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Serbian Biochemical Society
Tenth Conference

with international participation

24.09.2021. Kragujevac, Serbia

"***Biochemical Insights into Molecular Mechanisms"***

PROGRAMME

10:00-10:15	Opening ceremony
10:15-10:50	Samo Kreft Faculty of Pharmacy, University of Ljubljana, Slovenia **Phytochemical, pharmacological and clinical investigations of the extract from the branches of *Abies alba*** (FEBS3+ Lecture)
10:50-11:10	Dragica Selaković Faculty of Medical Sciences, University of Kragujevac **Molecular mechanisms of platinum-based chemotherapeutics-induced behavioral manifestations of neurotoxicity – a beneficial role of antioxidant supplementation**
11:10-11:30	Simeon Minić University of Belgrade - Faculty of Chemistry **Bioactive properties of Spirulina-derived phycobiliproteins and phycobilins**
11:30-12:00	Coffee break
12:00-12:20	Nikola Gligorijević Institute for Application of Nuclear Energy, University of Belgrade **Ligand binding to fibrinogen influences its structure and function**
12:20-12:40	Ivan Čapo Faculty of Medicine, University of Novi Sad **New aspects of vitamin C during prenatal period of development**

12:40-13:00	Milan Dragićević Institute for Biological Research "Siniša Stanković", University of Belgrade **Arabinogalactan protein mining and diversity - the case of *Centaurium erythraea*** **Poster session** Cocktail / Lunch break Miloš Prokopijević University of Belgrade - Institute for Multidisciplinary Research **Natural polymers: suitable carriers for enzyme immobilization** Ana Ninić University of Belgrade - Faculty of Pharmacy **Link between resistin, low-grade systemic inflammation and obstructive sleep apnea** Branka Đorđević Faculty of Medicine, University of Niš **Blood-retinal barrier breakdown in diabetic retinopathy – the protective role of melatonin** Coffee break Poster awards and closing ceremony
13.00-14.00	
14.00-15.00	
15:00-15:20	
15:20-15:40	
15:40-16:00	
16:00-16:30	
16:30-17:00	

Poster Session

Tamara Antonić
Faculty of Pharmacy, University of Belgrade
Prognostic capability of uric acid on the development of hypertensive complications during pregnancy

Miloš Avramov
Faculty of Sciences, University of Novi Sad
Protein disorder determination and expression of hsp genes during diapause and cold acclimation in the European corn borer *Ostrinia nubilalis* (Hbn.)

Dejana Bajić
Faculty of Medicine, University of Novi Sad
Levothyroxine: biochemical aspects and consumption in Serbia

Ana Marija Balaž
Institute of Chemistry, Technology and Metallurgy, University of Belgrade
Horseradish peroxidase C1A wild type gene and its variants expressed in *Pichia pastoris* KM71H strain

Vedrana Bazović
Faculty of Chemistry, University of Belgrade
Screening of potential inhibitors of recombinant S-adenosyl-L-homocysteine hydrolase from banana

Sofija Bekić
Faculty of Sciences, University of Novi Sad
Heterologous expression, purification and enzymatic activity of aldo-keto reductase 1D1

Stefan Blagojević
Faculty of Science, University of Kragujevac,
Impact of gold(III) and ruthenium(II) complexes on miRNA expression involved in metastasis on primary ovarian cell culture isolated from ascites

Dragica Bulajić
Faculty of Medicine, University of Novi Sad
Cytotoxicity of pulp-capping materials NeoMTA Plus, ProRoot MTA, and Biodentine on human deciduous dental pulp stem cells

Sunčica Buljević
Faculty of Medicine, University of Rijeka
The impact of CD26 deficiency on peptide YY and substance P expression patterns in Crohn's disease

Marina Crnković
Faculty of Agriculture, University of Novi Sad
Effect of soil liming on phenolic compounds in soybean

Jelena Danilović Luković
University of Belgrade - Institute for Multidisciplinary Research
The effect of increased nickel concentrations on *Chlorella sorokiniana* culture

Srđana Đorđievski
Faculty of Sciences, University of Novi Sad
The effect of spermidine on the malondialdehyde levels in honey bees (*Apis mellifera* L.)

Nevena Đukić
Faculty of Science, University of Kragujevac
Accumulation of malondialdehyde in different varieties of *Triticum aestivum* L. under conditions of heat stress

Jovana Drljača
Faculty of Medicine, University of Novi Sad
Glioblastoma-associated microglia as a new target and strategy to fight with

Yaraslau U. Dzichenka
Institute of Bioorganic Chemistry of National Academy of Sciences, Minsk
Modified steroids as modulators of cholesterol level in humans by acting on CYP7A1 and CYP7B1

Sonja Filipović

Faculty of Sciences and Mathematics, University of Niš

The unanticipated monoterpene quantities variation in the Montenegro *J. oxycedrus* essential oil specimens

Sonja Filipović

Faculty of Sciences and Mathematics, University of Niš

The first antifungal activity assessment of *M. juliana* essential oil against crop pathogen *Colletotrichum acutatum* J.H. Simmonds

Azra Guzonjić

Faculty of Pharmacy, University of Belgrade

Relative telomere length and oxidative stress in patients with idiopathic pulmonary fibrosis, mesothelioma and non-small cell lung cancer

Jasmina Ivanišević

Faculty of Pharmacy, University of Belgrade

Redox status in patients with obstructive sleep apnea

Anđelka M. Isaković

Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade

ADAM10, CEA, and CA19-9 as triad biomarkers of colorectal cancer response to neoadjuvant chemoradiotherapy

Jovana D. Jagodić

Faculty of Chemistry, University of Belgrade

Elemental composition of healthy adrenals and adrenal adenomas: whole blood and solid tissue analysis

Natalija Jonić

Institute for Biological Research "Siniša Stanković, University of Belgrade

Promoting the pro-inflammatory phenotype in macrophages by blocking the aryl hydrocarbon receptor

Jelena S. Katanić Stanković

Institute for Information Technologies Kragujevac, University of Kragujevac

Meadowsweet extract-mediated green synthesis of silver nanoparticles: A promising bioactive tool

Teodora Knežić

Biosense Institute, University of Novi Sad

Expression of cs, ast, alt and ldh genes during diapause of the European corn borer *Ostrinia nubilalis* (Hbn.)

Milica Kojadinović

Institute for Medical Research, University of Belgrade

The supplementation with fish and evening primrose oils improves fatty acid status in breast cancer patients receiving aromatase inhibitors

Jelena Korać Jačić

University of Belgrade - Institute for Multidisciplinary Research

The impact of pH on coordination chemistry of tetracycline and Fe(III) in water

Lela Korićanac

Vinča Institute of Nuclear Sciences, University of Belgrade

Antitumor effect of Ru(II) complexon A375 and HeLa cell growth, migration and adhesion ability

Ana Kostić

Institute for Biological Research "Siniša Stanković", University of Belgrade

Sclareol, a natural compound, inhibits P-glycoprotein activity in cancer cells

Iva Lakić

Faculty of Biology, University of Belgrade

Rat brown adipose tissue thermogenic markers are modulated by estrus cycle phases and short term fasting

Milica Lazarević

Institute for Biological Research „Siniša Stanković", University of Belgrade

The effect of novel rosmarinic acid derivative on the pathogenesis of experimental autoimmune encephalomyelitis in rats

Ivana Lukić
Institute of Virology, Vaccines and Sera – Torlak, Belgrade
***Lactobacillus reuteri* alleviate the severity of chemically-induced colitis in mice**

Jovan Luković
Faculty of Medical Sciences, University of Kragujevac
Cytotoxic effect of chalcone analogue on tumor cells of murine and human origin

Tatjana Majkić
Faculty of Sciences, University of Novi Sad
Hypoglycemic effect of Cabernet Sauvignon wine

Aleksandra Margetić
University of Belgrade – Institute of Chemistry, Technology and Metallurgy
Antibacterial activity of polypiridinearene ruthenium(II) complexes

Marija Marin
Faculty of Biology, University of Belgrade
Effect of the *Thymus lykae* Degen et Jav. methanol extract on the erythrocyte membrane

Stefan Marković
Faculty of Science, University of Kragujevac
Heat-induced accumulation of proline and yield components in different cereal varieties

Miloš Matić
Faculty of Science, University of Kragujevac
Effects of cadmium on oxidative metabolism and motility of human placental cells in chemically-induced hypoxia

Nenad Mićanović
Faculty of Agriculture, University of Belgrade
Antioxidant and antidiabetic potential of hawthorn (*Crategus mopnogyna* Jacq. L.) fruits originating from Serbia

Tijana Mićović
Institute for Medicines and Medical Devices of Montenegro, Podgorica
Promising anti-inflammatory potential of methanol extracts of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (*Lamiaceae*)

Vladimir Mihailović
Faculty of Science, University of Kragujevac
Selected medicinal plant extracts as a potential preservative in cosmetic products

Marija Mihajlović
Faculty of Pharmacy, University of Belgrade
Relationship between single nucleotide polymorphism TNF-α -308 G>A (rs1800629) and *TNF-α* gene expression levels in colorectal cancer patients

Nemanja Mijin
Faculty of Chemistry, University of Belgrade
Amyloid fibril formation of ovalbumin in the presence of heavy metal ions, lead and cadmium

Marijana Milanović
Medical Faculty of the Military Medical Academy, University of Defense, Belgrade
Exogenous alpha-ketoglutarate impair differentiation and maturation of human dendritic cells

Sonja Milić Komić
Institute for Multidisciplinary Research, University of Belgrade
Efficient production of highly purified Late Embryogenesis Abundant (LEA) protein from *Arabidopsis thaliana* by recombinant DNA technology

Milorad Miljić
Faculty of Sciences, University of Novi Sad
The profile of carotenoids and phenolic compounds in 20 different varieties of pumpkin pulp cultivated in Serbia

Sara Milojević
Faculty of Science, University of Kragujevac
Evaluation of pathophysiological effects of clinical concentrations of proinflammatory interleukin-6 detected in patients with COVID-19 on homeostasis of human trophoblast cells

Jelica Milošević
Faculty of Chemistry, University of Belgrade
Bioinformatic analysis of ovalbumin and HEWL amyloidogenic peptides

Maja Milošević
Faculty of Technology, University of Novi Sad
Use of acid-extracted fiber from butternut squash pulp for complex coacervate preparation

Marija Milošević
Faculty of Science, University of Kragujevac
Testicular toxicity induced by chlorpyrifos and imidacloprid: Comparative study

Tamara Milošević
Clinical Hospital Center „Zvezdara“, Belgrade
High lactate dehydrogenase activity is significant mortality predictor in a 6 months study on end stage renal disease patients

Milan Mišić
Faculty of Chemistry, University of Belgrade
Antioxidant potential and protein interactions of four tea plant extracts

Maja Mladenović
University of Belgrade – Faculty of Chemistry
Optimization of expression, purification and HRMS characterization of recombinant N-protein fragment from SARS-CoV-2

Jovana Muškinja
Institute for Information Technologies, University of Kragujevac
Synthesis of ferrocenyl-based pyrazoline derivatives and their biological evaluation

Aleksandra Nikezić

Faculty of Science, University of Kragujevac

Anticancer potential of *Alchemilla vulgaris* L. on triple negative breast cancer cell lines

Ivana Nikolić

Faculty of Medical Sciences, University of Kragujevac

Biological effects of vanillin derivatives (enones) on HeLa cells in vitro

Ana Obradović

Faculty of Science, University of Kragujevac

Molecular mechanisms of antitumor activity 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin in human breast cancer MDA-MB-231 cells

Miloš Opačić

Institute for Multidisciplinary Research, University of Belgrade

Distribution of high-affinity copper transporter CTR1 in sclerotic hippocampi of mTLE patients

Dejan Orčić

Faculty of Science, University of Novi Sad

A novel HPLC-DAD method for quantification of dominant lignans in cow parsley (*Anthriscus sylvestris* (L.) Hofm.)

Ana Pantelić

Faculty of Chemistry, University of Belgrade

Characterization of the late embryogenesis abundant (LEA) proteins family in hydrated and desiccated *Ramonda serbica* Panc. leaves

Jelena Pantović

Faculty of Chemistry, University of Belgrade

Expression, purification and characterization of recombinant L-phenylalanine dehydrogenase

Milica G. Paunović

Faculty of Science, University of Kragujevac

Estimation of redox potential of novel Pt(IV) complexes in the blood of rats

Kasja Pavlović

Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia

Response of muscle cells to metformin depends on glucose availability in cell culture medium

Marija Pavlović

Vinča Institute of Nuclear Sciences, University of Belgrade

Highly active pectinases from newly isolated *Aspergillus tubingensis* strain

Milica Pavlović

Faculty of Science, University of Kragujevac

Bioaccumulation coefficient as an indicator of the ability to accumulate heavy metals of plant species on tailings

Milica Perović

Faculty of Technology, University of Novi Sad

Preparation and characterization of protein nanoparticles from chickpea protein isolates

Nevena Planojević

Faculty of Science, University of Kragujevac

The influence of newly synthesized Au(III) and Ru(II) complexes on gene expression on primary ovarian cancer cell culture from ascites

Lena Platanić Arizanović

University of Belgrade - Faculty of Chemistry

Atypical antipsychotics clozapine, sertindole, ziprasidone, and erythrocytes: Friends or foes?

Dušica J. Popović

Faculty of Medicine, University of Novi Sad

Inhibition of tumor vasculature production by metformin treatment of cancer inoculated to hamsters

Kosta J. Popović
Faculty of Medicine, University of Novi Sad
Nitroglycerin reduces no production in hamster fibrosarcomas

Aleksandra Popović
Faculty of Medicine, University of Novi Sad
The effects of Karnozin EXTRA® on mitochondrial bioenergetics in healthy fibroblasts

Iva Potočnjak
Faculty of Medicine, University of Rijeka
Oleanolic acid induces mitophagy and chemosenzitises human colon carcinoma cells

Tanja Radisavljević
Faculty of Science, University of Novi Sad
Chemical characterization and antioxidant capacity of decaffeinated coffee extracts

Milena Rašeta
Faculty of Sciences, University of Novi Sad
Phenolic profile and biological activity of fungal species *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo 2011

Marina Ristović
Institute of Chemistry, Technology and Metallurgy, University of Belgrade
Diffusion screening method for estimation potential fungal producers of xylanase responsible for xylooligosaccharides production

Dragana Robajac
Institute for the Application of Nuclear Energy, University of Belgrade
Changes in serum glycans in pregnant women with gestational diabetes mellitus

Milena Simić
Faculty of Pharmacy, University of Belgrade
Activity and phenotype of paraoxonase 1 enzyme in pediatric patients on hemodialysis

Miron Sopić
Faculty of Pharmacy, University of Belgrade
Short telomeres in peripheral blood leukocytes are associated with the presence and the severity of lung cancer

Nikola Z. Srećković
Faculty of Science, University of Kragujevac
Green synthesis, characterization, and biological evaluation of silver nanoparticles synthesized using *Salvia pratensis* L. extracts

Ana Stančić
Institute for Biological Research "Siniša Stanković", University of Belgrade
Ferroptosis as a novel determinant of β-cell death in diabetic conditions

Marija Stanišić
Faculty of Chemistry, University of Belgrade
Biomimetic mineralisation of periodate oxidized glucose oxidase

Marijana Stanojević Pirković
Faculty of Medical Sciences, University of Kragujevac
Cytotoxicity of enone sodium salts on human colon cancer cells (HCT-116) *in vitro*

Aleksandra Stefanović
Institute for Oncology and Radology of Serbia, Beograd
Methylenetetrahydrofolate reductase C677T and A1298C polymorphic variants in rectal cancer: significance for cancer risk and response to chemoradiotherapy

Olgica Stefanović
Faculty of Science, University of Kragujevac
Phenolic compounds content and antibacterial activity of selected medicinal plants growing in Serbia

Olgica Stefanović
Faculty of Science, University of Kragujevac
Antibacterial and cytotoxic activity of *Tanacetum macrophyllum* extracts

Branko Subošić
University Children's Hospital, Belgrade
Childhood obesity: metabolic disorder and liver function

Emilija Svirčev
Faculty of Sciences, University of Novi Sad
Identification of compounds with antioxidative properties in alpine bistort (*Bistorta vivipara* (L.) Delarbre) ethanol extracts

Biljana Šmit
Institute for Information Technologies Kragujevac, University of Kragujevac
Synthesis and antimelanogenic activity of *N3*-aryliden-2-thiohydantoin derivatives

Marinela Šokarda Slavić
Faculty of Chemistry, University of Belgrade
Production of polyglutamic acid by *Bacillus* sp: strains selection, optimization, batch fermentation and characterization

Marinela Šokarda Slavić
Faculty of Chemistry, University of Belgrade
A search for nature's robust proteases with zein as a substrate

Filip Štrbac
Faculty of Agriculture, University of Novi Sad
Chemical composition of thyme essential oil and its anthelmintic properties

Jovana Šućur
Faculty of Agriculture, University of Novi Sad
Allelopathic effect of ragweed (*Ambrosia trifida* L.) on soybean phenolic compounds

Miloš Šunderić
Institute for the Application of Nuclear Energy, University of Belgrade
Interaction between alpha-2-macroglobulin and phycocyanobilin – structural and physiological implications

Marija Takić
Institute for Medical Research, University of Belgrade
Associations between zinc intake and status with biochemical and antropometric parameters in hemodialysis patients

Nada Tokodi
Faculty of Sciences, University of Novi Sad
Biochemical, molecular and histological insight into cyanobacterial effects on *Cyprinus carpio* from Kis-Balaton, Hungary

Jovana Tubić Vukajlović
Faculty of Science, University of Kragujevac
The effect of acetone extract of *Lenzites betulinus* mushroom on peripheral blood lymphocytes genome in patients with acute coronary syndrome *in vitro*

Mirela Ukropina
Faculty of Biology, University of Belgrade
Possible protective role of probiotic bacteria on intestinal epithelium after chronic $CdCl_2$ intake

Iva Uzelac
Faculty of Sciences, University of Novi Sad
Expression of heat shock protein genes in response to unusually high temperatures during winter diapause in the European corn borer *Ostrinia nubilalis* (Hbn.)

Snežana Vojvodić
Institute for Multidisciplinary Research, University of Belgrade
Redox changes in microalga *Chlorella sorokiniana* exposed to high concentrations of Mn(II)

Sanja Vujčić
University of Belgrade - Faculty of Pharmacy
Triglyceride-glucose index and oxidative stress biomarkers in patients with type 2 diabetes mellitus

Srđan Vujinović
Faculty of Sciences, University of Novi Sad
Chemical composition and antioxidant activity of pomegranate (*Punica granatum* L.) peel and pulp

Iva Vukelić
Faculty of Medicine, University of Rijeka
Luteolin ameliorates experimental colitis in mice through ERK-mediated suppression of inflammation, apoptosis and autophagy

Anđela Vukojević
University of Belgrade - Institute for Biological Research "Siniša Stanković",
Effects of fermented food on the body weight and behavior after repeated LPS administration in mice

Irina Vulin
Faculty of Sciences, University of Novi Sad
Effect of caffeine on cell proliferation and key elements of neurotransmitter pathways in human neuroblastoma SH-SY5Y cell line

Milan Zarić
Faculty of Medical Sciences, University of Kragujevac
Selective cytotoxicity of melittin against human cervical cancer cells

Gordana Zavišić
Faculty of Pharmacy, University of Academy Business, Novi Sad
Effect of probiotic consumption on serum glucose and lipid levels in mice

Milica Zeković
Institute for Medical Research, University of Belgrade
Omega-3 index and anthropometric indices among testicular cancer survivors

Milena Zlatanova
Faculty of Chemistry, University of Belgrade
Small molecules attenuate activation of the NF-κB signaling in epithelial cells by Act d 1 kiwifruit allergen

Nenad Zlatić
Faculty of Science, University of Kragujevac
Monoterpenes variability in *Teucrium montanum* L. essential oils

Alma Zuković
Faculty of Pharmacy, University of Belgrade
Biomarkers of oxidative stress in pediatric patients with type 1 diabetes mellitus

Dragan Živančev
Institute of Field and Vegetable Crops, Novi Sad
The use of the handheld grain quality analyzer for determination protein content in wheat cultivars

Katarina Živić
Institute for Oncology and Radiology of Serbia, Belgrade
Prevalence of somatic *BRCA1* gene methylation in patients with ovarian cancer

Kristina Živić
Institute for Oncology and Radiology of Serbia, Belgrade
Anti-migratory properties of Nischarin agonist rilmenidine in pancreatic ductal adenocarcinoma

Foreword

Dear Colleagues,

It is a distinct pleasure to welcome you to the 10th Conference of the Serbian Biochemical Society, entitled '*Biochemical Insights into Molecular Mechanisms*'.

In contrast to the 9th Conference, when we had one year results presented in three conference days, this time we have two years results presented in only one day. To some surprise, the latter was more abundant, reflecting high spirit and resilience of biochemists in this time of crisis. The participation of Professor Samo Kreft from Ljubljana, who is our FEBS3+ guest, and eight invited lecturers from all four major universities in Serbia, mark the winter of our discontent and the return to homeostasis.

I would like to thank again all the participants for their valuable contributions and to the Organizing Board and the Scientific Board for their efforts invested into this meeting.

Editor of the Proceedings
Ivan Spasojević

Invited Lectures

Phytochemical, pharmacological and clinical investigations of the extract from the branches of *Abies alba*

Samo Kreft

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Abies alba (silver fir) is a tree native to the mountains of Europe. It has only weak traditional use in phytotherapy. Its bark, wood and particulary brancehs are rich in antioxidative polyphenols among which the lignans (taxiresinol, 7-(2-methyl-3,4-dihydroxytetrahydropyran-5-yloxy)-taxiresinol, secoisolariciresinol, lariciresinol, hydroxymatairesinol, isolariciresinol, matairesinol and pinoresinol) are the most characteristic [1,2]. In our group, we optimised the extract under the name Belinal. We performed several pharmacological, toxicological and clinical studies. The extract was shown to prevent atherosclerosis in guinea pigs [2], and to have cardioprotective effect in isolated rat hearts [1]. It was found to reduce the post-prandial glycemic response in healthy volunteers [3]. Its topical use improved the appearance and function of the skin. We found no influence on liver and kidney function. The production of the extract and its use was patented and in collaboration with the partners from the industy, it was put on the market as food supplement.

References

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Molecular mechanisms of platinum-based chemotherapeutics-induced behavioral manifestations of neurotoxicity – a beneficial role of antioxidant supplementation

Dragica Selaković[1*], Igor Kumburović[1], Rade Vuković[2], Jelena S. Katanić Stanković[3], Vladimir Mihailović[4], Gvozden Rosić[1]

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For decades, platinum-based chemotherapeutics have been widely used in therapeutic protocols for numerous malignancies. However, despite their undisputed therapeutic potentials, there are numerous limitations for their clinical usage due to serious adverse effects. With its variety in clinical manifestations, neurotoxicity is considered as one of the most frequent side effects of platinum-based compounds. Those clinical entities have common underlying pathophysiological mechanisms that include oxidative damage and pro-apoptotic action. Therefore, it was implicated to estimate the potential role of antioxidant supplementation in treatment of platinum-based drugs-induced neurotoxicity. Indeed, very promising results were achieved with a variety of antioxidant-rich compounds. Our results also confirmed that both synthetic and natural products with high antioxidant content may successfully attenuate cisplatin-induced neurotoxicity by prevention of oxidative damage and apoptosis in brain. The beneficial role of antioxidant supplementation was additionally confirmed by reversing the behavioral deterioration induced by cisplatin.

Bioactive properties of Spirulina-derived phycobiliproteins and phycobilins

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Arthrospira (Spirulina), photosynthetic, filamentous cyanobacteria, has been used as food for centuries. It is one of the richest known natural source of proteins and essential amino acids, an excellent source of vitamins, macro-and micro-elements, pigments, essential fatty acids, glycolipids, and polysaccharides. C-phycocyanin (C-PC), the most abundant protein of Spirulina, is a highly fluorescent and water-soluble heterodimeric phycobiliprotein. Its blue colour arises from covalently attached (via thioether bond) tetrapyrrole chromophore phycocyanobilin (PCB). Numerous studies have shown C-PC exhibit significant anti-inflammatory, immunomodulatory and anticancer effects which could be ascribed to the powerful anti-oxidative activities of PCB. Indeed, studying the digestion of C-PC in simulated gastrointestinal conditions revealed that obtained chromopeptides possess significant anticancer, anti-oxidative and metal-binding properties. Furthermore, it has been shown that various proteins (human and bovine serum albumins, bovine catalase, bovine lactoglobulin) can bind PCB, which could influence pharmacokinetic behaviour, bioavailability and oxidative stability PCB. On the other hand, the presence of tetrapyrrole chromophore increases the thermal, oxidative and digestion stability of these proteins. This work reviews the recent findings of bioavailability, distribution and bioactive properties of C-PC and its chromophore, which could be of fundamental significance and applicative potential.

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Ligand binding to fibrinogen influences its structure and function

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Fibrinogen is a plasma protein most susceptible to oxidation. Through this chemical modification, fibrinogen acquires thrombogenic characteristics in different pathophysiological conditions. Increased carbonyl content and reduced porosity impair the degradation of formed fibrin mediated by plasmin. Fibrinogen is capable of interacting with many proteins, ions, and small molecules. These interactions can modify the functions of this protein. The discovery of new binding partners that may protect fibrinogen from harmful oxidation and, thus, preserve its normal function is essential. Some of the newly detected interactions between fibrinogen and small, natural bioactive molecules, together with the influence of these interactions on the structure and function of fibrinogen, will be presented in this text.

Acknowledgements

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New aspects of vitamin C during the prenatal period of development

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Every vitamin deficiency leads to a cascade of reactions that ultimately result in the development of the disease. Vitamin C is synthesized from glucose in the liver of most mammals, while in humans, non-human primates and guinea pigs (*Cavia porcelus*), this is not the case. Namely, due to the evolutionary loss of the gene for the synthesis of L-gulonolactone oxidase (Gulo gene), the endogenous synthesis of vitamin C was stopped so that these mammals have to take vitamin C to survive. Vitamin C is directly involved in collagen synthesis and severe disturbance in the survey, representing a postnatal form of this vitamin deficiency. Although a consequence of postnatal vitamin C deficiency is relatively clear, there is still insufficient data for the prenatal period. Recent data indicate the importance of collagen in basal membrane integrity and possible its disturbance in vitamin C depletion. We developed a novel guinea pig model of prenatal pial basal membrane disturbance during prenatal deprivation of vitamin C. Results indicated that disturbance of collagen synthesis induced breaches in the pial basement membrane. Consequentially Bergmann glia connection was lost, followed by neuron migration disturbance and dysplasia of cerebellar cortex that are found in Lissencephaly type II. The fact that neither humans nor guinea pigs can synthesize vitamin C creates an opportunity for further research into the impact of prenatal deprivation of vitamin C in developing neuron migration disorders.

Arabinogalactan protein mining and diversity - the case of *Centaurium erythraea*

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Centaurium erythraea (common centaury) is a medicinal plant with extraordinary developmental plasticity *in vitro*, used as a model organism for studying *in vitro* morphogenesis in our lab. Several experimental lines of evidence have identified arabinogalactan proteins (AGPs) as one of the key players involved in century morphogenesis; however, the role of specific genes has yet to be determined. AGPs are ubiquitous plant cell surface glycoproteins associated with various physiological functions. AGP sequences are characterized by the presence of non-continuous hydroxyproline residues which serve as O-glycosylation anchor sites for branched arabinogalactans. Due to biased amino acid composition rich in disorder-promoting amino acids, AGP sequences lack a stable structure and consequently have lessened evolutionary constraints. Therefore, homology-based approaches to AGP sequence mining have limited success. We have recently developed a bioinformatics pipeline for AGP sequence mining, ragp, which exploits their key feature – the presence of hydroxyprolines[1]. This pipeline combines estimation of proline hydroxylation based on local sequence context by a machine learning model with a flexible motif search. After applying this pipeline to the centaury transcriptome, AGP regions were found to associate with a variety of conserved domains. Here we introduce a streamlined way to train models for prediction of Pro hydroxylation, analyze important protein sequence features determining Pro hydroxylation status, present some of the AGP types found in centaury and discuss model limitations and future prospects.

Acknowledgements

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Natural polymers: suitable carriers for enzyme immobilization

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Enzyme immobilization onto support carriers is a method used to overcome some of the limitations of soluble enzymes in practical applications. Various different materials have been used as carriers, such as inorganic matrixes, natural and synthetic polymers [1]. Production of carriers from natural biopolymers and their derivatives are the focus of numerous research worldwide, and a summary of their applications for enzyme immobilization is presented in this paper. Enzymes or cells are entrapped inside a three-dimensional polymeric network that is able to retain water in large amounts, called hydrogel. This network could be formed by chemical cross-linking, ionotropic gelling in the presence of cation or in thermo reverse polymerization, depending on the polymer in use and its characteristics. Most frequently used biopolymers as carriers for immobilization include alginate, cellulose, chitosan, collagen, xylan, pectin, starch, and others [2].

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Link between resistin, systemic low-grade inflammation and obstructive sleep apnea

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Obstructive sleep apnea is manifested by airway obstruction due to a decrease or complete cessation of airflow in upper parts of respiratory system, despite inhalation efforts. It is linked to cardiometabolic diseases, obesity and dyslipidemia. Adipose tissue, as recently recognised endocrine organ, when exposed to systemic inflammation and chronic intermittent hypoxia undergoes structural and functional changes. These result in proinflammatory cytokine release by adipocytes and vascular stromal cells. Resistin is a cysteine-rich peptide expressed mainly by bone marrow, immune cells, and macrophages. It promotes inflammation enhancing its own and transcription of other proinflammatory cytokines. It was speculated that resistin was associated with development and progression of insulin resistance, type 2 diabetes, metabolic syndrome, and cardiovascular diseases. The connection between obstructive sleep apnea, as a chronic low-grade proinflammatory state, and resistin was tried to explain by inflammation processes which might deteriorate airflow and muscular functions of the upper parts of respiratory system [1].

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Blood-retinal barrier breakdown in diabetic retinopathy - the protective role of melatonin

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Diabetes mellitus and its complications represent a global socioeconomic burden with more than 450 million people affected worldwide. The integrity of the blood-retinal barrier (BRB) is essential for retinal neuronal health. Barrier breakdown results in fluid accumulation in the retina, macular edema, neuronal death, and vision loss. BRB breakdown may result from a disruption of the tight junctions, an up-regulation of vesicular transport across the inner or outer BRB, or by degenerative changes to the endothelial cells, the pericytes, and glia. The present review aims to discuss mediators of BRB dysfunction and molecular mechanisms of BRB breakdown in diabetes mellitus and the emerging evidence that patients with diabetic retinopathy might benefit from the melatonin treatment. Relevant articles included in this review were identified by searching the PubMed database (last 10 years) and reference lists of relevant articles. The data suggest that melatonin might protect ocular tissues by decreasing the production of reactive oxygen species and pro-inflammatory mediators implicated in BRB breakdown, such as vascular endothelial growth factor, tumor necrosis factor-α, and interleukin-1β [1,2]. Therefore, melatonin might be considered in the therapy of ocular diseases characterized by BRB, although, the topic remains under investigation.

Acknowledgements

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Posters

Prognostic capability of uric acid on the development of hypertensive complications during pregnancy

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More attention is directed towards the early identification of women at high risk of hypertensive complications in pregnancy. Models used for risk assessment include maternal demographic, clinical, and various biochemical markers, but few have clinical applications. The significance of hyperuricemia, observed in women with preeclampsia, has not yet been clarified [1]. The aim of this study was to examine the role of uric acid (UA) in hypertensive complications prediction. A total of 129 pregnant women participated in the study, of which 38 had uncomplicated, 61 high-risk pregnancies, and 30 women developed hypertensive complications of pregnancy. Women with preeclampsia or gestational hypertension were, on average, older ($p<0.05$) and with a higher body mass index (BMI) ($p<0.001$). These women had higher concentrations of total ($p<0.001$) and LDL-cholesterol ($p<0.05$) and triglycerides ($p<0.001$) compared to healthy pregnant women. UA was higher throughout gestation in women with hypertensive complications compared with the other two groups ($p<0.001$). Maternal age, BMI, and the UA stood out as significant independent predictors of the hypertensive outcome of pregnancy. UA could be used to distinguish between women who will develop and those who will not develop hypertensive complications (AUC=0.700, $p<0.05$). However, adding UA to the parameters (maternal age, BMI, cholesterol, triglycerides) used in assessing the risk of developing, primarily preeclampsia, does not significantly increase the diagnostic potential.

Acknowledgements

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Protein disorder determination and expression of *hsp* genes during diapause and cold acclimation in the European corn borer *Ostrinia nubilalis* (Hbn.)

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Diapause and cold acclimation are intertwined phenomenons that allow many insects, such as the economically important European corn borer in the 5th larval instar, to survive harsh winter conditions by modifying their metabolism and accumulating protective compounds. Of particular interest for the study of these mechanisms are intrinsically disordered proteins which are unstructured (or contain unstructured regions) under native conditions, making them resilient against stressful factors. They also play important roles in signalling pathways and interact with many partners. Stress-related heat shock proteins (HSPs) display such interactions. Several HSPs (HSP20.1, HSP20.4, HSP70, HSP90 and HSC70) were chosen for disorder determination using their amino acid sequences and the IUPred disorder predictor. Relative expression of genes encoding these proteins was determined in non-diapausing (ND, control group) and diapausing (D) 5th instar larvae exposed to different acclimation temperatures (15°C, 5°C, –3°C, –16°C). IUPred analysis has shown that HSP20.1 and HSP20.4 are intrinsically disordered (50% and 79% disorder content, respectively), while HSP70, HSP90 and HSC70 are partially disordered (23%, 19% and 20%, respectively). Expression of all genes is progressively upregulated as temperature decreases and diapause progresses. Additionally, *hsp20.4*, *hsp70*, *hsp90* and *hsc70* are generally upregulated during D compared to ND, while *hsp20.1* is greatly downregulated. The exception is the D(15) group where *hsp90* is significantly downregulated in comparison to non-diapause and other diapausing, cold-acclimated groups, which is in connection with hormonally induced arrest of development at the larval stage.

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Levothyroxine: biochemical aspects and consumption in Serbia

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Thyroid hormones play a vital role in the regulation of numerous metabolic processes and they are essential for normal growth and development. The thyroid gland produces, storages and releases iodine-containing hormones thyroxine (T4) andtriiodothyronine (T3).The predominant hormone produced by the thyroid gland is T4, with approximately 70–90 µg of T4 and 15–30 µg of T3 produced daily. They make certain effects byentering the cell nucleus and binding to DNA-bound thyroid receptors, which regulate gene transcription and synthesis of proteins involved in metabolism of proteins, carbohydrates and lipids. Tissue-specific modulation of the thyroid hormone activity depends on thyroid hormone secretion, plasma transport, transmembrane transport, activation/inactivation, and interaction with nuclear receptor isoforms and their co-regulators. Naturally, the hormones secreted by the thyroid are regulated by the hypothalamic–pituitary–thyroid axis through a negative feedback system [1]. Inadequate or decreased synthesis of any of these hormones requires appropriate replacement treatment. Hypothyroidism is one of the most common endocrine disordersand diagnosis is based on statistical reference ranges of the relevant biochemical parameters (thyroxine and TSH level). Levothyroxine is synthetic thyroxine hormonethat is biochemically and physiologically indistinguishable from the natural form produced normally in body. It is commonly used for treatment of primary (thyroidal), secondary (pituitary) and tertiary (hypothalamic) hypothyroidism, as well as for euthyroid goiters including thyroid nodules, subacute or chronic lymphocytic thyroiditis, multinodular goiter or for thyroid cancer patients who have undergone thyroidectomy.Biochemical findings of subclinical hypothyroidism can be found in asymptomatic population [1]. According to data obtained from website for Medicines and Medical Devices Agency of Serbia for 2009 and 2019, it is noticed remarcable change in average annual consumption of approved levothyroxine medicines (doses per tablet 25, 50, 75, 100, 125, and 150 µg). An increasing trend in the number of people that need thyroxine replacement is evident. To facilitate the ability to compare consumption information between countries a defined daily dose (DDD) is introduced. It represents “the assumed

average maintenance dose per day for a drug used for its main indication in adults". This parameter is fixed unit of measurement independent of price, currencies, pack size and strength, enabling trends in drug utilization to be assessed and compared between population group in different countries. In 2009, total annual consumption of levothyroxine-sodium was 6.19 DDD/1000 inhabitants/day, and in 2019, it was 19.26 DDD/1000 inhabitants/day. This indicates growing population with diagnosed thyroid deficiency and increased consumption of hormone replacement in therapy [2,3].

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Horseradish peroxidase C1A wild type gene and its variants expressed in *Pichia pastoris* KM71H strain

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Enzyme immobilization enables maintenance of enzyme activity and structural stability even in adverse conditions [1]. Structural changes in enzymes that can occur due to the action of organic solvents, inhibitors or increased temperature can be prevented by immobilization of the enzymes in metal–organic frameworks (MOFs). It is reported that several enzymes, such as cytochrome c and horseradish peroxidase (HRP) have been successfully incorporated into MOFs [2]. The aim of this work is to produce wild type horseradish peroxidase, isoform C1A, and several mutants specially designed to increase the activity and stability of HRP while immobilized within selected MOFs. Wild type and its variants were produced in metalotrophic yeast, *Pichia pastoris* KM71H strain, their activity and basic kinetic parameters were determined and compared prior imobilization.

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Screening of potential inhibitors of recombinant *S*-adenosyl-*L*-homocysteine hydrolase from banana

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S-adenosyl-*L*-homocysteine hydrolase (SAHH) catalyses hydrolysis of *S*-adenosyl-*L*-homocysteine to adenosine and homocysteine. It regulates all *S*-adenosylmethionine dependent methylations and is one of the most conserved proteins across different kingdoms of life [1]. Presented study focused on a recombinant SAHH from banana. After a successful production in the BL21 cell culture, it was purified by immobilized metal affinity chromatography, with a yield of 1.5 mg of protein per 30 mL of cell culture. Inhibitory potential of three phenolic compounds: vanillyl alcohol (VA), cinnamic acid (CA) and ferulic acid (FA) was tested. Preliminary i*n silico* and *in vitro* screening revealed that FA is the most potent inhibitor and the only of the tested compounds with a positive drug likeness coefficient, predicted by Osiris Property Explorer [2]. After only 15 minutes of incubation 250 nM FA reduced the activity of SAHH by 50%, while VA and CA caused a moderate inhibition at the concentrations ≥500 nM. *In vitro* inhibition potential of the tested compounds is closely correlated to the discrete structural differences, as showed by the structure activity relationship analysis. Since a large number of different metabolic pathways depend on the activity of SAHH, studies regarding its inhibition could aid in the treatment of a wide range of disorders [3].

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Heterologous expression, purification and enzymatic activity of aldo-keto reductase 1D1

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Aldo-keto reductases (AKR) are a superfamily of NADPH-dependent oxidoreductases that function in the metabolism of carbonyl-containing endogenous compounds and detoxification of xenobiotics. AKR 1D1 (AKR1D1), also known as Δ4-3-ketosteroid 5β-reductase, is the only enzyme shown to catalyze stereospecific reduction of double bonds at C5 in Δ4-3-ketosteroids, a unique reaction in steroid metabolism. It also attracts considerable attention as one of the key enzymes in bile acid synthesis. The aim of this study was to express and purify His-tagged AKR1D1 in *E.coli*, and to determine its enzymatic activity by a fluorimetric method. Plasmid construct pET16b-AKR1D1 was kindly provided by Dr. Trevor M. Penning from University of Pennsylvania [1]. We achieved high protein yield and purity using a combination of affinity and size exclusion chromatography. Reductase activity of purified recombinant AKR1D1 toward 9,10-phenanthrenequinone was monitored by measuring the decrease in NADPH fluorescence intensity over the time, while no change in fluorescence signal was detectable in the absence of the enzyme. To confirm that the assay is applicable for evaluation of the inhibiton potential of compounds of interest, a known inhibitor, ursodeoxycholate, was tested and found to reduce the activity of AKR1D1 isoform. We expressed and purified active recombinant human AKR1D1, and optimized an assay for testing new substrates and/or modulators of this enzyme. Identification of AKR1D1 ligands is important because its expression is associated with the development of several types of cancer and bile acid defficiency syndrome, a serious disease affecting newborns.

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Impact of gold(III) and ruthenium(II) complexes on miRNA expression involved in metastasis on primary ovarian cell culture isolated from ascites

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Ovarian cancer is one of deadliest gynecological tumors [1]. The key problem in cancer therapy is the ability of cells to metastasize. During the process of metastasis, altered ovarian cells through migration, adhesion and invasion form multicellular aggregates in ascites. Micro RNAs (miRNAs) are small non-coding RNA molecules, which function as guide molecules during the silencing of mRNA molecules [1]. The focus of this research was metastasis-associated miRNAs expression (miR-200a, miR-210, miR-126 and miR-21) after treatment with synthesized gold (Au - $Au_3(NH_2(CH_2)_6NH_2)_2Cl_8]Cl$) and ruthenium, complexes (Ru1 - $[\{RuCl(bpy)_2\}_2(\mu\text{-pzn})][PF_6]_2\cdot 2H_2O$ and Ru2 - $[\{RuCl(phen)_2\}_2(\mu\text{-pzn})][PF_6]_2\cdot 2H_2O$), on primary ovarian cell culture. Relative gene expression of miRNAs was performed by qRT-PCR assay. All miRNAs showed decreased gene expression after treatment with chemical complex compared to control (untreated cells), apart from miR-200a which showed increased gene expression in treatment with Ru complexes. Our data suggest that all tested chemical treatments may have an antimetastatic impact on cells used in this research through reduced expression of miRNAs. Further research should be directed towards the quantification of protein targets that have a role in metastasis for tested miRNAs, in order to validate miRNA interference.

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Cytotoxicity of pulp-capping materials NeoMTA Plus, ProRoot MTA, and Biodentine on human deciduous dental pulp stem cells

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Direct pulp capping procedures involve the application of a medicament, dressing, or dental material to the exposed pulp in cases of pin-point pulp exposure, in an attempt to preserve its vitality [1]. Calcium silicate cements, like Biodentine and mineral trioxide aggregate (MTA), are dental biomaterials with the ability to raise the number and odontogenic differentiation of human dental pulp cells in vitro [2]. In this study, we assessed and compared the biocompatibility of various pulp capping materials - NeoMTA Plus (Avalon Biomed), ProRoot MTA (Dentsply Tulsa Dental Specialties), and Biodentin (Septodont) on human deciduous dental pulp stem cells (SHED). SHEDs were isolated and their phenotypes were evaluated by flow cytometry. Afterwards, they were cultured in the eluates of the above-mentioned pulpotomy materials (aged 24h, 7 and 14 days) for 24h. Cell viability was determinate by MTT assay. All groups showed cell viability mathematically similar to the control group, at all time point. Statistical analysis showed significant differences between experimental groups and control group. All three materials showed adequate cytocompatibility with SHEDs.

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The impact of CD26 deficiency on peptide YY and substance P expression patterns in Crohn's disease

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Crohn's disease (CD) is a multifactorial condition characterized by the destructive immune response that failed to be attenuated by common regulatory mechanisms which reduce inflammation and promote intestinal healing[1]. Present research on CD confirmed the impact of proteases on inflammation onset and the course of the disease, especially the role of dipeptidyl peptidase 4 (DP4). This multifunctional glycoprotein, also known as the CD26 molecule, is expressed on epithelial and immune cells of the gut. The involvement of CD26 in the pathogenesis of CD is effected through proteolytic cleavage of various types of immunomodulatory substrates and its costimulatory function[2]. Particularly, DP4 directly controls activation/inactivation or changes receptor specificity of numerous cell signaling mediators, including peptide YY (PYY) and substance P (SP). The neuropeptide-receptor interaction represents one of the triggers in some of the key pathways which are dysregulated in CD, so we aimed to investigate the impact of CD26 deficiency on the expression of SP and PYY in experimental CD in CD26 deficient mice. By qPCR and immunodetection, we assessed changes of CD26, SP, and PYY in inflammation, healing, and recovery as well as quantified DP4 activity. We found that CD26 deficiency caused an increase in PYY mRNA expression in inflammation paired with a decrease in its colon concentration, while at the same time SP mRNA, as well as its protein expression, were decreased. DP4 activity in the colon and serum was decreased in the peak of inflammation. It can be concluded that experimental CD affects the expression patterns of both PYY and SP which implicates its involvement in the neuroimmune response in CD pathogenesis.

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Effect of soil liming on phenolic compounds in soybean

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Soil acidity correction is related to soil reaction influence to a whole range of chemical, biological and physical soil properties. Thus, liming represents common amelioration measure for conditioning of excessive acid soil reaction [1]. The aim of this study was to investigate the effect of sugarcane press mud on the phenolic compounds (total phenolics (TP), total tannins (TT) and total flavonoids (TF)) in soybean grown on the black meadow soil. Furthermore, radical scavenging activity of soybean extracts was examined using ABTS assay. Results showed that the amount of TP and TF was higher in soybean leaves grown on black meadow soil with the press mud treatment (10 t ha^{-1}) (TP 100.19±2.15 mg GAE/g DW; TF 12.71±0.12 mg RE/g DW), compared to control soybean plants (grown on untreated soil). On the other hand, the amount of TT was lower in soybean plants grown on treated soil. The highest antioxidant capacity was obtained in soybean leaves grown on black meadow soil with the press mud treatment (10 t ha^{-1}) (17.41±0.44 mg tocopherol equivalents/g DW) compared to control (15.28±0.66). From the results obtained for soybean antioxidant capacity it might be concluded that the application of press mud had positive effect on plants due to its liming effect.

Acknowledgements

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The effect of increased nickel concentrations on *Chlorella sorokiniana* culture

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Microalgae show significant biotechnological potential for remediation of wastewaters polluted with metals, including nickel [1]. Ni enters aquatic ecosystems from fuel combustion, smelting, mining, and electroplating endeavors, and represents a particularly important problem for the access to safe drinking water [2]. It is noteworthy that microalgae utilize Ni as a co-factor of urease. Herein we analyzed the impact of high levels of Ni(II) on freshwater microalga *Chlorella sorokiniana* in the stationary phase of culture growth. In the concentration range 0.5 to 30 mM, Ni induced a drop in cell density and biomass after 7 days of incubation, whereas significant negative impact was present as early as 2 days for 30 mM. High concentrations also induced a decrease in the level of chlorophylls (*a* and *b*) in biomass. In contrast, microalgae that were exposed to moderate stress (1 and 2 mM of Ni) appear to increase photosynthetic activity, indicating the activation of some adaptive mechanism. Scanning electron microscopy showed that *C. sorokiniana* does not release significant amounts of mucilage polymers in response to Ni, in contrast to the response to some other metals [3]. Further, Ni at 1 mM induced an increase in the intracellular production of reactive oxygen species, which appears to reach a plateau after 1 h. The concentration of free thiol groups showed a gradual drop during 24 h of incubation with the same Ni concentration. On the other hand, the decrease in the concentration of reduced glutathione that was observed after 1 h was reversible for 1 mM Ni at 24 h. Ni at 5 mM had a more lasting impact on glutathione. It is important to stress out that the concentrations of reduced glutathione and total glutathione showed very similar trends. This implies that free thiols and reduced glutathione may be 'lost' due to glutathionylation of proteins and synthesis of phytochelatins. Our results demonstrate that the response of *C. sorokiniana* to high Ni levels involves multiple components that may be different than for other metals. Redox regulation and protection of thiol (switches) may represent a crossroad between adaptation and death.

Acknowledgements

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The effect of spermidine on the malondialdehyde levels in honey bees (*Apis mellifera* L.)

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Honey bees (*Apis mellifera* L.) are one of the most important pollinators worldwide; however, their numbers have declined over the past few decades. Oxidative stress and the consequent pathophysiological states are one of the possible reasons that eventually lead to bee loss. Oxidative stress can be accessed by measuring malondialdehyde (MDA) levels as MDA is the end product of lipid peroxidation. Spermidine is a natural polyamine compound with various metabolic functions. External supply of spermidine can extend lifespan in model organisms including yeast, nematodes, flies, and mice. Increased uptake of spermidine with food also had beneficial health effects in humans, indicating that this agent may act universally [1]. The aim of this study was to evaluate malondialdehyde (MDA) levels in honey bees after a 20-day oral spermidine supplementation. Four experimental groups were set up in controlled conditions: C fed with 50% sucrose; and S1, S2, S3 whose diet was supplemented with 1, 0.1 and 0.01 mM spermidine, respectively. The results showed that spermidine at given concentrations was neither attractive nor repulsive for honey bees. Further, spermidine supplementation for 20 days in all three analyzed concentrations reduced MDA levels. Lower MDA levels reflect decreased oxygen radical production and consequently lower oxidative stress. The obtained results indicate that spermidine has an antioxidant effect in bees, however the exact mechanism by which spermidine acts in the honey bee remains to be determined.

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Accumulation of malondialdehyde in different varieties of *Triticum aestivum* L. under conditions of heat stress

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High temperatures in plants lead to oxidative stress, which creates reactive oxygen species that are specific to cause damage to the cell membrane [1]. An indicator of lipid membrane damage by free radicals is the accumulation of malondialdehyde (MDA) [2]. The aim of this study was to determine the effect of heat stress on the degree of lipid peroxidation in leaves of 10 wheat cultivars, in order to select cultivars more resistant to heat stress. The degree of lipid peroxidation was determined spectrophotometrically by measuring the concentration of MDA. In the examined wheat varieties, the average concentration of MDA in normal physiological conditions is 0.786 μmol/g of fresh weight (f.w), while the average concentration of this substance is significantly increased under conditions of heat stress to 1.474 μmol/g of f.w. The largest increase in MDA under heat stress was observed in the cultivars Renesansa, Carica and Vlajna. These varietes are the most sensitive to heat stress than the tested varieties. On the other hand, the cultivar Talas showed the smallest increase in MDA content in conditions of high air temperature and therefore it was characterized as cultivar that is more resistant to heat stress.

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Glioblastoma-associated microglia as a new target and strategy to fight with

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Glioblastoma (GBM) is one of the deadliest primary brain tumors, heavily infiltrated with tumor-associated microglia/macrophages (TAM), which has received a great deal of interest. Due to the negligable number of peripheral macrophages by the 14th day, in our study TAM were referred to as microglia. We evaluated histopathological characterization of TAM and kinetics of their infiltration in U87 orthotopic GBM in immunosupressed Wistar rats, a commonly used model in preclinical research. To mimic different stages of GBM growth, we evaluated three-time points. Our data showed that the highest areal density of TAM was 7 days after GBM inoculation, with ability to proliferate early after initiation of GBM growth. The areal density of TAM within the tumor correlated with GBM growth and proliferation. Moreover, microglia underwent substantial morphological changes upon exposure to GBM cells. A transition from ramified morphology in peritumoral area to ameboid shape with larger soma and shortened, thick branches in the tumor core was observed. Higher areal fraction of blood vessels also correlated with the areal density of TAM. Given these pro-invasive features of microglia, this GBM model represents a good basis for further testing of microglia as a target for treatment.

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Modified steroids as modulators of cholesterol level in humans by acting on CYP7A1 and CYP7B1

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It is well known that cholesterol homeostasis in the human body depends on the complex interactions of a number of enzymes. Among the most important pathways of cholesterol metabolism are those connected with human cholesterol-7α-hydroxylase enzymes (CYP7A1 and CYP7B1)[1]. Dysfunctions of the enzymes (due to inhibition or point mutations) can lead to various pathological processes. In present study a panel of modified estrane and androstane steroids and bile acid derivatives – among which some are perspective molecules with antiproliferative activity – including widely used anticancer steroidal drug abiraterone, was tested against human CYP7 enzymes. Among tested compounds both substrate-like and inhibitor-like ligands were detected. It was found that CYP7B1 is more selective and binds only three compounds from the tested library with the affinity similar to the corresponding values for the essential substrates of the enzyme. Binding of abiraterone to the human cholesterol-7α-hydroxylase (CYP7A1) with Type I spectral response (substrate-like binding, K_d=13.6±4.3 μM), while in case of CYP7B1 with Type II spectral response (inhibitor-like binding, K_d=2.0±0.3 μM) was detected, which could be possible reason of increasing of blood cholesterol during treatment with abiraterone. Analysis of the enzymes activity toward novel ligands showed that only few compounds (androstane and estrane derivatives, abiraterone) are hydroxylated by human CYP7. The data obtained could be useful for the design of novel compounds with antiproliferative activity and reduced side effects.

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The unanticipated monoterpene quantities variation in the Montenegro *J. oxycedrus* essential oil specimens

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Genus *Juniperus* L. (Cupressaceae, Gymnosperms) represents typical boreal element of flora native across the Mediterranean region, around the Black Sea and Middle East with the altitudinal range going from sea level to 2200 m. Among ca. 67 species and 34 varieties associated with grass and shrub vegetation consisting this genus, prickly juniper, *Juniperus oxycedrus* L. (Cupressaceae Bartlett), is recognizable by its needle-like leaves and red bark. The diversity in its essential oil composition is significant since the intraspecific variation in the secondary metabolite content of J. *oxycedrus* was reported, along with known morphological variation and debatable taxonomic status. Herein, we present the results of detailed analyses of the essential-oil (0.1%, w/w) constituents obtained from dried *J. comunis* leaves, collected during the summer period from the peninsula Luštica, Montenegro. Subsequent GC and GC-MS analyses enabled the identification of more than 60 constituents, among which germacrene D (10.3%), σ-cadinene (9.2%), (*E*)-caryophyllene (9.0%), limonene (8.9%), caryophyllene oxide (6.6%), α-humulene (6.0%) and γ-cadinene (3.4%) were the dominant ones, differing significantly from the samples previously reported from the Adriatic coast. The present study was performed to infer the variation in the chemical composition of the isolated essential oil, knowing the significance of the correlation between the terpene compositions of different populations with respect to the geographic distribution. The importance of the result would be in respect to the biogeography and chemotaxonomy knowing that there are numerous cryptic plant species differing mutually only in secondary metabolite profile content.

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The first antifungal activity assessment of *M. juliana* essential oil against crop pathogen *Colletotrichum acutatum* J.H. Simmonds

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Micromeria (Bentham, 1829), Lamiaceae, comprises perennial aromatic species naturally grown in the rocky areas of the Mediterranean region. *M. juliana* (L.) Bentham ex Reichenb., is a medicinal, essential oil bearing plant species of this genus traditionally recognized as an effective digestive, carminative, and stimulant. Essential oils have a long history of use as antimicrobial agents inhibiting the growth of a wide range of microorganisms. Increasing requires for healthy food limits the use of synthetic pesticides and provides an impetus for further attempts to overcome problems of crop pathogens resistance, harmful effect on non-target organisms and the environment leading to the pesticides of herbal origin as a prolific source of a variety of bioactive compounds. Antifungal activities of the essential oils from *M. dalmatica*, *M. albanica*, *M. thymifolia*, *M. cilica*, *M. barbata* and *M. fructicosa* were evaluated so far, displaying strong fungitoxicity against various fungal strains. According to the available literature, among the *Micromeria* genus, *M. juliana* antifungal potential has not yet been estimated. This work was aimed to evaluate the agricultural plant protection potential of *M. juliana* by assessing sporulation intensity and mycelia growth of *Colletotrichum acutatum* J.H. Simmonds C.A.2 isolates, causative of strawberry anthracnose infecting both the root and crown, causing necrosis at all stages of development, leading to the yield reductions of more than 80%.

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Relative telomere length and oxidative stress in patients with idiopathic pulmonary fibrosis, mesothelioma and non-small cell lung cancer

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Since chronic oxidative stress plays a major role in the pathophysiology of various lung diseases, it is hypothesized that telomere length is reducing at a faster rate during oxidative stress [1]. Pertinent to this, we measured relative telomere length (rTL) using the qPCR method and we determined some redox status parameters by appropriate spectrophotometric methods. Our study included 9 patients with idiopathic pulmonary fibrosis, 12 patients with mesothelioma and 15 non-small cell lung cancer (NSCLC) patients. There are multiple common genetic, molecular, and cellular processes such as oxidative stress that connect lung fibrosis with lung cancer, especially with NSCLC [2]. The results showed that oxidative stress parameters (advanced oxidation protein products, superoxide anion radical, pro-oxidant-antioxidant balance (PAB), total oxidant status) were increased in these patients compared to 30 healthy subjects, while parameters of antioxidant status (total antioxidant status, concentration of sulfhydryl groups (SHG)) and rTL were decreased. A negative correlation between rTL and PAB ($p<0.05$) as well as rTL and SHG ($p<0.05$) were observed in IPF patients.

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Redox status in patients with obstructive sleep apnea

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Obstructive sleep apnea (OSA) is a chronic respiratory disorder characterized by distrurbed airflow leading to intermittent hypoxemia and subsequent reoxygenation which result in disturbances of redox status [1]. The aim of our study was to explore redox status in OSA patients and the ability to predict the existence of disease. A total of 96 patients with OSA and 51 healthy subjects were included. Redox status parameters: total antixodant status, total sulfhydryl (SH) groups, superoxide dismutase (SOD), paraoxonase 1 (PON1), total oxidant status (TOS), advanced oxidation protein products (AOPP), ischemia modified albumin (IMA) and prooxidant-antioxidant balance (PAB) were determined in serum samples. Patients with OSA had significantly higher concentrations of TOS, IMA, AOPP and SH groups ($P<0.001$) and significantly lower SOD activity ($P<0.001$) when compared to control group. The differences between PAB, TAS and PON1 did not reach significance. TOS, SOD, PON1 and SH groups had the ability to predict the existence of OSA ($P<0.05$). OSA patients were characterized by imbalance in redox status. Redox status parameters had the ability to predict the existence of disease.

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ADAM10, CEA, and CA19-9 as triad biomarkers of colorectal cancer response to neoadjuvant chemoradiotherapy

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The aim of this study was to analyze ADAM10 as novel, together with CEA and CA19-9 as traditional biomarkers, as triad predictors of response to neoadjuvant chemoradiotherapy (nCRT: 45-50.4 Gy + 5- fluorouracil) in patients with colorectal cancer (CRC). This study included 21 CRC patients receiving nCRT in the Clinical Centre of Serbia, Belgrade, in the period March 2019 – March 2020. The study was approved by the Ethical Committee of the Clinical Centre of Serbia and all patients provided their informed consent prior to obtaining a blood sample. Based on the initial MRI staging before nCRT and pathohistology after surgery, patients were categorized as with advanced locoregional disease (T3N0/T4N0 or TxN1/TxN2) or without it. The cut-off values for ADAM10, CEA, and CA19-9 were 258.7 pg/mL (median value), 5 ng/mL, and 37 U/mL, respectively. Statistical analysis was performed in SPSS, version 22 (IBM Corporation). The study group included 13 male and 8 female patients, the youngest was 34 years old and the oldest was 83. The majority of patients (11/21) were 61-70 years old. Initially, all patients were with advanced disease; 9 of them (43%) responded well to nCRT. Patients who responded well to nCRT had lower initial values of all three biomarkers; however, not statistically significant. In general, 5 patients had CEA above 5 ng/mL, none had CA19-9 above 37 U/mL, and 9 patients had ADAM10 above 258.7 pg/mL. All patients with CEA above 5 ng/mL remained with advanced disease after nCRT ($p<0.05$). In summary, ADAM10, CEA, and CA19-9 were insignificant triad predictors of the response to nCRT in the multivariant logistic regression model among patients in this study.

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Elemental composition of healthy adrenals and adrenal adenomas: whole blood and solid tissue analysis

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Adrenal adenomas (AAs) are benign tumors that most often develop on the adrenal cortex. An adrenal mass can be discovered in roughly 5% of patients over the age of 50, and the incidence of AAs increases with age [1,2]. The elemental profile of healthy and pathologically altered adrenal tissues has yet to be elucidated. The aim of this study was to examine the composition of micro- (Mn, Co, Cu, Zn, As, Se, Cd, Pb, U) and macroelements (Na, K, Mg, Ca) in healthy adrenal tissue (HAT) and adenomatous adrenal tissue (AAT) samples collected from patients with diagnosed AAs (n = 50). Healthy blood (HB) and adenomatous blood (AB) were also analyzed. The concentration of elements was determined using ICP-based techniques. We found a significant difference in the elemental composition of AAT samples compared to HATs. AATs had significantly higher amounts of Mn, Cu, Zn, Se, Pb, K, and Mg than HATs. Opposite results were obtained in the AB samples, which could indicate the withdrawal of these elements from the bloodstream by AATs. This first comprehension of the clinically significant micro and macroelements in HAT, AAT, HB, and AB samples could provide a new in-depth insight in which target metallome should be considered as initiating/modifying factor of AAs.

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Promoting the pro-inflammatory phenotype in macrophages by blocking the aryl hydrocarbon receptor

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A novel way of regulating the function of immune cells has been discovered and it is mediated by targeting the activation of the aryl hydrocarbon receptor (AhR) [1]. AhR is a ligand-activated transcription factor that responds to various aromatic compounds - exogenous such as plant flavonoids, polyphenolics and indoles and endogenous such as kynurenine [2]. By inhibiting its activation a pro-inflammatory immune response is promoted, whereas its activation exerts an opposite effect [1]. Therefore, we have tested a selection of plant-derived indol derivatives for their AhR-binding activity. According to the inhibition of mRNA expression of Cytochrome P450 Family 1 Subfamily A Member 1 (Cyp1a1), a down-stream effector of AhR activity, a potent AhR antagonist was selected under the code C46. This compound was further tested on mouse peritoneal macrophages for its ability to modulate macrophage function. Macrophages were exposed to the compound C46 *in vitro* in concentrations ranging from 250 to 1000 ng/mL for 48 h. By using flow cytometry we established that C46 significantly and dose-dependently up-regulated the proportion of M1 macrophages ($F4/80^+CD40^+$) and not only that, but it affected only M1 macrophages, while the proportion of M2 ($F4/80^+CD206^+$) remained stable throughout the exposure to different concentrations of C46. In further analysis with DAF-FM staining, it was found that C46 increased the cytocidal function of macrophages, since their content of nitric oxide was increased. With intraperitoneal administration of C46 the results were similar - the proportion of M1 macrophages in the peritoneum was up-regulated, 72 h after the treatment, while the proportion of M2 macrophages remained unaltered. In conclusion, by blocking the AhR signaling pathway with C46, a pro-inflammatory immune response could be achieved by promoting the M1 macrophage phenotype and it may as well be a promising approach for future testing in animal models of cancer.

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Meadowsweet extract-mediated green synthesis of silver nanoparticles: A promising bioactive tool

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Filipendula ulmaria (L.) Maxim. (meadowsweet) is a perennial herb belonging to the Rosaceae family. It has a long history of use as a medication for its anti-inflammatory, antipyretic, and analgesic properties. *F. ulmaria* aerial part and root extracts showed impressive antioxidant and anti-inflammatory effects *in vitro* and *in vivo*, but also good antimicrobial properties [1-3]. Presented research aimed to evaluate the biological potential of silver nanoparticles synthesized using an aqueous extract of meadowsweet aerial parts (Fu-AgNPs) as stabilizing and capping agent. The characterization of Fu-AgNPs was done using UV–Vis and FTIR spectroscopy, X-ray powder diffraction, DLS, and SEM/EDX analyses. Fu-AgNPs showed remarkable antibacterial activity towards most of the tested bacterial species (MIC < 39.1 μg/mL) while antifungal activity was moderate. Fu-AgNPs exerted high antioxidant potential in ABTS assay (IC_{50} value 59.8 μg/mL). The results of this research showed promising antimicrobial and antioxidant properties of Fu-AgNPs for use in biomedical applications and are worthy of further investigation.

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Expression of *cs*, *ast*, *alt* and *ldh* genes during diapause of the European corn borer *Ostrinia nubilalis* (Hbn.)

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Many insect species overcome unfavourable environmental conditions by entering diapause, a type of hypometabolic resting state which features accumulation of energy reserves, lowered oxidative metabolism rates, synthesis of protective metabolites and expression of specific genes. In order to better understand the changes occuring at the transcriptional level during diapause of the economically important European corn borer *Ostrinia nubilalis* (Hbn.), the expression of genes encoding selected metabolic enzymes was measured in this study. The selected enzymes are markers of aerobic (citrate synthase, aspartate aminotransferase, alanine aminotransferase) and anaerobic metabolism (lactate dehydrogenase). Total RNA was isolated from whole-body non-diapausing (ND) and diapausing (D) 5th instar larvae acclimated to different temperatures (15°C, 5°C, –3°C, –16°C) due to a close link between diapause and cold hardiness in this species. QPCR was performed and relative gene expression was determined using *rps3* as the reference gene and the ND group as control. Relative expression of *cs* gene did not differ between groups, suggesting that this enzyme is regulated posttranslationally. Relative expressions of *ast* and *alt* genes were significantly higher in the ND group compared to diapausing larvae, while the expression of *ldh* was higher in the diapausing groups. A temperature effect on relative gene expression of *cs*, *ldh* and *alt* was determined. Higher *cs* expression was recorded in diapausing larvae acclimated to 5°C compared to D(15°C), probably compensating for lowered CS enzyme activity. Cold acclimation leads to higher expression of *ldh* in diapausing larvae, especially in D(–3°C) group, as expected. Lastly, acclimating diapausing larvae to 5°C and –3°C resulted in higher expression of *alt* in these groups compared to D(15°C) larvae, leading to an increase of the cryoprotective alanine synthesis.

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The supplementation with fish and evening primrose oils improves fatty acid status in breast cancer patients receiving aromatase inhibitors

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Breast cancer is one of the biggest public health challenges in the 21st century. Most breast cancers express estrogen (ER+) and/or progesterone receptors (PR+), which require estrogen deprivation therapy[1]. Aromatase inhibitors **(**AIs) are considered as standard of care for adjuvant treatment of postmenopausal women with hormone receptor-positive breast cancer[2]. This study aimed to evaluate the efficacy of fish oil (rich in EPA and DHA), and evening primrose oil (rich in GLA) on serum lipids and fatty acid in breast cancer patients receiving adjuvant hormonal therapy. In this randomized control trial, we included 37 postmenopausal women, ages 50-65, with ER+ and/or PR+ breast cancer history, who received AI therapy (1 mg anastrozole, 2.5 mg letrozole) for at least 2 years. The first group of 19 patients had been taking, daily after meals, two gel capsules of Omega-3 Cardio (containing 300 mg of DHA and 200 mg EPA) and two gel capsules of evening primrose oil (containing 117 mg of gamma-linolenic acid (GLA) (Oenothera biennis, seed, Natural Wealth®, New York, USA) in a period of 12 weeks with regular therapy. The second group of 18 patients had been taking a placebo capsule, containing mineral oil. Serum lipids (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol) levels were determined on the same day the samples were collected using a clinical chemistry automated analyzer COBAS c-111 and commercial Roche diagnostics kits, according to the manufacturer's instruction. Fatty acid profiles were analyzed by gas chromatography. The results of this study showed that lipid profiles stay unchanged after 12 weeks of supplementation of n-3 and n-6 FA in patients receiving AI therapy. On the other hand, we showed significantly increasing levels of EPA, DPA, and DHA ($p<0.05$) and decreasing n-6/n-3 ratio ($p<0.05$), in the intervention group. Despite GLA supplementation, the level of this FA did not change after the intervention period.This study demonstrate that fish and primrose oil supplementation can improve fatty acid status in breast cancer patients receiving AI but have no influence on serum lipid profile. However, longer-term studies with a higher number of patients are needed to assess these effects.

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The impact of pH on coordination chemistry of tetracycline and Fe(III) in water

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Tetracycline (TC) is a common pollutant of waters that shows specific environmental matrix due to formation of complexes with metal ions [1]. Although the interaction with iron is probably one of the most assiduously investigated TC-metal system, the coordination chemistry in water is not fully understood. Herein, we analyzed the interactions of TC with Fe^{3+} at different pH values. UV-Vis spectra of TC in the presence of Fe^{3+} showed that two peaks arise at 440 and 530 nm at pH ≤ 5. At higher pH these peaks are not observed. At pH ≥ 7 peak at λ_{max} = 356 nm which corresponds to the absorption of TC BCD rings is bathocromically shifted to 374 nm due to phenolic-diketone moiety (pK_{a2} = 7.78) deprotonation. The appearance of additional peaks at 440 nm and 530 nm as well as broad absorption band in the range of 300–400 nm at pH ≤ 5 indicates formation of Fe^{3+}–TC complex. It appears that Fe^{3+} may bind to TC through phenolic-diketone moiety. The stability constants of Fe^{3+}–TC complex at different pH were calculated using Benesie-Hildebrand equation [2]. Cyclic voltammogram of TC in the presence of Fe^{3+} implicated TC stabilization by coordination to Fe^{3+} since the oxidation peak of TC (E_{pa} = 1090 mV) was not observed. The reduction potential of Fe^{3+} in the complex was shifted to lower values. This implies that TC acts as an electron donor ligand to Fe^{3+}. From the perspective of environmental chemistry, it is important to point out that the coordination of TC and Fe^{3+} shows pH dependence in relation to both, TC deprotonation and iron solubility.

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Antitumor effect of Ru(II) complexon A375 and HeLa cell growth, migration and adhesion ability

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Platinum-based complexes represent the mainstay of treatment for various cancer types. However, their usage is often restricted by numerous side effects or intrinsic and acquired resistance. Therefore, significant research efforts have focused on developing therapeutics based on other transition metals, such as ruthenium [1,2]. In this study, effects of transition metal complex, cis-dichlorobis (2,2′-bipyridyl-4,4′-dicarboxylic acid)ruthenium(II) (Ru(II) complex) were analyzed on A375 human melanoma and HeLa cell growth, adhesion ability andmigration. Cell viability assay indicated significant antitumor activity of Ru(II) complex on A375 (~60% of control) up to 72 h after treatment, but not on HeLa cells. However, analysis by clonogenic assay showed that growth of both cell lines was decreased 7 days after treatment. Growth inhibition was followed by G_1 phase cell cycle arrest (5–10% G_1 increase for A375 and 5–8% for HeLa cells compared to control). Moreover, Ru(II) complex increased adhesivity of A375 and HeLa cells by 11 and 16 % respectively and decreased cell migration, as shown by scratch assay. The obtained results indicate that the analyzed Ru(II) complex is a promising metallodrug, as itinduced growth inhibition of A375 and HeLa cells through induction of G_1 arrest and decreased metastatic potential of these cells through the increase ofadhesivity and decrease of cell migration.

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Sclareol, a natural compound, inhibits P-glycoprotein activity in cancer cells

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P-glycoprotein (P-gp) is often expressed at the cellular membrane of cancer cells where it plays a significant role in protecting cancer cells from extracellular assault. It works as an export transporter for many substrates - xenobiotics including chemotherapeutics. Several generations of P-gp inhibitors have been developed and studied but they have not yet been introduced into clinics. The most promising fourth-generation comprises natural compounds. In this study, we evaluated the potential of sclareol, a naturally occurring labdane diterpene, to inhibit P-gp activity in human glioblastoma (U87, and its resistant variant U87-TxR with P-gp overexpression) and non-small cell lung carcinoma (NCI-H460, and its resistant variant NCI-H460/R with P-gp overexpression) cell lines. To that end, we used the accumulation assays of fluorescent P-gp substrates (rhodamine 123 and doxorubicin) that were analyzed by flow cytometry. An increase in the accumulation of the P-gp substrate corresponds to the level of P-gp activity suppression. Our results showed that simultaneous application of sclareol (20 μM and 50 μM) with either rhodamine 123 (5 μM) or doxorubicin (20 μM) significantly increased their accumulation in resistant cells (U87-TxR and NCI-H460/R) than in their corresponding sensitive cells (U87 and NCI-H460). The doxorubicin accumulation was also considerably increased in sensitive U87 cells implying that sclareol may interact with doxorubicin through other mechanisms in glioblastoma cells (not only by P-gp inhibition). Further investigations are envisioned to reveal the mechanisms behind sclareol and doxorubicin interaction in glioblastoma cells.

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Rat brown adipose tissue thermogenic markers are modulated by estrus cycle phases and short term fasting

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Brown adipose tissue (BAT) thermogenic activity, contributes to the total energy expenditure and its modulation may become an attractive strategy to combat current obesity epidemic[1]. However, data concerning sex specific role of BAT function in the systemic energy homeostasis is lacking. Thus, we investigated BAT thermogenic markers in female rats during two phases of estrus cycle (diestrus and proestrus) under mild metabolic stress (six hours fasting). Female rats were divided into four groups (n=6) with respect to the estrus cycle phase (diestrus and proestrus) and treatmet (*ad libitum* access to food, and fasting). Proestrus and diestrus were chosen due to the highest and the lowest circulating levels of sex hormones, respectively. Expressions of uncoupling protein 1 (UCP1) and tyrosine hydroxylase (TH) were analyzed in interscapular BAT using Western blot analysis and immunohistochemistry. Our results show that UCP1 content in rat interscapular BAT was modulated by estrus cycle stages. Specifically, UCP1 amount was decreased in interscapular BAT of females during proestrus compared to diestrus. On the other hand, selected estrus cycle phases and mild metabolic stress interactively modulated TH content in interscapular BAT of female rats. Notably, depending on the phase of estrus cycle, short term fasting exerted distinct effects on TH protein expression. Overall, our results imply that during diverse stages of the estrus cycle, BAT thermogenic activity is modified in order to adjust total energy expenditure to create optimal conditions for potential onset of implantation.

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The effect of novel rosmarinic acid derivative on the pathogenesis of experimental autoimmune encephalomyelitis in rats

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Rosmarinic acid is a polyphenolic compound, abundantly presentin herbs of the Lamiaceae family. The aim of the study was to evaluate a recently developed rosmarinic acid derivative (RAd), with an enhanced ability of diffusion through biological membranes [1], in preclinical settingsof the central nervous system autoimmunity. To this extent, experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis was used. EAE was induced in DA rats by subcutaneous injection of autologous spinal cord homogenate [2], while treatment with RAd (30 mg/kg) started at 7 day post immunization and lasted for 15 days. Subcutaneous RAd administration successfully ameliorated EAE, leading to abbreviation of the disease duration and reducement of maximal, cumulative and meanclinical score. Also, RAd effects on draining lymph node cells (DLNC) isolated in the inductive phase of EAE and spinal cord immune cells (SCIC) obtained at the peak of the diseasewere evaluated. *In vitro* treatment with RAd (5 μM) reduced production of major encephalitogenic cytokines, *i.e.*interferon (IFN)-γ and interleukin (IL)-17, both in DLNC and SCIC. The reduction of IFN-γ and IL-17 production under the influence of RAd was also detected in the $CD4^+$ T cells purified fromDLNC, thus suggesting that RAd had a direct effect on $CD4^+$ T cells. Additionally, the effects of *in vitro* treatment with RAd were examinedon macrophages (Mf), immune cells with important role in EAE pathogenesis. Treatment of peritoneal Mf,obtained from non-immunized DA rats, with RAd (25 μM) led to reduction of NO and IL-6 production, exterted no effect on IL-1beta production, and elevated tumor necrosis factor production in Mf. Expression of MHC II and co-stimulatory molecule CD80, the phagocytic ability and the production of reactive oxygen species in RAd-treated Mf were also downregulated. Our results imply that RAd possesses anti-inflammatory and anti-encephalitogenicproperties. Thus, further studies on the mechanisms behind the observed effects and their relevance for the therapy of multiple sclerosis are warranted.

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Lactobacillus reuteri alleviate the severity of chemically-induced colitis in mice

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Inflammatory bowel diseases (IBDs) are associated with reduced diversity and decreased contribution of probiotic bacteria within the comensal microbiota. A range of probiotic bacteria, including the genera *Lactobacillus*, could be protective in IBDs due to their capacity to trigger anti-inflammatory mechanisms [1]. The aim of the study was to explore immunomodulatory impact of probiotic bacteria. *Lactobacillus reuteri* (LR) and its contribution to the alleviation of severity of a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. Colitis was induced in outbred Intor Swiss:Albino mice by a single administration of TNBS dissolved in 50% ethanol (day 0). LR in phosphate buffer solution ($5x10^6$ CFU/mL, p.o.) was given daily, 7 days prior and/or 7 days after day 0 (n=10 per treatment). Mice subjected to the induction of colitis without LR treatment were referent. A significant reduction in disease severity was noticed only in group treated by LR prior and upon colitis induction. The alleviation of disease severity correlated with lessening of local infiltration of leukocytes, a decrease in local inflammatory response (myeloperoxidase activity, production of superoxide ions, IL-6, and TNFα), and an increase in local production of IL-10. Presented results show that LR triggers a regulatory mechanism which alleviates severity of TNBS-induced colitis in mice, implying that it is worth further evaluation in prevention and treatment of IBDs.

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Cytotoxic effect of chalcone analogue on tumor cells of murine and human origin

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Chalcones represent a type of flavonoids which are present in vegetative and reproductive organs of plants. They are metabolic progenitor molecules for several flavonoids and isoflavonoids. The results of many studies have shown that the chemical structure of chalcones is responsible for their antitumor, antioxidant and anti-inflammatory effects [1-3]. Changes in the chemical structure of chalcones have produced many chalcone derivatives and analogues that can be used to develop new anticancer therapeutics. According to their chemical structure, chalcones are α, β – unsaturated ketones with one aromatic ring bonded to the carbonyl group and another aromatic ring bonded to the olefin group. However, compared to other chalcone analogues in previous studies, the chalcone analog in this study does not contain the aromatic A ring. The aim of our research was to investigate the cytotoxic effect of the (E)-1-(3-methoxy-4-propoxyphenyl)-5-methylhex-1-en-3-one on tumor HCT-116 cells (human colon cancer cell line) and CT26 cells (murine colon cancer cell line). The viability of the treated cells was evaluated using MTT assay after 48h of treatment. The concentrations of the tested chalcone analogue were 3, 10, 30, 100 and 300 μM. Morphological changes of both control and treated HCT-116 and CT26 cells were visualized and compared under phase contrast microscopy. The results of this study showed that chalcone analogue exhibits strong cytotoxic effect on colon tumor cells. Also, our results indicated a significant reduction in the number of treated HCT-116 and CT26 cells compared to the number of control cells, and intensity of morphological changes of the treated cells is directly dose dependent. The chalcone analogue is a good agent for future in vivo research due to its effective cytotoxic effect on CT 26 cells.

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Hypoglycemic effect of Cabernet Sauvignon wine

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There is a growing awareness of the influence of lifestyle and diet on the development of coronary heart disease, atherosclerosis, diabetes, neurodegenerative disorders, ageing and cancer. A market analysis has shown that consumers believe that wine is healthier than other alcoholic beverages [1]. Therefore, wine is one of the most popular drinks, which is consumed for hedonistic, but also health reasons. As a part of a broad investigation about the bioactivity of wines from Serbia, the aim of this study was to compare the hypoglycemic effect of wines from Serbia, Macedonia, France and Italy. The inhibition potential of Cabernet Sauvignon wines was evaluated in *in vitro* assays related to the activity of two digestive enzymes involved in carbohydrate metabolism: α-amylase and α-glucosidase [2]. The HPLC-UV/VIS technique was applied to elucidate differences in the phenolic profile of samples. All analysed wines manifested some hypoglycemic potential: IC_{50} ranged from 2.38 to 5.02 mg/mL (α-amylase) and 0.17 to 1.40 mg/mL (α-glucosidase). Compered with acarbose, a well-known inhibitor of α-amylase and α-glucosidase, all samples had lower activity. The phenolic profile showed a dominance of gallic acid (41.5-103 mg/L) and catechin (22.9-57.6 mg/L). Malvidin-3-*O*-glucoside (5.50-80.0 mg/L) was leading anthocyanin, while the content of resveratrol ranged from 0.80 to 3.70 mg/L. Obtained results showed that Cabernet Sauvignon wines from Serbia have a comparable hypoglycemic effect of renowned European wines, such as French Cabernet Sauvignon.

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Antibacterial activity of polypiridinearene ruthenium(II) complexes

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Few studies concerning antibacterial activity of ruthenium complexes have been published but all of them have been performed on small limited number of strains [1,2]. In this study complex 1 ([(η^6-toluene)Ru(ppf)Cl]PF_6) and complex 2 ([(η^6-*p*-cymene)Ru(ppf)Cl]PF_6) where ppf is pyrido[2′,3′:5,6]pyrazino[2,3-f][1,10]phenanthrolinewere investigated as antibacterial agents. Previous study proved the cytotoxicity of these compounds [3]. The structural difference between 1 and 2 reflected through the presence or absence of isopropyl group onto one of the ligand (toluene), resulted in significant different activity against melanoma cells [3]. Five strains of Gram-positive bacteria (*C. sporagenes, M. flavus, B. subtilis, S. lutea and S. aureus*), and four strains of Gram-negative bacteria (*S. enteritidis, P. vulgaris, P. aeruginosa and E. coli*) were used for study of antibacterial activity of 1 and 2. While 2 did not show activity against most strains, complex 1 showed good results against all strains, but the best against *Clostridium sporagenes* and *Proteus vulgaris*. The obtained antibacterial activity of the complexes was in accordance with the nuclease activity obtained by plasmid DNA cleavage study. Complex 2 showed higher damaging effect to supercoiled DNA, than complex 1. Minor structural modifications of arene moiety resulted in major difference in activity of the complexes.

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Effect of the *Thymus lykae* Degen et Jav. methanol extract on the erythrocyte membrane

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Thymus lykae Degen et Jav. is endemic aromatic species from central Balkan. In previous studies compounds which were identified from essential oil of *T. lykae* shown significant antimicrobial and antioxidant activities [1]. The aim of this study was to determine the possible effect of the different concentrations of methanol extracts of leaves of *T. lykae* on the erythrocyte membrane. The rats red blood cells were treated with *T. lykae* methanol extract at concentrations of 50, 100 and 200µg/mL and incubated at 37°C for 1h [2]. The erythrocytes which were incubated with phosphate buffered saline was used as controls. Blood smears were stained with May-Grunwald-Giemsa, rinsed with buffer and dried. It was shown that methanol extracts with increasing concentrations caused changes on the erythrocyte membrane [3]. The most significant changes, the increased of echinocytes forms, were observed for methanol extract at concentration of 200µg/mL. It could be assumed that constituents of the methanol extracts of *T. lykae* bind to erythrocytes surface, cause formation of echinocytes, and protect red blood cells from potential oxidative stress and damage.

Acknowledgements

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Heat-induced accumulation of proline and yield components in different cereal varieties

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Growth, development, and yield of cereals are influenced by high temperature. Recent studies have shown that an important factor in plant adaptation to high temperature can be the proline accumulation. Proline is an amino acid involved in a series of metabolic processes in plants, such as protein stabilizer, antioxidant and osmolite. The aim of this research was to determine the effect of high temperature on the content of proline and yield elements, in different cereal varieties during two vegetative seasons, to compare it with the content of proline in moderate temperature conditions and to isolate varieties more resistant to conditions of heat stress. For research, plant material of 10 genetically divergent cereal varieties was used. The results during the first experimental year showed that under moderate air temperature conditions proline content was 0.66 µmol/g fresh plant (fp), and in the second experimental year 0.77 µmol/g fp. Under conditions of heat stress proline accumulation increased during both years. In the first year, the average content of proline was 2.17 µmol/g fp, and in the second experimental year the average content was 2.51 µmol/g fp. The obtained results showed that heat stress led to increased proline accumulation in analyzed cereal varieties. Compared with other cereal varieties, wheat varieties Zvezdana and Pobeda were characterized by higher contents of proline and yield in conditions of heat stress. Statistical analysis and correlation of the results of proline accumulation and yield elements showed that more resistant varieties to heat stress conditions have higher proline accumulation andhigher yields.

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Effects of cadmium on oxidative metabolism and motility of human placental cells in chemically-induced hypoxia

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Elevated cadmium concentrations are indicated as serious risk factor in the pathogenesis of various pathological forms of pregnancy related to an impaired migratory and invasive potential of trophoblasts, such as preeclampsia. The aim of this study was to investigate *in vitro* effects of increasing cadmium concentrations on human trophoblast cells under chemical hypoxia, the state that corresponds to physiological conditions in first trimester of pregnancy and evaluate theirpotential contribution to metabolic and trophoblast motility disorders under hypoxia, leading to a better understanding of role of cadminum in the pathogenesis of gestational disorders. Cell viability, migration capacity and the parameters of oxidative metabolism (such as superoxide anion radical and nitrites) were evaluated. We examined the effects of cadmium treatment in four concentrations (0.1, 1, 5, and 10 μM) at two periods of incubation (24 and 72 h) under normoxia and conditions of chemically induced hypoxia on human JEG-3 choriocarcinoma cell line. In conditions of chemical hypoxia, cadmium shows particularly intense effects on the disturbance of metabolism and mobility of human trophoblasts in relation to normoxic conditions, where its effect on the examined parameters is insignificant. In hypoxic conditions, cadmium significantly reduces the production of superoxide anion radicals, which indicates a strong decline in the mitochondrial trigger metabolism and correlates with reduced trophoblast viability. Cadmium induced a strong decrease in nitrite production in hypoxia, and also showed a promigratory effect, which in combination with severely disturbed redox balance and disturbed nitite signaling may indicate its contribution to inadequate trophoblast motility. These results indicate a high degree of sensitivity of human trophoblasts to cadmium in the early stages of embryonic development and placental formation, when proper proliferation and invasiveness of trophoblasts is crucial for establishing optimal levels of trophic exchange with the endometrium.

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Antioxidant and antidiabetic potential of hawthorn (*Crategus mopnogyna* Jacq. L.) fruits originating from Serbia

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Hawthorn (*Crategus mopnogyna Jacq.* L.) is an endemic member of the *Rosaceae* family that grows in Europe, Africa, and Asia. Its small dark-red fruit, which ripens in mid-autumn, is used for different culinary purposes. It has been demonstrated that hawthorn fruit possesses potent antioxidant and free radical scavenging activities, due to the presence of different bioactive compounds, such as phenolic compounds mainly flavonoids which shows anti-diabetic, anti-carcinogenic and anti-inflammatory effects [1]. In this study, ripe hawthorn fruits originated from a rural area of South Banat District in the Republic of Serbia were studied for antioxidant properties and α-glucosidase (α-Glc) inhibitory activity. For the determination of total phenolic content (TPC) and total flavonoid content (TFC) in ethanolic extract of *C. mopnogyna* fruits spectrophotometric methods were used. The antioxidant activity was evaluated using $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging potential assays [2], whereas α-Glc inhibitory activity of the extract was examined as decribed previously [3]. The content of TPC and TFC in *C. mopnogyna* fruits extract were 14.9 ± 0.7 mg GAE/g and 3.51 ± 0.05 mg RUE/g, respectively. The total antioxidant capacity of the extract was significant (100.4 ± 6.7 mg AAE/g) while the IC_{50} values for $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activity were not that prominent, especially compared to the standard antioxidants. In α-Glc inhibitory activity assay, *C. mopnogyna* extract with an IC_{50} of 335.71±6.68 μg/mL showed lower activity when compared with acarbose, used as a standard drug. Due to the presence of significant naturally occurring bioactive compounds with therapeutic properties, hawthorn is a valuable wild-growing plant. Further research and clinical trials are needed to evaluate the link between the chemical compositions of hawthorn, and the mechanisms of action in the treatment of various diseases [1].

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Promising anti-inflammatory potential of methanol extracts of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (*Lamiaceae*)

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Hyssop (*Hyssopus officinalis* L.) is a well-known aromatic plant that is used in traditional medicine, as well as raw material for the food and cosmetics industry. This study aimed to assess the anti-inflammatory properties of characterized hyssop methanol extracts. The *in vitro* anti-inflammatory activity of six *H. officinalis* methanol extracts was evaluated using cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme assays. In addition, an *in silico* study of the inhibitor efficiency of the main compounds of methanol extracts (rosmarinic acid - RA and chlorogenic acid - CA) towards COX-1 and COX-2 receptors, was performed using molecular docking. All analysed *H. officinalis* extracts at a concentration of 20 μg/mL were able to inhibit COX-2 enzyme activity (54.04 - 63.04%). The preliminary computer simulation results indicate that main compounds of methanol extracts (RA and CA) have a better inhibitory activity to the COX-1 and 2 receptors than ibuprofen. Results of the current study support traditional use of hyssop in the treatment of different inflammatory conditions, but also call for further investigation of its mechanism of action.

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Selected medicinal plant extracts as a potential preservative in cosmetic products

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This research aims to evaluate the potential of three commonly used medicinal plants in natural skincare products, *Calendula officinalis* L. (marigold), *Salvia officinalis* L. (sage), and *Hypericum perforatum* L. (St John's-wort), as a natural preservative in a topical cream formulation.[1] Ethanolic extracts of plant species were prepared and after evaporation of ethanol their antimicrobial activity against different bacterial and fungal species was determined. The cream formulations used for the determination of the preservative potential of extract were prepared using sterile demineralized water, glycerin, olive, almond sweet and coconut oils, glyceryl stearate/cetearyl alcohol/cetyl alcohol as emulsifying agents, and extracts at a concentration of 4% (w/w). Cream formulations were stored at room temperature or 37°C and after two months of continuously simulated usage, diluted cream samples were transferred to microbiological agar plates for the determination of effectiveness of extracts as preservatives in creams. The effect of extracts was compared with samples prepared without any added antimicrobial substances and with control samples with benzyl alcohol/dehydroacetic acid (1%) as a synthetic preservative. The results showed that examined extracts inhibited the bacteria and fungi growth in cream formulations during 60 days compared with samples without any preservative added. The antimicrobial effects of extracts were comparable with a tested synthetic preservative in a cream formulation. These results indicate that under the test conditions, selected extracts could be considered as a natural alternative for preservatives in cosmetic formulations.

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Relationship between single nucleotide polymorphism *TNF-α* -308 G>A (rs1800629) and *TNF-α* gene expression levels in colorectal cancer patients

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Tumor necrosis factor α (TNF-α) is among the most studied immunomodulatory and proinflammatory cytokines with ambigous role in colorectal cancer (CRC) development. TNF-α dual effects are further complicated by the existence of *TNF-α* single nucleotide polymorphysm (SNP rs1800629). Studies have shown that G/A substitution at the -308 position of the gene promoter leads to changes in the circulating TNF-α levels, while being related to increased risc for CRC development as well. The aim of our study was to examine *TNF-α* gene expression levels in peripheral blood mononuclear cells (PBMCs) in CRC patients and healthy individuals. We also aimed to inspect the influence of *TNF-α* (rs1800629) polymorphic variants on *TNF-α* mRNA levels. Presented study included 73 patients with CRC and 80 healthy subjects. Routine PCR method was employed to determine *TNF-α* gene expression levels, as well as for genotyping purposes. Reduction in *TNF-α* mRNA levels was observed in CRC ($p<0.050$), while genotype distribution between patients and controls did not differ ($p=0.614$). The following relative frequencies of genotypes were obtained: CRC group [GG = 0.79 GA = 0.21 AA = 0]; control group [GG = 0.80 GA = 0.19 AA = 0.01]. Given the low prevalence of the risk genotype in the presented groups, the GA and AA genotypes were merged in order to assess the difference in *TNF-α* mRNA levels between GG and GA+AA genotypes separately in CRC and control group. *TNF-α* gene expression levels didn't differ across the genotype groups, neither in patients, nor in controls ($p= 0.445$, $p = 0.656$, respectively). This study did not establish an association between *TNF-α* SNP rs1800629 and the risk for CRC development, or TNF-α gene expression levels.

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Amyloid fibril formation of ovalbumin in the presence of heavy metal ions, lead and cadmium

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Due to the marginal thermodynamic stability of the native state of proteins, many peptides undergo conformational and structural changes while in a more thermodynamically destabilizing environment. The conditions of such an environment, as well as the amino acid sequence of the protein therein, ultimately determine the final structure of said protein. Under specific, highly destabilizing conditions, protein aggregation becomes much more common and the formation of highly-ordered, repeating, and thermodynamically stable structures known as amyloid fibrils becomes favorable [1]. The extent of fibrillation is further determined by environmental conditions, including the presence of certain agents or metal ions. The effect of the latter on the fibrillation process of ovalbumin was investigated. Ovalbumin samples were incubated at high temperatures and under highly acidic conditions, in the presence of heavy metal ions, cadmium and lead. The formation of amyloid fibrils was subsequently monitored using Thioflavin T and 8-anilinonapthalene-1-sulfonic acid fluorescence, attenuated total reflectance Fourier-transform infrared spectroscopy, as well as microscopic techniques. The obtained results show a notable dependency of the formation of amyloid fibrils on the presence of cadmium and lead salts, which is further pronounced with the increase of the metal ions' concentration.

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Exogenous alpha-ketoglutarate impair differentiation and maturation of human dendritic cells

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Alpha-ketoglutarate (AKG) is a crucial intermediate in cell metabolism. Exogenous AKG has been shown to extend the lifespan by regulating the cellular response to calorie restriction. However, how the exogenous AKG affects immune responses is unknown, particularly those mediated by critical immunoregulatory and dendritic cells (DC). Using a model of human monocyte-derived DC, we found that exogenous AKG (10 mM or 50 mM) does not induce autophagy in DC (according to LC3II expression) but impairs the differentiation of DC from monocytes (according to CD1a and CD14 co-expression). Additionally, AKG inhibited LPS/IFN-γ-induced upregulation of NLRP-3, IL-1β, HLA-DR, CD83, CD86, CD40, CCR7, CD209, IL-33, IL-10, and IL-12p70 expression in DC, but potentiated the capacity of these cells to express TGF-β, CD73, and IL-23 in a dose-dependent manner. Although AKG-treated DC displayed a lower ability to stimulate proliferation of alloreactive T cells than control mature DC, the normalized number of $CD4^+ROR\gamma t^+IL17^+$ (Th17) and $CD4^+GATA3^+IL\text{-}4^+$ (Th2) cells was higher. The number of $CD4^+$T-$bet^+IFN\text{-}\gamma^+$ (Th1) and $CD8^+IFN\text{-}\gamma^+GranzA^+Perf^+$ (CTL) was lower in co-cultures with AKG-treated DC compared to corresponding control DC. Moreover, AKG increased the capacity of DC significantly to induce $CD4^+CD25^{hi}CD127^-FoxP3^+$ Tregs in a dose-dependent manner. These results suggested that, while exogenous AKG could have beneficial effects on lifespan, the quality of life might be compromised due to its immunomodulatory effects related to the reduction of Th1 mediated immune responses.

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Efficient production of highly purified Late Embryogenesis Abundant (LEA) protein from *Arabidopsis thaliana* by recombinant DNA technology

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Late embryogenesis abundant (LEA) proteins are induced in cellular dehydration, such as freezing, drought, or desiccation. They can be involved in antioxidative defense, ion sequestration, and structural stabilization of both membranes and enzymes during freezing or drying, while by forming intracellular proteinaceous condensates they increase structural integrity and intracellular viscosity of cells during desiccation [1]. The genome of the model plant *Arabidopsis thaliana* contains 51 genes encoding LEA proteins[2]. The majority of these LEA proteins (35%) belongs to Pfam LEA_4 (PF02987) family. *In silico* analysis suggested that these proteins are highly hydrophilic proteins with significant intrinsically disordered protein (IDP) properties. In order to evaluate structural properties and possible functions of LEA_4 protein family under different water content, a representative AtLEA25 protein (At2g42560, 635 aa), naturally located in the cytoplasm of seeds[3] was obtained in *Escherichia coli* by recombinant DNA technology. Although this technology has been traditionally used to over-express and purify various globular proteins, numerous reports have shown that the IDPs, due to their structural plasicity are naturally highly susceptible to proteolytic cleavage. To conduct structural and functional studies we developed a robust method to produce highly purified (>95% pure) AtLEA25 with no detectable amount of protein breakdown products.

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The profile of carotenoids and phenolic compounds in 20 different varieties of pumpkin pulp cultivated in Serbia

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High nutrition value, amount of bioactive compounds and non-demanding cultivation make pumpkin pulp and its products a great and valuable plant food candidate in human nutrition and medical purpose. Pumpkins are characterized by the presence of carotenoids as the most dominant and studied compounds. This plant also contains phenolics that can contribute to biological activity. Consumption of food that is rich in both, carotenoids and phenolics has been correlated with beneficial physiological and immunological effects which results in lower risk of various diseases.In this study, pumpkin pulp derived from 20 different varieties of pumkins grown at the Institute of Field and Vegetable Crops in Novi Sad, Serbia were analysed for their polyphenol and carotenoid content. These varieties belong to two basic species: *Cucurbita maxima* and *Cucurbita moschata*. Phenolic content of pumpkins was determined by the LC-MS/MS technique. The most present phenols were quinic acid (QA), p-hydroxybenzoic acid (PHBA) and amentoflavone (AF). The highest content of phenolics was found in Mo 29-1 (QA – 28.74 ng/mg; PHBA – 162.39 ng/mg; AF – 40.08 ng/mg), Max 1 (QA – 36.48 ng/mg; PHBA – 23.21 ng/mg; AF – 100.18 ng/mg) and MAX 105 (QA – 33.71 ng/mg; PHBA – 65.18 ng/mg; AF – 57.37 ng/mg). The carotenoid composition was determined by HPLC-DAD technique. In *C. moschata* α-carotene was the most dominant carotenoid. The highest content of α-carotene was found in Mo 39 (0.659 mg/g) thran β-carotene, lutein and zeaxanthin were dominant carotenoifd in Mo 39 (0.499 mg/g), Mo 31 (0.004 mg/g) and Mo 31 (0.004 mg/g), respectively.In *C. maxima* β-carotene was the main carotenoid. The highest content of β-carotene was found in Max 118-2 (0.548 mg/g) and the greatest amount of α-carotene lutein and zeaxanthin was found in Max 113 (0.014 mg/g), Max 65-1 (0.038 mg/g) and Max 65-1 (0.407 mg/g), respectively. The above results indicate that poorly studied pumpkin pulp is a great source of bioactive compounds can find a signifacant place in food industry and could have important effect on selection in plant breeding.

Evaluation of pathophysiological effects of clinical concentrations of proinflammatory interleukin-6 detected in patients with COVID-19 on homeostasis of human trophoblast cells

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Proliferation, survival and invasion of extravillous trophoblasts on maternal tissue are the key processes for successful establishment and propagation of the fetal development. COVID-19 represents current major health issue globally affecting hundred of millions of people worldwide and causing the damaging of large number of tissues. Proinflamatory cytokines are one of the underlying mechanisms of COVID-19 inflamatory complications.The aim of this study was to investigate the potential pathophysiological effects of concentrations of proinflamatory interleukin-6 (IL-6) present in clinical cases of COVID-19 on physiology of JEG-3 trophoblast cells. The experiment was performed in normoxia and atmospheric hypoxia (3% of O_2) conditions, which suit oxygen environment of trophoblast in the first trimester of gestation. Cell viability, migration capacity and the parameters of oxidative metabolism, superoxide radical anion ($O_2^{\cdot -}$), nitrites and gluthatione were evaluated. $O_2^{\cdot -}$ production and levels of both oxidized and reduced glutathione were significantly increased after several applied IL-6 concentrations, implying the induction of intensive redox imbalance. Significant reduction of NO levels compared to optimal physiological values may indicate disturbance in NO signaling pathways which could refelct on perturbances of cell motility, regulation of vascular reactivity and control of blood flow through uteroplacental tissue. The applied treatments induced the elevation in migratory capacity of trophoblast cells compared to physiological concentrations. The results indicate that IL-6 in concentrations present in COVID-19 complications has disruptive effects on redox homeostasis, migration potential and cell viability in first trimester trophoblasts. The obtained data suggest the potentially pathological effects of IL-6 in pregnant women after hospitalisation caused by COVID-19.

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Bioinformatic analysis of ovalbumin and HEWL amyloidogenic peptides

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The tendency to form amyloid fibrils depends on the amino acid sequence of a peptide. The application of bioinformatics enabled searching for patterns among known amyloidogenic sequences and the development of tools for their detection in novel sequences. Regions with sequences prone to amyloid aggregation seem to be widespread among proteins. The results are supporting their organization in amyloid fibrils under optimal conditions regardless of the rest of the polypeptide. Three amyloid prediction tools were applied for screening of amyloidogenic sequences of ovalbumin and hen egg-white lysozyme (HEWL) – proteins reported to readily form amyloid fibrils. ZipperDB tool is based on fitting amino acid sequence in known amyloid structure characteristic for sequence NNQQNY[1], TANGO and PASTA predict the propensity to form aggregation-specific β-sheet based on interactions between amino acid residues. The analysis of ovalbumin and HEWL amyloidogenic regions predicted using ZipperDB, TANGO and PASTA were applied to examine the reliability of these methods compared to experimentally determined amyloid-core sequences. Comparative analysis showed that various parameters result in different peptides predicted as amyloidogenic. Nevertheless, common peptides are found using two or even all bioinformatics methods, and some of them appear to match with experimentally determined. The best match with amyloidogenic core regions of ovalbumin was found using the ZipperDB tool, but there are still many false-positive results. In the case of HEWL, the essential region involved in amyloid core formation was not in good correlation with predicted amyloidogenic regions. It led to a conclusion that prediction tools are efficient in the case of amyloid fibrils formed around β-sheet existent in the native state of protein as was shown for ovalbumin.

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Use of acid-extracted fiber from butternut squash pulp for complex coacervate preparation

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The interest in production of non-toxic, highly biocompatible biopolymer-based systems, such as complex coacervates, is ever growing. These systems can be formed between polyanions and polycations due to electrostatic interactions, and can be used for drug encapsulation and delivery. Encapsulation by coacervation is an innovative method for the controlled release of bioactive molecules due to its possible high loading capacity and the ease of delivery of encapsulated content induced by the change in pH, mechanical stress, or temperature. Pectic polysaccharides are among the most frequently used negatively charged coacervate constituents. As the market demand for pectic substances continually increases, butternut squash pulp can be used as unconventional source of fiber rich in pectic substances. The acid-extracted fiber from butternut squash could therefore be used as a constituent in oral drug delivery systems. The aim of this study was to evaluate pectin-rich fiber from butternut squash pulp for potential application in the formulation of complex coacervates. Pectin-rich fiber was extracted by the means of conventional hot acid extraction, precipitated with ethanol and dried. Extraction yield amounted to 59.1 mg/g_{DW}, and extracted squash fiber was characterized in respect to galacturonic acid content (29.0%, w/w), degree of methoxylation (76.6%, n/n), degree of acetylation (3.9%, n/n) and molecular weight (11.2 kDa). In complex coacervation formulation, squash fiber was used as a polyanionic component, while bovine gelatin was used as a polycation. Formed coacervates showed good structure strength and good stability in simulated gastrointestinal conditions, making acid-extracted squash fiber a suitable component for complex coacervate preparation with possible use for controlled drug delivery.

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Testicular toxicity induced by chlorpyrifos and imidacloprid: Comparative study

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Various toxicants from the environment could affect male reproductive system. Toxic effects of pesticides on non-target species have become a global problem. In the present study, testicular toxicity of two widely used insecticides chlorpyrifos and imidacloprid was evaluated. Male rats were divided in three groups, control and experimental groups treated with chlorpyrifos (35 mg/kg b.w.) and imidacloprid (70 mg/kg b.w.) two times a week for one month via gavage. The mortality was noted throughout the experiment in group treated with chlorpyrifos. The exposure of chlorpyrifos and imidacloprid both stimulated superoxide dismutase and catalase activities in testis. No significant changes were found in activities of glutathione peroxidase and glutathione reductase in both experimental groups, while glutathione s-transferase was significantly higher in chlorpyrifos group. Lipid peroxidation was induced in experimental groups but was more pronounced in the group administered with chlorpyrifos. Reduced glutathione and the activity of cholinesterase did not significantly differ among the groups. The concentration of vitamin C was higher in rats treated with imidacloprid. The results from the present study showed that chlorpyrifos and imidacloprid both induced oxidative stress in rat testicular tissue. The use of these insecticides should be limited due their deleterious effects on the living world, including humans and should be replaced with less harmful insecticides.

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High lactate dehydrogenase activity is significant mortality predictor in a 6 months study on end stage renal disease patients

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Chronic low-grade inflammation, oxidative stress, cardiovascular disease (CVD), anemia, hypertension, hyperphosphatemia are hallmarks of the end-stage renal disease which could be even complicated with hemodialysis (HD) procedure [1]. Lactate dehydrogenase (LDH) enzyme is cellular and ubiquitous molecule which occurence in circulation is a marker of organ damage [2]. LDH increase in HD patients is connected with increased risk of all-cause and CVD mortality [3]. The aim of our study was to find biochemical parameters - mortality predictors in a group of 130 HD patients. During the 6 months period of study extent 13 patients died (10%) and Kaplan-Meier survival analysis was performed to find significant mortality predictor among all measured parameters. Results indicated increased LDH activity is significant mortality predictor (Log rank=7.9, p=0.005); average survival time for deceased patients was 3.0 (2.8-3.8) months. Risk LDH value was considered upper tertile value for this HD patients group (375 U/L). 30% of patients in this study, and 9 of 13 deceased patients had LDH above this cut-off value. Deceased patients had LDH activity averagely 387 (345- 478) U/L, while survivors had significantly lower values 333 (289-387) U/L, p=0.012 (Mann-Whitney U test). LDH activity increase in HD patients could be a warning sign for clinicians, reflecting the more extensive kidney or cardiovascular system damage, pronounced inflammation and consequence of hemodialysis technique. Serum LDH activity monitoring in HD patients could help recognizing subjects who are at risk of disease exacerbation.

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Antioxidant potential and protein interactions of four tea plant extracts

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Polyphenols are a large group of natural organic compounds mainly found in plants with diverse protective and metabolic functions. Phenolic compounds are well known for their antioxidant properties and the name "antioxidant" is mostly associated with them[1]. It's also known that phenolic compounds, especially tannins, interact with proteins in various significant and distinct ways. This study was focused on examining these characteristics on aqueous extracts of tea plants local to Serbia (freshly picked *Satureja montana*, *Mentha spicata*, *Salvia officinalis* and *Matricaria chamomilla*), which are known to have various amounts and types of phenolic compounds. We examined total concentration of phenolic compounds, tannins, flavonoids, antioxidant activity and interactions with bovine serum albumin and whey protein. It was concluded that *S. montana* extract had the highest concentration of polyphenols and tannins. Flavonoid concentration was measured using the aluminium-chloride method and it was concluded that *S. officinalis* had the highest flavonoid content. Antioxidant activities were measured using DPPH, FRAP and ABTS, which are Single Electron Transfer (SET) mechanism-based antioxidant tests and ORAC which is a Hydrogen Atom Transfer mechanism-based antioxidant test. It was concluded that SET mechanism-based antioxidant activities correspond to the total concentration of polyphenols and tannins, which meant that *S. montana* extract had the highest antioxidant activity. *S. officinalis* extract exhibited the highest antioxidant activity measured by the ORAC assay, which corresponded to the highest Flavonoid concentration. Interactions between plant extracts and bovine serum albumin were measured via spectrophotometric and spectrofluorimetric titrations. It was concluded that *S. montana* exhibited most pronounced interactions with the protein. Nature of such interactions is still unknown but using SDS-PAGE it was concluded that proteins exhibit significant structural changes after interacting with plant extracts.

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Optimization of expression, purification and HRMS characterization of recombinant N-protein fragment from SARS-CoV-2

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Nucleocapsid (N) protein is the most abundant SARS-CoV-2 virus derived protein and strong immunogen which can be used as a component of the immunological tests for the diagnosis of SARS-CoV-2 infection. Recombinant fragment of N-protein (58–419 aa) was expressed in *E. coli* in a soluble form using developed optimized protocol of expression (16-18h, 37 °C, 0.4 mM IPTG). After lysis of cells, N-protein from soluble fraction of lysate was purified using optimized protocol for purification by immobilized metal affinity chromatography on Ni-Sepharose in two repeated steps under different elution conditions. Obtained fraction of N-protein after the second chromatography was desalted and concentrated using phosphate buffer solution and ultrafiltration. The purity of isolated N-protein was deterrmined by SDS PAGE, while high resolution mass spectrometry was used for its characterization. Isolated N-protein was over 90% purity and identified as the most intense and abundant protein fragment, with PEAKS PTM score of 508 and sequence coverage of over 70%, including 173 unique peptides.

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Synthesis of ferrocenyl-based pyrazoline derivatives and their biological evaluation

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The medicinal application of ferrocene is currently an active area of research, and many reports show its activities. Ferrocene moiety is widely applied in biological systems [1] and in the design of novel drugs [2]. Incorporation of ferrocene instead of the aromatic core usually causes unexpected changes in the biological properties of many well-known drugs [3]. Herein, we synthesized new ferrocenyl compounds with similar structure as chalcones, chalcone analogues, as substrates for the next synthetic step. In reaction of these compounds with hydrazine hydrate in formic or propionic acid, new ferrocenyl-based pyrazolines were synthesized. The structures of the new compounds were determined by IR and NMR methods. Synthesized pyrazoline derivatives were tested for their biological activity and demonstrated moderate *in vitro* antimicrobial activity towards different strains of bacteria and fungi.

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Anticancer potential of *Alchemilla vulgaris* L. on triple negative breast cancer cell lines

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Breast cancer is still a major concern among cancers occurring in women, as it is a second leading cause of death after lung cancer [1]. Considering that plants can modulate numerous signaling pathways in cancer cells, the aim of the study was to analyze the anticancer potential of *Alchemilla vulgaris* L. methanol extract on human triple negative MDA-MB-231, MDA-MB-468 breast cancer cell lines and healthy MRC-5 cells. Cytotoxic activity was analyzed using MTT assay. Relative expression of target genes involved in apoptosis, biotransformation of xenobiotics, cell-cell junction and migration process was tested with qRT-PCR method. According to obtained results, methanol extract of *A. vulgaris* significantly reduced cancer cell viability of MDA-MB-231 cells, IC50 value after 24 h was 261.87 µg/mL. They were more sensitive compared to MDA-MB-468 who reacted on continual "chronic" treatment; IC50 value after 72 h was 268.43 µg/mL. There was no significant cytotoxic effect on normal MRC-5 cells suggesting selective effect of tested treatment. Methanol extract inhibited the relative expression of all analyzed genes, *Bax, Bcl-2, Vimentin, E-cadherin, MMP9,* and *CYP1b* in breast cancer cell lines. The MDA-MB-468 cells were generally more sensitive to treatment compared to MDA-MB-231 cells. There is an evident cell specificity of treatment between healthy and cancer cells. The results showed anticancer potential of *A. vulgaris* via modulation of target molecular pathways in triple negative breast cancer cells.

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Biological effects of vanillin derivatives (enones) on HeLa cells in vitro

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The research over the last decades has focused on the synthesis of compounds that exhibit efficient antitumor effect selectivity, and represent analogs and derivatives of natural products [1,2]. To this group of compounds belong analogs of curcumin and vanillin - enones that contain unsaturated double bond, and differ in the substituent on the carbonyl C-atom. Vanillin derivatives show numerous pharmacological effects, including antitumor activity. Due to the wide range of mechanisms of action of these newly synthesized enones, these compounds represent ideal agents for examination and potential implementation in the treatment of malignancy. The aim of this study was to examine the cytotoxicity of enones: (E)-2-(2-methoxy-4-(3-oxobut-1-en-1-yl) phenoxy) acetic acid and (E)-2-(2-methoxy-4-(4-methyl-3-oxopent-1-en-1-yl) phenoxy) acetic acid on human cervical cancer cells (HeLa) and healthy lung fibroblasts (MRC-5) after 48 h of treatment. The cytotoxicity of the enones was assessed using the MTT assay. The tested concentrations of the enones were 3, 10, 30, 100 and 300 μM. Changes in the morphology of treated cells relative to untreated HeLa cells were assessed using phase contrast microscope. The results of the study showed that two enones exhibit powerful cytotoxic effect on human cervical cancer cells, while their effect on healthy MRC-5 cells was not statistically significant. The effective cytotoxic effects of the tested enones was confirmed by significant reduction in the number of HeLa cells, cell rounding and the loss of normal cells shape.

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Molecular mechanisms of antitumor activity 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin in human breast cancer MDA-MB-231 cells

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Breast cancer is one of the most common cancer types and represents the leading cause of women mortality worldwide.The aim of this study is to investigate molecular mechanisms of antitumor effects of 3-(4-chlorobenyzl)-5-isopropyl-5-phenylhydantoin on human breast cancer MDA-MB-231cells. The cells were treated with increasing concentrations of compound (0.01 to 100 μM) during 24 h and 72 h to evaluate the cytotoxic effect by determining lactate dehydrogenase release and oxidative/antioxidative status.The investigated compound induced decrease in levels of oxidative stress parameters suggesting their significant antioxidative effects. The concentrations with optimal cell inhibiting effects (1 and 10 μM) were selected for further examination of this compound to cell cycle regulation parameters, ERK and JNK signaling. After 72 h treatment the expression of anti-apoptotic and pro-apoptotic proteins was detected by Western blot analysis. Both treatments led to an increase in the number of cells in the S phase of the cell cycle compared to non-treated cells, with the most significant antiproliferative effect in 72 h treatment at concentration of 10 μM, where more than 48% of cells were arrested in S phase. The obtained results of immunoblot analysis show significant decreaseexpressionin the total ratio of ERK2 and increased expression of JNK after treatment, compared to values in non-treated cells. This results certainly indicate that the treatment with the tested3-(4-chlorobenyzl)-5-isopropyl-5-phenylhydantoin significantly attenuate the signaling pathway mediated by ERKs as the most common signaling target of antitumor therapeutics, although other stress-activated MAPK signaling pathways as well as metabolic and epigenetic factors may also influence the modulation of tumor cell responses to chemotherapeutics.

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Distribution of high-affinity copper transporter CTR1 in sclerotic hippocampi of mTLE patients

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Disruption of copper homeostasis has been documented in sclerotic hippocampi (HS) of patients with mesial temporal lobe epilepsy (mTLE), the single most frequent human focal epilepsy. Cu concentration is decreased in the hippocampal structural areas heavily affected by sclerosis and shows a positive correlation with neuron density [1]. In addition, the function of some copper metalloproteins, such as cytochrome c oxidase, is disrupted in HS [2]. However, the integrity of copper transport machinery in HS is poorly understood. Herein, we analyzed the distribution of CTR1, protein that mediates cellular Cu uptake, in hippocampi of drug-resistant mTLE patients with HS type 2. The relative level of CTR1 in hippocampal tissue was significantly decreased in HS. Cytoplasm of pyramidal neurons in the subiculum, stratum pyramidale (SPy) in CA1-CA3, and CA4 stained positive against CTR1 protein. In contrast, CTR1 positive reaction in granular neurons in gyrus dentatus was located closer to membrane area. The part of the CA1 region with total neuron loss showed evenly distributed CTR1-positive deposits, which might represent damaged neurons or glial cells. These deposits were also noticed in SPy of CA3 and CA4, located among normal-appearing CTR1-positive neurons. CTR1 has uniform distribution in the cytoplasm of neurons in the subiculum, a region adjacent to the SPy but generally not affected by sclerosis. On the other hand, preserved neurons in CA1 and CA2 showed accumulation of CTR1 in perinuclear and apical dendrite zones. Decreased expression of CTR1 protein, and occurrence of CTR1 deposits may negatively affect regular Cu transport in hippocampal tissue, which supports the hypothesis that Cu homeostasis represent an active contributor to the pathological mechanisms of HS.

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A novel HPLC-DAD method for quantification of dominant lignans in cow parsley (*Anthriscus sylvestris* (L.) Hofm.)

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Anthriscus sylvestris, known as cow parsley, is herbaceous biennial plant from Apiaceae family, native to Europe, western Asia and northwestern Africa. The plant is considered as an invasive weed and spreads easily along roads and the edges of woods and fields. Dried roots of the plant are used in Chinese traditional medicine to treat various pathological conditions including bronchitis, cough, fever and pain. The root accumulates high amounts of lignans, which are the most responsible for biological activity of this plant.

In this study, a novel HPLC-DAD method for simultaneous quantification of 13 dominant lignans (nemerosin, podophyllotoxone, guayadequiol, yatein, podophyllotoxin, isokaerophyllin, isochaihulactone, dimethylmatairesinol, 5'-demethoxypodophyllotoxin, isopicropodophyllotoxone, deoxypodophylotoxin, picropodophyllotoxone, acetylpodophyllotoxin) in 80% methanol extracts of *A. sylvestris* was developed and optimized. The analysis was performed on Agilent 1100 Series HPLC with DAD detector. The standard compounds were previously isolated and chemically characterized in our lab. The best separation and the shortest retention time were achieved with Zorbax Eclipse XDB-C18 (150 mm x 4,6 mm, 5 μm) and mobile phase A (0.1% HCOOH) and B (acetonitrile/methanol, v/v 65%:35%) with the gradient mode: 0 min 35% B, 8 min 72% B, 9 min 100% B, 11 min 100% B, *post run* 3 min, the flow 1 mL/min. The detection was done at 280 nm. The sample injection volume was 5 μL and concentrations of standards were in the range from 4 to 1000 μg/mL. The obtained calibration curves were linear. The method for simultaneous quantification of 13 lignans developed in this study can be successfully applied for further routine chemical characterisation of different *A. sylvestris* samples which are of potential interest for the pharmaceutical industry.

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Characterization of the late embryogenesis abundant (LEA) proteins family in hydrated and desiccated *Ramonda serbica* Panc. leaves

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Endemic plant species, *Ramonda serbica* is a resurrection plant that can tolerate extreme dehydration (desiccation, loss of 95% of cellular water) even over months. The accumulation of late embryogenesis abundant proteins (LEAPs) is a crucial step in the mechanism of desiccation tolerance. The role of LEAPs is not completely resolved, but they are accepted as intrinsically disordered proteins (IDPs). Based on previously established *de novo* transcriptome database of *R. serbica* leaves we identify around 160 members of LEA gene family. Identified LEAPs were classified into six groups: LEA 1-5 and seed maturation proteins (SMPs) according to protein family (Pfam) database. Based on multiple sequence alignment, secondary structure prediction and 3D structure modeling, we conducted LEA protein structure analysis. We showed that more than 50% of identified LEAPs exhibited a high propensity to form α-helices. As predicted by several bioinformatic tools, more than 70% of identified LEAPs were found to be highly disordered. Thus, these proteins are predicted to be disordered in solution, but they acquire a secondary, predominantly α-helical structure during drying, in contrast to globular proteins, which most often causes the loss of structure upon dehydration. By using molecular dynamic simulations, we identified the most favorable conformations of representative LEAPs and we have studied conformational transitions driven by the water scarcity. Structural characterization of LEAPs is a key to understand their function and regulation of their intrinsic structural disorder-to-order transition during desiccation as a requirement for biological function, in order to promote development of new therapeutic strategies in neurodegenerative disorders, cell preservation technology and the improvement of crop drought tolerance.

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Expression, purification and characterization of recombinant L-phenylalanine dehydrogenase

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L-Phenylalanine dehydrogenase (L-phenylalanine: NAD+oxido-reductase, deaminating (EC 1.4.1.20), PheDH), catalyzes the reversible NAD+-dependent oxidative deamination of L-Phe to phenylpyruvate [1]. Even though PheDH is an acknowledged industrial biocatalyst, it is generally known as key component in diagnostic kits and biosensors for detection and disease monitoring of phenylketonuria (PKU), an autosomal recessive inborn error of L-Phe metabolism. Phenylketonuria is characterized by increased levels of L-Phe in biological fluids, which accumulates in central nervous system (CNS) where its toxic effect is manifested. Incidence of classic PKU (L-Phe blood level ≥ 1.2 mmol/L) in Republic of Serbia varies between 1:18732 and 1: 39338 [2]. Untreated PKU leads to severe and irreversible intellectual impairment, neurological and behavioral problems. Timely diagnosis, a life long special diet with controlled L-Phe intake and regular monthly monitoring of L-Phe level, are crucial for disease control and prevention of CNS damage. From 1966 a mandatory PKU screening for all neonates is introduced in Serbia at the Institute of Maternal and Child Healthcare „Dr Vukan Čupić" in Belgrade. Recombinant PheDH of bacterial origin was successfully expressed in BL21 strain and characterized. Enzyme is purified to homogeneity by immobilized metal affinity chromatography to the yield of 2.4 mg. Molecular mass, determined by SDS PAGE, is approximately 40 kDa. Maximum catalytic activity in oxidative deamination of L-Phe was exhibited at 37°C and pH 10. Recombinant enzyme was stable in wide pH range of 5.5-11.5, and in temperature range of 25-37°C. Km and V_{max} for L-Phe were 3.3 mmol/L and 0.18 μmol/min, respectively. Furthermore, a standard curve for L-Phe determination with recombinant PheDH showed linearity and high goodness of fit (R^2=0.9963) for the L-Phe concentrations up to 1.5 mmol/L, which encompass both physiological and pathological values of the analyte. These preliminary results indicate that PheDH from our study is a promising candidate for further development of recombinant PheDH assays with real biological samples and PKU biosensor design.

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Estimation of redox potential of novel Pt(IV) complexes in the blood of rats

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Pt(IV) complexes offer the potential to overcome the cancer cell resistance to the most commonly used chemotherapeutic drug, cisplatin, with possible reduced adverse effects on healthy tissues. Novel Pt(IV) complexes with some esters of ethylenediamine-N,N'-di-S,S-(2,2'-dibenzyl)acetic acidpreviously showed cytotoxic effect stronger than cisplatin on chronic lymphocytic leukemia cells, but also a hepatotoxic effect. The present study investigates the effects of three novel Pt(IV) complexes containing ethyl-, propyl- and butyl-esters of the ethylenediamine-N, N'-di-S, S- (2,2'-dibenzyl) acetic acid compared to cisplatin, on hematological and oxidative stress parameters measured in the blood of rats. The 30 rats were randomly divided into five groups. Experimental groups received a single dose of Pt(IV) complexes (10 mg/kg) or cisplatin (7.5 mg/kg) intraperitoneally, while the control group received saline in the same manner. All three investigated complexes caused changes in measured hematological parameters. A decrease in the eythrocytes, hemoglobin, and hematocrit values and increase of mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and red cell distribution width compared to control were recorded. Cisplatin provoked an increase only of mean cell hemoglobin, mean cell hemoglobin concentration, and red cell distribution width. Further, used complexes significantly elevated the production of $O_2^{\cdot-}$, lipid peroxides and GSH, while the concentration of NO_2^- and H_2O_2 were decreased. Cisplatin also caused disturbance of redox homeostasis, but did not significantly increase the production of lipid peroxides and GSH. Investigated complexes showed stronger prooxidative potential than cisplatin. The obtained results indicate the ability of novel Pt(IV) complexes to induce oxidative stress in erythrocytes, leading to their dysfunction and destruction. These findings may be useful in further researches involving elucidation of the mechanisms of action of novel Pt(IV) complexes.

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Response of muscle cells to metformin depends on glucose availability in cell culture medium

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Metformin is a first-line drug in type 2 diabetes treatment, but despite being widely used in clinical practice for over 60 years, the molecular mechanisms of action are still a matter of debate. Proposed mechanisms include inhibition of mitochondrial respiratory chain complex I and AMP-activated protein kinase (AMPK) activation. These have been studied and confirmed in numerous *in vitro* studies, but different treatment concentrations (most studies use significantly higher concentrations than plasma concentrations found *in vivo*) and cell types used make it difficult to draw straight conclusions about the properties of these mechanisms. Taking into account that metformin is a glucose lowering agent and that the proposed mechanisms of action are essential for glucose metabolism, it is of relevance to consider the effect of glucose availability in cell medium. In this study, we explored the effects of therapeutic and higher metformin concentrations used in most *in vitro* studies on muscle cells grown in cell culture mediums with different glucose concentrations. C2C12 mouse myoblast were grown in low glucose (5.5 mM) or high glucose DMEM (25 mM) and treated with either therapeutic (50 μM) or experimental (5 mM) metformin concentrations, for 24 h. Cell viability was measured by acid phosphatase, crystal violet and MTT viability assays. Activity of signalling pathways was assessed by measuring the abundance of phosphorylated forms of signalling proteins by Western blot. Mitochondrial membrane potential was measured by flow cytometry, and mitochondrial function was analysed by high-resolution respirometry (Oroboros O2k). Cells grown in low glucose medium were more sensitive to 5 mM metformin treatment than cells grown in high glucose medium – they showed a larger decrease in cell viability and more pronounced mitochondrial inner membrane depolarisation and complex I inhibition (observed as decreased respiration using complex I-linked substrates and ADP). Low glucose medium – grown cells also exhibited AMPK pathway activation, as observed by a marked increase in AMPK and ACC (acetyl-CoA carboxylase) phosphorylation, and this effect was not observed in cells grown in high glucose medium. Metformin 50 μM treatment did not cause any of these effects. All of this leads to the conclusion that therapeutic metformin concentrations do not cause complex I inhibition or AMPK activation in muscle cells, and

that further investigation is needed to explore the mechanisms that cause the therapeutic effects of metformin. Also, glucose concentration could play a significant role in the mechanisms of action of metformin, which should be kept in mind when conducting *in vitro* studies, as many cell lines are grown in cell culture media with high glucose concentrations.

Highly active pectinases from newly isolated *Aspergillus tubingensis* strain

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Pectinolytic enzymes represent a large group of enzymes that catalyze the reactions of depolymerization and deesterification of pectin polysaccharides[1]. Saprophytic fungi produce pectinases on a large scale for industrial purposes. These enzymes have a various biotechnological application and their global annual production represents 25% of total industrial enzymes [1,2]. Agro-waste is widely used as economical substrate for the production of pectinases by solid state fermentation [3]. In this study, sugar beet pulp, as a good source of pectin[3], was used as a substrate for enzyme production by *Aspergillus tubingensis*. This strain was isolated from the quince fruit and identified by the molecular DNA marker calmodulin (*CaM*). SSF was performed with this strain on sugar beet pulp (80%) in combination with wheat bran (20%), a potent substrate for pectinase production [3]. The obtained high pectinolytic activity (15 U/mL), determined by 3,5-dinitrosalicylic acid reagent, was in the range of commercial pectinases. Zymography detection, using Ruthenium Red to visualize endo-pectinase activity and pectin-methyl esterase activity revealed several pectinase activity bands. Hydrolysis of different pectin substrates with the obtained pectinase complex was analyzed by thin layer chromatography in order to detect different products such as pectic oligosaccharides, which are emerging prebiotics superior to intact pectin.

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Bioaccumulation coefficient as an indicator of the ability to accumulate heavy metals of plant species on tailings

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Man with his activities has the greatest impact on environmental pollution. The development of heavy industry has both positive and negative impacts. In the metallurgical industry, a huge mass of ore material is excavated, but a small part is exploited. All unused ore material accumulates in the form of waste (tailings ponds, ash, slag) [1]. The aim of this study is to determine the content of heavy metals in the soil and roots of the examined species (*Melilotus officinalis*, *Sanguisorba minor*, *Echium vulgare*, *Achillea millefolium*, *Robinia pseudoacacia*), as well as the ability of plant species to accumulate heavy metals from the soil. The content of heavy metals (Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb, Cu) which was analyzed in the soil and underground organs, was collected from the site Kosovska Mitrovica (tailings pond). Plant material was subjected to wet digestion. For the determination of heavy metals in the soil, the destruction of the soil was performed with nitric and perchloric acid [2]. The results were read on an atomic spectrophotometer. Based on the middle values of the metal concentration in the soil, we can align them in the following sequence: Fe> Mg> Ca> Mn> Pb>Cr>Ni>Zn>Cu, and the values are within the remediation values (Regulation No. 30/2018 and 64/2019) [3]. Soil in which large amounts of metals and metalloids are found within the limits of remediation values has a seriously impaired physical-chemical composition. On such soils remediation measurements have to be applied, in order to restore the quality of the soil. Bioconcentration factor (BCF) value of Ca and Mg was >1, but the other metals had BCF value <1. Based on the obtained results, we can conclude that the examined species are hyperaccumulators of Ca and Mg.

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Preparation and characterization of protein nanoparticles from chickpea protein isolates

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Nanotechnology has increasingly gained attention as a great tool for numeruos applications in food industry. It is well known that nanotechnology plays an important role in incensement of flavor, extension of shelf life, improvement of rheological, textural and organoleptic properties of food. Nanotechnology implies nanoscale level of materials with the size from 1 to 100 nm followed with their unique properties. A variety of proteins, from animal and plant origin, could be used as a source of material for production and design of protein-based nanostructures. Preference is given to plant proteins due to their low toxicity, cost-effectiveness, availability and better hydrophobic properties compared to animal proteins. The aim of the present work was production of protein nanoparticles from different chickpea protein isolates by heat treatment and evaluation of their properties. Protein isolates were extracted from defatted chickpea seed under alkaline conditions with or without enzymatic pretreatments (arabinofuranosidase). The influence of heating time and pH on particle size, linoleic acid binding capacity and ABTS scavenging activity was analyzed. Results showed that nanoparticles fabricated from chickpea protein isolates varied in size, approximately from 28 to 290 nm. The smallest particles originated from protein isolate extracted after pretreatment of arabinofuranosidase, and at higher values of pH. Furthermore, nanoparticles prepared from this isolate showed the highest linoleic acid binding capacity of 58.14% at pH 7, and 69.39% at pH 9.3. Results of ABTS scavenging activity showed that protein extracted with the assistance of enzyme produced nanoparticles with enhanced antioxidant potencial compared to protein from alkaline extraction only.

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The influence of newly synthesized Au(III) and Ru(II) complexes on gene expression on primary ovarian cancer cell culture from ascites

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The primary cell culture isolated from ascites of patients with ovarian cancer is a heterogeneous cell population that composes the patient's tumor. Primary cell culture as a model system is essential for the individualization of therapy. The influence of a newly synthesized gold(III) (Au - $Au_3(NH_2(CH2)_6NH_2)_2Cl_8]Cl$) and ruthenium(II) complexes (Ru1 - $[\{RuCl(bpy)_2\}_2(\mu\text{-pzn})][PF_6]_2 \cdot 2H_2O$ and Ru2 - $[\{RuCl(phen)_2\}_2(\mu\text{-pzn})][PF_6]_2 \cdot 2H_2O$) on primary ovarian cell culture, is the main goal of this research. Monitoring of key processes such as apoptosis, biotransformation and metastasis under the treatment can be important in patients' survival. MTT test was performed to determine the cytotoxicity of the treatment. Determination of relative gene expression of target genes was performed by qRT-PCR assay. Au-complex shows slightly lower cytotoxicity with 61.1 μM after 24 h and 82.89 μM after 72 h compared to cisplatin whose IC_{50} value after 24 and 72 h was 36.37 and 2.87 μM, respectively. Ru-complexes didn't show a cytotoxic effect. Our results of gene expression for *Bax*, *Bcl-2* and *Caspase 9* suggest a potential participation of the internal apoptotic pathway (except for Cisplatin). Increased gene expression of *CYP1A2, CYP1B1,* and *GSTP1* suggest that xenobiotics are being processed by these enzymes as well as the elimination of xenobiotics from the cell. Different structures showed different effects on the expression of *E-cadherin, HIF-1α, VEGF, PTEN,* and *MMP9* genes involved in metastasis. Increased gene expression of *E-cadherin* suggests that treatments can contribute to maintaining cellular integrity. Tested substances show potential to suppress metastasis in various pathways.

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Atypical antipsychotics clozapine, sertindole, ziprasidone, and erythrocytes: Friends or foes?

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Atypical or second-generation antipsychotics are far less likely to cause extrapyramidal adverse effects than typical antipsychotic agents. Still, they are known to cause, to a different extent, weight gain, type II diabetes mellitus, hyperlipidemia, QTc interval prolongation, myocarditis, and sexual problems due to hormonal changes [1]. Location in the body, its number, and simplicity of structure make erythrocytes a useful model system for examining the pleiotropic effects of all drugs. Herein we examined the effects of three widely used atypical antipsychotics, clozapine, ziprasidone, and sertindole, on erythrocytes under *in vivo* and *ex vivo* experimental setup. After four-week treatment of adult 3-month-old male Wistar rats with recommended daily dose of selected drugs, clozapine decreased superoxide dismutase and glutathione reductase activity in erythrocytes. At the same time, sertindole and ziprasidone exerted no marked effect on antioxidant enzyme function (spectrophotometric assays). Using paramagnetic membrane spin probes 5-doxyl stearate and 16-doxyl stearate as labels, electron paramagnetic resonance study revealed that only ziprasidone influenced the fluidity of human erythrocyte membrane. Finally, all three drugs efficiently protected 2,2′-azobis(2-methylpropionamidine) dihydrochloride-induced hemolysis of human erythrocytes (spectrophotometric assay), in correlation with their antioxidant potency in spectrofluorimetric oxygen radical absorbance capacity and hydroxyl radical antioxidant capacity tests. The obtained results and differences confirm the ability of the tested drugs to modulate different homeostatic parameters and mechanisms in the body.

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Inhibition of tumor vasculature production by metformin treatment of cancer inoculated to hamsters

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We investigated the anticancer antineovascular effect of metformin on solid tumor model of fibrosarcoma in hamsters. Metformin inhibits growth of various cultured cancer cell lines. Metformin has the role in the prevention and treatment of a variety of cancers [1-3]. 40 Syrian golden hamsters of both sexes (20 males and 20 females), were randomly allocated to 3 experimental and 1 control groups. BHK-21/C13 cells ($2x10^6$ in 1 mL) were injected subcutaneously into the animal's back in all 4 groups. The first experimental group started peroral treatment with metformin 750 mg/kg daily via a gastric probe 7 days before tumor inoculation, the second 3 days before inoculation and the third immediately after inoculation. After 17 days, when the tumors were approximately 2-3 cm in the control group, all animals were sacrificed. The blood was collected for glucose and other analyses. The tumors were excised and weighed and their volume (by water displacement method) and diameters were measured. The tumor samples were histologically and immunohistologically assessed and the main organs toxicologically analyzed. Tumor volume was also determined using the formula $LxS^2/2$, where L was the longest and S the shortest diameter. CD34-positive areas in the tumor samples were quantified. Statistical significances of differences in tumor weight, volume, of CD34-positive areas and other parameters were determined by the one way ANOVA. Metformin inhibited fibrosarcoma and tumor vasculature growth in seven-day pretreated hamsters without toxicity and without influence on blood analyses. Inhibition of tumor vasculature production as an anti-tumor strategy might be an effective and safe therapeutic approach in novel nontoxic therapies and relapse prevention for human cancers.

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Nitroglycerin reduces no production in hamster fibrosarcomas

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We investigated the effect of nitroglycerin on fibrosarcoma in hamsters. Nitroglycerin is converted to NO. NO has vasodilatator, antimicrobial, antimalarial (plasmodium infections) and tumorocide actions. It contributes to the pathogenesis and control of malarial megaloblastic anaemia, infectious diseases, autoimmune processes and chronic degenerative diseases. NO has a wide and complex role in the immune system [1-3]. The 20 Syrian golden hamsters of approximately 100 g, both sexes, were randomly allocated in experimental and control groups of 10 animals in each. $2x10^6$ BHK-21/C13 cells in 1 mL were injected subcutaneously on the back of animals. The experimental group started peroral treatment with nitroglycerin 50 mg/kg daily via gastric probe 3 days before tumor inoculation. After 18 days, when tumors were approximately 2-3 cm in control group, all animals were sacrificed, blood collected for glucose and other analyses, tumors excised, weighed, diameters measured, tumor samples pathohistologically and immunohistochemically (iNOS) assessed and main organs toxicologically analyzed, including control animals. Tumor volume was determined using the water displacement method and formula $LxS^2/2$, L - the longest, S - the shortest diameter. iNOS-positive cells in the tumor samples were quantified. Statistical significances were determined by the one way ANOVA. The nitroglycerin treatment inhibited fibrosarcoma growth and NO production in hamsters without toxicity. Administration of nitroglycerin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment and relapse prevention antitumor therapy.

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The effects of Karnozin EXTRA® on mitochondrial bioenergetics in healthy fibroblasts

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Carnosine is known as a natural dipeptide, which inhibits the proliferation of tumor cells throughout its action on mitochondrial respiration and cell glycolysis. However, not much is known about its effects on the metabolism of healthy cells. We explored the effects of Karnozin EXTRA® capsule with different concentrations of L-carnosine, on the cell viability and the expressions of intermediate filament vimentin (VIM) and superoxide dismutase (SOD2) in normal fibroblasts BHK-21/C13. Furthermore, we investigated its action on the energy production of these cells. Cell viability was quantified by the MTT assay. The Clark oxygen electrode (Oxygraph, Hansatech Instruments, England) was used to measure the "intact cell respiration rate", state 3 of ADP-stimulated oxidation, maximum oxidation capacity and the activities of complexes I, II and IV. Results showed that Karnozin EXTRA® capsule in concentrations of 2 and 5 mM of L-carnosine did not induce toxic effects and morphological changes in treated cells. Our data revealed a dose-dependent immunofluorescent signal amplification of VIM and SOD2 in the BHK-21/C13 cell line. This supplement substantially increased the recorded mitochondrial respiration rates in the examined cell line. Due to the stimulation of mitochondrial energy production in normal fibroblasts, our results suggested that Karnozin EXTRA® is a potentially protective dietary supplement in the prevention of diseases with altered mitochondrial function.

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Oleanolic acid induces mitophagy and chemosenzitises human colon carcinoma cells

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Oleanolic acid (OA) has been shown to possess numeous beneficial health effects. However, the effect of OA on mitophagy in colon cancer cells is unknown. The current study aimed to investigate the mechanism of autophagy/mitophagy in the anticancer activity of OA in HCT116 human colon carcinoma cells. OA dose-dependently reduced viability of HCT116 cells, with IC_{50} 29.8 μM. The expression of cleaved caspase-3 and PARP1 increased after OA treatment, suggesting that OA induces apoptosis in HCT116 cells. Concurrently, OA induced autophagy in cancer cells, evidenced by the increase in expression of Beclin-1, Atg5 and LC3B-II, which played a prosurvival role. The induction of mitophagy was suggested by increased expression of p62 and PINK1 and reduced expression of TOMM20, which colocalized with LC3B. OA also induced nuclear accumulation of FOXO3a and p-FOXO3a. The cytotoxic activity of OA coincided with downregulation of the PI3K/Akt and ERK1/2 pathways and activation of AMPK, JNK1 and p38. In addition, OA enhanced the cytotoxicity of 5-FU. Our results showed concomitant induction of apoptosis and survival autophagy/mitophagy in HCT116 cells by OA via modulation of key signaling pathways involved in tumorigenesis. Additionally, we showed chemosensitization of HCT116 cells to 5-FU by OA.

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Chemical characterization and antioxidant capacity of decaffeinated coffee extracts

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Coffee plant is classified in the family Rubiaceae. There are a lot of types of coffee plant described, however economically the most important and worldwide loved coffee plant, the source of coffee drink, belongs to the genus *Coffea* L [1]. The most famous species of coffee plant are *Coffea arabica* L. and *Coffea canephora* L [2]. Decaffeination is a process of removing caffeine from coffee beans. During the decaffeination process, coffee beans lose aroma, change size and structure and lose weight. As a result of this process coffee beans can contain solvent residue [3,4]. The aim of this study was to examine the chemical composition and antioxidant activity of decaffeinated coffee. The content of total phenols, flavonoids and tannins was determined by spectrophotometric methods. Antioxidant capacity was examined regarding potential of decaffeinated coffee to inhibit lipid peroxidation and neutralize $HO^{\bullet}$ and $DPPH^{\bullet}$ radicals, as well as by FRAP assay [5]. The results showed significant antioxidant capacity of decaffeinated coffee due to presence of mentioned compounds in significant amounts. Decaffeinated coffee exhibited a lower antioxidant activity when compared to caffeinated coffee.

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Phenolic profile and biological activity of fungal species *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo 2011

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Mushrooms have tremendous potential in production of bioactive compounds with diverse bioactivities while the biochemical potential of some specific mushroom strains (autochthonous for the region) in production of specific bioactive agents may be of the main importance in a continuous search for novel strains with supreme activities all over the world [1]. The aim of this research was to study the chemical characterisation, antioxidant, antidiabetic and anti-obesity activity of six extract types of edible fungal species *V. gloiocephalus*. Phenolic profile was quantified using LC/MS-MS technique and standard total phenol content. Hydroxybenzoic acids, cinnamic and *p*-coumaric acids were identified in higher amount in relation to other used phenols. The highest scavenging activity and reducing power has been observed in polysaccharide (DPPH, ABTS and FRAP assay) and methanolic extracts (LP and NO assay). The results were obtained on α-amylase and lipase. This study introduced *V. gloiocephalus* as potentially valuable resource of antidiabetic and anti-obesity agents. In conclusion, this study showed composition and antioxidative performace of rare edible fungal species *V. gloiocephalus*, and it could be of potential interest as new sources of strong natural antioxidants as well as antidiabetic and anti-obesity agents in the future.

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Diffusion screening method for estimation potential fungal producers of xylanase responsible for xylooligosaccharides production

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Wild-type microorganisms from the environment represent a wide source of potential enzyme producers. In order to determine whether an isolated microorganism produces an enzyme of interest, various screen tests have been developed [1-3]. A new screening method for detection of endo and exo-xylanase activity including short time growth of fungal strains on a minimal medium containing xylan (inducible substrate) as a carbon source is developed and used for testing 58 fungal isolates from genus *Aspergillus*. The test is based on the diffusion of samples (fermentation extracts) in polyacrylamide gel incorporated by xylan. Endoxylanase activity is detected as enlightenment in the gel after staining of xylan with Congo Red. Exoxylanase activity was visualized as a precipitate after staining of reduction oligosaccharide ends with NBT. Selected isolate *A. tubigensis* was grown on SSF where corn cob served as an inducible substrate. In order to examine the influence of nitrogen sources on endoxylanase production and fungal growth, two sources (peptone and urea) were varied in 3 concentrations (1, 5 and 10 g/L). There were statistically significant differences in the obtained activities. The increase in activity compared to the screening medium was ~250 times. The obtained enzymes with high specific activity were further used for the production of xylooligosaccharides in high yield which showed that the selection of strain *A. tubingensis* was good.

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Changes in serum glycans in pregnant women with gestational diabetes mellitus

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During gestation, the body is affected by various changes induced by placenta and growing fetus. With glucose homeostasis being seriously tackled, gestational diabetes mellitus (GDM) is accounted as the most frequent pregnancy-related metabolic disorder. GDM is characterized as any degree of glucose intolerance diagnosed in the 2nd or the 3rd trimester of pregnancy. Women diagnosed with GDM are at several times higher risk of developing DM type 2 later in life than women without GDM history and, unless controlled, GDM may affect development of fetus. GDM diagnosis is based on the results of oral glucose tolerance test (OGTT) whose procedure is inconsistent in different populations, the reason why the methodology of OGTT is debatable and novel biomarkers of GDM are needed. Herein, we investigated changes in serum glycome in pregnant women without and with GDM. Using 15 lectins in lectin microarray, a difference was found only in relation to the response to phaseolus vulgaris erythroagglutinin, which was lower in GDM group. In the same group, the most positive correlations were found between datura stramonium response and levels of insulin, glucose, triglycerides, ratios cholesterol/HDL and triglycerides/HDL. The most negative correlations were detected for aleuria aurantia responses and levels of insulin, glucose, triglycerides and ratio triglycerides/HDL. To some extent these correlations were observed in the nonGDM group. These data suggest that serum levels of core N-acetylglucosamine (GlcNAc) and (core)fucose might be associated with glycemic markers and atherosclerotic factors that are related to pregnancy, and not exclusively to gestational diabetes, while lower levels of biantennary N-glycans with bisecting GlcNAc seem to accompany GDM.

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Activity and phenotype of paraoxonase 1 enzyme in pediatric patients on hemodialysis

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The level of oxidative stress is elevated in all stages of chronic kidney disease (CKD), together with the decline of renal function and it increases with the hemodialysis [1]. It is shown that there is a significant drop in paraoxonase 1 enzyme (PON1) activity in CKD, especially in children on hemodialysis [2]. Our research aimed to compare paraoxonase (POase) and arylesterase (ARE) activities, distribution of PON1 phenotypes, and parameters of redox status between patients before and after hemodialysis and the control group. Based on the paraoxonase/arylesterase activity ratio we divided our patients into phenotype groups. POase and ARE activity of PON1 were higher in patients after hemodialysis (POase: 281 IU/L vs 305 IU/L, p=0.009; ARE: 69.21 IU/L vs 82.58IU/L, p=0,017), and ARE activity was higher in the control group than in patients (86.62 IU/L vs 69.21IU/L, p=0.06). Redox status parameters in patients were higher than referent values for the same population. The distribution of phenotypes didn't differ between examined groups (p>0.05). Based on phenotype distribution, we concluded that the largest number of patients had Q192 aloenzyme, which is recognized as a protective aloenzyme in terms of protecting low density lipoprotein from oxidation.

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Short telomeres in peripheral blood leukocytes are associated with the presence and the severity of lung cancer

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Lung cancer is considered one of the cancer types with the highest mortality rates due to its high invasion and rapid metastasis[1]. If the ability of immune cells to adequately respond to tumorogenesis is compromised then tumor cells can more easily escape immune surveillance and thrive. Telomere shortening may contribute to the reduced ability of immune cells to mount a strong and sustained immune response[2]. We aimed to explore if shorter telomere length (TL) in peripheral blood leukocytes is associated with the presence and the severity of lung cancer. This study included 89 patients with the lung cancer (LC) and 77 healty subjects designated for control group (CG). TL was measured with qPCR (modified Cawthon method). TL was significantly shorter in LC compared to the CG ($P<0.001$). Shorter telomers were also observed in patients with metastasis compared to those without metastasis ($P=0.013$), patients in stadium IV compared to those with stadium I, II or III. Patients with complete response to therapy had significantly longer telomers comperd to those with progressive disease, partial response or stable disease ($P=0.039$). Furthere more, patients on any therapy regime had longer telomers compared to those without therapy ($P=0.008$). Short telomeres in peripheral blood leukocytes are associated with the presence and the severity of lung cancer suggesting that that telomere loss influenced tumorigenesis.

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Green synthesis, characterization, and biological evaluation of silver nanoparticles synthesized using *Salvia pratensis* L. extracts

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Biosynthesis of silver nanoparticles using medicinal plants has been considered as a promising approach to obtain nanoparticles enriched with biologically valuable phenolic compounds [1]. The study aims to synthesize silver nanoparticles using *Salvia pratensis* L. aerial part (SPA-AgNPs) and root (SPR-AgNPs) aqueous extracts. *S. pratensis* is a flowering plant belonging to the genus *Salvia* that has been widely used in the food, fragrance, and drug industry [2]. Synthesized nanoparticles were characterized using UV-vis, FTIR, SEM/EDS, and XRPD. Obtained SPA-AgNPs have shown significantly higher free radical scavenging (IC_{50} 22.18 μg/mL), while SPR-AgNPs possessed better antimicrobial properties. The lowest tested concentration (39.10 μg/mL) of SPR-AgNPs inhibited the growth of most studied microorganisms. Synthesized nanoparticles did not show hemolytic activity on red blood cells when applied in concentrations up to 150 μg/mL. The presented results suggest that silver nanoparticles may be eco-friendly synthesized using *S. pratensis* aerial part and root aqueous extracts and potentially applied as antimicrobial and antioxidant agents, not provoking cell hemolysis.

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Ferroptosis as a novel determinant of β-cell death in diabetic conditions

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Diabetes is a complex metabolic disorder which incidence rises in the epidemic fashion, suggesting the urgent need for new therapies. Its main pathological hallmark is loss of functional β-cells, and to date, several types of β-cell death have been described – necrosis, apoptosis, and autophagy. However, the role of ferroptosis in reducing β-cell population in diabetes remains elusive. In this study we aimed to examine whether and how this type of cell death is implicated in regulation of β-cell destiny in diabetes. For that purpose, Rin-5F insulin-producing pancreatic cells were treated with diabetes-mimicking factors – high glucose (HG) and H_2O_2, as well with commonly used diabetogenic agent streptozotocin (STZ). Results showed that HG, H_2O_2 and STZ induce the death of Rin-5F cells along with the accumulation of reactive oxygen species, lipid peroxides and iron; inactivation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and decrease in glutathione peroxidase 4 expression. This is consistent with the effect of the treatment with RSL-3, a well-known inducer of ferroptosis. Ferrostatin-1, a ferroptosis inhibitor, diminished above-stated effects and rescued cells from death. Our data revealed that β-cells underwent ferroptotic cell death under diabetogenic conditions. Results also implicate HG and H_2O_2 as contributing factors to ferroptosis of β-cells and suggest the novel mechanism of STZ diabetogenic action. Furthermore, the results shed a new light on antidiabetic strategy based on Nrf2 activation, putting it into the anti-ferroptotic context. In close, targeting ferroptosis in diabetes might be a new promising therapeutic approach based on preservation of β-cell population.

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Biomimetic mineralisation of periodate oxidized glucose oxidase

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Glucose oxidase (GOx) is an enzyme that belongs to a group of oxidoreductases. This enzyme catalyzes the oxidation of glucose to gluconic acid using molecular oxygen as an electron acceptor. Glucose oxidase contains carbohydrates in its structure, most often mannose and glucose (11-13%) [1]. Durability of GOx in harsh conditions can be enhanced by encapsulation within metal–organic frameworks via a process called biomimetic mineralisation. We demonstrate that chemical modification of carbohydrate parts on the protein surface by periodate oxidation is an effective method for control of biomimetic mineralisation by zeolitic imidazolate framework-8 (ZIF-8). Obtained GOx-ZIF-8 biocomposite had the higher half-life at 65^{o}C, and higher specific activity than native GOx.

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Cytotoxicity of enone sodium salts on human colon cancer cells (HCT-116) *in vitro*

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The enone salts by their chemical composition represent sodium salts of carboxylic acids (Na-carboxylates) and differ in substituents on the *C*-atom of the carbonyl group. Enone salts were de novo synthesized and their biological activity was investigated for the first time. Accordingly, the results of studies of similar compounds showed that sodium or potassium salts had effective antimicrobial and anti-inflammatory effects [1,2]. Different acetates (Na or K - carboxylates) have been shown to prevent the proliferation of colon cancer tumor cells, HCT-15 and RKO [3]. The aim of this study was to investigated the cytotoxic effect and morphology changes of enone sodium salts: sodium (E)-2-(2-methoxy-4-(3-oxobut-1-en-1-yl) phenoxy) acetate and sodium (E)-2-(2-methoxy-4-(4-methyl-3-oxopent-1-en-1-yl) phenoxy) acetate on human cancer colon (HCT-116) and human fibroblast cells (MRC-5) cells. The cytotoxic effect of the tested enone salts was determined by MTT test after 48h of treatment. Investigated concentrations of the two enone Na-salts were 3, 10, 30, 100 and 300 μM. Using phase contrast microscope, morphological changes of treated HCT-116 cells compared to untreated cells were assessed. The results of this study showed extremely high selectivity of Na-salt of enones to colon cancer tumor cells (HCT-116), without a significant effect on healthy human fibroblast cells. Also, our results showed that the cytotoxic effect of the enone Na-salt is consistent with the morphological changes of the cells.

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Methylenetetrahydrofolate reductase C677T and A1298C polymorphic variants in rectal cancer: significance for cancer risk and response to chemoradiotherapy

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Methylenetetrahydrofolate reductase (MTHFR) is a folate-metabolizing enzyme that influences DNA methylation and synthesis. The aim of this study was to analyze the association of *MTHFR* C677T and A1298C single nucleotide polymorphisms that affect the activity of MTHFR with the risk of rectal cancer in Serbia as well as the response to preoperative chemoradiotherapy. A total of 102 patients with locally advanced rectal cancer and 119 healthy controls were included in this case-control study. Restriction fragment length polymorphism analysis was used for *MTHFR* genotyping. Using both dominant and recessive models, it was found that the *MTHFR* 667C allele and the 1298A allele were significantly associated with rectal cancer as low-penetrant factors. No significant association was confirmed with the response to chemoradiotherapy. The protective effect of the 677T and 1298C that code for a more thermolabile MTHFR might be achieved through a more reliable DNA synthesis and repair which overcomes the inadequate DNA methylation induced by this variant of the enzyme. To the best of our knowledge, this is the first study of this type performed on the Slavic population in the Western Balkan area which might be useful for future meta-analyses and construction of genetic cancer risk prediction panels, as various population-based factors might also be significant in this setting.

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Phenolic compounds content and antibacterial activity of selected medicinal plants growing in Serbia

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Having in mind the current progress of antibiotic-resistance spreading and difficulties in treatment of bacterial infections, the search for new antibacterial compounds and development of new alternative strategies in combating bacterial infections is of growing interest[1]. In this study, the *in vitro* antibacterial activity of ethanol extracts of 13 medicinal plants was evaluated, such as *Melissa officinalis*, *Teucrium chamaedrys*, *Teucrium montanum*, *Origanum vulgare*, *Agrimonia eupatoria*, *Melilotus albus*, *Melilotus officinale*, *Cichorium intybus*, *Urtica dioica*, *Mentha piperita*, *Allium flavum*, *Gentiana asclepiadea* and *Clinopodium vulgare*. The inhibitory effect was determined against six strains of human pathogenic bacteria using the microdilution method and shown by the minimum inhibitory concentration (MIC). The total phenol content and total flavonoid content were determined spectrophotometrically. The tested extracts showed antibacterial activity in the range of 0.078 mg/mL to 20 mg/mL. The susceptibility of gram-positive bacteria was significantly higher than the susceptibility of gram-negative bacteria. The growth of *B. subtilis* was inhibited in the range of 0.156 to 20 mg/mL. The effect on this bacterium was shown by all 13 tested plants. *S. aureus* strains showed susceptibility to 12 tested plants. MICs ranged from 0.078 to 10 mg/mL. Of the gram-negative bacteria, *P. mirabilis* showed greater susceptibility than *E. coli* strains and *P. aeruginosa*. The range of the tested extracts action was 1.25 – 20 mg/mL. Among the tested plants, the most active were *O. vulgare*, *A. flavum* and *A. eupatoria*. Measured amounts of total phenols (17.04 – 159.84 mgGA/g) and flavonoids (8.22 – 159.84 mgRU/g) have indicated that phenolic compounds determine the antibacterial activity of plant extracts.

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Antibacterial and cytotoxic activity of *Tanacetum macrophyllum* extracts

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Plants produce a great number of bioactive compounds which possess different biological activities and can be seen as potential sources of novel antibacterial and anticancer agents. *Tanacetum macrophyllum* (Waldst. & Kit.) Sch.Bip., a member of family Asteraceae, is a perennial, herbaceous shrub-like plant widely distributed in Central and Southeast Europe. The biological activities of this plant are insufficiently investigated. Therefore, the *in vitro* antibacterial and cytotoxic activity of *T. macrophyllum* ethanol and acetone extract was examined. Antibacterial activity, expressed as minimum inhibitory concentration (MIC), was investigated against 12 bacterial strains including human-pathogenic and food-spoilage bacteria using a broth microdilution method with resazurin. Cytotoxic activity was evaluated on healthy human lung fibroblast cell line (MRC-5) and human colorectal carcinoma cell line (HCT-116). The cell viability was assessed using MTT assay, after 24 and 72 hours of treatment. In addition, total phenolic, flavonoid and tannin contents were determined spectrophotometrically. The antibacterial activity of tested extracts varied depending on the bacterial strains and concentration of the extract. In general, the extracts showed low antibacterial activity, the MIC values were in the range from 10 mg/mL to >20 mg/mL. The most sensitive were bacterial strain of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. Both extracts of *T. macrophyllum* induced proliferation of normal MRC-5 cells at a dose of 1-100 µg/mL after 72 hours and reduced the viability below 50% on the MRC-5 cell line at both incubation times only at the 1000 µg/mL, while the concentrations higher than 250 µg/mL reduced HCT-116 cell viability below 50%. The cytotoxic effects of *T. macrophyllum* extracts, expressed as IC_{50} value, were higher than 300 µg/mL on MRC-5 cells, while they showed high cytotoxicity on HCT-116 colon cancer cells (81.11 µg/mL for acetone extract after 72 hours of treatment). Acetone extract of *T. macrophyllum* shows potential anticancer effects. The higher concentration of phenolic compounds was measured in acetone than in ethanol extract.

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Childhood obesity: metabolic disorder and liver function

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In the recent decades, obesity has become an epidemic issue due to the number of patients diagnosed with this condition. The aim of this study was to estimate obesity type and non-alcoholic fatty liver disease existence in obese children by calculating several indexes and to compare it with healthy control normal weight children.This study included 59 children, 33 girls and 26 boys whose BMI was larger than 95^{th} percentile of children matched by age and gender. The concentrations of glucose, total cholesterol, HDL -cholesterol, triglycerides and insulin were measured as well as activities of ALT and AST with routine biochemical methods. LDL-cholesterol concentration was calculated using Friedewald equation. Index of central obesity (ICO), children lipid accumulation product (CLAP), hepatic steatosis index (HIS) and De Ritis ratio (AAR) were calculated using primary parameters. The obtained results suggested that patients have central obesity (ICO cut-off >0,53) and metabolic disorder (CLAP cut-off >2.20 and >2.25, girls and boys respectively), although all children had HIS under cut-off value (<36,0). Metabolic disorder, seen through the insulin and lipid parameters, is correlated with liver function. This connection is evident through the positive correlation of insulin and triglycerides with HIS ($\rho = 0.35$ $p = 0.006$; $\rho = 0.26$ $p = 0.047$, respectively). Despite normal HIS values, all patients had AAR<1 suggesting that this index could be useful in categorization of children with high cardiometabolic risk [1].

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Identification of compounds with antioxidative properties in alpine bistort (*Bistorta vivipara* (L.) Delarbre) ethanol extracts

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Bistorta vivipara (L.) is an arctic-alpine plant species from Polygonaceae family which has been used in folk medicine to treat tonsillitis, pharyngitis, dysentery and gastrointestinal disorders. It is known that plants from Polygonaceae family are rich in phenolic compounds. However, compared to other *Bistorta* species, *B. vivipara* is much less studied concerning its chemical composition and biological activity. Therefore, the aim of this study was to determine phenolic profile and antioxidant potential of 80% ethanolic extract of the aerial parts of the plant. Plant samples were collected from four different localities in Montenegro (Durmitor Mountain). For phenolic profile determination various chromatographic (LC-MS-MS, LC-DAD-MS, 1D-TLC and 2D-TLC) and spectrophotometric techniques were used. Antioxidant potential was evaluated spectrophotometrically, using DPPH and FRAP assays. Combined TLC-DPPH and TLC-FRAP assays were used to identify the compounds with the most potent antioxidant activity. Based on the obtained results, the content of total phenolics, and total flavonoids in prepared extracts were: 81-105 mg *gallic acid* eq/g *dry extract* and 31-54 mg q*uercetin* eq/g *de*, respectively. Among 33 quantified secondary metabolites the most abundant (given as mg/g de) were: 5-*O*-caffeoylquinic acid (>12.2) and quercetin glycosides (rutin: 3.3-10.7; hyperoside: 2.2-9.0, isoquercitrin: 1.3-2.7). Based on MS2Scan chromatograms the dominant peak is quercetin-3-*O*-glucuronide. The extracts exhibited high DPPH scavenging activity (IC_{50}=28.2-36.5 μg/mL) and Fe^{3+} reducing ability (59.3-79.4 mg *ascorbic acid* eq/g de). It was found that flavon-3-ol mono- and di-glycosides, isomers of chlorogenic acid and other caffeic acid derivatives are responsible for extracts' antioxidant activity. Our study supports needs for further *in vitro* and *in vivo* studies of *B. vivipara* as a valuable source of biologically active compounds.

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Synthesis and antimelanogenic activity of *N3*-aryliden-2-thiohydantoin derivatives

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The main goal of this study was to synthesize novel potent and safe inhibitors of melanogenesis based on the 2-thiohydantoin moiety for therapeutic application in the treatment of skin hyperpigmentation disorders in humans (freckles, chloasma, melasma, solar lentigo, senile lentigines, ephelides and melanoderma). A series of 15 *N3*-aryliden-2-thiohydantoin derivatives was synthesized, fully characterized, and subjected to extensive in vitro and in vivo biological evaluation. All compounds have been tested on the zebrafish model, a powerful and highly predictive animal platform used for toxicity assessment and biological activity evaluation of novel bioactive molecules, thus simplifying the path to clinical trials and reducing the failure at later stages of testing. Their antimelanogenic activity and toxicity were compared to those of kojic acid and hydroquinone, well-known depigmenting agents used for the treatment of skin hyperpigmentation disorders. Most of the compounds reduced body pigmentation of the treated zebrafish embryos with different efficacy. Derivatives which exhibited the best melanogenesis inhibitory activity exerted much better therapeutic profile than kojic acid and hydroquinone, the former of which was non-toxic and poorly effective and the latter highly effective but extremely toxic. The presented results of biological activity evaluation *in vivo* clearly demonstrate that compounds with 2-thiohydantoin moiety could present a novel effective and safe antimelanogenic compounds with a large potential for further clinical evaluation and therapeutic application in humans.

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Production of polyglutamic acid by *Bacillus* sp: strains selection, optimization, batch fermentation and characterization

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Polyglutamic acid (PGA) is an anionic, non-toxic natural polymer that consists of D- and L-glutamic acid. Glutamic acids that makeup PGA create bonds between α-amino and γ-carboxyl groups. PGA can be found on the surface of many different bacteria, usually as a part of their capsule. Bacteria use polyglutamic acid to survive adverse environmental conditions. PGA has found multiple potential applications as a thickener, drug carrier, biological adhesive, heavy metal and basic dye adsorber, etc. Its biodegradability is especially useful in the fields of food, cosmetics, medicine and water treatment[1]. The aim of this study was to find the best polyglutamic acid producer from the selection of 50 different *Bacillus* sp strains originating from Serbia, as well as the optimal medium composition. It was discovered that the best PGA producing strain was 17B and it was selected for further fermentation medium optimization. Optimization was carried out using Design of Experiment, specifically Box Behnken design. Results were analyzed using response surface methodology. PGA that was produced during this process was analyzed using SDS PAGE and basic dye adsorption was attempted as well. The best PGA producer, *Bacillus* sp strain 17B, was used for PGA production in batch fermenter [2]. PGA isolated from the fermentation broth was purified using gel filtration and further characterized using SDS PAGE, FTIR spectroscopy and direct-infusion MS.

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A search for nature's robust proteases with zein as a substrate

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Zein is produced in large quantities as a byproduct of corn starch manufacturing since it constitutes a majority of the total protein of maize seed (44–70%). Enzymatic treatment of zein significantly improves its aqueous solubility and provides peptides that are used as animal feed, functional food, or biologically active carriers for other bioactive molecules. Moreover, zein-derived peptides exhibit antioxidant, anti-inflammatory, antihypertensive, anticancer, and antimicrobial activities in human organisms [1]. Few attempts up to this day have been made to screen for microorganisms that are capable of zein degradation. Available protocols for proteases identification almost exclusively rely on screening on casein, skim milk, and gelatin agar in limited experimental conditions. We have screened different *Bacillus* sp strains isolated from across Serbia for zein-degrading proteases. To do so we developed an inexpensive, simple, and reproducible way of high throughput functional screening of zein-degrading proteases on zein-containing gels. Besides detecting proteases with specificity towards zein, a developed diffusion assay was designed to support screening for naturally occurring robust proteases with high potential for industrial application. By using classical methods of protein purification, we isolated an alkaline thermostable protease from *Bacillus amyloliquefaciens* strain 12B that is resistant to the presence of detergents, organic solvents, and high salt concentrations.

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Chemical composition of thyme essential oil and its anthelmintic properties

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Thyme (*Thymus vulgaris* L.) is a well-known member of the genus *Thymus* with healing properties known since ancient times. Thyme has application in traditional medicine and pharmaceutical preparations, but also in the food and cosmetic industry. The aim of this study was to examine the chemical composition and *in vitro* anthelmintic potential of thyme essential oils from Serbia and compare it with sample from France[1]. Chemical characterization of the samples was done by GC-MS and antihelmintic potential of tested samples was evaluated using *in vitro* egg hatch test on sheep gastrointestinal nematodes (concentration range 0.049-50 mg/mL) for each sample. The most represented compounds in sample from Serbia were: p-cymene (41.72%), thymol (31.59%), α-terpineol (11.71%), linalool (4.37%) and α-pinene (2.47%) and it inhibited egg hatchability for 95.25-100% and in sample from France thymol (54.48%), p-cymene (21.01%), γ-terpinene (8.11%), carvacrol (3.95%) and linalool (2.77%) were the dominant compounds and inhibited egg hatchability for 98.5-100%. The results indicate that chemical composition of thyme essential oil is dependant on geographic origin but regardless it poseses antihelmintic potential and may be used in veterinary medicine as anthelmintic agent.

Acknowledgements

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Allelopathic effect of ragweed (*Ambrosia trifida* L.) on soybean phenolic compounds

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Giant ragweed (*Ambrosia trifida* L.) invades cultivated fields, interfering with crops, particularly soybean. Competition is an important interference mechanism that is responsible for the giant ragweed infestation, but the allelopathy of the giant ragweed in which released allelochemicals inhibit the growth of crops, would also be an important mechanism [1]. The aim of this study was to determine the allelopathic influence of the giant ragweed on soybean (*Glycine max* L.) plants growth to explore the effect of released allelochemicals through *A. trifida* root on crops. The effect of released allelochemicals of *A. trifida* on total flavonoids (TF) and radical scavenging activity of soybean extracts were examined. The radical scavenging activity was investigated using DPPH assay. Seeds of crop and *A. trifida* were sown in pots 40x30 cm, at three-term ratio 1:1, 3:1, 1:3 (crop-weed), for each crops separately, while the control pots contained crops only. Evaluation was conducted 7, 10 and 14 days after emergence. The highest amount of TF (6.79±0.46 mg RE/g DW) and the lowest antioxidant capacity were obtained in soybean leaves at a ratio weed:crop 3:1, 7 days after the sowing. On the other hand, the lowest amount of TF (3.96±0.75 mg RE/g DW) and the highest antioxidant capacity were obtained in soybean leaves at the same ratio 14 days after the sowing. From the obtained results, it can be concluded that greatest oxidative stress occurred in soybean plants 7 days after the sowing at a ratio weed:crop 3:1. After 14 days, it seems that soybean plants established a defense system against the negative allelopathic effect of weed.

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Interaction between alpha-2-macroglobulin and phycocyanobilin – structural and physiological implications

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In this study, the interaction between phycocyanobilin (PCB)[1], a bioactive chromophore of blue-green algae Spirulina's phycobiliproteins, and alpha-2-macroglobulin (α2M)[2], a universal anti-proteinase, was investigated under simulated physiological conditions using spectroscopic techniques and α2M activity assay. Using spectrofluorimetric measurements, we found that α2M binds PCB with a moderate affinity, with a binding constant of 6.3×10^5 M^{-1} at 25°C. The binding of PCB to α2M does not cause any significant change in the secondary structure of the protein (circular dichroism measurements). Besides, PCB protects α2M from structural oxidative alterations under AAPH-induced free radical overproduction. Further, PCB binding effectively preserves α2M anti-proteinase activity. Since α2M is involved in controlling the action of enzymes during the inflammatory process, the protection that PCB expresses could indirectly influence the intensity and direction of body response to impaired homeostasis, especially under oxidative stress.

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Associations between zinc intake and status with biochemical and antropometric parameters in hemodialysis patients

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Zinc plays an important role in numerous biological processes, so dietary zinc intake and its status could be associated with biochemical and anthropometrics parameters [1]. It has also been noted that serum copper (Cu)/Zn ratio reflects their reciprocal reaction better than serum Cu or Zn concentrations alone [2]. The aim of this study was to explore relationships of dietary Zn intake, its serum status and serum Cu/Zn ratio with some important biochemical and anthropometric parameters in chronic kidney patients undergoing hemodialysis (HD) treatment. We studied 40 hemodialysis patients and their serum hemoglobin, iron, C-reactive protein (CRP), urea, creatinine, albumin, total cholesterol, LDL cholesterol, HDL-cholesterol, triglycerides, intact parathyroid hormone (iPTH) and vitamin D. The anthropometrics parameters BMI, muscular arm circumfluence (MAC), visceral fat area (VFA) and visceral fat index (VAI) were measured. Dietary zinc intake was significantly associated with LDL cholesterol ($r = -0.332$, $p = 0.036$), iPTH levels ($r = -0.317$, $p = 0.046$), MAC ($r = 0.449$, $p = 0.004$) and VAI ($r = -0.317$, $p = 0.046$). We noted the significant inverse association between serum Cu/Zn ratio and serum albumin ($r = -0.404$, $p = 0.009$), iron ($r = -0.351$, $p = 0.026$), iPTH ($r = -0.332$, $p = 0.036$)) concentration, Kt/V ($r = -0.317$, $p = 0.046$), HD period ($r = 0.355$, $p = 0.023$), CRP ($r = 0.315$, $p = 0.048$), BMI ($r = 0,384$, $p = 0.014$) and VFA ($r = 0.327$, $p = 0.040$). In conclusion dietary intake and status of zinc could play an important role in iPTH concentration regulation as well as weight mass and distribution control, but also albumin, iron homeostasis and inflammation. Moreover, it seems that serum Cu/Zn ratio is increased

by HD procedure, both its duration and efficiency, and so importance of this ratio monitoring in HD patients is also emphasized by this study.

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Biochemical, molecular and histological insight into cyanobacterial effects on *Cyprinus carpio* from Kis-Balaton, Hungary

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Cyanobacterial blooming is a worldwide phenomenon that nowadays affects most aquatic ecosystems. Fish can be exposed to the harmful effects of cyanobacterial blooms, primarily toxic metabolites called cyanotoxins. Therefore, examination of several different biomarkers could elucidate cyanotoxin effects on fish tissues. Previous research has shown occurrence of severe blooming and the presence of several microcystin congeners, cyanotoxin synthetizing genes and histopathological alteration in investigated fish species from Kis-Balaton, Hungary during 2018 [1]. Further research has been performed on the same aquatic ecocystem in June of 2019 that included assessment of cyanobacteria, cyanotoxins, as well as several biomarkers (biochemical, molecular and histopathological) of *Cyprinus carpio*. Investigation showed presence and bloom of potentially toxic *Microcystis aeruginosa*, although cyanotoxins were not detected. Furthermore, DNA fragmentation in organs was not noted, however, changes in biotransformation (cytochrome P450 1A, glutathione-S-transferase) and antioxidative (lipid peroxidation, catalase, total superoxide dismutase, glutathione peroxidase and glutathione reductase) enzyme activity in liver, gills and gonads indicated occurrance of an oxidative stress in

fish. Additionally, histopathological alterations in liver, kidneys and gills were recorded. Albeit cyanotoxins were not detected during the investigation in 2019, they have been confirmed previously which is supported by the present cyanobacterial species. Described biomarkers depict mostly acute and reversible changes, thus, further research of Kis-Balaton in connection to cyanobacterial blooming and its consequences on aquatic life is warranted.

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The effect of acetone extract of *Lenzites betulinus* mushroom on peripheral blood lymphocytes genome in patients with acute coronary syndrome *in vitro*

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Cardiovascular diseases are the leading cause of mortality, which is why the need to identify all possible factors that reduce primary and secondary risk is increasing. Mushrooms are important source of compounds with protective and antioxidative effects, such as phenolics and flavonoids. Thus, the aim of this study was to evaluate the potential genoprotective effect of acetone extract of *Lenzites betulinus* mushroom in patients with acute coronary syndrome (ACS). The study included 10 healthy controls (55.40 ± 5.56) years and 15 ACS patients (56.73 ± 4.89 years), which are treated with standard drug therapy (ACE-inhibitors, beta-blockers, anti-inflammatory, diuretic, statin and anticoagulant in various combinations). The genoprotective effect of different concentrations of the extract (50, 100, 150, and 200 μg/mL) was determined using comet assay on peripheral blood lymphocytes of ACS patients, while the total phenolic and flavonoid contents were measured by spectrophotometry. Our results showed that ACS patients have an increased level of DNA damage (expressed as genetic damage index, GDI) comparing to the healthy controls (1.29 ± 0.11 vs. 0.37 ± 0.04, $p < 0.001$). Analyzing the effects of *L. betulinus* extract in healthy controls, all tested concentrations of extract caused no significantly increased GDI values (from 0.38 ± 0.05 to 0.66 ± 0.08) comparing to the negative control (without treatment, 0.37 ± 0.04). In ACS patients, extract decreased GDI values in all tested concentrations, but significantly ($p < 0.001$) in concentrations of 100 μg/mL (0.86 ± 0.10), 150 μg/mL (0.71 ± 0.09) and 200 μg/mL (0.55 ± 0.08) comparing to their basal frequencies (1.29 ± 0.11). In healthy control, Pearson's correlation coefficient showed that the extract increased GDI values in a dose dependent manner ($r = 0.825$, $p < 0.01$), while in ACS patients, GDI values decreased with the increase of extract concentration ($r = -0.925$ $p < 0.001$). The total phenolic content was 45.83 ± 5.83 mg GAE/g, while total flavonoid content was 21.43 ± 0.14 mg RUE/g of the acetone extract. We can conclude that acetone extract of mushroom *L. betulinus* is rich in polyphenolic composition. Acetone extract of *L. betulinus* was not genotoxic in tested concentrations in human peripheral blood lymphocytes of healthy controls, while in ACS

patients it manifested genoprotective effect, so the use of this mushroom can be recommended in order to reduce the degree of genome damage in ACS patients.

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Possible protective role of probiotic bacteria on intestinal epithelium after chronic $CdCl_2$ intake

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The epithelium of the small intestine is involved in the absorption of nutrients and fluids, at the same time preventing the passage of any harmful substances from the intestinal lumen to the circulation. The main cell type of the intestinal epithelium are enterocytes, columnar cell originating from the stem cells located deeply in the intestinal crypts. Newly formed enterocytes migrate from the base of the crypt toward the tip of the villus where they finally slough off into the lumen, thus ensuring fast and continuous renewal of the epithelium. During migration, enterocytes differentiate, mature and gradually increase their height[1,2]. Cd is a heavy metal without known biological function, present in small amounts in soil, water, and air. After entering the human body, usually through ingestion of contaminated food and water, it can cause adverse effects on human health depending on dose, duration and way of exposure and individual body response[3]. Given that intestinal epithelial cells are the first line of defence against various orally ingested pathogens and toxins, the purpose of this study was to investigate the effects of orally ingested cadmium compound (cadmium-chloride, $CdCl_2$) on the height of epithelial cells in small intestinal villi and crypts and to assess possible protective effects of probiotic bacteria. Twenty four male Wistar rats were divided into 4 equal groups and treated as follows: control group - tap water (TW) and commercial pelleted food (CF), *ad libitum*; PB group - TW, CF, probiotic (PB) (PROBIOTIC®, Ivančić i sinovi, d.o.o., $5x10^8$ cfu/g of food); Cd group - TW with dissolved $CdCl_2$ (Fisher Scientific, 70 ppm) and CF; PB+Cd group - TW with dissolved $CdCl_2$ and CF supplemented with PB. After 5 weeks, jejunum was dissected and routinely processed for light microscopy. Tissue sections stained with alcian blue/nuclear fast red were analyzed and photographed. Morphometry was performed using ImageJ analysis software. Statistical analysis was performed by a two-tailed Student's t-test, with data reported as means ± s.e.m. and statistical significance set at $P<0.05$. The height of epithelial layer in villi and crypts (μm) was as follows: control 35.3±1.1 and 22.9±0.7; PB 30.4±1.03 and 22.2±0.93; Cd 28.8±0.67 and 24.3±0.79; PB+Cd 32.7±0.74 and 24.1±0.76. Expectedly, epithelial height in crypts was lower than that of villi in all groups. None of the treatments affected crypts since there were no differences in epithelial height in any treatment group relative to control. On the contrary, treatments with PB and Cd each decreased height of villar epithelium while after combined treatment it approximated the control value.

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Expression of heat shock protein genes in response to unusually high temperatures during winter diapause in the European corn borer *Ostrinia nubilalis* (Hbn.)

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In recent decades, global climate changes have lead to an increase in average annual temperatures and have often caused mild winters, during which temperatures rarely fall below 0°C. In the European corn borer *Ostrinia nubilalis* (Hbn.), one of pest moth species, many protective mechanisms important for acquiring cold resistance develop only after gradual exposure to low temperatures during early stages of diapause. It is not clear how the ecophysiology and maintenance of diapause in this cold-adapted species are affected by milder winters. Thus, the aim of this study was to contribute to a better understanding of diapause-related heat stress tolerance in fifth instar larvae of *O. nubilalis*, through monitoring the changes in gene expression of five selected heat shock protein genes: *hsp90*, *hsp70*, *hsc70*, *hsp20.1*, *hsp20.4*. Using RT-qPCR, we analysed the relative expression of these genes in non-diapausing (ND) larvae as well as in two groups of diapausing larvae – one which was placed outside and exposed to field conditions (FC) and the other which was warm acclimated (WA) to room temperatures (12–20°C), both monitored and sampled each month, from November to April. The results have shown that *hsp70* and *hsp20.4* genes were strongly upregulated during the entire diapause, both in the FC and WA groups, in comparison to ND larvae, which suggest their importance in both thermal stress response and survival in the dormant state. The expression of the *hsp90* gene was in increasing trend until January and then started to fall in both FC and WA groups toward the end of diapause. This is in contrast to *hsp20.1*, which was only upregulated in March, during diapause termination. The constitutively expressed *hsc70* gene was downregulated from November until February in both FC and WA groups and then upregulated in March and April, suggesting well-balanced and coordinated energy expenditure for *hsp* gene expression – from suppressed during general metabolic depression in early diapause, to a more intensive expression ahead of metamorphosis.

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Redox changes in microalga *Chlorella sorokiniana* exposed to high concentrations of Mn(II)

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Microalgae can be implemented in the remediation of mining and other metal-rich wastewaters as pioneer species. On the other hand, metals affect aquatic ecosystems through the negative impact on microalgae which are the primary producers of O_2 and biomass. Redox processes represent an important component of the mechanisms of interaction of microalgae with transition metals. We analyzed the redox changes in *Chlorella sorokiniana* culture that are induced by high levels of Mn(II). Mn is the key metal pollutant, with five main oxidation forms that can bind to a variety of different ligands. Mn (1 mM) induced a significant increase in the intracellular production of reactive oxygen species. The boost appears to show two phases – the first is very fast (observed after 15 min), whereas the second starts after 1 h reaching a plateau at 24 h. The concentration of reduced thiols, which represent important targets of oxidation, appears to parallel this trend. Total glutathione concentration shows a drop at 1 h and recovery at 24 h. This implicates that either a glutathionylation of proteins or a synthesis of phytochelatins - sulfur-rich short-chain peptides that sequester metals, takes place early in the response to Mn. Further, FTIR analysis showed that Mn induced a decrease of C=C levels and CH_2/CH_3 ratio implicating increased lipid peroxidation. Finally, Mn ions that were accumulated in the cells were extracted with nitric oxide and analyzed by cyclic voltammetry. Two redox forms were detected - Mn(II) and Mn(IV). The latter appears to prevail at higher manganese concentrations and longer periods of incubation. These results demonstrate that redox response of *C. sorokiniana* to high Mn levels involves at least two phases. Initially, Mn(II) enters the cells and induces pro-oxidative changes that are mitigated by glutathione-based antioxidative defense. Later on, redox homeostasis is reestablished with concomitant inactivation of Mn in the more stable redox form.

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Triglyceride-glucose index and oxidative stress biomarkers in patients with type 2 diabetes mellitus

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Insulin resistance and oxidative stress play pivotal roles in the pathogenesis of diabetes mellitus type 2 (DM) [1]. Triglyceride-glucose (TyG) index is a useful surrogate marker of insulin resistance [2]. The study aimed to compare levels of TyG between DM patients and healthy individuals. Additionally, the TyG connection with oxidative stress (OS) biomarkers was assessed. The study included 42 DM patients and 40 controls. AOPP, TOS, PAB, IMA, PON, and TAS were measured as biomarkers of OS. Biochemical parameters were determined by standardly used laboratory methods. TyG index was significantly higher in the DM group in comparison to the control group ($P<0.01$). AOPP and PAB levels were significantly higher in the DM group ($P<0.001$), while TAS concentrations were lower ($P<0.001$) which indicate increased production of free radicals and reduced antioxidant defense in DM patients. HbA1c was in positive correlation with the TyG index. On the other hand, a negative correlation was observed between HbA1c and TAS levels ($P<0.05$) in the DM group. In conclusion, the TyG index is increased in DM patients reflecting the impairment of insulin action. TyG and OS parameters are higher in poorly regulated glycemia.

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Chemical composition and antioxidant activity of pomegranate (*Punica granatum* L.) peel and pulp

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Pomegranate has been recognised in folk medicine as food with anthelmintic, anti-coccidial and anti-inflammatory activities. Nowadays, popularity of pomegranate as functional food is rising since it displays a diversity of health benefits, such as antioxidant activity [1,2]. In this study, 80% ethanol extracts of pomegranate peel and pulp were used to determine their chemical composition and antioxidant activity. Total phenolics, flavonoids, tannins, anthocyanidins and vitamin C were determined by spectrophotometric methods. Antioxidant activity was determined by FRAP and DPPH tests, as well as inhibition of lipid peroxidation. Pomegranate peel was richer in total phenolics, flavonoids, tannins and vitamin C, whilst pomegranate pulp was richer in anthocyanidins. Results showed drastically higher antioxidant activity of pomegranate peel in comparison to pomegranate pulp and slightly lower activity of peel in comparison to synthetic antioxidants PG and BHT. Therefore, pomegranate peel, which is often discarded, could be a promising raw material for cosmetic, pharmaceutical and food industry, while both peel and pulp could be used in diet as a rich source of antioxidants.

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Luteolin ameliorates experimental colitis in mice through ERK-mediated suppression of inflammation, apoptosis and autophagy

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Ulcerative colitis (UC) is one of the major forms of inflammatory bowel disease with an increasing incidence and prevalence worldwide. It is a chronic remitting and relapsing disease that occurs spontaneously characterized by gastrointestinal symptoms, and significantly affects the quality of life[1]. Currently used treatments for UC are unsatisfactory due to various side effects, failure in the induction of remission and prevention of relapse, as well as high economic costs[2]. The use of natural compounds has become increasingly attractive in the prevention and treatment of various diseases, including UC. Luteolin (3′,4′,5,7-tetrahydroxyflavone; Lut) is a common flavonoid present in many edible plants that has been shown to have beneficial effects on various diseases[3], however, its effect on UC has been poorly studied. In this study, we investigated the effect of Lut in the posttreatment and cotreatment of experimental colitis induced by dextran sulfate sodium (DSS) in mice. Lut attenuated clinical symptoms of DSS-induced colitis in mice by diminishing body weight loss and improving disease activity index, alleviated colon tissue damage, and reduced inflammation, apoptosis and autophagy. This effect was more pronounced when Lut was applied simultaneously with DSS. Additionally, the role of extracellular signal-regulated kinases 1/2 (ERK1/2) in the mechanism of action of Lut in experimental colitis was investigated as it was shown that Lut increased the activity of ERK1/2. Inhibition of ERK1/2 exacerbated the symptoms of DSS-induced colitis and diminished the protective effects of Lut as evidenced by worsening of inflammation and increased apoptosis and autophagy. The results of this study contributed to the elucidation of the underlying mechanism of action of Lut in experimental colitis and provided new mechanistic details underlying the antiinflammatory, antiapoptotic and antiautophagic effects of Lut through the activation of the ERK signaling pathway. In conclusion, the results suggest that Lut could be an effective therapeutic candidate for the treatment of UC, or a supplement to conventional therapy, although further clinical studies are required.

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Effects of fermented food on the body weight and behavior after repeated LPS administration in mice

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Non-communicable chronic diseases are largely driven by chronic inflammation, which can be in relation with poor diet and toxic products of commensal bacteria in guts. Diet intervention can change gut microbiota function and composition [1]. The fermented foods containing microorganisms able to remodel host microbiota, can improve inflammatory status of the organism, including brain. Sauerkraut, produced by spontaneous fermentation of cabbage with lactic acid bacteria (LAB), is an important dietary ingredient, but studies of its effects are rather scarce [2]. Here, we aim to examine whether sauerkraut brine is able to change physiological and behavioral response to systemic inflammation in mice induced by lipopolysaccharides (LPS), a constituent of the Gram-negative bacteria cell wall [3]. Three-month-old C57BL/6 mice were given 150 μL of sauerkraut brine (SB) or pasteurized sauerkraut brine (PSB) for 5 weeks by oral gavage. Control animals (CON) received the equivalent amount of water. During last week, animals were challenged by 5 injections of LPS (0.5 mg/kg, i.p.) Before and after LPS, behavior of animals was tested by open field, light-dark box, Y-maze, tail-suspension and rota rod tests. Food consumption and body weight were measured throughout the experiment. Quality analysis of in-home made sauerkraut produced by tradiotional spontaneous fermentation show that sauerkraut brine counts $2x10^5$ colony forming units (CFU/mL) of LAB. SB and PSB treatments did not influence body weights and behavior compared to CON mice. LPS induced sick behavior characterized by weight loss and decreased food intake, where the fastest recovery was observed in the SB group. Behavioral analysis revealed similar response to LPS challenge between groups. However, further intra-group analysis and molecular screening is required to assess the possible subtle impact of sauerkraut on the mice behavior and immune status.

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Effect of caffeine on cell proliferation and key elements of neurotransmitter pathways in human neuroblastoma SH-SY5Y cell line

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Being an ingredient of many beverages, food products and pharmaceuticals, caffeine is the most widely consumed stimulant in the world. It acts as a stimulant to the central nervous system mainly by antagonism of adenosine receptors. It has been detected in wastewater, surface water and groundwater worldwide, so there is a concern for its adverse impact on nontarget organisms, including wildlife and humans. We investigated the effect of caffeine using human neuroblastoma SH-SY5Y cells as an experimental model. Caffeine did not disturb cell proliferation (SRB assay), the activity of mitochondrial dehydrogenase (MTT assay) and mitochondrial membrane potential (TMRE assay). The effect of caffeine (10 ng/L, 10 µg/L and 10 mg/L) on some of the key elements of neurotransmitter (acetylcholine, serotonin, dopamine and GABA) pathways was analysed using quantitative real-time PCR analysis and enzyme activity assays. The results showed significant downregulation of genes encoding protein involved in exocytosis of neurotransmitters – synaptotagmin 10 (*SYT10*), serotonin receptor 3A (*HTR3A*) and GABA transaminase (*ABAT*). A trend of upregulation of dopamine receptor D2 (*DRD2*) and downregulation of monoamine oxidase B (*MAOB*) was also noticed, while the investigated elements of the acetylcholine pathway were unaffected by the treatment. The results represent a contribution to the mechanistic knowledge on coffein effects on humans, other than its primary mode of action. They may failitate the development of new biomarkers of effects of neuroactive compounds and contaminants in complex environmental mixtures.

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Selective cytotoxicity of melittin against human cervical cancer cells

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Melittin is a small cationic peptide consisting of 26 amino acid residues. Melittin displays significant wide-spectrum antitumor and cytotoxic effects on multiple types of tumor cells, including genitourinary, system, nervous, skeletal, respiratory, digestive and others system [1-3]. Our study aimed to examine the cytotoxicity of melittin on human cervical carcinoma HeLa cells and human fibroblast MRC-5 non-cells and to determine the selectivity index of melittin. Melittin was purified from honey bee venom collected from *Apis mellifera carnica* using HPLC. Cells were maintained in an atmosphere containing 5% CO_2 in absolute humidity at 37°C and tested at the exponential growth phase. Cytotoxicity of melittin was determined after 48 h in concentrations of 0.3, 1, 10, 30, and 100 μM. Selectivity index was calculated as a ratio of IC_{50} (half maximal inhibitory concentration) for non-cancer cells (MRC-5) versus IC_{50} for cancer cells (HeLa). The results showed high anticancer activity of melittin against HeLa cells after 48 hours with IC_{50} of 56.2±4.7 μM. Moreover, melittin displayed low cytotoxicity against MRC-5 with IC_{50} of 984.7±12.5 μM. The calculated selectivity index was 17.5 demonstrating very high selectivity of melittin against human cervical cancer cells. In conclusion, our results recommend melittin as a promising therapeutic candidate in patients with cervical cancer, as it not only has a highly potent activity at lower concentrations but also it exhibits a high degree of selectivity towards cervical cancer cells.

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Effect of probiotic consumption on serum glucose and lipid levels in mice

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The purpose of present study was to examine the effect of probiotic administration on the level of glucose and lipids in mice after exposure to metabolic stress by glucose. The probiotic was composed from two commercial strains *Lactobacillus rhamnosus* Rosell 11 and *Lactobacillus helveticus* Rosell 52 (Probiodrops®). The levels of the selected metabolites were monitored in the serum of high fat diet mice and the serum of mice on a standard diet [1]. Eight-week-old male C57BL/6J mice, with initial body weight 18-22g were randomly distributed among five groups (n=8 mice/ group) [2]. Three experimental groups were exposed to high-fat food and sucrose as drinking solutionand treated by gavage with probiotic mixture in three different concentrations (10^7, 10^8, 10^9 CFU/mL). Positive control group was exposed also to high fat food and sucrose, whilenegative control group was on standard diet and tap water. Average food, water intake and body weight gain were recorded on weekly basis. Throughout experimental period it was not observed difference beetwen groups in food consumption, water and weight gain. The serum glucoselevel were determined on monthly basis, whileserum lipids level and glucose tolerence test were performedat the end of experiment. After two months of oral administration of *Probiodrops*® in concentration 10^9 CFU/daywas shown reduction of serum glucose and triglycerides level (~30%), and significantly improvement of glucose tolerance (approx. 35%). Therefore, *L. rhamnosus* Rosell-11 and *L. helveticus* administration in dose 10^9 CFU/day demonstrated promising results and might be good candidate for further research for better glucose tolerance and triglycerides level lowering.

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Omega-3 index and anthropometric indices among testicular cancer survivors

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Due to toxicity of cisplatin-based chemotherapy and potential testosterone deficiency testicular cancer survivors are considered to be at increased risk of unfavorable cardiometabolic status. Red blood cell (RBC) fatty acid (FA) composition, and, in particular, the Omega-3 Index, have been reported to provide predictive information concerning the risk for cardiovascular diseases and metabolic syndrome. In this cross-sectional study sample of patients undergoing post-treatment follow-up program at the Clinic of Urology, University Clinical Center of Serbia was recruited from May to August 2021. RBC membrane FA panel was determined by gas chromatography. The Omega-3 index (%) was computed as the sum of RBC eicosapentaenoic acid and docosahexaenoic acid, and risk categories were determined as high, <4%; intermediate, 4–8%; and low, >8%. Based on weight, height, waist, and hip circumference additional anthropometric indices were calculated including: a body shape index (ABSI = waist circumference (cm) / ($BMI^{0.66}$ x height $(m)^{0.5}$), body mass index (BMI = body weight (kg) / height $(m)^2$), waist to hip ratio (WHR), waist to height ratio (WHtR), and waist to hip to height ratio (WHHR). Bioimpedance analysis was applied for body fat percentage (BF%) determination. A total of 24 men aged 26-58 ($\bar{x}$=38.54±8.18) years participated in the study . Based on BMI ($\bar{x}$=25.91±3.28) 58.33% were overweight or obese (BMI≥25.0kg/m2). Average omega-3 index value was 4.49±0.89% with 8 subjects being allocated in the high-risk group, 16 in the intermediate hazard zone and none reaching the low-risk threshold. Although the omega-3 index did not correlate with BMI and BF% estimates, statistically significant inverse associations were found with abdominal adiposity surrogate markers including WC (r=-0.468, p<0.05), WHR (r=-0.572, p<0.01), WHtR (r=-0.451, p<0.05), WHHR (r=-0.453, p<0.05), and ABSI (r=-0.512, p<0.05). Findings of this study warrant further research and long-term intervention trials to examine the clinical significance of increased intake of long chain omega-3 polyunsaturated FAs via food sources and/or supplementation in the prevention and management of abdominal adiposity and subsequent cardiometabolic consequences among testicular cancer survivors.

Small molecules attenuate activation of the NF-κB signaling in epthelial cells by Act d 1 kiwifruit allergen

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Many inflammatory events are regulated by the NF-κB signaling pathway including the allergic response to food allergens. Small molecule NF-κB inhibitors can enable better regulation of the inflammation process, prevent unwanted side effects and increase safety of vaccines [1–3]. The aim of this study was to explore the inhibitory potential of selected small molecules on NF-κB signaling pathway to decrease the inflammatory effect of food allergens on epithelial cells (HEK293 and Caco-2 cell line). Besides kiwifruit allergen Act d 1, LPS was used as pro-inflammatory stimuli for the cell treatment. Fluorescent microscopy and flow cytometry were employed to confirm the activation of NF-κB in HEK293 cells after transfection with reporter NF-κB-GFP plasmid. LPS induced a very low inflammatory effect on HEK293 and Caco-2 epithelial cell lines. On the other hand, after Act d 1 treatment gene expression of pro-inflammatory cytokines in HEK293 cells significantly increased and showed a typical cytokine profile of allergic sensitization. Cells that were treated with vanillyl alcohol or lauric acid previous to the Act d 1 exposure, showed decreased expression of cytokines (IL-1b, IL-6, IL-25, IL-33, TNFα). The transcription factor was activated upon allergen treatment and subsequently attenuated by the small molecules.

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Monoterpenes variability in *Teucrium montanum* L. essential oils

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The aim of this study was to determine the qualitative variability and relative presence of oxygenated monoterpenes in essential oils from *Teucrium montanum* L. collected from different habitat substrates. Plant material was sampled from twenty different populations with serpentinite and calcareous habitat substrate. *T. montanum* essential oils were obtained by hydrodistillation of the aboveground plant parts and analysed by GCxGC-MS chromatographic technique. The sum of the total relative presence of the oxygenated monoterpenes in the essential oil samples from serpentinite and calcareous habitats was 5.1% and 2.1% respectively. Limonen-10-ol (2.4%) was the most abundant compound from the group of oxygenated monoterpenes. The results also indicate that decanol (0.7%) and limonen-10-ol (0.5%) are the oxygenated monoterpenes with the highest relative presence in the analysed *T. montanum* samples from the calcareous substrate, as well as limonen-10-ol (2.0%) and mentha-1(7),8-diene-2-ol (0.8%) in the samples from serpentinite substrate. Carvone and 3-caren-2-ol were monoterpenes identified only in the *T. montanum* essential oil samples from serpentinite habitats. The determined variability indicates a significant influence of the geological substrate on *T. montanum* essential oil synthesis. One of the important reasons for such variability is the difference between the chemical characteristics of serpentinite and calcareous soils [1]. Soils formed on a serpentinite substrate are characterized by a lack of macroelements and higher content of heavy metals which has a positive effect on terpene synthesis.

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Biomarkers of oxidative stress in pediatric patients with type 1 diabetes mellitus

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Type 1 diabetes mellitus (T1DM) is the result of cellular-mediated autoimmune destruction of insulin-secreting β-cells of the pancreas. A defect in insulin secretion in T1DM results in hyperglycemia that initiates non-enzymatic glycation and oxidative stress (OS), two main processes involved in the development of diabetic complications [1,2]. In the presented study, we analyzed levels of OS biomarkers in 97 pediatric patients with T1DM, as well as their association with parameters of inflammation, lipid profile, and metabolic control. We observed a significant correlation between the concentrations of C reactive protein and prooxidant-antioxidant balance, advanced oxidation protein products, total antioxidant status, and superoxide dismutase activity. The levels of advanced oxidation protein products were positively associated with the levels of LDL-cholesterol ($P<0.01$), triglycerides ($P<0,01$), total cholesterol ($P<0.01$), and glycated hemoglobin ($P<0.05$). Our results showed that low-grade inflammation in T1DM is associated with the intensive production of free radicals. In addition, the observed link between OS and dyslipidemia suggests an increased cardiometabolic risk of the pediatric population with T1DM.

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The use of the handheld grain quality analyzer for determination protein content in wheat cultivars

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Determination of different quality traits in plant breeding program often requires fast, reliable and non-destructive analytical techniques. During the last two decades, near-infrared (NIR) spectroscopy has been most often used for measuring of chemical composition: protein, moisture, oil and other parameters [1]. The NIR techniques possess a number of advantages, but also a disadvantage that these devices are relatively large and weight more than 8 kg. The aim of the study was to examine possible using of handheld grain quality analyzer for protein content examination in wheat samples. The protein and water content of 13 wheat cultivars was determined by standard methods of Kjeldahl digestion and drying methods, respectively. Protein content was calculated on dry matter. Furthermore, this quality trait was also determined by handheld GrainSense Analyzer (Oulu, Finland) which weight is 820 grams without batteries [2]. The results of the study showed that the correlation between commonly used method and handheld grain quality analyzer is positive with r = 0.951. From results it can be concluded that handheld analyzer can be successfully used for protein determination in wheat samples

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Prevalence of somatic *BRCA1* gene methylation in patients with ovarian cancer

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Ovarian cancer is a disease with a significant mortality rate, and Serbia has the second highest incidence of ovarian cancer in the world. Early diagnostics, screening and targeted therapy, are still the greatest need in the fight against this disease. A considerable number of ovarian cancercases arises as a result of underlying aberations in *BRCA1/2* genes, where germline mutation and methyilation of promoter are the most crucial ones and thought to be mutually exclusive [1]. There is a growing need for testing methylation of *BRCA1/2* genes because positive result provides an indication for treatment with poly (adenosin diphosphate-ribose) polymerase (PARP) inhibitors [2], and also, the level of DNA methylation coresponds to the stage of the disease and tumor progression [3]. We were investigating the prevalence of promoter methyilation of *BRCA1* gene in pathogenesis of sporadic ovarian cancer. The study included the analysis of FFPE samples from 20 patients, who had previously been screened for *BRCA1* and *BRCA2* mutations. Bisulfite conversion of cytozine residues in CpG islands was followed by PCR amplification to evaluate the methylation pattern. We performed two methyl specific PCR reactions for each bisulfite converted sample, one with primers designed for methylated sequence in the promoter region and the other with primers designed for unmethylated sequence. Following the completion of these experiments and statistical analysis of the results, we were able to determine the amount of methylation in the promoter region of the *BRCA1* gene as well as the prevalence of somatic *BRCA1* gene mutation in this small group of patients. Based on the findings, we calculated the percentage of patients with a totaly methylated, intermediately methylated, or non-methylated region of the *BRCA1* gene. *BRCA1* mutation was found in 10% of cases, or two patients in this investigation, one with no methylaton detected and one with detected intermediary methylation profile. Complete promoter methylation was detected in a small number of cases, but no matching somatic mutation was found in any of them. Using Fisher's exact test, we examined the relation between the totaly methylated promoter and the mutational status of the *BRCA1* gene. In this small group of patients there was no evidence to reject the hypothesis that the prevalence of methylation of the *BRCA1* gene promoter is independent of its somatic mutation. More research is needed in this field to acquire more consistent results and to increase the number of examined samples. In the future, we will expand the study to include the *BRCA2* gene and compare the results to those found in this study, so that we

can have a more accurate clinical picture of cancer patients and the findings can be considered clinically relevant.

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Anti-migratory properties of Nischarin agonist rilmenidine in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of cancer, with the five-year survival rate of just 6%. Research on PDAC is limited and progress in patient survival has not been made in the last 40 years. PDAC tumors are extremely chemo and immunotherapy resistant due to the early metastatic dissemination, rich desmoplastic stroma that limits drug delivery, the presence of highly resistant cancer stem cells and the immunosuppressive infiltrate. Drugs that target any of these mechanisms/components are potential candidates for PDAC treatment. The objective of our project was to examine the expression of Nischarin (NISCH) in PDAC and its potential as a target for drug repurposing. NISCH is an Imidazoline-1 receptor (IR1) protein that has been described as a tumor suppressor in breast and ovarian cancers [1], and there are several clinically approved agonists for this receptor. We performed Gene Set Enrichment Analysis (GSEA) on several publicly available PDAC datasets and found that NISCH was expressed in both cancer cells and associated stroma, and that its expression was associated with cell adhesion, migration and invasion potential.We found that NISCH was expressed in a panel of PDAC cell lines and that treatment with the most potent NISCH agonist rilmenidine had effects on the organisation of the actin cytoskeleton, cell adhesion and migration of cancer cells. With these anti-migratory and potentially anti-metastatic effects of rilmenidine, our study lays a ground for more extensive examination of the role of NISCH in PDAC and implies that its agonists may be good candidates for drug repurposing in this type of cancer.

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