antibacterial activities as disinfectants. These compounds were prepared by reacting triphenyltin(IV) hydroxide (**1**) with 4-aminobenzoate and 4-nitrobenzoate acids as the liganda. Compound **2** was obtained as yellow solid with a yield of 84.09% and compound **3** as a white solid with a yield of 80.70%. These compounds were characterized mainly with UV-Vis spectrometry and FT-infrared spectrometry. The bioactivity test as disinfectant were tested against *Salmonella typhosa* and *Staphylococcus aureus*. The activity was carried out by measuring the optical density of the compounds tested with concentration of 5×10^{-3} , 1×10^{-3} , and 5×10^{-4} M in methanol and 5% dimethyl sulfoxide (DMOS). A commercial Wipol (2.5% pine oil added with Aquades) was used as apositive control. The observation was monitored at contact time of 10, 20, and 30 min.. The result indicated that the triphenyltin (IV) compounds tested were active against the bacteria compared to the positive control with compound **3** was found to be more active than compound **2**.

Keywords: antibacterial, disinfectant, *S. typhosa*, *S. aureus*, triphenyltin(IV) compounds

**BIOACTIVE AND PROTECTIVE EFFECTS OF PHENOLIC COMPOUNDS FROM
CURCUMA LONGA L. RHIZOME EXTRACTS AGAINST OXIDATIVE STRESS**

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ABSTRACT

Oxidative stress is involved in several chronic diseases. In this sense, plant antioxidants from herbal play a key role in reducing oxidative stress and eliminating free radicals involved in the pathogenesis of many chronic diseases. The aim of the paper is to evaluate the free radical scavenging effects of *Curcuma longa L.* rhizome extracts and the total phenolics compounds content. The extracts were obtained by employing the Soxhlet extraction method, procedure followed by filtration at normal pressure and concentration the crude extract by roto-evaporation under vacuum. By UV-VIS (Jasco, Japan, V-570 spectrophotometer) spectrometric method were determined the amount of curcuminoids content (spectral scan λ -max of curcumin was found at 421 nm), expressed as curcumin equivalent/g (mg/CR g^{-1}), the total phenolic contents (TPC) expressed as gallic acid equivalent/g (mg/GAE g^{-1}) using the Folin-Ciocalteu reagent, total flavonoid contents (TFC) expressed as rutin equivalent/g (mg/RE g^{-1}), by aluminum chloride colorimetric assay and the caffeic acid derivatives content (CAD_C) expressed as mg caffeic acid equivalent/g dry extract (mg/CAG^{-1}) by using Arnows' reagent. Antioxidant activity were evaluated by in vitro non cellular assays using the chemiluminescence technique in an luminol hydrogen peroxide system, DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and ABTS (2,2'azinobis-(3-ethylbenzthiazoline-6- sulfonic acid) methods. The results indicated a significant free radical scavenging capacity for all the three methods applied: chemiluminescence (CL), DPPH and ABTS, and highlighted a good correlation between phenolic contents and antioxidant protective effects evaluated in vitro, against oxidative stress.

Keywords: oxidative stress, chemiluminescence, antioxidant activity, curcumin, *Curcuma longa L.*

**THE SERIES SOLUTION METHOD SOLVING VOLTERRA INTEGRAL
EQUATIONS OF THE FIRST KIND USING MAPLE**

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ABSTRACT

In this paper, The Series Solution Method method (SSM) is used in this study to solve Volterra Integral Equations of the First kind using Maple. Volterra's integral equations were presented to the public by the scientist Vito Volterra and the scientist Triene Laliscu explained them in his 1908 book "Sur les équations de Volterra" and was written under the supervision of the French scientist Charles Emile Piccard. In 1911, Lalexo wrote his first book on Integral Equations. The Series Solution Method method (SSM) is a strong tool for approximation analytic solutions and numerical simulations. By demonstrating several instances, the Series Solution Method (SSM) is used to solve Volterra Integral Equations of the First kind using Maple. For several examples, the findings are presented in the form of tables and graphs. The findings are presented in the form of tables and graphs for several examples. The contrast between the precise and numerical solutions demonstrates the effectiveness of the Maple program solution, as well as the ease and speed with which it was obtained.

Keywords: The Series Solution Method, Volterra Integral Equations of the First kind, Maple.

**METHODS OF FORMATION OF COGNITIVE ACTIVITY IN THE PROCESS OF
TEACHING BIOLOGY IN THE SUBJECT INFORMATION AND EDUCATIONAL
ENVIRONMENT**

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ABSTRACT

Information is characterized by redundancy, diversity, mosaic perception. When working with extended information bases, it is important that students have self-learning skills. Formation of cognitive training activities, including methods of handling information through means of information and educational environment of the subject is one of the current problems of education.

The biology teacher is faced with the task of forming cognitive educational actions, including ways to handle information through the means of the information and educational environment of his subject.

The main goal in the activity-type SIEE (subject-based information and education environment) is not only to master knowledge, but also to form ways of mastering, meaning the development of cognitive interest and creative abilities of students.

Methodological issues of the construction of SIEE, which is considered as the field of interaction between subjects of education, are devoted to the studies of G. I. Zakharova, O. A. Ilchenko, Yu. G. Korotkov, A. A. Kuznetsov, B. P. Saykov.

In the theory and methodology of teaching, the solution to the problem of the formation and development of cognitive educational actions in SIEE is not sufficiently justified.

In the practice of teaching biology, many teachers have difficulty analyzing the didactic apparatus of existing educational and methodological complexes, choosing innovative educational resources that allow them to comprehensively develop cognitive learning activities.

Therefore, it is often difficult for students to apply their knowledge and translate it into specific educational situations.

Keywords: education, subject-based information, education environment, methodology, teaching.

**DRUG UTILIZATION PATTERN OF ANTIBACTERIAL INCLUDING
PIPERICILLIN+TAZOBACTUM IN TERTIARY CARE HOSPITALS:
ASSESSMENT OF THERAPEUTIC OUTCOMES AND BENEFITS**

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ABSTRACT

Background: Utilization management is a set of treatment reviews and cost minimizing techniques used by health care practitioners and health planners. Commonly utilized management systems for prescription drugs include: previous authorization, step therapy, quantity bounds, and compulsory generic substitution.

Objective: To assess the drug utility pattern of potent antibiotic used in ICU settings in adult patient cohort. To foresee the prescribing details and Therapeutics outcome of this drug with respect to various diseases concerning.

Methodology: a prospective study was carried out to evaluate the Drug utilization evaluation (DUE) of Piperacillin+Tazobactam. A well-constructed form was structured that addressed information regarding demographic data of the patients, medical use, implication, culture sensitivity measures, effects of therapy, renal impairment events of dose modifications and suitable usage during regular practice in various wards of different tertiary care hospitals in Karachi, Pakistan.

Results: Results of this investigation indicates insignificant association for drug utilization as single component ($p=0.446$) and in combination ($p=0.111$) in comparison to gender. With respect to treatment outcome geriatric patients with age group of 61-70 years were observed more prone to therapeutic failure (45.5%) in comparison to effective therapy (9.37%) and

significantly higher ratio of mortality (50%). Moreover, partially negative correlation pattern was observed in the light of standard practice and guidelines.

Conclusion: Since drug utilization evaluation includes a complete survey of prescription details of patients' and drug information previously, during and subsequent to administration resulting in proper medicines management and better positive patient centered results, the outcome of such studies in quality affirmation measure. DUR programs give remedial activity, feedback of prescriber and further assessments avenues. Prescribing gaps may be addressed in such studies and local population needs and related practices may be worked out in optimal manner to improve patient safety parameters and provide them with better health benefits.

SYNTHESES OF TRISPIROCYCLOTRIPHOSHAZENE COMPOUNDS
TRİSPİROSİKLOTRİFOSFAZEN BİLEŞİKLERİNİN SENTEZİ**Yasemin Tümer**Dr., Karabük Üniversitesi, Fen Fakültesi, Kimya Anabilim Dalı
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ÖZET

Kloro siklofosfazenler, anorganik halkalı bileşiklerin dikkat çekici bir grubudur. Halka iskeleti, fosfor ve azot atomlarının ardışık olarak tek ve çift bağ ile bağlanmasıyla meydana gelmiştir. Ayrıca her bir fosfor atomuna iki klor atomu bağlıdır. Halka dışı fosfor-klor bağları oldukça aktiftir ve bu bağların süstitüsüyonu ile farklı özelliklere sahip yeni bileşikler sentezlenebilmektedir [1].

Hekzaklorosiklotrifosfazen ($N_3P_3Cl_6$; trimer), tek ya da çok dişli reaktiflerle nükleofilik süstitüsyon reaksiyonları ile yer değiştirilebilen altı adet Cl atomu içermektedir. Günümüze kadar yapılan çalışmaların çoğunda, trimer ile tek dişli reaktifler arasındaki Cl yer değiştirme reaksiyonları ile kısmen ve tamamen süstitüe bileşikler elde edilmesi üzerine yoğunlaşmıştır. Ayrıca, İki veya çok dişli reaktifler ile $N_3P_3Cl_6$ ün kondenzasyon reaksiyonu sonucu spiro-, dispiro-, trispiro-, ansa-, bino-, spiro-ansa- ve spiro bino-spiro fosfazenler de sentezlenebilir.

Bu çalışmada, *cis*- ve *trans*-dispirosiklik ferrosenilfosfazen bileşiklerinin, iki dişli reaktif olan bifenil türevi ile reaksiyonundan (stokiometrik oran 1:1) trispirosiklik fosfazen bileşikleri sentezlenmiştir. Sentezlenen yeni bileşiklerin yapıları element analizi, FTIR, 1H , ^{13}C ve ^{31}P NMR yöntemleri ile aydınlatılmıştır.

Anahtar Kelimeler: Hekzaklorosiklotrifosfazen, Ferrosenilfosfazen, Bifenil

1. Vadapalli Chandrasekhar and Amit Chakraborty. Phosphazenes, (2020) Volume 49, Pages 349 – 376. Organophosphorus Chemistry: (Editors: David W Allen, David Loakes, Lee J Higham, John C Tebby)

ABSTRACT

Chlorocyclophosphazenes are a remarkable group of inorganic ring compounds. The ring skeleton is composed of phosphorus and nitrogen atoms sequentially linked by single and

¹ Bu çalışma; **Proje No:** KBÜBAP-21-DS-77 ile desteklenmiştir.

double bonds. In addition, two chlorine atoms are attached to each phosphorus atom. Phosphorus-chlorine bonds are very active and new compounds with different properties can be synthesized by substitution of these bonds [1].

Hexachlorocyclotriphosphazene ($N_3P_3Cl_6$) contains six Cl atoms that can be replaced by nucleophilic substitution reactions with single or polydentate reagents. Most of the studies carried out to date have focused on obtaining partially and completely substituted compounds by Cl displacement reactions between trimer and monodentate reagents. In addition, the condensation reactions of $N_3P_3Cl_6$ with bidentate or polydentate reagents can produce the spiro-, dispiro-, trispiro-, ansa-, bino-, spiro-ansa- and spiro bino-spiro phosphazenes.

In this study, trispirocyclic phosphazene compounds were synthesized from the reaction of cis- and trans-dispirocyclic ferrocylphosphazene compounds with a bidentate reactive biphenyl derivative (stoichiometric ratio 1:1). The structures of the newly synthesized compounds were elucidated by elemental analysis, FTIR, 1H , ^{13}C and ^{31}P NMR methods.

Keywords: Hexachlorocyclotriphosphazene, Ferrocylamine, Biphenyl

1. Vadapalli Chandrasekhar and Amit Chakraborty. Phosphazenes, (2020) Volume 49, Pages 349 – 376. Organophosphorus Chemistry: (Editors: David W Allen, David Loakes, Lee J Higham, John C Tebby)

METAL-CATALYZED SYNTHESIS OF FURAN USING YINON DERIVATIVES

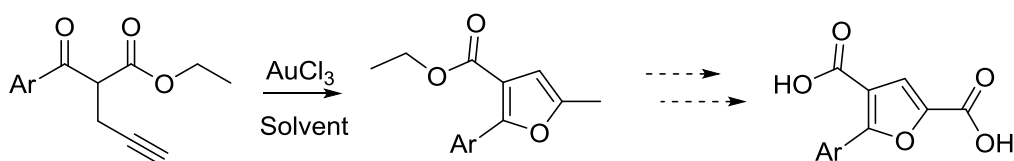
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ABSTRACT

Compounds containing alkyne and carbonyl compounds, known as yinon compounds, have attracted the attention of researchers in recent years and continue to attract. [1] C-C coupling, C-H bond activation and gold chemistry reactions of compounds containing alkyne groups in their structure have been researched and continue to be investigated by numerous researchers around the world. [2] We can say that metal catalysts activate the triple bond of the alkyne group either through an intermediate such as the cyclopropane ring or by making a coordinate covalent bond with the metal. [3]. As a matter of fact, endo-dig or exo-dig alkyne cyclizations allow the formation of more than one product, and products formed as a result of alkyne cyclization have been the subject of extensive research. [4].



Scheme 1. Cyclization reactions

This study was previously carried out on α -propargyl acetophenone derivatives with the catalysis of a toxic metal $\text{Hg}(\text{OTf})_2$ furan derivatives were obtained [5]. In this study, we are trying to develop a more environmentally friendly approach. In this study, α -propargyl- β -ketoesters will be synthesized as starting compounds. The cyclization reactions of the obtained α -propargyl- β -ketoesters derivatives with AuCl_3 will be investigated. Then, the synthesis of derivatives with carboxylic derivatives in the C2 and C4 positions and the advanced reactions of these derivatives will be investigated.

Keywords: Alkyne, Cyclizations, Furan, Yinon

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LC-TOF/MS ANALYSIS OF ORGANIC ACIDS WITH DIFFERENT COLUMNS

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ABSTRACT

Lactic acid, butyric acid, valeric acid, succinic acid, fumaric acid, and propionic acids are all produced during lignocellulosic biomass fermentation. Chromatographic techniques such as HPLC, GC are commonly used to determine organic acids. In this study, traditional organic acid (87H3), aqua and hilic columns were tested for organic acid analysis with LC-TOF/MS. The results showed that an excellent linear range of the three columns, with correlation coefficient (R^2) values of 0.99–1.00. Organic acids are separated with good peak shape using the aqua column. It has an effect on valeric, butyric, and succinic acid retention time and sensitivity in hilic separation. The ion exclusion column 87H3 may separate weak acids with varying efficiencies and selectivities. The mobile phase in this study for the 87H3 column was 0.5 % formic acid solution, which was suitable for detector selection. Peak resolutions and residence durations are greatly influenced by chromatographic parameters. Using an 87H3 column and a TOF/MS detector, the mobile phase showed no improvement in extracted ion peak geometries or retention for valeric, butyric, and propionic acid.

Keywords: Organic Acids, TOF/MS, Hilic Column, Biomass

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**BAKIRIN ASİDİK ÇÖZELTİDEKİ KOROZYONUNA 4-AMİNO-N-(1,3) –
THIAZOLE-2-YL BENZEN SÜLFONAMİDE'İN İNHİBİSYON ETKİSİNİN
İNCELENMESİ**

INVESTIGATION OF INHIBITION EFFECT OF 4-AMINO-N-(1,3) – THIAZOLE-2-YL
BENZENE SULFONAMIDE ON CORROSION OF COPPER IN ACIDIC SOLUTION

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ÖZET

Bakır ve alaşımları son derece yüksek elektriksel ve termal iletkenliğe sahip olmaları, güçlü korozyon direnci ve mekanik işlenebilirlikleri nedeniyle endüstriyel sistemlerde yaygın olarak kullanılan malzemelerdir [1]. Özellikle elektronik endüstrisinde, iletişimde birçok uygulamada, evsel ve endüstriyel su hizmetleri için boru hatlarında yaygın olarak kullanılmaktadır. Bu nedenle, yüksek klorür içeren ortamlarda bakırın korozyonu ve inhibisyonu, birçok araştırmacının dikkatini çekmiştir [2-4]. Metal yüzeyine adsorplanarak metallerin korozyonunu önleyen inhibitör kullanımı tercih edilmektedir. Kullanılacak olan inhibitörlerin çevreyle dost olması ve zehirli olmamasıda önem arz etmektedir. Bu çalışmada bakırın 0,5 M HCl çözeltisindeki korozyonunu önlemek için çevreyle dost (10^{-2} - 10^{-4} M) 4-Amino-N-(1,3) – thiazole-2-yl benzen sülfonamide 'in inhibisyon etkisi incelenmiştir. İnhibitörün asidik çözeltilerdeki bakır korozyonu üzerindeki etkisi, tafel polarizasyon ölçümleri, kronoamperometrik ölçümler ve empedans ölçümleriyle incelenmiştir. Ayrıca 4-Amino-N-(1,3) – thiazole-2-yl benzen sülfonamide'in metal yüzeyindeki adsorpsiyonu incelenmiş, inhibitörün bakır yüzeyine fiziksel olarak adsorplandığı belirlenmiştir. Adsorpsiyon mekanizması Langmuir adsorpsiyon izotermi ile açıklanmıştır. Ayrıca yüksek derişimlerde 4-Amino-N-(1,3) – thiazole-2-yl benzen sülfonamide 'in bakır korozyonunu % 84 oranında inhibe ettiği belirlenmiştir.

Anahtar Kelimeler: Bakır, Asidik Çözelti, Korozyon.

ABSTRACT

Copper and its alloys are widely used materials in industrial systems due to their extremely high electrical and thermal conductivity, strong corrosion resistance and mechanical workability[1]. It is widely used in many applications, especially in the electronics industry, communications, pipelines for domestic and industrial water services. Therefore, the corrosion and inhibition of copper in high chloride environments has attracted the attention of many researchers [2-4]. It is preferred to use inhibitors that prevent corrosion of metals by adsorbing on the metal surface. It is important that the inhibitors to be used are environmentally friendly and non-toxic. In this study, the inhibition effect of environmentally friendly (10^{-2} - 10^{-4} M) 4-Amino-N-(1,3)-thiazole-2-yl benzene sulfonamide was investigated to prevent corrosion of copper in 0.5 M HCl solution. The effect of the inhibitor on copper corrosion in acidic solutions was investigated by Tafel polarization measurements, chronoamperometric measurements and impedance measurements. In addition, the adsorption of 4-Amino-N-(1,3)-thiazole-2-yl benzene sulfonamide on the metal surface was investigated, and it was determined that the inhibitor was physically adsorbed on the copper surface. The adsorption mechanism is explained by Langmiur adsorption isotherm. In addition, it was determined that 4-Amino-N-(1,3)-thiazole-2-yl benzene sulfonamide inhibited copper corrosion by 84% at high concentrations.

Keywords: Copper, Acidic Solution, Corrosion

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**TİROİD FONKSİYON TESTLERİNİN ÖLÇÜM BELİRSİZLİĞİNİN
DEĞERLENDİRİLMESİ**

**EVALUATION OF MEASUREMENT UNCERTAINTY FOR THYROID FUNCTION
TESTS**

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ÖZET

Klinik biyokimya laboratuvarında ölçümü yapılan testlerin referans değerden ya da aynı numuneyi analiz eden başka laboratuvarların ortalama değerinden olası en yüksek sapmasını gösteren değere ölçüm belirsizliği adı verilir. Doğru teşhis için ölçümü yapılan testlerin ölçüm belirsizliğinin hesaplanıp gerektiğinde raporlanması ISO 15189 standartlarında önerilmektedir. Bu çalışmada, Muğla Eğitim ve Araştırma Hastanesi biyokimya laboratuvarında çalışılan tiroid fonksiyon testlerinin (TFT) ölçüm belirsizliğinin hesaplanması ve müsaade edilen hata oranları ile karşılaştırılması amaçlanmıştır. Roche COBAS E801 immünoanalizöründe elektrokemilüminesans yöntemle çalışılan Serbest T3, Serbest T4 ve Tiroid Stimulan Hormon (TSH) testlerine ait ölçüm belirsizliği Nordtest kılavuzuna uygun olarak iç ve dış kalite kontrol (EQAS) sonuçları kullanılarak hesaplanmıştır. Ölçüm belirsizliği Serbest T3, Serbest T4 ve TSH için sırasıyla %11,78, %13,55 ve %11,73 olarak hesaplandı. Elde edilen sonuçlar RILIBAK limitleri (<%24,0) dahilinde yer almaktadır. Sigma düzeyleri sırasıyla 7,30, 5,95 ve 6,92 olarak hesaplandı. Sonuç olarak laboratuvarımızda ölçülen TFT analitik performansı oldukça iyi durumdadır. Biyokimyasal test sonuçları raporlanırken uluslararası standardizasyon kuralları içinde ölçüm belirsizliklerini hesaplamak gerekmektedir. Ölçüm belirsizliği ile sunulan bir sonuç, klinik karar verme noktasında klinisyene ve nihayetinde hastalara verilen sağlık hizmetine çok önemli katkı sağlayacaktır.

Anahtar Kelimeler: Ölçüm belirsizliği, tiroid, hormon, biyokimya

ABSTRACT

The measurement uncertainty is the value that represents the maximum deviation of the tests measured in the clinical biochemistry laboratory from the reference value or the mean value of other laboratories that analyze the same sample. It is recommended in ISO 15189 standards to calculate and report the measurement uncertainty for the biochemical tests. In this study, it was aimed to calculate the measurement uncertainty for the thyroid function tests (TFT) in Muğla

Training and Research Hospital and to compare them with the total allowable error. Free T3, Free T4 and Thyroid Stimulating Hormone (TSH) were performed by electrochemiluminescence method on Roche COBAS E801 immunoanalyzer. In accordance with the Nordtest guideline, the results of the internal quality controls and the bias value derived from the external quality control (EQAS) results was used to calculate the measurement uncertainty. The measurement uncertainty was found 11.78%, 13.55% and 11.73% for FT3, FT4, and TSH, respectively. The results were within the RILIBAK limits (<24.0%). Six sigma values were calculated as 7.3, 5.95, and 6.92 for FT3, FT4, and TSH, respectively. In conclusion, TFT performed in our laboratory had a good analytical performance. The biochemical test results reported with measurement uncertainty can make a very important contribution to the clinician and ultimately to the patients at the point of clinical decision making.

Keywords: Measurement uncertainty, thyroid, hormone, biochemistry

**BIOSYNTHESIS OF SILVER NANOPARTICLES USING ETHYL ACETATE
EXTRACT OF *ORIGANUM ONITES* LEAVES: CHARACTERISATION AND
ANTIOXIDANT ACTIVITY**

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ABSTRACT

Aromatic and medicinal plants have been used effectively for food and medicinal purposes since ancient times. In this study, *Origanum onites* leaves were extracted with hexane and ethyl acetate sequentially. The ethyl acetate extract was used for synthesis of silver nanoparticles (AgNPs). The ethyl acetate extract of *O. onites* leaves (2.0 g) was dissolved in deionized water (100 mL) and silver nitrate deionized water solution (2.0 mM, 100 mL) was added to the extract solution slowly. The reaction mixture was heated at 60°C for 2 hours. The colour changing from yellow to dark brown indicated the formation of silver nanoparticles. In addition, extensive spectroscopic study proved the nanoparticles formation. The maximum absorption was observed at 425 nm in Ultraviolet-Visible (UV-Vis) spectrophotometer. The functional groups of bioactive compounds responsible for the reduction of silver ions were determined by Fourier Transform Infrared Spectroscopy (FTIR). X-Ray Diffraction (XRD) pattern revealed that the structure of nanoparticles was face centered cubic crystal. Scanning electron microscopic (SEM) analysis proved the desired product with an average size of 25.63 nm. Antioxidant activity of AgNPs and extract was carried out using DPPH[•] free radical scavenging, ABTS^{•+} radical cation, and FRAP assays. AgNPs and extract revealed the considerable antioxidant activity.

Keywords: Nanostructures, *Origanum onites* leaves, spectroscopy, antioxidant activity

INTRODUCTION

Due to the significant secondary metabolites content, medicinal plants gain considerable interest in folk medicine as well as drug development [1-4].

Nanotechnology refers to the physicochemical and biological methods used to produce nanoparticles with dimensions of less than 100 nm and with unique properties [5]. Nanotechnology is widely used in a variety of areas, including nanomedicines, biomaterials, nanoelectronics, imaging, agriculture, and environmental protection. It has been widely used in medicine for illness diagnosis and therapy, as well as drug delivery and innovative drug

compositions [6]. Physical and chemical methods have been used commonly for the synthesis of nanoparticles. However, due to the toxicity and pollution of corresponding methods, the green synthesis approaches gain great interest [7]. Natural compounds, plant extracts, algae, enzymes, vegetables, seaweed, and arthropods have been employed for synthesis of nanoparticles [8].

Origanum genus includes twenty-four species and six hybrids, sixteen of which are endemic for Turkey [9]. *Origanum* species have been used extensively for traditional medicine for years as well as spicy food. Phytochemical research on *Origanum* species resulted in the isolation of bioactive compounds responsible for biological activities [10-13].

Herein, silver nanoparticles were synthesized using ethyl acetate extract of *Orignaum onites* and their antioxidant activity was evaluated using the DPPH[•] scavenging, ABTS^{•+} radical cation scavenging, and reducing power assays.

MATERIALS AND METHODS

Plant material

Origanum onites was cultivated in Aromatic and Medicinal Plant Field of Tokat Gaziosmanpasa University.

Synthesis of silver nanoparticles

Air dried *O. onites* leaves (40 g) was extracted with hexane and ethyl acetate sequentially. After the removing of the solvent by rotary evaporator, crude hexane extract and ethyl acetate extract were yielded. Ethyl acetate extract (2.0 g) was dissolved in deionized water (100 mL) and silver nitrate deionized water solution (2.0 mM, 100 mL) was added to the extract solution slowly. The reaction mixture was heated at 60°C for 2 hours and the colour change was observed from yellow to dark brown. After centrifugation, AgNPs were dried by lyophilization [14].

Identification of silver nanoparticles

The structure of synthesized AgNPs was identified using spectroscopic methods. The maximal absorption of silver nanoparticles as well as antioxidant activity analyses were determined using a UV-2600 spectrophotometer. XRD analysis was executed by Malvern Panalytical diffractometer. The functional groups of compounds capped and stabilized the silver were established by Fourier to transform infra-red, FTIR 4700 spectrometer. SEM image was obtained by Quanta Feg450.

Antioxidant activity

DPPH[•] free radical scavenging activity

DPPH[•] free radical scavenging activity was carried out by the method described in the literature [2]. Firstly, stock solutions (1 mg/mL) were prepared. DPPH[•] solution in ethanol (1.0 mL, 0.26

mM) was added to each solution. After the vortex process, the solution was incubated for 30 minutes at room temperature, then absorbance measurement was executed with a spectrophotometer at 517 nm [15].

Ferric reduction antioxidant power (FRAP) assay

Ferric ion reduction antioxidant power (FRAP) activity test was executed according to the reported work. The analyte (100 μ L, 50-100 μ g/mL) was mixed with the phosphate buffer (1.15 mL, 0.20 M, pH 6.7), potassium ferric cyanide (1.0%, 1.25 mL) and the mixture was incubated for 30 min at 50°C, then FeCl₃ and trichloroacetic acid were added to the mixture. After the vortex process for 5 min absorbance measurement was executed on a spectrophotometer (700 nm) [16].

ABTS^{•+} radical cation activity

The reaction of ABTS solution (2.0 mM) with the sodium persulfate (2.45 mM) produced the ABTS^{•+} kept in dark for 5 h at rt. ABTS^{•+} solution was treated with sodium phosphate buffer (0.1 mM, pH 7.4). The samples (AgNPs and extract) were treated with ABTS^{•+} at various concentrations. The absorbance was measured at 734 nm [17].

Statistical analysis

The statistical analysis of antioxidant activity was carried out using GraphPad Prism (version 8.00) with one-way ANOVA software.

RESULTS AND DISCUSSION

UV-Vis spectral analysis of silver nanoparticles

The colour change from yellow to dark brown indicated the formation of AgNPs. The maximum absorption peak was observed at 426 nm in UV-Vis spectrum (Fig. 1). After the oxidation-reduction reaction of ethyl acetate extract of *O. onites* leaves with silver nitrate solution yielded the silver nanoparticles.

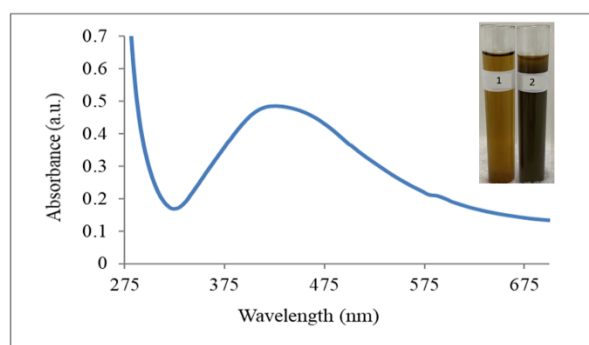


Fig. 1. UV-Vis spectrum of AgNPs. The aqueous extract solution (1) and AgNPs solution (2)

Fourier-transform infrared spectroscopy

FTIR analysis revealed the functional groups of the compounds responsible for reducing, capping, and stabilizing of the silver. The signal observed at 3262 cm^{-1} belonged to the O-H stretching of alcohol and the peak at 2931 cm^{-1} could be attributed to C-H stretching. The sharp peak at 1595 cm^{-1} was due to the double bond of alkene. The peak at 1521 cm^{-1} belonged to the N-O stretching. The signals at 1392 cm^{-1} and 1259 cm^{-1} could be due to the C-H bending and C-O stretching respectively (Fig 2).

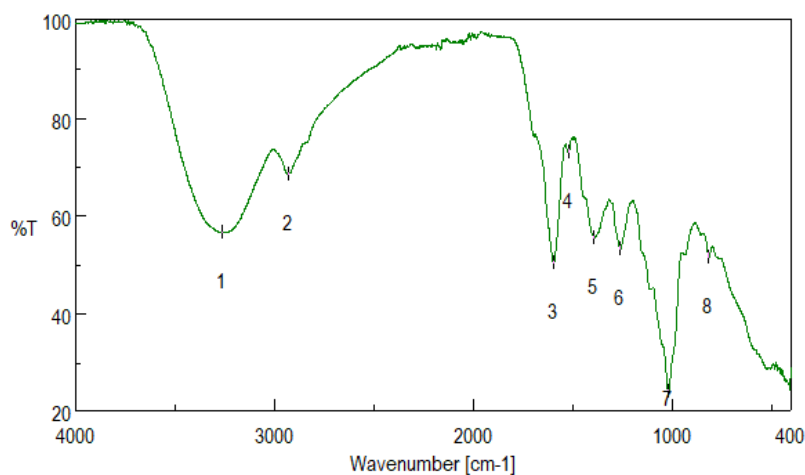


Fig. 2. FTIR spectrum of AgNPs. 1: 3261, 2: 2930, 3: 1594, 4: 1521, 5: 1393, 6: 1259, 7: 1018, 8: 814

X-ray diffraction

XRD pattern indicated the crystal structure of AgNPs. Diffraction peaks (2θ) at 38.1° , 44.3° , 64.4° , and 77.4° can be indexed to the 111, 200, 220 and 311 facets showing the face-centered cubic crystal structure. Debye-Scherrer formula was employed to calculate the average crystal size. The impurity diffraction peaks were caused by the plant materials. The particle size was calculated as 25.63 nm (Fig. 3).

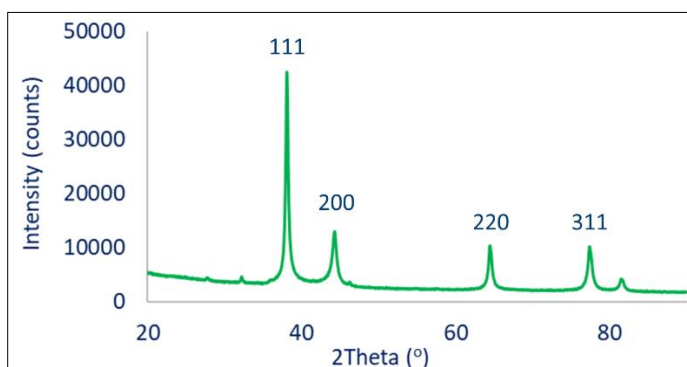


Fig.3. XRD pattern of AgNPs

Scanning electron microscopy analysis

SEM analysis showed the nanoparticles morphology as well as agglomerated clusters of nanoparticles (Fig.4).

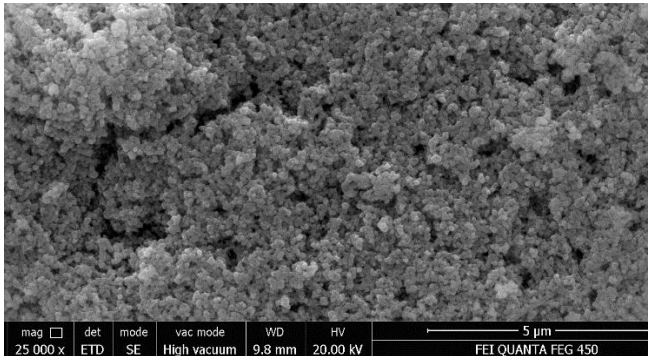


Fig.4. SEM image of AgNPs

Antioxidant activity

Antioxidant activity of extract and AgNPs were carried out using the 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH[•]), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS^{•+}), and ferric reducing antioxidant power assays (Fig.5). The extract and AgNPs revealed the good antioxidant activity. Hence, both extract and AgNPs have the potential to be antioxidant agents in the food and pharmaceutical industries.

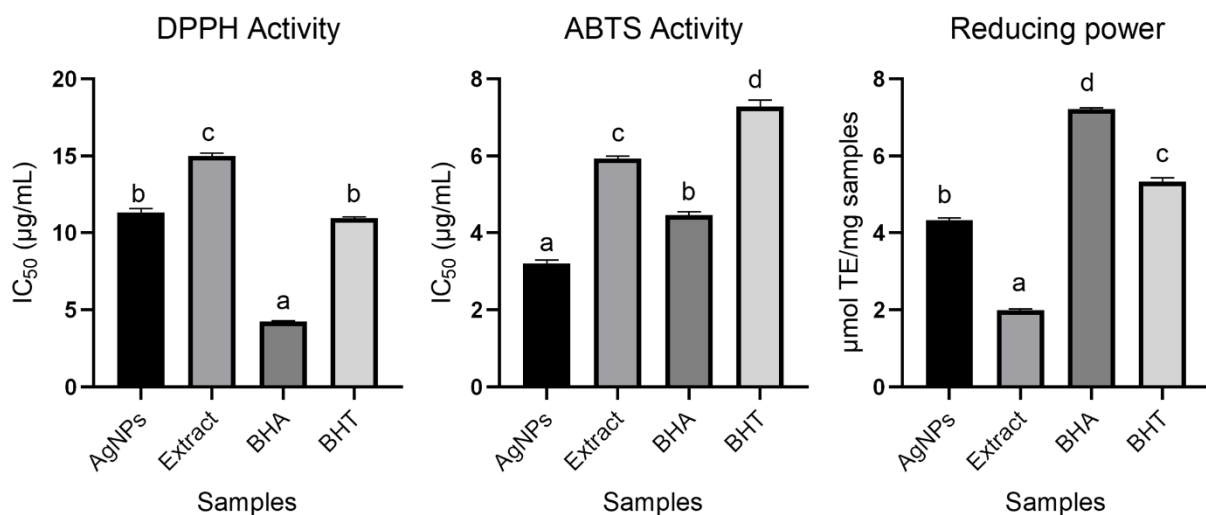


Fig.5. Antioxidant activity of extract and AgNPs

CONCLUSION

Origanum onites is the medicinal plant and includes bioactive compounds and this plant has been used in food and perfumery. Silver nanoparticles were synthesized from the ethyl acetate extract of leaves. Both extract and AgNPs revealed the considerable antioxidant activity. Therefore, these materials could be antioxidant agents for food and pharmaceutical industries.

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CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL OF *ECHIMUM VULGARE*

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ABSTRACT

Essential oils (EOs) derived from plants have been used effectively in the foods, pharmaceuticals, and cosmetics. Moreover, volatile oils from several medicinal plants were reported to exhibit significant biological activities. *Echium vulgare* belonging Boraginaceae family is represented by nine species in the flora of Turkey. This plant has been used as a folk medicine for treatment of wound healing. *E. Vulgare* is known as a honey plant. Phytochemical investigation on this plant revealed the isolation of pyrrolizidine alkaloids, flavonoids, and phenolic acids. In this study, the essential oil of *E. vulgare* was isolated by the hydrodistillation method, and the chemical compounds were determined by gas chromatography mass spectrometry (GC/MS). The major essential oil components were linalool (31%), trans-geraniol (10.8%), α -Terpineol (10.7%), trans-geraniol acetate (9.7%).

Keywords: *Echium vulgare*, essential oil constituents, CG-MS analysis, natural products.

INTRODUCTION

Medicinal plants have been used efficiently since ancient times [1]. After the development of spectroscopy and chromatography in 19th century, plants became the subject of science, and many bioactive compounds were isolated and identified from plants and their biological activities were investigated [2]. Therefore, with the understanding of the importance of plants, it attracted the attention of pharmaceutical companies, and active molecules isolated from plants began to be used in pharmacognosy as drug-active substances [3]. The EOs generated from aromatic plants are commonly used in food, pharmaceuticals, and cosmetics. A large variety of biological activities of essential oils such as antibacterial, antioxidant, antifungal, acaricidal, anticancer, antidiabetic, antiviral was reported [4-6].

Echium vulgare L originated in the Mediterranean and was later domesticated in Africa, America, Asia, and Europe [7]. This plant has been widely used as a folk medicine to treat many ailments such as inflammatory, cough, asthma, heart, brain, depression, insomnia, wound, ulcer, bruising, pulled muscles, ligaments, and sprains. *E. vulgare* was reported to consist of phenolic acids, flavonoids, naphthoquinones, steroids, polysaccharides, and unsaturated fatty

acids [8]. In addition, this plant revealed the considerable antioxidant, antiulcer, anticancer, antidiabetic, antidepressant, anti-inflammatory activities [9].

MATERIALS AND METHODS

Plant material

Echium vulgare L. was collected from Unye-Akkus, at the coordinate 40°42'59.3"N37°01'08.1"E. Taxonomic identification was carried out by Prof.Dr. Ozgur Eminagaoglu, Artvin Coruh University.

Isolation of the essential oils

Aerial parts of *Echium vulgare* L (120 g) were dried at shade and then powdered by grinder and added to a flask with distilled water (900 mL). The mixture was subjected to steam distillation using a Clevenger apparatus for 4 hours. The essential oil was kept in the fridge until further usage [10].

GC and GC/MS analysis

Essential oil analysis was carried out on a GC/MS PerkinElmer, Clarus 500 Series (Shelton, USA), in divided mode (50:1), equipped with a flame ionization detector (FID) and a mass spectrometer. DB-5 capillary column (30 m × 0.25 mm, 0.25 µm i.d) was used. Helium was used as a carrier gas (Split 1/20). The injection volume was 5 µL and the oven temperature was 220°C. Ionization energy was 70 eV, and the temperature of the transfer line was at 250°C of the mass spectrometer.

RESULTS AND DISCUSSION

The essential oil was isolated from *Echium vulgare* by hydro-distillation method using a Clevenger apparatus. The major compounds were presented in table. GC/MS analysis resulted in the identification of compounds including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpene. The major constituents in the EO of *E. vulgare* were linalool (31.1%), α -terpineol (10.7%), trans-geraniol (10.8%), trans-geraniol acetate (9.7%).

Table. Chemical compounds of EO from aerial parts of *E. vulgare*

No	Compounds	Retention time	Concentration (%)
1	Linalool	18.04	31.09
2	Camphor	20.02	2.03
3	endo-Borneol	21.03	3.22
4	α -Terpineol	22.20	10.66
5	trans-Geraniol	25.06	10.83
6	Lavandulyl acetate	26.62	4.33
7	cis-Geranyl acetate	29.79	5.21
8	trans-Geraniol acetate	30.61	9.72
9	Caryophyllene oxide-isomer	38.14	2.77
10	α -Bisabolol	41.21	4.75

CONCLUSION

The GC-MS analysis of EO of *E. vulgare* aerial parts exhibited the presence of linalool, α -terpineol, trans-geraniol, trans-geraniol acetate as the major constituents as well as minor compounds. *E. vulgare* essential oil includes the significant bioactive compounds as the major products. Hence, this plant could be a source of corresponding compounds. Moreover, the EO of *E. vulgare* can be a promising agent for cosmetic and perfumery industries.

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**SARS-COV ENFEKSİYONUNUN YAYILMASINDA RESEPTÖRLERİN VE
OKSIDATIF STRESİN ROLÜ**

**THE ROLE OF CELL RECEPTORS AND OXIDATIVE STRESS IN THE SARS-COV
INFECTION SPREAD**

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ÖZET

SARS CoV enfeksiyonu, son 100 yılın en yaygın ve ekonomik olarak zarar veren ve yaşamı tehdit eden enfeksiyonlarından biridir. Bununla mücadele etmek için her şeyden önce gelişimini teşvik eden mekanizmaları incelemek gerekir. Bu amaçla, SARS-CoV virüsünün yayılmasında genomik değişikliklerin ve oksidatif stresin rolü araştırılmaktadır. Bu hastalığın yayılması sırasında akciğer hasarının, aktif oksijen formlarının neden olduğu bir “sitokin fırtınası” altında meydana geldiği bulunmuştur. SARS virüsü, anjiyotensin dönüştürücü enzim-2 (ACE2) konakçı hücre reseptör aracılığıyla hedef hücrelere bağlanır. Hastalığın erken evrelerinde SARS virüsleri, virüse karşı inflamatuvar yanıtın oluşumunda öncü rol oynayan NLRP3-inflammasomun birikmesine ve aktivasyonuna neden olur. Sitokin fırtınası aşamasında interlökinler, monositik hematopoietik protein MCP-1, makrofaj inflamatuvar protein MIP-1a, TGF, CCL2, CXCL10, CNXL9, TNF-a artmaya devam eder.

COVID virüsünün SARS-CoV-3b proteini ve yapısal olmayan proteini 10 (nsp10) mitokondride oksijenin aktif formlarının oluşumunu teşvik eder. 3CLpro proteazın ayrıca oksijenin aktif formlarının oluşumunu artırarak insan promonositlerinde apoptoza neden olduğu bilinmektedir. NF-κB transkripsiyon faktörünün oksidatif stres yoluyla aktivasyonu ciddi akciğer hasarına yol açabilir. Doğal olarak, oksidatif strese yanıt olarak, antioksidan sistem aktive olur ve bu da sistemin enzimatik ve enzimatik olmayan dallarının tükenmesiyle sonuçlanır.

Şu mekanizma ile tanıştıktan sonra, ACE-2 blokerleri ile tedavi edilen hipertansiyonlu hastalarda SARS COV enfeksiyonunun yayılmasının gecikme nedeni bilinmektedir. Antioksidanların bu hastalığındaki tedavi edici etkisi de bir gerçek olarak kabul edilmektedir.

Anahtar Kelimeler: hücre reseptörleri, oksidatif stres, SARS-CoV, sitokin fırtınası

ABSTRACT

SARS-CoV infection is one of the most common economically damaging and life-threatening infections of the last 100 years. To combat it first of all, it is necessary to study the mechanisms that promote its development. For this purpose, the role of genomic changes and oxidative stress in the spread of SARS-CoV virus is investigated. During the spread of this disease, lung damage has been found to occur under a "cytokine storm" caused by active forms of oxygen. SARS virus binds to target cells via angiotensin converting enzyme-2 (ACE2) cell receptors. In its early early stages, SARS viruses stimulate formation and cause activation of the NLRP3-inflammasome, which plays a leading role in the development of the inflammatory response. Cytokine attacks, interleukins, monocytic hematopoietic protein MCP-1, macrophage inflammatory protein MIP-1a, TGF2, CCL10, CNXL9, TNF-a continue to raise. In mitochondria, SARS-CoV-3b protein and non-structural protein 10 (nsp10) of virus bring the active forms of oxygen to life. It is known that 3CLpro protease cause apoptosis in human promonocytes by increasing the formation of oxygen active forms. Activation of the NF-kB transcription factor by oxidative stress can lead to severe lung injury. Naturally, in response to oxidative stress, the antioxidant system is activated, resulting in the depletion of the enzymatic and non-enzymatic branches of this system.

After acquaintance with this mechanism, the reason for delay of SARS-CoV infection spread in patients with hypertension treated with ACE-2 blockers is known. The therapeutic effect of antioxidants in this disease is also accepted as a fact.

Keywords: cell receptors, cytokine storm, oxidative stress, SARS-CoV

ANTİMİKROBİYAL PEPTİTLERİN SENTEZİNİN TEMEL İLKELERİ
BASIC PRINCIPLES OF SYNTHESIS OF ANTIMICROBIAL PEPTIDES

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ÖZET

Son zamanlarda antibiyotiklere direncin artması nedeniyle bulaşıcı hastalıkların yayılması ve tedavi süreleri uzamıştır. Bulaşıcı hastalıklardan ölümlerde de artışa neden olmuştur. Bu nedenle mikroorganizmaların direnç oluşturamadığı yeni maddelerin uygulanmasına ihtiyaç vardır. Bu tür maddeler, antimikrobiyal peptitleri (AMP) içerir. Doğal AMP'ler bazen talebi karşılayamadığından, sentetik analoglarının elde edilmesi tıp biliminin acil sorunlarından biri haline gelmiştir. Bu amaçla, AMP'lerin genel özellikleri, ayrıca bireysel alanlarının aktivite ve toksisite üzerindeki etkisi, membranı geçme yeteneği araştırılmaktadır. AMP'deki hidrofobik amino asitlerdeki bir artışın, bunların patojenik organizmaların zarlarına bağlanmasını artırabileceği gösterilmiştir. Peptidlere pozitif yüklü amino asitlerin eklenmesi, zarda bulunan negatif yüklü fosfolipidlere ve asitlere bağlanma yeteneklerini artırır. Cari dönemde AMP'lerin mikroorganizmalar üzerindeki yıkıcı etkilerini açıklayan üç düşünce sunulmaktadır. AMP'lerin sentezi sırasında mikroorganizmaların zarlarına karşı afinitelerini artırmak, ayrıca bu peptitlerin sadece fizikokimyasal özellikleri dikkate alınarak içlerindeki patojenlerin büyümesini durdurabilecek diziler elde etmek mümkündür. Bu amaçla sentetik AMP'lerin kimyasal yapısındaki değişikliklerin aktiviteleri üzerindeki etkisini ele almak onların etkisini talep olunan ve istenen yönde yönlendirmeye hizmet eder. Şu malumatlar, AMP sentezi yapan laboratuvarlar için büyük önem taşımaktadır.

Anahtar Kelimeler: antimikrobiyal peptitler, iyon yükü, hidrofobiklik, toksik etkiler

ABSTRACT

Due to the increase in resistance to antibiotics, the spread of infectious diseases and the duration of their treatment have been prolonged. It has also caused an increase in deaths from infectious diseases. For this reason, there is a need for the application of new substances microorganisms cannot form resistance. Such substances include antimicrobial peptides (AMP). Since natural AMPs sometimes fail to meet the demand, obtaining their synthetic analogues has become one of the pressing problems of medical science. For this purpose, the general properties of AMPs, as well as the effect of their individual domains on their activity and toxicity, the ability to cross

the membrane are investigated. It has been shown that an increase in hydrophobic domains by incorporation of non-polar amino acids in AMP structure can increase their binding to the membranes of pathogenic organisms. Adding positively charged amino acids to peptides increases their ability to bind to negatively charged phospholipids and acids present in the membrane. Three ideas are presented to explain the destructive effects of AMPs on microorganisms in the current period. Taking into account the physicochemical properties of these peptides during the synthesis of AMPs, it is possible to increase their affinity for the membranes of microorganisms, as well as to obtain sequences that can inhibit the growth of pathogens in them. To achieve this goal, considering the effect of changes in the chemical structure of synthetic AMPs on their activity serves to direct their effect in the desired direction. The following information is of great importance for laboratories that synthesize AMP.

Keywords: antimicrobial peptides, hydrophobicity, ion charge, toxic effects

***N,N*-BİS(4-AMİNOFENİL)PİRİDİN-4-AMİN SENTEZİ VE KARAKTERİZASYONU:
TD-DFT ÇALIŞMALARI VE MOLEKÜLER DOKİNG ANALİZİ
N,N-BIS(4-AMINOPHENYL)PYRIDINE-4-AMIN SYNTHESIS AND
CHARACTERIZATION: DFT STUDIES AND MOLECULAR DOKING ANALYSIS**

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ÖZET

Son zamanlarda moleküler doking ve ilaç tasarımı çalışmalarında ligand olarak sıkça tercih edilen diammin molekülleri çalışmaya öncülük etmiştir. Burada dimetil sülfoksit (DMSO) çözücü ortamda piridin-4-amin, 4-fluronitrobenzen ve sezyum florür varlığında ısı ve karıştırma ile *N,N*-Bis(4-aminofenil)piridin-4-amin molekülü sentezlenmiştir. Sentezlenen molekülün FT-IR, ¹H-NMR ve ¹³C-NMR spektroskopileri ile karakterizasyonları gerçekleştirilmiştir. Aynı zamanda molekülün doğal reaktivitesini nicel olarak tanımlayan global indisleri hesaplamak için yeterli bir teorik bilgi sağlayan quantum kimyasal hesaplamalarından zamana bağlı yoğunluk fonksiyonel teorisi (TD-DFT) çalışması yapılmıştır. Molekülün geometri optimizasyonları TD-DFT B3LYP/6-311G (d,p) ve TD-DFT B3PW91/6-311G (d,p) seviyesinde gerçekleştirilmiştir. Moleküler doking, özellikle hedef proteinin 3D yapısı mevcut olduğunda en çok uygulanan sanal tarama yöntemlerinden biridir. Bu yöntem, hem ligand ve protein arasındaki bağlanma afinitesini hem de molekülün ilaç potansiyeli araştırmada yararlı bilgiler olan protein-ligand kompleksinin yapısını tahmin edebilir. Moleküler doking otuz yılı aşkın bir süredir uygulanmaktadır ve buna göre çok sayıda yeni ilaç keşfedilmiş ve geliştirilmiştir. Moleküler doking çalışmasında reseptör olarak asetilkolinesteraz (AChE) (PDB ID: 4EY7), butirilkolinesteraz (BChE) (PDB ID: 6SAM) ve SARS-CoV-2 (PDB ID: 7RN4) enzimleri kullanılmıştır. Research Collaboratory for Structural Bioinformatics (RCSB) protein veri tabanından (PDB) elde edilen enzimlerin kristalografik yapıları (bkz. <http://www.rcsb.org/pdb>) elde edilmiştir. Moleküler doking çalışmasında doking skorunun -7.534 kcal/mol ile AChE en yüksek skoru vermiştir.

Anahtar kelimeler: Diamin, sentez, DFT, moleküler doking.

ABSTRACT

Recently, diamine molecules, which are frequently preferred as ligands in molecular docking and drug design studies, have pioneered the study. Here, *N,N*-Bis(4-aminophenyl)pyridin-4-amine molecule was synthesized by heat and stirring in the presence of pyridine-4-amine, 4-fluoronitrobenzene and cesium fluoride in the presence of dimethyl sulfoxide (DMSO) solvent.

Characterizations of the synthesized molecule were performed by FT-IR, ¹H-NMR and ¹³C-NMR spectroscopies. At the same time, a time-dependent density functional theory (TD-DFT) study has been performed from quantum chemical calculations, which provides sufficient theoretical information to calculate global and local indices that quantitatively describe the natural reactivity of the molecule. Geometry optimizations of the molecule were performed at the TD-DFT B3LYP/6-311G (d,p) and TD-DFT B3PW91/6-311G (d,p) levels. Molecular docking is one of the most applied virtual scanning methods, especially when the 3D structure of the target protein is available. This method can predict both the binding affinity between ligand and protein and the structure of the protein-ligand complex, which is useful information in investigating drug potential of the molecule. Molecular docking has been practiced for over three decades, and accordingly, numerous new drugs have been discovered and developed. Acetylcholinesterase (AChE) (PDB ID: 4EY7), butyrylcholinesterase (BChE) (PDB ID: 6SAM) and SARS-CoV-2 (PDB ID: 7RN4) enzymes were used as receptors in the molecular docking study. The crystallographic structures of the enzymes (see <http://www.rcsb.org/pdb>) were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database (PDB). In the molecular docking study, AChE gave the highest score with the docking score of -7.534 kcal/mol.

Keywords: Diamine, synthesis, DFT, molecular docking.

GİRİŞ

Bilindiği üzere çok iyi biyolojik aktiviteye sahip bileşikler grubundan biri, diaminlerdir (Hamidian, Rahimi, and Hosseini-Kharat 2021; Sedenkova et al. 2018; Toviwek et al. 2021). Literatür, diamin bileşiklerin antibakteriyel, antifungal, antikonvülsan, antitüberküler, antioksidan, antelmintik ve antikanser aktivite sergilediği bilgisini vermektedir tıbbi kimya ve farmakoloji için çok çekicidirler (Das, Das, and Banik 2021; Sahoo et al. 2020). Bu nedenle, diamin sistemler, özellikle tıp ve eczacılık alanında çok popüler organik bileşiklerdir. Değerli biyolojik özelliklere sahiptirler, örneğin antibakteriyel veya antifungal. Antibiyotiklerde, antienflamatuar ve antifungal ilaçlarda aktif maddeler olarak yaygın olarak kullanılırlar (Toviwek et al. 2021). Diamin bileşikler yüksek biyolojik aktivite gösterenler, günümüz hastalıklarının tedavisinde olası kullanımları nedeniyle hala araştırılmaktadır. Diaminlerin biyolojik sistemlerdeki yaygınlığı ve dolayısıyla canlı organizmalarda meydana gelen süreçlere katılımları nedeniyle bu bileşiklerin yapıları ve çeşitli ortamlarda ve diğer bileşiklerle reaksiyon mekanizmaları önemli ve temel bir konudur (Gazizov et al. 2021). Diaminlerin diğer bir avantajı da, elde etme reaksiyonu genellikle basit, hızlıdır ve yüksek verimle ilerler. Diamin türevleri, elektroaktif özellikler, ışık yayıcılar ve fotoiletkenler gibi potansiyel uygulamalar nedeniyle büyük ilgi görmüştür (Meng et al. 2017). Diamin bileşikleri sentez kimyasında faydalı ara maddelerdir. Moleküler mimarinin tasarımında bir ara bileşen olarak çok önemlidir (Liu,

Chao, and Yao 2014). Hesaplmalı kimya, özellikle kimyanın birçok alanında organik ve biyokimyasal yapıların araştırılmasında destekleyici olarak iyi bir düzeye ulaşmıştır. Teorik kimyasal hesaplamalar, molekülün yapılarını, özelliklerini, mekanizmalarını ve reaksiyon seçiciliğini aydınlatmak için gerekli hale gelmiştir. Teorik kimya, kimyanın matematiksel olarak tarifidir. Teorik hesaplama yöntemlerinden birisi olan yoğunluk fonksiyonel teorisi (DFT) son yıllarda çok popüler oldu (Jeazet et al. 2016; Mondal, Banerjee, and Chattopadhyay 2022). Bu, benzer doğruluğa sahip ve diğer yöntemlerden daha az hesaplama hassasiyete Bu nedenle, bu teori ile henüz keşfedilmemiş problem sınıfları vardır, bu da bilinmeyen sistemlere uygulanmadan önce metodun doğruluğunu test etmeyi çok daha önemli hale getirmiştir. Asetilkolinesteraz (AChE) ve butirikolinesteraz (BChE), sinir sistemindeki iki tip kolinesteraz anlamına gelir. AChE enziminin insan beyinde baskın olduğu ve BChE enziminin asetilkolin seviyesinin düzenlenmesi ile ilişkili olduğu belirlenmiştir (Scacchi, Ruggeri, and Corbo 2011; Taha et al. 2021). Alzheimer hastalığı, yaşa bağlı bilişsel bozulma ve izlenebilir hafıza kaybı ile ifade edilen nörodejeneratif bir hastalıktır. AChE, asetilkolini hidrolize edebildiği düşünülen periferik sinir sistemindeki kolinerjik sistemin bileşenleridir (Scacchi, Ruggeri, and Corbo 2011; Taha et al. 2021). Alzheimer hastalığının erken evrelerinde AChE aktivitesi artarken, bu hastalığın ileri evrelerinde BChE aktivitesi arttı. Bu nedenle, bu iki enzim kolinerjik eksikliğin tedavisinde önemli hedeflerdir. Ligand bağlanmasında etkili olan SARS-CoV-2, enzim inhibitörlerinin performansını belirlemek için kullanılan bir ilaç proteinidir (Fourati et al. 2022; Omar et al. 2021).

Burada diamin molekülünün karakterizasyonu $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ ve FT-IR gibi spektroskopik analizlerle gerçekleştirildi. Molekülün optimizasyon temel seti B3LYP/6-311G (d,p) ve B3PW91/6-311G (d,p) olan elektronik özelliklerine dayalı detaylı bir TD-DFT çalışması sunuldu. Ek olarak, HOMO-LUMO enerji boşluğu ve Haritalı moleküler Elektrostatik Potansiyel (MEP) yüzeyleri gibi moleküler orbital hesaplamaları da yapıldı. Hesaplama HOMO-LUMO analizine dayalı enerji boşluğu (Δ), iyonizasyon potansiyeli (I)Elektron ilgisi (A), Küresel Sertlik (η), Kimyasal Potansiyel (μ), Elektrofillik (ω), Elektronegativite (χ) ve Polarize edilebilirlik (α) gibi parametreler elde edildi. Moleküler doking çalışmasında ise reseptör olarak AChE (PDB ID: 4EY7), BChE (PDB ID: 6SAM) ve SARS-CoV-2 (PDB ID: 7RN4) enzimleri kullanıldı. Sentezlenen diamin molekülünün doking skoru araştırıldı.

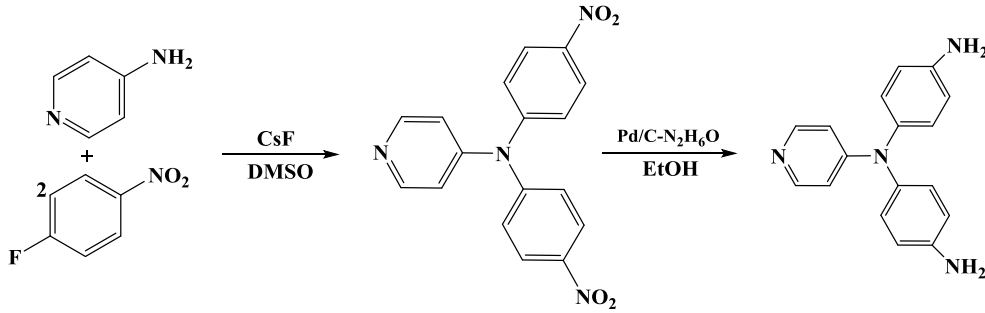
YÖNTEM

Materyal

Piridin-4-amin (Sigma-Aldrich), 4-fluronitrobenzen (Sigma-Aldrich), sezyum florür (Sigma-Aldrich), DMSO (Sigma-Aldrich), etanol (Sigma-Aldrich), Pd/C (%10) (Sigma-Aldrich), asetik asit (Sigma-Aldrich), hidrazinmonohidrat (Sigma-Aldrich).

N,N-bis(4-aminofenil)piridin-4-amin Sentezi

Üç boyunlu 250 mL'lik cam bir balona argon akışı altında sırasıyla 1.17 g (12.5 mmol) piridin-4-amin, 3.52 g (25 mmol) 4-fluoronitrobenzen ve 3.79 g (25 mmol) sezyum florür ve 50 mL DMSO de çözülerek 110 °C'de 12 saat boyunca karıştırıldı. Balon ve muhteviyatı oda sıcaklığına kadar soğutulduktan sonra 400 mL etanol içinde çöktürüldü. Sarı renkteki çökelek Gouch hunisi yardımıyla süzülerek vakum etüvünde kurutuldu ve asetik asit ile kristallendirildi. Ardından iki boyunlu 250 mL'lik cam bir balona sırasıyla 3.36 g (10 mmol) N,N-bis(4-nitrofenil)piridin-4-amin ve 0.1 g Pd/C (%10) konularak üzerine 80 mL EtOH ilave edildi. Balon ve muhteviyatı kaynama sıcaklığına kadar ısıtıldıktan sonra 1.16 g (30 mmol) hidrazinmonohidratın 20 mL etil alkol içindeki çözeltisi damla damla ilave edildi ve 18 saat süre ile geri soğutucu altında kaynatıldı. Reaksiyon karışımı oda sıcaklığına kadar soğutuldu. Pd/C (%10) süzgeç kâğıdı yardımı ile süzülerek alındı. Çözücü döner-buharlaştırıcıda vakum altında uçuruldu. Daha sonra vakum etüvünde kurumaya bırakıldı. FT-IR (cm⁻¹): 1620 (C=N), 1586 (C=H), 1506 (aromatik C-H), 1303 (aromatik C=C), 1182 (p-ikameli benzenin C=C), 1110 (C-N gerilmesi). ¹H-NMR (400 MHz, DMSO-d₆) δ 8.04-7.94 (m, 1H), 7.26-7.08 (m, 3H), 6.98-6.87 (m, 1H), 6.89-6.77 (m, 3H), 6.75-6.63 (m, 2H), 6.61-6.50 (m, 3H), 5.29 (s, 1H), 3.51 (s, 1H), 2.60-2.51 (m, 1H), 2.49-2.37 (m, 3H), 1.61-1.51 (m, 2H), 1.54-1.44 (m, 3H), 1.39-1.22 (m, 15H), 0.99-0.83 (m, 6H), 0.85 (s, 1H). ¹³C-NMR (101 MHz, DMSO-d₆) δ 154.13, 146.16, 140.02, 129.56, 129.42, 128.96, 128.45, 128.25, 126.10, 125.39, 116.67, 114.98, 114.69, 114.04, 40.37, 34.80, 34.54, 31.53, 31.29, 31.21, 30.96, 28.55, 28.44, 22.19, 22.13, 13.86.



Şekil 1. N,N-bis(4-aminofenil)piridin-4-amin sentezi.

Karakterizasyon

Bileşiklerin yapısal aydınlatması için ¹H-NMR Bruker Avance III HD 600 Mhz model cihaz (Almanya) kullanıldı. NMR spektroskopik gerekli veriler, ¹H-NMR için döteryumsuz sinyal dahili standartları takip edilerek DMSO'ya kaydedildi. IR spektrumu, kaymaları ifade etmek için bir FT-IR spektrofotometre üzerinde bir Perkin-Elmer spektrumunda elde edildi.

Simülasyon Çalışmaları

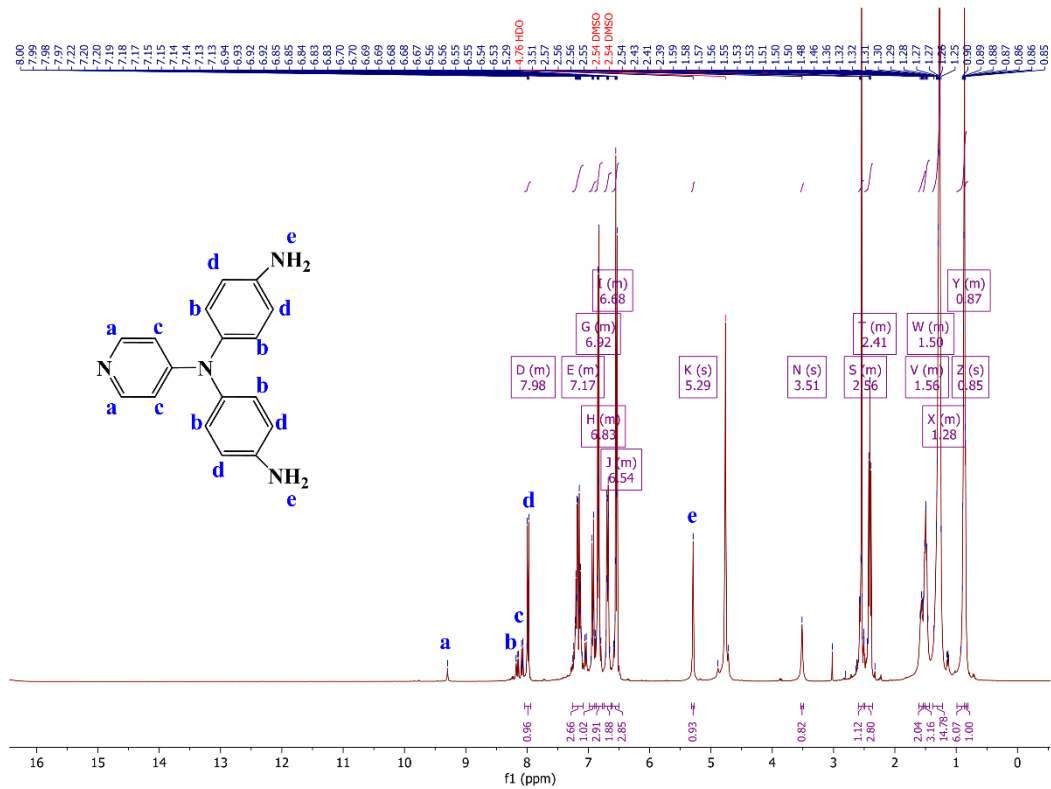
Diamin molekülü ilk olarak gaussian 09 programında TD-DFT yöntemi ile minimize edilmiştir. TD-DFT çalışması B3LYP ve B3PW91 yönteminde 6-311G (d,p) temel seti üzerinden

hesaplanmış ve her bir hesaplamanın (Geometri optimizasyonu, HOMO ve LUMO analizi, Mulliken atomik yükleri ve dipol momenti, MEP analizi) görüntüleri alınmıştır. ligand-protein etkileşimlerinin tam bağlanma yeri ve bağlanma mekanizması. Schrödinger'in Maestro Moleküler Modelleme platformu (sürüm 11.8). LLC modeli, moleküler yerleştirme yaklaşımında uygulandı. Protein Data bank'tan Asetilkolinesteraz (AChE) (PDB ID: 4EY7), butirilkinesteraz (BchE) (PDB ID: 6SAM) ve SARS-CoV-2 (PDB ID: 7RN4) enzimler indirildi. Elde edilen reseptör 3D etkileşimleri, Discovery Studio 2016 istemcisi (Visualizer 2005) ile görselleştirildi.

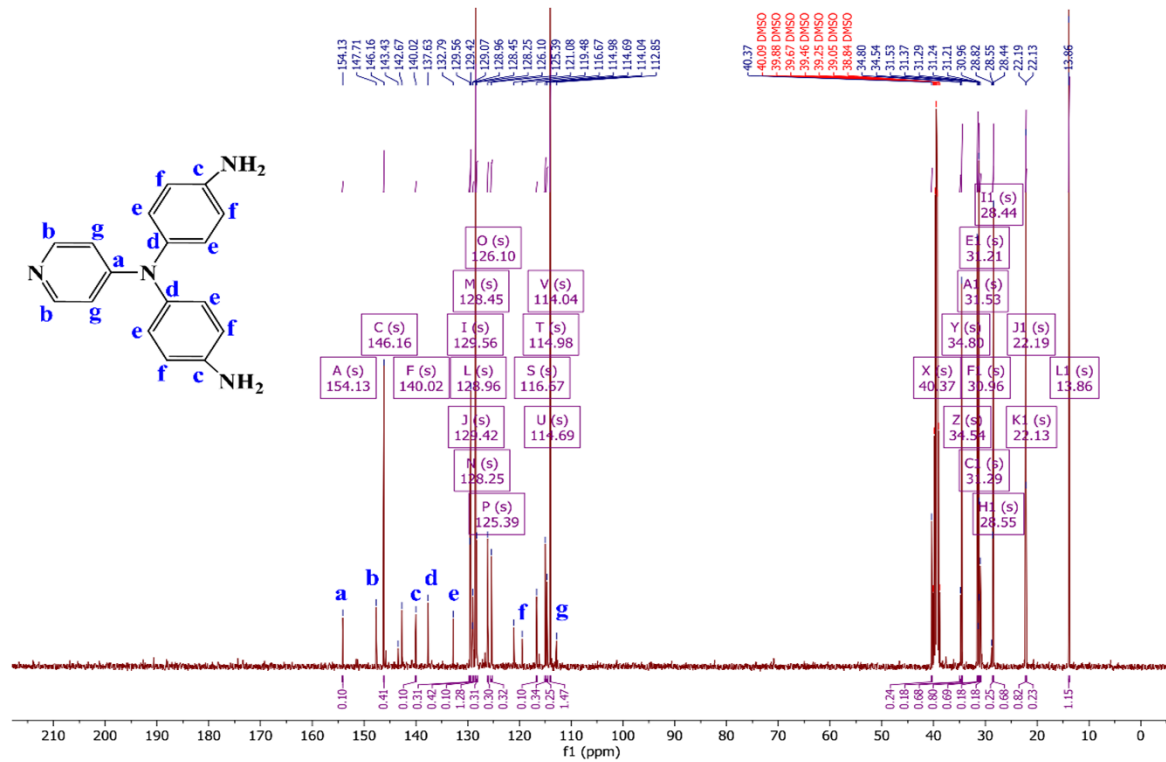
BULGULAR

¹H-NMR, ¹³C-NMR ve FT-IR Analizleri

¹H-NMR, enerji transferi, depolarizasyon akımı gibi gelişmiş yöntemler için çok uygundur. ¹H-NMR spektrumları, 400 MHz frekanslarında standart olarak DMSO ile kaydedilir. Analiz için bileşikler, önceden sülfonatlanmış forma dönüştürülür. ¹H-NMR ayrıca proton hareketliliği ve hidrojen bağı hakkında bilgi verir. Burada, halka sistemi içeren komplekslerin oluşumu ile tutarlı yapıları ortaya çıkardı. NH protonuna karşılık gelen rezonanslar, nitrojen atomlarından birinin koordine olduğunu yansıttı. Şekil 2'deki ¹H-NMR spektrumu, 9.10 (N-CH), 8.04-7.94 (HC=CH), 7.26-7.08 (aromatic), 5.29 (-NH₂) ppm'de sinyaller gösterdi. Böyle bir kimyasal kaymanın değeri, bu NH₂'nin sp³ hibritleştirilmiş atomlara bitişik olduğunu gösterdi. ¹³C-NMR spektroskopik analizi, SPI'de bulunan karbonun çeşitli izotopik doğrulaması için yapılırdı. Şekil 3, diamin bileşiğinin ¹³C-NMR spektroskopisini göstermektedir. 154.13-146.16 ppm aralığındaki zirve, amin bağlı karbonlara aittir. 140.02 ppm'de C-NH₂ karbonlarına ait pikleri sunar. 140.0- 114.98 ppm aralığındaki zirveler -C=C- karbonlarını yansıtır.

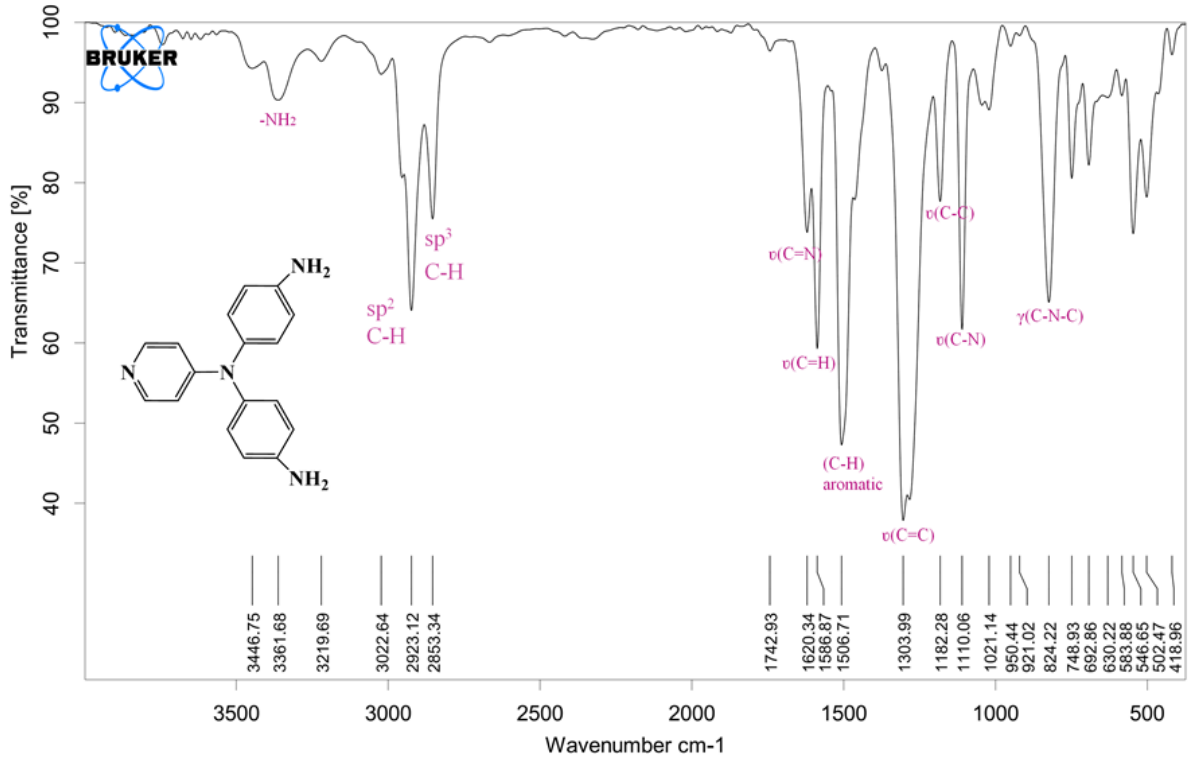


Şekil 2. Diamin molekülünün 1H-NMR spektrumu



Şekil 3. Diamin molekülünün 13C-NMR spektrumu

Sülfonik asit grubunun hidrofilik özelliklerini ve fonksiyonel grupların gerilme titreşimlerini belirlemek için FT-IR analizi yapılır. Ayrıca sentezin başarılı olup olmadığı FT-IR spektrumundan da anlaşılabilir. Şekil 4’deki FT-IR spektrumunda, 1620 cm^{-1} ’de bir tepe ($\text{C}=\text{N}$ simetrik gerilme) ve 1586 cm^{-1} ’de diğer bir tepe ($\text{C}=\text{H}$ simetrik gerilme) ortaya çıkmıştır. 1506 cm^{-1} ’de (aromatik CH, yüksek ton aromatik), 1303 cm^{-1} (aromatığın $\text{C}=\text{C}$ gerilmesi), 1182 cm^{-1} (p-ikameli benzenin $\text{C}=\text{C}$ gerilmesi), 1110 cm^{-1} ($\text{C}-\text{N}$ gerilmesi) tepeleri görünmüştür.

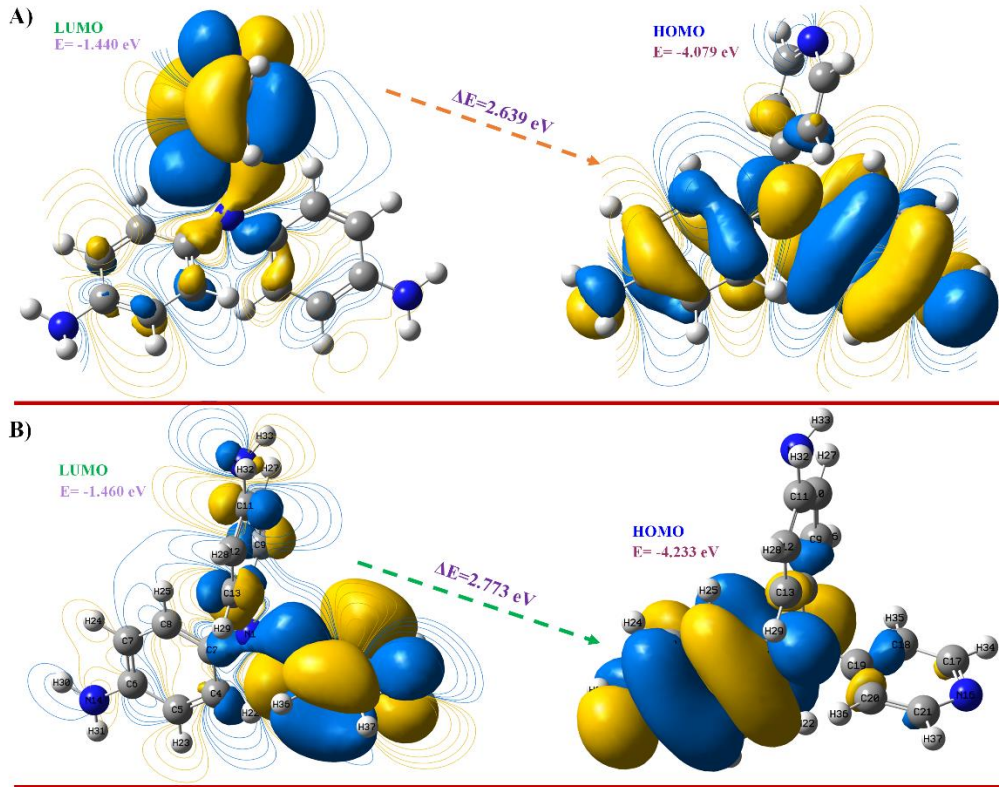


Şekil 4. Diamin molekülünün FT-IR spektrumu

DFT Çalışmaları

Frontier molekül orbitalleri (HOMO – LUMO)

Bir moleküldeki orbitallerle ilgili ana elektriksel parametreler, en yüksek dolu moleküler orbital (HOMO) ve en düşük boş moleküler orbital (LUMO) ve enerji boşluklarıdır. HOMO, elektron donörü olarak işlev görebilen en dıştaki (en yüksek enerjili) yörünge elektronlarıdır. LUMO, elektronları kabul etmek için yeterli alana sahip ve elektron vurgusu olarak işlev görebilen en içteki (en düşük enerjili) yörüngedir. HOMO ve LUMO orbitalleri, molekülün diğer tiplerle etkileşimini belirtir (Janeoo et al. 2022; Şahin and Dege 2021). Yoğunluk diamin molekülü için HOMO ve LUMO'nun yörünge gösterimi Şekil 2'de gösterilmiştir. HOMO ve LUMO orbitalleri, molekülün diğer türlerle nasıl etkileştiğini düzenler. Küçük bir sınır, yörünge boşluğu olan bir molekülün polarizasyonunu, sertliğini, elektronegatifliğini ve diğer reaktivite alımlarını gösterir. Tablo 2, kimyasal reaktivite indekslerini göstermektedir.



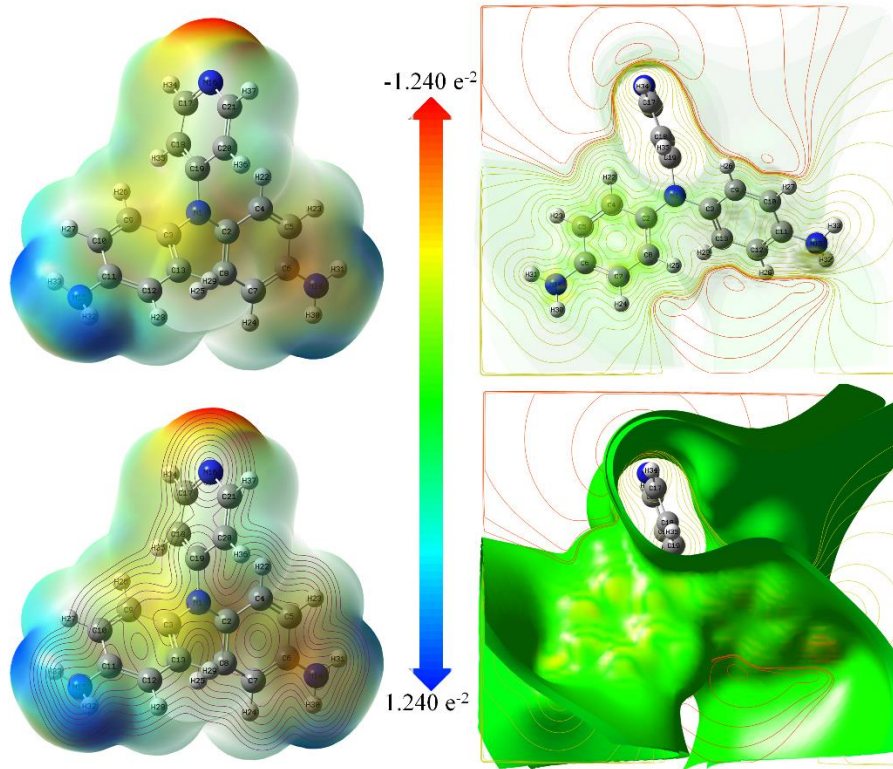
Şekil 5. Diamin molekülü için TD-DFT yöntemi ile A) B3LYP ve B) B3PW91 temel seti kullanılarak hesaplanan HOMO-LUMO enerji haritaları ve bant genişliği.

Tablo 1. HOMO, LUMO, enerji boşlukları (HOMO-LUMO) ve diamin bileşiğinin özelliklerinin karşılaştırılması

Moleküllerin Enerjisi	TD-DFT B3LYP/6-311G (d,p)	TD-DFT B3PW91/6-311G (d,p)
E_{LUMO}	-1.4400	-1.4604
E_{HOMO}	-4.0793	-4.2339
E_{LUMO+1}	-0.6090	-0.6171
E_{HOMO-1}	-5.8571	-5.5915
Enerji Boşluğu (Δ)= $ E_{HOMO} - E_{LUMO} $	2.6393	2.7734
İyonlaşma Potansiyeli ($I = -E_{HOMO}$)	4.0793	4.2339
Elektron ilgisi ($A = -E_{LUMO}$)	1.4400	1.4604
kimyasal sertlik ($\eta = (I - A)/2$)	1.3196	1.3867
kimyasal yumuşaklık ($s = 1/2 \eta$)	0.6598	0.6933
Kimyasal potansiyel ($\mu = -(I + A)/2$)	-2.7596	-2.8471
Elektronegatiflik ($\chi = (I + A)/2$)	1.2200	1.2302
Elektrofilite indeksi ($\omega = \mu^2/2 \eta$)	2.8854	2.9228

Moleküler elektrostatik potansiyel (MEP)

Molekölün boyut ve elektrostatik potansiyel deęerleri ve diamin molekölü için hesaplanmıřtır. Moleküler elektrostatik potansiyel (MEP) haritası, moleküler yapının fizikokimyasal özelliklerinin araştırılmasında çok faydalıdır (Buvaneswari et al. 2021; Shukla and Yadava 2022). Negatif elektrostatik potansiyele sahip bazı moleküller elektrofilik saldırıya karşı hassastır. MEPS haritasındaki mavi ve kırmızı bölgeler, pozitif ve negatif potansiyel bölgelere yanıt verir ve sırasıyla elektronca zengin ve elektronca fakir olan bölgelere atıfta bulunur. Yeřil renk, nötr elektrostatik potansiyeli gösterir. Bu çalışmada, diamin molekölü için moleküler elektrostatik potansiyel (MEP) haritaları çıkartıldı. Şekil 3'te gösterildięi gibi Diamin molekölünün nitrojen atomlarının etrafındaki nispeten daha büyük bir bölge, en negatif potansiyel bölgeyi (koyu kırmızı) belirtir ve etkileşime izin verilir. Hidrojen atomu, pozitif yükün (koyu mavi) maksimum gücünü taşır. Aromatik halka bölgesinin çoęu yeřil ile temsil edildięinden, neredeyse nötr bir potansiyel gösterir.



Şekil 6. Diamin molekölünün çeřitli MEP haritaları

Moleküler Doking Çalışmaları

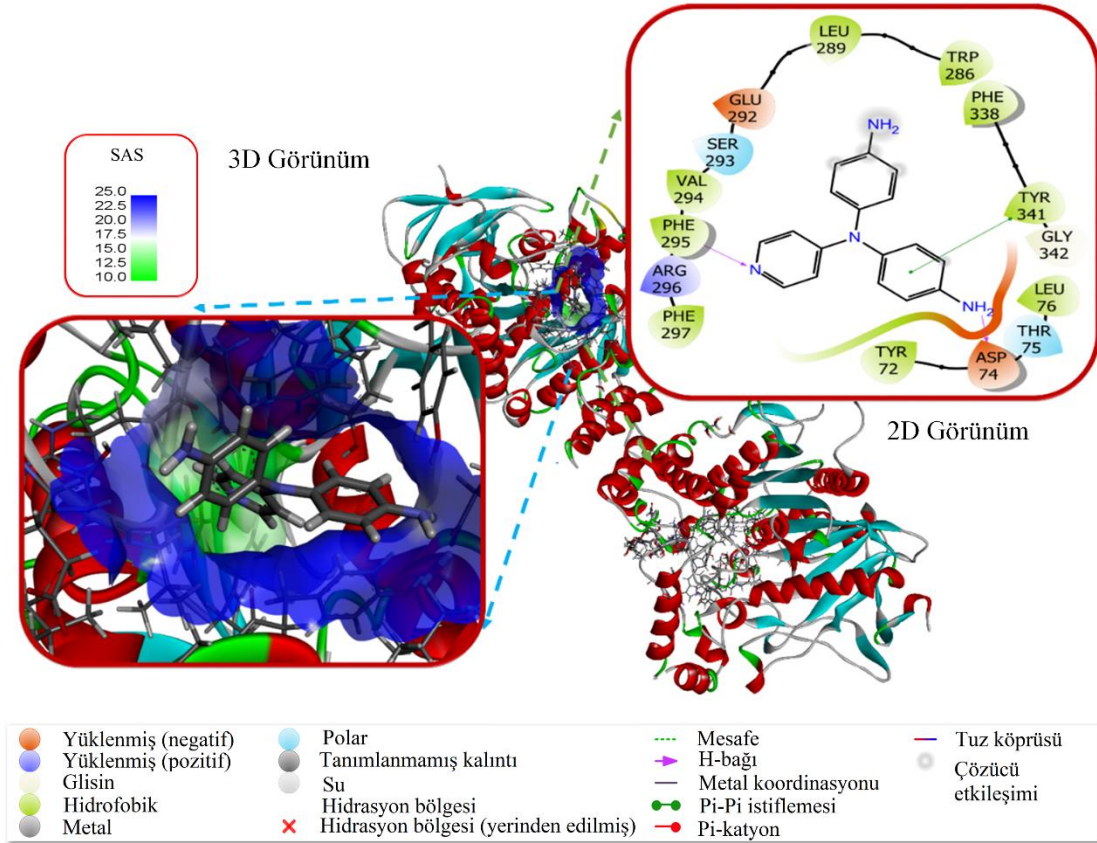
Bu inhibitör tasarımı çalışmasında reseptör olarak butirilkolinesteraz (PDB ID: 6SAM), asetilkolinesteraz (PDB ID: 4EY7) ve SARS-CoV-2 (PDB: 7RN4) reseptörleri kullanılmıřtır. Protein yapıları indirildi (RCSB). (see<http://www.rcsb.org/pdb>). Maestro version 11.8. Maestro

yazılımının Protein Prep., Grid Gen ve Lig Prep modülleri kullanılarak yerleştirme hazırlıkları yapıldı. Ligand-protein etkileşimi ile ilgili literatüre göre yerleştirme çalışmaları yapıldı (Alshehri et al. 2022; Fatriansyah et al. 2022; Kalaimathi et al. 2021). Maestro programında Glide yerleştirme alt programı ile moleküler yerleştirme analizleri yapılmıştır. In silico çalışma görüntüleri Discovery Studio 2017 ile sunuldu (Biovia 2017).

Docking yerleştirme, Alzheimer hastalığında yer alan AChE ve BChE proteinlerindeki bağlanma pozlarını kullanarak bir enzim inhibitörü olarak performansını belirlemek için faydalıdır.(Cheng et al. 2019; Larik et al. 2020; Aras et al. 2021). COVID-19 pandemisinde yer alan esnek SARS-CoV-2 proteinleri (Alghamdi et al. 2021; Hall-Swan et al. 2021). Ayrıca teorik olarak covid proteininin etkisinin belirlendiği in silico çalışması yapılarak iyi bir docking skoru elde edildi. Bu COVID-19 ve Alzheimer hastalığı tedavileri için reseptörlerine bağlanma gerçekleştirilmiş olup, yerleştirme sonuçları Tablo 4'te verilmiştir.

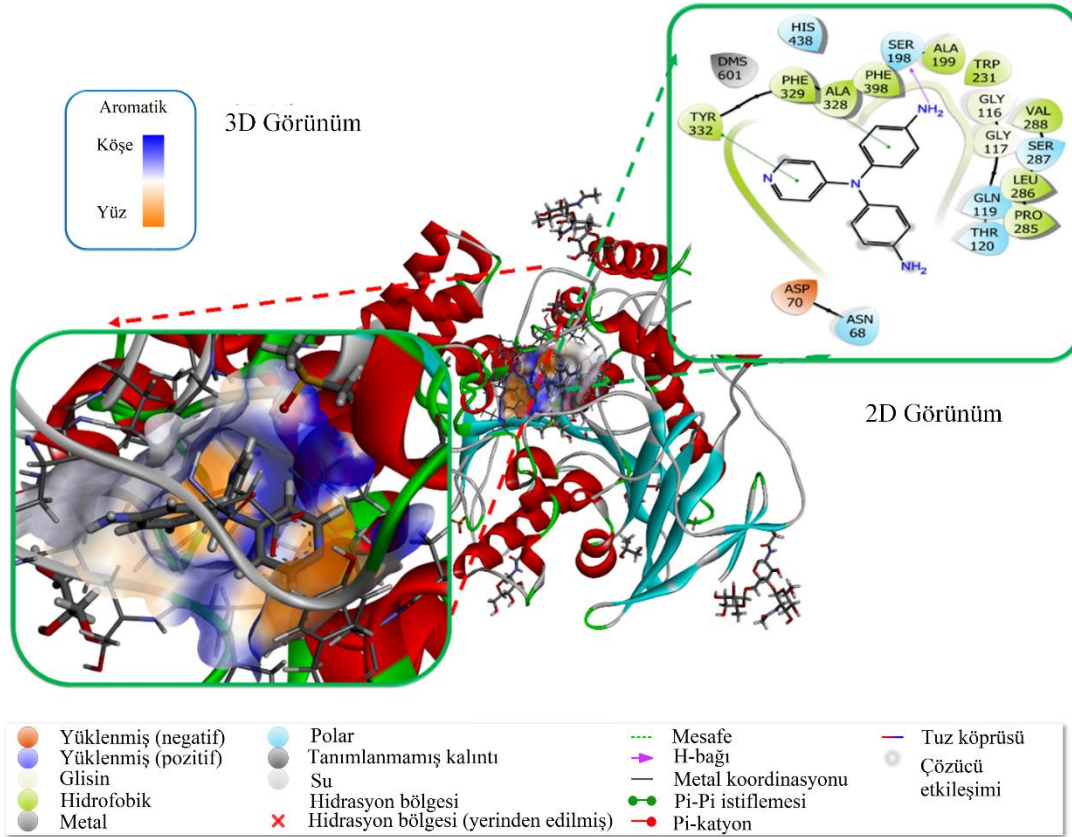
Tablo 4. Ligand-protein bağlanma afinite skorları (kcal/mol).

Bileşik	Docking Skorları (kcal/mol)		
	AChE (PDB: 4EY7)	BChE (PDB: 6SAM)	SARS-CoV-2 (PDB: 7RN4)
3	-7.534	-6.653	-6.428



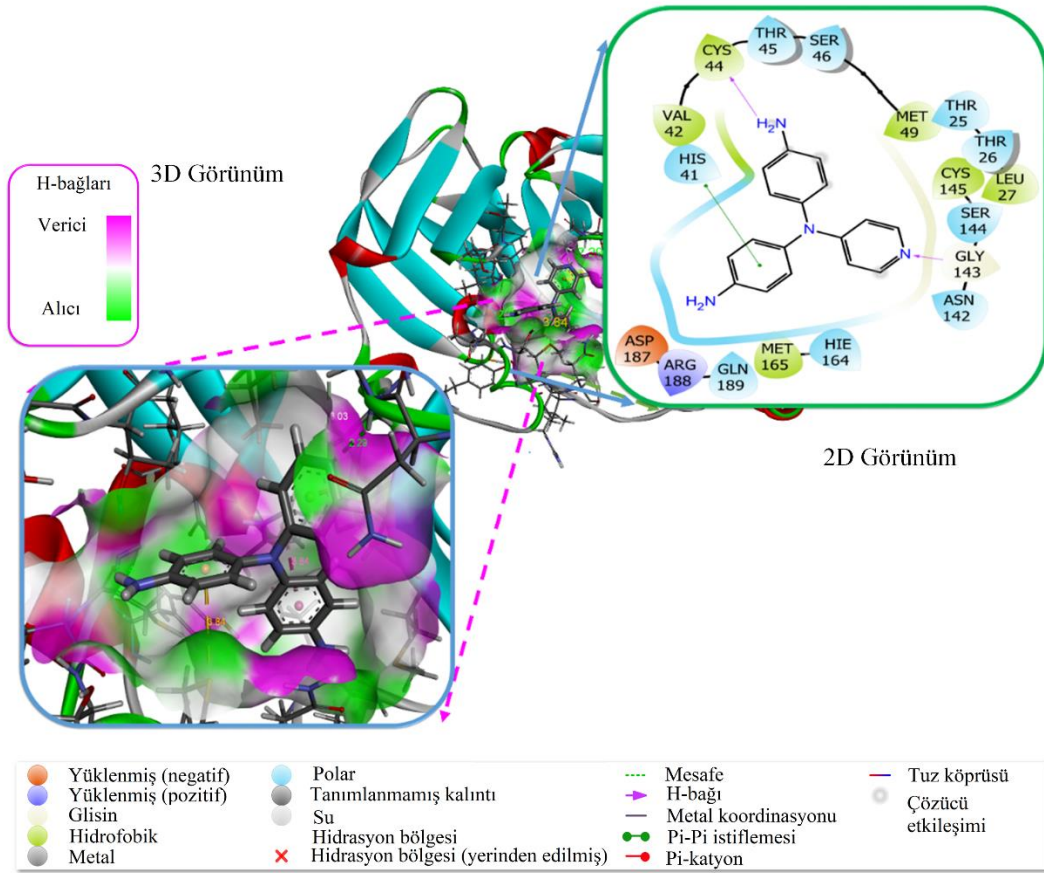
Şekil 7. 3D Görünüm ve 2D görünüm piridin diamin etkileşim modunu -AChE protein (4EY7).

Asetilkolinesteraz (asetilkolinesteraz) (AChE) inhibitörleri genellikle Alzheimer hastalığının neden olduğu demans semptomlarını iyileştirmek için kullanılır (Franklin et al. 2016; Adiguzel et al. 2021; Yildiko et al. 2021). Ftalonitrilin AChE inhibitörü olarak değerlendirdiğimiz çalışmamızda iyi bir bağlanma performansı göstermiştir ve -7.534 kcal/mol doking skoru hesaplandı. Bu bağ, VAL 294, GLY 342 LEU 76 ve THR 75 van der Waals, ASP 74 3.35 VE PHE 295 5.39 geleneksel Hidrojen Bağı, ARG 296 5.21 karbon-hidrojen bağı, , TYR 341, 4.86 π - π bağlanma ve SER 293 6.36 π T şeklindedir ve TRP 286 6.29-6.27 amide- π yığılmıştır. Bileşik 3 ve AChE proteininin aromatik yüzey yoluyla en iyi pozda bağlanması haritalandı ve Şekil 8'de gösterildi.



Şekil 8. 3D Görünüm ve 2D Görünüm Diamin Piridin - BChE arasındaki etkileşim modunu (6SAM)

Alzheimer hastalığına karşı yapılan çalışmalarda, Triptofan bazlı seçici nanomolar butirilkinesteraz (BChE) proteini olarak birçok kristal yapı bulunmaktadır (Meden et al. 2019; Yildiko et al. 2021). Sentezlenen Diamin Piridin bileşiğinin insan BChE'si ile yeni bağlanma modunu gösteren bir yerleştirme çalışması yapıldı ve -6.653kcal/mol 'lük bir yerleştirme puanı hesaplandı. Bağlanma performansı, bileşiğin in siliko inhibisyonu için çok başarılı bir sonuçtur (Atalar et al. 2021). Alzheimer hastalığına karşı semptomatik tedavi için bir ligand olarak kabul edilebilir. Protein-ligand etkileşimi TRP 231 $3.41 \pi-\pi$ yığılmış, TYR 332 4.30 ve PHE 329 $6.76 \pi-\pi$ T-şekilli, SER 198 3.80 karbon-hidrojen bağı, DMS 601 5.29π -sülfür bağlanması, ALA 199, GLY 117, LEU 286 THR 120 VE ASN 68 van der Waals bağları ligand yerleştirme ile hesaplandı. Bir inhibitör olarak çalışan ve BChE omurgasına bağlanan diamin piridin, hidrojen bağları protein yüzeyi ile eşleştirilir ve Şekil 9'da gösterilir.



Şekil 9. 3D Görünüm ve 2D Diamin Piridin-SARS-CoV-2 enziminin (7RN4) etkileşim modunu görüntüleyin.

Tüm dünyada etkili olan ve şiddetli akut solunum yolu sendromu gösteren Coronavirüs 2 (SARS-CoV-2) proteinlerine uygun küçük moleküllü antivirallerin sentezlenmesi bu hastalıkla mücadelede çok önemli COVID-19. Ligand bağlanmasında etkili olan SARS-CoV-2, enzim inhibitörlerinin performansını belirlemek için kullanılan bir ilaç proteindir. Enzimin substrat bağlama bölgeleri üzerinde yerleştirme çalışmaları yaptık. Diamin Piridin bileşiği ve SARS-CoV-2 ana proteaz enzimi yerleştirme puanı -6.428 kcal/mol'lük değerle etkileşime girdi. Bu etkileşimler sırasıyla; Ligand-protein etkileşimi, GLY 143 2.29 ve CYS 44 2.29 geleneksel hidrojen bağ, HIS 41 3.71 π - π yığılı, CYS 145 ve MET 49 3.84 π -sülfür bağlanma, LEU 24, THR 25, ASP 187 van der Waals bağlamaları yapıldı. Şekil 10'da proteinin katalitik bölgesine esnek bir şekilde bağlıdır.

TARTIŞMA VE SONUÇ

Bu çalışmada *N,N*-Bis(4-aminofenil)piridin-4-amin bileşiğinin sentezi ve teorik çalışmaları yapılmıştır. THBF molekül yapısı için kuantum kimyasal hesaplamalar kullanılarak detaylı araştırma yapılmış, bileşiğin elektronik titreşim frekansları B3LYP/6-311G(d,p) ve B3PW91/6-

311G(d,p) temel seti ile TD-DFT yöntemi ile hesaplanmıştır. Yapısal parametreler (bağ uzunlukları, bağ açıları ve dihedral açıları) teorik olarak belirlendi. Doğrusal olmayan optik özellikler araştırıldı. Son olarak, incelenen bileşiğin, MEPS'in yanı sıra doğrusal olmayan optik (NLO) malzeme olarak kullanılabilmesi sonucuna varılmıştır. HOMO-LUMO haritaları ve mulliken görselleştirilirken ücretler görselleştirilir. Sentez için iyi bir ara malzeme olarak mevcuttur. Bu nedenle gelecekteki sentez çalışmaları için bu verileri dikkate almak gelecekte sentezlenecek moleküllerin kimyasal tüketimlerinin en aza indirilmesi ve gerekli öngörülerin yapılması moleküllerin sentezi için önemli kolaylıklar sağlayacaktır. COVID-19 pandemisinde yer alan esnek SARS-CoV-2 proteinleri ile inhibisyon etkisinin belirlendiği in silico çalışması yapılarak iyi bir docking skoru elde edildi.

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**EFFECTS OF EXOGENOUS SOME POLYAMINE BIOSYNTHESIS INHIBITORS
ON SOME PHOTOSYNTHETIC PARAMETERS UNDER DROUGHT STRESS**

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ÖZET

Kuraklık stresi altındaki mısır fidelerinde poliamin biyosentez inhibitörlerinin fotosentez üzerindeki etkileri incelenmiştir. Bunun için, arginin dekarboksilaz (ADC) ve ornitin dekarboksilazın (ODC) yarışmalı iki inhibitörü olan DL-a-diflorometilarginin (5 uM, DFMA) ve DL-a-diflorometilornitin (5 uM, DFMO) ve tüm gruplarda kuraklık etkisi olarak polietilen glikol (%3, PEG₆₀₀₀) kullanıldı. Kuraklık stresi altında fotosentez hızı (A), terleme hızı (E), hücreler arası CO₂ konsantrasyonu (C_i) ve stoma iletkenliği (gsw) gibi gaz değişim parametrelerinin içeriklerinin azaldığı görüldü. PEG ile karşılaştırıldığında DFMA+DFMO uygulaması gaz değişim parametrelerinin seviyelerini daha da azalttığı belirlendi. Bununla birlikte, fotosentetik enerji dönüşümünün etkili kuantum verimi (ΦPSII) ve PS II'nin maksimum kuantum verimi (F_v/F_m) kuraklık stresi ile azaldı ve bu azalış DFMA+DFMO uygulamasıyla da daha önemli hale geldi. Sonuçlar, kuraklık stresi altında dıştan DFMA+DFMO uygulamasının fotosentetik parametreleri, poliamin içeriğini azalttığını ve bu inhibitörlerin fotosentez mekanizması üzerinde önemli derecede olumsuz etkiye sahip olduğunu göstermektedir.

Anahtar Kelimeler: DL-a-diflorometilarginin, DL-a-diflorometilornitin, fotosentez, klorofil floresan, poliaminler

ABSTRACT

The effect of polyamine biosynthesis inhibitors on the photosynthesis response of maize seedlings was investigated under drought stress. For this, DL- α -difluoromethylarginine (5 μ M, DFMA) and DL- α -difluoromethylornithine (5 μ M, DFMO), two competitive inhibitors of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC), were used. Polyethylene glycol (% 3, PEG₆₀₀₀) was used as the drought effect in all groups. The changes in gas exchange parameters including photosynthesis rate (A), transpiration rate (E), intercellular CO₂ concentration (C_i), and stomatal conductance (gsw) decreased under drought. The

DFMA+DFMO application further reduced levels of the gas exchange parameters compared with the PEG. Moreover, the effective quantum yield of photosynthetic energy conversion (Φ_{PSII}) and maximum quantum efficiency of PS II (Fv/Fm) decreased with the drought stress, and this decrease was highly significant in the applications with DFMA+DFMO. The results suggest that under drought stress, exogenous DFMA+DFMO application decreased the photosynthetic parameters, polyamine content and the inhibitors have a strong negative effect on the photosynthesis mechanism.

Keywords: DL- α -difluoromethylarginine, DL- α -difluoro methyl ornithine, chlorophyll fluorescence, photosynthesis, polyamines

GİRİŞ

Agro-meteorolojik tehlike, dünyanın birçok bölgesinde bitki büyümesi ve gıda üretimi için ana sınırlayıcı faktördür. Tarımsal meteorolojik tehlikeler arasında verim stabilitesi üzerinde en büyük etkiye kuraklık sahiptir (Vinocur ve Altman, 2005; Li vd., 2000). Ortalama verimi %50 veya daha fazla azaltarak tahıl kalitesini ve tahıl eldesini ciddi şekilde etkileyebilir (Wang vd., 2003). Kurak ve yarı kurak iklimlerde, toprak su kaynağındaki azalma veya yüksek terleme oranı bitkilerde kuraklığa neden olabilir (Somerville vd., 2001). Kuraklık stresi, hücre içinde meydana gelen bir dizi metabolik reaksiyonu olumsuz yönden etkilemesinin yanı sıra (Ashraf ve Foolad, 2007), fotosentez, kuraklıktan büyük ölçüde etkilenen en önemli metabolik süreçlerden biridir (Kalaji vd., 2016). Sonucunda, bitkilerde stoma iletkenliğinin (gs) azalması ve stomaların kapanması durumu meydana gelir (Ali ve Ashraf, 2011). Hem stomaların kapanması hem de metabolik bozuklukların meydana gelmesi fotosentez için önemli bir sınırlama olduğu bilinmektedir. Fotosentez engellendiğinde, bitkinin fotosentetik karbon fiksasyonu yoluyla tüketilebilecek olandan daha fazla ışık enerjisi emmesiyle sonuçlanması muhtemeldir (Jedmowski vd., 2013). Bu aşırı enerji, PSII ve PSI arasındaki elektron taşıma zincirinin aşırı azalmasını tetiklemektedir.

Kuraklık sırasında fotosentetik aygıtın ışık reaksiyon sistemlerinde hasar olup olmadığını belirlemek mümkündür (Jones, 2005). Klorofil floresansının ölçülmesi bu anlamda fotosentez hakkında hızlı kalitatif ve kantitatif bilgi elde etmede çok kullanışlı bir teknik haline gelmiştir (Roháček, 1999). Fotosistem II (PSII) reaksiyon merkezinin yapısı ve işlevi arasındaki ilişki hakkında bilgi sağlayabilmektedir (Rosenqvist ve Van Kooten, 2003). Bununla birlikte, farklı ortamlarda PSII'nin işlevindeki değişiklikleri değerlendirmek için yararlı, güçlü ve güvenilir bir tekniktir (Colom, 2003; Mauchamp ve Methy, 2004). Fotosistemlerin kompozisyonunu ve organizasyonunu, uyarma enerjisi transferini, fotokimyası ve çeşitli streslerin bitkiler üzerindeki etkilerini kontrol edebilir (Yang, 2009). Klorofil floresansı, kuraklık stresi altındaki birçok bitkinin fotosentetik performansı hakkında faydalı bilgiler sağlar (Baker ve Rosenqvist,

2004). Yapılan çalışmalar kuraklığa maruz kalan mısır yapraklarında fotosentez hızı, terleme hızı, stoma iletkenliği ve fotokimyasal verimin önemli ölçüde azaldığını göstermiştir. (Song vd., 2019).

Poliaminler (PA'lar), birçok bitki fizyolojik sürecine ve çevresel stres faktörlerinin cevabına aracılık eden içsel bitki büyüme düzenleyicileridir (Yang vd., 2008). Putresin (put), spermidin (spd) ve spermin (spm) gibi poliaminler, yüksek bitkilerde birçok fizyolojik süreçte yer alan temel nitrojen bileşikleridir (Bouchereau vd., 1999, Martin-Tanguy 2001). Put biyosentezinde arginin dekarboksilaz (ADC) ve ornitin (Orn) dekarboksilaz olmak üzere iki ana sentez enzim görev almaktadır. Put biyosentezinin ardından, Spd sentaz, Spm sentaz tarafından Spm'ye dönüştürülen triamin Spd üretimini katalize eder (Chen vd., 2019; Yu vd., 2019). Çeşitli çevresel stress koşulları altında PA içeriğindeki değişiklikler birçok türde rapor edilmiştir (Flores 1991). *Arabidopsis thaliana* model bitkisinde, çevresel stres toleransının poliamin içeriği ile ilişkili olduğu bulunmuştur (Cuevas vd., 2008, Alcázar vd., 2010). PA'ların biyosentezi, sırasıyla ADC ve ODC'nin geri dönüşümsüz rekabetçi inhibitörleri olan diflorometilarginin (DFMA) ve diflorometilornitin (DFMO) tarafından inhibe edilebilir (Grossi vd., 2016; Yamamoto vd., 2017). Poliaminlerin abiyotik stresle olan ilişkilerini incelemek için, dışarıdan poliamin uygulaması veya poliamin biyosentezini bloke eden inhibitörlerin uygulanması gibi birçok yaklaşımlar da mevcuttur (Groppa ve Benavides, 2008; Moschou vd., 2012). Bu nedenle bu çalışmada poliamin biyosentez inhibitörlerinden diflorometil arjinin (DFMA) ve diflorometil ornitin (DFMO) kullanılarak mısır fidelerinde fotosentetik mekanizma üzerindeki olası etkisi araştırılmıştır.

MATERYAL ve METHOD

Bitki Büyüme Koşulları ve Uygulamalar

Denemelerde bitkisel materyal olarak kullanılan Akpınar mısır çeşidi (*Zea mays* L.) tohumları, Karadeniz Tarımsal Araştırma Enstitüsü'nden temin edildi. Tohumlar, her saksıya 6 tohum olacak şekilde toprak içeren saksılara (25×18×12 cm) ekildikten sonra, dört hafta süreyle , % 55-60 nem, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ışık şiddeti, 25 °C \pm 2 sıcaklık ve 16 saat aydınlık; 8 saat karanlık periyodunda bitki büyütme odasında yetiştirildi. Bitkilere dört hafta süreyle iki günde bir 200 ml su verildi. Dört haftalık süre sonunda, mısır fideleri topraktan 2 cm yükseklik olacak şekilde gövdelerinden kesilerek saf su içeren cam tüplere aktarıldı. Yaralanma stresinin etkilerini yatıştırmak amacı ile bir saat boyunca saf suda bekletilen fideler kuraklık etkisini oluşturmak için 6 saat boyunca polietilen glikole (%3'lük PEG 6000 ve -0.3 MPa) maruz bırakıldı. Kontrol grubu olarak saf su kullanıldı ve diğer gruba PEG'e ek olarak poliamin sentezini inhibe eden diflorometil arjinin (DFMA) ve diflorometil ornitin (DFMO) inhibitörleri 5 μM 'lık

derişimlerde birlikte uygulandı. Örneklemeler için tabandan itibaren seçilen 3. yapraklar, sonraki çalışmalar için sıvı azottan geçirilerek -80 °C de saklandı.

Poliamin Tayini

Poliamin içeriđi Flores ve Galston, (1982) metoduna göre ölçüldü. Taze yaprak dokusu (5 g) 10 ml 0,4 M perklorik asit ile homojenize edildi. Homojenat, 4 °C de 3000 g de 10 dk santrifüj edildi. Süpernatant toplandı, üzerine 10 ml 0,4 M perklorik asit ilave edildi ve tekrar santrifüjlendi. Süpernatantlar birleştirildi ve son hacim perklorik asit ile 25 ml'e tamamlandı. Süpernatant Whatmann filtre kađıdı ile süzüldü. Süpernatantın 1 ml'si üzerine 200 µl 1 M NaOH ve 300 µl sodyum hidrojen karbonat ilave edildi ve karışım 30 saniye vorteksle karıştırıldı. Bu karışımın üzerine 2 ml dansil klorit ilave edildi. Karışım 40 °C sıcaklığında 45 dk inkübe edildi. Reaksiyon 100 µl % 25 amonyum hidroksit ile durduruldu. Karışım 30 dk oda sıcaklığında bekledikten sonra hacim asetonitril ile 5 ml'e tamamlandı. Bu karışım 2500 g' de 5 dk santrifüj edildi. Süpernatant 0,22 µm'lik filtrelerden geçirildi ve HPLC'ye yüklendi. Poliamin içeriđi UV/VIS detektör ile HPLC cihazıyla (Shimadzu, LC 20 AT/Prominence, Japan) okundu. Çözücü miktarları amonyum asetat: asetonitril (65:35 v/v), akış hızı 0,70 ml dk-1 ve 50 °C biçiminde olacak şekilde kullanıldı. 20 µl ekstre, C18 (4.6 × 250 mm) kolonuna enjekte edildi ve 254 nm dalga boyunda belirleme yapıldı. Piklerin alanları kaydedildi ve bir bilgisayar yazılımı ile putresin, spermin ve spermidin konsantrasyonları hesaplandı. Sonuçlar gram taze ağırlık başına µg olarak ifade edildi.

Gaz Deđişim Parametrelerinin Ölçümü

Fotosentetik hız (A), stoma iletkenliđi (gsw), terleme (E) ve hücreler arası CO₂ konsantrasyonu (Ci) gibi fotosentetik gaz deđişim parametreleri taşınabilir bir fotosentez sistemi (LI-6400 XT, LI-COR Inc. ABD) kullanılarak ölçüldü. PPF (1000 µmol m⁻²s⁻¹), CO₂ konsantrasyonu (400 ± 10 µmol mol⁻¹), hava sıcaklığı (20 ± 2 °C), bađıl nem (RH) (%60–70) ve hava akış hızı (500 µmol m⁻² s⁻¹) deđerleri örnek başlık içinde sürdürüldü.

Klorofil Floresans Parametrelerinin Ölçümü

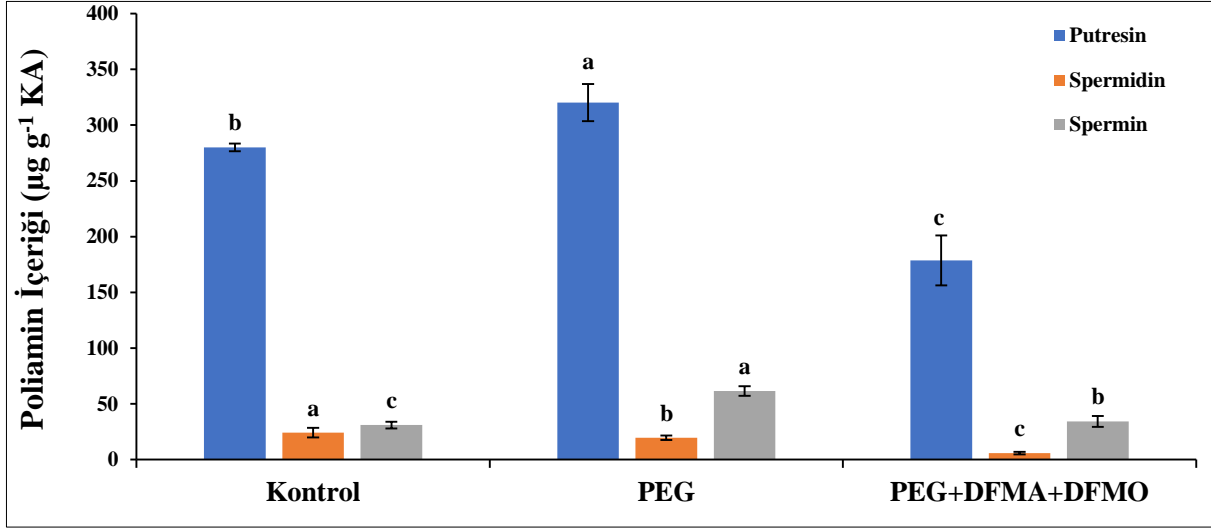
Klorofil floresansı, bir florometre (OS1-FL, OptiScience Corporation, Tyngsboro, MA, ABD) kullanılarak ölçüldü. PSII fotokimyasının (Fv/Fm) maksimum kuantum verimi ile PSII fotokimyasının (ΦPSII) etkili kuantum verimi van Kooten ve Snel (1990) tarafından açıklanan yöntem kullanılarak ölçülmüştür.

İstatistik Analizler

Üç tekerrürlü olarak gerçekleştirilen ekstraksiyon ve analizlerin sonucunda elde edilen verilerin varyans analizi Statistical Package for Social Sciences (SPSS for Windows 16.0) paket programı içerisinde yer alan Duncan Çoklu Karşılaştırma Testi'ne göre belirlendi.

BULGULAR**Dıştan uygulanan DFMA+DFMO'nun Poliamin İçeriği Üzerine Etkisi**

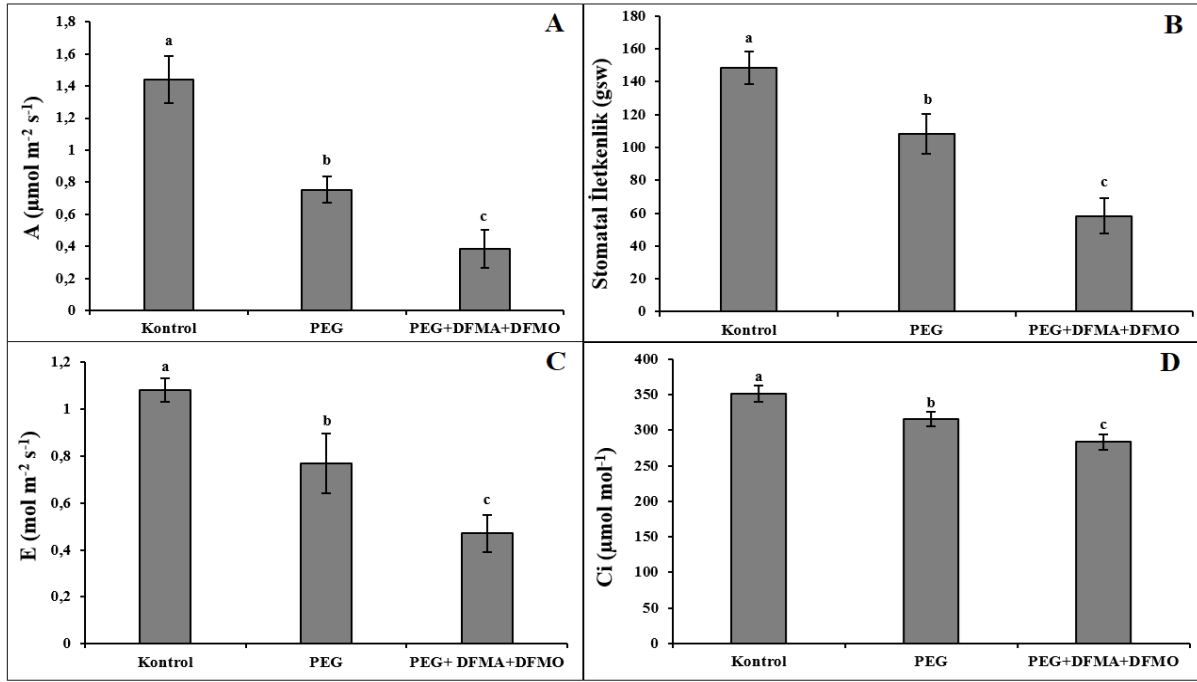
Kontrole göre, PEG uygulamasının poliamin içeriklerinden özellikle putresin ve spermin içeriğini önemli derecede arttırırken, spermin içeriğini azalttığı görüldü. DFMA+DFMO uygulamasının ise PEG grubuna kıyasla poliamin içeriklerini (putresin, spermidin, spermin) düşürdüğü belirlendi (Şekil 1).



Şekil 1. 5 µM DFMA+DFMO uygulamasından sonra poliamin içeriğindeki değişiklikler

Dıştan uygulanan DFMA+DFMO'nun Gaz Değişim Parametreleri Üzerine Etkisi

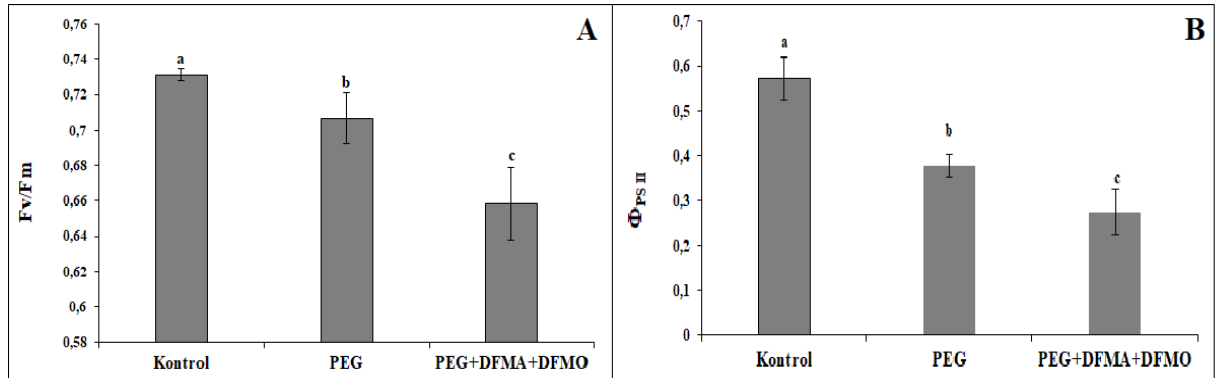
Kontrol grubuna kıyasla tüm PEG uygulanmış fidelerde net fotosentez hızı (A), stoma iletkenliği (gs), terleme (E) ve hücreler arası CO₂ konsantrasyonunun önemli derecede düştüğü tespit edildi. Bununla birlikte 5 µM DFMA+DFMO uygulaması, PEG grubuna kıyasla A, gs, E ve Ci değerlerini daha da azalttığı belirlendi (Şekil 2ABCD).



Şekil 2. Kuraklık stresi altında 5 μM DFMA+DFMO uygulamasının gaz değişim parametreleri üzerine etkisi

Dıştan uygulanan DFMA+DFMO'nun Klorofil Floresansı Üzerine Etkisi

Kontrol grubuna kıyasla PEG uygulanan fidelerde F_v/F_m 'de ve Φ_{PSII} 'de bir azalış görüldü. Bununla birlikte PEG grubuna göre 5 μM DFMA+DFMO uygulanmış fidelerde de F_v/F_m ve Φ_{PSII} değerlerinde önemli azalış kaydedildi (Şekil 3A, 3B). En fazla azalış DFMA+DFMO uygulanmış fidelerde belirlendi.



Şekil 3. Kuraklık stresi altında 5 μM DFMA+DFMO uygulamasının klorofil floresans üzerine etkisi

TARTIŞMA

Fotosentez, bitkilerin organik bileşiklerin sentezini yürütmek için ışık enerjisini kullandığı fiziko-kimyasal süreçtir ve bitki üretiminin temelidir (Xu ve ark. 2014). Kuraklık, fotosentezi engelleyen ciddi bir çevresel strestir. Stoma kapanmasının neden olduğu karboksilasyon bölgesine ortamdaki CO₂ difüzyonunun sınırlandırılması, genellikle su stresi altında fotosentez hızındaki düşüşün ana nedeni olarak kabul edilir (Chaves vd., 2009). Bu çalışmada, kuraklığa maruz kalan mısır fidelerinde sırasıyla poliamin içeriğini arttırdığı, ancak fotosentetik hızı (A), stoma iletkenliği (gsw), terleme (E) ve hücreler arası CO₂ konsantrasyonu (Ci) gibi fotosentetik gaz değişim parametrelerinin içerikleri ile Fv/Fm ve PSII'nin etkili kuantum verimini önemli derecede azalttığını göstermektedir. DFMA+DFMO uygulaması sonucu mısır fidelerinde poliamin seviyeleri baskılandığı ve gaz değişim parametreleri ile klorofil floresans parametrelerinin içeriklerini daha da düşürerek inhibitörlerin su eksikliği stresi altındaki fidelerde fotosentezin etkinliğini veya fotosentetik hasarı olumsuz etkileyebileceği belirlenmiştir. Çalışmamıza benzer olarak, literatürde de farklı stress koşulları altında DFMA+DFMO inhibitör uygulamasının fotosentez etkinliğini azalttığı çalışmalar mevcuttur. Örneğin, Yamamoto vd., (2017) yaptıkları bir çalışmada tuz stresi altında pirinç fidelerinde DFMA+DFMO uygulamasının PSII'nin verimini azalttığı tespit etmiştir.

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**VERSATILE SLIDING MODE CONTROL OF PARTICULAR SELF-
RECONFIGURABLE SPACE APPARATUS WITH TIME DEFER ASSESSMENT BY
AI TECHNOLOGY**

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ABSTRACT

The multiplication control of disconnected self-reconfigurable space mechanical assembly is tended to using an adaptable sliding mode control scheme reliant upon time-defer evaluation advancement. It can be measures through MARS Mission by taking information and that were instead of the ground, the underpinning of the self-reconfigure is floating when assembled in circle, achieving a strong one of a kind coupling sway. A period concedes appraisal advancement system with extraordinary showing up at regulation is expected to achieve high-precision worked with control between the rocket base and the robotized arm. Time-concede appraisal advancement is used by the controller to compensate for coupling terms and weaknesses, while self reconfigured can increment and further foster time-defer evaluation development's power. To cover time-concede appraisal advancement bumbles and crash chattering, one more flexible regulation is made to change gain limits web, ensuring quick strong response and high after accuracy. The latest man-made mental ability approach shows that the accompanying botches are reliably finally restricted finally; the on-circle gathering pattern of adaptable sliding is reproduced to endorse the sufficiency of the proposed control plan. The propagation results show that the proposed control technique can unequivocally complete the goal module's on-circle get together, with insignificant inconveniences to the rocket's mindset. In the meantime, it has a critical level of force and can effectively discard chattering.

Keywords: Multiplication, Time-Defer, Coupling, Adaptable Sliding, Chattering.

PAKISTANI MARITIME AND AERONAUTICAL REMOVAL RESOURCES CAN BE USED FOR THE MARINE AND SPACE EXPERIENCES BY AI TECHNOLOGY

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ABSTRACT

This paper presents another vision overall arranging structure in Pakistan for adaptable robot by removal airplane can be using and made modest type of room transport by the assistance of SUPARCO Engineer group as the fantasy can be valid when it will be finished by the assistance of Government support on that stage, inertial sensors, and dynamic sign. The proposed framework precisely closes versatile robot position and bearing involving various techniques for relative and overall position measures, and turns the camera towards the objective during speed by acoustic and Aerial climate for the appraisal through Machine Learning and Artificial Neural Network. Among the executed sensors, the encoder information gives decently positive robot improvement data next to while controls lose your equilibrium. The issue of give and take of sloppy information, while dynamic reference centers is slow when wandered from different sensors. The organized construction repays the sensors objectives and slip bungle by exchanging between two Matrices procedures, worked for slip and no-slip cases. Every framework utilizes various sensors mix and checks robot improvement freely. The slip identifier is utilized to see the slip condition by separating the information from the gas pedal and encoder to pick the either Matrix as the result of the framework. The paper is related on the audit set up and this individual based with respect to the proposed sensor mix technique, a dream overall arranging framework is executed on a two-wheeled robot. Exploratory outcomes portray that proposed framework can notice robot position inside an overall sense diminished position bungles and useful after of the objective for different circumstances and robot advancement conditions.

Keywords: Vision, Assistance, Equilibrium, Sloppy, Dynamic, Matrices Procedures.

**IMPROVING THE INFRASTRUCTURE OF A COMMUNITY PLAYGROUND
THROUGH CONSTRUCTION WORK**

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ABSTRACT

The purpose of this project was to build cafeteria at a local playground. 20 voluntary visitors were interviewed in order to elicit their feedback on the cafeteria particularly on the aspects of the structure that required improvements. The two primary objectives of this project were to build a concrete floor and to supervise the construction of tall pillars. The total cost of this project was RM 6098.00 and the actual cost of supervising the construction of the concrete pillars was RM 1655.5. The design of the project was developed via AutoCAD of 2010 version. The job specifications for the project were predetermined and agreed upon by the contractor and the client prior to its commencement. The schedule and list of duties were done in accordance with the project's Gantt Chart. The construction of the project began with the process of putting up barriers around the designated spot for the structure, information board and clearance of the area where the construction would take place in. This was followed by a routine procedure until the project was completed. Based on the assessment of the structure, the pillars were considered durable and resilient that they were strong enough to support the roof and there was no crack with the concrete floor. Overall, the project was a success and the construction process was consistent with the design and job specifications that had been agreed on by the contractor and the client. It was recommended that concrete mixer should be used in any construction work in order to reduce the time and effort that the bricklayers had to spend on preparing the concrete through manual method.

Keywords: Autocad, playground, cafeteria

**PRELIMINARY CHARACTERIZATION AND EVALUATION OF THE
POTENTIAL USE OF CLAY MATERIALS FROM NORTH-EAST MOROCCO IN
THE CERAMIC INDUSTRY**

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ABSTRACT

This study focuses on the characterization and evaluation of the potential use of clay-rich raw materials in the region of Nador (northeast, Morocco) to manufacture ceramic products. In this regard, physical (particle size distribution, organic matter), chemical (X-ray fluorescence, XRF), mineralogical (X-ray diffraction, XRD), and geotechnical (Atterberg limits) analyses were carried out on the materials studied. The results show that the main minerals in the samples were feldspars and cristobalite, while carbonates are ubiquitously present. Smectite was present as the dominant clay phase and minor phases of smectite kaolinite, illite and kaolinite, including chlorite appeared in some samples. The results showed that the studied clay materials require the addition of another type of clay or less plastic materials to improve their properties and to be suitable for the production of structural ceramics.

Keywords: clay, raw materials, ceramics, smectite.

**MINERALOGY AND GEOCHEMISTRY OF RHYOLITIC DOMES AND PERLITES
ROCKS IN THE TIDINIET MASSIF, (NADOR, NORTHERN MOROCCO)**

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ABSTRACT

Volcanic rocks of Tidiennit massif are located some 18 km southwest of Nador, Morocco. Volcanic product of the area exposed as acidic rocks include Miocene perlite and rhyolite and Messinien bentonite clay. Tectonic setting of the study area results from the convergence between the African and Eurasian lithospheric plates since the Cretaceous period. The rhyolites of Tidiennit are exposed in the form of domes as well-known as lava flows. All samples were subjected to a series of analytical methods for the determination of their mineralogical, petrography and geochemical characteristics: XRD, FTIR, optical microscopy, XRF. Mineralogically the perlitic and rhyolitic rocks include feldspar, plagioclase, quartz, biotite, hematite and illmenite. Petrography study show that the samples studied composed mainly of the sanidine, quartz, plagioclase and opaque minerals with microlithic and spherulitic texture. Geochemically indicate the rocks of Tidiennit calc-alkaline nature. Taken into consideration Statistical analysis show the correlations between the mineralogical and chemical composition related to perlite and rhyolite.

Keywords: Volcanic rocks, perlite, rhyolite, Cretaceous, African lithospheric plates.

**GAUSS HYPERGEOMETRIC GAMMA AND BETA FUNCTIONS WITH
PROPERTIES CONNECTING TO INTEGRAL TRANSFORMS**

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ABSTRACT

Special functions have extensive applications in pure mathematics and applied mathematics such as electrical current, fluid dynamics, heat conduction, solutions of wave equations, moments of inertia and quantum mechanics. These functions include the logarithmic, exponential, trigonometric, gamma, beta, zeta, and many other functions. The vast field of these functions contains many formulae and identities used by mathematicians, engineers, physicists and statisticians. The hypergeometric function lies in the heart of special function theory in which all classical special functions can be expressed in terms of this powerful function. Hypergeometric functions have explicit series and integral representations and thus provide ideal tools for establishing useful summation and transformation formulae. Also applied problems frequently require solutions of a function in terms of parameters, rather than in terms of variables, and such solutions are perfectly provided for by the parametric nature of the hypergeometric function. Consequently, the hypergeometric function can be used to solve physical problems in diverse areas of applied mathematics.

The gamma function was first introduced by the Swiss mathematician Leonhard Euler (1707 – 1783) in his goal to generalize the factorial to non integer values. Later, because of its great importance, it was studied by other eminent mathematicians like Adrien – Marie Legendre (1752-1833), Carl Friedrich Gauss (1777-1855), Christoph Gudermann (1798-1852), Joseph Liouville (1809-1882) Karl Weierstrass(1815-1897), Charles Hermite(1822-1901), ... as well as many others.

The Gamma function belongs to the category of the special transcendental functions and we will see that some famous mathematical constants are occurring in its study.

It also appears in various areas as asymptotic series, definite integration, hypergeometric series, Riemann zeta function, number theory.

In my research paper a Gauss hypergeometric Gamma and Beta functions have been defined.

Relationships of these functions with different integral transforms are evaluated. Also, differentiation formulas, recurrence relation and continuity are derived.

Keywords: Gauss hypergeometric function, Gamma and Beta functions, Laplace and Mellin Transforms.

**A MODIFIED BIPOLAR SOFT SETS BASED MODEL FOR SOLVING DECISION-
MAKING PROBLEMS**

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ABSTRACT

Bipolar soft sets (BSS) and rough sets (RS) are two practical approaches to deal with uncertainty. In 2018, Karaaslan and Cagman introduced the idea of bipolar soft rough sets (BSRSs), a hybridization of RS and BSS. There are certain shortcomings with BSRS, that violates Pawlak's RS theory. To address these deficiencies, this thesis introduces the notion of the modified bipolar soft rough set (MBSRS). Additionally, we analyze this concept by doing a careful inspection of the key properties using illustrative examples. Moreover, several key MBSRS-related metrics are included. Finally, we present an application of the MBSRS to decision making (DM) problems and give an algorithm for it, along with a practical example, that determines the optimal alternative among several possibilities. Additionally, we conduct a comparison study of the proposed model with BSRS.

Keyword: Bipolar soft set, Bipolar soft rough set, MBSR-approximations, MAGDM.

XENOTRANSPLANTATION

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ABSTRACT

To review the progress in the field of xenotransplantation with special attention to most recent encouraging findings which will eventually bring xenotransplantation to the clinic in the near future.

Starting of early 2000, with the introduction of genetic cloning in pigs, prolonged survival especially in heart and kidney xenotransplantation was recorded. However there are several modification done in pigs heart, to prevent rejection. Standard immunosuppressant used in human-to-human organ transplant aren't effective if immune system makes lots of antibodies. Most recently CRISPR technology made the production of genetically-engineered pigs easier and available to more researches. Today, the survival of pig-to-nonhuman primate heterotopic heart, kidney and islet xenotransplantation reached >900 days,>600 days,>400 days , respectively. The availability of multiple-gene pigs (5 or 6 genetic modification) significantly contributed to this success. Now the field is ready for more trials with an international consensus. Clinical trials for xenotransplantation are getting closer and in next decade will show us new achievements.

Keywords: Clinical, CRISPR, Genetic Cloning, Genetic Engineered, Xenotransplantation

BIOTECHNOLOGY

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ABSTRACT

Biotechnology is multidisciplinary field which has major impact on our lives and technology that utilizes biological system, living organisms or part of this to develop or create different product. Biotechnology is applied in every field such as agriculture, industry, medicine. Many of the problem facing humanity are same recurring challenges that man tackled for centuries. Biotechnology is a boon or a bane is topic which has been discussed for a considered period of time, right from the days of making wine to the recent days of BT crop .India has skewed distribution of natural resources, like land, biological divers, wild life forest, water etc. And hence Indian economy is highly independent on other geographical zone , for basic amenities like food and Electricity .A balanced approach on biotechnology provides pragmatic alternative and sustainable solution. Biotechnology- a fusion of life science and technology offers various various solutions. Over the years, judicious use of biotechnology has enriched food quality , agriculture productivity, miracle drugs, vaccines etc. and by large the impact has been positive in ecological stewardship , medical progress and social justice. One of the major areas of biotechnology contribution which has impact at global level is agriculture biotechnology which has given new hope to solve global food crisis. Biotechnology is emerging platform. The potential benefit include solving world food shorta ge and improvement in medicine and agriculture and Veterinary science. Based own our experience we see a great transformation in the way industrial chemical are being manufactured with conscious effort of reducing chemical toxics. . For the medical perspective there will always be more to learn and more advanced medical technology. The biotechnology industry greatly contribute to Research and Development prior to product manufacturing and marketing and research effort after the product is manufactures and approved by the New drug application.

Keywords: Multidisciplinary, Agriculture, Medicine, Geographical Zone, Research, Development, Veterinary.

**MEDICAL ETHICS AND MEDICAL EDUCATION: A NEW CHALLENGE IN
OUTGOING PANDEMIC**

Anisha Chauhan

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ABSTRACT

The main purpose of this paper is to analysis the importance of Medical ethics and Medical Education. Medical ethics improves the quality of patient care by identifying, analysing, and attempting to resolve the ethical problems arise in practice. Ambitious and diverse goals have been proposed for medical ethics education, including increased awareness of ethical issues; a cultivation a basic ethical commitments; more human medical practice; tolerance of conflicting views; development in analytic skill in moral reasoning; enhanced intellectual development in ethics and the humanities; positive attitudes toward patients. Medical education is , broadly, how we education physicians to care for patients. Medical education and training varies considerably across the world. Various teaching methodologies have been used in medical education, which is an active area of educational research.Both medical education and research are primarily directed at providing benefits to society as a whole. With research, society benefits from contributions to medical knowledge; with education, it benefits from the production of well trained doctors. Both medical education and research May also benefit participating individuals. Patients May benefit from participating in research by gaining access to experimental treatments and from closer follow up.

Keywords: Medical , Medical education, Health, medical ethics, Doctors.

BIOTECHNOLOGY AND ITS APPLICATIONS: A REVIEW

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ABSTRACT

This paper aimed at evaluating biotechnology with respect to its applications. Major areas of applications identified in the literature are environment, medicine, agricultural, food processing and industry. It was observed that the areas and scope of application of biotechnology would broaden with the respect to advancement in science. It was concluded that as the scope of biotechnology application broadening, research works should focus on the risks and challenges identified, especially in agricultural application. Biotechnology is the use of living organisms to develop or make useful products, or any technological application that uses biological systems used for improvements. The exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms for the production of antibiotics, hormones, etc. This technology harnesses cellular and biomolecular processes to develop technologies and products that help improve our lives and the health of our planet.

Technique #1. Recombinant DNA technology

Genetic engineering deals with synthesis of artificial gene, repair of gene, combining of DNA from two organism and manipulating the artificial gene together with the recombinant DNA for the improvement of microbes, plants, animals and human beings.

Isolation of DNA segment

The DNA to be used as vehicle or passenger is taken out from the cell by lysing it with a suitable enzyme. The DNA is isolated from other cell contents. Then both vehicle and passenger are cleaved by using the same restriction endonuclease so that they have complementary sticky ends.

Formation of Recombinant DNA

The complementary sticky ends pair and their ends are sealed with ligase. This produces a recombinant DNA.

The use of biotechnology in animals production has advanced more quickly than its applications in plant production. Worldwide, more than one -half of all biotechnology research and development expenditures are in the field of human health. At the experimental stage, a large number of drugs, diagnostic probes, vaccines and so on are frequently applied in livestock production prior to becoming available for use by humans. Development in the pharmaceutical

industry, therefore, have had considerable ramifications for animal production since many innovations in this area are also applicable to animal

Keywords: Biotechnology application, environment, medicine, agriculture, food processing and industry

**AN ANALYTICAL STUDY OF HEALTH TOURISM INDUSTRY – A STUDY ON
POTENTIALITY OF INDIAN HEALTH TOURISM PERSPECTIVE.**

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ABSTRACT

Health tourism is an emerging trend in tourism received greater attention from the health tourists. Health tourism industry has witnessed a steady growth in the recent decades globally. As the number of health seeking population becomes more aware of wellness center and healthcare options and as quality healthcare rises as a priority in the minds of the majority ages, patients are bound to pursue cross border healthcare. A significant and increasingly upcoming segment of this multibillion dollar health and wellness tourism industry which has recorded remarkable growth in India from past few years. Several features such as cost-effective and advanced healthcare systems, availability of specialized and skilled healthcare professionals, and increasing popularity of traditional healing systems and alternative medicine, have all positioned India as a favourable destination for wellness and health care in the global scenario. However, India enjoys less than two percent share of the global wellness market which was not so significant when the health resources are concern so this paper attempts to analyse the past and present health tourism growth in India. The comparisons of health tourism growth rate of India reveals that Indian health tourism is remarkably increasing and it has succeeded to receive attention from the global health tourists. This study is based on the secondary data collected from various reports and articles and analysis was made on every bit of secondary data to come in to conclusion.

Keywords: Health tourism, wellness tourism, cost-effectiveness, Traditional healing system.

GENE THERAPY AND IT'S FUTURE

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ABSTRACT

Gene therapy is understood as the capacity for gene improvement by means of the correction of altered (mutated) genes or site-specific . modifications that have therapeutic treatment or target . research continue into the ways this therapy can treat cancer and other conditions. most often, gene therapy works by introducing a healthy copy of a defective gene into the patient's cell .Injection or intravenous (IV) infusion introduces vectors into the body .in some cases , doctors collect cells from a patient, add vectors in a laboratory and return the vector containing cells to the patient's body through injection or IV fusion. clinical trials are investigating gene therapy for the treatment of cancer, age-related macular degeneration and other eye disease, certain genetic conditions .but there is not enough evidence about gene therapy as a whole to determine all the possible risks .some gene therapy reasech indicates gene therapy may cause then to last longer. Additional , complications of certain gene therapies may include cancer , toxicity and inflammation.

Keywords: Gene Therapy, Genetic Therapy, Vectors, Injection Or Intravenous IV Infusion

EVOLUTIONARY BIOLOGY

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ABSTRACT

The purpose of this paper is to analyze the Evolutionary Biology . Evolutionary Biology is the subfield of biology that studies the evolutionary process such as natural selection, common descent and speciation that produced the diversity of life on Earth. It can be also be defined as the study of the history of life forms on Earth. Evolution here plays a very important role in studying Evolutionary biology. Evolution is based on the theory that all species are related and they gradually changes over time. Genetics variations is also among a major important topics which affects the physical characteristics i.e., phenotypes of an organism . evolutionary biology is mainly to understands the principles behind the origin and extinction of species.

Evolution here can be divided into various ways , such as biological organization which is from molecular to cell , organism to population . An earlier way is by perceived taxonomic group, with field such as Zoology, botany , and microbiology ,reflecting what was once seen as the major divisions of life. A third way is by approaches , such as field biology , theoretical biology , experimental evolution , and paleontology . More recently , the merge between biological science and applied sciences gave birth to new fields that are extensions of evolutionary biology , including evolutionary robotics , engineering , algorithms , economics, and architecture. The research generated in these applied fields , in terms, contributes to progress , especially thanks to work in evolution in computer science and engineering fields such as mechanical engineering.

There are different types of evolution , some of them are Convergent evolution , co-evolution and Adaptive evolution which can be discussed later on main papers. We should discussed more about what evolution means and what is the mechanism of evolution focus mainly on Mutation , genetic drift , gene flow , non-random mating and natural selection. In evolutionary developmental biology the different processes of development can play a role in how a specific organism reaches its current body plan.

Microbiology too is becoming an evolutionary discipline, now that microbial physiology and genomics are better understood. The quick generation_time of bacteria and viruses such as bacteriophages makes it possible to explore evolutionary questions. Current research in evolutionary biology covers diverse topics and incorporates ideas from diverse areas, such as molecular genetics and computer science.

Here, Firstly some fields of evolutionary research try to explain phenomena that were poorly accounted for in the modern evolutionary synthesis. Second, we need to be straightforward to

our evolutionary question: "what happened and when?". Third, the modern evolutionary synthesis was devised at a time when nobody understood the molecular basis of genes. We need to answers the question such as how many genes are involved, how large are the effects of each gene, how interdependent are the effects of different genes, what do the genes do, and what changes happen to them.

An evolutionary approach a is key to much current research in organismal biology and ecology. Newly discovered molecular phenomena have been easily accommodated in the past by elaborating orthodox evolutionary theory , and it appears that the same holds today for phenomena such as epigenetic inheritance . In several of these areas, empirical evidence is needed to evaluate enthusiastic speculation . Evolutionary theory will continue to be extended, but there is no sign that is requires emendation.

Keywords: Introduction , Evolutionary Biology , Evolution , Natural selection , Genetic Drift, Paleontology, Experimental evolution and Research

GENETIC SCREENING

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ABSTRACT

Current approaches to genetic screening include new-born screening to identify infants who would benefit from early treatment, reproductive genetic screening to assist reproductive decision making, and family history assessment to identify individuals who would benefit from additional prevention measures. Although the traditional goal of screening is to identify early disease or risk in order to implement preventive therapy, genetic screening has always included an atypical element-information relevant to reproductive decisions. New technologies offer increasingly comprehensive identification of genetic conditions and susceptibilities. Tests based on these technologies are generating a different approach to screening that seeks to inform individuals about all of their genetic traits and susceptibilities for purposes that incorporate rapid diagnosis, family planning, and expediting of research, as well as the traditional screening goal of improving prevention. Use of these tests in population screening will increase the challenges already encountered in genetic screening programs, including false-positive and ambiguous test results, over diagnosis, and incidental findings. Whether this approach is desirable requires further empiric research, but it also requires careful deliberation on the part of all concerned, including genomic researchers, clinicians, public health officials, health care payers, and especially those who will be the recipients of this novel screening approach.

Keywords: Genetic Screening, New technologies, genomic researchers, clinicians.

TOPIC- ON-OFF SWITCH FOR INHIBITING BACTERIAL GROWTH

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ABSTRACT

Researchers have discovered an antitoxin mechanism that seems to be able to neutralize hundreds of different toxins and may protect bacteria against virus attacks. The mechanism has been named Panacea, after the Greek goddess of medicine whose name has become synonymous with universal cure. The understanding of bacterial toxin and antitoxin mechanisms will be crucial for the future success of so-called phage therapy for the treatment of antibiotic resistance infections, the researchers say. So-called toxin-antitoxin systems, a kind of on-off switch in many bacterial DNA genomes, are increasingly being found to defend bacteria against attack by bacteriophages -- viruses that infect bacteria. Activation of toxins allows bacterial populations to go into a kind of lockdown that limits growth and therefore the spread of the virus.

Keywords: Virus attack, bacterial toxin, antitoxin mechanisms, bacteriophages.

BIOTECHNOLOGY ABSTRACT

Bhavika Bisht

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ABSTRACT

Biotechnology is technology based on biology – biotechnology harnesses cellular and biomolecular processes to develop technologies and products that help improve our lives and the health of our planet. We have used the biological processes of microorganisms for more than 6,000 years to make useful food products, such as bread and cheese, and to preserve dairy products. The biotechnology industry was dominated by recombinant DNA technology, or genetic engineering. This technique consists of splicing the gene for a useful protein (often a human protein) into production cells—such as yeast, bacteria, or mammalian cells in culture—which then begin to produce the protein in volume. In the process of splicing a gene into a production cell, a new organism is created.

The five branches into which modern biotechnology is divided — human, environmental, industrial, animal and plant — help us fight hunger and disease, produce more safely, cleanly and efficiently, reduce our ecological footprint and save energy.

Biotechnology has numerous applications, particularly in medicine and agriculture. Examples include the use of biotechnology in merging biological information with computer technology (bioinformatics), exploring the use of microscopic equipment that can enter the human body (nanotechnology), and possibly applying techniques of stem cell research and cloning to replace dead or defective cells and tissues (regenerative medicine). Companies and academic laboratories integrate these disparate technologies in an effort to analyze downward into molecules and also to synthesize upward from molecular biology toward chemical pathways, tissues, and organs.

Genetically modified food can be touted as the most radical transformation in our diet since the invention of agriculture a thousand decades ago. Experts say that selective plant breeding has brought food security, greater nutrition, and increased biodiversity, while at the same time protecting food systems against hard times, like natural or economic disasters. On the other hand if biotechnology provides more harm than benefits to the surroundings then it would definitely be a part of a much bigger problem. A balanced approach on biotechnology provides pragmatic, alternative and sustainable solutions. India has skewed distribution of natural resources, like land, biological diversity, wild life, forests, water etc., and hence Indian economy is highly interdependent on other geographical zones, for the basic amenities like food and water to electricity. This uneven distribution of resources makes the society highly

vulnerable to extreme cases of natural disaster like flood, disease, famine etc. With shrinking “food bowls” unable to match the food demand or with ever increasing disease resistance, one looks at biotechnological solutions. Biotechnology- a fusion of life science and technology offers various solutions. Over the years, judicious use of biotechnology has enriched food quality, agricultural productivity, miracle drugs, vaccines etc., and by and large the impact has been positive in ringing in ecological stewardship, medical progress and social justice. Biotechnology is an emerging platform. The potential benefits include solving world food shortages, and improvements in medicine, agriculture, and veterinary sciences. Based on our own experience, we see a greater transformation in the way industrial chemicals are being manufactured with conscious efforts of reducing the toxic waste. We expect more biotechnological solutions to many essential industrial processes that currently produce toxic effluents. An increasing role for biotechnology in environmental management will be systematically adapted and implemented. Like any new technology, there needs to be judicious use and vigilance and Biotechnology is no exception and with the concerted efforts of all agencies involved, Biotechnology can be harnessed for sustainable social transformation.

COVID-19 PANDEMIC AND LIFE THROUGH IT

Shrishty gupta

Banasthali university

ABSTRACT

The coronavirus family has significant human and animal pathogens. At the end of December 2019, a novel coronavirus was recognized as the reason for a group of pneumonia cases of unidentified etiology in Wuhan, a city in the Hubei Province of China. The novel coronavirus has rapidly become widespread, resulting in an epidemic throughout China, followed by a pandemic, an increasing number of cases in various countries throughout the world. Coronavirus disease 2019 (COVID-19) is spread through large droplets produced during coughing and sneezing by symptomatic patients, as well as asymptomatic individuals before starting of their symptoms. The incubation period of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is assumed to be 14 days succeeding exposure, mostly around four to five days. Individuals of all ages may acquire SARS-CoV-2 infection, although middle age and older individuals are the majority. The usual clinical characteristics involve fever, dry cough, fatigue, sore throat, rhinorrhea, conjunctivitis headache, myalgia, dyspnea, nausea, vomiting and diarrhea. Hence, there are no unique clinical features that yet dependably differentiate COVID-19 disease from other upper/lower airway viral infections. In a subgroup of cases, by the end of the first week, COVID-19 disease may develop to pneumonia, pulmonary failure and death.

Keywords: Coronavirus, COVID-19 disease, SARS-CoV-2 infection.

GENE-EDITING TECHNOLOGY

Vidha Pandey

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ABSTRACT

This paper includes the different technologies assisted with the editing of the gene. It is a process of versatile technologies that has motivated researchers and scientist to edit the genes to sequence genes according to the modified genome sequence. At the molecular level, alternation DNA of interest can be executed with the implementation of different gene-editing methodologies which includes CRISPR CAS 9 (clustered regularly interspaced short palindromic repeats which are associated with TALEN (transcription activator-like effectess nuclease), and ZFN (Zinc Finger Nuclease).

These gene-editing tools are frequently used in the field of molecular biology that allows research and scientists to make changes in the gene to produce different precaution and treatment for different health diseases especially conch. With the help of such tools & technologies, the genetic information of any virus and DNA of interest can be studied.

Keywords: CRISPR CAS-9 , TALEN , ZFN , gene-editing tool.

RISK MANAGEMENT IN DISASTERS

AFETLERDE RİSK YÖNETİMİ

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ABSTRACT

Disasters pose a serious risk, considering both social and economic activities and population density in residential areas. Even though earthquakes are a natural event, they turn into disasters, that is, real disasters, when they occur in residential areas where the population is high and economic and social activities are intense. Within the scope of risk management in disasters, road maps are created by institutions and organizations from time to time in order to ensure the resilience of the people against disasters and to reduce disaster damages. Within the scope of these roadmaps, the disaster risk reduction framework; Hyogo Action Plan (2005-2015) has been prepared. Later, the Sendai Action Plan (2015-2030), which is the continuation of this plan, was accepted. In disaster risk management, Hyogo Action Plan; It is emphasized that governments, national and regional organizations, private sector, volunteers, non-governmental organizations should be involved in this process. It aims to reduce disaster risks and reduce the loss of life, environmental, social and economic losses caused by disasters. It covers monitoring, evaluation, determination of risks and keeping negative situations to a minimum by developing intervention methods for them. It is in the form of integrating the minimization of disaster losses into sustainable policies and plans, developing and strengthening institutions, capacities and mechanisms to create sensitivity and awareness towards disasters, and systematically participating in the disaster and emergency preparedness, response, and recovery processes of these approaches. "using all possible information, raising awareness and creating a culture of resistance" are among the priority actions in reducing disaster damage.

Keywords: Disaster, risk, risk management

ÖZET

Yerleşim alanları hem sosyal ve ekonomik faaliyetler hem de nüfus yoğunluğu gibi özellikler göz önüne alındığında, afetler ciddi bir risk oluştururlar. Özellikle depremler, bir doğa olayı olsa da nüfusun fazla, ekonomik ve sosyal aktivitelerin yoğun olduğu yerleşim alanlarında gerçekleştiğinde felakete yani gerçek bir afete dönüşmektedir. Afetlerde risk yönetimi

kapsamında, halkın afetlere karşı olan dirençliliğinin sağlanması ve afet zararlarının azaltılması amacıyla, belirli zamanlarda kurum ve kuruluşlar tarafından yol haritaları oluşturulmaktadır. Bu yol haritaları kapsamı içerisinde afet risk azaltma çerçevesi olan; Hyogo Eylem Planı (2005-2015) hazırlanmıştır. Daha sonra ise bu planın devamı özelliğinde olan Sendai Eylem Planı (2015-2030) kabul edilmiştir. Afet risk yönetiminde, Hyogo Eylem Planında; hükümetlerin, ulusal ve bölgesel organizasyonlar, özel sektör, gönüllüler, sivil toplum kuruluşlarının bu süreç içerisinde olmasının gerekliliği vurgulanır. Afet risklerinin azaltılmasını ve afetlerin sebep olduğu can kayıpları, çevresel, sosyal, ekonomik kayıpların azaltılmasını hedefler. Risklerin izlenmesi, değerlendirilmesi, belirlenmesi ve bunlara yönelik müdahale yöntemlerinin geliştirilerek meydana gelebilecek olumsuz durumları en az düzeyde tutmayı kapsar. Afet zararlarının en aza indirgenmesinin sürdürülebilir politika ve planlarına bütünleştirilmesi, afetlere karşı duyarlılık ve bilincin oluşturulması için kurumların, kapasite ve mekanizmalarının geliştirilmesi ve güçlendirilmesi, bu yaklaşımların afet ve acil durumlara hazırlık, müdahale, iyileştirme süreçlerine sistematik olarak katılımı biçimindedir. Afet zararlarının azaltılmasında öncelikli eylemler arasında ‘mümkün olan her türden bilgiyi kullanıp, farkındalığın artırılması ve direnç kültürünün oluşturulması’ yer almaktadır.

Anahtar Kelimeler: Afet, risk, risk yönetimi

GİRİŞ

Yönetimsel kurumlar ve talimatları kullanarak, politika ve strateji uygulama imkanı aracılığıyla, afetlerin etkileri ve meydana gelme risklerini en aza indirmeye yönelik mücadele yöntemlerini kapsayan süreçtir. Bu süreç, zarar azaltıcı, önleyici ve afetlere hazırda bulunmayı kapsayan bir dizi tedbir ve faaliyetlerdir. Meydana gelen zarar verici durumların, kalıcı olabilecek etkilerini en aza indirmeyi ya da bunlardan uzak kalmayı hedefler (Çilingir ve Güler, 2020). Risk yönetimi afet yönetiminin en önemli safhasıdır. Afet yönetiminde temel amaç, kayıpların sonradan telafisi yerine, risk yönetimi ile muhtemel zararları en aza indirmek ya da tamamen engellemek olmalıdır (Ekşi, 2016). Bir afetin gerçekleşmesinin tanımını da veren risk, aşağıdaki faktörlerden meydana gelir. Bu faktörler; afet tehlikesinin gerçekleşme olasılığı, afetin etkileri altında kalan toplum yapısı unsurlarının dağılımı ve bu unsurların afetlere ne derece maruz kaldığını belirleyen zarar görülebilirlik seviyesidir.

Yerleşim alanları hem sosyal ve ekonomik faaliyetler hem de nüfus yoğunluğu gibi özellikler göz önüne alındığında, afetler ciddi bir risk oluştururlar. Özellikle depremler, bir doğa olayı olsa da nüfusun fazla, ekonomik ve sosyal aktivitelerin yoğun olduğu yerleşim alanlarında gerçekleştiğinde felakete yani gerçek bir afete dönüşmektedir (Uzunçibuk, 2005).

AFETLERDE RİSK YÖNETİMİ

Afetlerde risk yönetimi kapsamında, halkın afetlere karşı olan dirençliliğinin sağlanması ve afet zararlarının azaltılması amacıyla, belirli zamanlarda kurum ve kuruluşlar tarafından yol haritaları oluşturulmaktadır. Bu yol haritaları kapsamı içerisinde afet risk azaltma çerçevesi olan; Hyogo Eylem Planı (2005-2015) hazırlanmıştır. Daha sonra ise bu planın devamı özelliğinde olan Sendai Eylem Planı (2015-2030) kabul edilmiştir (Varol ve Kırıkkaya, 2017). Afet risk yönetiminde, Hyogo Eylem Planında; hükümetlerin, ulusal ve bölgesel organizasyonlar, özel sektör, gönüllüler, sivil toplum kuruluşlarının bu süreç içerisinde olmasının gerekliliği vurgulanır (Varol ve Kaya, 2018). Afet risklerinin azaltılmasını ve afetlerin sebep olduğu can kayıpları, çevresel, sosyal, ekonomik kayıpların azaltılmasını hedefler (Karaman ve Altay, 2016). Risklerin izlenmesi, değerlendirilmesi, belirlenmesi ve bunlara yönelik müdahale yöntemlerinin geliştirilerek meydana gelebilecek olumsuz durumları en az düzeyde tutmayı kapsar (Akpınar Bektaş ve Ceran, 2020). Afet zararlarının en aza indirgenmesinin sürdürülebilir politika ve planlarına bütünleştirilmesi, afetlere karşı duyarlılık ve bilincin oluşturulması için kurumların, kapasite ve mekanizmalarının geliştirilmesi ve güçlendirilmesi, bu yaklaşımların afet ve acil durumlara hazırlık, müdahale, iyileştirme süreçlerine sistematik olarak katılımı biçimindedir (Günaydın ve ark., 2017).

Hyogo Eylemi'nin devamı olan Sendai Eylem Planında (Gerdan, 2019) etkin müdahale ve iyileştirme aşamasında iyi bir alt yapının oluşturulması amacıyla afete yönelik hazırlıkları geliştirmede yerel ve ulusal seviyede, afet olasılığı bulunan alanlarda yaşayan kişilerin tahliyesi için yerel yönetimin kapasitesini artırmak ve afet sonrası oluşabilecek bütün ihtiyaçların (geçici barınma, gıda, tahliye) karşılanmasını sağlayacak tatbikat ve hazırlıkların düzenli bir biçimde yapılmasını, hayatı sürdürebilmenin sağlıklı yürütülmesinin gerekliliği ve önemini ortaya koymaktadır (Yücel, 2017).

Afet zararlarının en aza indirgenmesinin sürdürülebilir politika ve planlarına bütünleştirilmesi, afetlere karşı duyarlılık ve bilincin oluşturulması için kurumların, kapasite ve mekanizmalarının geliştirilmesi ve güçlendirilmesi, bu yaklaşımların afet ve acil durumlara hazırlık, müdahale, iyileştirme aşamalarına sistematik olarak katılımı biçimindedir (Günaydın ve ark., 2017).

AFET YÖNETİMİ AŞAMALARI

Gelişim hızları ve kökenleri ne olursa olsun, bütün afetlerle ilgili çalışmalar, 5 ana aşamadan oluşur. Yapılan çalışmalar birbirlerini takip ederek iç içe girmiş bulunmakta ve önceki aşamada yürütülen çalışmalar bir sonraki aşamada yürütülecek olan çalışmaları etkilemektedir.

1.Zarar Azaltma Aşaması

Acil durum ve afetlerin meydana gelen etkilerini azaltmaya dair çalışmaları kapsamaktadır. Olası bir afet ve acil durumlarda mal ve can kaybının en az düzeye indirilebilmesi için zarar azaltma faaliyetlerinin verimli ve etkin bir şekilde yürütülmesi sağlanmalıdır. Riskleri

oluşturabilecek tehlikeler önceden belirlenip tehlikenin meydana gelmesini önlemek veya ortaya çıkabilecek zararları engellemeye yönelik önlemleri içerir (Yılmaz ve Yıldırım, 2020).

2.Hazırlık Aşaması

Hazırlık aşamasında yapılması gereken faaliyetlerin amacı, tehlikenin toplumlar için zararlı etkiler ortaya çıkarabilecek sonuçlarına karşı tedbirler alarak, zamanında, uygun bir şekilde, etkin yöntem ve organizasyon ile müdahaleye hazırlanabilmektir. Afet veya acil durumlara karşı hazırlıklı olma destek kaynakların düzenlenmesini, yetki ve sorumlulukların tanımlanmasını içerir (Kadıoğlu ve Özdamar, 2008). Afetlerin meydana gelmesinden sonra toplumlar üzerindeki öldürücü ve yıkıcı etkiyi azaltmaya dair çalışmaları kapsamaktadır. Hazırlık aşamasında planlama, eğitim, yönetim, organizasyon, ölçüm, değerlendirme, gerekli araç ve ekipmanların temininin sağlanması ve tüm bunların koordinasyonunu içermektedir. Bu amaçla; sürekli eğitim programları yürütülmeli, STK'da görev almakta olan gönüllülerde bu eğitimlere dahil edilmeli, sürekli tatbikatlar yapılmalı, tahliye planları oluşturulmalıdır. Gerekli ihtiyaç kaynaklarının zamanında hazır bulundurulması için acil haberleşme bağlantıları oluşturulmalıdır. Gerekli araç ve ekipmanların stoğu bulundurulmalıdır (Adaş ve ark., 2012).

3.Müdahale Aşaması

Acil ve afet hallerinde mal ve can kurtarma, sağlık, ibate, iaşe, güvenlik, çevre ve mal koruma, psikolojik ve sosyal destek hizmetlerinin karşılanmasına yönelik çalışmaları içerir. Afetin meydana geldiği ilk aşamadır. Arama-kurtarma, tıbbi sağlık, ilk yardım, barınma, giyim, gıda, su ve arıtma tedariki hizmetleri gibi toplumun acil gereksinimlerinin sağlanması; yardım kaynaklarının koordinasyonu, hasar tespiti çalışmaları gibi hususlar bu evrede gerçekleştirilir. Bu evre, ilk müdahaleyle başlayan, durumun gidişatına göre kısa ya da uzun vadede devam eden bir süreçtir. Afete müdahale veya acil müdahale olarak da tanımlanır (Yılmaz, 2015). Afetin gerçekleşmesinden hemen sonra hızlı ve etkin bir biçimde insan yaşamını kurtarmak ve afetin sebep olduğu zararları minimize edebilmek, barınma problemi olanların ve yaralıların ihtiyaçlarının tedarik edilmesi gibi hedefler için yapılan faaliyetlerin tamamı müdahale aşaması kapsamındadır. Bu aşamada yapılacak çalışmalar, toplumun sahip olduğu tüm güç ve imkanların hızlı ve etkin bir biçimde afetin meydana geldiği alanda uygulanabilirliği ile mümkündür. Müdahale aşaması için iyi bir plana ve koordinasyona gereksinim duyulmakta ve olağanüstü haller için uygulanma mecburiyeti bulunan olağanüstü yetki ve hazırlık gerekmektedir. Bu aşamada gerçekleşen aksaklıklar afetlerin olumsuz etkilerinin artmasına sebep olacaktır. Bundan ötürü müdahale aşamasının uygulanabilir olması oldukça önemlidir. Bu aşama, bir plan ve düzen içinde olmalıdır. Önceden hazırlanmış bir plan veya düzenlemenin olmaması, toplumda kargaşaya neden olur. Bu kargaşa, müdahale aşamasının başarısızlıkla sonuçlanmasına sebep olacaktır (Bulat, 2020).

4. İyileştirme Aşaması

Birey ve toplumların, devlet kurumlarının ve iş yerlerinin kendi kendilerine faaliyet göstermeleri, normal hayata dönmeleri ve gelecekte meydana gelebilecek tehlikelere karşı savunmalarını sağlayacak biçimde yeniden yapılandırılmasıdır (Arca, 2012). Bu aşamada; kayıp, hasar ve ihtiyaçların tespitini yapılması, stratejilerin belirlenmesi, finansal değerlendirmelerin yapılması, iyileştirme ve yeniden inşa faaliyetlerinin uygulanması, uygulamaların izlenip değerlendirilmesi hususları öne çıkmaktadır (Memiş ve Babaoğlu, 2020). Afetzedelere geçici barınma alanlarının sağlanması gerekir. Uzun ve orta süreli maddi hasara uğrayan alanlarda afetin izlerinin silinmesine yönelik çalışılmaktadır. Bu aşama, rehabilitasyon çalışmaları, acil yardım barınaklarının yapımından kalıcı konutların yapımına kadar olan süreci kapsamaktadır (Limoncu ve Atmaca, 2018).

5. Yeniden İnşa Aşaması

Afetlerden etkilenen veya hasar gören bütün insan faaliyetlerinin afet öncesi durumlarından daha ileri bir seviyede karşılanabilmesidir (Kemaloğlu, 2015: 138). Bu aşamada amaç, afete maruz kalan alanların temel ihtiyaçları, eğitim, ulaşım, haberleşme, elektrik, kanalizasyon, kalıcı konut yapımı, sosyal ve ekonomik yaşamın normal düzene dönmesi için ihtiyaç duyulan tüm faaliyetleri yapmaktır. Yeniden yapılanma çalışmaları afet yönetiminin en uzun dönemi kapsayan evresidir (Şahin ve Üçgül, 2019).

SONUÇ

Afet zararlarının azaltılmasında öncelikli eylemler arasında “Mümkün olan her türden bilgiyi kullanıp, farkındalığın artırılması ve direnç kültürünün oluşturulması” yer almaktadır. Birey ve toplumları acil durum ve afetlere karşı korumak için zamanında ve etkin davranmalarına olanak tanıyacak biçimde haberdar edebilmek amacıyla gerekli faaliyetleri yürüten kurum ve kuruluşlar arasında iş birliği ve koordinasyonu sağlayarak, ülke düzeyinde standartların tespit edilmesini, denetlenmesini ve politikaların üretilip uygulanmasını sağlamak gerekir (Kadıoğlu, 2008).

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PREPAREDNESS FOR DISASTERS

AFETLERE HAZIRLIK

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ABSTRACT

Disasters do not generally appear as completely preventable events. The main responsibilities of disaster management stages are; social factors such as government institutions, local governments, universities, non-governmental organizations and individuals. Preparedness for disasters; It is handled under two main headings as individual-social preparation and institutional preparation. As the knowledge and education levels of individuals about disasters increase, the measures of material and moral damages caused by disasters, the preparations of individuals against disasters, their attitudes and behaviors also change. Individuals and institutions such as families, schools, educators, oral and written media are effective in raising awareness of individuals against disasters. The most effective method applied to raise awareness about disasters is education in schools. Incorporating appropriate skills and knowledge about disaster risk reduction and hazards into school curricula, starting at an early age, will make a significant contribution to ensuring a safe society and minimizing disaster risks. It is vital to equip the individuals of the future with knowledge and skills for the construction of a safe society in terms of disasters. The European Center for Natural Disasters Education has activities to create a "Public Education Campaign" on the issues of preparing, printing and distributing training programs to be applied to different target groups for the reduction and prevention of disaster damage, with publications and seminars. Disasters are preventable events. By being prepared for disasters, loss of life and property can be prevented.

Keywords: Disaster, individual-social preparation, institutional preparation.

ÖZET

Afetler genel anlamda tamamen önlenbilir olaylar olarak karşımıza çıkmamaktadır. Afet yönetim aşamalarının temel sorumluları; devlet kurumları, yerel yönetimler, üniversiteler, sivil toplum kuruluşları ve bireyler gibi toplumsal etkenler olarak tanımlanmaktadır. Afetlere hazırlık; bireysel-toplumsal hazırlık ve kurumsal hazırlık olarak iki ana başlıkta ele alınır. Afetlere yönelik bireylerin bilgi ve eğitim düzeyleri arttıkça, afetlerin sebep olduğu maddi ve manevi zararların ölçüleri, bireylerin afetlere karşı hazırlıkları, tutum ve davranışları da değişir.

Bireylerin afetlere karşı bilinç sağlamasında aileler, okullar, eğitimciler sözlü ve yazılı basın gibi kişi ve kurumlar etkili olmaktadır. Afetler ile ilgili farkındalığı artırmak amacıyla uygulanan en etkili yöntem okullarda alınan eğitimidir. Erken yaşlarda başlanılarak afet risk azaltma ve tehlikeler ile ilgili uygun beceri ve bilgilerin okul müfredatlarına dahil edilmesi güvenli toplumun sağlanmasına ve afet risklerinin en aza indirgenmesine önemli katkı sağlayacaktır. Afetler açısından güvenli bir toplumun inşası için bilgi ve becerilerin geleceğin bireylerine kazandırılması hayati önemdedir. Avrupa Doğal Afetler Eğitim Merkezi, basın yayınlar ve seminerler ile “afet zararlarının azaltılması ve önlenmesine yönelik farklı hedef gruplarına uygulanacak eğitim programlarının hazırlanması, basın ve dağıtımına yönelik konularda Halkın Eğitimi Kampanyası” oluşturma faaliyetleri bulunmaktadır. Afetler önlenebilir olaylardır. Afetlere hazır olarak can ve mal kayıpları önlenebilir.

Anahtar Kelimeler: Afet, bireysel-toplumsal hazırlık, kurumsal hazırlık

GİRİŞ

Afet yönetim aşamalarının temel sorumluları; devlet kurumları, yerel yönetimler, üniversiteler, sivil toplum kuruluşları ve bireyler gibi toplumsal etkenler olarak tanımlanmaktadır. Bu etkenlere baktığımızda bireyler en somut muhatap olarak karşımıza çıkar. Diğer etkenleri oluşturan, toplumsal olgu haline getiren ve yöneten bireylerdir. Böylelikle afetlerde bireysel hazırlık aşamaları tam anlamıyla gerçekleşmediği sürece diğer etkenler hazırlık konusunda istenilen düzeyde başarılı olamazlar (Kaya, 2020).

Afetlerle mücadele kapsamında bireylerin eğitim düzeyleri oldukça önemlidir. Afetlere yönelik bireylerin bilgi ve eğitim düzeyleri arttıkça, afetlerin sebep olduğu maddi ve manevi zararların ölçüleri, bireylerin afetlere karşı hazırlıkları, tutum ve davranışları da değişir. Bireylerin afetlere karşı bilinç sağlamasında aileler, okullar, eğitimciler sözlü ve yazılı basın gibi kişi ve kurumlar etkili olmaktadır (Koç, 2013).

Afet yönetimi idarenin ve yönetimlerin görevidir. Afet yönetiminin sağlanmasından, rol ve sorumlulukların belirtilmesinden, yerine getirilmesinden, denetim ve kontrolünden devlet sorumludur. Afetler önlenebilir olaylardır. Afetlere hazır olarak can ve mal kayıpları önlenebilir.

AFETLERE HAZIRLIK

1. Bireysel ve Toplumsal Hazırlık

Bireysel afet yönetimi faaliyetleri yürütülerek yaşam alanlarımızda meydana gelen hasarları en aza indirmeye, yaralanmaları önleme ve afetler sonrası ilk 72 saatte yardım almadan hayatta kalmaya dair konularda hazırlıklı olunmalıdır (Oral ve Yıldırım, 2020).

Afetlerin ülkemizde sık sık meydana gelmesi sebebiyle toplumlar afetlere karşı bilgilendirilerek afetlerin yoğun olarak meydana geldiği alanlarda öğrencilere öncelik verilmek üzere bilinç sağlanmalıdır. Afetler ile ilgili farkındalığı artırmak amacıyla uygulanan en etkili yöntem okullarda alınan eğitimidir (Sapsağlam, 2019). Örgün eğitim, kişilerin afetlere hazırlık düzeylerini artıracak bilgi, beceri ve uzmanlıkları edinmelerini sağlayan temel yol olarak kabul edilir. Erken yaşlarda başlanılarak afet risk azaltma ve tehlikeler ile ilgili uygun beceri ve bilgilerin okul müfredatlarına dahil edilmesi güvenli toplumun sağlanmasına ve afet risklerinin en aza indirgenmesine önemli katkı sağlayacaktır. Ayrıca bir toplumun çocukları o toplumun geleceği ve geleceğin karar vericileri konumunda olan yetişkin bireyleridir. Afetler açısından güvenli bir toplumun inşası için bilgi ve becerilerin geleceğin bireylerine kazandırılması hayati önemdedir (İnal ve ark., 2018).

2. Kurumsal Hazırlık

Afet yönetiminin etkin ve verimli bir şekilde sağlanması ise kurum ve kuruluşların rol ve sorumluluklarını yerine getirmeleri, bunları toplumlara mal etmeleriyle, yaymalarıyla, sivil toplum kuruluşları, gönüllülerle ve toplumun tüm kesimiyle paylaşım sağlamalarıyla mümkündür. Afetlere müdahale olağandışı hallerde, normal koşulların bulunmadığı hallerde, kargaşa ortamında birçok işin oldukça kısa bir zamanda yapılmasını zorunlu kılmaktadır. Bunlar ertelenmesi mümkün olmayan işler olup toplum yaşamını etkileyen, can kaybına sebep olabilen faaliyetlerdir. Bu sebeptendir ki, kurumların altından kendi kendine kalkabileceği olaylar değildir. Şüphesiz orada yaşayan kişilerin yardımına gereksinim duyulmaktadır (Çakacak, 2008).

2009 yılında afet yönetimine yönelik en önemli ulusal girişim Afet ve Acil Durum Yönetim Başkanlığı'nın (AFAD) kurulmasıdır. AFAD, ülkemizde afet yönetiminin tüm safhalarını koordine etmekle sorumludur. Ayrıca Türkiye Deprem Vakfı, STK, Türk Kızılay'ının yanı sıra özel sektör ve meslek örgütleri de toplumu bilgilendirici eğitim faaliyetleri yürüterek destek sağlamaktadır. Afet yönetim döngüsünün bütün safhalarında başarılı olabilmek amacıyla afet çalışmalarında görevde bulunacak personellerin eğitimi, geliştirilmesi ve yetiştirilmesine gerekli önem verilmelidir. Afet ekibinde rol alacak personellerin temel eğitimlerinin yanında hizmet içi eğitim faaliyetleri, kurs vb. etkinlikler ile afetlerdeki rollerine yönelik devamlı olarak eğitilmeleri gerekir. Bu eğitimlerin uygulamalı şekilde olması, özellikle tatbikatlarla desteklenmesi afet esnasında daha başarılı olma yolunda etkilidir. Ayrıca eğitim faaliyetlerinin güncel gelişmeler doğrultusunda devamlı olacak şekilde yeniden yapılandırılması gerekir. Eğitim programlarına hazırlık yapılırken personellerin rollerine yönelik konuların yanında, ekip çalışması, liderlik, motivasyon, stres yönetimi, iletişim becerileri, çatışma yönetimi, zaman yönetimi, iş güvenliği, yasal düzenlemeler gibi konularda bireylerin afet gibi karmaşık olaylarda ekip ruhuyla çalışmalarının sağlanması yönünden oldukça önem taşımaktadır (Taşkiran ve Baykal, 2018). Bölgesel ve yerel görevliler ve ilgili uzmanlar deneyimlerin

paylaşılmasında rol üstlenmelidirler. Bu sebeple olayların afetlere dönüşmemesi için, kurumlara, sivil toplum kuruluşlarına, idarecilere ve üniversitelere sorumluluklar düşmektedir (Varol, 2007).

Afetler hayat boyu eğitime konu olmakta, üniversite eğitiminde hem afetlere karşı farkındalığı yüksek kişiler yetiştirilmesi hem de afet yönetim aşamalarında sorumluluk alacakları bilgiye ve deneyime sahip olmaları açısından oldukça önemli göreve sahiptir. Bir kurum olarak üniversitenin rolü, afetin tüm safhalarını kapsar. Bir araştırma ve eğitim kurumu olan üniversite, topluma geniş açıda katkıda bulunmak amacıyla görevlendirildiği için tüm safhalara dahil edilmek zorundadır. Üniversiteler çoğunlukla acil gönüllü destek ve fon sağlama hizmetleri ile sınırlıdır. Sınırlamayı göz önüne alacak olursak, üniversite, genellikle, afetlerle ilgili eğitim ve araştırma enstitüsü, kamu rolü üstlenmek, politika önerileri, yeniden yapılanma aşamasına dahil olmak gibi akademik katkılarda yardım sağlayabilecek bir niteliktedir. Üniversitelerin bu görevlerinin yanında afet yönetimi eğitimi de müfredata geniş bir biçimde dahil edilmelidir (Şengün ve Küçükşen, 2019).

Milli Eğitim Bakanlığı eğitim müfredatına “Deprem Haftası” dahil etmiştir. Bakanlığımız “eğitim yoluyla afet kültürü oluşturulması” konusunda önemli çalışmalar yürütmektedir. Çevre ve Orman Bakanlığı, Sivil Savunma Genel Müdürlüğü gibi kurum ve kuruluşlar hem internet üzerinde hem de kitap, broşür, dergi gibi basılı yayınlarla birey, toplum ve kurumlara yönelik bilinçlendirme faaliyetlerini sürdürmektedirler. Avrupa Doğal Afetler Eğitim Merkezi (AFEM), basım yayınlar ve seminerler ile “afet zararlarının azaltılması ve önlenmesine yönelik farklı hedef gruplarına uygulanacak eğitim programlarının hazırlanması, basım ve dağıtımına yönelik konularda Halkın Eğitimi Kampanyası” oluşturma faaliyetleri bulunmaktadır. AFEM tarafından yürütülen eğitimler, hedef kitlenin oldukça geniş olması sebebi ile öncelik olarak “Eğiticilerin Eğitimi” tarzında yapılmaktadır. Seminer, kurs, yayın-baskı yolu ile bilgilerin yaygınlaştırılmasının yanı sıra kitle iletişim araçları (radyo, televizyon, video, sinema vb.) ile izleyenlerin dikkatini çekmeye yönelik metotlara da ağırlık verilmeye çalışılmaktadır (Varol, 2007).

SONUÇ

Afetler önlenabilir olaylardır. Afetlere hazır olarak canımızı, malımızı ve çevremizi koruyabiliriz. Afet yönetimi idarenin ve yönetimlerin görevidir. Afet yönetiminin sağlanmasından, rol ve sorumlulukların belirtilmesinden, yerine getirilmesinden, denetim ve kontrolünden devlet sorumludur. Afetlerle mücadele kapsamında bireylerin eğitim düzeyleri oldukça önemlidir.

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**AĞRI DAĞI VOLKANİK KAYAÇLARINDAKİ ARSENİK-MOLİBDENİN ULTRA
SAF SULARDA ÇÖZÜNME ORANI⁵**
**DISSOLVED RATE OF ARSENIC-MOLYBDENUM IN MOUNT ARARAT VOLCANIC
ROCKS IN ULTRAPURE WATERS⁶**

Servet AŞKIN

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ÖZET

Iğdır ili ve ilçelerindeki içme suları, Ağrı dağı volkanik kayalarındaki toksik elementler çözünerek canlı organizmalarda semptomlar oluşturabilmektedir. Toksik mineral bileşimindeki arsenik ve molibdeninin içme sularındaki çözünmüş haldeki derişimi Su Kalite Standartlarının üstünde olduğunda insan sağlığına ciddi hastalıklara yol açmaktadır. İnsanlarda ki bu hastalıklar karaciğer, akciğer, böbrek, mesane ve kalp-damar kanserleri gibi sayılabilir. Bunlardan arsenik ise arsenat- AsO_4^{3-} , arsenit- AsO_3^{3-} , arsenik oksiti- As_4O_6 , arsenit asidi- H_3AsO_3 , arsenat asiti-(H_3AsO_4) alkali arsenit tuzları Na_3AsO_3 ve arsenopyrite- $FeAsS$, ve molibden su içinde daha çok molibdat MoO_4^{2-} , MoS_4^{2-} , $[P(Mo_2O_7)_6H_4]^{4-}$, $[Si(Mo_2O_7)_6H_4]^{4-}$ ve $[As(Mo_2O_7)_6H_4]^{3-}$ kompleksleri şeklinde çözünebilmektedir.

Bu araştırmada Iğdır ve ilçelerinde volkanik kayalarındaki arsenik ve molibdenin ultra saf suda çözünen derişimi ile bölgede içme sularındaki As ve Mo konsantrasyonları karşılaştırılmıştır. Bu incelemede daha önce içme sularındaki arsenik- Molibden korelasyonunu destekleyen bulgulara ek olarak volkanik kayadan ultra saf sudaki çözünen As ve Mo konsantrasyonları arasında da tespit edilmiştir. Ağrı Dağı volkanik kayalarından numuneler toplandıktan sonra, öğütülmüş ve sabit kütleye getirilmiş, eşit hacimdeki ultra saf suda, manyetik karıştırıcılarda 60 dakika boyunca çözülmüştür. Daha sonra santrifüjlendikten sonra ile çözeltideki As ve Molibden ICP- MS' te tespit edildi.

Volkanik kayadan ultra saf suya geçen As ve Mo maksimum değerleri sırasıyla 0,614 ppb ve 6,351 ppb minimum değerleri ise 0,0034ppb ve 0,076 ppb tespit edildi. Ayrıca bölgedeki içme suyu numunelerinde maksimum As ve Mo ölçümleri ise sırasıyla 101,723 ppb, 7,901256 ppb minimum ölçümler ise 7,406 ppb ve 1,064161 ppb tespit edilmiştir.

⁵ Bu Çalışma İGDIR ÜNİVERSİTESİ BAP Birimince desteklenen (2016-FBE-B04) "Ağrı Dağı Volkanik Kayaların Yeraltı ve Yerüstü Sularına Etkisi" adlı proje kapsamında hazırlanmıştır.

⁶ This study was carried out within the scope of the project "The Effect Of Mount Ararat Volcanic Rocks On Underground And Local Waters" at IGDİR UNIVERSITY, Scientific Research Project Unit (2016-FBE-B04).

Volkanik Kayaçlardaki, molibden ve arseniğin ultra saf suda çözünen konsantrasyonlarının korelasyon halinde olduğu ve ayrıca bölgedeki içme sularındaki As ve Mo derişimleri arasındaki korelasyonu desteklediği ispatlamıştır.

Anahtar kelimeler: Arsenik, Molibden, Su Kalitesi, Volkanik Kayaç, Toksik Element

ABSTRACT

It is being caused some symptom at the organisms to dissolving in the drinking water toxic elements of the volcanic rocks the mount Ararat. It is being caused serious diseases to human health when the dissolved concentration of arsenic and molybdenum in the toxic mineral composition in drinking water exceed the Water Quality Standards (WQS). They are the cancers the liver, lung, kidney, bladder and cardiovascular etc. They could dissolved in drinking water molybdenum and arsenic respectively, as arsenate AsO_4^{3-} , arsenite - AsO_3^{3-} , arsenic oxide- As_4O_6 , arsenic acid- H_3AsO_3 , arsenate acid- H_3AsO_4 , alkaline salts- Na_3AsO_3 , and molybdate- MoO_4^{2-} , Molibtiyonat - MoS_4^{2-} , other form complexes of molybdenum the $[P(Mo_2O_7)_6H_4]^{4-}$, $[Si(Mo_2O_7)_6H_4]^{4-}$, $[As(Mo_2O_7)_6H_4]^{3-}$.

In this paper, were compared the soluble concentration in ultrapure water of arsenic and molybdenum in volcanic rocks and theirs concentrations in these region drinking waters of in Iğdır and its districts. It was also determined between the dissolution As and Mo concentrations in ultrapure water from volcanic rocks in addition to the consequence previously supporting the As-Mo correlation in drinking water. After collected the samples of mount Ararat volcanic rocks, ground and have got been to constant mass were dissolved in an equal volume of ultrapure water, it's different masses for 60 minutes in magnetic stirrers. They were measured As and Mo in the solution with ICP-MS after centrifugation.

They were determined Maximum values of As and Mo dissolving from volcanic rock in ultrapure water respectively as 0.614 ppb and 6.351 ppb, and minimum values of 0.0034 ppb and 0.076 ppb. In addition, they were determined maximum As and Mo measurements in drinking water samples in the region as 101,723 ppb, 7.901256 ppb, and minimum measurements of 7,406 ppb and 1,064161 ppb, respectively.

It has been proven, both correlated the soluble concentrations of molybdenum, arsenic in the volcanic rocks in ultrapure water, and supporting the correlation with theirs concentrations in drinking water in the region.

Keywords : Arsenic, Molybdenum, Water Quality, Volcanic Rock, and Toxic Elements

VOLKANİK KAYAÇLAR VE KUMTAŞLARINDAKİ DEMİR, KOBALT VE NİKEL DERİŞİMLERİNİN ICP-MS İLE ÖLÇÜMLERİNİN KARŞILAŞTIRILMASI
COMPARİSON IRON, COBALT, AND NICKEL IN VOLCANIC ROCKS AND SANDSTONES CONCENTRATION MEASUREMENTS OF WITH ICP-MS.

Servet AŞKIN

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ÖZET

Andezik ve bazaltik türlerden oluşan volkanik kayalar ve kumtaşları bileşimlerinde birçok toksik mineral olduğu gibi teknolojik olarak birçok yeni malzemelerin geliştirilmesinde kullanılan değerli nadir elementler de içermektedir. Bu elementlerin önemlilerinden olan: demir kobalt ve nikel ferromanyetik nitelikte olup birçok bilimsel çalışmada kullanılmaktadır. Bu ferromanyetik elementlerin yeryüzündeki dağılımları arasında korelasyon olduğu yapılan birçok çalışma ile tespit edilmiştir.

Ferromanyetik nitelikteki bu elementler bileşiklerinde $Fe^{2+,3+,6+}$, $Co^{2+,3+}$ ve Ni^{2+} yükseltgenme basamaklarında bulunmaktadır. Bu elementlerin volkanik kayalar ve kumtaşlarında; hematit- Fe_2O_3 , enstati- $Al_{0.03}Fe_{0.15}Mg_{1.82}O_6Si_{1.97}$, hedenbergite- $CaFeO_6Si_2$, diopside- $CaMg_{0.26}Ni_{0.74}O_6Si_2$, magnetite- Fe_3O_4 , pigeonite- $Al_{0.02}Ca_{0.121}Fe_{1.008}Mg_{0.871}O_6 Si_{1.98}$, pyroxene- $Al_{1.388}Ca_{0.742}Fe_{0.162}Mg_{0.016}O_6Si_{1.5}$, apuanite $As_{0.4} Fe_{4.56} O_{12} S_{0.84} Sb_{3.84}$, aerugite $As_3 Ni_{8.499}O_{16}$, periclase- $Fe_{0.037}Mg_{0.963}$, qenite- $CoSbAs$, cobaltneustadtelite- $Bi_2Fe^{3+}(Co, Fe^{3+}) (AsO_4)_2 (O/OH)_4$ Cobaltlotharmeyerite - $CaCO_2(AsO_4)_2 \cdot 2H_2O$ mineraller tespit edildi.

Bu çalışmada volkanik kayalar ve kumtaşları arasında karşılaştırmanın ölçümü ICP- MS ile yapılmış, korelasyon ve ferromanyetik maddelerin derişimi ppm seviyesinde tespit edilmiştir.

Volkanik kayaların öğütülerek 300 mesh elekaltı toz numunelerinin sabit tartıma getirildikten sonra derişik asitte mikrodalga fırında yakılıp çözeltiye geçen ICP-MS ile ölçülen Fe, Co ve Ni elementlerinin maksimum derişimleri sırasıyla: 8.64 ppm, 156.89ppb ve 131.21 ppb, minimum derişimler ise sırasıyla: 3.80 ppm, 54.11 ppb ve 39.90ppb tespit edilmiştir. Kumtaşı numunelerinde Fe, Co ve Ni elementlerinin maksimum derişimleri sırasıyla; 26.06 ppm, 247.52 ppb ve 429.33ppb en az derişimler ise sırasıyla 22.20 ppm, 216.44 ppb ve 228.01 ppb olarak tespit edildi. Bu sonuçlar volkanik kayalar ve kumtaşı numunelerindeki ferromanyetik elementler arasında korelasyonun varlığına ek olarak kumtaşındaki derişimlerinin volkanik kayalara göre Fe, Co ve Ni sırasıyla % 201.7 , % 57,8 ve % 227.2 oranlarında arttığı tespit edildi.

Anahtar kelimeler: Ferromanyetik Elementler, Korelasyon, Volkanik Kayalar ve Kumtaşı

ABSTRACT

They have got contain volcanic rocks and sandstones consisted of andesitic, basaltic both valuable rare elements used be invention materials of innovation, and many toxic minerals in their composition. They are being used in many scientific studies, were ferromagnetic iron, cobalt, and nickel, among the most important of these elements. It has been determined correlation between the distributions of these ferromagnetic elements on earth by many studies. There are oxidation states of $\text{Fe}^{2+,3+,6+}$, $\text{Co}^{2+, 3+}$, and Ni^{2+} in their compounds these ferromagnetic elements. The minerals containing ferromagnetic elements in their composition in the volcanic rocks and sandstones, were detected hematit- Fe_2O_3 , enstati- $\text{Al}_{0.03}\text{Fe}_{0.15}\text{Mg}_{1.82}\text{O}_6\text{Si}_{1.97}$, hedenbergite- $\text{CaFeO}_6\text{Si}_2$, diopside- $\text{CaMg}_{0.26}\text{Ni}_{0.74}\text{O}_6\text{Si}_2$, magnetite- Fe_3O_4 , pigeonite- $\text{Al}_{0.02}\text{Ca}_{0.121}\text{Fe}_{1.008}\text{Mg}_{0.871}\text{O}_6\text{Si}_{1.98}$, pyroxene- $\text{Al}_{1.388}\text{Ca}_{0.742}\text{Fe}_{0.162}\text{Mg}_{0.016}\text{O}_6\text{Si}_{1.5}$, apuanite $\text{As}_{0.4}\text{Fe}_{4.56}\text{O}_{12}\text{S}_{0.84}\text{Sb}_{3.84}$, aerugite $\text{As}_3\text{Ni}_{8.499}\text{O}_{16}$, periclase- $\text{Fe}_{0.037}\text{Mg}_{0.963}$, qenite- CoSbAs , cobaltneustadtelite- $\text{Bi}_2\text{Fe}^{3+}(\text{Co}, \text{Fe}^{3+})(\text{AsO}_4)_2(\text{O}/\text{OH})_4$, and Cobaltlotharmeyerite - $\text{CaCo}_2(\text{AsO}_4)_2 \cdot 2\text{H}_2\text{O}$.

This paper, was determined the ppm concentration of ferromagnetic elements the comparison a between volcanic rocks and sandstones, with ICP-MS. After ground the volcanic rocks the 300 mesh sieve down powder samples, while have been constant weight, and than burning in a microwave oven in concentrated acid, their's concentrations were been detected with ICP-MS, the maximum 8.64 ppm, 156.89 ppb, 131.21 ppb, and the minimum 3.80 ppm, 54.11 ppb, 39.90 ppb, the Fe, Co and Ni, respectively. The concentrations of Fe, Co, and Ni elements were detected in sandstone samples the maximum, 26.06 ppm, 247.52 ppb, 429.33 ppb, and the minimum 22.20 ppm, 216.44 ppb 228.01 ppb, with same preceding process respectively. In addition to it was being determined correlation between ferromagnetic elements both in the volcanic rocks and sandstone samples, increased the concentrations Fe, Co and Ni in sandstone by 201.7%, 57.8% and 227.2%, respectively, compared to volcanic rocks.

Keywords: Ferromagnetic Elements, Correlation, Sandstone, and Volcanic rocks

**TİROİDEKTOMİ HASTALARINDA LABORATUVAR TESTLERİNİN KULLANIM
SIKLIKLARI**

FREQUENCY OF LABORATORY TESTING IN THYROIDECTOMY PATIENTS

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ÖZET

Dünya genelinde sağlık harcamalarının her geçen gün artmaktadır. Sağlık harcamalarının artışı, sağlık hizmetleri sunumunda tanı testlerinin yaygın kullanımının etkisi bulunmaktadır. Bu araştırmanın amacı, tiroidektomi operasyonu yapılan hastalarda laboratuvar testlerinin kullanım sıklıklarını belirlemektir. Ayrıca test kullanım sıklığının hastanın yaşı, cinsiyeti ve yatış süreleri ile ilişkisinin olup olmadığının belirlenmesi amaçlanmıştır. Araştırmada tiroidektomi operasyonunun seçilmesinin nedeni, sık uygulanan cerrahi işlemlerden birisi olmasıdır. Araştırma verileri, Ankara'da faaliyet gösteren bir eğitim araştırma hastanesinde, 2016 yılı içerisinde tiroidektomi operasyonu yapılan 594 hastayı kapsamaktadır. Araştırmada tanımlayıcı analizler ve bağımsız gruplarda iki ortalama arasındaki farkın önemlilik testi kullanılmıştır. Analizlerde anlamlılık düzeyi $p < 0,05$ olarak kabul edilmiştir. Araştırmada kullanım sıklığı en yüksek olan 23 adet laboratuvar testi incelenmiştir. Elde edilen sonuçlara göre; 594 hastaya toplam 28174 adet laboratuvar testi yapılmıştır. Analize alınan 23 testin toplam sayısı 23532 olarak bulunmuştur. Hasta başına uygulanan test sayısı ortalama 53,45 bulunmuştur. En fazla yapılan test, %17,16 (4834) ile hemogram testidir. Uygulanan test sayıları genel olarak erkeklerde kadınlara göre daha yüksek olup, 10 test için fark istatistiksel olarak da anlamlı bulunmuştur. 50 ve üzeri yaş grubu hastalarda test sayıları, gençlere göre daha yüksek olup, 15 testte farklılık istatistiksel olarak anlamlıdır. Yatış sürelerine göre ise, tüm testler dört günün ve üzeri gün yatış süresi olan hastaların tüm testler daha sık kullanılmıştır ve istatistiksel olarak anlamlıdır. Sonuç olarak, hastalara yapılan test sayıları hasta özelliklerine göre farklılık göstermektedir.

Anahtar Kelimeler: Laboratuvar testleri, tiroidektomi, sağlık harcamaları.

ABSTRACT

Health expenditures are increasing constantly worldwide. The widespread use of diagnostic tests, in the provision of health services, has an impact on the increase in health expenditures. The aim of this study is to determine the frequency of use of laboratory tests in patients who

underwent thyroidectomy procedures. In addition, it was aimed to determine whether there is a relationship between the frequency of test use and the age, gender, and length of stay of the patient. The reason for choosing thyroidectomy is that it is one of the most frequently performed surgical procedures. Research data includes 594 patients who underwent thyroidectomy in 2016 in a training and research hospital in Ankara, Turkey. The descriptive analysis and the independent samples t-test were performed in the study. $p < 0.05$ was accepted as the level of significance in the analysis. The most frequently used 23 laboratory tests were examined in the study. It was found that a total of 28,174 laboratory tests were performed on 594 patients. The average number of tests per patient was 53.45. The most frequently performed test is the hemogram test with 17.16% (4834). The number of tests applied was generally higher in men than in women, and the difference was statistically significant for 10 tests. The number of tests in patients aged 50 and over is higher than younger ones, and the difference in 15 tests is statistically significant. According to the length of hospitalization, all tests were used more frequently in patients with a four-day or longer hospitalization and were statistically significant. In conclusion, the number of tests performed on patients varies according to patient characteristics.

Keywords: Laboratory tests, thyroidectomy procedure, health expenditures.

GİRİŞ

Sağlık harcamalarının ve ülke bütçelerindeki paylarının artması harcamaların düşürülebilmesi gerekliliğini ortaya çıkarmaktadır. Genel olarak laboratuvar ve radyoloji testleri, ilaçlar, tanı ve tedavi işlemleri, enfeksiyon oranları, komplikasyonlar, tekrarlayan yatışlar vb. gibi birçok etken sağlık harcamalarını artıran etkenler olarak sıralanabilir(Qaseem et al., 2012). Hastaneler sağlık harcamaları içinde en yüksek paya sahip kurumlardır ve buralarda verilen sağlık hizmet harcamaları içerisinde görüntüleme ve laboratuvar hizmetlerinin de önemli bir yere sahip olduğu bilinmektedir(Abbott et al., 2014; Morgen & Naugler, 2015; Vegting et al., 2012). Laboratuvar testleri gerek poliklinik hastaları için gerek yatan hastalar için sık kullanılmaktadır. Kan testleri hastalara hem teşhis koyabilmek hem de tedavilerin sonuçlarını değerlendirmekte hekimler için önemli başvuru kriterlerindedir(Thakkar et al., 2015). Sağlık harcamalarında her geçen yıl artış olduğu bilinmekle birlikte laboratuvar ve görüntüleme testlerinin artış oranının toplam sağlık harcamaları artış oranından daha yüksek olduğu bilinmektedir(Feldman et al., 2013; Uwe E. Reinhardt: *Fees, Volume and Spending at Medicare - The New York Times*, n.d.). Laboratuvar testleri her ne kadar teşhis ve tedavi süreci için tıbbi gerekliliğe sahip olsa da bazı nedenlerden dolayı aşırı kullanımların olduğu bilinmektedir. Aşırı test kullanımı hastalara zarar verebileceği gibi sağlık hizmetleri maliyetlerini de artırmaktadır(Harb et al., 2019).

Tiroidektomi dünya genelinde en sık yapılan cerrahi işlemlerden biridir ve işlem sonucunda ortaya çıkan komplikasyonlar dikkat çekici oranlarda yüksek olabilmektedir(De Andrade Sousa et al., 2010; Rosato et al., 2004). İşlem sırasında paratiroid bezlerinin zarar görmesi hastalarda önemli sağlık sorunlarına yol açabilir (Singer et al., 2012). Total tirodektomi sonrası en sık görülen komplikasyonların kanama, vokal kord paralizisi ve hipokalsemi olduğu bildirilmiştir(Mercante et al., 2019). Cerrahi işlem yapılacak hastalara yatmadan önce ve yatış sırasında, cerrahi işlemler öncesi ve sonrasında, hastanın tanısına, yapılacak tıbbi işleme, hastanın tıbbi durumuna ve yaşına ve hekimin de uygun gördüğü birçok duruma göre bir dizi laboratuvar testi yapılmaktadır. Laboratuvar testleri yatış öncesi, cerrahi işlemin yapıldığı yatış süresi içerisinde ve taburculuk sonrası dönemlerde tıbbi kontrol amacıyla, hekimin gerek görmesi halinde yapılabilmektedir.

Bu araştırmada; bir eğitim araştırma hastanesinde, 2016 yılı içerisinde yapılmış olan tüm tiroidektomi hastalarına ait, yatış hazırlık işlemleri laboratuvar test sayıları ve cerrahi işlemin yapıldığı yatış süresinde yapılan laboratuvar test sıklıklarının belirlenmesi ve test sıklığının hastanın yaşı ve cinsiyeti, yapılan cerrahi işlem türü ve yatış süreleri ile ilişkilerinin incelenmesi amaçlanmıştır.

YÖNTEM

Araştırmada bir eğitim araştırma hastanesinde, 2016 yılı içinde Tiroidektomi işlemi yapılan 603 hastadan verilerine ulaşılabilen 594 hastaya ait veriler kullanılmıştır. Veriler Excel ortamında temin edilmiştir. Test sayıları ve tekrarları ile ilgili çalışmalar için hastaların tiroidektomi işlemi için yatışına karar verilen ve gerekli testlerin istendiği preop poliklinik işlemlerine ait testler ve işlemin yapıldığı yatışa ait test verileri kullanılmıştır. Hasta verileri manuel olarak tek tek kayda alınmış ve excel formatında düzenlenmiştir.

Hastalar cinsiyet, yaş (<50, ≥50), Tiroidektomi işlem grupları ve yatış süreleri (<4, ≥4) gruplarına göre incelenmiştir. Yaş ve yatış süreleri araştırmaya dahil edilen hasta grubu ortalamalarına göre gruplanmıştır. Toplam hastaların yaş ortalaması 49.4 olduğun için hastalar <50 yaş ve ≥50 yaş olarak gruplanmıştır. Hastaların yatış süreleri ortalaması 4.2 olup yatış süreleri <4 gün ve ≥4 olarak gruplanmıştır.

Araştırmaya en sık yapılan 23 laboratuvar testi dahil edilmiştir. Bunlar dışında kalan laboratuvar testlerinin diğer başlığı altında sadece sayıları verilmiştir. Araştırmaya dahil etme koşulu olarak sıklık, yatış prosedürüne ilişkin testler ve tiroidektomide sık kullanılan testler tercih edilmeye çalışılmıştır. Laboratuvar test sayıları cinsiyet, yaş ve yatış süresi hasta gruplarına göre karşılaştırılmıştır.

Araştırma verilerinin analizinde tanımlayıcı analizler ve bağımsız gruplarda iki ortalama arasındaki farkın önemlilik testi (Independent Samples t Test) kullanılmıştır. Araştırmada

güven aralığı %95, önemlilik değeri $p < 0,05$ olarak belirlenmiştir. Verilerin analizinde, SPSS statistic 23 (IBM, 2016) programından yararlanılmıştır.

BULGULAR

Araştırmaya dahil edilen 594 hastaya ait Tiroidektomi işlem türü ve cinsiyet ve yaş grupları dağılımı Tablo 1’de verilmiştir. Elde edilen sonuçlara göre hastaların çoğunluğu (%75.3) kadın hastalardan oluşmaktadır. En çok yapılan işlem % 87.0 oranıyla “Tiroidektomi total, iki taraf” işlemidir. Yaş gruplarına göre Tiroidektomi işlem türü dağılımlarına bakıldığında, cinsiyet gruplarında da olduğu gibi, her iki yaş grubunda da en çok yapılan işlem türü “Tiroidektomi total, iki taraf” işlemi olmuştur. Yaş gruplarına göre hasta sayılarının hemen hemen eşit dağıldığı (%49.8’e karşı %50.2) tespit edilmiştir. Tiroidektomi (Tamamlayıcı, total) işlemi (%3.9) üçüncü en çok yapılan işlem olarak bulunmuştur. Hastaların çoğunluğu (%67.8) 4 günden daha az yatmıştır.

Tablo 1. Tiroidektomi İşlem Türlerinin Cinsiyet, Yaş ve Yatış Süresi Gruplarına Göre Dağılımı

İşlem Türü	Cinsiyet (n / %)		Yaş (n / %)		Yatış Süresi (n / %)		Toplam
	Kadın	Erkek	<50	≥50	<4	≥4	
Substernal tiroidektomi, intratorasik	1 / 0.2	2 / 1.4	1 / 0.3	2 / 0.7	1 / 0.2	2 / 1.0	3 / 0.5
Tiroidektomi (Tamamlayıcı, total)	19 / 4.3	4 / 2.7	8 / 2.7	15 / 5.0	10 / 2.5	13 / 6.8	23 / 3.9
Tiroidektomi (Tek taraf total ve karşı taraf subtotal)	0 / 0.0	1 / 0.7	0 / 0.0	1 / 0.3	0 / 0.0	1 / 0.5	1 / 0.2
Tiroidektomi subtotal, iki taraf	1 / 0.2	1 / 0.7	0 / 0.0	2 / 0.7	2 / 0.5	0 / 0.0	2 / 0.3
Tiroidektomi subtotal, tek taraf	3 / 0.7	1 / 0.7	1 / 0.3	3 / 1.0	4 / 1.0	0 / 0.0	4 / 0.7
Tiroidektomi total, iki taraf	394 / 88.1	123 / 83.7	264 / 89.2	253 / 84.9	353 / 87.6	164 / 85.9	517 / 87.0
Tiroidektomi total, tek taraf	29 / 6.5	15 / 10.2	22 / 7.4	22 / 7.4	33 / 8.2	11 / 5.8	44 / 7.4
Toplam (n / %)	447 / 100.0	147 / 100.0	296 / 100.0	298 / 100.0	403 / 100.0	191 / 100.0	594 / 100.0
Toplam %	75.3%	24.7%	49.8%	50.2%	67.8%	32.2%	100.0%

Tiroidektomi hastalarına toplam 186 farklı laboratuvar testi yapılmış olup 594 hastaya toplam 28174 adet laboratuvar testi yapılmıştır. Tablo 2’de görüldüğü gibi en fazla yapılan ilk 23 laboratuvar testleri toplam testlerin %83.5’ini oluşturmaktadır. En sık yapılan laboratuvar testi %17.16 oranı ile Hemogram testidir.

Tablo 2. Tiroidektomi Hastalarına Yapılan Laboratuvar Testleri

Test Adı	Test Sayısı	(Test sayısı içinde) %	Ortalama Test Sayısı
Tam Kan (Hemogram)	4834	17.16%	8.14
Kalsiyum (Ca)	1623	5.76%	2.73
Albümin	1481	5.26%	2.49
Potasyum	1027	3.65%	1.73
Sodyum (Na) (Serum ve vücut sıvılarında, herbiri)	1023	3.63%	1.72
Kreatinin	1018	3.61%	1.71
Protein (Serum ve vücut sıvıları, herbiri)	1003	3.56%	1.69
Alanin aminotransferaz (ALT)	975	3.46%	1.64
Kan üre azotu (BUN)	974	3.46%	1.64
Aspartat transaminaz (AST)	950	3.37%	1.60
Bilirubin (Total,direkt), her biri	921	3.27%	1.55
Glukoz	901	3.20%	1.52
Gamma glutamil transferaz (GGT)	752	2.67%	1.27
Protrombin zamanı (Koagülometre)	732	2.60%	1.23
Alkale fosfataz	727	2.58%	1.22
Kan gazları	681	2.42%	1.15
TSH	665	2.36%	1.12
APTT	632	2.24%	1.06
Serbest T4	544	1.93%	0.92
Gluko test (Hastabaşı, glukometrik)	534	1.90%	0.90
HCV (ELISA)	514	1.82%	0.87
HBsAg (ELISA)	511	1.81%	0.86
Anti HIV (ELISA)	510	1.81%	0.86
Diğer Laboratuvar Testleri	4642	16.48%	7.81
Toplam	28174	100.0%	47.43

Tablo 3. Cinsiyet, Yaş ve Yatış Süresi Hasta Gruplarına Göre Laboratuvar Test Sayılarının Karşılaştırması

Laboratuvar testleri	Cinsiyet		Yaş		Yatış Süresi (gün)	
	Kadın (K) (n=447)		<50 (n=296)		<4=403	
	Erkek (E) (n=147)		≥50 (n=298)		≥4=191	
	Ort. / SS	p	Ort. / SS	p	Ort. / SS	p
Tam kan hemogram	K	7.58 / 23.136	<50	5.84 / 20.170	<4	4.50 / 13.602
	E	9.84 / 24.059	≥50	10.42 / 25.995	≥4	15.82 / 35.006
Kalsiyum	K	2.59 / 2.073	<50	2.47 / 1.798	<4	2.06 / 0.703
	E	3.16 / 3.351	≥50	2.99 / 2.957	≥4	4.15 / 3.856
Albumin	K	2.34 / 1.858	<50	2.24 / 1.779	<4	1.87 / 0.765
	E	2.95 / 3.308	≥50	2.74 / 2.725	≥4	3.81 / 3.595
Potasyum	K	1.55 / 1.823	<50	1.45 / 1.497	<4	1.13 / 0.607
	E	2.27 / 3.342	≥50	2.00 / 2.881	≥4	2.99 / 3.682
Sodyum	K	1.55 / 1.816	<50	1.45 / 1.479	<4	1.13 / 0.609
	E	2.26 / 3.335	≥50	1.99 / 2.879	≥4	2.97 / 3.671
Kreatinin	K	1.53 / 1.833	<50	1.45 / 1.504	<4	1.11 / 0.607
	E	2.26 / 3.354	≥50	1.98 / 2.895	≥4	2.98 / 3.697
	K	1.58 / 1.560	<50	1.59 / 1.531	<4	1.31 / 0.883

Protein (serum ve vücut sıvıları herbiri)	E	2.02 / 2.440		≥50	1.78 / 2.075		≥4	2.50 / 2.789	
Alanin_aminotransferaz_ALT	K	1.49 / 1.699	0.023*	<50	1.48 / 1.433	0.069	<4	1.15 / 0.584	<0.001*
	E	2.10 / 3.026		≥50	1.80 / 2.623		≥4	2.68 / 3.421	
Kan_üre_azotu_BUN	K	1.48 / 1.787	0.023*	<50	1.40 / 1.458	0.008*	<4	1.08 / 0.587	<0.001*
	E	2.12 / 3.245		≥50	1.88 / 2.810		≥4	2.82 / 3.612	
Aspartat_transaminaz_AST	K	1.45 / 1.687	0.021*	<50	1.43 / 1.373	0.047*	<4	1.12 / 0.565	<0.001*
	E	2.05 / 2.955		≥50	1.77 / 2.601		≥4	2.61 / 3.376	
Bilirubin_total_direkt_herbiri	K	1.39 / 2.086	0.019*	<50	1.32 / 1.836	0.019*	<4	0.97 / 0.830	<0.001*
	E	2.05 / 3.163		≥50	1.78 / 2.856		≥4	2.77 / 3.807	
Glukoz	K	1.38 / 1.528	0.022*	<50	1.36 / 1.312	0.047*	<4	1.08 / 0.589	<0.001*
	E	1.94 / 2.819		≥50	1.67 / 2.402		≥4	2.44 / 3.126	
Gamma_glutamils transferaz_GGT	K	1.16 / 1.691	0.061	<50	1.10 / 1.185	0.034*	<4	0.86 / 0.627	<0.001*
	E	1.58 / 2.491		≥50	1.43 / 2.441		≥4	2.12 / 3.109	
Protrombin_zamanı_Koagülometre	K	1.24 / 0.867	0.726	<50	1.18 / 0.776	0.129	<4	1.14 / 0.528	<0.001*
	E	1.21 / 0.813		≥50	1.29 / 0.923		≥4	1.43 / 1.275	
Alkalen_fosfataz	K	1.14 / 1.462	0.118	<50	1.09 / 1.152	0.070	<4	0.88 / 0.615	<0.001*
	E	1.47 / 2.374		≥50	1.35 / 2.160		≥4	1.95 / 2.798	
Kan_gazları	K	1.09 / 7.214	0.740	<50	0.50 / 3.511	0.025*	<4	0.28 / 3.244	0.001*
	E	1.31 / 6.343		≥50	1.79 / 9.213		≥4	2.97 / 11.221	
TSH	K	1.09 / 0.881	0.161	<50	1.12 / 0.802	0.955	<4	1.03 / 0.624	0.003*
	E	1.21 / 0.916		≥50	1.12 / 0.972		≥4	1.31 / 1.263	
APTT	K	1.07 / 0.649	0.624	<50	1.05 / 0.525	0.539	<4	1.00 / 0.399	0.008*
	E	1.04 / 0.691		≥50	1.08 / 0.770		≥4	1.20 / 0.996	
Serbest_T4	K	0.90 / 0.904	0.431	<50	0.91 / 0.758	0.776	<4	0.80 / 0.624	<0.001*
	E	0.97 / 0.847		≥50	0.93 / 1.006		≥4	1.16 / 1.248	
Glukotest	K	0.78 / 2.193	0.224	<50	0.30 / 1.215	<0.001*	<4	0.32 / 0.858	<0.001*
	E	1.26 / 4.577		≥50	1.50 / 3.926		≥4	2.12 / 4.875	
HCV_Elisa	K	0.87 / 0.367	0.967	<50	0.90 / 0.399	0.043*	<4	0.92 / 0.362	<0.001*
	E	0.86 / 0.492		≥50	0.83 / 0.400		≥4	0.74 / 0.450	
HBsAg_Elisa	K	0.86 / 0.371	0.913	<50	0.90 / 0.402	0.034*	<4	0.92 / 0.368	<0.001*
	E	0.86 / 0.483		≥50	0.83 / 0.397		≥4	0.74 / 0.441	
Anti_HIV_Elisa	K	0.86 / 0.373	0.960	<50	0.89 / 0.404	0.106	<4	0.92 / 0.361	<0.001*
	E	0.86 / 0.468		≥50	0.83 / 0.392		≥4	0.73 / 0.444	

*p<0,05

En sık yapılan 23 laboratuvar test sayılarının cinsiyet, yaş ve yatış süresi gruplarına göre karşılaştırması Tablo 3'te özetlenmiştir. Hemogram, Kalsiyum, Gamma glutamil transferaz GGT, Protrombin zamanı (Koagülometre), Alkalen fosfataz, Kan gazları, TSH, APTT, Serbest T4, Gluko test, HCV (Elisa), HBsAg (Elisa) ve Anti HIV (Elisa) testlerinin cinsiyet grupları arasındaki fark istatistiksel olarak anlamlı bulunmamıştır. Ancak, Albümin, Potasyum, Sodyum, Kreatinin, Protein (Serum ve vücut sıvıları, herbiri), Alanin aminotransferaz (ALT), Kan üre azotu (BUN), Aspartat transaminaz (AST), Bilirubin (Total,direkt), her biri ve Glukoz test sayıları erkekler lehine yüksek ve istatistiksel olarak anlamlı bulunmuştur. Yaş gruplarına göre; Protein (Serum ve vücut sıvıları, herbiri), Alanin aminotransferaz (ALT), Protrombin zamanı (Koagülometre), Alkalen fosfataz, TSH, APTT, Serbest T4 ve Anti HIV (Elisa) testi

dışındaki laboratuvar test sayıları “ ≥ 50 ” yaş grubu hastalar lehine yüksek ve istatistiksel olarak anlamlı bulunmuştur. Araştırmadaki en çarpıcı bulgu yatış süreleri gruplarının test sayıları arasındaki farklılıklarda görülmüştür. Analize dahil edilen tüm testlerin test sayıları yatış süresi ≥ 4 gün olan hastalarda yatış süresi < 4 gün olan hasta grubuna göre çok açık bir şekilde yüksek çıkmış ve bu sonuçlar istatistiksel olarak anlamlı bulunmuştur.

SONUÇ

Araştırma grubunda en çok yapılan tiroidektomi işlemi “Tiroidektomi total, iki taraf” işlemidir. Tiroidektomi işlemi yapılanların 3/4’ü kadın hastalardır. Hastaların yarıdan fazlası ≥ 50 yaş grubu hastalardan oluşmaktadır. Hastaların yarıdan fazlasının yatış süresi 4 günden daha düşüktür. En fazla yapılan test Tam Kan (Hemogram) testi olup hasta başına ortalaması diğer laboratuvar test ortalamalarına göre oldukça yüksektir. Bu hastalara cerrahi işlemi yapılmadan kontrol amacıyla istenen testlerden biri olması ve işlemden sonra klinik kontrol amacıyla sık kullanılan testlerden biri olması ile açıklanabilir. Ancak hasta başına ortalama hemogram test sayısının bu kadar yüksek olmasını etkileyen sebeplerin ayrıca incelenmesinin tıbbi bakım kalitesini artırmak ve maliyetlerin düşürülebilmesi için etkili olabileceği söylenebilir. Araştırma grubu çoğunluğu kadın hastalardan oluşmasına karşın, genel olarak erkek hastalarda test sayıları kadınlara göre daha yüksek bulunmuştur. Araştırmadaki hastalarda kronik hastalıkların varlığına yönelik bilginin olmamasının getirdiği kısıtlılıktan dolayı bunun nedenlerine yönelik ayrıntılı çalışma yapılamamıştır. Tiroidektomi hastalarında 50 yaş ve üzeri hasta grubunda test sayılarının genel olarak daha yüksek olduğu görülmüştür. Bu sonucun yaşlanmaya ve yaşlanmanın beraberinde getirdiği hastalıklara bağlı olabileceği düşünülmektedir. Ancak yatış süresi incelendiğinde; 4 gün ve daha uzun süre hastane yatışı laboratuvar test sayılarını dikkat çekici miktarlarda artırmaktadır.

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**DIGITAL MARKETING IN HEALTH TOURISM: A RESEARCH FOR PRIVATE
HEALTH INSTITUTIONS**

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ABSTRACT

In recent years, both the changes and transformations in technology, the developments in the field of health, the change in the patient-physician relationship, the increasing demands in the field of preventive health services, and the fact that the health status of the patients has begun to follow more closely increase the interest of health institutions in marketing. In addition, the emergence of new technologies increases society's expectations of health services. In addition to these, both the health sector and health tourism are a sector that operates with different dynamics from other sectors. All these developments also increase the costs in health institutions and push them to research different digital marketing techniques and use them more effectively, taking into account the special conditions of the sector. In this study, secondary data were used. In the research, the use of digital marketing channels of the first five private health institutions, which have the internationally valid JCI accreditation certificate in the health sector and have the highest number of followers on youtube, which is the most used social media channel in Turkey in 2021, was evaluated. As a result; Although there are some developments in private health institutions' websites, mobile applications, and blogs, it has been determined that they are not at the desired level. In line with the results obtained, suggestions have been developed for health institutions and researchers working in the relevant literature.

Keywords: Health Tourism, Digital Marketing, JCI Certificated Health Institutions, Youtube

ÖZET

Son yıllarda gerek teknolojideki değişim ve dönüşümler gerekse sağlık alanında yaşanan gelişmeler, hasta-hekim ilişkisinde değişim, koruyucu sağlık hizmetleri alanındaki artan talepler ve hastaların sağlık durumlarını daha yakından takip etme başlaması sağlık kurumlarının pazarlamaya olan ilgisini de artırmaktadır. Ayrıca, yeni teknolojilerin ortaya

çıkmasıyla toplumun sağlık hizmetlerinden beklentilerini de arttırmaktadır. Bunların yanı sıra gerek sağlık sektörü gerekse sağlık turizmi diğer sektörlerden farklı dinamiklerle işleyen bir sektördür. Tüm bu gelişmeler sağlık kurumlarında maliyetleri de arttırmakta sektörün özel şartlarını da gözetenek farklı dijital pazarlama tekniklerini araştırmaya ve bunları daha etkin kullanmaya da itmektedir. Bu çalışmada, ikincil veriler kullanılmıştır. Araştırmada sağlık sektöründe uluslararası geçerliliğe olan JCI akreditasyon belgesine sahip ve Türkiye de 2021 yılında en çok kullanılan sosyal medya mecrası olan youtube'da en çok takipçi sayısı olan özel sağlık kuruluşlarından ilk beş kuruluşun dijital pazarlama kanallarını kullanımı değerlendirilmiştir. Sonuç olarak; özel sağlık kuruluşlarının web sitesi, mobil uygulamalar ve blog konularında bazı gelişmeler olmakla birlikte istenilen düzeyde olmadığı tespit edilmiştir. Elde edilen sonuçlar doğrultusunda sağlık kurumlarına ve ilgili literatürde çalışmalar yapan araştırmacılara yönelik öneriler geliştirilmiştir.

Anahtar Kelimeler: Sağlık Turizmi, Dijital Pazarlama, JCI Belgeli Sağlık Kurumları, Youtube

INTRODUCTION

With globalization, with the cooperation of countries, from the world traveling in the world of travel, from the world traveling from the other world to a more tremendous success than the quality of the health service in the world, from the service to the world, from the world traveling to travel to the whole world, from the whole world, from the quality of the health service from a world more significant than the quality of health care in the world from a world more significant than the quality of health care in the world (Jenner, 2008).

From the perspective of the health sector; From now on, no more information is given about the health care services that are being referred to patients with interest. It can be designed to be delivered in health services, benefit from the services, and benefit from possible health services. However, new days to health services are increasing and benefit from health care applications. In this regard, they know the education and use modern marketing techniques in health services (Karaçor and Arkan, 2014). In addition, information and research on health problems have become observable through digital platforms (İlgün and Uğurluoğlu, 2019).

CONCEPTUAL FRAMEWORK

Health Tourism, Quality and Digital Marketing

In the most general sense, health tourism is tourism created by people who travel from their residence to other places for health purposes. For Turkey to get the share it wants from the health tourism cake, health institutions need to improve their service quality and be accredited, become sufficient in terms of physical and medical equipment, and be cheaper. In addition to providing treatment, they should also use other instruments (Tengilimoğlu, 2013). Researchers

Huang and Xu (2018) and Marković et al. (2014) state that although health tourism has been a developing market in recent years, it is an area that focuses on and researches gain significance. With this, the primary purpose of health tourism marketing is the “announcement of our personality with its outstanding features such as education, experience, and competencies, superior service such as general systems of hospitals and communication methods to target audiences” (Tontus,2018). Moreover; the quality, accessibility, and cost of the service provided to the patient and patient candidate, who can be considered as the target audience in the health sector, are among the most critical issues today (Akalın and Verenyurt, 2020).

Quality studies and the concept of accreditation come to the fore in the health sector. *Accreditation* is defined as a quality infrastructure created to verify the evaluation of the studies by conformity assessment bodies and the validity and reliability of the conformity documents issued based on the studies (Karaca at all., 2020).

Digital marketing can generally be defined as the marketing of goods or services using classical marketing methods and digital marketing tools and channels (Kannan, 2017). Healthcare organizations are expanding their existing markets through their websites (Watson et al., 2000). Well-designed websites and having sufficient content allow accessible communication with patients and ensure that marketing activities are carried out more effectively (Gruca and Wakefield, 2004).

Deloite (2022) states that according to data, there are more than 260 thousand health-related applications worldwide and more than seventy percent of patients use at least one of the applications mentioned in order to manage their health conditions (Deloite,2022). As a result of these developments, a digital ecosystem is formed in health. At the institutional level, a study states that approximately half of the budget allocated to health services marketing in the USA is devoted to digital marketing (Horner, 2017). This helps to strengthen further the message that health organizations want to reach their target audience (Ngoqo, 2018).

RESEARCH METHOD

In this study, to investigate the digital marketing research that health institutions who have JCI (Joint Commission International) accreditation and who are in the top 5 on youtube channel (Boomsocial, 2022), which is the most preferred social media channel in Turkey, can have health tourists.

The websites of the received health service have been evaluated to the extent needed and used by the review reviews in the literature (Gruca and Wakefield, 2004; Bildir and Buzcu,2004; Huang and Chang, 2014; Uğurluoğlu, 2009; Zingmond et al., 2001). In a examined website kitchen, features or absence of features related to the checklist, which includes a total of 36 different features under 4 headings, were determined. The mobile website and mobile applications were not evaluated due to content evaluation.

“JCI is a worldwide prestigious institution that determines the most successful practices in the field of quality and patient safety in the field of health, and measures all dimensions such as accreditation, patient safety and quality care (JCI, 2022). Within the scope of the research, the private health services organizations that have the most followers on Youtube and have JCI accreditation are listed in the table below.

Digitalization in health services has transformed marketing activities along with the service provided. Digital marketing applications to healthcare providers; Compared to traditional marketing practices, it offers the opportunity to reach more individuals in a much shorter time and provides various advantages such as competitive advantage, cost savings, and more effective patient-physician relationship development (Demirci and Uğurluoğlu, 2020). This study it is aimed to evaluate the use of digital marketing channels by private health institutions. For this purpose, the use of digital marketing channels of the first five private health institutions, which have the internationally valid JCI accreditation certificate in the health sector and have the highest number of followers on youtube, which is the most used social media channel in Turkey in 2021, has been evaluated (Table 1). In the literature, website, search engine optimization, social media, mobile website, mobile application, health-related blogs, and various video sharing sites are among the digital marketing tools of a healthcare organization (Chaffey and Smith, 2013; Demirci and Uğurluoğlu, 2020; Dahiya and Gayatri, 2018; Yalçınkaya, 2018; Kannan, 2017; Taiminen and Karjaluo, 2015; Key, 2017). The use of digital marketing tools by private health care institutions has been examined within the framework of these identified titles. In this study; The use of digital marketing channels of private health institutions was examined through the titles of website, mobile website and mobile application, health-related blog use.

Table 1. Private Health Services Organizations Included in the Research

Acıbadem Hospital
NP İstanbul Brain Hospital
Yeditepe University Hospital
Florance Nightingale Hospital
Memorial Hospital

FINDINGS

When the websites of the five health institutions included in this study were examined in terms of content and features, it was determined that NP Istanbul Brain Hospital was the health institution that met the criteria determined most, with 83.3%. NP Istanbul Brain hospital is followed by Yeditepe University Hospital with 80.5%, Acıbadem Hospital with 77.7%,

Memorial Hospital with 77.7 %, and Florence Nightengale Hospital with 66.6% (Table 2). When the results regarding the general information in Table 2 are examined, the fee information, parking information and Patient satisfaction survey were not shared in all hospitals included in the study. In addition, some health institutions' virtual tour, performance information, Current News, online support, patient satisfaction, information on patient relatives/visitors, hospital admission/exit procedures, and content related to international patients; It has been determined that the interactive service and health information does not include membership to the website, evaluation of the use of the website, online access to the physician, and health event news.

Table 2: Analysis of Hospitals and Websites Included in the Study

Website Features	Acibadem Hospital	NP İstanbul Brain Hospital	Yeditepe University Hospital	Florence Nightingale Hospital	Memorial Hospital
General Information					
History	+	+	+	+	+
Mission/Vision Statements	+	+	+	+	+
Current News/Announcements	+	+	+	+	-
News in the Press	-	+	+	-	+
Hospital logo	+	+	+	+	+
Parking information	-	-	-	-	-
Virtual tour Location	-	-	-	-	+
List of medical units	+	+	+	+	+
Performance (operation, inpatient, treated etc. patient) information	-	-	-	-	+
Accreditation Information	+	+	+	+	+
ISO 9001:2000 Quality Management System Certificate	+	+	+	+	+

Address information	+	+	+	+	+
Phone number	+	+	+	+	+
Target Group and Stakeholder					
Information List of physicians	+	+	+	+	+
Information on physicians	+	+	+	+	+
Patient satisfaction survey	-	-	-	-	-
Patient rights	-	+	+	+	+
Fee information	-	-	-	-	-
Information on patient relatives/visitors	+	+	+	+	-
Information on hospital admission/exit procedures	+	+	+	-	-
Content related to international patients	+	+	+	-	+
Interactive Service Information					
Online Appointment	+	+	+	+	+
Online support	+	+	-	-	+
Online access to audit results	+	+	+	+	+
Website membership	+	-	-	-	+
Foreign language support	+	+	+	-	+
Information request by e-mail	+	+	+	+	+
Online wish complaint notification	+	+	+	+	+
Evaluation of website usage	+	+	-	-	+
Search option	+	+	+	+	+

Physician online access	+		+		+		+		+	
Access to hospital web pages through the institution's website	+		+		+		+		+	
Health information										
General health information	+		+		+		+		+	
Link for more information	+		+		+		+		+	
Health event news	+		+		+		-		+	
Total of Satisfying Specified Features	n	%	n	%	n	%	n	%	n	%
	28	77,7	30	83,3	29	80,5	24	66,6	28	77,7

The news in the press, parking information for those who reach the hospital with their vehicles, the performance information of the health institution, the salary information, and the certificates obtained by the health institution is essential in terms of reaching the target audience, promoting the health institution and reaching the information they need (Yurdakul and Öksüz, 2007). When we look at the studies examining the websites of health institutions, similar to this study; It is observed that there are deficiencies in interactive services such as general information, performance, and wage information, sharing health event news, online access to the physician, and evaluation of the website (Zingmond et al., 2001; Birdir and Buzcu, 2014; Uğurluoğlu, 2009; Demirci and Uğurluoğlu, 2020). While 62.5% of the world uses the internet and the rate of internet usage globally is 65.5%, this rate is 82%. (www.recrodigital.com, 2022). The data above shows that it can access system systems. The fact that these websites have services for their intended use is more than the purpose of use and reaches a more comprehensive patient audience.

In Table 3, the status of private health institutions having a mobile website, blog, and mobile application. It has been determined that all health institutions have mobile websites (Mobile-Friendly Test, ND). When the status of having a blog is examined, it is determined that Acıbadem, NP Brain Hospital and Yeditepe University Hospital are health institutions. Blogs are an essential marketing tool for healthcare organizations in terms of improving the interaction between the patient and the healthcare institution (Özüdoğru, 2014). In addition, it was determined that Acıbadem and Memorial health institutions have just mobile applications. Mobile applications for health workers include making an appointment for users' electronic records and health-related information, duties in the office, and announcing health-related news (Seabrook, at all., 2014).

Table 3. Use of Mobile Websites, Blogs and Mobile Applications by Health Institutions Participated in the Research

Private Health Institutions	Mobile Website	Blog	Mobile Applications	
			Android	Los
Acıbadem	+	+	+	+
NP İstanbul Brain Hospital	+	+	-	-
Yeditepe University Hospital	+	+	-	-
Florance Nightingale Hospital	+	-	-	-
Memorial	+	-	+	+

CONCLUSION AND DISCUSSION

This study has been tried to determine whether private health institutions use digital marketing channels effectively or not. It is a result of the findings; it has been seen that the health institutions within the scope of the research have some deficiencies in the use of digital marketing channels. It has been determined that some of the features that health institutions should have on their websites are not provided, also within the scope of the research have a blog, Acıbadem Hospital, NP Brain Hospital and Yeditepe University Hospital have mobile applications. As a result of these determinations, it is thought that it would be beneficial to establish digital marketing units as sub-units of marketing departments in private health institutions to use digital marketing channels more effectively. In addition, it is thought that it will be valuable and practical to focus on more specific issues such as finding web pages and managing these pages effectively, having an electronic satisfaction survey for patients on the website, creating a web page where discharged patients can share their thoughts. More comprehensive health institutions can be subjected to in-depth analysis in future studies, and their mobile applications can be analyzed.

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**KARABAĞ SAVAŞ QAZİLERİNİN TIBBİ DOĞRU ZAMANDA UYGULANMIŞ
REABİLİTASYON PROGRAMININ SONUÇLARI**
SOME RESULTS OF THE MEDICAL REHABILITATION PROGRAM OF KARABAKH
WAR VETERANS

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ABSTRACT

Anavatanımızın özgürlüyü için Karabağ savaşına katılan gazilerimizin sağlığına kavuşması bugün Azerbaycan Halk Sağlık Bakanlığının en önemli vazifelerinden biridir. Bu hastalarda askeri çöl koşullarında alınan kapalı kafa travmaları, kör şarapnel yaraları, ekstremitelerin çeşitli yerlerindeki kırıkları ve diğer yaralanmalar rehabilitasyon ve fizyoterapi yöntemleri ile tedavi edilmektedir.

Araştırmaların amacı: Ulusal spor ve tıp rehabilitasyon enstitüsünde tedavi gören 60 gazinin tıbbi reabilitasyon programının bazı sonuçlarının incelenmesidir.

Ulusal spor ve Rehabilitasyon Enstitüsünde tedavi alan qazilerin tedavi-randevu kartları incelendi. Bu hastalar iki gruba ayrıldı.

İlk aşamada esas (birinci) grup tedaviye daha erken, doğru zamanda başlayan 36 hastanı, ikinci karşılaştırma grupta daha geç süre zarfında rehabilitasyon tedavisi gören 24 hastayı içeriyordu.

Kullanılan tedavi yöntemleri Manyetik terapi, Elektroforez, Kısa dalgalı ultrason tedavisi, Lazer tedavisi, Robotik yürüme cihazı--GEO, Tedavi yönümlü egzersizler, "Artromot" cihazı.

Çalışmamızın sonucuna göre, birinci (esas grup) gruptaki hastalarda iyileşme ikinci (kıyaslama grubu) gruba göre daha erken ve iyileşme sayısı toplam hasta sayısının büyük kısmını oluşturmaktadır. Birinci grupta kulak zarı barotravması tanısı alan 4 hastanın 3-ü (%8.3) ilk

olarak iyileşmeyi bildirdi. Bu rakam ikinci grupta (%4.6) eşitti, birinci grupta 4 hastanın 3-ünde (%8.3) ikinci gruptaki iki hastanın birinde (%4.16) sinir travmasından (n.tibialis) iyileşme bildirildi. Hem erken, hem de geç dönemde fizyoterapiye katılan hastalarda hemen-hemen aynı prosedürler reçete edildi. Ancak fizyoterapiye başlanıldığında birinci grupta daha erken pozitif rehabilitasyon sonuçları (kas-iskelet sistemi, bilişsel, görsel, işitsel işlevlerde iyileşme) gözlemlendi.

Anahtar kelimeler: Askeri çöl yaraları, fizyoterapi, rehabilitasyon yöntemleri

ABSTRACT

Restoration of the health of our veterans who fought in the Karabakh war for the freedom of our homelands is one of the most important tasks of Azerbaijan`s health today.

In these patients closed head traumas blind shrapnel wounds, fractures of various parts of the extremities and other injuries received in military—field conditions can be successfully treated with rehabilitation—physiotherapeutic methods.

The purpose of study is preliminary results of the medical rehabilitation program of 60 veterans treated at NSMRI (National Sport and Medical Rehabilitation Institute). Information of treatment appointment cards of 60 patients aged 23-48 treated at NSMRI.

These patients were divided into two groups: In the first phase, the main (first) group includes 36 patients who started treatment earlier, at the right time, the second comparison group included 24 patients who received rehabilitation therapy later in the day.

The methods of treatment used magnetic therapy, electrophoresis, short-wave ultrasound therapy, laser therapy, robotic gait, massage, Geo-therapy, therapeutic gymnastics, "Artromot" device.

As can be seen, patients in group 1 recover faster than group 2 and recover more than the total number of patients.

For example, 3 (8.3%) of the 4 patients in the first group with a diagnosis of ear barotrauma had an initial recovery compared to 1 (4.6%) in the second group; 3 (8.3%) of the 4 patients in the first group recovered from nerve injuries, 1 (4.16%) recovery from 2 patients in the second group. Almost the same procedures were prescribed to patients who joined physiotherapy in both early and late stages, but earlier than those who started physiotherapy early. Signs of rehabilitation (improvement of musculoskeletal system, cognitive functions, vision, hearing) were observed.

Keywords: Military-field injuries, physiotherapy, rehabilitation methods.

**RETROSPECTIVE ANALYSIS OF PATIENTS PRESENTING TO THE
EMERGENCY DEPARTMENT WITH POISONING AFTER ORAL DRUG
INGESTION**

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ABSTRACT

In cases of intoxication with oral medication, especially for suicide, the patient takes very high doses of medication, and often a life-threatening situation occurs. This situation is an important part of emergency department (ED) admissions. In this study, we tried to examine the treatment and prognosis of patients who were poisoned by taking oral drugs intentionally or accidentally and applied to our emergency department for 2 years.

In our study, we retrospectively reviewed the files of 257 patients who applied to our ED with the complaint of accidentally or intentionally taking oral drugs between January 2018 and December 2020 and were diagnosed with intoxication. The demographic characteristics of these patients, the medications they took, their symptoms at admission, the treatments applied, and their prognosis were evaluated.

In our study, archiving data were analysed retrospectively and 257 records were included. It was determined that the most frequent admissions to the ED were between 16:00 and 00:00 and in July (12%). It was observed that women (54.9%) most often attempted suicide and that they did so mainly by taking analgesic drugs (32.2%). 51.8% of the patients were asymptomatic, 30.8% were treated in the ED, and 2 (0.8%) of 27 patients who were referred to another hospital died.

Treatment and monitoring of intoxication cases are difficult and often uncertain processes. Patients' prognosis varies according to several factors. In our study, we obtained data compatible with the literature and, we observed that the prognosis was good. Therefore, we believe the transfer of data to documentation will facilitate the follow-up of the patient by the emergency physician and the clinician who follows the patient. In addition, we hope that the length of stay will be standardized and that the capacity of the hospital will be used effectively.

Keywords: Emergency Department, Intoxication, Epidemiology

INTRODUCTION

Poisoning is when a substance negatively impacts the functioning of any system in the body. If exposure is high, each substance can become poison. Although poisoning usually occurs orally, it can also occur through many routes such as inhalation, breathing, skin, mucous membranes, or injection. The primary referral center for these patients is generally the Emergency Department (ED). The nature and quantity of the substance taken, the time elapsed between exposure and admission to hospital, is of great importance for the first intervention and treatment in hospital. Although the rate of ED visits due to poisoning varies widely around the world, it typically accounts for 5-10% of all patients admitted to the ED (1). While admission due to accidental intoxication is more prevalent in children and older people, it is more prevalent in adolescence and adulthood due to suicidal intoxication.

The first symptoms of an oral poisoned patient are usually in the form of vomiting caused by irritation caused by gastrointestinal disruption (GIS). The aim of interventions in the emergency department should be to ensure that as few toxic substances as possible, remain in the body and reduce GI absorption, similar to the physiology. Gastric lavage and activated charcoal are usually used first. Excretion should be accelerated by hydration, a symptomatic approach should be taken based on the type of toxic substance taken, and an antidote should be applied where appropriate.

In this study, we tried to analyse the demographic characteristics, treatments and, prognosis of patients who applied to our ED with the complaint of poisoning after accidental or deliberate oral drug intake.

MATERIAL AND METHOD

The Ethics Committee of the Institute of Health Sciences of Kırklareli University (klusabe-PR0306R01/1) has approved our study. In our study, we included patients who had signs of intoxication after oral medications and entered the X44 ICD (International Statistical Classification of Diseases and Related Health Problems) code in the system between 2018 and 2020 in the ED of our hospital. Patient files have been analysed retrospectively from the archive. In the files, we examined demographic characteristics of the patients, type of exposure (accidental or suicidal), admission complaint, time and duration of admission to the ED, amount and type of taken drugs, vital signs at admission, treatment method applied, duration of treatment, outcomes, mortality, etc.

Statistical analytics were performed using SPSS 19.0 for Windows. Descriptive criteria are presented as mean and standard deviation, percentage distribution. The Kolmogorov-Smirnov test verified that the data conformed to the normal distribution. Student t-test was used to compare continuous variables, and chi-square analysis was used to examine distributions among groups, $p < 0.05$ level of significance.

RESULTS

In our study, which included patients who took drugs orally and had signs of intoxication, 265 emergency room patient files were scanned. Since we could not access the information in any files, we included 257 patient files in the study. 116 (45.1%) patients were male, 141 (54.9%) were female. 74 patients (28.7%) were under the age of 18 years old (Figure-1). The mean age of all patients was 25.8 ± 17.3 years. It was determined that patients applied to our ED most frequently between 16:00 and 00:00 and within an average of 2.43 ± 3.23 hours after taking the drug. 180 (70.1%) of the patients were admitted for suicidal purposes, 77 (29.9%) of the patients were admitted with accidental drug intake. Adults constituted 24 (13.5%) of those who took drugs accidentally, and children constituted 53 (66.2%). 19 (7.4%) patients stated that they took alcohol with the drug (Table-1). According to the application times, the most frequent applications were in summer ($n=79$, 31%) (Figure-2). As a result of the analysis, it was determined that those who took drugs for the purpose of suicide were statistically higher in patients over the age of 18 and in the female gender ($p<0.001$) (Table-2) The mean age of those who took drugs for suicidal purposes was statistically significantly higher than the average age of those who took drugs by accident ($p<0.001$) (Table-3).

Figure 1. Distribution of patients by gender

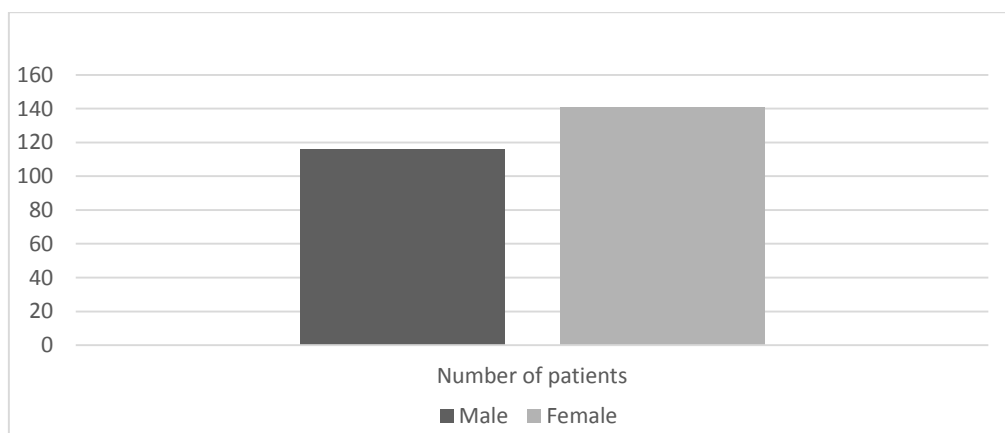


Table 1. Distribution of patients' admission times, reasons for taking medication, amounts of drugs taken, and alcohol use histories

	Number of patients (n)	Ratio (%)
Admission Time		
08:01-16:00	68	26.5
16:01-00:00	132	51.3
00:01-08:00	57	22.2
Type of exposure		
Accidental	77	29.9
Suicidal	180	70.1

Drugs taken		
Single	164	63.8
Multiple	93	36.2
Taken with alcohol		
No	238	92.7
Yes	19	7.3

Table 2. The relationship between the type of exposure and age, gender

Type of exposure

	Accidental n (%)	Suicidal n (%)	p value
<18 years old	53 (68.8)	21 (11.7)	0.001
18 ≥ years old	24 (31.2)	158 (88.3)	
Male	48 (62.3)	67 (37.4)	0.001
Female	29 (37.7)	112 (62.6)	

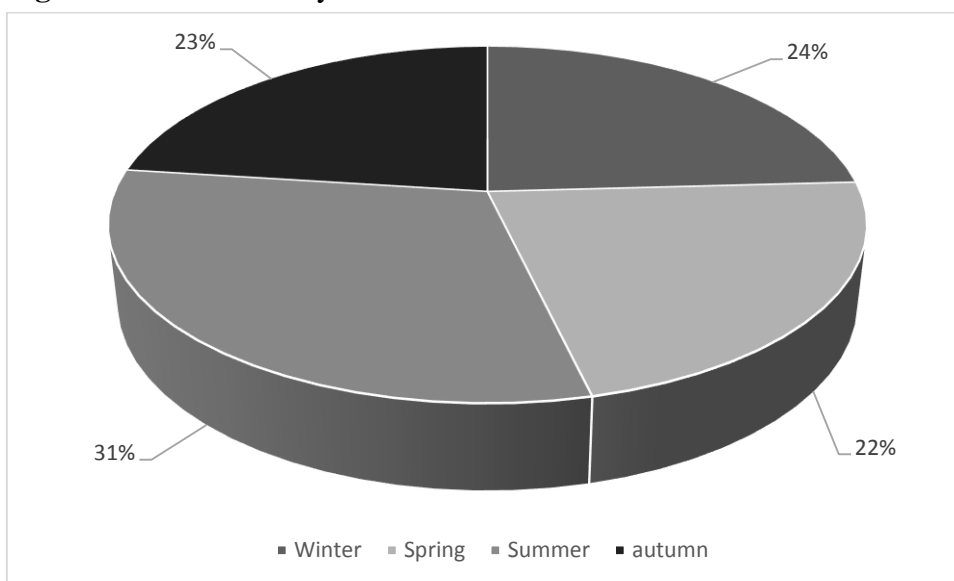
n: Patient number

Table 3. Comparison of the mean age of those who took drugs accidentally and for suicidal purposes

	Accidental Mean±SD	Suicidal Mean±SD	p value
Age	15.8±20,4	30.0±13,8	0.001

SD: Standard Deviation

Figure 2. Distribution by seasons



GCS (Glasgow Coma Scale) of 233 (90.7%) patients was 15. It was found that blood pressure was normal in 237 (92.2%) patients, respiration was normal in 254 (98.4%), and pulse was normal in 246 (95.7%) patients (Table-4).

Table 4. Distribution of patients, according to examination findings

	Number of patients (n)	Ratio (%)
Consciousness		
Awake	233	90,7
Confused	21	8,2
Coma	3	1,2
Blood pressure		
Hypotensive	9	3,5
Normotensive	237	92,2
Hypertensive	11	4,3
Respiration		
Bradypnea	0	0
Normal	254	98,4
Tachypnea	3	1,1
Pulse		
Bradycardia	2	0,8
Normal	246	95,7
Tachycardia	9	3,5
Fever		
No	249	96,9
Yes	8	3,1

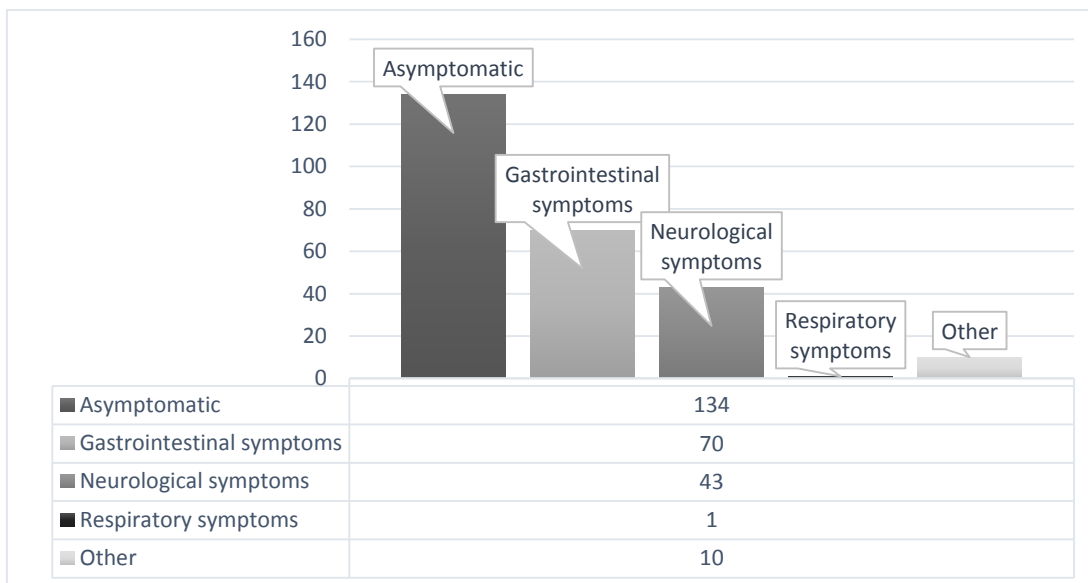
When classification was made according to the type of drug, analgesics were the most common cause of drug poisonings (32.3%), and neuropsychiatric drugs (27.6%) were in the second place (36.2% of the patients took more than one type of drug). In some patient files, it was noted that the patient did not know the name of the drug he/she took or, the package was not brought from his/her relatives (n = 9, 3.5%) (Table-5). One of the 2 patients who died took a chemical agent (age 15) and, the other took an antidepressant (age 54).

Table 5. Distribution by type of drugs

Type of drug	Number of patients (n)	Ratio (%)
Analgesic drugs	83	32.3
Neuropsychiatry drugs	71	27.6
CVS drugs	34	13.2
Antibiotics	34	13.2
GIS drugs	16	6.2
Endocrine drugs	11	4.3
Chemical agents	24	9.3
Unknowns	9	3.5
Others	11	4.3

CVS: Cardiovascular System, GIS: Gastrointestinal system, Others: Supplements, herbal ingredients, narcotic, iron complexes, pesticide, cigarette, detergent

Gastrointestinal symptoms such as nausea, vomiting, stomach ache, etc. Were found as the most common occurred, symptoms (27.1%, n=70) but, a large part of the patient was asymptomatic (51.9%, n=134) (Figure-3).

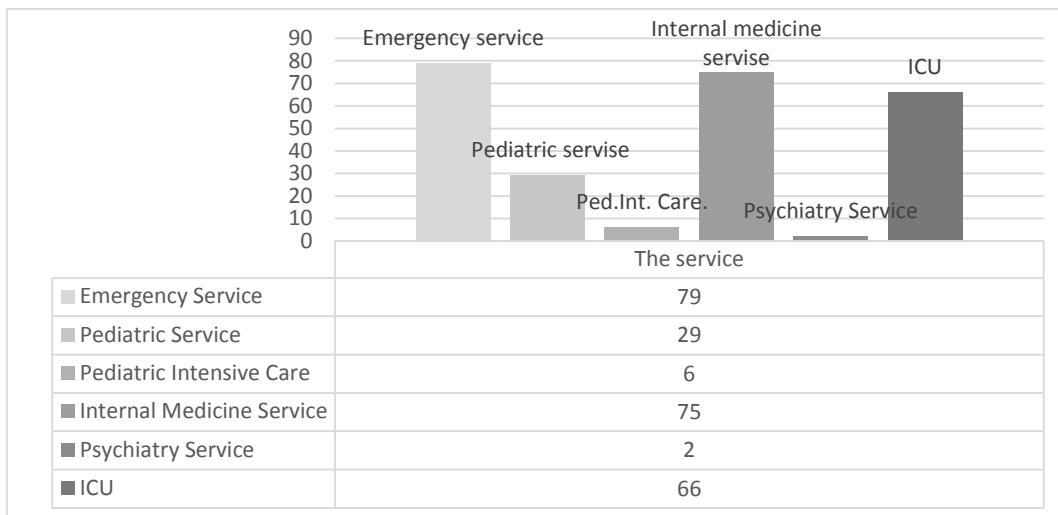
Figure 3. Distribution by presenting symptoms

Symptoms such as weakness, sore throat, itching, rash are stated under the heading 'other'.

It was noted that gastric lavage and activated charcoal (1gr/kg) were administered in %58 (n=149) of patients who applied within one hour after taking the drug. Most of the patients (98.1%) received symptomatic treatment and, the patients, mostly (30.8%, n=79) received treatment in the ED (Figure-4). 109 of all patients (42.4%) were administered psychiatric

consultation during their follow-up in the ED. 185 patients (72%) were discharged with recovery, and 2 (0.8%) of 27 patients who were referred to other centers died. It was determined from the records that there were 12 (4.7%) patients with recurrent admissions to the ED, and 25% of these patients attempted suicide with the drugs they used in their own treatment (Table-6).

Figure 4. Distribution according to the departments where treatments are given



Ped. Int. Care: Pediatric Intensive Care, ICU: Intensive Care Unit

Table 6. Treatment modalities and patient outcome

	Number of patients (n)	Ratio (%)
Activated charcoal and gastric lavage		
No	108	42.0
Yes	149	58.0
Intubation		
No	255	99.2
Yes	2	0.8
Psychiatric consultation		
No	148	57.6
Yes	109	42.4
Treatment modality		
Symptomatic	252	98.1
Further treatment	5	1.9
Mortality		
No	252	98.0

Yes	2	0.7
Not known	3	1.3
Outcome		
Recovery	185	72.0
Referral to other centres	27	10.5
Left against medical advice	45	17.5
Repetitive admission		
No	245	95.3
Yes	12	4.7

DISCUSSION

Intoxicated patients constitute an important part of emergency department admissions. In the studies conducted in our country, this rate ranged from 0.46% to 1.57% (2). In the two-year period, we conducted our study, it was determined that 471,461 patients were admitted to our ED and the rate of cases of intoxication was 0.05%. There were 7,011 patients followed as inpatients, and 178 (2.5%) of these inpatients were diagnosed as intoxicated. Contrary to the literature, we attribute this low rate of intoxication cases to our province's low population rate. We attribute the high rate of hospitalization in our hospital, as it is the largest hospital in our province and the only one in the city centre.

Health care professionals are responsible for both determining the need for emergency response and ensuring that the judicial process is initiated. If the patient is brought to the emergency room by ambulance, the ambulance team should inform the emergency doctor if there is any suspicious situation in the environment where the patient is taking. Especially intoxicated patients who attempt suicide may not want to give an accurate and sufficient anamnesis. In this case, the emergency physician may need to go for an exam for a diagnosis, just like a detective.

In the literature, it was determined that suicidal intoxicated patients generally took the drugs orally, and it was observed in the female patient group at a higher rate. In a study conducted by Çetin et al., 46% of ED patients were found to be taking suicidal oral drugs, 73.9% of whom were female (3). In a study by Kaya et al., 100% of patients with suicidal intoxication had oral medicines, and 70% of these patients were female (4). In our study, 100% of cases of intoxication were poisoned by oral, and patients who took drugs to commit suicide were more numerous than the group of patients who took drugs accidentally. These patients were mostly women. We think the most important reason why the level of oral intoxication is so high is the low level of rational drug use in our country. For this reason, the patient does not continue the current treatment and stores a lot of drugs at home, thus making it easier for the person with suicidal ideation to access drugs at home. In addition, we believe that women's social status

problems and the fact that women are more burdened by the traditional family structure have increased attempts to commit suicide (5).

It was found that 4.7% of the patients in our study had recurrent emergency service admissions for the same reason, and all of these patients were followed up by a psychiatrist. In addition, most of these patients took the drugs that they used in their treatment also for suicide attempts and apply to ED. Christodoulou et al. Analysed articles written over 30 years and found that the suicidal trend increased in the spring and autumn. Based on this result, sunlight is thought to reduce depression, but serotonin receptors can be acute and rapidly affected during long sunny hours (6). In the study where Salib examined fatal suicide attempts in patients under the age of 65; it was determined that 45% of the patients attempted suicide during the summer period (7). In our study, we found that patients were more likely to apply in the summer. In addition, considering the application time in our study, we observed that suicidal thoughts were intense between 16:00 and 00:00. We think that the reason for this is that patients choose a time period when they try to be alone and do not want their relatives to reach them.

In cases of drug intoxication, there is multiple use of drugs, particularly when taking drugs for suicide. Analgesics and antibiotics are among these drugs. In a study by Yılmaz et al., it was seen that 52% of patients who took drugs for suicidal purposes took analgesics and antibiotics (8). In our study, contrary to the literature, the number of patients who took a single drug was higher than the number of patients who took multiple drugs. Similarly, the number of patients who took analgesic drugs was relatively higher. We think that the most important factor behind this situation may be the excessive use of non-prescription drugs (especially analgesics) in our society (9).

Cases of accidental oral intoxication are most commonly seen in children. In a study by Kondolot et al., 5.8% of patients under 5 years old with poisoning were poisoned by analgesics. In this age group, accidental intoxication is common due to increased hand-to-mouth activity with curiosity about research and learning. Furthermore, negligent and unconscious family members who leave drugs in easily accessible areas and keep them out of their packaging can also cause these situations (10, 11). There is not enough data in the literature on which drug is poisoned more by adult patients. The drugs specific to chronic diseases were examined one by one and it was found that people using anticoagulant, digital antiarrhythmic or synthetic drugs experienced poisoning due to drug metabolism (12). In our study, we observed that the patients who were poisoned by accident were mostly in the pediatric age group. Furthermore, cases of accidental poisoning have been poisoned by substances which we have classified under the other heading (supplements, herbal ingredients). The mean age of these patients was 15.8 ± 20.4 years.

The faster the poisoning cases are intervened, the better the prognosis. In the study conducted by Yeşil et al., the duration of admission to the ED after the oral drug intake was found to be 4.

77±4. 34 hours (13). In the study of Deniz et al., it was determined as 2.51±2.43 hours (14). No patients died in these studies. The early application time was found to be effective in this regard, but the generally good condition of the patient over the follow-up period also affected this situation. In our study, we found a result consistent with the literature in terms of admission time. There were no deaths at the hospital (referrals excluded).

Our study found that 79 (30.8%) patients were followed at the ED and 45 (17.5%) refused treatment and left the hospital. The number of patients monitored in internal medicine (29.2%) and ICU (28%) was higher than in other departments (inpatient services other than the emergency department). In the study of Ersoy et al., they suggested that intensive care follow-up is necessary and advanced treatment should be performed in the intensive care unit because of the possibility of developing multiorgan failure in intoxication cases (15). In our study, most of the patients had stable vital signs and good general condition. Therefore, not all patients were followed up in the intensive care unit. Since there is no psychiatry service in our hospital, patients with continuing suicidal thoughts were followed in the intensive care unit, which is a closed service, until they became metabolically stable. Patients without active suicidal thoughts were followed in the service. Therefore, we think that the number of hospitalizations in the internal service and adult intensive care unit may be close to each other. In our study, it was observed that 98.1% of the patients were treated with symptomatic treatment and 1.9% with advanced treatment. In further treatment, if available, the antidote of the drug is applied. If there is no response, treatments for the removal of metabolic toxins such as hemodialysis or hemofiltration, intravenous lipid emulsion are performed. In our study 2 (%0. 8) patients died. On arrival, these patients were conscious and, their vitals were stable. The general condition of these patients, who were referred to an external center, deteriorated during their intensive care follow-up and died. According to this result, we think that the vital signs and GCS level on admission are not prognostic indicators. Also, we think that the number of drugs taken, the physiology of the patient and, the care during the follow-up is effective. Since these patients died in the place of referral, we can claim that there was no mortality in our hospital in our study. We can say that the prognosis of the patients has progressed well due to many reasons such as the high level of symptomatic treatment and adequateness, the low average age of our patients, the absence of underlying organ dysfunction, and the low amount of medication taken. Consistent with the literature, the low mortality rate is definitely pleasing.

Poisoning cases in our country are reported to 114 Poison Information Centers (PIC, ZDM in Turkish) and recorded. The recommendations of the authorized health personnel in this unit are taken according to the Ministry of Health Intoxication Guide, the symptoms that may develop are learned, the antidotes that can be applied, and the patient follow-up period is tried to be determined. If there is an antidote and the application period of the drug taken in the literature, it is applied. If there is an unknown substance or a history of drug intake that has not been

reported in the literature, symptomatic treatment is usually applied and at least 24 hours of asymptomatic follow-up is recommended in addition to the treatment period. For this reason, it is of great importance to report, especially rare cases of poisoning to PIC and to share the experience. In this way, perhaps we can follow up in the hospital for a shorter time, prevent unnecessary patient hospitalizations, and reduce the loss of workforce.

In a study conducted by Satar et al. For 6 years in a city with a large population in our country, the duration of hospitalization was found to be 2.9 ± 1.8 days (16). In our study, we found this rate to be 1.8 ± 0.9 days in our hospital. We can attribute this to the fact that, for a while, the intensive care unit of our hospital only serves Covid patients and some patients were referred to an external center, since the time included in the study coincided with the pandemic period.

We found that the majority of the patients in our study did not describe symptoms at the ED admission. In a study by Burillo-Putze et al. In which they recorded data from 14 emergency departments, they stated that 44.6% of the patients did not have any symptoms at presentation. In our study, the majority of patients (51.8%) were asymptomatic. The development of symptoms related to the drug that patients take can give us a clue about the drug, especially for patients who do not want to talk about the drug they are taking (17). In addition, we think that patients who take drugs for suicidal purposes do not want to talk about their current symptoms (such as headache, dizziness, abdominal pain) if they do not have visible symptoms (such as vomiting, impaired consciousness). However, the fact that the prognosis is so good also raises doubts about whether the drug was actually taken. When the psychiatry consultation notes are examined, it is seen that the patients generally use sentences such as ‘in a moment of anger, because I want to punish those who upset me’. The opinion of psychiatrists that patients do not have active suicidal thoughts supports our opinion.

As a result, regardless of the cause, the follow-up and treatment of a patient with a history of excessive drug intake is a difficult process for the physician and the patient. Especially for patients whose prognosis cannot be predicted and who have active suicidal thoughts, follow-up in the intensive care unit is a safe choice. Since our study is the largest hospital in the city and the only hospital in the center, we think that the data obtained is important and should be included in the literature. When the statistics of the prognosis of intoxication cases are obtained with similar studies to be conducted across the country, we can predict that the possibility of following the patient in the right clinic in a sufficient time with the cooperation of the physician-patient will increase. In addition, we think that these data to be obtained can be a guide for emergency service health workers working with similar facilities in our province.

LIMITATIONS

There were some limitations in our study. First of all, we could not reach the records in some files, as it was a work in the form of archive scanning. In addition, if the patient was accidentally

poisoned, the diagnosis of his/her symptoms may have been entered into the system instead of the diagnosis of "poisoning". In this case, we think that the number of patients obtained in our study did not meet the actual data, but most of them were recorded.

During our study, we determined that some of the patients who came with poisoning were transferred out of the province for a while since the intensive care and services of our hospital only serve Covid patients. Therefore, the prognosis of some patients could not be determined. If the patients who were discharged after their treatment was readmitted to our hospital, they were detected in the system and were considered to be non-mortal. For patients who do not reapply to our hospital; The prognosis was recorded as uncertain. In addition, it could not be determined whether the symptoms of the patients who left the hospital with treatment refusal continued or whether there were sequels. The mortality of these patients was estimated by looking at the recurrent hospital admissions on the system.

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**POSTKOVID DÖNEMİNDE GASTRODUODENAL KANAMALARIN TEDAVİSİ VE
ÖNLENMESİNİN MODERN YÖNLERİ**

**MODERN ASPECTS OF THE TREATMENT AND PREVENTION OF
GASTRODUODENAL HEMORRHAGE IN POSTKOVID PERIOD**

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ÖZET

Tanıtım.: Covid-19 virüs enfeksiyonunun kompleks tedavisinde antikoagülan kullanımı sırasında gözlenen gastroduodenal kanamanın etkin tedavisine ilişkin sorunların çözümü modern cerrahide güncel tartışma konularından biridir. Günümüzde postkovid dönemde gastroduodenal kanama insidansı %7,4-16 gibi yüksek oranlarda kalmaktadır. Böyle yüksek bir yüzde, postkovid dönemde gastroduodenal kanamanın tedavisi ve önlenmesi alanında yeni araştırmaları gerektirmektedir.

Çalışmanın amacı kompleks tedavi ve önleme yöntemlerini geliştirerek postkovid döneminde gastroduodenal kanamanın sonucunu iyileştirmektir.

Gereç ve yöntem: 2020-2021 yıllarında Covid-19 virüs hastalığı sonrası gözlenen ülser kaynaklı gastroduodenal kanama tanısı ile tedavi edilen 42 hasta AMU Cerrahi Anabilim Dalında kontrolümüz altındaydı. Hastaların 29'u kadın, 13'ü erkekti. Hastaların yaş aralığı 20-80 idi. 9 hastada mide ülserinden kanama, 33 hastada 12 parmak ülserinden kanama olmuştur. Bu hastalarda tanı acil fibroözofagogastroduodenoskopi ile koyulmuş, hastaların kompleks tedavisinde hemostatik (aminokapronik asit 100ml x 3 kez intravenöz, disinon 2ml x 4 kez intramüsküler, transamin 5ml x 2 kez intravenöz, kleston 5ml x2 kez), antisekretor (histamin, H- histamin blokerler - ranitidin 50 mg x 3 kez, kvamatel 20 mg x 2 kez veya proton pompa inhibitörü (pantoprazol - 30 mg intravenöz x 1 kez), infüzyon-transfüzyon (eritrosit kütleli, plazma, protein preparatları, vb.), antihelikobakteriyal (klaritromisin 0,5x2 kez, amoksisilin 1.0x2 kez) tedavi için kullanıldı.

Tartışma: Bu tedavi rejimini alan hastaların çoğunda akut dönem ortadan kalktı ve kanama hızla durduruldu. 8 hastada kanamanın devam etmesi ve konservatif tedavinin etkisiz kalması nedeniyle endoskopik hemostaz (pıhtılaşma + skleroterapi) uygulandı ve kanama durduruldu. 2 hastada aşırı kanamanın devam etmesi nedeniyle endoskopik hemostaz yapılsa dahi kanamanın tekrarlama olasılığı yüksek olup acil cerrahi uygulanmış ve kanayan damar kapatılmıştır.

Sonuç: Gözlemlerimize göre, Covid-19 virüs hastalığı sonrası gastroduodenal kanama durumunda modern ilaçlarla kompleks tedavi, kanamayı hızla ortadan kaldırmayı, akut dönemi hızla durdurmayı ve hastaları hastaneden taburcu etmeyi sağlayan uygun bir yöntemdir. Mümkün olan en kısa sürede. FEQDS incelemesi gastroduodenal kanama tanısının yanı sıra tedavi amaçlı endoskopik hemostaz ile kanamayı durdurur ve böylece ameliyat riskini önemli ölçüde azaltır. Ayrıca Covid-19 virüs enfeksiyonunun kompleks tedavisinde gastrik lavaj kullanımını ameliyat sonrası kanamaların önlenmesinde büyük önem taşımaktadır.

Anahtar Kelimeler: Postkovid, Kanama, Tedavi

ABSTRACT

Introduction. The solution of problems of effective treatment of gastroduodenal hemorrhage observed during the use of anticoagulants in the complex treatment of Covid-19 virus infection is one of the current topics of discussion in modern surgery. At present, the incidence of gastroduodenal hemorrhage in the postkovid period remains high at 7.4-16%. Such a high percentage requires new research in the field of treatment and prevention of gastroduodenal hemorrhage in the postkovid period.

The aim of the study was to improve the outcome of postcovidial gastroduodenal hemorrhage by improving complex treatment and prevention methods.

Materials and methods: Between 2020-2021, 42 patients treated with the diagnosis of gastroduodenal bleeding of ulcer origin observed after Covid-19 virus disease were under our control on the Department of Surgical Diseases AMU. Of the patients, 29 were women and 13 were men. The age range of patients was 20-80. Bleeding from gastric ulcer in 9 patients, bleeding from ulcer of 12 fingers in 33 patients. In these patients, the diagnosis was made by emergency fibroesophagogastroduodenoscopy. In the complex treatment were used hemostatic(aminocaproic acid 100ml x 3 times intravenously, disinon 2ml x 4 times intramuscularly, transamine 5ml x 2 times intravenously, kleston 5ml x2 times), antisecretory(histamine, H-histamine blockers - ranitidine 50 mg x 3 times, kvamatel 20 mg x 2 times or proton pump inhibitor (pantoprazole - 30 mg intravenously x 1 time), infusion-transfusion (erythrocyte mass, plasma, protein preparations, etc.), antihelicobacterial (clarithromycin 0, 5 x 2 times, amoxicillin 1.0 x 2 times).

Outcome: In the majority of patients receiving this treatment regimen, the acute period was eliminated, and the bleeding was stopped quickly. In 8 patients, endoscopic hemostasis (coagulation + sclerotherapy) was performed because the bleeding continued and conservative treatment was ineffective, thus stopping the bleeding. In 2 patients, due to the continuation of profuse bleeding, even if endoscopic hemostasis is performed, there is a high probability of recurrence of bleeding, emergency surgery was performed, and the bleeding vessel was closed.

Conclusion: According to our observations, in the case of gastroduodenal hemorrhage after Covid-19 virus disease, complex treatment with modern drugs is a convenient method, which allows to quickly eliminate the bleeding, quickly stop the acute period, and discharge patients from the hospital as soon as possible. In addition to the diagnosis of gastroduodenal hemorrhage, FEQDS examination also stops the bleeding by endoscopic hemostasis for therapeutic purposes, thus significantly reducing the risk of surgery. Also, the use of gastric lavage in the complex treatment of Covid-19 virus infection is of great importance in the prevention of postoperative bleeding.

Keywords: Postkovid, Bleeding, Treatment

**COVID-19 NEDENİ İLE ACİL SERVİSE BAŞVURAN HASTALARDA YOĞUN
BAKIM YATIŞINI ÖNGÖRDÜREN FAKTÖRLER**
FACTORS PREDICTING TO INTENSIVE CARE HOSPITALIZATION IN PATIENTS
APPLYING TO THE EMERGENCY DEPARTMENT DUE TO COVID-19

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ÖZET

Covid-19 pandemisinde ilk vakalar 2019 aralık ayında Çin Wuhan kentinde görülmeye başlanmıştır. Dünya Sağlık Örgütü (WHO) tarafından ise Covid-19 Mart 2020'de pandemi olarak ilan edilmiştir. Artan vakalar nedeniyle sağlık sistemi zorlanmakta ve bu nedenle evde, serviste ya da yoğun bakımda takip edilmesi gereken hastaların belirlenmesi sistemin rahatlanması açısından önem kazanmaktadır. Bizde çalışmamızda yoğun bakım yatışı gerektiren vakaların klinik farklılıklarını belirlemeyi amaçladık.

Karaman Eğitim ve Araştırma Hastanesi Acil Servisine Covid-19 nedeniyle başvuran 489 hasta (K/E: 260/229, ortalama yaş: 48.69 ± 17.25) çalışmaya dahil edildi. Hastaların demografik ve klinik özellikleri kaydedildi. Ayrıca yoğun bakım yatış takibi gereken vakaların bulguları değerlendirildi. Acil servise başvuran hastaların 240' ı hastanede yatış yapılarak takip edilirken bu hastaların 33'üne yoğun bakım yatış endikasyonu konulmuştu. Hastalar yatış endikasyonu açısından gruplandırıldığında; gruplar arasında cinsiyet ve lenfosit sayıları açısından anlamlı fark yoktu. Yoğun bakımda yatış endikasyonu verilen grupta oksijen saturasyonu anlamlı olarak daha düşükken bu grupta hasta yaşı, ateş, kalp hızı, üre, kreatinin, nötrofil sayısı, nötrofil-lenfosit oranı, CRP ve D-Dimer anlamlı olarak yüksek bulundu (Tablo 1). Yoğun bakım ünitesinde yatış endikasyonu ile bağımsız olarak ilişkili değişkenleri

tanımlamak için binominal lojistik regresyon analizi yapıldı. Yaş, oksijen satürasyonu, ateş, kalp hızı, üre, kreatinin, nötrofil-lenfosit oranı, CRP ve D-Dimer bu modele dahil edildi. Çok değişkenli analizde oksijen satürasyonu ve nötrofil-lenfosit oranı yoğun bakım ünitesinde yatış endikasyonunun bağımsız öngördürücüsü olarak bulundu (Tablo 2).

Çalışmamızın sonucunda acil servise Covid-19 nedeni ile başvuran hastalarda özellikle düşük oksijen satürasyonu ve yüksek nötrofil-lenfosit oranına sahip kişilerin yoğun bakım takibi açısından değerlendirilmesi gerektiğini düşünüyoruz.

Anahtar Kelimeler: Koronavirüs Salgını, Yoğun Bakım, NLR, Oksijen Satürasyonu

ABSTRACT

In the Covid-19 pandemic, the first cases began to be seen in the Chinese city of Wuhan in December 2019. Covid-19 was declared a pandemic by the World Health Organization (WHO) in March 2020. Due to increasing cases, the health system is struggling, and therefore it is important to determine the patients who need to be followed at home, in the service, or in the intensive care unit, in terms of the relief of the system. In our study, we aimed to determine the clinical differences of cases requiring intensive care admission.

489 patients (F/M: 260/229, mean age: 48.69 + 17.25 years) admitted to the Emergency Service of Karaman Training and Research Hospital due to Covid-19 were included in the study. Demographic and clinical characteristics of the patients were recorded. In addition, the findings of the cases requiring intensive care hospitalization were evaluated. While 240 of the patients admitted to the emergency department were hospitalized and followed up, 33 of these patients were indicated for intensive care admission. When patients are grouped in terms of hospitalization indication after admission to the hospital there were no significant differences with respect to the following variables between groups, gender, and lymphocyte counts. The group that was given an indication for hospitalization in the intensive care unit had significantly lower oxygen saturation, while serum age, fever, heart rate, urea, creatinine, neutrophil count, neutrophil-lymphocyte ratio, CRP and D-Dimer were significantly higher in this group (Table 1). We also performed binomial logistic regression analysis to define variables that are independently associated with the indication of hospitalization in the intensive care unit (Table 3). Age, oxygen saturation, fever, heart rate, urea, creatinine, neutrophil-lymphocyte ratio, CRP, and D-Dimer were included in this model. In multivariate analysis, oxygen saturation and neutrophil-lymphocyte ratio were found to be the independent predictor of indication of hospitalization in the intensive care unit (Table 2).

As a result of our study, we think that patients who applied to the emergency department due to Covid-19 should be evaluated in terms of intensive care follow-up, especially those with low oxygen saturation and high neutrophil-lymphocyte ratio.

Keywords: Coronavirus Pandemic, Intensive care, NLR, Oxygen saturation

Table 1 Comparison of demographic, clinical, and biochemical characteristics of patients according to the outcome.

Parameters	Discharged (n=249) (Mean±SD), Median (IQR) or Frequency (n-%)	Service (n=207) Mean±SD), Median (IQR) or Frequency (n-%)	Intensivecare (n=33) Mean±SD), Median (IQR) or Frequency (n-%)	p
Age (years)	39.81 ± 12.65	55.86 ± 15.63	70.73 ± 16.75	0.000
Female/Male	134/115	107/100	19/14	0.719
Oxygen saturation (%)	95.9 ± 1.9	93.21 ± 3.98	79.8 ± 12.69	0.000
Fever (C°)	36.7 ± 0.58	36.9 ± 0.68	37 ± 1.03	0.01
Heart rate (bpm)	94.58 ± 15.1	99.49 ± 16.31	111.28 ± 38.27	0.000
Urea (mg/dl)	28 (12)	32 (20)	96.5 (109)	0.000
Creatinine (mg/dL)	0.93 ± 0.19	1.04 ± 0.35	2.01 ± 1.07	0.000
Lymphocyte count (10³/μL)	1.25 (1.16)	1.05 (0.78)	0.6 (0.43)	0.138
Neutrophil count (10³/μL)	3.59 (1.89)	4.23(3.62)	9.5 (4.67)	0.000
Neutrophil lymphocyte ratio	2.96 (3.27)	3.32 (5.32)	16.72 (29.54)	0.000
CRP (mg/L)	6 (16.8)	26.4 (41)	148 (176.3)	0.000
D-Dimer (μg/mL)	254 (195)	615 (468)	2169 (2581)	0.000

Table 2 Binomial Logistic Regression Analysis of Intensive care hospitalization and other parameters in patients with with COVID-19.

Parameters	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	pvalue	OR (95% CI)	pvalue
Age (years)	1.09 (1.062-1.119)	0.000	0.992 (0.950-1.036)	0.729
Oxygen saturation (%)	0.695 (0.632-0.764)	0.000	0.803 (0.721-0.894)	0.000
Fever (C°)	1.598 (1.010-2.529)	0.045	0.524 (0.246-1.116)	0.094
Heart rate (bpm)	1.036 (1.018-1.054)	0.000	0.986 (0.963-1.011)	0.276
Urea (mg/dl)	1.04 (1.034-1.058)	0.000	1.006 (0.971-1.041)	0.757
Creatinine (mg/dL)	13.130 (6.388-26.987)	0.000	1.680 (0.610-4.629)	0.316
Neutrophil lymphocyte ratio	1.224 (1.155-1.298)	0.000	1.095 (1.022-1.172)	0.01
CRP (mg/L)	1.023 (1.017-1.029)	0.000	0.998 (0.986-1.011)	0.817
D-Dimer (µg/mL)	1.001 (1.001-1.002)	0.000	1.00 (1.00-1.001)	0.098

**CERRAHİ HASTALARDA MALNÜTRİSYON RİSKİ VE BESLENME TARAMA
TESTLERİYLE DEĞERLENDİRİLMESİ**
MALNUTRITION RISK AND EVALUATION OF NUTRITIONAL SCREENING TOOLS
IN SURGERY PATIENTS

Sema KONATEKE

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ÖZET

Cerrahi hastalarda malnütrisyonla yatkınlık cerrahi strese bağlı olarak salınan birtakım hormonlarının etkisiyle artmaktadır. Malnütrisyon görülen cerrahi hastalarda ameliyat sonrasında kardiyak, solunum ve gastrointestinal sisteme ait birçok önemli komplikasyon görülmektedir. Ayrıca immün sistem baskılanmasıyla birlikte ameliyat sonrasında enfeksiyon gelişme riski artmakta, yara yeri iyileşmesi gecikmekte ve hastanede yatış süresi uzamaktadır. Hastanede yatış süresinin uzaması malnütrisyonla girmeyi daha da artırarak hastada mortalite ve morbidite riskinin artmasına neden olmaktadır. Malnütrisyonun cerrahi hastalarda yarattığı olumsuz sonuçlarının önüne geçmek için her hastanın ilk 24-72 saat içerisinde beslenmesinin değerlendirilmesi gerekmektedir. Beslenme değerlendirmesi; beslenme tarama testleri, antropometrik ölçümler, biyokimyasal değerlendirmeler ve immünolojik testlerle yapılabilmektedir. Yatan hastalarda hızlı, etkili ve pratik olması sebebiyle genellikle beslenme tarama testleri kullanılmaktadır. Geçerli ve güvenilir birçok tarama testi geliştirilmiş olup Avrupa Klinik Nutrisyon ve Metabolizma Derneği (European Society for Clinical Nutrition and Metabolism, ESPEN) hastanede yatan hastalar için Nütrisyonel Risk Skoru (NRS) 2002 ve Malnütrisyon Universal Tarama Aracı (MUST) kullanılması gerektiğini önermektedir. Bu tarama testlerinin dışında cerrahi hastalarında Subjektif Global Değerlendirme (Subjective Global Assessment, SGA) kullanımı da görülmektedir. Ameliyat öncesinde hasta kabulünde klinik hemşiresi veya diğer sağlık personeli hastaya uygun beslenme değerlendirme aracını kullanarak malnütrisyon riskini belirlemelidir. Riskli hastalarda ek nütrisyonel destek sağlanana kadar mümkünse ameliyat ertelenmelidir. Cerrahi hastasının protein enerji gereksinimi hesaplanarak hastaya özgül oral, enteral ya da parenteral nütrisyonel destekle ameliyat sonrasında iyileşme hızlandırılmalıdır.

Anahtar kelimeler: Cerrahi, malnütrisyon, beslenme değerlendirmesi, tarama testi

ABSTRACT

The susceptibility to malnutrition in surgical patients increases with the effect of some hormones released due to surgical stress. In surgical patients with malnutrition, many important complications related to cardiac, respiratory and gastrointestinal systems are seen after surgery. In addition, with the suppression of the immune system, the risk of post-operative infection increases, wound healing is delayed, and the length of hospital stay is prolonged. Prolongation of the hospitalization period further increases the risk of malnutrition and increases the risk of mortality and morbidity in the patient. In order to prevent the negative consequences of malnutrition in surgical patients, it is necessary to evaluate the nutrition of each patient within the first 24-72 hours. Nutritional evaluation can be done with nutritional screening tools, anthropometric measurements, biochemical evaluations and immunological tests. Nutritional screening tools are generally used in hospitalized patients because they are fast, effective and practical. Many valid and reliable screening tools have been developed, and the European Society for Clinical Nutrition and Metabolism (ESPEN) recommends the use of the Nutritional Risk Score (NRS) 2002 and the Malnutrition Universal Screening Tool (MUST) for hospitalized patients. Apart from these screening tools, the use of Subjective Global Assessment (SGA) is also seen in surgical patients. Admission of patients before surgery, the clinical nurse or other health personnel should determine the risk of malnutrition by using the appropriate nutritional assessment tool for the patient. If possible, surgery should be postponed until additional nutritional support is provided in patients at risk. By calculating the protein energy requirement of the surgical patient, recovery should be accelerated with patient-specific oral, enteral or parenteral nutritional support.

Keywords: Surgery, malnutrition, nutritional assessment, screening tools

GİRİŞ

Avrupa Klinik Nutrisyon ve Metabolizma Derneği (European Society for Clinical Nutrition and Metabolism, ESPEN) malnütrisyonu; protein, enerji ve diğer besinlerin yetersiz, aşırı veya dengesiz alınması sonucu vücut yapısı ve fonksiyonlarında klinik olarak ters etkiler gösteren bir beslenme hali olarak tanımlamaktadır (Cederholm et al., 2017; Elia, 2017). Malnütrisyon; yetersiz beslenme, fazla beslenme ya da dengesiz beslenmeyi kapsayan bütüncül bir terim olmakla birlikte günümüzde genellikle yetersiz beslenme ile eş anlamlı kullanılmaktadır (Elia, 2017). Malnütrisyon riskinin hastalığın başlangıç, ilerleme ve rehabilitasyon aşamalarında oldukça önemli olduğu ve her hasta için değerlendirilmesi gerektiği bilinmektedir (Karahan ve Çiftçi, 2020). Hastaneye yatan hastaların yaklaşık %20-50'sinde malnütrisyon görülmektedir (Güler ve Tireli, 2018). Cerrahi hastalarında bu oran %27-50 arasında değişmekte bu durum mortalite ve morbidite ile yakından ilişkilendirilmektedir (Dumlu ve ark., 2013; Cascio and

Logomarsino, 2018; Cortes et al., 2020). Ayrıca cerrahi hastalarda ameliyat sonrası hastanede yatış süresi uzadıkça bu oranın daha da arttığı belirtilmektedir (Palmer et al., 2021).

Hastalarda beslenme yetersizliği; beslenme tarama testleri, antropometrik ölçümler, biyokimyasal değerlendirmeler ve immünolojik testlerle belirlenebilmektedir (Güler ve Tireli, 2018; Miller et al., 2018). Cerrahi hastaların yeterli ya da yetersiz beslendiğini belirlemek için risk tanılama araçlarıyla taranması beslenme desteğinin belirlenmesi için önemlidir (Cortes et al., 2020; Langley-Evans, 2021). Hastalarda vücudun tekrar iyileşebilmesi ve yara onarımı için anabolik faaliyetlerin hızlandırılması, artan besin ve enerji gereksiniminin karşılanması önemlidir. Burada makro besinlere, vitaminlere ve eser elementlere duyulan ihtiyaç artmaktadır (Weimann et al., 2017). Metabolik faaliyetlerin devam etmesi ve doku onarımı için beslenme desteğinin sürdürülmesi gerekmektedir (Uzunköy, 2018). Bireyselleştirilmiş bir beslenme desteği uygulayabilmek için yatan her hastanın ilk 24-72 saat içerisinde beslenme durumu değerlendirilerek, riskli hastalara ek nütrisyonel destek verildikten sonra cerrahi operasyona alınması gerekmektedir (Mueller et al., 2011; Özer ve ark., 2016).

Cerrahi Hastalarda Beslenme Yetersizliğinin Etkileri

Hastalar cerrahi operasyon sonucunda protein enerji malnütrisyonuna girmeye eğilimlidirler. Cerrahi operasyon vücutta stres yaratarak katekolamin ve diğer stres hormonlarının salınımını sağlayarak inflamatuvar süreci başlatmaktadır (Weimann et al., 2017). Böylece vücutta metabolizma hızı artarak negatif nitrojen dengesi ortaya çıkmaktadır (Özer ve ark., 2016). Stres yanıtı ile salınan birtakım sitokinler katabolik aktiviteyi hızlandırarak protein yıkımına, besin alımının ve emiliminin azalmasına neden olmaktadır. İnflamasyonun katabolik etkisi, travmalar, ameliyata bağlı bulantı-kusma ve iştahsızlık gibi durumlar nedeniyle de beslenme yetersizliği gelişmektedir (Altundağ-Derin ve ark., 2018).

Hücrel beslenme yetersizliğinde kardiyak fonksiyonlarda bozulma görülmektedir (Dumlu ve ark., 2013). Kardiyak outputta düşmeyle birlikte santral venöz basınçta azalma, orta derece hipotansiyon, bradikardi ve oksijen tüketiminde azalma gibi ağır bir tablo ortaya çıkmaktadır. Ayrıca malnütrisyondaki hastalarda vital kapasite azalarak oksijenizasyon bozulmaktadır. Bu durum postoperatif dönemde görülen atelektazi ve pnömoni gibi solunumsal komplikasyonların artmasına neden olmaktadır. Beslenme yetersizliğine bağlı olarak plazma proteinleri, karaciğer ve sindirim enzimleri gibi bütün endojen enzimler harcanmaktadır. Bu durum bağırsak motilitesinde azalmaya neden olmaktadır (Erdim, 2007).

Malnütrisyonun diğer etkilerinden birisi immün sistemin inaktive olmasıdır. Bu durum yara yerinde enfeksiyon, anastomoz yerlerinde açılma ve iyileşmede gecikmeyle sonuçlanmaktadır (Cederholm et al., 2017; Taberna et al., 2019). Cerrahi öncesi dönemde yeterli beslenme sağlanamayan hastalarda ameliyat sonrasında iyileşme gecikmektedir. Bu durum hastada nozokomiyal enfeksiyonların artmasına ve çoklu organ yetmezliğine kadar birçok

komplikasyon gelişmesine yol açarak hastanede yatış süresini uzatmaktadır (Altundağ-Derin ve ark., 2018). Bu durum hasta bakım maliyetlerinin de önemli ölçüde artmasına yol açmaktadır (Dumlu ve ark., 2013; Uzunköy, 2018).

Cerrahi hastalarda malnütrisyonu artırılan ileri yaş ve kullanılan ilaçlar da ayrıca değerlendirilmelidir. Hastalarda kullanılan digoksin, diüretik ve antibiyotik gibi ilaçların yan etkilerine bağlı olarak da beslenme yetersizlikleri ortaya çıkmaktadır. Bunun yanı sıra yaşlı hastalarda yaşlanmanın getirdiği fizyolojik değişiklikler ile birlikte malnütrisyonu yatkınlık görülmektedir (Karahana ve Çiftçi, 2020).

Beslenme Durumunun Tarama Testleriyle Değerlendirilmesi

Beslenme tarama testleri beslenme yetersizliğini belirlemek için kullanılan basit kontrol listeleridir (Langley-Evans, 2021). Ameliyat öncesinde beslenme durumunun geçerli ve güvenilir risk tarama araçlarıyla değerlendirilmesi, risk altındaki hastalara ameliyat öncesi beslenme desteğinin sağlanması, ameliyat sonrasında hastanın iyileşmesini hızlandırarak görülebilecek komplikasyonların azalmasında etkilidir (Türkoğlu ve ark., 2015). Beslenme yetersizliğini belirlemek için birçok tarama testleri geliştirilmiştir. Seçilecek tarama testi duyarlılığı ve özgüllüğü yüksek, uygulanışı kolay, pratik ve zaman gerektirmeyecek şekilde olmalıdır (Cereda et al., 2018). Bu tarama testleri yatan hastalar, yaşlılar, evde bakım hastaları ve toplum taramaları gibi birçok amaçla kullanılmaktadır. Bu amaçla sıklıkla kullanılan tarama testleri; Anında Beslenme Değerlendirmesi (Instant Nutritional Assessment, INA), Prognostik İnflamatuvar ve Beslenme İndeksi (Prognostic İnflammatory and Nutritional Index, PINI), Beslenme Tarama Kontrol Listesi (Nutritional Screening İnitiation Checklist, DETERMINE), Mini Nütrisyonel Değerlendirme (Mini-Nutritional Assessment, MNA), Malnütrisyon Tarama Aracı (Malnutrition Screening Tool, MST), Basitleştirilmiş Beslenme İştahı Anketi (Simplified Nutritional Appetite Questionnaire, SNAQ), Geriatrik Beslenme Risk İndeksi (Geriatric Nutritional Risk Index, GNRI) olarak sıralanabilir (Taberna et al., 2019).

ESPEN hastanede yatan hastalar için Nütrisyonel Risk Skoru (NRS) 2002 ve Malnütrisyon Universal Tarama Aracı (MUST) kullanılması gerektiğini önermektedir. Bu tarama testlerinin dışında cerrahi hastalarında Subjektif Global Değerlendirme (SGA) kullanımı da görülmektedir (Türkoğlu ve ark., 2015; Gündüz ve ark., 2019).

Nütrisyonel Risk Skoru (The Nutritional Risk Screening, NRS) 2002

Beslenme durumundaki bozulma ve hastalık şiddetini değerlendiren iki ana değerlendirmeden oluşur. Beslenme durumundaki bozulma 0-3 puan arasında; son aylardaki ağırlık kaybının yüzdesi, vücut kütle indeksi (VKİ), besin alımındaki yüzdelerle değerlendirilir. Hastalık şiddeti ise 0-3 puan arasında; kırıklar, komplikasyonlar, travmalar gibi birçok duruma göre değişen puanlama ile değerlendirilir. Toplam puana hasta 70 yaş üzeri ise +1 puan eklenerek nütrisyonel risk skoru belirlenir. Bu skor 3 ve üzerinde olursa beslenme

yetersizliğinin olduğu ve beslenme planının başlatılması gerektiği belirtilir. 3 puanın altında olan cerrahi hastalarda haftada bir tekrar değerlendirme yapılması önerilir (Karahan ve Çiftçi, 2020).

Malnütrisyon Universal Tarama Aracı (Malnutrition Universal Screening Tool, MUST)

ESPEN'İN yanı sıra İngiltere Parenteral ve Enteral Beslenme Derneği (BAPEN) tarafından da yetişkin hastalarda kullanılması önerilen tarama aracıdır. Bu araçta değerlendirme 3 adımda yapılmaktadır. Birinci adımda; BKİ değerlerine göre 0-2 puan arasında, ikinci adımda; son 6 aydaki kilo kaybının yüzdesi 0-2 puan arasında, üçüncü adımda; oral alımdaki azalma 2 puan olarak değerlendirilir. Bu üç adım puanları toplanarak toplam malnütrisyon riski belirlenir. Sonuç düşük risk (0), orta risk (1) ve yüksek risk (≥ 2) şeklinde belirlenir. Riske göre nütrisyon tedavisi planlanır (Türkoğlu ve ark., 2015; Gündüz ve ark., 2019).

Subjektif Global Değerlendirme (Subjective Global Assessment, SGA)

Hastaları öykü ve fizik muayeneden elde edilen sonuçlarla değerlendiren bir araçtır. Hastalar vücut ağırlığı kaybı ve değişimi, yeme alışkanlıklarındaki değişim, gastrointestinal bulguları, fonksiyonel kapasite, stres düzeyi ve fizik muayene bulguları antropometrik ölçümleri de kapsayacak şekilde subjektif olarak değerlendirilir. Değerlendirme sonucunda hastaların iyi beslenmiş, orta ya da ciddi düzeyde malnütrisyonundan hangi kategoriye girdiği belirlenerek bir beslenme planı hazırlanır (Charney, 2008; Güler ve Tireli, 2018).

SONUÇ

Malnütrisyonun cerrahi hastaları için olumsuz etkileri bilindiğinden hastaneye yatan her hastanın hemşireler ve diğer tıbbi personeller tarafından beslenme riskinin belirlenmesi gerekir. Bunun için en etkili yol bir beslenme değerlendirme aracının kullanılmasıdır. Bu amaçla hastanelerde tarama araçları hasta kabul protokolünün bir parçası olmalıdır. Karar verilen tarama aracının sağlık personeli tarafından kolay uygulanır olması, zaman ve maliyet açısından ek yük getirmemesine dikkat edilmelidir. Bunun yanı sıra hastaları değerlendirmek için kullanılan beslenme tarama testleri arasında bir fikir birliği yoktur. Altın standart beslenme tarama aracı olmadığından testlerin çeşitli hasta gruplarında karşılaştırmaları yapılarak üstünlükleri ve eksiklikleri belirlenebilir.

Hastalara uygun olan risk değerlendirme aracı belirlendikten sonra yüksek riskli hastalara multidisipliner bir ekip tarafından önlemlerin alınması ve beslenme planının yapılması gerekmektedir. Riskli hastalarda ek nütrisyonel destek sağlanana kadar mümkünse ameliyat ertelenmelidir. Cerrahi hastalarda ameliyat öncesi ve sonrası oral, enteral ya da nütrisyonel destek sağlanarak yetersiz beslenmenin hasta sonuçlarına olumsuz etkilerinin önüne geçilmelidir.

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**AMELİYATHANEDE HASTA GÜVENLİĞİ SORUNU: YABANCI CİSİM
UNUTULMASI**

**PATIENT SAFETY PROBLEM IN THE OPERATING ROOM: RETAINED SURGICAL
ITEM**

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ÖZET

Yabancı cisim unutulması ameliyathanede hasta güvenliğini tehdit eden tıbbi bir hatadır. Kadın doğum-jinekoloji, genel cerrahi, ortopedi ve kardiyotorasik cerrahilerde yabancı cisim unutulmasına daha sık rastlanmaktadır. Ameliyat süresinin uzun olması, ameliyatın komplike olması, acil ameliyatlara ve birden fazla cerrahi ekip gerektiren ameliyatlarda yabancı cisim unutulma riski artmaktadır. Hastanın aşırı şişman olması ve ameliyat sırasında kan kaybının fazla olması riski artıran hastaya özgü faktörlerdir. Cerrahi alanda yabancı cisim unutulması hastada sepsis, visseral perforasyon, fistül oluşumu, enfeksiyon ve bağırsak tıkanıklığı gibi birçok komplikasyon gelişmesine neden olabilir. Hastanın tekrar ameliyat olması hastanede yatış süresini uzatmakta mortalite ve morbidite riskini artırmaktadır. Tampon, iğne ve cerrahi aletler gibi yabancı cisimlerin unutulmasını önlemek için tam ve doğru bir cerrahi sayım yapmak gerekir. Cerrahi sayım ameliyathane hemşiresinin kontrolünde olsa da sorumluluk tüm cerrahi ekibe aittir. Bu sorumluluk gereği ameliyata başlamadan önce, vücut boşluklarını kapatmadan önce, yarayı kapatmadan önce ve cerrahi işlem sonunda cilt kapatılmadan önce mutlaka cerrahi sayım yapılmalıdır. Kanıta dayalı rehberler; manuel sayım süreciyle kombine edilmiş standart prosedürler, barkod sistemi, radyofrekans yöntemi gibi yardımcı teknolojilerin kullanılması önermektedir. Ayrıca ekip içi iletişim geliştirilmesi, multidisipliner bir yaklaşımın sürdürülmesi, sayım için özel alanlar veya sayım kapları, cerrahi alet konteynerleri, görsel ve elektronik hatırlatıcıların kullanılması ve personelin bu konuda eğitilmesi cerrahi sayım sürecini kolaylaştırmaktadır.

Anahtar kelimeler: Yabancı cisim unutulması, önleme, hasta güvenliği, ameliyathane, hemşirelik

ABSTRACT

Retained surgical item is a medical error that threatens patient safety in the operating room. Retained surgical item is more common in obstetrics-gynecology, general surgery, orthopedics and cardiothoracic surgeries. The risk of retained surgical item in the body increases in the long duration of the operation, the complexity of the operation, emergency operations and operations that require more than one surgical team. Excessive blood loss during surgery and the patient's being overweight are patient-specific factors that increase the risk. Retained surgical item in the body may cause many complications such as sepsis, visceral perforation, fistula formation, infection and intestinal obstruction. Re-operation of the patient prolongs the hospitalization and increases the risk of mortality and morbidity. A complete and accurate surgical count is required to prevent retained surgical items such as tampons, needles, and surgical instruments. Although the counting surgical items is under the control of the operating room nurse, the responsibility belongs to the entire surgical team. Due to this responsibility, surgical items must count before starting the operation, closing the body cavities, closing the wound, and closing the skin at the end of the surgical procedure. Evidence-based guidelines recommends the use of assistive technologies such as standard procedures, barcode system, radiofrequency method combined with manual counting process. In addition, developing communication within team, maintaining a multidisciplinary approach, using special areas or counting containers for counting, surgical instrument containers, visual and electronic reminders, and training personnel on this subject facilitates the surgical counting process.

Keywords: Retained surgical item, preventive, patient safety, operating rooms, nursing

GİRİŞ

Dünya Sağlık Örgütü'nün (DSÖ) hasta güvenliği için belirlediği hedeflerden birisi güvenli cerrahinin sağlanmasıdır. Güvenli cerrahi içerisinde ameliyat sırasında cerrahi alanda unutilan yabancı cisimler oldukça önemlidir (Freitas et al., 2016). Kullanılan cerrahi malzemelerin hasta vücudunda unutulması ameliyat kaynaklı yapılan tıbbi bir hatadır (Taşdemir, 2015). Yabancı cisim unutulma sıklığı ameliyatın türüne göre farklılık gösterse de yaklaşık 1/5000-5500 olarak belirtilmektedir (Cima et al., 2008; Lutgendorf et al., 2011; Taşdemir, 2015). Bir grup araştırmacının 5 yıllık süre içerisinde unutilan 319 yabancı cisme yönelik yaptıkları çalışmada; unutilan cisimlerin çoğunluğunun pelvis-karın (%50,2) ve vajinada (%23,9) olduğu belirtilmiştir. Yabancı cisim unutulmasında en sık karşılaşılan uzmanlık alanları sırasıyla; kadın doğum- jinekoloji, genel cerrahi, ortopedi ve kardiyotorasik cerrahidir (Steelman et al., 2018).

Cerrahi alanın kapatılmasından sonra hastada unutilan yabancı cisme bağlı olarak kısa veya uzun dönemde çok ciddi komplikasyonlar gelişebilir (Kusler-Jensen, 2011). Komplikasyonlar uzun süre semptomsuz seyredebileceği gibi ani ölümle de sonuçlanabilir. Hastada enfeksiyon,

sepsis, fistül oluşumu, visseral perforasyon, bağırsak tıkanıklığı ve ölüm görülebilir (Gürer ve ark., 2011). Bu durum hastanın yeniden hastaneye yatmasına ve ameliyata alınmasına, hastanede yatış süresinin uzamasına, mortalite ve morbidite riskinin artmasına neden olur. Hasta güvenliği açısından oldukça tehlikeli olan bu durum hem hastaları ve hem de ilgili sağlık kuruluşunu sağlık bakım maliyeti konusunda olumsuz etkiler. Sağlık kuruluşlarına ve cerrahi ekibe tıbbi hatanın bir sonucu olarak malpraktis sorumluluk maliyetleri, mali para cezaları, yasal ücretler yüklenilmektedir (Taşdemir, 2015; Freitas et al., 2016; Steelman, 2019a). Bu nedenle yabancı cisim unutulması ameliyathanede birçok boyutuyla ele alınıp değerlendirilmesi gereken hasta güvenliği sorunudur.

Yabancı Cisim Unutulmasına Neden Olan Risk Faktörleri

Yapılan bütün cerrahi operasyonlarda cerrahi alanda yabancı cisim unutulma riski vardır. Fakat bazı durum ve ameliyatlarda bu risk daha da artmaktadır. Ameliyathanede yabancı cisim unutulma riski elektif olmayan acil vakalarda ya da ameliyat sırasında acil bir durum gelişmesiyle birlikte planlanmamış cerrahilerde artmaktadır (Çeçen, 2016). Ameliyatın karmaşık ve uzun sürmesi, ameliyat sırasında kapalı cerrahiden açık cerrahiye geçilmesi, çok fazla cerrahi alet ve malzeme gerektiren komplike ameliyatlarda bu riski daha da artırmaktadır (Taşdemir, 2015; Freitas et al., 2016). Ameliyat olacak hastanın beden kütle indeksi (BKİ) değerinin yüksek olması durumunda risk değerlendirmesi ayrıca yapılmalıdır (Freitas et al., 2016).

Bazı çalışmalarda birden fazla cerrahi ekip üyesi gerektiren ameliyatlarda cerrahi ekip üye sayısının artmasıyla birlikte yabancı cisim unutulması riskinin arttığı belirtilmiştir (Egorova et al., 2008; Rowlands 2012). Ameliyat sırasında tahmini kan kaybının 500 ml'den daha fazla olduğu ameliyatlarda, yanlış cerrahi sayım yapılması ve ameliyat sırasında hemşirenin değişmesi de riski artıran diğer durumlardır (Moffatt-Bruce et al., 2014).

Ameliyat ve hasta kaynaklı risk faktörlerinin yanı sıra ele alınması gereken başka faktörler de yabancı cisim unutulmasına neden olmaktadır. Bu faktörler kategorize edildiğinde; insan, liderlik ve iletişim yabancı cisim unutulmasına neden olan ana kategorilerdir (Endicott et al., 2020). İnsan faktöründeki sorunlara bakıldığında; personel akran değerlendirmesi, akreditasyon, oryantasyon, hizmet içi eğitim eksikliği, cerrahi ekibin sertifikalandırılmasındaki sorunlar, ekip üyelerinde dikkat dağınıklığı ve yorgunluk sayılmaktadır. Liderlik sorunları; hastane politikası, uygulanan prosedürler ve bunlara uyumdaki yetersizliklerdir. İletişim sorunları ise; ekip içi iletişim ve hekim ile iletişim eksikliği sayılmaktadır (The Joint Commission, 2013; Steelman, 2019a; Endicott et al., 2020).

Ameliyat Sırasında Unutulan Yabancı Cisimler

Ameliyat sırasında kullanılan her türlü alet, malzeme ve cisim cerrahi alan içerisinde unutulabilmektedir. Sıklıkla unutulmuş ve unutulma riski yüksek olan malzemeler; tamponlar,

iğne ve kesicilerdir. Cerrahi aletlerin unutulma riski diğerlerine göre daha az olmakla birlikte en sık ekartörler unutulmaktadır (Taşdemir, 2015; Çeçen, 2016). Yapılan çalışmalarda unutilan diğer yabancı cisimler; kataterler (vasküler, epidural, intratekal, torasik), drenler, bıçaklar, paketler ve implantlar olarak belirtilmektedir (Steelman et al., 2019b). Sıklıkla açık cerrahi girişimlerde yabancı cisim unutulmasıyla karşılaşılrsa da kapalı cerrahi girişimlerde veya girişimsel radyolojinin kullanıldığı kapalı cerrahilerde çeşitli aletlerin unutulma ve kırılma riskleri vardır. Bu ameliyatlarda genellikle kılavuz telin kopması ya da alanda unutulması ile karşı karşıya kalınmaktadır (Endicott et al., 2020).

Yabancı Cisim Unutulmasının Önlenmesi

Ameliyathanede yabancı cisim unutulmasını engellemek için sayım önerilerinde bulunan ve bununla ilgili çalışmalar yapan Perioperatif Hemşireler Birliği (Association of Perioperative Registered Nurses, AORN), Amerikan Cerrahlar Birliği (American College of Surgeons, ACS) rehber ve kılavuzlar yayınlamaktadır. Çalışmalar sonucunda yeni kanıtlarla güncellenen bu rehberler cerrahi ekibe öncülük etmektedir (Taşdemir, 2015; Endicott et al., 2020).

Tampon, alet ve diğer türdeki yabancı cisimler için tam ve doğru cerrahi sayım oldukça önemlidir. Çünkü ameliyatta yanlış cerrahi sayım yapıldığında yabancı cisim unutulma oranı 5/100 iken, doğru sayım yapıldığında bu oran 1/30.000' e kadar düşmektedir (Taşdemir, 2015). Kanıta dayalı rehberler; manuel sayım süreciyle kombine edilmiş standart prosedürler, barkod sistemi, radyofrekans yöntemi gibi yardımcı teknolojilerin kullanılmasını önermektedir (Freitas et al., 2016; Çeçen, 2016). Ayrıca ekip içi iletişim geliştirilmesi, multidisipliner bir yaklaşımın sürdürülmesi, sayım için özel alanlar veya sayım kapları, cerrahi alet konteynerleri, görsel ve elektronik hatırlatıcıların kullanılması ve personelin bu konuda eğitilmesi cerrahi sayım sürecini kolaylaştırmaktadır (Freitas et al., 2016).

Yapılan bazı çalışmalarda ameliyathanede beyaz tahta kullanılarak sayımların üzerine kaydedilmesinin ve kullanılmış tamponların kayıt için ayrı alana alınmasının yabancı cisim unutulma riskini azaltacağı belirtilmiştir (Chagolla et al., 2011; Lutgendorf et al., 2011). Kullanılan tamponların radyopak özellikte olması, tampon sayımlarında uyumsuzluk belirlendiğinde x-ray ile görüntüleme yapılması da öneriler arasında yer almaktadır (Lutgendorf et al., 2011).

Cerrahi girişim sırasında ve sonrasında aletlerin ve malzemelerin sayımı ameliyathane hemşiresinin görevi olsa da sorumluluk tüm cerrahi ekip üyelerini içermektedir (Taşdemir, 2015). Sayımlar için bir form oluşturulması scrap ve sirküle hemşire arasında çapraz sayımın yapılması ve tüm cerrahi ekibin dahil edilmesi gerekmektedir (Freitas et al., 2016).

Yabancı Cisim Unutulmasının Önlenmesinde Destekleyici Teknolojinin Kullanımı

Teknolojinin ilerlemesiyle birlikte yabancı cisim unutulmasının önüne geçmek için yeni teknolojik birtakım ürünler kullanılması önerilmektedir (AORN, 2019). Ameliyat sırasında

özellikle tamponların unutulmasını önlemek için radyofrekans yoluyla tespit edilebilen elektronik çipli tamponların kullanılması öneriler arasında yer almaktadır (Steelman et al., 2018) Radyofrekans yoluyla cerrahi bölgedeki tamponlar daha hızlı ve kolay bulunmaktadır (Steelman et al., 2019b). Tamponlar ve aletlerin cerrahi sayımı, oluşturulacak barkod sistemiyle yapılması yabancı cisim unutulma riskini azaltmaktadır (Goldberg and Feldman, 2013). Ameliyat sırasında kullanılan ve sıklıkla unutulan yabancı cisimlerin Amerikan Gıda ve İlaç Dairesi (U.S. Food and Drug Administration, FDA) onaylı olmasının yabancı cisim unutulmasının önlenmesinde önemli bir rolü vardır (Taşdemir, 2015).

Hadjiiski et al. (2015) tarafından yapılan bir çalışmada; sünger ve tamponların tespit edilmesi için 3D mikroetiketler kullanılması ve bilgisayar destekli teknoloji ile bunların tespit edilmesinin yabancı cisim unutulmasını önlemede etkili olduğu belirtilmektedir. Ayrıca hiçbir teknolojinin insan faktörünün yerine geçemeyeceği unutulmamalıdır. Bu nedenle personelin manuel sayımla geleneksel yöntemleri teknoloji ile destekleyerek risk en aza indirilmelidir (Freitas et al., 2016).

Yabancı Cisim Unutulması Önlemek İçin Sayım Önerileri (AORN, 2010; 2011; 2019)

- ✓ Ameliyata başlamadan önce, vücut boşluklarını kapatmadan önce, yarayı kapatmadan önce ve cerrahi işlem sonunda cilt kapatılmadan önce mutlaka cerrahi sayım yapılmalıdır.
- ✓ Multidisipliner bir cerrahi sayım açık ya da kapalı tüm ameliyatlarda yapılmalıdır.
- ✓ Tüm cerrahi ekip üyeleri yabancı cisim unutulmasının önlenmesinden sorumludur.
- ✓ Cerrahi sayım cerrahi ekip üyelerinden herhangi biri tarafından başlatılabilir.
- ✓ Steril alana açılan tüm malzemeler ve aletler sayılmalıdır.
- ✓ Sayma süreci boyunca gereksiz işlemler ve dikkat dağıtıcı aktivelerden uzak durulmalıdır. Sayım kesinlikle bölünmemelidir.
- ✓ Kullanılan tüm tamponlar, havlular ve tekstil ürünleri steril alana açılırken tek tek sayılmalı ve x-ray ışınları ile tespit edilebilecek radyoopak özellikte olmalıdır.
- ✓ Sayılan tamponlar bilinçli olarak cerrahi alanda bırakıldıysa ameliyat sırasında bırakılan tampon çeşidi, sayısı ve bırakılma nedeni cerrahında onayıyla kaydedilmelidir.
- ✓ Boyutları ne olursa olsun tüm iğneler tüm cerrahi operasyonlarda sayılmalıdır. Boş sütur paketleri sayım için kullanılmamalıdır.
- ✓ Cerrahi aletlerin unutulabileceği her zaman değerlendirilmelidir. Cerrahi aletler sterilizasyon için toplandığında tekrar sayılmalıdır. Cerrahi setlerin içeriği standartlaştırılmalıdır. Cerrahi alet setlerinde gereksiz ve fazla sayıda olan malzemeler kurum politikası gereği belirlenerek çıkarılmalıdır.
- ✓ Cerrahi alandan çıkarılan aletler bütünlük açısından kontrol edilmeli, kopan ya da kırılan bir parçası olup olmadığına bakılmalıdır. Kalan parçanın hasta için riskleri belirlenmelidir.

- ✓ Cerrahi ekip üyeleri manuel sayım prosedürlerine ek olarak barkod sistemi ve radyofrekans yöntemi gibi destekleyici teknolojiler kullanmalıdır.
- ✓ Cerrahi ekip üyeleri destekleyici teknoloji konusunda yetkin ve eğitilmiş olmalıdır.
- ✓ İntraoperatif dönemde sirküle hemşire tarafından tüm cerrahi sayım kayıtları tutulmalıdır. Kayıtta standart kayıt terminolojisi kullanılmalıdır.
- ✓ Cerrahi işlemden sonra son sayımda sayım tutarsızlıkları tespit edilirse kayıpları bulmak için cerrahi ekip harekete geçmelidir. Sirküle hemşire sayımda eksiklik tespit ederse cerraha ve cerrahi ekibe bilgi vermelidir. Cerrahi alan elle ve gözle incelenmelidir. Alandaki zemin ve çöpler kontrol edilmelidir. Sirküle hemşire odadaki steril olmayan alanları kontrol etmelidir. Eksik öğe bulunamamışsa intraoperatif x-ray görüntülüne yapılmalıdır.

SONUÇ

Ameliyathanede hasta güvenliğinin sağlanması ameliyat olan hastada gelişebilecek komplikasyonları önlemek için dikkat edilmesi gereken bir konudur. Yabancı cisim unutulması ameliyat olan hastada birçok komplikasyon gelişmesine ve hayatının tehlikeye girmesine neden olabilmektedir. Bu konuda sorumluluk tüm cerrahi ekibin olmakla birlikte ameliyathane hemşireleri cerrahi sayımda görevlidir. Ameliyatta hasta için kullanılan tüm alet ve malzemelerin ameliyattan önce ve sonra sayılması sayımlar bitmeden hastanın kapatılmaması gerekmektedir. Bunun için kanıta dayalı rehber önerileri doğrultusunda sayımlar yapılmalı, teknolojik aletlerin kullanımı yaygınlaştırılmalı, hastane politikaları geliştirilerek cerrahi ekibe eğitim verilmeli ve yabancı cisim unutulması durumunda yapılacak adımlar tek tek ele alınmalıdır. Yabancı cisim unutulmasını önlemek için yapılabilecekler için çalışmalar artırılarak kanıta dayalı rehberlere yeni kanıtlar sunulmalıdır.

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**ABOUT THE GLANDS GENERAL EXCRETORY DUCTS OF THE HUMAN
URINARY BLADDER IN NORM**

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ABSTRACT

The structure of the glands in the walls of different inner organs, the laws of their morphogenesis have been studied in sufficient details. In the literature, there are a large quantity of works devoted to age, individual and regional characteristics of the glands of tubular organs. According to the literature, the glands of the walls of some internal organs of the digestive, respiratory, urinary and reproductive systems form bulbs, ampoules. The general ducts of glands in the tubular organ wall opens into the epithelial layer of the mucus layer and hence the organ cavity and is the gateway for infection or foreign particles. In this sense, the data of the morphological features of the general duct of the glands, which shape differently in different age periods, are probably useful in influencing the course of urology disease.

In connection with this, to establish similar features in norm of the ducts, the purpose of the investigation is to learn the age, individual, regionally features of the general excretory ducts of a glands in walls of the human urinary bladder of postnatal ontogenesis.

In different age periods of postnatal ontogenesis, the glands general excretory duct of human urinary bladder walls are investigated by the macro-microscopically (with Sinelnikov metod) and by the histologically methods (Van Gizons, Kreybergs methods) of the 34 cadavers. Statistical data processing included calculation of arithmetic-mean values, their errors, confidential intervals.

Usually in the last periods of the postnatal ontogenesis, the general excretory duct of the urinary bladder walls glands has expansions, S-shaped form and form an ampoule. The quantity of these glands increases up to 19 times compared to newborns in old age. The individual minimum and maximum percentages of these glands gradually increase from the newborn to senile age. This connected with biological activity of the people in the definite degree. These glands are also characterized by regionality peculiarities. In all age groups of postnatal ontogenesis the quantity of these glands is more in the distal part of the urinary bladder, than in the middle and proximal parts.

In postnatal ontogenesis of the urinary bladder walls in norm, the glands with expansions, S-shaped curvature and ampoule are characterized by age, individually and regionally features.

Keywords: Postnatal ontogenesis, Human urinary bladder glands, General excretory ductus

RELATIONSHIP OF THE METOPIISM WITH FEATURES OF THE FRONTAL AND SPHENOID SINUSES

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ABSTRACT

Metopism has attracted the attention of morphologists and clinicians for a long time. Compared to other skulls, the absence of the frontal sinus is more common in metopic skulls. Also, the relationship of metopism to the development of the sphenoid sinuses remains poorly understood.

The material for the study was a male skull taken from the educational museum of the Department of Human anatomy and medical terminology of the Azerbaijan Medical University. The study used cranoscopic, craniometric, and computed tomography methods.

The results of the study showed that the metopic suture is represented by three segments. The first segment, 3.20 mm long, passed into the area where the suture was ossified. The length of this part was 13.35 mm. The third, longest segment of the metopic suture, 84.02 mm long, ends to the right of the bregma point. The aplasia of the right frontal sinus was identified. The height of the left frontal sinus was 1.84 cm, which corresponds to the average size of these sinuses. The width of the left frontal sinus was 1.54 cm, the anterior-posterior dimension was 8.02 mm; both sizes correspond to the small frontal sinuses. The anterior-posterior size of the right sphenoid sinus was 1.68 cm, the same size for the left sphenoid sinus was 1.58 cm. The width of the right sphenoid sinus was 1.02 cm; the same for the left was 1.26 cm. The height dimension for both sinuses was almost the same - 1.93 cm on the right, 1.98 on the left. The indicated sizes characterize the sphenoid sinuses of childhood; the incomplete development of the sphenoid sinuses was identified.

Metopism can be associated with underdevelopment of not only the frontal but also the sphenoid sinuses, the fact has both theoretical and clinical importance.

Keywords: Metopic suture, Metopism, Frontal sinus, Sphenoid sinus.