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Editor
Prof. Dr. Didem TURGUT COŞAN











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PROCEEDINGS BOOK

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UNIVERSITY OF PRISTINA "HASAN PRISTINA"
KOSOVO

Welcome

Dear colleagues and friends,

We are delighted to invite you to the 8th International Congress on Applied Biological Sciences 2023 (ICABS-2023) that will take place in Pristina, Republic of Kosovo, from September 13th to 16th. This congress was organized by researchers from Eskisehir Osmangazi University and the University of Pristina "Hasan Pristina".

Our field is developing extremely fast with important implications for basic science, biology, and medicine. ICABS-2023 aims to provide an interdisciplinary platform for significant scientific and professional activity in research findings, developments, and applications.

The scientific program includes invited international experts, selected presentations for young investigators, educational sessions, and poster presentations. Thus, the congress is a unique opportunity to get inspired for scientific work, share your results, and shape the future of the field.

ICABS-2023 will be a compact meeting and will be including some novel approaches to help make it extra rewarding and enjoyable, such as a 'stand-up' social evening for those interested in applied biological sciences.

This congress will serve as an excellent platform for participants to broaden their knowledge, enhance their professional networks, and gain new insights into the latest trends and developments in the basic sciences. For the ICABS-2023, we welcome researchers at all career stages, from a range of research fields to gather together in the Applied Biological Sciences for a better future.

We are looking forward to meeting you in Kosovo!

Prof. Dr. Didem TURGUT COŞAN
Congress President
Eskisehir Osmangazi University, Turkey

Prof. Dr. Ilir MAZREKU
Congress President
University of Pristina, Kosovo

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INVITED SPEAKERS

Prof. Dr. Yusuf ÖZTÜRK

"PATCH CLAMP TECHNIQUE AND APPLICATION AREAS"

Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskisehir, Turkey

E-mail: yozturk@anadolu.edu.tr

Academic ILIA MIKEREZI

"DIFFERENT GENETIC ANALYSIS REVEAL INTERESTING DATA ON GENETIC STRUCTURE AND RELATIONSHIPS OF ALBANIAN HUMAN POPULATION"

University of Tirana, Faculty of Natural Sciences, Department of Biology, Bulevardi Zog I, Tirane, Albania

CONGRESS PROGRAM

Day 1 (13.09.2023)		
09:00-10:00	**Registration**	
10:00-10:10	PROF. DR. QERIM QERIMI Rector of University Pristina	Opening remarks of the congress
10:10-10:20	PROF. DR. MUJGAN OZDEMIR ERDOGAN President of the congress organizing committee	Opening remarks of the congress
10:20-10:30	PROF. DR. SEFER AVDIAJ Vice Rector of University of Pristina	Opening speech and welcoming
10:30-10:40	PROF. DR. IDRIZ VEHAPI Dean of the FMNS	Opening speech and welcoming
10:40-10:50	PROF. DR. SUZANA MANXHUKA KERLIU Dean of FM	Opening speech and welcoming
10:50-11:00	PROF. DR. DIDEM TURGUT COSAN	Opening speech and welcoming
11:00-11:15	PROF. DR. ILIR MAZREKU & PROF. DR. ARMOND DACI	Opening speech and welcoming
Session I	**Cell Signalling**	**Chair: Prof. Avni Berisha**
11:15-12:15	PROF: DR: YUSUF OZTURK **Invited Speaker**	PATCH CLAMP TECHNIQUE AND APPLICATION AREAS
12:15-13:45	**Lunch break**	
Session II	**Cell Signalling**	**Chair: Prof. Avni Berisha**
13:45-14:05	TAULANT KUKAJ	EXPLORING MEMBRANE RECEPTORS USING OPTICAL METHODOLOGIES: A SPOTLIGHT ON G-PROTEIN COUPLED RECEPTORS (GPCRs)
14:05-14:25	FITIM KASTRATI	CHARACTERISATION OF CHLOROPHYLL FLUORESCENCE IMAGING AND PHOTOSYNTHETIC ACTIVITY OF GENUS RAMONDA IN KOSOVO
14:30-15:00	**Coffee break**	
15:00-17:00	**Poster area**	

CONGRESS PROGRAM

Day 2 (14.09.2023)		
Session I Genetic		**Chair: Prof.Asoc.Ramadan Sopi**
09:30-10:20	Academic ILIA MIKEREZI **Invited Speaker**	DIFFERENT GENETIC ANALYSIS REVEAL INTERESTING DATA ON GENETIC STRUCTURE AND RELATIONSHIPS OF ALBANIAN HUMAN POPULATION
10:20-10:50	Coffee break	
Session II Anatomy&Orthopedics		**Chair: Prof.Ass.Jeton Shatri**
10:50-11:10	MIRANDA BELLANICA	INFLUENCE OF GENDER AND AGE ON AVERAGE DIMENSIONS OF ARTERIES FORMING OF MIDDLE CEREBRAL ARTERY STUDYING BY CTANGIO
11:10-11:30	MENTOR SHATRI	THE DIAMETER OF POSTERIOR CEREBRAL ARTERY AND BASILAR ARTERY BY MAGNETIC RESONANCE ANGIOGRAPHY ON KOSOVO'S POPULATION
11:30-11:50	FULYA BAKILAN	THE RELATIONSHIP BETWEEN FRAGILITY FRACTURES AND GERIATRIC NUTRITIONAL RISK INDEX
11:50-12:10	BURCU ORTANCA	COMPARISON OF LABORATORY PARAMETERS OF INFLAMMATORY MARKERS IN PATIENTS WİTH FIBROMYALGIA AND LOCALISED MUSCULOSKELETAL PAIN
12:10-12:30	RONA KARAHODA	PLACENTAL TRYPTPHAN METABOLISM IN HEALTH AND DISEASE: FROM BASIC RESEARCH TO CLINICAL STUDIES
12:30-13:40	**Lunch break**	
Session III	**Ecotoxicology**	**Chair: Prof.Asoc.Ilir Mazreku**
13:40-14:00	MENTOR BICI	POLLUTION FROM SOME HEAVY METALS CAUSED BY MINING ACTIVITY AND THEIR EFFECT IN OXIDATIVE STRESS OF HEPATOPANCREAS OF SNAIL HELIX POMATIA. L
14:00-14:20	DONARD GECI	ERATIGENA MIRUSHA SP. NOV. (ARACHNIDA: ARANEAE), A NEW AGELENID SPECIES FROM KOSOVO WITH NOTES ON ITS ECOLOGY
14:20-14:40	NESADE MUJA-BAYRAKTARI	GEOGRAFICAL DISTRIBUTION OF CULEX PIPIENS s.s.L (CULICIDAE) IN KOSOVO
14:40-15:30	**Coffee break**	
15:30-17:00	**Poster area**	

CONGRESS PROGRAM

Day 3 (15.09.2023)		
Session I Biomedicine & Therapeutics		Chair: Prof.Assoc.Burim Neziri
10:00-10:20	HULYAM KURT	COULD PYROPTOSIS-ASSOCIATED GASDERMIN B PROTEIN LEVEL SERVE AS A POTENTIAL BIOMARKER IN THE PROSTATE CANCER
10:20-10:40	ZEHRA SEDA HALBUTOGULLARI	INVESTIGATION OF THE EFFECTS OF BONE MARROW MESENCHYMAL STEM CELL DERIVED MITOCHONDRIA (BM-MSC-MİT) THERAPY ON OVARIES IN OVARIAN PHYSIOLOGIC AGING (OPA) MODEL
10:40-11:10	Coffee break	
11:10-11:30	DONJETA KRASNIQI	VASORELAXANT EFFECTS OF ETHANOLIC EXTRACT FROM CYDONIA OBLONGA MILL. LEAVES ON ISOLATED RAT THORACIC AORTA AND POTENTIAL MECHANISM OF ACTION
11:30-11:50	GRESA HALIMI	NEW HORIZONS FOR BENZIMIDAZO[1,2-A]PYRIMIDINE DERIVATIVES
12:00-13:30	Lunch break	
Session II Biomedicine & Therapeutics		Chair: Prof.Assoc.Burim Neziri
13:30-13:50	FESTINA KUCI	THE IMPACT OF CERTAIN PROTOCOLS ON THE OUTCOME OF COVID-19 PATIENTS
13:50-14:10	GRANIT SALIHU	THERAPEUTIC DRUG MONITORING OF LEVETIRACETAM – CASE OF KOSOVO
14:10-14:40	Coffee break	
14:40-16:00	Poster area	

CONGRESS PROGRAM

Day 4 (16.09.2023)		
Session I Nanomedicine&Nanotoxicity		**Chair: Prof.Assoc.Armond Daci**
10:00-10:20	AYSE AK	IN VITRO BIOCOMPATIBILTY ASSESSMENT OF Ti45Nb ALLOY
10:20-10:40	AVNI BERISHA	2D CARBON NANOMATERIALS: POLLUTION'S NEW ADVERSARY
10:40-11:10	**Coffee break**	
11:10-11:30	TOSKË KRYEZIU	NANOEMULSION VS. LIPOSOME ENCAPSULATED ESSENTIAL OIL FROM WILD-GROWN ALBANIAN THYMUS CAPITATUS: A COMPARATIVE STUDY ON ANTICANCER ACTIVITY IN MCF7 HUMAN BREAST CANCER CELL LINE
11:30-11:50	FULYA DOGANER	EVALUATION OF THE ANTICANCER EFFECT OF SPINACIA OLERACEA LEAF EXTRACT ON HT-29
11:50-12:50	**Lunch break/Cocktail and finger food**	
12:50-14:00	**Congress Closing & Final Remarks**	

INVITED SPEAKERS

INVITED SPEAKER

PATCH CLAMP TECHNIQUE AND APPLICATION AREAS

YUSUF OZTURK[1*], FEYZA ALYU ALTINOK[1]

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Abstract

We present this oral presentation to highlight the significance of the patch clamp technique in biological sciences and drug research. As the founders of Turkey's inaugural patch clamp laboratory within a pharmacy faculty, our aim is to underscore its pivotal role. This technique has profoundly transformed electrophysiology by enabling precise investigations into ion channels and cellular electrophysiology. The patch clamp method, introduced by Neher and Sakmann in the late 1970s, involves attaching a micropipette to a cell membrane, facilitating the measurement and manipulation of ion currents. It offers two primary configurations: whole-cell and cell-attached, each providing unique insights into ion channel behavior and cellular dynamics. Patch clamp applications span diverse disciplines such as neurophysiology, cardiology, pharmacology, and cell biology. Within neurophysiology, this technique elucidates neuronal excitability, synaptic transmission, and plasticity, offering a comprehensive view of the nervous system's dynamic complexities. In cardiology, patch clamp informs cardiac electrophysiology, supporting insights into arrhythmias while serving as a vital platform for the detailed testing of novel pharmaceutical interventions. Moreover, in the realm of pharmacology, its capacity to revolutionize drug discovery remains intriguing, providing appproaches which interactions between drugs and ion channels can be reviewed, thus paving the way for the development of safer and more efficacious therapies. This technique's multidisciplinary significance underlines its pivotal role in advancing our understanding of cellular physiology and its applications across the scientific spectrum.

Keywords: Patch clamp technique, Pharmacology, Neurophysiology, Cardiology, Cell biology

ORAL PRESENTATIONS

EXPLORING MEMBRANE RECEPTORS USING OPTICAL METHODOLOGIES: A SPOTLIGHT ON G-PROTEIN COUPLED RECEPTORS (GPCRs)

TAULANT KUKAJ[1, 2*], KLAUS BENNDORF[1,2]

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Abstract

G-protein coupled receptors (GPCRs) constitute the largest family of membrane proteins, governing essential physiological and pathophysiological mechanisms within the human body. This pivotal family also serves as a focal point for numerous market-available drugs, with GPCRs being targeted by around 30-50% of these pharmaceutical compounds. The process of rational drug discovery commences with the identification and validation of biological targets. In this context, optical methods play a pivotal role in enhancing the comprehension and characterization of these targets. Our study centers on metabotropic glutamate receptors (mGluRs), positioned within the GPCR family C. These receptors hold profound significance for processes such as synaptic plasticity, learning, and memory. To delve into the activation mechanism, kinetics, and dimerization of mGluRs, we employed Fluorescence Resonance Energy Transfer (FRET).

Our methodology involved developing mGluR FRET sensors by incorporating fluorescent proteins into respective monomers. Subsequently, we expressed these constructs in Xenopus laevis oocytes and monitored activation using confocal patch-clamp fluorometry on membrane outside-out patches. Our investigation unveiled that the activation of mGluRs occurs sequentially in two distinct steps, unfolding within the millisecond timeframe. Notably, we also made the novel observation that mGluRs have the capacity to establish heterodimers. This discovery holds promise for shedding light on the intricate dynamics underlying synaptic plasticity, learning, and memory processes.

Keywords: G-protein coupled receptors (GPCRs), Fluorescence Resonance Energy Transfer (FRET), Metabotropic Glutamate Receptors (mGluRs), Confocal-patch-clamp fluorometry

Acknowledgement: Prof. Thomas Zimmer, Dr. Ralf Schmauder, and Dr. Christian Sattler

CHARACTERISATION OF CHLOROPHYLL FLUORESCENCE IMAGING AND PHOTOSYNTHETIC ACTIVITY OF GENUS RAMONDA IN KOSOVO

FITIM KASTRATI[1]*, BEKIM GASHI[1]

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Abstract

Ramonda nathaliae and Ramonda serbica are endemic and relict plant species of the Balkan Peninsula, which belong to a small group of angiosperms known as resurrection plants that can tolerate extreme dehydration. The special value of these endemic plants is the ability of "resurrection" and their high poikilohydric properties, in this case, their leaves withstand almost complete drying and manage to renew themselves by receiving small amounts of moisture. Photosynthesis is one of the primary processes in plants affected by various environmental conditions. The aim of this study is to compare the response and differences of chlorophyll fluorescence imaging and the photosynthetic activity of two Gesneriaceae species Ramonda nathaliae and Ramonda serbica. The experiment was conducted with mature leaves of both species of genus Ramonda. Plants were collected from their natural habitat and further cultivated under ex situ environmental conditions We have measured chlorophyll fluorescence imaging and the rate of gas exchange, where we used an infrared gas analyser (CIRAS 4, PP System, USA). CO_2 assimilation (A), stomatal conductance (gs) and intracellular CO_2 concentration (Ci) were analysed. The higher value of Fv/Fm was recorded in R. serbica compared to R. nathaliae. Stomatal conductance (gs) and CO_2 assimilation (A) were both higher in R. nathaliae, whereas we have found higher levels of concentration of intracellular CO_2 (Ci) in R. serbica. The results of our study showed that photosynthetic activity in R. nathaliae was higher compared to R. serbica.

Keywords: Ramonda nathaliae, Ramonda serbica, resurrection, dehydration, chlorophyll fluorescence, stomatal conductance

ORAL PRESENTATION

INFLUENCE OF GENDER AND AGE ON AVERAGE DIMENSIONS OF ARTERIES FORMING OF MIDDLE CEREBRAL ARTERY STUDYING BY CT ANGIO

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Abstract

Middle cerebral artery (MCA), is the closing branch of internal carotid artery (ICA). It divides at the junction of the carotice cistern and the Sylvi cistern at the level of the cisterna fossae lateralis cerebri. Along its way it splits into two, or more than trunks primary ends, from which it splits in seconds and from which the cortical branches will split 2. MCA is divided into four segments named from M1 to M4. The purpose of this research is to provide knowledge on the anatomy of the cerebral media artery, to present the clinical importance of the cerebral media artery, to present the obtained data on the diameter and length of the middle cerebral artery and to compare the obtained results with the results of the others authors. Our study included 30 patients examined with CT-Angio who determined the diameter and length of the M1 segment of the MCA based on the gender and age of the patients present in the study who ranged in age from 5 to 83 years. Patients who were referred to the Radiology Clinic, University Clinical Center of Kosovo, as part of their health screening, were included in this study. For comparative-statistical analysis, patients were divided into groups based on the gender and age of the patients. In our study conducted with 300 patients, the M1 segment had an average diameter of 2.77 mm by 2-3.4 mm while the average length was 19.4 mm by 11-25 mm and based on the age group of patients, the diameter and length of the M1 segment of the MCA is larger in the age group> 60 as opposed to the age group <60. From the results obtained in our research we conclude that our study presented differences in the length and diameter of the M1 segment of the MCA based on the gender and age of the patients and at the same time confirms a non-significant difference.

Keywords: Middle cerebral artery, diameter, height, age, sex.

ORAL PRESENTATION

THE DIAMETER OF POSTERIOR CEREBRAL ARTERY AND BASILAR ARTERY BY MAGNETIC RESONANCE ANGIOGRAPHY ON KOSOVO'S POPULATION

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Abstract

The basilar artery is part of the posterior cerebral circulation. It arises from the union of the right and left vertebral arteries at the base of the pons as they rise toward the base of the brain. The posterior cerebral arteries (PCA) are the terminal branches of the basilar artery and supply oxygen to the occipital lobes and the posteromedial part of the temporal lobes. The purpose of this research is to provide knowledge on the anatomy of the posterior cerebri artery and basilaris artery, to present the obtained data on their diameter, clinical importance and to compare the obtained results with the results found by other authors. This is an observative descriptive study performed at the University Clinical Center of Kosovo. A randomised sample of 513 angiographic examinations in adult patients of both sexes who were instructed to exploration is included. Were measured diameters of arteries forming the CAC and the correlation of diameters between to age and sex. The diameters and lengths measured in our study were comparable with other brain-cadaver studies especially those performed by MRA. All dimensions of the arteries are larger in male than female, except the diameter of PCoA that is larger in female ($p < 0.05$) and length of the ACoA ($p < 0.05$). Significant differences were found in diameters of arteries between the younger and the older age groups. From the results obtained in our research, we conclude that this study proves differences in the diameter of the posterior cerebri artery and basilaris artery according to age and gender, at the same time it also presents a non-significant difference based on different peoples and regions. Knowing the dimensions of the arteries of the circle of Willis has a great importance in interventional radiology as well as during anatomy lessons.

Keywords: MRA, 3D-TOF, diameters of arteries, posterior cerebral artery, basilari artery.

THE RELATIONSHIP BETWEEN FRAGILITY FRACTURES AND GERIATRIC NUTRITIONAL RISK INDEX

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Abstract

In previous studies, it has been shown that the “geriatric nutritional risk index” is an indicator in determining the nutritional need in patients with osteoporosis. However, in osteoporotic patients with fragility fractures, it has not been investigated whether this indicator has an effect on the fracture. The aim of this study is to investigate whether the “geriatric nutritional risk index” is different in osteoporotic patients with and without fragility fractures.

The file records of female patients, aged 60 years and over, who were registered to the "Osteoporosis Clinic" within the part of Physical Medicine and Rehabilitation Outpatient Service, between September 2022 and July 2023, and diagnosed with "osteoporosis", were reviewed retrospectively. Patients with secondary osteoporosis, using steroids, having any comorbidity that may cause cancer or malnutrition, or using any protein-containing drug were excluded and a total of 48 patients were included in this study. Twenty-four patients with fragility fractures according to radiography and file examinations were accepted as the 1st group. Twenty-four patients who did not have any fragility fractures were accepted as 2nd group. Bone mineral densitometry, radiographic datas, laboratory parameters including albumin, calcium, alkaline phosphatase, phosphorus, parathyroid hormone, calcium/creatine ratio in spot urine, 25 hydroxy vitamin D values and FRAX values were recorded. The “geriatric nutrition risk index” was calculated using the albumin values and the height and the weight of patients.

Comparison of two groups showed that, in patients with fragility fracture, the risk of FRAX major osteoporotic fracture was significantly higher ($p=0.004$), and the phosphorus value was significantly lower ($p=0.003$). Patients with fragility fractures were divided into 2 groups as those with vertebral fractures ($n=11$) and those with non-vertebral fractures ($n=13$), the corrected calcium value was significantly lower in those with non-vertebral fractures ($p=0.012$). Comparison of two groups showed that “geriatric nutritional risk index” and blood albumin levels were similar in both 1st and 2nd groups.

According to our study results, no relationship was found between “geriatric nutritional risk index” which is one of the malnutrition indicators, and fragility fractures in geriatric osteoporotic patients.

Keywords: Fragility, Malnutrition, Osteoporosis

COMPARISON OF LABORATORY PARAMETERS OF INFLAMMATORY MARKERS IN PATIENTS WITH FIBROMYALGIA AND LOCALISED MUSCULOSKELETAL PAIN

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Abstract

Fibromyalgia syndrome (FMS) is a disease characterized by chronic and widespread musculoskeletal pain, the pathogenesis of which has not been fully elucidated. It is thought that immunological changes that cause inflammatory state may play a role in its pathogenesis.

Our study was performed in order to compare C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neutrophil-lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/ lymphocyte ratio (MLR), mean corpuscular hemoglobin/ platelet distribution width ratio (MCH/PDW ratio), the platelet distribution width (PDW), the mean platelet volüme (MPV), the red blood cell distrubution (RDW) and the systemic immune-inflammation index (SII) which are the systemic inflammatory response (SIR) markers in patients with fibromyalgia and acute localised musculskeletal pain.

This study was conducted with 31 female patients diagnosed with fibromyalgia and 31 female patients with acute localized musculoskeletal pain in our Physical Therapy and Rehabilitation outpatient clinic between January 2022- June 2023. Demographic characteristic (age), CRP, ESR, NLR, PLR, MLR, MCH/PDW ratio, MPV, RDW and SII were recorded.

The mean platelet volume in the fibromyalgia group was higher than in the localised pain group($p=0.015$) ; No differences were found between the two groups in the other systemic inflammatory response markers. There was no statistical difference between the 2 groups in terms of the SII values ($p=0.894$).

The results of this study suggest that MPV may be a promising marker as an inflammatory marker in patients with fibromyalgia and may be useful in facilitating the diagnosis of FMS patients.

Keywords: Fibromyalgia, inflammatory markers, systemic immune-inflammation index

PLACENTAL TRYPTPHAN METABOLISM IN HEALTH AND DISEASE: FROM BASIC RESEARCH TO CLINICAL STUDIES

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Abstract

The placenta is an ephemeral organ inevitable for the successful course of pregnancy. The latest research has associated perturbations of maternal conditions (such as pharmacotherapy, malnutrition, diseases, stress, or inflammation) with alterations of the placentas' endocrine, transport, and metabolic functions. Of note is the placental utilization of the essential amino acid tryptophan, suggested as a potential mechanism contributing to the fetal programming of adulthood diseases. Nonetheless, a comprehensive characterization of the enzymes and transporters involved in the placental tryptophan metabolism in health and disease is still lacking. On these grounds, the aims of this work were manifold and included: 1) a detailed assessment of placental tryptophan during gestation in humans and rats, 2) an evaluation of the contribution of fetal organs to the prenatal tryptophan metabolism, 3) the effect of inflammation on placental tryptophan metabolism, and 4) the link between preterm birth and placental tryptophan pathway signature.

A wide range of methodological approaches was utilized, including ex vivo tissue explants isolated from human term placenta, gene expression analysis by Quantitative- and Droplet Digital PCR analysis, protein expression by western blotting, the metabolic activity of rate- limiting enzymes, and metabolomic profiling using UHPLC/MS. The clinical part of the study was conducted in a cohort of healthy term pregnancies (n = 39) and preterm deliveries (n =167) collected at the University Hospital Hradec Kralove.

We report that the placental homeostasis of tryptophan is subject to strictly regulated developmental changes during pregnancy. We show that placental production of kynurenine increases during pregnancy, with a low contribution of other fetal organs. On the other hand, placental tryptophan metabolism to serotonin is crucial in early-to-mid-gestation, with a subsequent switch to fetal brain and intestine serotonin synthesis. Importantly, inflammation severely affects placental tryptophan metabolism, particularly the kynurenine pathway. Using robust statistical analysis, we reveal putative relationships between maternal inflammation, preterm delivery, the placental tryptophan pathway, and gestational age at delivery. Altogether, our data provide the first comprehensive evidence of changes in placental homeostasis of tryptophan during healthy and pathological pregnancy (preterm birth, maternal inflammation). Elucidating these associations will help efforts to understand the complex processes involved in the biological roots of fetal programming.

Keywords: placenta, preterm birth, tryptophan, serotonin, kynurenine, fetal development

Acknowledgement: The study was supported by the Czech Health Research Council [NU20-01-00264].

POLLUTION FROM SOME HEALTY METALS CAUSED BY MINING ACTIVITY AND THEIR EFFECT IN OXIDATIVE STRESS OF HEPATOPANCREAS OF SNAIL *Helix pomatia*. L

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Abstract

In this research project, we measured the impact and distribution of the activity of minning "Ferronikel" on the concentrations of heavy metals (Pb, Zn, Ni) in the locality Drenas and their effect in oxidative stress of some parameters: carbonyl of proteins, malondyaldehid MDA and total proteins in hepatopancreas of snail *Helix pomatia*.L.

These heavy metals can caused oxidative stress in terrestrial animals such as snails which obtain these metals through food from storage plants in areas polluted by industry and coal burning.

120 soil samples and 12 snails collected through concentric circles from the pollution point at1 km, 2 km, 5 km. The dry sample of 0.3 g was treated with reagents $HN0_3$ 69% 8 20 2 30% in a ratio of 1:3 and then they \Ve re filtered and the content \Vas read in two types of flame absorbers Thermo and Contra AAA.

Snail hepatopancreas samples after measurement and homogenizatio in phosphate buffer 9:1 of the \Veight of]ive tissue, \Vere treated with relevant methods and \Ve re read in a spectrophotometer with \Vav e lengths of 450nm and 650nm for oxidative stress parameters.

Higher concentrations of Pb, Zn, and Ni \Vere recorded in the southern parts of the country compared to that control with significant differences ($p<O.O1$). Bioaccumulation levels of these heavy metals have been recorded in snail shells (Helix pomati.a, L.). These concentrations of these metals have influenced the increase in the levels of oxidative stress parameters: protein carbonylation, malondialdehyde MDA and total proteins in the hepatopancreas.

Keywords: heavy metals, pollution, o:xidative stress, hepatopancreas, snail.

Acknowledgement: Special thanks for the professional help in the idea and design of this scientific project Prof Dr. Kemajl Bislimi.

Eratigena mirusha sp. NOV. (ARACHNIDA: ARANEAE), A NEW AGELENID SPECIES FROM KOSOVO WITH NOTES ON ITS ECOLOGY

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Abstract

In this paper we describe a new species of the genus Eratigena Bolzern, Burckhardt & Hänggi, 2013 from the Koznik Mountain in Kosovo, based on molecular and morphological data. Males of *Eratigena mirusha* sp. nov. are most similar to *Eratigena agrestis* (Walckenaer, 1802) but exhibit differently shaped conductors and retrolateral tibial apophysis. The genetic divergence calculated based on the barcode region of the cytochrome c oxidase subunit I gene (COI) confirm the morphological distinctness and supports *Eratigena mirusha* sp. nov. as sister species of Eratigena agrestis.

Keywords: New species, Agelenidae, Western Balkans, COI

GEOGRAFICAL DISTRIBUTION OF *Culex pipiens* s.s.L (CULICIDAE) IN KOSOVO

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Abstract

Mosquitoes have a large spread all over the world and are adopted to different biotopes. Their impact on the health is very dangerous, because they are vectors of various pathogens that cause disease in animals and humans. In this paper are presented the results of a research for the *Culex pipiens* s.l. that was done in all the territory of the Republic of Kosovo, during the years 2016, 2017, 2018 in 98 localities, in 106 sampling stations. The density of *Culex pipiens* s.l. during the years of research has been different, which also varied on habitat types, temperatures, preferences for urban, rural areas, farms with animals ect.Our results show that *Culex pipiens* s.l has a wide distribution almost throughout the surface of Kosovo. In total, 1044 specimens were collected in 76 localities of 26 municipalities in all the teritory of Kosovo. The results of this research will be hopefull, because these datas for the geographic distribution of *Culex pipiens* s.l that is the vector of WNV could be used for implementions of entomological programs, focused on risk assessment in order to prevent the public health from potential epidemic.

Keywords: Mosquito, *Culex pipiens* s.l., WNV, Kosovo, geografical distribution

Acknowledgement: We would like to express our gratitude to Prof. Dr. Bulent Alten, and Prof. As.Filiz Gunay, for the molecular identification of Culex pipiens s.l. species in the Ecological Sciences Research Laboratory (ESRL) in Faculty of Science, Department of Biology, University of Hacettepe in Ankara and Enkelejda Dikolli-Velo, head of the Vector Control Unit, to the Department of Epidemiology and Infectious Diseases Control, Institute of Public Health, Tirana, Albania for her continuous support of our research.

COULD PYROPTOSIS-ASSOCIATED GASDERMIN B PROTEIN LEVEL SERVE AS A POTENTIAL BIOMARKER IN THE PROSTATE CANCER

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Abstract

Prostate cancer is one of the biggest and most important health problems for men. Unfortunately, the incidence of prostate cancer is increasing every day in all countries of the world. Age, race, family history are the most well-known factors that affect the development of prostate cancer. A large number of studies done in recent years have highlighted the importance of pyroptosis on cancer. Pyroptosis is an inflammatory cell death that affects the development and progression of prostate cancer. The aim of our study was to determine the relationship between Gasdermine B protein level and prostate cancer. For this study we had two groups: the control group with 25 healthy individuals and the group with 25 individuals with prostate cancer. The level of pyroptosis-related proteins was assessed in serum. Measurement of GSDMB protein level was performed by ELISA method.

Our study was conducted with the decision of the Ethics Committee for Non-Interventional Clinical Research of ESOGU number 2022-194/40. In this study, blood samples were taken from the two groups and the Elisa Kit was used to determine the GSDMB protein level in the serum. In our study, there was no statistically significant difference between prostate cancer and control groups for serum GSDMB protein level (p=0.197). It is worth noting that our study is the first study that makes the connection between gasdermone protein B and prostate cancer and will contribute to the new literature, and the creation of strategies for the treatment and new diagnosis of prostate cancer. It is necessary to conduct new and comprehensive studies in this direction where the number of samples is greater and the changes of genes and proteins are evaluated together.

Keywords- *Pyroptosis, Gasdermin, Prostate Cancer, GSDMB.*

EFFECTS OF BONE MARROW MESENCHYMAL STEM CELL DERIVED MITOCHONDRIA (BM-MSC-Mit) TREATMENT ON OVARIAN PHYSIOLOGICAL AGING (OPA) MODEL

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Abstract

There has been a significant increase in the number of older pregnancies owing to advances in technology, changes in social relationships, and differences in living conditions. With advancing age, it becomes more difficult for women to conceive because of decreased ovarian function, reduced follicle number, and endocrine disorders. Since the recent past, studies have been conducted on the use of mesenchymal stem cells in the treatment of ovarian aging along with their damage repair, immune regulatory, antiapoptotic effects, proliferation, and differentiation abilities.

Mitochondria are important in the regulation of stem cell activity, and it is thought that they may underlie age-related impairment in stem cell function and self-renewal in multiple tissues.

In this study, a three-dimensional (3D) treatment model was developed by encapsulating aged ovarian tissue, rat bone marrow mesenchymal stem cells (rBM-MSCs), and mitochondrial extracts of these cells with alginate. In addition to the possible curative effects of 3D gel capsules applied around the ovary on ovarian aging, the possible curative effects of 3D alginate capsules loaded with mitochondria applied around the ovary were investigated. Only "alginate gel" as control group, "alginate gel containing rBM-MSCs" and "alginate gel containing rBM-MSCs mitochondrial extract" in treatment groups were applied around the ovarian tissue of aged animals.To evaluate the efficacy of the treatments, E2, FSH, and AMH protein levels in serum were analyzed by ELISA, FshR, EsR, AmhR, and Casp3 genes in ovarian tissues were analyzed by qRT-PCR, and cCasp3 and pCNA antibodies in ovarian tissue sections were analyzed by immunohistochemistry.

In conclusion, significant differences were found in the decrease in apoptosis markers and improvement in ovarian function-related genes in the groups treated with mitochondria-containing alginate capsules compared to the aged control groups.

Keywords: Mesenchymal Stem Cell, mitochondria, aging

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VASORELAXANT EFFECTS OF ETHANOLIC EXTRACT FROM *Cydonia Oblonga* MILL. LEAVES ON ISOLATED RAT THORACIC AORTA AND POTENTIAL MECHANISM OF ACTION

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Abstract

Cydonia oblonga leaves are used in Medicine for cardiovascular, gastrointestinal, respiratory, and infectious diseases. In many studies, the ethanolic extract of *Cydonia oblonga* leaves (CydOL-EE) has shown useful pharmacological properties in cardiovascular disease. However, there is a lack of investigations of its direct effects on the vascular smooth muscle tone.

Therefore, this study aimed to test the vasodilator properties of CydOL-EE and its mechanism of action in rat thoracic aorta preparations. In the beginning, was investigated CydOL-EE phytochemical profile by HPLC-UV/DAD. The vasorelaxant activity is tested in the preens of L- Name (the NO synthase inhibitor) and ODQ (cyclic Guanosine Monophosphate inhibitor) to explore its vasorelaxation effect through NO-dependent pathways. With this mechanism of action, CydOL-EE has also shown a reduction in vasoreactivity caused by PE. The available data provide scientific evidence for further clinical and ex-vivo studies.

Keywords: *Cydonia oblongata* Mill., Leaves Ethanolic Extract, Aorta, vasorelaxation, vasoreactivity, nitric oxide

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NEW HORIZONS FOR BENZIMIDAZO[1,2-A]PYRIMIDINE DERIVATIVES

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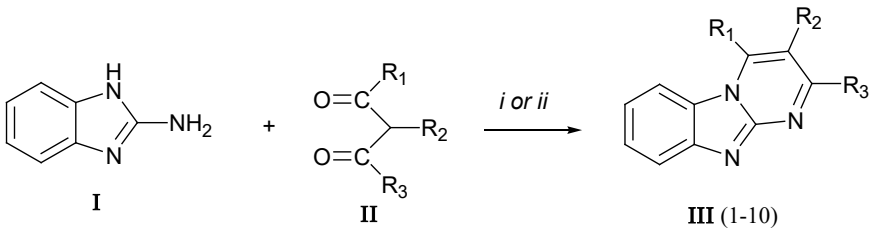
Abstract

Nitrogen-containing heterocycles are among the most important compounds present either in natural sources or created through synthesis. As a result, many researchers are motivated to devote significant efforts to exploring new strategies regarding various heterocycles, including nitrogen-containing ones, to be screened for biological activities.

Benzoimidazo[1,2-a]pyrimidines are biologically significant nitrogen-containing heterocyclic compounds that exhibit various pharmaceutical properties: including antifungal, antimicrobial, anti-inflammatory, antineoplastic, anticancer, antiviral, calcium channel blocker, antiparasitic, benzodiazepine receptor agonist, dihydroorotate dehydrogenase (DHODH) inhibitor (antimalarial), tumor pyruvate kinase M2 (PKM2) modulator and antiproliferative, copper corrosion inhibitor dipeptidyl peptidase-4 (DPP4) inhibitor (a class of oral hypoglycemic against Type 2 diabetes (T2DM)), P38 MAP kinase inhibitor, TIE-2 and/or VEGFR2 inhibitor, and T cell activation modulator, anticoccidial, GABA(A) receptor modülatör, antiinflammatory and anticancer**,** ADME prediction as antibacterial and cytotoxic agents. Beyond their biological functions, imidazo[1,2-a]pyrimidines exhibit intriguing optical properties and act as organic fluorophores.

Consequently, they can be used as biomarkers and photochemical sensors. In one of the recent studies, pyrimido[1,2-a]benzimidazole derivative (3a) had been evaluated as inhibitors of oncoproteins USP5 (ubiquitin-specific protease 5) and MYCN in the childhood cancer neuroblastoma. The most potent compound (DSM186) among the studied dihydroorotate dehydrogenase inhibitors has an imidazopyrimidine core and where direct comparisons between matched compounds were possible, the imidazopyrimidines were more potent than the triazolopyrimidines. Due to the elucidation of the new mechanisms of action of this ring system, we have focused on re-designing more efficient and promising compounds by re-evaluating compounds that we have already studied. We studied SwissADME predictions of previously and novel designed compounds' physicochemical properties, pharmacokinetics, drug-score and drug-likeness properties, on the present work. And bioavailability radar of designed compounds also have been displayed.

Keywords: Benzimidazo[1,2-a]pyrimidine; pyrimido[1,2- a]benzimidazole; SwissADME; bioavailability radar; drug-likeness; drug-score.



i) PhMe + MeOH or EtOH, reflux, 10-40h; (Method A) (-H_2O, -EtOH or -BnOH),
ii) PPA, 110-120 ^oC, 30 min; (Method B) (-H_2O)

II				III (1-10)			
R^1	R^2	R^3	Comp.	R^1	R^2	R^3	Comp.
BnO	H	Me	IIa	OH	H	Me	**1**
EtO	H	Ph	IIb	OH	H	Ph	**2**
EtO	H	n-Pr	IIc	OH	H	n-Pr	**3**
EtO	Ph	Me	IId	OH	Ph	Me	**4**
EtO	Me	Me	IIe	OH	Me	Me	**5**
Ph	H	Me	IIf	Ph	H	Me	**6**
Ph	H	Ph	IIg	Ph	H	Ph	**7**
Me	H	Me	Iıh	Me	H	Me	**8**
Me	Me	Me	IIj	Me	Me	Me	**9**
Me	Et	Me	IIk	Me	Et	Me	**10**

THE IMPACT OF CERTAIN PROTOCOLS ON THE OUTCOME OF COVID-19 PATIENTS

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Abstract

The COVID-19 pandemic, as rarely before, is characterized by quick preventive reactions that are later withdrawn. The same happened with the treatment protocols, which were modified almost on a monthly basis, starting with anti-inflammatory agents, antivirals, and even different antibiotics.

This is a single-center retrospective study. The outcomes of patients hospitalized with COVID-19 at the University Clinical Center of Kosovo's Infectious Diseases and Pulmonology Clinic were analyzed during 2020–2022. The study included 187 patients of different ages.

Based on the therapy protocols, the patients were divided into four groups: Treatment options include: fluoroquinolone; beta-lactam; fluoroquinolone and beta-lactam; and a combination of fluoroquinolone, beta-lactams, and remdesivir. The study's goal is to compare the disease's progression to the treatment strategy. Additional risk variables that affect the disease's progression were investigated.

The study's average age of those hospitalized patients with COVID-19 is 69 years old. Mortality was 16.57%. The mortality rate in the group treated with fluoroquinolone was 16.67%, while that of those treated with beta-lactam was 7.17%. Patients receiving fluoroquinolone and beta-lactam had a fatal outcome rate of 19.31%, whereas patients receiving remdesivir, fluoroquinolone, and beta-lactam had a fatal outcome rate of 33.33%.

Age (p=0.002), (RR=1.032), gender (p=0.005), (RR=1.157), comorbidities (p=0.030), (OR=6.413), antibiotic treatment, beta-lactam (p=0.021), (OR=0.718), (RR=0.316) and fluoroquinolone (p=0.012), (OR= 2.095), (RR=0.535) and some of the results of laboratory parameters such as CRP (p=0.001), AST (p=0.05), Urea (p=0.000), Creatinine (p=0.002), D-dimer (p=0.000), and RBC (p=0.005) have been some of the significant factors that have influenced the progress of the development of the disease. Antibiotics had a negotiable impact on influencing the course of the disease.

Keywords: COVID-19, fluoroquinolones, disease progression.

IN VITRO BIOCOMPATIBILTY ASSESSMENT OF Ti45Nb ALLOY

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Abstract

The evaluation of cytotoxicity is crucial when considering the biocompatibility of materials intended for biomedical applications. Ti45Nb alloy, a combination of titanium (54,69% wt) and niobium (45,11%) wt, has gained significant attention due to its potential use in medical implants. However, understanding its impact on cellular health is essential before clinical applications. In this study, we aimed to assess the cytotoxicity of Ti45Nb alloy on cells using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method.

Samples of the Ti45Nb alloy were cut at a thickness of 3 mm from a bar with a 15x15 mm square cross section. The cut samples were wet ground using emery sheets made of 80, 220, 400, 600, 800, 1200, and 2000 SiC, respectively. Ti45Nb alloy specimens were sterilized by autoclaving at 121°C for 30 minutes to ensure aseptic conditions. Cells were seeded into 12-well culture plates containing Ti45Nb alloy specimens at a density of $1x10^4$ cells per well. The cells were then incubated with the alloy specimens for 24, 48, and 72 hours. Cytotoxicity were evaluated using the MTT assay. After incubation, the culture medium was removed from each well, and MTT solution (5 mg/mL) was added. Following 3-hour incubation, the medium was replaced with dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. The resulting solution was transferred to a 96-well cell plate, and the absorbance at 570 nm was measured using an ELISA microplate reader (Multiskan GO-Thermo).

The MTT assay results indicated that cell viability was reduced at all hours incubation period when exposed to Ti45Nb alloy specimens.

While alloys containing niobium (Nb) are recommended for biomedical applications compared to those containing vanadium (V), the use of alloys with a high content of niobium, such as Ti45Nb, can lead to cytotoxicity in cells after long-term incubation. Therefore, it is recommended to research alloys with lower niobium content.

Keywords: Ti45Nb; cell cytotoxicity; MTT

2D CARBON NANOMATERIALS: POLLUTION'S NEW ADVERSARY

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Abstract

Today, the rising demand for clean water, driven by industrial growth, extended droughts, and rapid population expansion, has created a shortage of clean water resources. Swift and crucial water treatment measures are now imperative. The challenge of limited water resources has prompted the exploration of practical methods to increase usable water. Notably, industrial and agricultural wastewaters present promising sources. With various water treatment methods available, each method has its own limitations in terms of applicability, cost, and efficiency. Among these, utilizing an adsorption process to treat wastewater from industrial or other sources stands out as an appealing option. This process enhances water quality, making it suitable for further use in industries and agriculture.

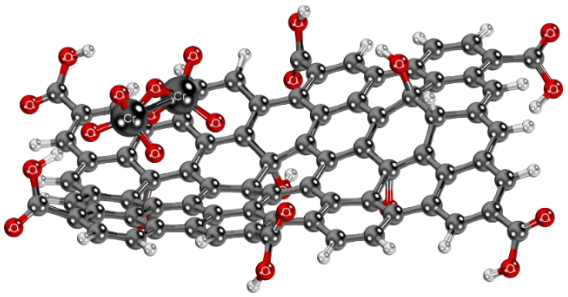


Figure 1. Adsorption of hexavalent chromium ions Cr(VI) using graphene oxide (GO)

Considerable research has been dedicated to identifying effective adsorbents for eliminating hazardous compounds like dyes, heavy metals, pesticides, phenols, and pharmaceuticals from water, ensuring water quality and averting long-term health consequences. In this context, carbon-based 2D nanomaterials materials have emerged as a remarkable choice for removing both conventional inorganic and organic pollutants. Graphene and Graphene Oxide have gained considerable attention in wastewater purification due to their adsorption, oxidation, and catalytic properties. Their properties, such as substantial pore volume, high conductivity, diverse surface chemistry, and a remarkably large aspect ratio, position them as ideal candidates for adsorbing the organic pollutants in wastewater. These materials exhibit a sheet-like structure with resonating polyaromatic π-systems, enabling significant interactions like π–π bonding, hydrogen bonding, and electrostatic interactions with various organic pollutants. These pollutants encompass dyes, pharmaceutical residues, and agricultural/industrial effluents containing reactive unsaturated aromatic rings and oxygen-rich functional groups.

Keywords: Graphene, Graphene Oxide, Pollutants, Adsorption.

NANOEMULSION VS. LIPOSOME ENCAPSULATED ESSENTIAL OIL FROM WILD-GROWN ALBANIAN THYMUS CAPITATUS: A COMPARATIVE STUDY ON ANTICANCER ACTIVITY IN MCF7 HUMAN BREAST CANCER CELL LINE

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Abstract

Cancer treatment remains a central concern in medical research, and the exploration of new therapeutic avenues is essential. In this study, we present a comparative analysis of nanoemulsions and liposome-encapsulated essential oil derived from Albanian wild-grown Thymus capitatus for the treatment of the MCF7 human breast cancer cell line. The essential oil of Thymus capitatus has been identified as a potent source of phytochemicals exhibiting anticancer properties. The aim of this research was to optimize the nanodelivery methods through nanoemulsion- and liposome-encapsulation, and to evaluate their comparative efficacy on the MCF7 cell line.

Both nanoemulsion- and liposome-encapsulated forms were carefully prepared, and their physicochemical properties were characterized using techniques such as Dynamic Light Scattering (DLS). The particle size, zeta potential, encapsulation efficiency, morphology, surface tension and rheological properties were analyzed to determine the optimal formulation.

The anticancer activity was assessed through the in vitro cytotoxicity MTT assay, underlying the action on MCF7 cells. The study demonstrated that both delivery systems effectively encapsulated the essential oil and displayed promising anticancer activities.

The findings of this study underscore the potential of Thymus capitatus essential oil as an novel therapeutic approach, providing insights into the optimization of its delivery using nanoemulsions and liposomes. While both nanodelivery systems exhibited therapeutic benefits, certain advantages and limitations of each method were identified. These findings contribute to the growing body of knowledge in cancer therapy and may guide future research in the development of targeted and efficient delivery systems for anticancer compounds.

Keywords: Essential oil, Nanoemulsion, Liposome, *Thymus capitatus*, MTT assay, MCF7, cancer, cell line.

EVALUATION OF THE ANTICANCER EFFECT OF *Spinacia oleracea* LEAF EXTRACT ON HT-29

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Abstract

The majority of deaths from colorectal cancer (CRC) are associated with the metastatic process. Many studies show that a diet rich in fruits and vegetables protects against many common chronic diseases, including cancer. Especially the nutrients and non-essential chemical compounds contained in green leafy vegetables are thought to promote health. Spinach *(Spinacia oleracea* L.), which is used in basic nutrition and contains phytochemicals, vitamins and minerals that support health, shows anti-cancer, anti-obesity, hypoglycemic and hypolipidemic properties.

In this study, the anti-cancer effect of *Spinacia oleracea* leaf extract on HT-29 colorectal cancer cell line was evaluated. For this purpose, cell proliferation (MTT), migration (wound healing assay) and apoptosis rate (Bax-Bcl-2 gene expression by qRT-PCR) were investigated on HT-29 cell lines exposed to this extract.

According to the MTT assay, *Spinacia oleracea* leaf extract was observed to greatly suppress the growth of HT-29 cell lines and significantly alter the Bax/Bcl-2 expression level. On the other hand, wound healing results showed that the metastasis ability decreased. The result of this study shows that *Spinacia oleracea* can be considered as a future chemotherapeutic agent and a suitable candidate for *in vivo* trial.

Keywords: Apoptosis, Migration, Colorectal cancer, *Spinacia oleracea* leaf extract

POSTER PRESENTATIONS

EFFECT OF THE COMBINATION OF CISPLATIN AND E-VINIFERIN ON OXIDATIVE STRESS IN HELA CELLS

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Abstracts

Cervical cancer is the fourth most common type of cancer among women worldwide, and is a global health problem. Cisplatin (CDDP) is the first platinum-group drug approved as an anticancer agent. It is effective for the treating various solid tumors, including testicular, ovarian, head and neck, bladder, lung, cervical, melanoma, and lymphoma. However, similar to other anticancer drugs, it damages normal cells. Combination treatments have attracted attention for reducing this effect. This study investigated the effects of CDDP, ε-Viniferin, and their combination on Hela cells. ε-Viniferin is a phenolic compound belonging to the stilbenes group. It has many anti-inflammatory, antioxidant, antineoplastic, anti-obesity, and cardioprotective effects. The MTT assay was used to determine the cytotoxic effects of CDDP and ε-Viniferin in the Hela cells. Different percentages of combination were created from the obtained IC_{50} doses and the combination index was calculated. In the continuation of the study, 10% (2.1 µM ε-Viniferin+0.75 µM CDDP) and 20% (4.2 µM ε-Viniferin+5.6 µM CDDP) that were synergistic and 40% (8.4 µM ε-Viniferin+11.2 µM CDDP) that were found to be antagonistic were selected. Oxidative Stress Index (OSI) was determined as a result of the measurement made using Total Oxidative Status (TOS) and Total Antioxidant Status (TAS) methods. 20% of the synergistic groups for TAS and 10% for TOS showed a significant difference compared to the control group. The OSI was calculated to be greater than 1 for all doses administered. Although it partially reduced CDDP-induced oxidative stress in the ε-viniferin combination groups, it did not reduce cell viability to the expected level.

Keywords: Hela cell line; Oxidative stress; Cisplatin; ε-Viniferin

Acknowledgement: This work was funded by a grant from the Anadolu University (Project No:2107S122)

EFFECT OF DIFFERENT CONCENTRATIONS OF Ni, Cr AND Pb ON SOME BIOCHEMICAL PARAMETERS AND ANTIOXIDANT RESPONSE OF MAIZE (*Zea mays* L.)

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Abstract

Heavy metals are normal components of soil and some of them are essential, but an increase in their concentration in soil directly attacks plants and causes phytotoxicity. The objective of this study is to evaluate the toxic effects of different concentrations of Ni, Cr and Pb by measuring some biochemical biomarkers and some indicators of the degree of oxidative stress in maize plants. Maize plants were grown in vegetation rooms and the substrate in which they were planted was separately poisoned with different concentrations of the metals Ni and Cr (50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg) and Pb (20 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg). All the parameters listed below were evaluated in the leaves of maize plants using appropriate methods. In general, with the increase in the concentrations of Ni, Cr and Pb, the decrease in the activity of δ-aminolevulinic acid dehydratase and total chlorophyll content, the increase in aminolevulinic acid content, the increase in glutathione and malondyaldehyde level were studied. Changes were also observed in total antioxidant capacity depending on the type of metal and exposure dose. Nickel, chromium, and lead have a toxic effect on the maize plant proportional to the increase in their concentration, decreasing the activity of the enzyme δ-aminolevulinic acid dehydratase and, consequently, the content of total chlorophylls. Under the stress of these metals, free radicals are formed in the plants, causing lipid peroxidation (formation of malondyaldehyde), and the enzymatic and non-enzymatic antioxidant protection mechanisms are activated.

Keywords: Ni, Cr, Pb, maize, biochemical parameters, antioxidant response

ONCOGENIC ROLE OF SEPTIN-9 GENE IN BLADDER CANCER CELLS

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Abstract

Although bladder cancer is usually treated surgically, due to its high prevalence, multiple recurrences, and invasive and metastatic character, health care costs associated with the treatment and active monitoring of bladder cancer exceed that of all other malignancies. Therefore, the development of effective treatment strategies for bladder cancer is important not only for patients but also for public health. The SEPTIN-9 (SEPT9) gene, the fourth component of the cytoskeleton, contains 17-exon and multiple transcript variants. These splice variants are the basis of multiple functions of septin proteins. One common feature of these variants is that different splice variants of the gene encode the same protein. Genetic and epigenetic changes in the SEPT9 gene are linked to proliferation, angiogenesis, cell invasion, apoptosis, autophagy, resistance to anti-cancer drugs, and the induction of tumor growth by the cancer-associated microenvironment. In recent years, the use of blood-based SEPT9 methylation screening and diagnostic kit for colon cancer, because of its noninvasive, high specificity, and cost-effective, has led to the focusing of studies on this gene. Although SEPT9 has been identified as a potential oncogene, it has also been shown to play a tumor suppressor role in several studies.

In the light of this information, in our project, the changes in the expression level of Vimentin, N-cadherin and E-cadherin genes in the EMT pathway after the knockdown of SEPT9 in T24 cells were investigated by qRT-PCR and western blot analysis at the transcriptional and translational levels, respectively. After suppression of SEPT9 gene, expression of vimentin and N-cadherin decreased and expression Ecadherin increased ($p<0.05$). It was also supported by migration assay in which EMT pathway was suppressed. However, the expression of the SEPT9 variants v1, v2 and v3 by qRT-PCR was compared with a healthy bladder tissue sample and it was determined that v1 and v3 were downregulated ($p<0.05$). When T24 cells were treated with 10 µM DNMT inhibitor DAC for 48 hours, the expression level increased in v1 and v2 variants.

This result shows that the expression level of these variants is mediated by methylation, further supporting our results by promoter hypermethylation of SEPT9 v1. As a result, it shows that SEPT9 may play a tumor suppressor role in bladder tumorigenesis. Therefore, our findings should be supported by bladder cancer tissues.

Keywords: SEPT9 Gene, Bladder Cancer, EMT Pathway

CYTOTOXIC EFFECTS OF VITEXIN IN A549 HUMAN NON-SMALL CELL LUNG CANCER CELLS

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Abstract

Lung cancer ranks highest as the primary cause of cancer-related deaths globally. There are two primary categories of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC makes up about 85% of all lung cancer cases. Currently, the standard treatment options for NSCLC in medical practice are chemotherapy and surgery. However, these approaches have limited efficacy for patients diagnosed with advanced NSCLC. It is crucial to recognize the significance of discovering new therapeutic agents to effectively treat this deadly form of cancer. Recently, researchers have been increasingly interested in natural products and especially compounds derived from plants, which have a high potential to be anti-tumor agents. Vitexin (apigenin-8-C-glucoside), a flavonoid, is one of them. Vitexin has recently been known to have anti-cancer, antioxidant, anti-inflammatory, anti-nociceptive, anti-hypertensive, anti-depressant-like effects and anti-viral activities.

In this study, the anticancer effects of vitexin were investigated on the A549 human lung adenocarcinoma cell line. Cisplatin was evaluated as a positive control. Cytotoxicity and IC50 values of vitexin in A549 cells were determined by MTT assay. Various concentrations of vitexin (15.62-2000 µM) effectively reduced cell viability in A549 cell line in a dose-dependent manner. IC50 values of vitexin and cisplatin were determined as 186,67 µM and 29,11 µM in A549 cells for 24h respectively. In addition, early phase apoptosis and caspase 3 activity also increased in a time-dependent manner. This results show that vitexin had selective anticancer activity in A549 cell line.

Keywords: Vitexin, Apoptosis, A549

THE EFFECT OF NATURAL COMPONENTS ON COFILIN AND ALPHA SYNUCLEIN PROTEINS IN THE PARKINSON'S DISEASE MODEL

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Abstract

Parkinson's disease (PD) is the second most common neurologic movement disorder after Alzheimer's in the world. Alpha-synuclein proteins or Lewy bodies block the connections of nerve cells and the exchange of neurotransmitters in the area where they accumulate. The inability to transmit information causes disturbances in functions such as movement and attention control. It has been revealed that cofilin levels are impaired in Parkinson's disease. Polyphenols such as curcumin and tannic acid are responsible for the color of many plants, fruits and vegetables and are known to have various biochemical properties, including anti-carcinogenic, antioxidant and anti-inflammatory effects, and can be potent proteasome inhibitors.

In this study, SH-SY5Y cells were differentiated into dopaminergic cells with Retinoic Acid (RA) and a Parkinson's Disease model was created with rotenone (ROT). Tannic acid (TA) and curcumin (CUR) were applied to determine their antioxidant effects on ROS production, which is one of the causes of Parkinson's disease. Then, alpha synuclein and cofilin protein levels were determined by ELISA method.

TA and CUR application showed a positive effect on cell viability. But their effects on Parkinson's-related proteins are still complex. Especially, studying the effect of CUR in terms of alpha synuclein protein may lead to the emergence of new therapeutic strategies.

Keywords: SH-SY5Y, Parkinson's, tannic acid, curcumin, cofilin, alpha synuclein

ASSESSMENT OF WELL WATER QUALITY IN SUHAREKA "XHAVIT SYLA" NEIGHBORHOOD: A COMPARATIVE ANALYSIS WİTH INTERNATIONAL STANDARDS

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Abstract

The purpose of this research is to assess the quality of well water in order to assure community safety in the "Xhavit Syla" area of Suhareka, Kosovo. It investigates physical-chemical and biological aspects and compares the results to worldwide standards. Temperature, conductivity, pH, iron, lead, and bacterial presence are all important goals. Contamination risks associated with urban and agricultural activities are also investigated. While temperature and conductivity levels are within permissible norms (25°C and 2500 S/cm, respectively), pH, iron, and lead concentrations exceed Kosovo and EPA regulations (pH: 6.5-9.5, iron: 0.02 mg/L, lead: 0.015 g/L). The presence of microbiological signs such as coliform bacteria, E. coli, and Pseudomonas indicates that well water supplies have been contaminated. Notably, 85% of the samples tested positive for E. coli, while 70% tested positive for Pseudomonas. To address contamination problems, immediate action is required, as water contaminated by chemical elements and microbiological indications is unfit for human consumption. Effective monitoring and comprehensive remedial procedures are required to address these issues.

This study underlines the importance of protecting the "Xhavit Syla" community's water resources, as well as the crucial function of severe water quality management measures in sustaining public health. The findings highlight the importance of strong remediation measures and continuous monitoring to return the well water to a safe and useable state, protecting the community's well-being and stressing the larger importance of water quality control for public health.

Keywords: Water quality, well water, contamination, physical-chemical parameters, biological indicators, monitoring.

GENUS *AUBRIETA (BRASSICACEAE)* IN THE FLORA OF KOSOVO

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Abstract

The genus *Aubrieta* includes perennial herbs native to SW Asia as well as southern and southeastern Europe, from Romania in the east to Italy in the west. In continental Europe, according to the EuroMed checklist (Euromed+ 2016:), there are a total of 19 recognized species with another 10 well-defined subspecies of the genus. In the literature there are different and rather confusing data on the taxonomy of the genus, indicating that *Aubrieta* in Europe has a total of only 12 species (Al-Shehbaz & al. 2006) or 20 species (Gustavsson 1986; Phitos 2002).

In Kosovo there are two taxa of *Aubrieta*, although the Flora Europaea database (Euromed+ 2016:) does not record any species of the genus for Kosovo. These two taxa in Kosovo are: *Aubrieta columnae* subsp. *croatica* (Schott & al.) Mattf. and *Aubrieta gracilis* subsp. *scardica* (Wettst.) Phitos. Both of these rare and fragile taxa in Kosovo, thrive on calcareous rocks and rocky surfaces and crevices in the alpine region of the Sharri Mountains. In addition, *A. columnae* subsp. *croatica* is classified as a critically endangered plant taxa at the national level (Millaku ed. 2013) because there is only one known and confirmed locality of it in Mt. Luboten, having a very limited area of distribution and the number of individuals recorded within this population is very small. *A. gracilis* subsp. *scardica*, on the other hand, is also known only from the Sharri Mountains, and its populations in the Maja e Zezë Mt, deserve further analysis, conservation action, and monitoring.

Understanding the distribution, ecology, and taxonomy of these endemic plant taxa of the Balkans is of great importance, especially for conservation efforts, ecosystem understanding, and subsequent informed management decisions.

Keywords: Floristics, taxonomy, Brassicaceae, distribution, Kosovo, plant ecology

HEMATOLOGY PARAMETERS OF MICE *(Mus musculus)* AFTER CYCLOPENTANONE DERIVATES TREATMENT

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Abstract

This study was designed to investigate differences in hematological parameters between mouses after cyclopentanones intoxitacion ((2E,5E)-2,5-Bis(4-methylbenzylidene)cyclopentanone; (2E,5E)-2,5-Bis (4-methoxybenzylidene) cyclopentanone; (2E,5E)-2,5-Bis[(4-dimethylamino) benzylidene cyclopentanone) and to investigate the coagulation time.

Objective: To investigate the effects of three different anticoagulation agents on heamogram and bleeding time of mice in a randomized controlled experiment.

Four groups of mice (n=5 per group) were treated with either a control solution, M2, M3, or M4 anticoagulation agents for 24 hours. The levels of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), basophils (BASO), and immature granulocytes (IG) were measured in the blood of the mice at the end of the experiment. The weight of lost blood and the time of bleeding were also measured after a standardized incision.

M2 significantly reduced the levels of RBC, HGB, HCT, PLT, and NEUT, but increased the levels of MCV, MCH, MONO, and IG, compared to the control group ($p<0.05$). M3 significantly increased the levels of WBC, RBC, HGB, HCT, PLT, NEUT, and BASO, but decreased the levels of MCV and MCHC, compared to the control group ($p<0.05$). M4 did not affect the levels of any heamogram parameters, compared to the control group ($p>0.05$). M2 and M3 significantly increased the weight of lost blood and the time of bleeding, while M4 did not affect them, compared to the control group ($p<0.05$).

M2 and M3 have different effects on heamogram and bleeding time of mice, and may have implications for blood clotting and hemostasis. M4 does not affect heamogram or bleeding time of mice, and may be a safer anticoagulation agent. Further studies are needed to elucidate the mechanisms and clinical relevance of these findings.

Keywords: Hematology Parameters, *Mus musculus*, Cyclopentanone, Intoxication, Blood Sample, Coagulation.

THE EFFECT OF AIR POLLUTION IN PHYSIOLOGICAL PARAMETERS OF LUNGS AND EYES

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Abstract

Environmental pollution is one of the main problems that is being confronted during this century, causing long term negative effects in public health. Factors that play important role in environmental pollution are the ones with natural origin, not natural and human origin. These pollutants are: solid fuels, chemicals, gases: SO_2, CO_2, NO, noise, radiation, waste etc. Urban pollution is more and more considered a serious problem for people living in urban areas.

Measurements of physiological lung parameters were made by Spirometry and for symptoms of eye surface problems was used OSDI test. Participants were informed before measurements and a consent form was signed by them. Age of the participants was 20-35 years old, and both genders were represented (50 females and 50 males). The region of Obiliq, Pristina and Fushe Kosovo were as a polluted area, and the village of Bllaca as a control group.

This research investigated the relationship between air pollution and health of lungs and eyes. The results suggest that the effect of air pollution in physiological parameters of lungs on the younger generation 20 to 35 are not detectable, but effects are more prone to be registered in the eye surface physiology. Our research results has shown that there were no significant changes in physiological lung parameters, but there was an correlation between air polluted areas and declared surface eye symptoms.

Results suggest that effects of air pollution can be detected faster by using surface eye symptoms compared to physiological lung parameters.

Key words: Environmental pollution, public health, Spirometer, OSDI test.

NOTES ON THE DIVERSITY OF ASCOMYCOTA FROM KOSOVO

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Abstract

This study explores the diversity and distribution of macrofungal species within the Ascomycota phylum in Kosovo. Utilizing extensive research and analysis, a total of 82 species were identified, with 39 species previously documented in scientific literature, and 43 species newly discovered, enriching the mycobiota of Kosovo. The taxonomic composition of the Ascomycota division was investigated, revealing 82 species distributed across 56 taxa or genera, further categorized into 34 families, 10 orders, six subclasses, five classes, and one subphylum. The Helotiales and Pezizales orders exhibited high diversity, with 12 and nine families, respectively, while other orders such as Xylariales and Hypocreales showed considerable variation. Additionally, several orders were represented by a single family each.

Keywords: Ascomycota, fungal diversity, Kosovo, macromycetes, taxonomy.

A COMPARISON BETWEEN THE EFFECTS OF HONEY, SNAIL MUCUS AND "JOMELOP" OINTMENT IN BURN WOUNDS

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Abstract

Throughout history honey and snail mucus have been used for wound healing and skin regeneration. In continuous studies they have shown good antimicrobial effects, including antibacterial, antiviral, and antifungal effects.

During this research, based on these effects, and others, we will test and compare the effects of honey, snail mucus and an ointment in superficial burn wounds on Ratus norvegicus Wistar individuals.

During this research we used 11 rats (Ratus norvegicus-Wistar) individuals, that we divided in four treatment groups: 3 honey, 3 snail mucus, 3 Jomelop ointment and 2 as a control. Animals were fed with a normal rat diet. Up to a week after the burn was caused, they were given pain medication (Paracetamol). The snail mucus is taken from Helix locorum and the honey was from European bee. Jomelop is a commercial plant-based ointment and with honey extract. Two burn wounds (1cm2) were created after fur removal from back pelvis region.

Our results show that groups treated with honey and Jomelop had significantly shorter healing time (25-26 days), compared with groups treated with snail mucus and control group. Skin regeneration and treatment with honey products has shown a promising result for creating a mixture that shorten the healing time in skin burn wounds.

Keywords: burn wounds, honey, mucus, healing

Acknowledgement: During this research we got support from our mentor, the laboratory worker and other colleagues.

EFFECTS OF CARBOXILATED GRAPHENE OXIDE IN RED BLOOD CELL OF COW AND PIG

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Abstract

Graphene oxide (GO) is a nanomaterial that has potential applications in biomedicine and biotechnology. However, its effects on red blood cells (RBC) of different animal species are not well understood.

Objective: To investigate the effects of GO on RBC of heparinized blood from cow and pig in different concentrations and exposure times.

Heparinized blood samples from cow and pig were treated with either a control solution or GO solutions with concentrations of 1 ppm, 0.5 ppm, or 0.25 ppm. The blood samples were then incubated in a water bath at 37°C with a rotation speed of 1 Hz for 4 or 24 hours. The RBC counts were measured in the blood samples after the incubation.

GO significantly reduced the RBC counts of both cow and pig blood in a concentration- and time-dependent manner, compared to the control group ($p<0.05$). The reduction was more pronounced in cow blood than in pig blood, and at 24 hours than at 4 hours. The lowest RBC counts were observed in the cow blood treated with 1 ppm GO for 24 hours (4.8 x 106/mm3), while the highest RBC counts were observed in the control groups of both cow and pig blood (7.6 x 106/mm3 and 9.2 x 106/mm3, respectively). The RBC counts of the blood samples treated with 0.25 ppm GO were not significantly different from the control group, except for the cow blood at 24 hours ($p<0.05$).

GO has detrimental effects on RBC of heparinized blood from cow and pig, and may have implications for hemolysis and anemia. The effects vary depending on the animal species, the concentration of GO, and the exposure time. Further studies are needed to elucidate the mechanisms and clinical relevance of these findings.

Keywords: Graphene oxide, nanoparticle, blood, cow, pig.

THE EFFECTS OF CERATINE AND LINOLEIC ACID ON LIVER ENZYMES OF MICE

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Abstract

Ceratine and linoleic acid are dietary supplements that may have beneficial effects on liver health and metabolism. However, their effects on liver enzymes of mice are not well understood. To investigate the effects of ceratine and linoleic acid on liver enzymes of mice in a randomized controlled experiment. Three groups of mice (n=10 per group) were fed with either a control diet, a diet supplemented with 0.5% ceratine, or a diet supplemented with 0.5% linoleic acid for four weeks. The levels of alanine aminotransferase (ALT), alkaline phosphatase (AP),gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and glucose (Glu) were measured in the serum of the mice at the end of the experiment. Ceratine significantly reduced the levels of AP, GGT, and AST, but increased the levels of LDH and Glu, compared to the control group (p<0.05). Linoleic acid significantly reduced the levels of AP and GGT, but did not affect the levels of ALT, LDH,AST, or Glu, compared to the control group (p<0.05). Ceratine and linoleic acid have different effects on liver enzymes of mice,and may have implications for liver health and metabolism. Further studies are needed to elucidate the mechanisms and clinical relevance of these findings.

Keywords: Ceratine, linoleic acid, liver enzymes

THE EFFECTS OF L-GLUTAMINE AND L-CARNITINE ON LIVER ENZYMES OF MICE

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Abstract

L-glutamine and L-carnitine are amino acids that may have beneficial effects on liver health and metabolism. However, their effects on liver enzymes of mice are not well understood. The objective was to investigate the effects of L-glutamine and L-carnitine on liver enzymes of mice in a randomized controlled experiment. Three groups of mice (n=10 per group) were fed with either a control diet, a diet supplemented with 0.5% L-glutamine, or a diet supplemented with 0.5% L-carnitine for four weeks. The levels of alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and glucose (Glu) were measured in the serum of the mice at the end of the experiment. L-glutamine significantly increased the levels of AP and AST, but decreased the levels of GGT and LDH, compared to the control group ($p<0.05$). L-carnitine significantly reduced the levels of GGT, but did not affect the levels of ALT, AP, LDH, AST, or Glu, compared to the control group ($p<0.05$). In conclusion L-glutamine and L-carnitine have different effects on liver enzymes of mice, and may have implications for liver health and metabolism. Further studies are needed to elucidate the mechanisms and clinical relevance of these findings.

Keywords: L-glutamine, L-carnitine, liver enzymes

COMPARATIVE ANALYSIS OF OLIVE OIL, FISH OIL, AND VASELINE EFFECTS ON WOUND HEALING TIME IN RATS

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Abstract

Wound healing is a complex and dynamic process that involves intricate cellular and molecular interactions. Natural compounds such as olive oil and fish oil have been recognized for their potential to accelerate wound healing due to their anti-inflammatory and antioxidant properties. This study aimed to investigate the effects of olive oil, fish oil, and vaseline on wound healing time in rats.

A total of 20 adult male rats were used for this experiment, divided into four groups: Control, Olive Oil, Fish Oil, and Vaseline. A full-thickness excisional wound was created on the dorsal skin of each rat, and the respective topical treatments were applied daily. Wound healing progression was monitored through regular assessments of wound size.

The findings revealed that the application of both olive oil and fish oil led to a significant reduction in wound size compared to the Control and Vaseline groups.

In conclusion, this study suggests that both olive oil and fish oil exhibit promising wound healing capabilities in rats. The observed effects on wound size reductionemphasize their potential to expedite the wound healing process. Further investigations are warranted to elucidate the underlying mechanisms responsible for these effects and to translate these findings into clinical applications for human wound care.

Keywords: wound, olive oil, fish oil, vaseline.

Acknowledgement: During this research we were helped by the mentor, laboratory assistant, and many colleagues.

EFFECTS OF GRAPHENE OXIDE EXPOSURE ON WHITE BLOOD CELL COUNT IN COW AND PIG BLOOD

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Abstract

Graphene oxide (GO), a nanomaterial with diverse applications, has gained attention due to its potential biomedical uses. This study investigates the impact of GO exposure on white blood cell (WBC) count in cow and pig blood, aiming to elucidate potential toxicity at varying concentrations and exposure times.

Heparinized blood samples from cows and pigs were treated with different concentrations of GO (1 ppm, 0.5 ppm, and 0.25 ppm) and subjected to a controlled environment at 37°C with a rotation speed of 1 Hz. WBC measurements were taken after 4 and 24 hours of exposure. Control samples, devoid of GO treatment, were also analyzed for comparison.

Results reveal a concentration- and time-dependent trend in WBC count alterations following GO exposure. In cow blood, after 4 hours, WBC counts decreased to 3.6, 4.4, and 6.0 at 1 ppm, 0.5 ppm, and 0.25 ppm concentrations, respectively, compared to the control count of 6.8. After 24 hours, similar patterns were observed with counts of 3.4, 3.6, and 5.2, respectively.

Similarly, pig blood showed reductions in WBC count. At 4 hours, counts were 6.8, 8.0, and 9.0 at 1 ppm, 0.5 ppm, and 0.25 ppm concentrations, respectively, compared to the control count of 9.8. After 24 hours, the counts were 6.0, 6.0, and 7.7, respectively.

These findings suggest that GO exposure may lead to decreased WBC counts in both cow and pig blood, indicating a potential impact on immune response. However, further studies are warranted to elucidate the mechanisms underlying these observations and to assess the broader implications of GO exposure on immune function and overall health.

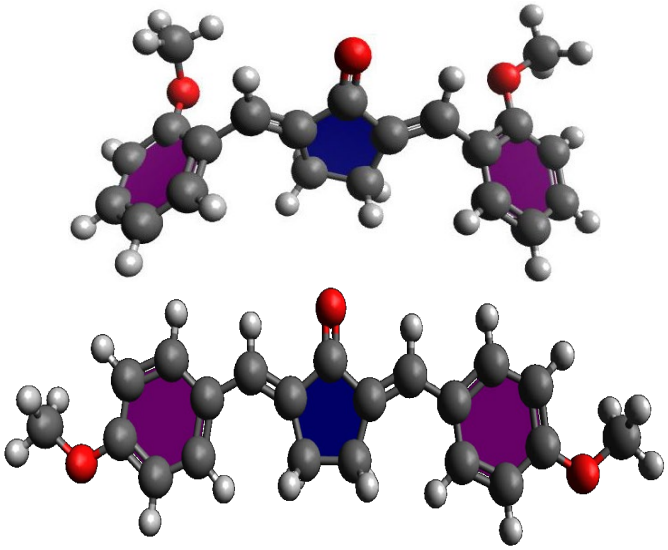
Keywords: graphene oxide, white blood cells, nanomaterial toxicity, immune response, cow blood, pig blood.

SYNTHESIS AND ANTICOAGULANT ACTIVITY OF (2E, 5E) -2,5-BIS METHOXYBENZYLIDENE CYCLOPENTANONE DERIVATIVES DETERMINED BY THE RAT TAIL BLEEDING METHOD

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Abstract

Synthesis of molecules: 2,5-diarylidenecyclopentanone derivatives of: (2E, 5E) -2,5-Bis (4-methoxybenzylidene) cyclopentanone and (2E, 5E) -2,5-Bis (2-methoxybenzylidene) cyclopentanone is the focus of this study. The characterization of the compounds was done by NMR, FTIR and UV-VIS spectroscopy. Due to low solubility in polar solvents, the compounds were dissolved in corn oil at concentrations of 1, 4 and 8 mg/ml. Six Mus musculus-Swiss albino mice (5-7 weeks, 24-36 g) were randomly and equally divided into two groups and weighed on analytical scales. By distributing the compounds in the stomach of the mouse by means of a gastric tube, they were monitored for 24h while being fed regularly. In this study we aimed to investigate the anticoagulant effects by measuring the bleeding time in a rat tail hemorrhage model. After 24 hours, the weight of the mice was measured, then by immersing the tail in a 37^0C water bath and then cutting a 10 mm part of the tail, the bleeding time was measured and the time was checked with a stopwatch. The compound with the methoxy group in the orto position had a longer time than the compound in the para position.

Keywords: Synthesis, benzylidenecyclopentanone, characterization, rat, bleeding time, hemorrhage.

INDUSTRIAL POLLUTION WITH HEAVY METALS AND BIOACCUMULATION BY IN EARTHWORM LUMBRICUS TERRESTRIS IN DRENAS-KOSOVO

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Abstract

The pollution of the environment with heavy metals and their bioaccumulation by living organisms with their harmful effects on various biochemical and physiological parameters.

In this paper, the concentration of heavy metals Pb, Zn and Ni in the soil of the Drenas locality has been investigated and the comparison has been made with the control region in the village of Brezne in the municipality of Dragash, the bioaccumulation of these heavy metals Pb, Zn and Ni has also been investigated in the earthworm, in the organic parts of the earthworm in the apical part and clitellum.

The samples were taken in Drenas in three concentric circles (1 km, 2 km and 5 km), a total of 15 individuals. They are treated according to the relevant methods. The samples were homogenized and then centrifuged at 3500 revolutions for 15 minutes. The tail samples of L. terrestris for the measurement of metals PB, Zn, Ni, were treated with 1 ml HNO3 ultra pure 69% (Merek Millipore Product) and H2O2 30% (Lachner product). The samples were placed in microwave teflon columns and 1 ml of HNO3 and 30% H2O2 were added and digested at a temperature of 200 o C for 45 minutes and then reading was done on the Analytic yena Contra AAA flame atomic absorber.

The results from this research show the implication of heavy metals from industrial pollution, and bioaccumulated from the soil to the earthworm (Lumbricus terrestris L).

From the obtained results of this research, it is established that the industrial pollution in the city of Drenas has influenced the environmental contamination with heavy metals.

Keywords: Bioaccumulation, earthworm, heavy metals, pollution

EFFECTS OF MICROPLASTIC POLLUTION IN HEALTH OF AQUATIC ORGANISMS

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Abstract

Microplastics, through their pollution, have diverse effects on both organism and ecosystem health. To date, numerous scientific studies have demonstrated that microplastics have negative impacts not only on the behavior of aquatic animals but also on their proper development, causing changes in fertility, inflammation, immune toxicity, oxidative stress, neurotoxicity, and even affecting metabolic pathways and gene expression. The ability of microplastics to interact with various xenobiotics, such as heavy metals and organic pollutants dissolved in water, has also been confirmed. Microplastics are highly resistant materials in aquatic environments, including oceans, and they should be considered extremely toxic to aquatic ecosystems. They pose significant challenges from ecological, economic, and toxicological perspectives.

Keywords: pollution, combined tixicity, mircoplastic, combined toxicity

CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF A DUAL-ACTING MOLECULE INHIBITING EGFR AND PI3 KINASE ON U87-MG GLIOMA CELLS

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Abstract

Glioblastoma multiforme (GBM) is one of the fatal tumors in adults and children. When the etiology of glioblastoma multiforme is examined, epidermal growth factor receptor (EGFR) gene amplification and overexpression are the most common genetic changes in primary glioblastoma. In this study, we aimed to investigate the cytotoxic and antiproliferative effects of MTX-211, a dual-acting molecule inhibiting EGFR and PI3 Kinase, in human U87-MG glioma cells. The cytotoxic effects of MTX-211 with the WST-1 method and its antiproliferative effects with the real-time cell analysis system (xCELLigence RTCA-DP) were studied.

Depending on the concentration of MTX211, an increase in the cytotoxic effect was determined in U87MG cells. Especially at both 24 and 48 hours, cell viability decreased significantly at 10 and 20 µM MTX concentrations.The antiproliferative effects of the MTX-211 concentrations (5, 10, 20 µM) on the cells were monitored simultaneously with a computer-connected system at certain time intervals, in real time, according to the concentration groups.

The cell proliferation curves of MTX-211 were evaluated compared to the control group and the cell proliferation curves were analyzed automatically by the device according to the cell index values during the 24 hour incubation period and the MTX IC50 values at the 24th hour were calculated as 9.2 µM. n conclusion, it was determined that MTX211 has cytotoxic and antiproliferative effects in malignant glioma U87-MG cell line.

Keywords: Glioma, MTX-211, anticancer

Acknowledgement: This study was supported by a numbered project (Project No: 2207S150) that was supervised by The Commission of Anadolu University Scientific Research Projects.

EFFECT OF URBAN POLLUTION IN PRISTINA AND FUSHË KOSOVË ON SOME BIOCHEMICAL AND ENZYMATIC PARAMETERS IN HEMOLYMPH, ALBUMIN GLANDS AND HEPATOPANCREAS OF SNAIL (*HELIX ASPERSA L*)

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Abstract

The aim of this study was the environmental pollution in urban areas has been treated, including the sources of this pollution at a global level, with special emphasis in Kosovo, namely in the cities of Pristina and Fushë Kosovë, as well as the impact of this pollution on living organisms, especially in the snail Helix aspersa L.

In this snail, the influence of urban pollution on several biochemical and enzymatic parameters in the hemolymph, albumin gland and hepatopancreas was investigated, in which case parameters such as: catalase activity in the albumin gland were analyzed; catalase activity in the hepatopancreas; glucose concentration in hemolymph; peroxidase activity in albümin glands; peroxidase activity in hepatopancreas as well as AST, ALP and GGT enzyme activity in hemolymph.

From the results obtained during this research, it is established that urban pollution in Pristina and Fushë Kosovë has affected the change of the parameters measured in the snail (Helix aspersa L), compared to the compared parameters of the snails of the control group of the village of Marinë Municipality of Skënderaj.

Urban pollution in these two cities has caused changes in the parameters of oxidative stress, respectively in the analyzed parameters of catalase and peroxidase compared to the control group. Also, urban pollution has caused changes in AST, ALP and GGT enzymes.

Keywords: Urban pollution, catalase, peroxidase, hemolymph, snail

FULL TEXTS PAPERS

PATCH CLAMP TECHNIQUE AND APPLICATION AREAS

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Abstract

We present this oral presentation to highlight the significance of the patch clamp technique in biological sciences and drug research. As the founders of Turkey's inaugural patch clamp laboratory within a pharmacy faculty, our aim is to underscore its pivotal role. This technique has profoundly transformed electrophysiology by enabling precise investigations into ion channels and cellular electrophysiology. The patch clamp method, introduced by Neher and Sakmann in the late 1970s, involves attaching a micropipette to a cell membrane, facilitating the measurement and manipulation of ion currents. It offers two primary configurations: whole-cell and cell-attached, each providing unique insights into ion channel behavior and cellular dynamics. Patch clamp applications span diverse disciplines such as neurophysiology, cardiology, pharmacology, and cell biology. Within neurophysiology, this technique elucidates neuronal excitability, synaptic transmission, and plasticity, offering a comprehensive view of the nervous system's dynamic complexities. In cardiology, patch clamp informs cardiac electrophysiology, supporting insights into arrhythmias while serving as a vital platform for the detailed testing of novel pharmaceutical interventions. Moreover, in the realm of pharmacology, its capacity to revolutionize drug discovery remains intriguing, providing appproaches which interactions between drugs and ion channels can be reviewed, thus paving the way for the development of safer and more efficacious therapies. This technique's multidisciplinary significance underlines its pivotal role in advancing our understanding of cellular physiology and its applications across the scientific spectrum.

Keywords: Patch clamp technique, Pharmacology, Neurophysiology, Cardiology, Cell biology

INTRODUCTION

The patch-clamp technique is a powerful and widely used electrophysiological method that allows researchers to study the electrical activity of individual cells, particularly excitable cells like neurons and muscle cells. Developed in the 1970s by Erwin Neher and Bert Sakmann, this technique has revolutionized our understanding of cellular physiology and ion channel behavior (Hamill et al., 1981).

In this study, we will shed light on the importance of the patch clamp technique in the field of biological sciences and its applications in drug research.

An overview of patch clamping

At its core, the patch-clamp technique involves creating a high-resistance seal between a glass micropipette and a small area of a cell membrane. This creates a patch of membrane that is isolated from the surrounding environment, allowing researchers to directly measure the flow of ions across the membrane and study ion channel activity (Hamill et al., 1981). The basic principles of patch-clamp recording involve creating a small, isolated patch of cell membrane, controlling the membrane potential, and measuring the resulting ion channel currents.

This technique has played a fundamental role in advancing our understanding of cellular physiology and has become an essential tool in drug research and various fields of neuroscience and biophysics.

The basic principles of patch-clamp recording can be summarized as follows (Hill and Stephens, 2021; Zheng and Trudeau, 2023):

Microelectrode and cell sealing: In patch-clamp recording, a glass microelectrode is filled with an electrically conductive solution. The tip of the microelectrode is brought into contact with the cell membrane, and a small amount of suction is applied to form a high-resistance seal between the microelectrode and the membrane. This creates a patch of membrane that is isolated from the surrounding environment.

Voltage or current clamp mode: After forming the seal, the patch-clamp setup can be operated in two primary modes: voltage clamp or current clamp. In voltage clamp mode, the membrane potential is controlled by the amplifier, allowing researchers to measure the ionic currents across the membrane. In current clamp mode, the membrane potential is left free to vary, and the amplifier records the currents required to maintain the cell at its natural membrane potential.

Ion channel currents: In voltage clamp mode, the patch-clamp technique allows researchers to study the flow of ions through ion channels. By controlling the membrane potential and measuring the resulting currents, researchers can determine the kinetics, conductance, and modulation of specific ion channels.

Single-channel recording: The patch-clamp technique is sensitive enough to measure the currents flowing through a single ion channel, known as single-channel recording. This level of precision provides valuable insights into the behavior of individual ion channels and their responses to various stimuli or drugs.

Configurations: Depending on the specific research objectives, different configurations of patch-clamp recording can be used:

Whole-cell patch-clamp: In this configuration, the researcher ruptures the membrane patch after establishing a seal, gaining complete access to the cell's cytoplasm. This allows for the study of various intracellular processes and whole-cell currents.

Cell-attached patch-clamp: The micropipette is sealed onto the cell membrane without breaking it. This configuration is particularly useful for studying ion channel activity in its native environment, without interference from the intracellular environment.

Inside-out and outside-out patch-clamp: These configurations involve pulling the pipette away from the cell membrane after a seal is formed, either exposing the cell's cytoplasm (inside-out) or isolating a small patch of membrane containing the ion channels (outside-out). These configurations are ideal for studying ion channel properties and their modulation by intracellular factors.

Data analysis: After recording ion channel currents, the data is analyzed to determine various properties, such as channel open and closed times, current amplitudes, and gating kinetics.

Role of patch-clamp in drug screening

Patch-clamp allows researchers to profile the activity of ion channels in response to various drug candidates. By studying the effects of compounds on specific ion channels, researchers can identify potential drug targets and assess their therapeutic relevance. Understanding how drug compounds interact with ion channels at the molecular level is essential for deciphering their mechanisms of action (Mathie et al., 2021).

With advancements in automated patch-clamp technology, high-throughput screening of drug candidates has become feasible. This enables researchers to test a large number of compounds against multiple ion channels rapidly, facilitating the identification of promising candidates for further investigation (Liu et al., 2019; Obergrussberger et al., 2021).

Assessing the potential side effects of drugs on cardiac ion channels is crucial for safety pharmacology (Alyu et al., 2021; Cao et al, 2010). Patch-clamp recordings help in evaluating drug-induced changes in cardiac ion channel currents, aiding in predicting and mitigating potential risks of drug-induced arrhythmias and cardiac toxicity.

Patch-clamp's precision in characterizing individual ion channels opens doors for personalized medicine approaches (Amuzescu et al., 2014). By examining how a patient's ion channels respond to specific drugs, clinicians can tailor treatments for enhanced efficacy and reduced side effects.

Investigating drug effects on ion channels

In the course of a patch-clamp experiment, various key steps are undertaken to investigate the effects of drug compounds on ion channels (Hill and Stephens, 2021; Molleman, 2003; Zheng and Trudeau, 2023). The initial phase involves the establishment of the patch-clamp configuration. Here, a patch-clamp electrode is meticulously brought into contact with the cell membrane containing the ion channel of interest. Depending on the specific research objectives, different patch-clamp configurations, such as whole-cell, cell-attached, inside-out, or outside-out patch, may be employed. Once the configuration is set, the experiment proceeds to record baseline currents, which represent the ion channel's activity before any drug intervention. These baseline measurements serve as the control against which subsequent drug-induced effects will be compared, enabling researchers to precisely quantify any alterations in ion channel behavior.

With the baseline established, the next step involves the application of the drug compounds under investigation. The drug can be introduced either to the entire cell or directly to an isolated membrane patch containing the ion channel. Delivery methods may vary, including the use of bath solutions or rapid solution exchange systems. Throughout this process, the patch-clamp amplifier continuously monitors ion channel currents in the presence of the drug. Researchers closely observe and

analyze changes in ion channel behavior, including variations in channel opening and closing, conductance, and kinetics. Dose-response studies are often conducted by applying different concentrations of the drug, offering insights into concentration-dependent effects.

Patch-clamp recordings also provide opportunities to delve into the mechanistic aspects of drug action. Researchers can explore whether the drug binds directly to the ion channel or modulates its activity through allosteric mechanisms. To assess the drug's specificity, researchers may employ specific blockers or activators of the ion channel, conducting control experiments to confirm whether the observed effects are indeed pharmacologically linked to the drug of interest.

Lastly, the reversibility of drug effects is assessed by washing out the drug application and recording ion channel currents once more to observe the return to baseline activity. Subsequently, the collected patch-clamp data is subjected to thorough analysis, yielding valuable quantitative measures, such as the half-maximal inhibitory concentration (IC50) or half-maximal activation concentration (EC50), which provide insights into the drug's potency and its impact on the ion channel's behavior.

Patch-clamp in neuropharmacology

One fundamental application of patch-clamp is the characterization of neurotransmitter receptors. This involves the study of receptors such as glutamate receptors, GABA receptors, and acetylcholine receptors, among others. By applying specific agonists and antagonists, scientists not only pinpoint the receptor subtypes at play but also delve into their pharmacological properties, including kinetics, desensitization, and modulation. Furthermore, patch-clamp empowers researchers to explore synaptic transmission by recording excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) at neuronal synapses. This capability facilitates investigations into synaptic strength, plasticity, and the impact of drugs on synaptic communication, offering crucial insights into the mechanisms governing neural network activity.

In the realm of neuronal excitability, patch-clamp recordings provide a window into the firing patterns and action potentials of individual neurons. This knowledge proves invaluable for understanding neuronal excitability and the influence of drugs on neural firing, shedding light on how specific compounds may alter neural network dynamics. Additionally, the technique aids in studying the modulation of various ion channels expressed in neurons, including voltage-gated sodium channels, potassium channels, and calcium channels. Unraveling how drugs affect these channels enhances comprehension of their impact on neuronal function and their potential therapeutic applications in neurological disorders.

Patch clamp in cardiac safety assessment

Researchers employ this technique to scrutinize various cardiac ion channels, encompassing voltage-gated sodium channels, different potassium channels (including delayed rectifier currents and inward rectifier currents), and calcium channels. A significant aspect of cardiac safety assessment is the evaluation of the drug's impact on the human ether-à-go-go-related gene (hERG) channel, a pivotal potassium channel responsible for cardiac repolarization (Rampe and Brown, 2013). Drugs that block hERG channels may prolong the QT interval on the electrocardiogram (ECG), elevating the risk of a perilous arrhythmia known as torsades de pointes. Utilizing patch-clamp, researchers assess the drug's propensity to block hERG channels, aiding in the identification of compounds that may pose a risk of QT interval prolongation.

Furthermore, patch-clamp permits the execution of dose-response studies, exposing cardiac ion channels to varying drug concentrations. This facilitates the determination of the drug's potency and empowers researchers to establish therapeutic concentrations that ensure cardiac safety. Additionally, patch-clamp studies provide valuable insights into the mechanism of action of the drug on cardiac ion channels. Researchers can investigate whether the drug directly interacts with the channel's pore or binding sites, or if it modulates the channel's gating behavior, thereby offering insights into potential risks and mechanisms underlying drug-induced arrhythmias. Furthermore, these recordings enable the assessment of the drug's impact on repolarization reserve, which reflects the heart's ability to recover its electrical activity after depolarization (Veerman et al., 2013). Drugs that impede repolarization reserve can precipitate hazardous arrhythmias, and patch-clamp data serves as a critical tool in identifying drugs that might compromise cardiac electrical stability.

Advancements and future directions

The development of automated patch-clamp systems, leveraging robotics and microfluidics, has revolutionized experimental efficiency (Suk et al, 2019). These systems have the remarkable capability to conduct multiple patch-clamp recordings simultaneously, enhancing throughput and precision in drug screening and ion channel studies. Furthermore, the emergence of planar patch-clamp technology, exemplified by platforms like the "giga-seal" and "inside-out" configurations, represents a significant leap forward (Milligan and Möller, 2013). It offers researchers enhanced stability and significantly higher success rates in obtaining high-quality patch-clamp recordings. This newfound stability empowers researchers to explore cells that were once considered rare or difficult to access. In addition to these advancements, high-speed patch-clamp recordings have become feasible, thanks to recent breakthroughs in electronics and data acquisition (Govendir et al., 2022). This innovation provides researchers with improved temporal resolution, making it possible to study fast ion channel kinetics and transient cellular events in unprecedented detail.

Another notable advancement is the fusion of optogenetics with patch-clamp, which enables precise control of neuronal activity through light stimulation (Ji et al., 2023). This approach offers unprecedented insights into the functional connectivity and synaptic plasticity of neural circuits. Another promising avenue is the coupling of patch-clamp recordings with single-cell transcriptomics, allowing simultaneous measurement of cellular electrophysiology and gene expression (Csillag et al. 2023). This breakthrough has the potential to unveil the intricate links between ion channel function and gene regulation, contributing to the development of personalized drug therapies and advancing precision medicine.

Advancements in nanotechnology hold the promise of miniaturized patch-clamp probes (Altalhi, 2023), capable of recording from nanometer-sized structures within cells, such as dendritic spines or subcellular compartments. This technology could offer profound insights into localized ion channel function, enhancing our understanding of neuronal signaling and cellular communication. Additionally, the creation of 3D patch-clamp arrays may enable researchers to record from multiple locations within complex neural circuits simultaneously, revolutionizing our understanding of brain information processing and facilitating the development of brain-machine interfaces (Chubinskiy-Nadezhdin et al., 2022; Kruskal et al., 2015). Further innovations include closed-loop experiments, where patch-clamp recordings interact with real-time feedback systems, enabling dynamic and interactive studies of neuronal excitability and synaptic plasticity (Suk et al., 2017). Advancements in virtual reality technology provide immersive environments for patch-clamp researchers, enhancing

training and experimental control (Noguchi et al. 2021). Lastly, the development of nanopore-based patch-clamp techniques could lead to label-free, single-molecule analysis of ion channels and biomolecules, with applications in personalized medicine, DNA sequencing, and drug screening (Bruhn, 2015; Kim et al., 2012).

CONCLUSION

Studying ion channels in drug research holds paramount importance due to their central role in regulating essential physiological processes and their involvement in numerous diseases. Ion channels play a crucial role in nerve signaling, muscle contraction, hormone secretion, and cardiac function, among other vital functions. Dysregulation or dysfunction of ion channels is implicated in a wide array of disorders, including neurological diseases, cardiac arrhythmias, epilepsy, and chronic pain.

The patch-clamp technique's high-resolution recordings allow for the detection of even the tiniest changes in ion flux, making it uniquely adept at scrutinizing subtle alterations in ion channel conductance and kinetics. Moreover, patch-clamp recordings are not only confined to global cell responses but extend to the study of individual ion channels, illuminating their open-close states and gating dynamics. This remarkable precision and sensitivity not only deepen our understanding of cellular physiology but also empower drug researchers to design more targeted and effective pharmaceutical interventions.

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COULD PYROPTOSIS-ASSOCIATED GASDERMIN B PROTEIN LEVEL SERVE AS A POTENTIAL BIOMARKER IN THE PROSTATE CANCER

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Abstract

Prostate cancer is one of the biggest and most important health problems for men. Unfortunately, the incidence of prostate cancer is increasing every day in all countries of the world. Age, race, family history are the most well-known factors that affect the development of prostate cancer. A large number of studies done in recent years have highlighted the importance of pyroptosis on cancer. Pyroptosis is an inflammatory cell death that affects the development and progression of prostate cancer. The aim of our study was to determine the relationship between Gasdermine B protein level and prostate cancer. For this study we had two groups: the control group with 25 healthy individuals and the group with 25 individuals with prostate cancer. The level of pyroptosis-related proteins was assessed in serum. Measurement of GSDMB protein level was performed by ELISA method.

Our study was conducted with the decision of the Ethics Committee for Non-Interventional Clinical Research of ESOGU number 2022-194/40. In this study, blood samples were taken from the two groups and the Elisa Kit was used to determine the GSDMB protein level in the serum. In our study, there was no statistically significant difference between prostate cancer and control groups for serum GSDMB protein level (p=0.197). It is worth noting that our study is the first study that makes the connection between gasdermone protein B and prostate cancer and will contribute to the new literature, and the creation of strategies for the treatment and new diagnosis of prostate cancer. It is necessary to conduct new and comprehensive studies in this direction where the number of samples is greater and the changes of genes and proteins are evaluated together.

Keywords- *Pyroptosis, Gasdermin, Prostate Cancer, GSDMB.*

INTRODUCTION

Among the cancers that affect men, prostate cancer has the second highest rate of incidence and the fifth highest mortality rate in the world (Banerjee, 2023). The development of prostate cancer is not equal in all men, a polygenic disease with great inheritance. In many researches the genetic contribution to prostate cancer has been proven. Family inheritance is one of the most predisposing genetic risk factors for prostate cancer (Termini, 2020).

Compared to men with no family history of prostate cancer, men with a brother or father affected by prostate cancer have a double risk of developing prostate cancer, this risk increases up to three to five

times if the first-degree relative had early disease (diagnosed at age ≤ 55 years) (Brook, 2023). Prostate cancer has a much higher incidence in men of African descent, followed by Asian and European men (DeSantis, 2019).

Patients in their 50s develop prostate cancer, which is a slow-growing tumor. Several factors can affect the development and progression of prostate cancer, such as genetic factors, aging, androgens, chronic diseases, local inflammation, and other infections. Factors such as lifestyle, exercise, diet, obesity are known as factors related to the development and progression of prostate cancer. (Matsushita, 2020). Based on both epidemiology and genetics, prostate cancer is a heterogeneous disease, the reduction in the estimated survival rate of prostate cancer for specific races is caused by the interactions between genetics and environmental and social influence and thus results in the observed differences in the epidemiology of prostate cancer in different countries (Hjelmborg, 2014).

Genomics research has identified molecular processes that result in certain cancer developments, such as chromosomal rearrangements. Prostate cancer is the most commonly diagnosed cancer in 112 countries and the leading cause of cancer death in 48 countries (Sung, 2021). It is worth noting that the burden of prostate cancer is assumed to increase due to population aging and economic growth (Culp, 2020).

Prostate cancer is diagnosed by prostate biopsy and analysis, prostate-specific antigen (PSA) testing, magnetic resonance imaging (MRI), digital rectal examination or physical examination (Sekhoacha, 2022). Often times, prostate cancer can be asymptomatic in the early stages and often has an indolent course, requiring minimal or no treatment. However, complaints such as difficulty urinating, increased frequency and nocturia may appear, all symptoms that can also arise from prostate hypertrophy.

Pyroptosis

Pyroptosis is inflammatory programmed cell death that is triggered by inflammasomes and executed by gasdermin proteins. Cell swelling, membrane perforation and release of cell contents are the main characteristics of pyroptosis, in normal physiology pyroptosis plays a critical role in the defense against pathogenic infections. Excessive pyroptosis can cause inappropriate and persistent inflammatory reactions that include the appearance of inflammatory diseases (Shi, 2015).

Pyroptosis may serve as a strategy for eliminating cancer by inducing the death of pyroptic cells by activating intensively antitumor immunity. Pyroptosis as a powerful tool to fight inflammatory diseases and cancer, and those that induce pyroptosis for cancer therapy. In many experiments, it has been discovered that the cleavage of gasdermin D (GSDMD) by caspase-1 results in the release of its N-terminal domain (GSDMD-NT), which then forms a pore in the cell membrane (Shi, 2015).

The gasdermin family consists of five members GSDMA, GSDMB, GSDMC, GSDMD, GSDME/DFNA5 and PVJK/DFNB59. With the exception of gasdermin DFNB59, all other members of this family have two conserved domains, an N-terminal effector domain and a C-terminal inhibitory domain. Binding of the N-terminal domain inhibits the N-terminal pore-forming activity (Rao, 2022). There are four signaling pathways that induce pyroptosis, including the canonical and non-canonical inflammasome pathways, the caspase-mediated apoptotic pathway, and the granzyme-mediated

pathway. In all these signaling pathways, gasdermin proteins are the final executioners that are cleaved by caspases or granzymes (Kesavardhana, 2020).

Gasdermin B

Gasdermin B is expressed in the bronchial epithelium of asthmatic lungs, the epithelium of the esophagus and the gastrointestinal tract (Feng, 2018). GSDMB has four splice variants, each variant encoding a protein of 35 - 50 kDa molecular weight (Tamura, 2007). An increase in the expression of Gasdermin B has been observed in patients with gastric cancer, breast cancer, cervical cancer (Feng, 2018).

The membrane binding of Gasdermin B differs from the membrane binding preferences of other Gasdermins. both the full-length domain and the N-terminal domain of Gasdermin B can bind to glycolipid phosphoinositides and sulfatides (Chao, 2017).

Difference the members of the gasdermin family, gasdermin B has the ability to interact with the extracellular sheet of the plasmatic membrane, based on its ability to bind to sulfatides. The binding ability of both the full-length domain and the N-terminal domain to membranes suggests that the C-terminal domain of gasdermin B is unable to inhibit the membrane binding ability of the N-terminal domain (Chao, 2017). However, the N-terminal domain of GSDMB, but not full-length GSDMB, can induce pyroptosis-like features in human HEK293T cells (Ding, 2016).

Thus binding of the full-length gasdermin B protein to cell membranes is not sufficient to induce pore formation, and activation or processing of the protein after membrane binding may produce the N-terminal domain that then catalyzes pore formation (Feng, 2018).

MATERIAL AND METHOD

For this study, 45 patients between the ages of 40-90 years who were diagnosed with prostate cancer and 45 healthy individuals between the ages of 25-90 years were taken, the samples were taken from the Research and Practice Hospital of the Eskişehir Osmangazi University Faculty of Medicine, Department of Urology. Blood samples from patients and control individuals were obtained and serum samples were used in the study.

This study was carried out with the decision of the ethics commission number 2022-194/40 of the ESOGU Non-Interventional Clinical Ethics Committee of Clinical Research. Each individual participating in the study was informed about the content, purpose and implementation of the study and written consent was obtained.

Determination of protein levels with the ELISA method

Enzyme-linked immunosorbent assay (ELISA) method and Human Gasdermin B Elisa Kit (BT-Lab Bioassay Technology Laboratory, Birmingham, United Kingdom) will be used to determine protein levels from isolated serum samples. In this phase, which will be developed in accordance with the kit procedure, the serum samples will be pipetted into the ELISA plate and after the incubation and washing phases, following the user's instructions, the level of Gasdermin B protein was measured in

spectrophotometrically with an ELISA reader. In the protein analysis stage; The Gasdermin B ELISA kit was used.

Preparation of samples, reagents and standards

Blood obtained from patients and controls was centrifuged at 2500 rpm at 4°C for 15 minutes and the supernatant fraction (serum) was separated. Samples were placed in eppendorf and stored at -80 °C until analysis. Before starting the Eliza method, the samples were centrifuged again to remove any particles that may have arisen during their stay at -80 °C. In addition, all standards and reagents were brought to room temperature before use. A standard reference sample was diluted with diluent. Serial dilutions were made from the stock solution obtained as a result of this dilution.

ELISA procedure

While 40 µL of standard solution was added to the first 6 wells, 40 µL of sample was added to the other wells, then 10 µL of Gasdermin B antibody was added to these sample wells, after the antibody was added to all wells and 50 µL of streptavidin-HPV. They were then incubated at 37°C for one hour. 300 µL of washing buffer was added to each well by aspiration and washing was repeated 3 times. These and other washing procedures were performed using a microplate washer (BioTek, ELx50). The plate was then dried with a blotter paper. After these procedures, solution A and solution B were added to each well, and the plate was then incubated at 37 °C for 10 minutes. After finishing my ink, 50 µL of stop solution was added. Finally, the optical density (OD) of each well was calculated simultaneously using a microplate reader (Thermo, MULTISKAN GO) set to 450 nm. Data were calculated according to the developed standard curve and expressed as ng/ml.

RESULTS

In our study, Gasdermin B protein levels were evaluated between patients with prostate cancer and control individuals, and no statistical difference was found (Table 1).

Table.1 Serum Gasdermin B levels in prostate cancer and control groups

Group	n	Gasdermin B (ng/ml)	Statistic
Control (M ± SD)	45	116.7373± 111.6685	p=0.197
Prostat Cancer (M ± SD)	45	89.9142± 82.1988	

DISCUSSION

In the study done on human breast cancer suggested that GSDMB is overexpressed in breast carcinomas and can be considered as a potential marker in these tumors. A reduced survival and increased metastatic disease has been shown in patients with increased expression of this molecule. GSDMB-2 isoform compared to other isoforms is significantly upregulated in a group of breast cancer tumors. These results suggest that GSDMB-2 may be responsible for the poor prognosis of GSDMB-overexpressing tumors (Hergueta-Redondo, 2014).

Gasdermin B isoforms have differential expression levels within tumors and normal tissue in gastric cancer. GSDMB is overexpressed in carcinomas of the esophagus, breast, liver, stomach, colon, and cervix, and transcription has been observed in normal proliferating epithelial cells (Hergueta-Redondo, 2014). Sun et al. in his experiment reported that GSDMB contributes to the development and progression of uterine CC. An increased level of GSDMB was observed in CC tissues compared to the adjacent cancerous tissues and the corresponding normal tissues (Sun, 2008).

Komiyama et al., in their study examining Gasdermin B expression in the ostrata of healthy and cancerous gastric tissues, from this study, it was determined that Gasdermin B has extremely low expression in healthy gastric tissues. Unlike healthy tissue, Gasdermin B was overexpressed in gastric cancer tissue. These results suggest that Gasdermin B may play a crucial role in regulating the spread of cancer cells (Komiyama, 2010).

Carl-McGrath et al show in another study that Gasdermin B can be overexpressed in hepatic carcinomas and colon cancer tissues (Saeki, 2009). GSDMB has a complex role in cancer where it is considered either as a potential oncogene or as a tumor suppressor gene However, two difficulties have so far prevented the determination of the exact cancer functions of GSDMB in vivo : a) the lack of preclinical mouse models, as rats and mice lack the GSDMB ortholog; b) Existence of four translated GSDMB isoforms that may play different roles in cancer and inflammatory diseases. (Sarrió, 2021).

CONCLUSION

At the end of the study, no statistically significant difference was found between the prostate cancer and control groups in terms of serum GSDMB protein levels (p=0.197). However, our study is the first to establish the link between GSDMB protein and prostate cancer, and to contribute to new literature, strategies for the treatment and new diagnosis of prostate cancer. Our study may contribute to new and comprehensive studies in which the number of samples is increased and gene and different protein changes are evaluated together.

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EVALUATION OF THE ANTICANCER EFFECT OF *Spinacia oleracea* LEAF EXTRACT ON HT-29

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Abstract

The majority of deaths from colorectal cancer (CRC) are associated with the metastatic process. Many studies show that a diet rich in fruits and vegetables protects against many common chronic diseases, including cancer. Especially the nutrients and non-essential chemical compounds contained in green leafy vegetables are thought to promote health. Spinach *(Spinacia oleracea* L.), which is used in basic nutrition and contains phytochemicals, vitamins and minerals that support health, shows anti-cancer, anti-obesity, hypoglycemic and hypolipidemic properties.

In this study, the anti-cancer effect of *Spinacia oleracea* leaf extract on HT-29 colorectal cancer cell line was evaluated. For this purpose, cell proliferation (MTT), migration (wound healing assay) and apoptosis rate (Bax-Bcl-2 gene expression by qRT-PCR) were investigated on HT-29 cell lines exposed to this extract.

According to the MTT assay, *Spinacia oleracea* leaf extract was observed to greatly suppress the growth of HT-29 cell lines and significantly alter the Bax/Bcl-2 expression level. On the other hand, wound healing results showed that the metastasis ability decreased. The result of this study shows that *Spinacia oleracea* can be considered as a future chemotherapeutic agent and a suitable candidate for *in vivo* trial.

Keywords: Apoptosis, Migration, Colorectal cancer, *Spinacia oleracea* leaf extract

INTRODUCTION

Cancer stands out as the 2nd biggest cause of death in the world and the cause of one in every 6 deaths. It is seen 2.5 times more frequently, especially in developed countries. Colorectal cancer (CRC) is the third most common diagnosis and second deadliest malignancy for both sexes combined. Colorectal cancer (CRC) occurs when abnormal cells develop inside the colon and were found one of the deadliest cancers widespread all over the world. CRC has both strong environmental associations and genetic risk factors (1-3).

Many studies show that a diet rich in fruits and vegetables is protective against common chronic diseases such as cancer, obesity and cardiovascular diseases. It is accepted that especially green leafy vegetables, their nutrients and chemical compounds have important health-promoting activities (4,5).

Spinach (*Spinacia oleracea L.*) contains many vitamins (A, B, C, D, E and K) and minerals (magnesium, calcium, copper, manganese, sodium, phosphorus and iron) (6,7)

In addition, it is a widely consumed food due to its phytochemicals and bioactive substances that support health as well as providing basic nutrition. Phytochemicals and bioactive substances derived from spinach can prevent oxidative damage by eliminating reactive oxygen species. It can also regulate the expression of genes involved in metabolism, proliferation, inflammation and antioxidant defense, and may reduce food intake by giving a feeling of fullness (8-10).

Spinach, which is used in basic nutrition and contains phytochemicals, vitamins and minerals that support health, has attracted the attention of researchers due to its anti-cancer, anti-obesity, hypoglycemic and hypolipidemic properties (11). Its anti-colorectal cancer capacity has not been well studied so far. In this study, anti-cytotoxicity, anti-migration and apoptosis induction power of Spinacia oleracea pure extract in the HT-29 CRC model cell line was evaluated.

MATERIAL AND METHODS

Spinacia oleracea Leaf Extract

Fresh spinach (*Spinacia oleracea*) leaves were washed several times with running water to get rid of the dust particles, and dried under sunlight to remove the residual moisture. Then, spinach leaves were washed with distilled water for any possible impurity. Typically; in a 250 ml vessel about 20 g of fresh spinach leaves dried up, and cut into very small pieces were added to 100 ml deionized water under vigorous stirring, and the reaction temperature was raised to 60 °C for 15 min. Then, the mixture was naturally cooled, and filtered using Whatman filter paper subsequently kept at 37 °C incubator for complete evaporation and stored at room temperature. For stock solution preparation 0.5 g of extract has been taken into 10 ml of RMPI-1640 culture medium solution (12). It is necessary to cover it with the aluminum foil to prevent oxidation.

Cell Culture

The HT-29 human colon cancer cell lines were obtained from the American Type Culture Collection and grown in DMEM (Dulbecco's modified Eagle's medium) (Lonza, Switzerland) with 1% antibiotics (100 IU/ml penicillin, 100 mg/ml Streptomycin) (Biowest, France) and 10% FBS (fetal bovine serum) (Biochrom KG). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells were harvested by incubation for 5 min with 0.05% (w/v) trypsin (Sigma) in PBS [10 mmol/L sodium phosphate, 4 mmol/L KCl, and 137 mmol/L NaCl (pH 7.4)] containing 0.3 mmol/L EDTA followed by the addition of 10% FBS to inactivate the trypsin. Cell numbers were determined by measuring tryphan blue exclusion under a light microscope.

Cytotoxicity Assay

Cytotoxicity effect of *Spinacia oleracea* aqueous extract on the cell was carried out in which HT-29 cell lines were settled into a 96-well culture plate and allowed to adhere for 48 h; cell lines were treated with different concentration of extracts (62.5, 125, 250, 500, and 1000 µg/ml, respectively) and control cells were treated with dimethyl sulfoxide (DMSO). The viability of cells was evaluated after

48 h of incubation by the addition of 50 µl of a 5 mg/ml MTT solution. Then the plate was further incubated at 37 °C for 4 h, and then all of the media was replaced with DMSO. The plate was shaken strongly half an hour for the dye to dissolve. After the dissolution of formazan crystals, OD read at 570 nm by ELISA reader (Biochrom, UK). The IC50 was calculated and analyzed by GraphPad Prism v. 8.

$$\left(\frac{OD\ treated}{OD\ untreated}\right) x\ 100 = \text{the \% inhibition}$$

Wound Healing Assay (Scratch Test)

A wound healing assay was performed to evaluate the cell migration ability of HT-29 cells. Cells were seeded in a 6-well culture plate and then all wells were grown to a monolayer of approximately 80-90%. A linear scratch was made with a sterile yellow pipette. The wells were washed to ensure that there were no debris or free-floating cells in the wells. Then, extract was applied to the cells at IC value. then the plate was incubated at 37 °C for 48 h. Wound healing in the scratched area was checked at 0, 24, 48 and 72 hours, and images were captured immediately using an inverted microscope.

Real Time-PCR and Detection of mRNA Expression

HT-29 cells were treated with IC50 value of *Spinacia oleracea* extract. After 24 h for Total RNA, the Trizol kit was used (Invitrogen, USA). The purified extracted RNA was quantitatively measured by NanoDrop, at wavelengths of 260 and 280 nm. cDNA was obtained using a random hexamer primer and a MultiScribe Reverse Transcriptase kit as described by the manufacturer (TaqMan RT Reagents; Applied Biosystems). Quantitative PCR reaction contained 1 µl cDNA, 7 µl water, 10 µl SYBR green premix (TaKaRa, Japan), and 1 µM of specific primer (Table 1).

Table 1. Sequences of the primers used in real time PCR assays

Name	5'-3'	Tm	PCR product size (bp)
Bax-F	GGTTGTCGCCCTTTTCTA	48.84	108
Bax- R	CGGAGGAAGTCCAATGTC	49.1	
β-Actin-F	GCGAGAAGATGACCCAGAT	50.87	88
β-Actin-R	GAGGCGTACAGGGATAGC	50.97	
Bcl-2-F	GATGTGATGCCTCTGCGAAG	65	93
Bcl-2-R	CATGCTGATGTCTCTGGAATCT	6	

The reaction was done under the following conditions: initial denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension for 30 s at 72 °C. Relative gene expressions were calculated with $2^{(-\Delta\Delta Ct)}$ method (13) using β-actin as a housekeeping gene (internal control). The primers used in this project are mentioned in the Table 2.

Table 2. QRT-PCR Conditions

PCR Condition	Temperature Time	Cycle
Denaturation	95 °C for 5 m	1
Denaturation	95 °C for 15 s	40
Annealing	60 °C for 30 s	
Extension	72 °C for 30 s	
Extension	72 °C for 7 m	1

STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation. Statistically, analysis and the T test were performed using the SPSS v. 25. The $P < 0.05$ was considered statistically significant.

RESULTS

Cell Cytotoxicity

MTT assay was used to determine the effect of *Spinacia oleracea* extract on HT-29 cell viability. The percentage of viable cells decreased compared with wild type cells in a dose-dependent manner and observed in 24, 48, and 72 h. The IC50 value was determined as 267.5 µg/ml after 48 h. P value considered as < 0.05 (Figure 1).

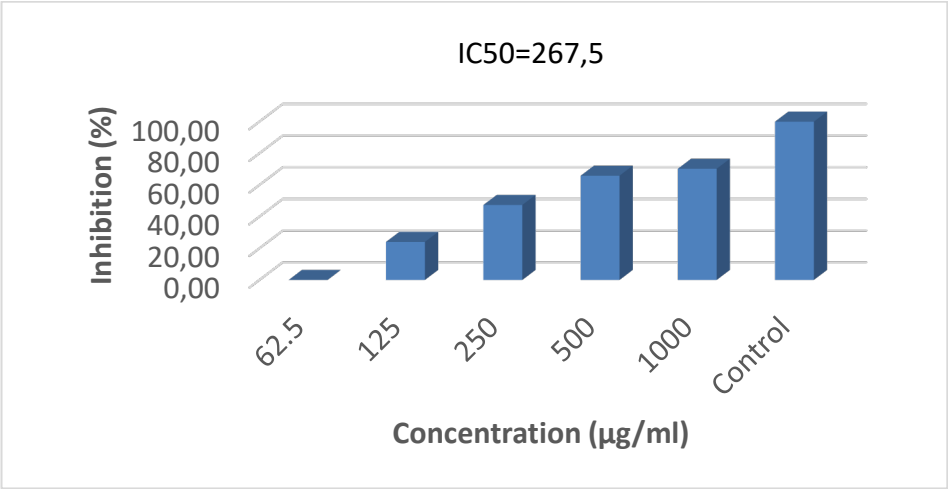


Figure 1. Spinach Extract induced apoptosis in HT-29 cells. Cells were treated with extract at 62.6, 125, 250, 500, and 1000 µg/ml, respectively. $P < 0.05$

Wound Healing Assay

The effect of Spinach extract on HT-29 cell motility was conducted using a wound-healing assay. Compared with the control group, *Spinacia oleracea* extract significantly decreased the number of migrated HT-29 cells. Outcomes in the present study indicated that higher extract treatment concentration resulted in a slower scratch wound healing process indicating *Spinacia oleracea* extract inhibits cell migration (Figure 2).

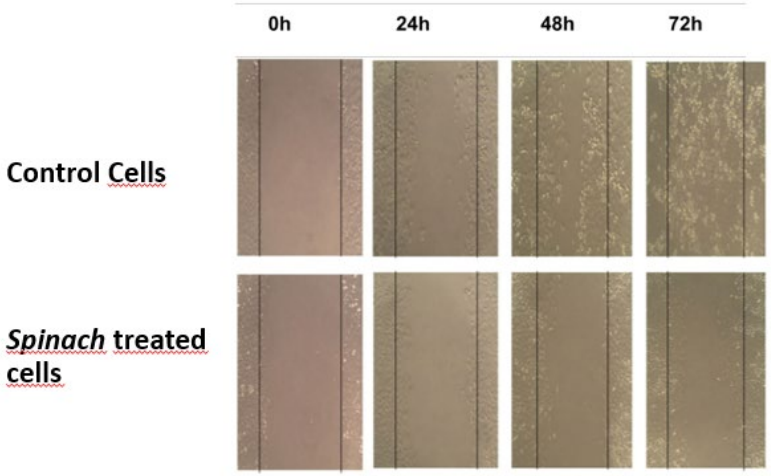


Figure 3. Effect of Spinach Extract on migration of human colon cancer HT-29 cells.

Gene Expression of Selected Apoptosis-Related Gene

According to qRT-PCR results, the increase in Bax expression indicates positive induction of apoptosis. Moreover, considering the anti-apoptotic properties of the Bcl-2 gene, the decrease in Bcl-2 expression proves that apoptosis occurs. (Figure 3). Bax gene has been upregulated and anti-apoptosis gene of Bcl2 has been downregulated. Bax gene upregulated by 14.227 fold and Bcl2 gene downregulated by 3.242 fold. Considering the P value of 0.05, the upregulation of the pro-apoptotic gene of Bax was considered significant and consequently, spinach extract was observed to induce apoptosis.

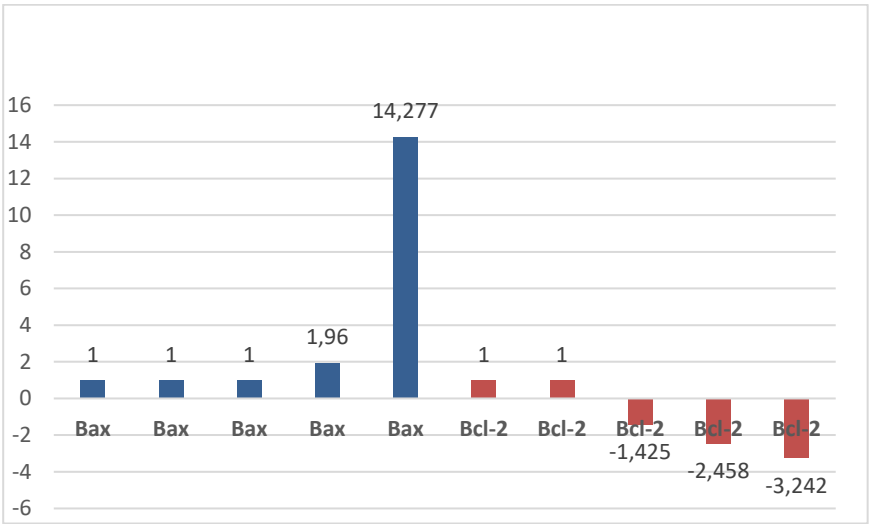


Figure 4. Gene Expression of Bax and Bcl-2.

DISCUSSION

Spinach is a rich source of water-soluble antioxidants compounds that exert anti-ageing, anti-inflammatory and anti-cancer activity. Despite increasing advances in modern therapeutic methods such as gene therapies and genome editing (14, 15), herbal medicine and secondary metabolites have maintained their role in the treatment and prevention of various diseases especially for chronic diseases and cancer (16). A substance in its natural nature has potential curative effects on a variety of diseases (17). Organic products are considered as a promising source of anti-tumor properties (18). While a dose-dependent inhibition effect of spinach extract was observed in prostate, lung, stomach and liver cancers, a significant inhibition was observed in colon cancer (19-22).

Its extract inhibitory effect on the cell proliferation and growth of breast cancer cells has been reported. In a previous study [23], have shown the ability of spinach leaf juice to inhibit the proliferation of the human HT29 colon cancer cell line in a time and dose-dependent manner. The juice significantly also reduced the damage induced by a known oxidant agent up to 80%.

CONCLUSION

Spinacia oleracea contributes several valuable components that have potent, antioxidant, and anticancer activities. The results of this study support the view that this plant material demands further investigations of its biological properties and toxicity in future studies. Since the results revealed the growth inhibitory and apoptotic effect and anticancer properties of the water extract of *Spinacia oleracea* we can suggest further research on this topic.

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