# Serbian Biochemical Society

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## Proceedings


**Editor:** Ivan Spasojević
**Technical support:** Jelena Korać Jačić
**Cover design:** Zoran Beloševac
**Publisher:** Faculty of Chemistry, Serbian Biochemical Society
**Printed by:** Colorgrafx, Belgrade

# Serbian Biochemical Society
# Eleventh Conference

Scientific meeting of an international character

September 22nd and 23rd, 2022, Novi Sad, Serbia

"***Amazing Biochemistry"***

# PROGRAMME

**Day 1 – Thursday, September 22nd 2022**

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| 9:00 – 10:00 | Participants registration and posters posting |
| 10:00 – 10:15 | Opening ceremony |
| 10:15 – 11:00 | István Zupkó<br>University of Szeged<br>**PL1: Cancer metastasis: methodological challenges and pharmacological possibilities**<br>FEBS3+ Lecture |
| 11:00 – 12:15 | Poster Session 1 & Coffee Break |
| 12:15 – 12:45 | Andrej Veljković<br>University of Niš - Faculty of Medicine<br>**IL1: Mechanisms of oxidative stress and antioxidant protection in carcinogenesis** |
| 12:45 – 13:00 | Marija Maksimović<br>MDPI - Multidisciplinary Digital Publishing Institute<br>**SL1: Open Access publishing: Trends and perspectives** |
| 13:00 – 13:15 | Yaraslau Dzichenka<br>Insitute of Bioorganic Chemistry of National Academy of Sciences of Belarus<br>**OP1: Novel promising fluorescent ligands of human CYP17A1** |

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|---|---|
| 13:15 – 13:30 | Dragana Pap<br>Students Health Protection Institute Novi Sad<br>**OP2: Antioxidant defense, obesity, type 2 diabetes in students** |
| 13:30 – 13:45 | Tamara Antonić<br>Faculty of Pharmacy, University of Belgrade<br>**OP3: HDL functionality in women with preeclampsia** |
| 13:45 – 14:00 | Mirjana Radomirović<br>University of Belgrade - Faculty of Chemistry<br>**OP4: Development and comparison of Western blot, dot blot and ELISA for mussels tropomyosin quantification** |
| 14:00 – 15:00 | Cocktail / Lunch break |
| 15:00 – 15:30 | Roman Jerala<br>National Institute of Chemistry, Slovenia<br>**IL2: Coiled-coil modules for designed protein folds and cellular logic circuits** |
| 15:30 – 16:00 | Jelena Danilović Luković<br>University of Belgrade - Institute for the Application of Nuclear Energy<br>**IL3: Extracellular vesicles in a maze of glycomic complexity** |
| 16:00 – 23:00 | Social events (guided tour and Conference dinner) |

**Day 2 – Friday, September 23rd 2022**

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|---|---|
| 9:00 – 10:00 | Participants registration and posters posting |
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| 10:45 – 12:00 | Poster Session 2 & Coffee Break |
| 12:00 – 12:30 | Sonja Milić Komić<br>University of Belgrade - Institute for Multidisciplinary Research<br>**IL4: Late Embryogenesis abundant proteins: Structural characterization and interaction with α-synuclein** |
| 12:30 – 13:00 | Miloš Matić<br>University of Kragujevac - Faculty of Science<br>**IL5: The role of interleukin-6 in placenta and possible pregnancy risks in patients with COVID-19** |
| 13:00 – 13:15 | Aleksandra Milenković<br>University of Niš, Faculty of Technology, Leskovac<br>**OP5: Chemical composition and antioxidant activity of Frankincense essential oil** |
| 13:15 – 13:30 | Filip Štrbac<br>Faculty of Agriculture, University of Novi Sad<br>**OP6: Chemical composition of sage (*Salvia officinalis* L.) essential oil and its anthelmintic properties against sheep gastrointestinal nematodes** |

| | |
|---|---|
| 13:30 – 13:45 | Dragica Mićanović<br>Institute for Biological Research "Siniša Stanković, University of Belgrade<br>**OP7: Effects of chokeberry fruit water extract on immune system in mouse models of infection and melanoma** |
| 13:45 – 14:45 | Cocktail / Lunch break |
| 14:45 – 15:15 | Miron Sopić<br>University of Belgrade, Faculty of Pharmacy<br>**IL6: Change of transcriptomic signature in subcutaneous adipose tissue induced by weight loss** |
| 15:15 – 15:45 | Dejan Orčić<br>University of Novi Sad - Faculty of Sciences<br>**IL7: Phytochemical and biochemical studies of wild chervil (*Anthriscus sylvestris*)** |
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# Foreword

Dear Colleagues,

It is a distinct pleasure to welcome you to the 11th Conference of the Serbian Biochemical Society, entitled '*Amazing Biochemistry*', and to the city of Novi Sad, European Capital of Culture in 2022.

In the shadow of global crisis it is very important for us scientists to present the results of constructive efforts that are focused on life, health, environment and other things that are really important. By helping to understand the world around us, we can re-establish the fate in an amazing future.

I would like to thank all the participants for their valuable contributions and to the Organizing Committee and the Scientific Board for their time dedicated to this meeting.

*Editor of the Proceedings*
*Ivan Spasojević*

# Plenary Lectures

# Cancer metastasis: methodological challenges and pharmacological possibilities

**István Zupkó**

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Currently, cancer is the second major cause of mortality globally. Still, a transition in the predominant causes of death is detected in high-income countries: mortality from cancer has become more common than cardiovascular disorders. Moreover, in solid tumors cases, most cancer-related mortality is due to the consequences of metastases. Therefore, antimetastatic pharmacological interventions may have a crucial impact on overall mortality. Metastasis formation is a complex and well-organized procedure, including the infiltrating growth of cancer cells through the extracellular matrix, migration, and initiation of colonies in distant organs. Therefore, identifying innovative antimetastatic compounds requires in vitro methods of relatively high throughput. The presentation aims to summarize the currently available antimetastatic methods, including different types of cell-based migration and invasion assays. Pathways play determining role in the metastasis formation and endogenous molecules representing targets for potential pharmacological interventions such as matrix metalloproteinases or tyrosine kinase receptors will be also discussed. Some preliminary results obtained at our Institute will be additionally presented.

## Acknowledgements

Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

# Design of proteins with photocontrollable structure and function and their applications

**Brankica Janković**

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Nature employs a very sophisticated strategy of using light to control certain biological processes such as vision or photosynthesis. If we borrow that concept from Nature and make it universally applicable to a wide variety of proteins, we would be able to control many aspects of life on a molecular level. In the group of Prof. Dr. Peter Hamm, one of our goals is to design proteins to be specifically photo-responsive, so that we use light to trigger the conformational changes and study them by real-time spectroscopy. Such a combination of tools enabled the studies of interesting biochemical phenomena such as protein binding mechanisms and allostery. More precisely, the design of a protein-peptide interaction that can be light-controlled, enabled the detailed kinetics investigation of their binding mechanisms. Furthermore, the design of a bidirectionally controlled allostery made it possible to directly investigate the speed of allosteric signaling within the single protein domain. Many other proof-of-concept studies and possible applications of photocontrollable proteins will be discussed.

# Invited Lectures

# Extracellular vesicles in a maze of glycomic complexity

**Jelena Danilović Luković*, Tamara Janković, Ninoslav Mitić, Sanja Goč**

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The carbohydrate portion of proteins and lipids mediates a variety of biological processes. Revealing its underlying principles isa challenging task that couldcontribute to a better understanding of many patho/physiological conditions. On the other hand, the interest in extracellular vesicles (EVs) has increased in recent years due to their involvement in intercellular communication leading to an array of functional and structural changes in recipient cells. Their characterization uncovered an exceptional diversity in size, morphology, and membrane and cargo content. Monitoring/analysis of surface glycosylation of EVs originating from the prostate termed prostasomes revealed their substantial contribution to the complexity of seminal plasma glycome. Surface glycans heterogeneity confirm the existence of several prostasome subpopulations. Presentation of surface glycans on prostasomal membrane isstrongly affected by co-localized membrane-associated glycoproteins and tetraspanins. They appear to be organized in established/regular distribution patterns on membrane domains. Surface glycans are a component of EVs membrane of importance for its functionality and potentially a distinction marker of prostasome subpopulations. Further understanding of the complex composition of glycans on EVs might explain the relation of their structure with functional alterations in distinct patho/physiological conditions.

# Coiled-coil modules for designed protein folds and cellular logic circuits

**Roman Jerala**

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Coiled-coil (CC) dimers are ubiquitous building modules in natural proteins. Rules that define interactions in CC dimers are relatively well known, which allows us to design new CC dimers with selected stability and selectivity. CC dimers can be concatenated into longer chains for the construction of new modular protein folds based on topological principles distinct from natural proteins. Coiled-coil protein origami - CCPO structures have interesting properties and are highly designable, enabling multiple use of the same building modules, design of the folding pathways and other functional properties[1-3]. Although CC dimers occur frequently in natural proteins, designed CC dimers can also be used for cell regulation. We have designed new CC pairs that can fused to other proteins, enabling new type of regulation of biological processes, including localization multiplexing within cells, augmented transcriptional response based on chemical regulators – CCCtag, and faster kinetics based on combination of split proteases with coiled-coil modules (SPOC logic)[4,5]. Those building blocks have also been used to construct two genetically encoded orthogonal secretion systems for fast secretion of proteins based on the pool of ER-retained proteins[6].

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5. Lebar et al. A tunable orthogonal coiled-coil interaction toolbox for engineering mammalian cells. Nat Chem Biol 2020;16:513-19.
6. Praznik et al. Regulation of protein secretion through chemical regulation of endoplasmic reticulum retention signal cleavage. Nat Commun 2022;13:1323.

# Adjuvant-free animal model for studying CNS autoimmunity

**Bojan Jevtić**

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Multiple sclerosis is a chronic inflammatory, demyelinating, and neurodegenerative disorder of the central nervous system. More than 2.5 million people suffer from this disease worldwide. It is assumed that the autoimmune response to myelin antigens in the CNS is the main cause of the disease. Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for studying MS. However, EAEmodels resemble only particular aspects of the MS pathogenesis. EAE is classically induced with the CNS antigens emulsified in complete Freund's adjuvant (CFA). CFA consist of paraffin oil supplemented with *Mycobacterium,* and its application potentiates innate immune response, prolongs the presence and effective transport of antigen in the lymphatic system. However, CFA has a confounding influence on the results and the translational capacity as a multiple sclerosis model. Our group has successfully excluded CFA from immunization regime. In a recent study, we compared clinical, histological, cellular and molecular properties between spinal cord homogenate (SCH) and SCH+CFA immunized Dark Agouti rats. We have observed higher clinical score in rats without CFA and greater number of immune cell infiltrates at the peak of EAE in the same animals. Further, stronger myelin basic protein-specific T cell immune response is evokedin the draining lymph nodes of CFA-free compared to CFA immunized rats. In the CNS, high abundance of CD8+T cells is detected at the onset of disease. Also, enrichment in CD8+ and CD4+ macrophageswas observed in the CNS during EAE. Therefore, CFA-free EAE is a reliable model for studying CNS autoimmunity.

# The physiological role of interleukin-6 in placenta and its pathological potential in pregnancy

**Miloš Matić*, Ana Obradović, Milica Paunović, Marija Milošević, Sara Milojević, Nevena Planojević, Branka Ognjanović**

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Interleukin-6 (IL-6) is pleiotrophic cytokine that has both pro-inflammatory and anti-inflammatory functions. Placentation and pregnancy progression depend on adequate interaction between the processes of proliferation, apoptosis and invasion of trophoblast cells into the endometrium. Various cytokines and growth factors play an important roles in the regulation of these processes, where IL-6 represents one of the major regulatory molecules influencing the trophoblast phenotype. In physiological circumstances, IL-6 is involved in angiogenesis and remodeling of endometrial blood flow, stimulates the production of placental hormones, and is one of the main regulators of inflammation response and immune homeostasis in placenta. Elevated levels of IL-6 are indicated in women with infertility, preeclampsia and placental neoplastic processes. Hypoxic conditions in placenta play a pivotal role in modulating differentiation, invasion and redox homeostasis of trophoblast and also seems to have a significant contribution to IL-6 effects, while IL-6 itself affect oxidative state of trophoblasts, although the mechanisms of these outcomes are yet to be fully understood. Our experiments suggest importance of hypoxic conditions in determining the effects of IL-6 in trophoblasts and differential reactivity of JEG-3 cells in response to this cytokine. Additionally, increased levels of IL-6 in different systemic pathological states induce various disturbances of trophoblast cell homeostasis and could be one of risk factors in development of pregnancy disorders. Recently, increased IL-6 levels are detected in COVID-19 cases, as one of the major actors of the cytokine storm, raising the concerns of infection regarding the effects on placenta and the offspring.

# Late embryogenesis abundant proteins: Structural characterisation and interaction with α-synuclein

**Sonja Milić Komić[1*], Sonja Veljović Jovanović[1], Ana Pantelić[2], Marija Vidović[2]**

*[1]University of Belgrade - Institute for Multidisciplinary Research, Department of Life Science, Belgrade, Serbia*
*[2]Institute of Molecular Genetics and Genetic Engineering, Laboratory for Plant Molecular Biology, University of Belgrade*

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Ressurection plants are extraordinary because of their ability to withstand long periods without water, enter a state of anhydrobiosis, and fully recover upon water arrival. *Ramonda serbica* is a relic and endemic species that belong to a very small group of desiccation-tolerant plants in Europe. Underlying physiological, molecular and morphological mechanisms that enable these plants to survive harsh environmental conditions have been an appealing subject to many researchers. Most of the genes responsible for this amazing ability are present in other plants, and this path of research where those genes could be activated in crops is growing much more attention because of the imminent crisis regarding food supplies in the near future. Key components involved in the response to dehydration in *R. serbica* plants were analysed through a comprehensive transcriptomic, proteomic, metabolite and photosynthetic study. Late embryogenesis abundant proteins play a significant role in the complex defence processes involved in desiccation tolerance. Defining physicochemical characteristics and specific physiological functions of late embryogenesis abundant proteins – LEAPs may lead to their applicability in other areas of research.

## Acknowledgements

This research was funded by the Science Fund of the Republic of Serbia-RS (PROMIS project LEAPSyn-SCI, grant no. 6039663) and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200053 and 451-03-68/2022-14/200042).

# Phytochemical and biochemical studies of wild chervil (*Anthriscus sylvestris*)

**Dejan Orčić*, Sanja Berežni, Neda Mimica-Dukić**

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Plants represent important sources of bioactive molecules that can be used directly as medications, or as industrial precursors thereof. Podophyllotoxin, that was first isolated from mayapple (*Podophyllum peltatum* L.), and related natural and semi-synthetic lignans, are important antiviral, antihelmintic and especially antitumor agents, used in traditional and official medicine. Since the exploitation of common sources, such as *Sinopodophyllum hexandrum*, becomes unsustainable, new species are being investigated. One of the most promising is wild chervil (*Anthriscus sylvestris* (L.) Hoffm.), a widely distributed wild-growing Apiaceae species, commonly considered a noxious weed, that is known to be rich in lignans[1–3], especially aryltetralins (such as deoxypodophyllotoxin, podophyllotoxin and podophyllotoxone) and dibenzylbutyrolactones (including yatein and nemerosin), but also in various phenylpropanoids and terpenoids. This paper provides an extensive overview of *A. sylvestris* chemical composition investigations conducted thus far, with special focus on recent comprehensive profiling studies based on hyphenated techniques. Additionally, a detailed account of the bioactivity studies of both extracts and isolated compounds, confirming their antioxidant, anti-inflammatory and antiproliferative activity, is given.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021–14/200125).

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# Change of transcriptomic signature in subcutaneous adipose tissue induced by weight loss

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Obesity is a chronic disease underlined as one of greatest public health challenges in 21st century that significantly increase the risk of developing diabetes, cardiovascular diseases, liver disease and cancer. Current strategies in obesity prevention focus on the lifestyle changes, calorie-restriction diets, pharmacological and surgical interventions. Although obese subjects share some phenotype characteristics, others can be significantly different. For example, up to 30% of obese patients are metabolically healthy and do not display the "typical" metabolic obesity-associated complications. These phenotype difference also lead to variation in success rate of treatments among different individuals. Since obesity cannot be looked at as one simple pathological entity,we need novel tools to define different phenotypes of obesity in order to improve stratifications of patients and facilitate the development of personalized treatments. The use of next-generation sequencing enables comprehensive view on interaction between different genes, and the discovery of novel pathways that are dysregulated in different pathophysiological processes. So far, this approach has identified novel coding and non-coding regions of DNA that are implicated in the development and progression of obesity and help to identify potential tissue-specific biomarkers that could be used for successful predictions of intervention outcomes. Another interesting layer of information that recently is being explored is related to the modifications of RNA and its implications in adipose tissue physiology. Animal as well as human studies have confirmed critical role that some RNA modification play in the dysfunctionality of adipose tissue in obesity. Future efforts should aim to integrate transcriptomics data with other omics (genomics, epigenomics, proteomics and metabolomics) through the use of machine learning algorithms in order to get more holistic view and deeper understanding of different obesity phenotypes.

# Xanthine oxidase activity and its relation with oxidative stress in human colorectal and prostate cancer

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Colorectal cancer (CRC) and prostate cancer (PC) are one of the most often diagnosed cancers and the main reason for mortality connected with tumor diseases[1,2]. There is still a shortage of examination including the influence of xanthine oxidase (XO) activity in progressiveness and invasion of the cancers, so the present study investigated the role of XO activity, in correlation to TBA-reactive substances (TBARS) and AOPP as a markers of oxidative stress in progression and invasion of human CRC and PC. We took tissue specimens from 30 patients with CRC and 18 patients with PC, in all four TNM clinical stages of the disease. They were divided in 3 groups: cancer tissue, tissue surrounding the tumor and healthy control tissue group. We made 10% homogenates in which we conducted the study with proper methods. The activity of XO in tumor tissue and tissue adjacent to the tumor was significantly higher when compared to healthy colon tissue. Tumour tissue of PC was significantly higher compared to healthy prostate tissue. The highest activity of XO is in T2 and T3 tumor stadiums. TBARS and AOPP also have higher concentration when compared to control healthy tissue in both tumours. There is a positive correlation between XO activity and PSA levels in PC. Presented results suggest that one of the possible causes of oxidative stress in CRC and PC could be high XO activity. It may include this enzyme in the malignant transformation, progression and invasion of human CRC and PC. We could use this enzyme as theranostic biomarker, since using XO inhibitors such as allopurinol as adjuvant therapy could be promising treatment.

## Acknowledgements

This study was supported by Science Fund of the Republic of Serbia (IDEAS), project number: 7750154 (NPATPETTMPCB).

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## Posters

# The immunosuppressive therapy and the outcome of COVID-19 infection in patients with rheumatoid arthritis

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The interaction between COVID-19 infections and rheumatic diseases is still unknown. Is the bad outcome of the infection a consequence of the patient's immunocompromised condition or does the application of antirheumatic therapy alleviate the hyperinflammatory response? Do all drugs used to treat autoimmune diseases cause the same effect on the course and outcome of COVID-19? These are some of the many questions that rheumatologists face in their daily work. The aim of this study was to analyze the impact of therapy on the course of the disease (DMARD) and the outcome of the COVID-19 infection in patients with rheumatoid arthritis (RA). Retrospective data analysis, obtained by filling out web questionnaires, included 112 patients with RA who had confirmed SARS-CoV-2 infections in a period of July 16 to November 22 2021. The diagnosis of RA was made on the basis of 2010 ACR/EULAR criteria, while statistical analyses were performed using the statistical software SPSS Statistics 22.0. All presented p-values were two-sided and $p<0.05$ was considered statistically significant. In our group of patients with RA, 21 patients were hospitalized due to COVID-19 infections. 19 patients needed oxygen support, while 6 patients died intrahospital. The results indicate that Azathioprine and Rituximab increase the risk of hospitalization due to COVID-19, while Leflunomide and Rituximab are risk factors for fatal outcomes from COVID-19 infections. Therapy with Tocilizumab was proved to be protective of hospitalization. The use of anti-TNF drugs does not increase the risk of hospitalization. Based on our results, we can conclude that regular use of anticytokine biological DMARD therapy (anti-TNF and anti-IL-6 therapy) is not associated with a bad outcome of COVID-19, unlike B-cell depressants which contribute to higher hospitalization and death rates of the COVID-19 infection by weakening the humoral immune response.

# Transcriptional changes induced by di-2-ethylhexyl phthalate in FSH-stimulated human granulosa cells

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Di-2-ethylhexyl phthalate (DEHP) is an endocrine disruptor widely used in many industrial and consumer products. Animal studies suggest a possible association between DEHP exposure and disrupted function of the female reproductive system[1]. Other studies demonstrated adverse effect of DEHP on human granulosa cells[2]; however, the effect of DEHP on the follicle-stimulated hormone (FSH)-treated human granulosa cells (hGC) is not known. The goal of this study was to identify novel DEHP target genes in FSH-stimulated hGC. The primary culture of hGC obtained from women undergoing *in vitro* fertilization procedure was exposed to 100 ng/mL FSH and 25 μM DEHP for 48 h and the total mRNA was analyzed using the DNBSEQ platform. In FSH-stimulated hGC, DEHP exposure caused an increase in the expression of 47 genes and a decrease in the expression of 198 genes. The upregulated genes are involved in steroid hormone synthesis and metabolism of xenobiotics by cytochrome C, whereas the downregulated genes belong to the cell cycle pathway. Some of the genes that are regulated by FSH, but also affected by DEHP exposure, are the genes encoding the water channel proteins aquaporins 2 and 5, angiotensin-converting enzyme, and low-density lipoprotein receptor class A. These results, for the first time, identify novel FSH-regulated genes that are DEHP targets in hGC.

## Acknowledgements

This study was supported by the Science Fund of the Republic of Serbia, program PROMIS, project DETOX, grant number 6062573.

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# HDL functionality in women with preeclampsia

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High-density lipoprotein (HDL) role in pregnancy is often overlooked. HDL cholesterol concentration is thought to rise during a healthy pregnancy, while this adaptive change was not noticed in preeclampsia. Our objective was to identify the pattern of changes in HDL particles functionality during high-risk pregnancy and connect particular alterations with preeclampsia development. HDL functionality markers were longitudinally assessed in 90 women with high-risk pregnancies, 20 of whom developed preeclampsia by the end of pregnancy. As expected, women with preeclampsia did not experience a rise in HDL cholesterol concentration, while a protective increase was observed in women without preeclampsia despite being at risk. Additionally, a significant increase in triglycerides was observed in both groups, while the levels were higher in women with preeclampsia in both, the 1$^{st}$ and 3$^{rd}$ trimesters. This increase was accompanied by a decrease in cholesterol-ester transfer protein (CETP) activity in both groups. The activity of the paraoxonase, the most potent antioxidative component of HDL, was higher in women with preeclampsia ($p<0.05$ in 1$^{st}$ and 3$^{rd}$ trimester), probably as a response to the increased oxidative stress. Although levels of Monocyte Chemoattractant Protein-1 (MCP-1), which expression is inhibited by HDL, decreased during the testing period, levels of this inflammatory protein were higher in both test points in preeclamptic patients. In conclusion, preeclampsia seems to be followed by altered HDL-cholesterol levels and impaired HDL functionality, *i.e.*, impaired maturation of the particle, and poor anti-inflammatory and anti-oxidant activity.

## Acknowledgements

This study was supported by the Science Fund of the Republic of Serbia, #7741659, HIgh-density lipoprotein MetabolOMe research to improve pregnancy outcome – HI-MOM.

# Effect of FITC labeling on enzymatic activity and antigenicity of food allergen Act d 1

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Some food allergens due to their proteolytic activity can disrupt and pass through the epithelial monolayer and induce sensitization, the first phase of food allergy development. Therefore, it is of interest to have a simple in vitro method for detecting the protein leaking below the epithelial monolayer[1]. Labeling individual allergens with fluorescent molecules can enable easy analysis of the protein trajectory, as long as it doesn't have a negative effect on its activity and antigenicity. For this purpose in our research, we used Act d 1 as a model allergen, since it is a major kiwifruit allergen that has cysteine protease activity. This study aimed to purify and label Act d 1 with FITC, as well as to see if the binding of FITC has any effect on its proteolytic activity and reactivity to Act d 1-specific sera. After successful purification, to capture the effect of different amounts of FITC, three different concentrations were used for the labeling reactions. The labeled proteins were first characterized by determining the FITC/protein molar ratio. Unlabelled actinidin was used as a positive control in the western blot and ELISA as well as in the activity assays. It was shown that with higher amounts of FITC we can expect a better molar ratio and higher fluorescence signal, but more importantly that none of the used amounts of FITC affect the protein activity or its reactivity to actinidin-specific sera.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of Serbia, and the Erasmus+ EU program for education, training, youth, and sport

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# The impact of albumin/globulin ratio on COVID-19 mortality

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Albumin is the most abundant protein in blood and low concentration is associated with decreased production or increased loss. Globulins are more diverse group of proteins, and some of them such are immunoglobulins are specifically involved in acute and chronic infections. Albumin/globulin ratio (AGR) is a biomarker with potentially prognostic value in COVID-19 patients with sepsis[1]. The aim of research is to compare AGR value at survivors vs non-survivors, and note if there are differences among women and men.The study retrospectively enrolled 88 confirmed COVID-19 patients with sepsis (aged 22-84 years; 59 men, 29 women), who were hospitalized in a tertiary care hospital from November 2020 to January 2022. Demographic data and laboratory values were collected from medical records (the following variables for each patientage, sex, serum albumin level, globulin level, total serum proteins, albumin/globulin ratio and 28-day mortality).Patients were divided into 2 groups acording to outcome: survivors (33 patients) and non-survivors(55 patients). Normality test (Shapiro-Wilk), descriptive statistic and statystical analysis were performed by software IBM SPSS® Statistics 23.0. Differences among groups and subgroups(sexes) were estimated using the indipendend T-test. P-value <0,05 had been considered significant. Mean AGR was not much higher in survival ($\bar{x}$=1,23) than in non-survival group ($\bar{x}$=1,14) and there are no significant difference among sexes. But graphical presentation of data (scatter plot and box plots) showed better correlation between low albumin and death at men. Thus, a larger study with more patients is needed for more exact correlation.

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# Potential changes in the estrous cycle of female mice exposed to arsenic(III)-oxide during three consecutive generations

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Previous research indicates that arsenic is a reproductive toxicant and a significant endocrine disruptor[1]. Exposure to relatively low concentrations of arsenic, which can pass through the placenta, leads to premature births, fetal death, and stillbirth[2]. This study aimed to investigate arsenic effects in the estrous cycle (proestrus, estrus, metestrus, and diestrus) through the three consecutive generations of mice**.** In the vivarium of the Institute for Antirabies Protection - Pasteur Institute, Novi Sad, *Mus musculus*, strain NMRI female mices, were bred.The control group received water from the water supply network, while the experimental group received water with a dissolved concentration of 106 mg/l arsenic(III)-oxide for 2 months. Histological examinations were performed on Gimza-**s**tained sections.The results were shown the histological differences in each phase of theestrous cycle. The epithelial cells with a visible nucleus were predominantly noticed in the proestrus, while the epithelial cells without the nucleus were presented in the phase of the estrus. The metestrus was characterized by the presence of neutrophilic granulocytes and keratinized cells. The presence of neutrophilic granulocytes and epithelial cells with a nucleus were observed in the phase of the diestrus. No significant histological differences were observed in the estrus cycle of the experimental group, through the three consecutive generations, compared to the control group, but in the experimental group, was detected prolongated of the diestrus phase of the cycle.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development (Republic of Serbia), grant number 451-03-68/2022-14/ 200178.

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# Antioxidative properties of progesterone in striatum of permanently occluded adult male Wistar rats

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Progesterone (P4), a naturally occurring gonadal hormone in the brain, and its metabolites, are proposed as potential therapeutic agents in various neurodegenerative animal models given that their neuroprotective properties might be associated with amelioration of oxidative stress. Since P4 actions in rat striatum upon permanent ligation of both common carotid arteries are still ambiguous, the present study aimed to evaluate whether 7 days lasting P4 treatment could modulate the levels of several striatal oxidative stress indicators, including prooxidant/antioxidant balance (PAB), advanced oxidation protein products (AOPP) and products of lipid peroxidation (LPO). For the purpose of the experiment, adult male Wistar rats (n = 12) were divided into 3 groups: sham-operated animals subjected to vehicle (commercial flax oil, 1 mg/kg, s.c., Sham + V), occluded animals treated either with vehicle (2VO + V) or P4 (dissolved in commercial flax oil, 1.7 mg/kg, s.c., 2VO + P4). Rats were sacrificed 4 h following the last treatment[1] and striatal synaptosomal fraction was used for further biochemical analyses[2]. Our results demonstrate that investigated oxidative stress indicators are affected to the different extents by P4 treatment. Namely, in comparison to the Sham + V group, PAB level was elevated in 2VO + V rats, while in 2VO + P4 animals it was downregulated to the levels observed in the Sham + V group. In parallel, 2VO-induced alteration of AOPP was decreased following P4 treatment whereas LPO level was still slightly elevated. Overall, our findings suggest that P4 might manifest antioxidative features in the striatum of hypoperfused rats.

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This study was supported by MPNTR, grant 451-03-68/2022-14/200017.

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# Antioxidative effects of carotnoids in doxorubicin cardiotoxicity

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Doxorubicin is an anthracycline group antibiotic. It is believed that doxorubicin cardiotoxicity is caused by DNA and myocardium damage through induction of oxidative stress[1]. In order to prevent the side effect of this drug, we tested mixture of carotenoids extracted from pumpkin. The biologically significant role of carotenoids is reflected in their antioxidant activity, improvement of immunity, regression of malignant lesions[2]. In total, 54 animals were randomly divided into 6 groups. According to study design, animals were given saline (K), solvent (NADES – natural deep eutectic solvents, N, 1 mL), carotenoids (B, 900 µg/kg), doxorubicin alone (single dose) (D, 1.5 mg/kg) or pretreated with solvent (ND) or carotenoids (BD). Tissue slides were microscopically analyzed for the presence of indicators of myocardial damage. The level of lipid peroxidation and specific antioxidative enzyme (AOE) activity were determined in heart tissue. Doxorubicin increases the intensity of lipid peroxidation, while in the BD group the intensity of lipid peroxidation is significantly lower. Doxorubicin reduced the activity of AOE compared to the K group. Carotenoids attenuated doxorubicin's prooxidative effect, compared to the D group. In the D group, tissue indicators of myocardial damage were more pronounced and frequent. Carotenoids have high potential for preventing doxorubicin cardiotoxic effects, through lowering lipid peroxidation, inducing AO activity, and leading to reduction of myocardial damage.

## Acknowledgements

This study was supported by the Autonomous Province of Vojvodina (Project No 142-451-2543/2021-02)

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# Mesoporous SBA-16/hydroxyapatite nanocomposite and demineralized dentin particles have *in vitro* osteoinductive potential on human dental pulp stem cells?

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Tissue engineering is a multidisciplinary field with a focus on the combination of cells or proteins with biomaterials to generate new tissue[1]. Critical in this procedure is the ability of a synthetic material that closely mimics bone structure in order to stimulate stem cell migration, proliferation, and differentiation. Dentin has become a significant potential biomaterial for bone tissue engineering, as a scaffold for hard oral tissue, along with materials based on hydroxyapatite[2]. In this study, we used dental pulp stem cells from deciduous teeth (SHED), due to their ability to osteoblast differentiation. Consequently, the present study aimed to compare the osteoinductive potential of three synthetic materials, in three-time groups, on SHED. The in vitro capability of the scaffold to promote osteogenic differentiation was assessed by quantitative real-time PCR for genes coding for bone markers including *Runx2* and *ALPL*. Altogether, the in vitro results of alizarin red and immunofluorescence staining demonstrate SHED ability to differentiate into bone cells. Moreover, the osteoinduction potential showed only dentin particles. Bio-oss® and SBA-16/HA did not express positively osteogenic markers. Henceforth, these results manifest the dominance of dentin particles in osteoinduction efficiency *in vitro*-feature that suggests such a biomaterial may find use in bone regeneration.

## Acknowledgements

This work was supported by the Ministry of Science and Technological Development, Republic of Serbia, Grant No: 451-03-9/2021-14/200114.

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# Influence of cholic acid on viability and germination of maize and sunflower seeds

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The aim of this study was to evaluate the effect of cholic acid on seed viability and germination, as well as morphological characteristics and oxidative status of sunflower and maize shoot and/or root. Seeds were treated with four concentrations of cholic acid in Hoagland's solution: 20, 40, 60 and 80 mg/L, and the control seeds were grown either just in distilled water or in Hoagland's solution. Starting 36 h after the treatment, until the 7$^{th}$ day, the seed germination was monitored[1]. After the 7$^{th}$ day, the seedlings were sampled and the following tests were conducted: morphological characteristics (shoot and root lenght, shoot fresh weight and vigour index) and lipid peroxidation in shoot. The results showed that none of the tested morphological characteristics or germination of the treated seeds is statistically significantly different from those of the control seeds, except for the root lenght of maize treated with 60 and 80 mg/L (3.1±0.5 cm, 4.1±0.7 cm, respectively), compared to the control (6.2±0.7 cm). The amount of end product of a lipid peroxidation – malondialdehyde (MDA), was found to be lower in maize seed treatment with 80 mg/L (42.4±2.6 nmol MDA g$^{-1}$ FW) of cholic acid, compared to the control (54.0±5.7 nmol MDA g$^{-1}$ FW) and higher in sunflower seed treatment with 20 and 40 mg/L (56.9±4.1 and 57.6±5.3 nmol MDA g$^{-1}$ FW, respectively), compared to control (44.0±3.4 nmol MDA g$^{-1}$ FW). In a conclusion, cholic acid mostly does not affect viability and germination of maize and sunflower seeds, while slightly affects oxidative status of shoot.

## Acknowledgements

This study was supported by the Ministry of Education, Sciences and Technological Development of the Republic of Serbia (451-03-68/2022-14/200117 and 451-03-68/2022-14/200125).

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# Oral supplemetation with sodium butyrate increases antioxidative capacity of honey bee

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Honey bees are the most prominent group of pollinators, with great impact on economy and biodiversity. Recently, decline in numbers of managed honey bees' colonies had been reported, due to influence of various environmental and anthropogenic factors. Sodium butyrate (SB) is short-chain fatty acid and one of fermentation end-products of intestinal bacteria. Beneficial effect of SB on human health and lifspan on Drosophila has been described. Hu et al. (2017) showed that SB up-regulate genes involved in anti-pathogen and detoxification pathways, increasing tolerance to imidacloprid, and additionally strengthening the immune response of honey bees to infections[1]. Proposed mechanism of SB action is protective antioxidative activity and influence on gene expression through epigenetic changes, acting as histone deacetylase inhibitor. In the present study, we examined the effect of supplementation with SB at concentration of 10 mM during ten days on antioxidative capacity of honey bee. We measured the antioxidant capacity by FRAP assay, and contents of protein thiol groups and reduced glutathione (GSH). Results showed that SB-supplemented honey bees had increased antioxidative capacity compared with unsupplemented control. Also, contents of protein thiols and GSH increased. Our results indicated beneficial effects of SB on oxidative status of honey bees. Based on these results, a potential application of SB supplementation in beekeeping practice could be considered.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2022-14/200125).

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# The effect of spermidine on antioxidative capacity of honey bee (*Apis mellifera* L.)

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Honey bees (*Apis mellifera* L.) have become major topic due to their economically important role as pollinators and decrease in number of their colonies in the last few decades. Oxidative stress and the consequent pathophysiological states are one of the possible reasons that eventually lead to bee loss. Under normal conditions, there is a balance between reactive oxidative species generation and their removal by antioxidant defence. Spermidine, a naturally occurring polyamine shows various metabolic functions, such as antioxidative function[1]. The aim of this study was to evaluate antioxidant function of spermidine by measuring ferric reducing antioxidant power (FRAP) value, in honey bees whose food was suplemented with spermidine for 20 days. Four experimental groups were set up: control C fed with 50% sucrose and S1, S2, S3 groups whose diet were supplemented with 1, 0.1 and 0.01 mM spermidine, respectively. The results showed significant increase in FRAP value in all supplemented groups. Higher FRAP values are related to higher antioxidative capacity, which decrease 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 remains to be determined.

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# Activity of antioxidant enzymes of different varieties of cereals under conditions of heat stress

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Antioxidative enzymes participate in the catalytic transformation of reactive oxygen species and their by-products into stable non-toxic molecules, which is important defense mechanism against cell damage caused by stress[1]. In the coming years, a rising temperature is predicted, so its influence will be increasingly significant, and it can have negative effect on crops worldwide[2]. The goal of this research was to examin the effect of heat stress on the activity of the antioxidant enzymes catalase, ascorbate peroxidase and guaiacol peroxidase in ten different cereals varieties. The activity of the analyzed enzymes was increased under conditions of heat stress and varied among the analyzed cereals. A significant increase in catalase activity was found in the oat variety Jadar and was also observed in wheat varieties Hystar and Pobeda. High increase in the activity of the ascorbate peroxidase was observed in the wheat variety Hystar and Zvezdana. A significant increase in guaiacol peroxidase activity was found in the triticale variety Odisej and in wheat variety Avenu. A significant increase in the activity of catalase, ascorbate peroxidase and guaiacol peroxidase was recorded under conditions of heat stress, whereby higher enzyme activity was found in varieties that were more tolerant to heat. This research opens up the possibility of using new knowledge in the efficient selection of commercial varieties of cereals, as well as the possibility of creating new heat-tolerant varieties of cereals through the application of biotechnology and breeding programs.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2020-14/200122).

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# Mesoporous silica nanoparticles improve the antitumour activity of cisplatin-acetylated caffeic acid conjugate

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Severe side effects and drug resistance are the main obstacles in clinical usage of cisplatin. The preparation of platinum(IV) prodrugs and the use of nanoparticles might be potential paths for overcoming the problem of toxicity. Caffeic acid is plant metabolite with many pharmacological effects such as antiinflammatory, anticancer, and hepatoprotective[1]. In this study, a hybrid molecule build up from cisplatin and acetylated caffeic acid, free and loaded into nanoparticles, SBA-15, was evaluated as an anticancer agent. Cytotoxic studies revealed that free conjugate possessed similar activity as cisplatin alone against 4T1 cell line, while upon imobilisation in SBA-15, much improved cytotoxicity was noticed. Further investigation showed that these compounds induced caspase-dependent apoptosis and an accumulation of cells in the subG compartment of the cell cycle. Intensive production of oxygen and nitrogen radicals was also observed. Also, survived clones lost their dividing potential. Mode of action of this cisplatin-caffeic acid conjugate against 4T1 cells makes it valuable in futher research, from the side of enhancement of its antitumour activity upon mobilisation into nanoparticles and potential reduced toxicity *in vivo.*

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200007.

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# Diazepam exerts an antagonistic effect in concomitant therapy with temozolomide in glioblastoma

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It has been reported that diazepam (DIA) is widely adopted, among antiepileptic drugs, to treat *status epilepticus* in patients with glioblastoma (GBM). Besides, DIA is the first-choice drug to alleviate anxiety and can inhibit chemotherapy-associated delayed emesis in GBM patients, as well. Having in mind its pleiotropic spectrum of effects, DIA is frequently prescribed drugs in GBM patients. Even though temozolomide (TMZ), an alkylating agent, and DIA could be found as possible combination therapy in clinical practice, there are no reports of their combined effects in GBM. The U87 human GBM cells were used to examine the effects of combined TMZ and DIA treatment. Following the treatment for 24h, we examined the cell viability using the MTT assay, the cooperative index, long-term cell survival using the colony formation assay, and the level of apoptosis using qRT-PCR for the *Bcl2/BAX* ratio and immunocytochemical staining with anti-Bcl2 antibody. The cooperative index showed the presence of antagonism between TMZ and DIA, which was confirmed on long-term observation. Moreover, the level of apoptosis after the TMZ treatment was significantly decreased when administered with DIA ($p<0.001$). Data reported that DIA elicits cell cycle arrest in the G0/G1 phase, which precedes the S phase, thus blocking the DNA replication and favoring senescence. Given that TMZ exerts its effects through DNA methylation, finally resulting in G2/M cell cycle arrest, these results reveal that DIA diminishes TMZ efficacy in concomitant use in the treatment of GBM and should not be administered together.

## Acknowledgment

This study was supported by the Autonomous Province of Vojvodina, Republic of Serbia, Grant No: 142-451-3176/2020-01.

# Novel promising fluorescent ligands of human CYP17A1

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At the moment identification of novel drugs against cancer is one of the perspective tasks of the medicinal chemistry. Prostate cancer (PC) is on the second place among all oncological disorders. The main molecular target used for the development of novel high efficient drugs against PC is cytochrome P450 17A1[1]. This enzyme localizes in endoplasmic reticulum and takes part in metabolism of androgens. Using of the fluorescent compounds is one of the convenient approaches in the screening of novel ligands for the given target because of high sensitivity and relative simplicity of the method. At present work we performed complex investigation of interaction of pregnenolone, containing NBD (nitrobenzoxadiazole) group, with human CYP17A1. It was found for the first time that 20α-NBD-pregnenolone and 20β-NBD-pregnenolone bind with human CYP17A1 with $K_d$ values 19.8±1.8 μM and 21.5±3.1 μM, respectively. In both cases binding mode corresponds to substrate molecule, but no products of enzymatic reaction were detected during activity studies in reconstituted system. According to the data obtained only in case of 20α-NBD-pregnenolone binding of the ligand results in significant change in the intensity of fluorescence spectrum of the compound that is, probably, connected with fluorescence quenching by heme. Stern-Volmer plots obtained for the quenching of ligands fluorescence by $I^-$ ions also points out on the different binding mode of the tested compounds. Results of molecular docking simulation showed that binding mode of the tested compounds and binding energy correspond to the localization and binding energy calculated for the pregnenolone, but presence of bulky polar group at $C_{20}$ results in unfavorable interactions with key residues.

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# Advanced oxidation protein products (AOPP) are positive predictors of non-alcoholic fatty liver disease

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Although the pathophysiology of Non-alcoholic fatty liver disease is still unknown, a number of variables have been identified to be implicated, with oxidative stress thought to be a key player. When oxidative stress occurs, chlorinated oxidants act to create advanced oxidation protein products (AOPP), which can cause the progression of fatty liver to further stages[1]. We aimed to determine if the serum AOPP levels in our subjects are suitable for predicting the risk of developing steatosis. This study included 158 subjects from Zemun Clinical Hospital Center. Ultrasound confirmed the presence of steatosis in 101 patients, while the remaining 57 were in the control group. AOPP levels were determined in the serum samples of all subjects. They were statistically significantly higher in patients compared to the control group. Univariate binary logistic regression analysis showed a positive association between AOPP and the presence of steatosis (OR=1.143, 95% CI 1.082-1.207; P<0.001). A model in which the following attributes were included: AOPP, body mass index, presence of diabetes and presence of cardiovascular disease identified AOPP remained positively associated with the presence of steatosis (OR=1.085, 95% CI 1.023-1.151; P=0.007). The redox status marker, AOPP, showed a positive prediction of the presence of steatosis in our subjects.

## Acknowledgement

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-68/2022-14/200161.

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# Developing reversible immuno-affinity capture for extracellular vesicles purification

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Extracellular vesicles (EVs) are a group of cell-secreted supramolecular structures that play a role in important physiological and pathological processes. EVs are present in all bodily fluids. EVs represent unique source of clinically relevant and easily accessible biomarkers. Accordingly, increasing research interests in EVs field is advancing toward their use in precision medicine with particular focus of Liquid Biopsy. Present EVs isolation approaches are very inefficient, time-consuming and expensive. The application of immune-based capture could represent an effective alternative. Further, as an alternative to conventional antibodies, single-domain antibodies (VHH) obtained by direct panning on EVs can enable purification of EVs from different sources (human plasma or conditioned culture medium) with significantly reduced costs[1]. The aim of this work was to combine single domain antibody based affinityfor high performance scalable EV capture. In this work, we develop system VHH-methacrylate-based copolymer for purification[2]. VHHs used in this work (H1, H6, D5, B1 and G2) were isolated from a naïve pre-immune library by direct panning against EVs from the supernatant of cultured human cells. VHHs cloned with eGFP and 6xHis tag, were produced in E. coli cells, purified and immobilised on polymer and used for immunocapture purification of EVs from tissue culture medium and human plasma. Biochemical and morphological features of the isolated EVs were determined using different methods. Methacrylate-based copolymer was used as a porous solid support, the chemical versatility of which enables its efficient functionalization with VHHs. The combined analyses of morphological features and biomarkers (CD9, CD63 and CD81) presence indicated that the recovered EVs were exosomes. The lipoprotein markers APO-A1 and APO-B were both negative in tested samples. This is the first report demonstrating the successful application of spherical porous methacrylate-based copolymer coupled with VHHs for the exosome isolation from biological fluids. This

inexpensive immunoaffinity method has the potential to be applied for the isolation of EVs belonging to different morphological and physiological classes.

## Acknowledgements

This study was supported by the Grant No. 172049 from Ministry of Education, Science and Technological Development of the Republic of Serbia.

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# Antioxidant and acetylcholinesterase inhibitory activity of willow bark and leaf extracts (*Salix* L.)

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Oxidative stress, resulting from the imbalance between free radical production and antioxidant defenses is thought to be involved in the development of many diseases, including neurodegenerative such as Alzheimer's disease (AD). Moreover, AD is associated with low levels of acetylcholine in the brain. Current therapy of AD is based on inhibition of acetylcholinesterase. Willow bark (*Salix* L., Salicaceae) is traditionally used for its anti-inflammatory properties, which is associated with its antioxidant effects. The aim of this study was to evaluate the antioxidant and acetylcholinesterase inhibitory activity of bark and leaf extracts of two species of the genus *Salix* L. Bark and leaf extracts of *S. amplexicaulis* Bory and *S. purpurea* L. were obtained by microwave-assisted extraction. Total phenolic, flavonoid content, hydroxyl (OH) radical scavenging and anticholinesterase activity were determined by spectrophotometric methods. Leaf extract of *S. purpurea* exhibited the strongest OH radical scavenging activity and was the richest in total flavonoids. The highest amount of total phenolics was found in bark of *S. amplexicaulis*. Statistically significant correlation between total flavonoid content and OH radical scavenging potential was observed. Extracts of *S. amplexicaulis* exhibited stronger acetylcholinesterase inhibitory activity than those of *S. purpurea*.

# Quercetin, resveratrol, alpha-lipoic acid and coenzyme Q10 binding to the major circulatory proteins

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Antioxidants as food supplements are prevalent today, and some of them are also medically prescribed as therapies for pathological conditions. Quercetin, resveratrol, alpha-lipoic acid and coenzyme Q10 are popular and widely used antioxidants. They can be bought as supplements from different companies, usually in the form of pills with several hundred milligrams per pill. Poor bioavailability and reduced stability are the main drawbacks to efficiently utilising many naturally occurring antioxidants, so their binding to circulatory proteins is essential. These interactions may increase the solubility and stability of antioxidants, thus improving their beneficial potential. In this work, we investigated whether some major human circulatory proteins (transferrin, alpha-2-macroglobulin and fibrinogen), besides albumin, bind the mentioned antioxidants. Our previous work demonstrated that fibrinogen binds both resveratrol and alpha-lipoic acid with affinity constants in the range of $10^4$ $M^{-1}$. In the present study, it was shown that fibrinogen binds quercetin with a similar affinity. Both transferrin and alpha-2-macroglobulin bind resveratrol, and transferrin binds quercetin and alpha-lipoic acid as well. Coenzyme Q10 binds poorly to all tested proteins (Ka ~ $10^2$ $M^{-1}$). The results of this study show that the distribution of tested antioxidants between proteins in the circulation can be altered, together with their mechanisms of action, under conditions that affect albumin concentration and/or function. Direct consequences of ligand binding on tested proteins will be further analysed.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-9/2022-14/200019.

# Immobilisation of phycocyanin on alginate beads and application in mercury removal

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Phycocyanin (CPC), a blue, light-harvesting protein from cyanobacteria Spirulina (*Arthrospira Platensis*), is well known for a wide range of beneficial effects, including antioxidative, anti-inflammatory, anti-tumour and immunomodulatory. This protein also has several industrial applications, the most important being its usage as a food colourant. Phicobiliproteins also have important applications as fluorescent markers in biomedical research. One attractive characteristic of CPC is the ability to bind heavy metals with high affinity. This study aimed to produce stable immobilised CPC in alginate beads and test it for mercury removal. CPC was immobilised using 0.5, 1 and 2% alginate slurry containing 10% CPC and dropping into 2% $CaCl_2$ solution at several pH values. CPC was successfully immobilised in alginate beads at pH 4.0. Above this pH, significant leakage of CPC was observed. For mercury removal analysis, the experiment was performed by incubating alginate beads with and without CPC in a 1.7 ppm $HgCl_2$ solution made in deionised water. Only alginate beads made from 0.5% alginate showed the ability to absorb mercury ions, even after 30 min of incubation. The more extended incubation period, for 48 h, resulted in almost complete 97% mercury removal. Further optimisation will be required to optimise mercury removal using this procedure.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-68/2022-14/200168.

# Changes in lipid status and liver enzyme activity in individuals on statin therapy

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In this paper, the goal was to monitor the influence of statins on the lipid profile of patients (HDL, LDL, cholesterol, triglycerides) during therapy with the same, as well as on the activity of liver enzymes (AST, ALT, GGT). Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA-reductase). They are structural analogues of HMG-CoA and have the role of preventing the action HMG-CoA-reductase on natural substrate[1]. The obtained results were compared with the lipid profile and activity of liver enzymes in people not on statin therapy. The data were collected in the Busovač Health Center and divided into three groups: control group (normal lipid values and liver enzyme activities), subjects with elevated lipid values and who are on statin therapy, and subjects with elevated lipid values and who are not on statin therapy. Based on the obtained results, we concluded that statins lower the level of cholesterol and triglycerides in patients with a high lipid profile, that they have no positive effect on the level of HDL in the blood, that they have a positive effect in lowering LDL in patients with a high lipid profile, and that they lead to an increase in the activity of liver enzymes.

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# Redox status of kidney disease patients on different types of hemodialysis treatment

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Hemodialysis (HD) treatment is necessary to substitute renal function in the last stage of kidney disease (KD). Different types of HD are available: bicarbonate treatment, hemodiafiltration (HDF), Theranova treatment etc. HDF and Theranova have been reported to provide much better removal of metabolic toxins than conventional bicarbonate treatment. KD patients also manifest excessive oxidative stress due to retention of metabolic toxins[1]. The aim of our study was to estimate redox status in KD patients receiving bicarbonate and HDF or Theranova treatment. Patients on bicarbonate treatment (group A; n = 88) and patients receiving HDF or Theranova treatment (group B; n = 38) were included. Redox status parameters: total sulfhydryl (SH) groups, paraoxonase 1 (PON1) and malondialdehyde (MDA) were determined in serum samples. Group B had significantly higher concentrations of SH groups and PON1 activity, but also significantly higher levels of MDA when compared to group A. SH groups and PON1 ($P<0.05$) showed the ability to discriminate KD patients on different HD treatments in univariate analysis whereas SH groups retained this potential in multivariate analysis ($P<0.05$). KD patients receiving different HD treatments had different redox status. Redox status showed the ability to discriminate patients with different HD treatments.

## Acknowledgements

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-9/2021-14/200161.

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# Transplacental transfer of redox-active trace elements during pregnancy

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With an ever-increasing concern for human health and well-being, the prenatal phase of development necessitates special consideration since fetuses can be exposed to many elements via the mother[1,2]. As a result, this study examined umbilical cord (UC) serum, maternal serum, and placental tissue samples from 92 healthy women with normal pregnancies in order to determine their status of selected toxic (Pb, Cd, Ni, As, Pt, Ce, Rb, Sr, U) and essential trace metals (Mn, Co, Cu, Zn, Se). Another objective was to investigate the possible transplacental transfer of these trace elements. All of the trace metals were found to cross the placental barrier and reach the fetus. Substantial variations across all three types of clinical samples were found in levels of toxic Ni, As, Cd, U, Sr, Rb, and essential Mn, Cu, and Zn. A correlation analysis revealed As a metal whose levels varied significantly across all three types of clinical samples. Cd, Mn, Zn, Rb, Ce, U, and Sr were shown to be the most important trace metals in differentiating placenta from maternal and UC serum samples using principal component analysis. In conclusion, the findings of this study could help to improve knowledge of transplacental transfer of these redox-active metals, as well as provide insight on overall levels of trace element exposure in the population of healthy pregnant women and their fetuses.

## Acknowledgements

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia Contract Number:451-03-68/2022-14/200288.

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# 2D-HPTLC visualization of major differences in phenolic profiles of plants belonging to 5 different *Polygonaceae* genera: *Rumex* L., *Polygonum* L., *Bistorta* Adans., *Persicaria* Mill., *Fagopyrum* Mill.

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Plant species from *Polygonaceae* family are widely distributed all over the world. In the Flora of Serbia, six genera of this family were described, with a total of 43 species. The notable members of the family are cultivated species: rhubarb (*Rheum rhabarbarum* L.) and buckwheat (*Fagopyrum esculentum* Moench.). Therapeutic application of these species is based on the presence of bioactive phenolic compounds. For example, *Rumex* species are used as laxatives due to their high content of anthraquinones or anthraquinone glycosides, while *Polygonum aviculare* is used in urinary infections due to proantocyanidin. The aim of this study was to develop fast and simple method for screening of phenolic profiles of *Polygonaceae* species. 2D-HPTLC was used for separation of components of 80% ethanol extracts of aerial parts of 19 different species. Cellulose precoated aluminium sheets were used as a stationary phase and ↑TBA (t-BuOH:$CH_3COOH$:$H_2O$=3:1:1) and → 6% acetic acid as mobile phases. After developing, plates were derivatised by spraying with 1% natural product reagent solution in methanol and 5% PEG solution in ethanol. The $R_F$ values and colors of the spots gave information on chemical structure of the compounds. One hundred thirty different zones were detected in chromatograms of 19 investigated extracts. It was possible to identify different classes of polyphenols (flavonoids, phenylpropanoic acid derivatives and hydroxybenzoic acid derivatives dominated), and to observe differences between investigated species. The chemical composition was in good correlation with the taxonomic classification of species. *B. officinalis* and *B. vivipara* extracts contained the most diverse types of secondary metabolites. It was concluded that applied 2D-HPTLC method is suitable for screening of phenolic profile and differentiation of *Polygonaceae* species.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development, Serbia, Grant No. 451-03-9/2021-14/200125.

# Antitumor properties of *Eryngium amethystinum* extract

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*Eryngium amethystinum* is herb from *Apiaceae* family. It has been used as a food or in traditional medicine for the treatment of various diseases[1]. In the present work, we have evaluated its cytotoxic effect on a panel of human cancer cells (human breast carcinoma MCF7, human malignant melanoma A375, human colon carcinoma HCT116 and human lung cancer A549). Treatment with *Eryngium amethystinum* ethanol extract decreased viability of all cancer cell lines in a dose-dependent manner after 72 h. For further investigation of potential mechanism of action A549 cell line was selected. The observed viability decrease was followed by loss of dividing potential after the treatment. Aditionaly, 90% of A549 cells were subjected to programmed cell death − apoptosis which was not followed with caspase activation. In paralel with this, typical apoptotic morphology of treated cells was observed by fluorescent microscopy. Apart from this decreased cell viability was due to triggered autophagic cell death. Taken together, the shown effect of *Eryngium amethystinum* makes this plant worthwhile for further evaluation in the field of oncology.

## Acknowledgements

This study was supported by: Grant: Ministry of Education, Science and Technological development of the Republic of Serbia No. 451-03-9/2021-14/200007 and joint funding of Development and research projects of the Republic of Serbia and the People's Republic of China.

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# Nitrogen-containing heterocycle steroids induce MDA-MB-231 breast cancer cells death caused by reactive oxygen species production

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Triple-negative breast cancers (TNBC) are very aggressive subgroup of breast cancers. They demonstrate the absence of estrogen and progesterone receptors, as well as human epidermal growth factor receptor 2. TNBC are resistant to hormonal therapy, due to deficiency of the appropriate hormone receptors. Consequently, TNBC are managed with standard treatment, including cytotoxic agents; however, this kind of therapy has a lot of side effects, and it is often followed by local and systemic relapse. These facts, as well as the fact that some steroidal compounds are known for their anticancer properties, prompted us to test novel modified steroids for their anticancer activity in order to recognize potential new therapeutics with low side effects. Two N-containing heterocycles with high potential for growth inhibition of TNBC MDA-MB-231 breast cancer cells (compounds with low $IC_{50}$ concentrations) and selectivity were tested for the mechanism underlying this antiproliferative effect. After the treatment with equitoxic concentrations of selected steroids, specific dyes were added and cells were analyzed by flow cytometry. This tool was used for counting cells which underwent some specific change, caused by treatment with steroids. Steroidal heterocycles did not induce apoptosis or necrosis in significant extent. They did not change mitochondrial membrane potential (MMP) or influence cell cycle of treated cells in high manner. The highest impact on treated cancer cells steroidal compounds exerted via inducing production of reactive oxygen species, with no clear evidence about type of cell death or cell cycle arrest for 72 h treatment period. Accordingly, further search for a mechanism of cell growth inhibition is needed.

## Acknowledgements

Financial support for this study was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

# C-Phycocyanin from cyanobacteria *Artrhorspira platensis*: Binding of selected food-derived ligands

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To minimize the impact of artificial food coloring (*e.g.*, in drinks) on health, chemical dyes are increasingly replaced by natural ones. C-phycocyanin (C-PC), hexameric light-harvesting phycobiliprotein from cyanobacteria *Artrhorspiraplatensis,* has been proposed as an alternative. The intensive blue color of C-PC arises from phycocyanobilin (PCB), the covalently attached tetrapyrrolechromophores. The presence of PCB chromophores gives C-PC a broad range of bioactive effects (antioxidant, anticancer, and immunomodulatory ones), substantially increasing their potential for applications in food industry. However, C-PC is sensitive to temperature, and its color significantly diminishes by thermal treatment, limiting the use. Hence, improving C-PC stability is the major challenge for successful application in food and beverage coloring. It is well known that binding small, high-affinity ligands significantly improveprotein stability. Therefore, selecting food-derived ligands (such as vitamins, polyphenols, sugars, etc.) with the ability to bind C-PC firmly could be a promising strategy to increase the stability and preserve color. The aim of this study was to characterize the binding of selected food-derived ligands (including quercetin, coenzyme Q10, gallic acid, vanillic acid,vanillin,resveratrol, glucose, fructose, sucrose, vitamin K, menthol, anddihydrolipoic acid) to C-PC by UV/VIS absorption spectroscopy, spectrofluorimetry, and CD spectroscopy. Quercetin showed the strongest binding affinity to C-PC ($K_a$~$3.7x10^5$ $M^{-1}$), and its effects on C-PC structure and stability were further investigated. CD spectroscopy revealed that quercetin induces stabilization of the protein secondary structure under simulated physiological conditions, while the conformation of the PCB chromophore is altered uponquercetin binding.Furthermore, quercetin binding increases the thermal stability of C-PC. Overall, our study revealed the ability of high-affinity, food-derived ligands to increase the stability of C-PC, which may enhance its application potential in the food industry.

## Acknowledgments

This work was supported by ANSO (Project No. ANSO-CR-PP-2021-01), and the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-68/2022-14/200288.

# Production of a novel opine dehydrogenase

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Opine dehydrogenases are a family of NAD(P)H dependent oxidoreductases, which catalyze the reductive condensation of an α amino group from an amino acid with an α-keto acid during anaerobic glycolysis by regenerating NAD[1,2]. They are widespread in cephalopods and mollusks. Opines are associated with crown gall tumor pathogenesis caused by *A. tumefaciens* providing nutrients to the pathogen, and novel opine compounds acting as metallophores have been identified.[1] Besides, opine-type secondary amine dicarboxylic acids are chiral intermediates of angiotensin-converting enzyme inhibitors[3]. A novel enzyme originating from an extremophile bacterium, with assumed opine dehydrogenase function was successfully expressed in Escherichia coli STAR cells and purified by affinity chromatography. Molecular mass determined by SDS-PAGE was approximately 40 kDa. The activity was measured by using pyruvate and alanine as substrates, by which proved that it has opine dehydrogenase activity.

## Acknowledgments

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia. [Grant No. 451-03-68/2022-14/200026, University of Belgrade-Institute of Chemistry, Technology and Metallurgy; Grant No. 451-03-68/2022-14/200168, University of Belgrade-Faculty of Chemistry; and Grant No. 451-03-68/2022-14/200053, University of Belgrade-Institute for Multidisciplinary Research].

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# Validation of insect protein extraction method from native polyacrylamide gel

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Insects are protein-rich and some edible species are used worldwide as food and feed. Therefore, insect proteins are of great interest for cellular agriculture (CA), i.e. tissue engineering for food production, particularly for cultivated meat production. Because of their significant role in amino acid metabolism, storage proteins stand out. However, they have not been sufficiently investigated, hence it is crucial to characterize them. The first step is the successful isolation of individual proteins of interest. In our previous work, the optimization of the method isolating individual hemolymph proteins from native polyacrylamide gel was shown using diapausing 5$^{th}$ instar larvae of the economically important *Ostrinia nubilalis* as a model system. The main objective in this study was the validation of the described method by isolating specific proteins of interest from *Tenebrio molitor* and *Zophobas morio* larvae hemolymph, as well as from the whole-body samples of *Plodia interpunctella* larvae. Similarly to *O. nubilalis*, *P. interpunctella* is an economically important pest species, while *T. molitor* and *Z. morio* larvae have been declared edible. Total protein concentrations were determined by Bradford protein assay and the results showed that the samples of *Z. morio* are the richest in total proteins, followed by *T. molitor* and *P. interpunctella*, respectively. Proteins were separated by discontinuous native polyacrylamide gel electrophoresis (nPAGE) and protein fractions of interest were cut from the nPAG and eluted overnight from the gels. To confirm that the proteins were well isolated, the individual fractions were run on discontinuous nPAGE. In total, six protein fractions were successfully isolated – two from every analysed insect species, respectively, and in sufficient amounts for downstream applications in CA.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant nos. 451-03-68/2022-14/200358, 451-03-68/2022-14/200125 and 451-03-68/2022-14/200117, MSCA-RISE H2020 project IPANEMA grant agreement no. 872662 and European Union's Horizon 2020 research and innovation program under grant agreement no. 739570 (ANTARES).

# Long-term low-level dibutyl phthalate exposure causes endothelial dysfunction in EA.hy926 cells

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Vascular endothelial cells operate as a semi-permeable barrier in blood vessels. Infection and inflammation can trigger dysfunction of this monolayer, which can further lead to atherosclerosis and cardiovascular diseases (CVDs). While genetics and lifestyle are primarily responsible for CVDs, a great share of scientific focus is now placed on environmental chemicals, such as phthalates, as the contributors to CVDs. Our aim was to determine whether a long-term low-level exposure to dibutyl phthalate (DBP) causes endothelial dysfunction. To mimic a "real-life" exposure, human vascular endothelial cell line EA.hy926 was exposed to three environmentally relevant concentrations of DBP ($10^{-9}$, $10^{-8}$, $10^{-7}$ M) or vehicle (0.05 % DMSO) for 12 weeks. We performed assays after 3, 6, 9, and 12 weeks of exposure to determine changes in metabolic activity, endothelial permeability, monocyte adhesion, and gene and protein expression. AlamarBlue™ assay showed no change in metabolic activity after 3 and 12 weeks of DBP exposure, while $10^{-7}$ M DBP and $10^{-8}$ M DBP decreased metabolic activity of EA.hy926 cells after 6 and 9 weeks, respectively. Endothelial permeability was determined using fibronectin-coated transwell units by measuring the passage of fluorescently-labeled dextran through the EA.hy926 cell monolayer. A transient increase in endothelial permeability was observed after 3 and 6 weeks of exposure to $10^{-8}$ M DBP and $10^{-9}$ M DBP, respectively. In contrast, $10^{-8}$ and $10^{-7}$ M DBP groups showed a decrease after 6 and 9 weeks, whereas all DBP-exposed groups showed no change in endothelial permeability after 12 weeks of exposure. Expression of two tight junction proteins, occludin and ZO-1, supported these findings. To assess the extent of endothelial dysfunction, we next performed adhesion studies using calcein AM-labeled human monocytic cell line U937. Exposure to $10^{-8}$ and $10^{-7}$ M DBP caused sustained increase in monocyte adhesion in all investigated time points. Relative mRNA expression of the three genes encoding cellular adhesion molecules ICAM-1, VCAM-1, and SELE supported these results. Obtained results indicate that repeated long-term exposure to DBP alters proliferation and metabolic activity of EA.hy926 cells and has a significant impact on endothelial permeability and monocyte adhesion, leading to endothelial dysfunction. Further research on DBP in CVDs is of high importance.

## Acknowledgements

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

# Cisplatin-ibuprofen conjugate free and immobilised in mesoporous silica nanoparticle SBA-15 indicate high antiproliferative potential on mouse cancer cell lines

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From its discovery, cisplatin therapy has widely been associated with toxicity and severe side effects. Platinum(IV) complexes, as well as immobilising them in nanomaterials could help to overcome these problems. Cyclooxygenase-2 (COX-2) is involved in cancer progresssion,[1] which encourages the development of inhibitors of COX enzymes in antitumour therapy. To determine the potential cytotoxic effect, a cisplatin-ibuprofen conjugate in free form, as well as loaded into SBA-15 nanomaterial, was tested on 4T1, CT26, B16 and MC38 cell lines. The results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and crystal violet viability assays showed that both agents dose-dependently decreased the number of viable cells of all tested cell lines. Flow cytometric analysis revealed significant decrease in the division potential of B16-treated cells. In further investigations, activation of caspases proved by ApoStat assay was noticed; however, apoptosis was not identified by flow cytometry in culture of treated B16 cells. Finally, light microscopy evaluation revealed the presence of enlarged cells with prominent heterochromatin foci in nuclei upon the treatment indicating that cells entered senescent state. High antitumour potential defined at the nanomolar concentration on mouse melanoma cells make cisplatin-ibuprofen a suitable candidate for further research.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200007

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# Coordination interactions between $Fe^{3+}$ and doxycycline in water at different pH

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The understanding of drug-iron interactions is of interest in the context of iron metabolism and the use of iron supplements[1]. The binding to iron may impact the stability and pharmacological activity of the drug[2]. Doxycycline (DOX) possesses several ionizable groups potentially available for metal ions coordination: oxygen atoms at the C10–C12 phenolic-diketone system, tricarbonylamide moiety at ring A (enolic oxygen at C3, carbonyl oxygen at C1 and carboxamide group) and dimethylamine nitrogen atom at C4. Pertinent to this, herein we investigated the interactions of $Fe^{3+}$ with DOX in water at different pH values. The UV-Vis spectroscopy results showed pH dependent coordination of $Fe^{3+}$ with DOX, most likely in relation to the solubility of Fe(III) species and deprotonation of DOX. The optimal conditions for $Fe^{3+}$-DOX complex formation were pH = 4 and $[Fe^{3+}]/[DOX]$ = 6 molar ratio. HESI-MS results showed that $Fe^{3+}$- DOX complex has 1:1 stoichiometry. UV-Vis and Raman spectra of $Fe^{3+}$- DOX complex confirmed that both tricarbonylamide moiety at ring A and phenolic-diketone system at BCD rings represent $Fe^{3+}$ binding sites. At pH 4 tricarbonylamide moiety can be considered as the main $Fe^{3+}$ binding site due to its deprotonation.

## Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract no. 451-03-68/2022-14/200053, 451-03-68/2022-14/200168).

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# Prooxidative and antimigratory effects of cerium oxide nanoparticles on melanoma and pancreatic cancer cells

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The development of new types of nanoparticles has become the focus of biomedical research in recent years. Cerium oxide nanoparticles (CONP) have shown particularly promising results as antitumor agents with a selective effect on tumor and normal cells. On the other side, melanoma and pancreatic carcinoma are among the most aggressive types of cancer with no satisfactory therapy[1,2]. Considering that, they represent important model systems for studying new treatment approaches. In this study, the antitumor potential of CONP (size below 10 nm) was studied on human A375 melanoma and PANC-1 pancreatic carcinoma cells. The obtained results indicated that analyzed CONP significantly inhibited clonogenic survival, with the number of colonies reduced on ~30% in A375 cells, while treated PANC-1 cells didn't form colonies. Growth inhibition was followed by $G_2$ cell cycle arrest (9% for A375, 17% for PANC-1). Percent of apoptotic PANC-1 cells was 38%, whereas ROS production increased for 78%. CONP significantly reduced metastatic potential through the decrease in cell migration and the increase in cell adhesiveness (up to 30 and 40% for A375 and PANC-1 respectively). These findings emphasize the significant CONP antitumor potential, based on the increase in ROS production, as well as a reduction of A375 and PANC-1 metastatic potential.

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This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No: 451-03-68/2022-14/200017).

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# Improvement of lipid metabolism regulation by low-intensity exercise in fructose-fed rats

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Excessive dietary fructose consumption in parallel with limited physical activity contributes to the global increase in prevalence of metabolic disorders. Metabolic syndrome represents a collection of cardiometabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia, and it is undoubtedly linked to increased risk for two global maladies, type 2 diabetes, and cardiovascular diseases. Fructose-rich diet is accompanied by the development of insulin resistance in the heart, and it could change the use of cardiac energy substrates towards increased fatty acid (FA) uptake, and catabolism. Exercise may be beneficial in prevention and treatment of the metabolic syndrome. The aim of this study was to analyse the impact of low-intensity exercise on protein expression of nuclear transcription factors involved in regulating FA β-oxidation in a heart of fructose fed rats. Male Wistar rats were divided into control group, and two groups that received 10% fructose for 9 weeks, one which was sedentary and one which was additionally exposed to low intensity exercise. The protein expression of important transcriptional regulators of fatty acid β-oxidation PPARα, and FOXO1, and coregulators Lipin1, PGC-1, and SIRT1 are analyzed in cardiac lysate and/or nuclear fraction by Western blot. Gene expression of ACADL, the enzyme that catalyzes the initial step of mitochondrial β-oxidation, was quantified by real-time PCR. Fructose-rich diet decreased nuclear PPARα compared to control. Exercise increased nuclear PPARα, nuclear FOXO1, lysate PGC1, and nuclear Lipin1 in fructose-fed rats compared to sedentary fructose-fed rats. Exercise increased lysate PPARα, lysate and nuclear FOXO1, lysate PGC1, lysate and nuclear SIRT1, and nuclear Lipin1 in fructose-fed rats compared to control. In conclusion, running at low intensity is accompanied by increased expression of key regulators of fatty acid oxidation. The results indicate that exercise achieves its effect by increasing the nuclear content of PPARα, Lipin1, and FOXO1.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia.

# Anticancer potential of xanthohumol loaded into SBA-15 mesoporous silica particles against B16-F10 cells

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Prenylflavonoid from hops - xanthohumol (XN) has been shown to possess diverse biological properties, including strong anticancer activity. One of the possibilities for improving delivery and effectiveness of drugs is the use of mesoporous silica nanoparticles such as nontoxic SBA-15. The aim of this study was the evaluation of the *in vitro* anticancer potential of XN loaded with different amounts into SBA-15 particles against malignant mouse melanoma B16-F10 cells. Our data indicate that SBA-15 containing XN showed a loading rate–activity dependence. Importantly, immobilization of XN into SBA-15 preserved and even potentiated its antitumor potential, in comparison to its free form. Also, by loading into SBA-15 carrier, XNs' anticancer mode of action converted from predominantly cytotoxic to cytostatic, resulting in a reduction of dividing potential. In addition, contrasting the previously observed apoptotic-inducing property of free XN, immobilized XN induced autophagic cell death that might be important for disabling tumor repopulation in response to apoptotic-induced cell proliferation, a mechanism often asscociated with therapy failure of advanced forms of cancer[1].

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-68/2022-14/20007, No. 451-03-68/2022-14/200116) and German Academic Exchange Service (DAAD) (No. 57448219).

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# Edible mushrooms from Novi Sad (Serbia) locality: phenolic profile, antioxidant and cytotoxic properties

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The use of mushrooms in human diet is reflected in their high nutritional value and their positive impact on human health. The edible mushroom species *Volvopluteus gloiocephalus* (Fr.) Gillet and *Fistulina hepatica* (Schaeff.) With., collected and determined in Novi Sad (Serbia) locality, have been selected for this study. This research sought to quantify phenolic compounds in four extracts (acetone, methanolic, chloroform, water) of these mushrooms and to determine their antioxidant (DPPH, ABTS, lipid peroxidation, NO, FRAP assays) and cytotoxic properties. LC/MS-MS analyses revealed the dominant presence of phenolic acids, with cinnamic, *p*-coumaric, and quinic acids as the main phenolics identified in all extracts, highlighting a cinnamic acid concentration found in the chloroform and acetate extracts of both species. The aqueous and methanolic extracts of both mushrooms showed the best antioxidant activity, except for the chloroform extract of *F. hepatica*, which showed the best effect against NO radicals ($IC_{50}$=32.22±0.31 μg).Among all tested extracts, only the chloroform extract of *F. hepaticum* showed cytotoxic effects in the CRRF-CEM leukemia cell line. The extracts were also tested on MRC healthy cell lines, where none of them showed cytotoxic activity, which is encouraging information for some future investigations. Our findings suggest that *V. gloiocephalus* and *F. hepatica* mushroom extracts have potent antioxidant and cytotoxic characteristics and may be used as a promising source for isolation of active lead compounds.

# Inhibition of AKR1C4 by 19-modified steroids

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Steroids are a large group of naturally occurring and synthetic compounds with a broad spectrum of activities. They possess anticancer, anabolic, antimicrobial, anti-inflammatory activity, etc. Behind each of these activities is the interaction of compounds with different protein targets, mainly receptors or enzymes. Thus, steroids are a constant subject of medicinal chemistry research. We have previously synthesized six novel 19-modified androstane derivatives in order to investigate their anticancer potential. These compounds include 6β,19-epoxy derivatives with halogen atoms on the C5 or $\Delta^4$-3-one system, and 19-hydroxy derivatives. Their inhibitory effect on the steroid-metabolizing enzyme aldo-keto reductase (AKR) 1C4, which is involved in the reduction of carbonyl groups to alcohols for a broad range of substrates, was tested using fluorimetric detection of NADPH consumption. In this preliminary screen, some of the tested 19-modified steroids showed moderate AKR1C4 inhibitory properties. Our results suggest that these compounds deserve further study to investigate their potential for use in the design of pharmaceutical treatments.

## Acknowledgements

This study was supported by Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: New steroid derivatives - potential chemotherapeutics, No. 142-451-2667/2021].

# Influence of FeJuice™ nutritional formula on anemia blood parameters *in vivo*

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According to WHO more than 30% of the world population is affected with iron defficiency anemia (IDA). Consequences of IDA can be detrimental and contribute to the premature birth, impaired physical and cognitive development in children and reduced productivity in adults. The most common cause of IDA is low rate of iron absorption from food in gastrointestinal track[1]. The standard therapy for IDA is iron supplementation which is suboptimal and has a number of side-effects[2]. The aim of this study was to evaluate the influence of innovative and 100% natural FeJuice™ nutritional formula, made of fruits and vegetables, on iron absorbtion rate by measuring anemia blood parameters in vivo. After becoming anemic Wister male rats were treated orally during 27 consecutive days by FeJuice™ nutritional formula. Blood samples were taken from tail vein and by cardiac puncture, before and after the treatment with FeJuice™ nutritional formula, respectively and hemoglobin, serum iron and transferrin saturation (TFS) levels were measured. According to results 27-days long treatment with FeJuice™ nutritional formula increased iron absorbtion rate since it caused increase in hemoglobin, serum iron and TFS levels by at least 10% per each parameter compared with control group. According to doses applied in rats it was calculated that human equivalent dose is 28.8 mL, which is 10 times smaller then amount which could be consumed daily (approx. 300 mL). Thus, regular consumption of FeJuice™ nutritional formula has a great potential to be beneficial for humans and prevent and treat IDA without side effects.

## Acknowledgements

Innovation Fund of Republic of Serbia, Technology Transfer Program (Grant No. 1078).

## Conflict of Interest

The authors have no conflicts of interest to declare.

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# Biochemical characterization of newly designed H1sD2 glycoforms as potential therapeutics for HDM allergy

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Allergen-specific immunotherapy (AIT) is the only curative approach for the treatment of allergies. However, this therapy is not always effective, so innovative approaches are urgently needed to potentiate its efficacy by the employment of novel adjuvants[1], such as for example virus-like particles[2]. Hemagglutinin (HA) is the surface glycoprotein and the major antigen in the host immune response to the Influenza virus[3]. As such, HA is a promising adjuvant candidate in AIT. By using *in silico* approach glycoforms of H1sD2 chimera composed of the receptor-binding domain of hemagglutinin, $HA_{63-286}$ (H1s), and Der p 2 allergen (D2) were designed. The aim of this study is to characterize those glycoforms in order to explore the effects of the glycan profile of chimeric HA glycoforms on the activation of the innate immunity and modulation of the immune response in house dust mite allergy. Recombinant glycoforms of H1sD2 were produced in the *Pichia pastoris* expression system and purified by immobilized metal affinity chromatography in combination with ion-exchange chromatography. For the structural characterization, SDS PAGE and MS were employed. Also, IgE reactivity of the glycoforms was tested in immunoblot assay using the serum of patients suffering from house dust mite allergy.

## Acknowledgements

Ministry of Education, Science and Technological Development of Republic of Serbia, Contract number: 451-03-9/2021-14/200168

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# Inhibition of type 3 3α-hydroxysteroid dehydrogenase activity by novel bile acid derivatives: combination of *in vitro* and *in silico* approaches to studying protein-ligand interactions

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Human type 3 3α-hydroxysteroid dehydrogenase (3αHSD-3), aka aldo-keto reductase 1C2 (AKR1C2) is a member of the aldo-keto reductase superfamily (AKRs). AKRs are involved in the reduction of a broad spectrum of substrates of endogenous or exogenous origin, including prostaglandins, steroids and xenobiotics. 3αHSD-3 is involved in the development and progression of several pathological conditions, including certain types of cancer, where 3αHSD-3 over expression is linked to resistance to chemotherapy drugs. The aim of this study was to describe interactions between 3αHSD-3 enzyme and bile acid derivatives with a tetrazole ring condensed to homo- B or -C rings. 3αHSD-3 enzyme was expressed and purified from E. coli BL21 (DE3) cells and purified by nickel affinity chromatography and gel filtration. Inhibition of 3αHSD-3enzymatic activity was monitored using an NADPH consumption assay. The structural bases of inhibition were examined by molecular docking. Potential off-target interactions with the glucocorticoid receptor were measured using a fluorescence assay in yeast. Enzymatic activity tests indicate binding of several compounds to 3αHSD-3. Molecular docking simulations suggest that hydrophobic interactions are important between the steroid core and non-polar residues, together with hydrogen bonds formed between polar groups of steroid compounds and amino acid residues. In conclusion, compounds tested in this work have potential to serve as leads in for structure-aided drug design, which could yield better inhibitors of AKR1C2 that might be used in the treatment of pathological conditions related to AKR1C2 over expression or impaired function.

## Acknowledgements

This study was supported by Ministry of Science, Education and Technological Development of the Republic of Serbia (Grant No. 172021).

# Pro-senescent effects of hyper-harmonized hydroxylated fullerene water complex in melanoma

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Antioxidant and anticancer properties of fullerene $C_{60}$ and especially of its polyhydroxylated, water soluble derivatives (fullerols) make them appealing for biomedical applications. In order to analyse antitumor effects of Hyper-Harmonized Hydroxylated Fullerene Water Complex (3HFWC)[1], second generation of fullerol, melanoma cells of different intracellular features and invasive potential (B16, B16-F10, A375) were treated with 3HFWC in various concentrations (0.19-100 μg/ml) for 24, 48 and 72h. Subsequently, syngeneic murine melanoma model was used (oral 3HFWC intake, 0.15 g/l). The most prominent effect of 3HFWC, both *in vitro* and *in vivo*[2], was induction of cell senescence, followed by decreased proliferative capacity and tumor growth inhibition. Senescent cells remained viable *in vitro*, but lost ability to divide and decreased metabolic activity, due to mitochondria alterations. Our findings demonstrate pro-senescence approach in antitumor therapy which is suggested to be less aggressive than the conventional strategies based on cancer cell killing, frequently followed by compensatory proliferation and subsequent tumor progression.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of Serbia, # 451-03-9/2021-14/200007, and by Zepter International Company.

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# BBIBP-CorV elicits lower, while BNT162b2 elicits higher anti-RBD IgG titres compared to COVID-19-recovered individuals

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COVID-19 is a disease caused by a novel coronavirus identified as SARS-CoV-2[1]. On March 11, 2020, WHO declared COVID-19 a global pandemic[2]. Vaccination remains one of the most effective ways of limiting transmission and providing protection of immunocompromised and other risk groups against severe forms of the disease[3]. Our cross-sectional study included 206 participants; 73 recovered, 71 vaccinated and 62 vaccinated with past COVID-19. In total, 78 participants were vaccinated with BNT162b2, 32 with BBIBP-CorV and 23 with AZD1222. The titres of anti-SARS-CoV-2 S1-RBD IgG antibodies were measured in the sera of participants using the CMIA method. Recovered participants had the lowest antibody levels compared to vaccinated participants or vaccinated participants with past COVID-19. The only exception was the BBIBP-CorV vaccine. Participants fully vaccinated with this vaccine had lower antibody levels compared to recovered participants, but those who also had past COVID-19 had much higher antibody levels. BNT162b2 elicited the highest titres of neutralizing antibodies compared to all other vaccines. Participants, vaccinated and with past COVID-19 had the highest titres compared to all other groups. Antibody levels correlated positively with age in the recovered group (r=0.059), negatively in the vaccinated group (r=-0.146), and positively in the vaccinated with past COVID-19 group (r=0.146). There was no statistically significant difference in the antibody levels based on gender. The mRNA vaccine (BNT162b2) elicits higher anti-RBD IgG antibody titres compared to inactivated/recombinant vaccines (BBIBP-CorV and AZD1222) and compared to naturally acquired immunity. Therefore, we conclude that mRNA vaccines should be used to immunize naïve individuals as well as COVID-19-recovered individuals. Participants vaccinated with two doses of BBIBP-CorV or AZD1222 should also receive a third booster shot with BNT162b2, unless they have already contracted the disease.

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# Effects of chokeberry fruit water extract on immune system in mouse models of infection and melanoma

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Chokeberry (*Aronia melanocarpa*) fruit extracts (CE) are rich in polyphenols and usually exhibit cardioprotective, anti-viral and anti-bacterial properties[1]. Our aim was to investigate the effects of CE on the immune response *in vivo* and *in vitro*, which have been only sporadically assessed. CE, administered orally to healthy mice, exerted immunomodulatory effects in the gut, evidenced by the altered proportion of macrophages (Mφ), dendritic cells (DC) and T cells. CE-pretreated BALB/c mice readily eradicated orally ingested *Listeria monocytogenes* due to higher proportions of Mφ and CD8 T cells both in the gut and spleen. Additionally, phagocytosis, ROS production and the proportions of activated Mφ and DC, as well as perforin$^+$ cells were enhanced in CE-pretreated infected mice. Also, CE pretreatment of C57BL/6 mice inoculated with B16 cells delayed melanoma appearance and increased infiltration of immune cells in the tumor microenvironment (TME). The TME of CE-treated mice contained more IFN-$\gamma^+$ cells and a less of tumor-promoting CCR5$^+$ MDSC. *In vitro*, CE displayed no direct cytotoxicity to B16 cells. Splenocytes isolated from CE-treated animals exerted strong cytotoxic effect on B16 cells and this effect was diminished by neutralization of IFN-γ. In conclusion, the CE exhibits strong immunomodulatory properties and should be consumed with care.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development, the Republic of Serbia, contract No. 451-03-9/2021-14/200007.

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# Anticancer properties of cisplatin-naproxen conjugate: free and loaded in SBA-15

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To develop anticancer drugs with higher activity and reduced toxicity, cisplatin was used as a scaffold to bear the anti-inflammatory drug naproxen and this conjugate was loaded into silica nanoparticles, SBA-15. In this study, the cytotoxic effect of the free conjugate and the one loaded in SBA-15 was evaluated on different cancer cell lines of mouse origin (B16, 4T1, CT26 and MC38). Treatment with free, as well as with SBA-15-bound conjugate, dose-dependently decreased viability of all cancer cell lines. The viability decrease of B16 cells after treatment with both agents was not caused by apoptosis, but it was followed by caspase activation. On the other hand, treatment with both agents caused significant decrease of B16 cells division rate, indicating the primary cytostatic effect of these agents. Additionally, it was shown that treatment with the free conjugate caused intensified autophagy, while the conjugate loaded into SBA-15 did not show this effect. Since the viability of cells recovered upon the exposure to 3-methyl adenine, detected autophagy serves as a cell death mechanism. Overall, these results indicate that both nacked and immobilized conjugates show great potential for cancer treatment.

## Acknowledgements

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# Dual CD73/$A_{2A}$R blockade reduces migration in C6 glioma cell line

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Ecto-5'-nucleotidase (CD73) is a membrane-bound ecto-enzyme, which converts AMP to adenosine (Ado) and interacts with extracellular matrix molecules. The enzyme is involved in cell adhesion and migration. Ado acts via four subtypes of adenosine receptors, linked to stimulation ($A_1$R, $A_3$R) or inhibition ($A_{2A}$, $A_{2B}$) of adenylyl cyclase[1]. The enzyme is critically involved in cell adhesion, proliferation, invasion and angiogenesis of several tumors[2]. Here we assessed the impact of dual blockade of CD73 and $A_{2A}$R on cellular functions in rat C6 glioma cell line. C6 cells were treated with specific CD73 inhibitor (APCP) and selective $A_{2A}$R antagonist (istradefylline) for 24 h. The release of proinflammatory cytokine interleukin-1β (IL-1β) into the medium was assessed by ELISA, and cell migration was assessed in a migration assay, using bright-field micrography. The values were compared between untreated and treated C6 cells. The results demonstrate that dual CD73/$A_{2A}$R blockade reduces IL-1β release in culture medium and decreases the rate of C6 cell migration, indicating that attenuation of CD73/$A_{2A}$R-adenosine signaling represents promising target in the treatment of glioma.

## Acknowledgments

This study was supported by the Ministry of Education, Science, and Technology of the Republic of Serbia (Grant No. 451-03-68/2022-14/ 200178).

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# Clustering of ovalbumin amyloid fibrils induced by the preferential binding of lead and cadmium ions

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As the native fold of proteins is generally thermodynamically unfavorable, mild changes in their immediate environment tend to induce conformational changes which may adversely affect the structure and overall function of proteins. These changes include factors such as temperature, pH value, ionic strength, as well as the presence of various denaturing agents. Such denaturing agents include heavy metal ions, which readily cause protein misfolding and aggregation, and their accumulation has been linked to several neurodegenerative diseases. The main factor in these illnesses is the development of highly ordered and thermodynamically stable protein structures – amyloid fibrils[1]. The propagation of the amyloid fold has been discovered to be positively influenced by the presence of certain metal ions[2]. In this work, the effects of increasing concentrations of heavy metal ions, lead(II) and cadmium(II), on hen egg white ovalbumin and its conversion to the amyloid fold are researched, including the determination of potential heavy metal ion binding sites within the structure of ovalbumin, as well as their differing effects on the formation of amyloid oligomers and clusters. This conversion was monitored via Thioflavin T fluorescence measurements, Fourier-Transform infrared spectroscopy, atomic force microscopy, dynamic light scattering, and computational analyses.

## Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract number: 451-03-68/2022-14/200168.

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# Chemical composition and antioxidant activity of Frankincense essential oil

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Frankincense is an aromatic resin widely used in incense and perfumes, obtained from trees of the genus *Boswellia*. This study was designed to examine the chemical composition and *in-vitro* antioxidant activity of Frankincense essential oil. Essential oil was obtained by hydrodistillation under defined operational conditions (time: 20 h, hydromodule: 1:5 m/v), from the material collected in monasteries on the territory of Skopje (North Macedonia). The qualitative and quantitative composition of collected essential oil was determined by a combination of GC/MS and GC/FID. The antioxidant activity of essential oil was determined spectrophotometrically by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Obtained essential oil yield was 7%. Twenty-six compounds were identified from essential oil, categorized into the following groups: aliphatic esters (30.2%), oxygenated diterpenes (29.0%), diterpene hydrocarbons (25.2%), oxygenated sesquterpenes (9.6%), oxygenated hydrocarbons (1.0%), monoterpene hydrocarbons (traces) and others (3.4%). Frankincense essential oil contained larger amounts of octanol acetate (29.9%) and incesole (16.1%) and lower amounts of incesole acetate (11.8%), 13-epi-dolabradiene (10.1%) and (*Z*)-nerolidol (9.6%). The concentration of essential oil, required for neutralization of 50% of initial DPPH radical concentration (EC50 value), was 25.33 ± 0.065 mg/ml, after 60 minutes of incubation. The obtained results indicate that Frankincense essential oil showed moderate antioxidant activity. Future research should focus on determining other biological activities of Frankincense essential oil, as well as the influence of the identified active components of the essential oil on biological activities.

## Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the Program of financing scientific research work, number 451-03-68/2022-14/200133. Aleksandra Milenković is Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

# Neutrophil-to-lymphocyte ratio as a prognostic marker in patients with COVID-19

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The pandemic caused by the SARS-CoV-2 virus has affected countries all over the globe. COVID-19 has claimed over 6 million lives and has had a profound public health impact worldwide[1]. The clinical progression of COVID-19 infection can be asymptomatic as well as progressing from mild to severe, or critical. It is observed that there is a high mortality rate, especially among elderly patients or those with underlying diseases[2]. Routine blood tests for the assessment of inflammatory processes are one of the most performed in clinical practice because its simple, rapid, and economical use, mainly in the early diagnosis of COVID-19 and assessing the disease's prognosis. Multiple studies have shown that neutrophil-to-lymphocyte ratio (NLR) indirectly reflect a patient's inflammatory state and can be a crucial predictor of the clinical progression of severe patients[3]. The aim of the study was to investigate the predictive value of NLR to distinguish between patients with severe and non-severe COVID-19, specifically in the Serbian population. The predictive findings may assist clinicians to identify and follow-up patients with a higher risk for progression. All patients involved in the study were 18 years of age or older, and diagnosis was confirmed by a positive real-time polymerase chain reaction showing SARS CoV-2. Data were collected from February to April 2021, from digital medical records. Patients with severe disease had higher NLR values compared to non-severe cases. It was noticeable that the increase of NLR meant the progressive increase of neutrophils, and the decrease of lymphocytes. The expansion of neutrofils usually suggests an underlying bacterial infection, while the reduction of lymphocytes means a compromised immune system. These results propose that it is obligatory to look after the COVID-19 patients with increased NLR. This study may be helpful for diagnosis and prevention of potential complications in severe patients with COVID-19.

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This study was supported by Laboratory of Biochemistry and Hematology, Health Center in Niš.

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# Status of paraoxonase 1 in renal patients with *diabetes mellitus* type 2

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Between numerous risk factors for chronic kidney disease epidemiological studies emphasized diabetes as one of the most dominant factor leading to the development and progression of the disease[1]. In the course of this, we examined the status of paraoxonase 1 (PON1) enzyme by determining the activity and concentration of the enzyme in serum and within high density lipoprotein (HDL) in renal patients with diabetes type 2 (RDM), renal patients without diabetes type 2 (RP), and healthy subjects (CG). The study involved 76 renal patients (21 RDM and 55 RP) and 19 CG. Enzyme activity was determined using kinetic method, while enzyme concentration was measured using the enzyme immunoassay technique. The arylesterase activity of PON1 within HDL2 and HDL3 were determined by a special staining technique[1]. In both patients groups, activity and concentration of PON1 were lower when compared to CG. The arylesterase activity of PON1 in HDL2 and HDL3 subclasses were lower in RP compared with CG. In RDM, PON1 activity on HDL2 and HDL3 were lower than in RP. Both patient groups had significantly lower PON1 concentration in HDL particles compared to healthy subjects. Determination of PON1 enzyme status clearly demonstrates significantly impaired antioxidant potential of HDL particles in patients with impaired renal function, especially in renal patients with diabetes.

## Acknowledgements

This work was supported by a grant of the Ministry of Education, Science and Technological Development, Republic of Serbia (grant no. 451-03-68/2022-14/200161).

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# Cabernet Franc and Dionis wines as effective inhibitors of oxidative stress *in vitro*

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It is well-known that oxidative stress is associated with numerous disorders, such as hypertension, diabetes mellitus, obesity and cardiovascular diseases. Oxidative stress is result of intense production of reactive oxygen species (ROS). ROS can react with all biomolecules in the cell, leading to their structural changes and dysfunction. ROS are usally neutralized by antioxidants and cellular antioxidant systems. The most important natural antioxidants are polyphenols[1]. Wine, the most famous grape product, is a very rich source of polyphenols. The aim of this study was to compare the content of total polyphenols, flavonoids, tannins and monomeric anthocyanins in ten red wines (five Cabernet Franc and five Dionis wines) and to evaluate their potential to inhibit oxidative stress in *in vitro* cell-based model (human monocytes, U937 cell line). The content of total polyphenols, flavonoids, tannins and monomeric anthocyanins was measured by spectrophotometric methods. The oxidative stress in U937 cells was induced by 2,2′-azobis [2-amidinopropane] dihydrochloride, while dichlorofluorescein diacetate was used to monitor intracellular level of oxidative stress[2]. The results were presented as μg trolox equivalents per mg of dry extract (μg Trolox eq/mg DW). Chemical analysis showed that all wines had significant amounts of total polyphenols, flavonoids, tannins and monomeric anthocyanins. Regarding inhibition of oxidative stress, the activity range varied from 9.46 to 22.45 μg trolox eq/mg DW, implicating that examined wine samples are effective inhibitors of oxidative stress.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

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# The response of enzymatic biomarkers in Mediterranean mussels to anthropogenic pollution in Boka Kotorska bay

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Anthropogenic pressures on marine ecosystems caused by discharge of waste waters, activities of shipyards, maritime traffic, tourism, agriculture, etc. are notable for enclosed seas and bays. In Boka Kotorska Bay accumulation of pollutants is increased due to very slow exchange of water masses. The Mediterranean mussel (*Mytilus galloprovincialis*) was proved as a sentinel species to study effects of pollutants in the seawater. Gills and hepatopancreas can accumulate variety of pollutants and their effects are studied at different leves. Oxidative stress (CAT, SOD, TBARS), neurotoxic effect (AChE activity) and content of metalothioneins were assessed in the Mediterranean mussel collected in early spring of 2014 from their natural beds in the Bay of Kotor (ST1) and from the mussel culture in Herceg Novi Bay (ST2). No significant differences were determined in AChE activity (4.16 and 4.44 nmol/min/mg proteins at ST1 and ST2, respectively). SOD inhibition rate was 95% at ST1, and 97% at ST2, measured in digestive gland. TBARS was in range from 13 to 15 nmol/mg protein measured in hepatopancreas at ST1 and ST2, respectively. The extent of oxidative stress was high, but in the same range between ST1 and ST2. The content of metallothineins was higher at ST1 (117 µg/mg w.w. *vs* 96 µg/mg w.w. at ST2) which pointed towards higher heavy metals loads at ST1. Results based on summerised biomarker responses showed that both sampling sites are uder high pollutant stress which could come from the industrial and touristic pressure.

## Acknowledgements

This study was done in scope of project No BI-ME/ 14- 15 -017 financed by research core funding No. P1-0237 Coastal Sea Research of the Slovenian Research Agency.

# MiR133a is associated with β-blocker therapy in chronic kidney disease patients with heart failure

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MiR-133a is a muscle-enriched miRNA, which plays a key role in skeletal and cardiac muscle function[1]. Chronic kidney disease (CKD) is a major risk factor for heart failure (HF)[1]. The aim of this study was to investigate whether plasma miR133a levels were associated with HF in CKD patients. This study included 44 CKD patients from Special hospital for endemic nephropathy, Lazarevac, Serbia. Patients were divided according to The New York Heart Association (NYHA) classification (NYHA-I=12 patients; NYHA II+III=32 patients). Plasma miR133a-3p levels were measured by qPCR. MiR-133a was significantly upregulated in NYHA-II+III compared to NYHA-I (P=0.030). Furthermore, miR133a was upregulated in patients using β-blocker therapy (P=0.037). It is documented that miR-133a regulates several genes in the beta1-adrenergic receptor signalling cascade, while carvedilol (a β-blocker) protects cardiomyocytes against oxidative stress induced apoptosis by up-regulating miR-133a2. These findings suggest that miR133a is affected by β-blockers, and that upregulation seen in NYHA-II+III compared to NYHA-I could be influenced by β-blockers therapy.

## Acknowledgements

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-68/2022-14/200161.

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# Antimicrobial potency of Ru(II) arene based pyridil complexes

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Discover a new class of ruthenium-based complexes that were investigated as potential antimicrobial agents: dinuclear polypyridil ruthenium(II) complexes exhibited excellent growth inhibition, and Ru(II) arene complexes with acetyl pyridine ligands exhibited moderate antimicrobial activity in the panel of bacteria[1]. Here we have synthesized 14 new Ru(II) arene complexes with pyridine-based ligands and examined their antimicrobial potency, trying to correlate their structure and biological activity. Reported complexes were obtained in a reaction of $[Ru(\eta^6\text{-benzene})Cl(\mu\text{-}Cl)]_2$ or $[Ru(\eta^6\text{-toluene})Cl(\mu\text{-}Cl)]_2$ with halogen derivatives of picolinic acid or pyridine dicarboxylic acids in a 1:2 molar ratio in ethanol. The complexes were soluble in DMSO and water. Their structural characterization included IR and NMR spectroscopy and MS spectrometry, and purity was confirmed by elemental analysis. In this report, we demonstrate the activities of these novel compounds against six typical gram-negative and two gram-positive bacteria. A micro-well dilution assay was used to determine the minimum inhibitory concentration (MIC), and minimum bactericidal concentration. Streptomycin and chloramphenicol, commercial antibiotics, were used as a positive control. The best activity of all tested bacteria was observed against *E. coli*, with a MIC value of 1.25 mg/mL, for C3, C6, and C10 complexes. Also, all synthesized complexes showed the same activity against *C. albicans.*

## Acknowledgments

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract numbers: 451-03-68/2022-14/200288 and 451-03-68/2022-14/200168.

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# Antitumor activity and impact on redox homeostasis of the essential oils of *Orlaya grandiflora*

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Cancer represents a group of persistent diseases with high mortality. Despite the extensive use of multimodal chemotherapies, their efficiency is limited, emphasizing the need for novel therapeutic approaches with higher cytotoxicity against malignant cells and acceptable harmful outcomes in healthy tissues[1]. Abiotic components can affect biosynthetic pathways of certain volatile compounds in plant metabolism. The aim of this study was to investigate antitumor activity of essential oils obtained from *Orlaya grandiflora* (L.) Hoffm. collected from two localities in Serbia (continental climate) and Montenegro (mediterranean climate). The possible antitumor mechanisms of essential oils were examined on human colon cancer HCT-116 and breast cancer MDA-MB-231 cell line by assessment of the cell viability and redox potential. The impact on proliferation rate appeared to be dose-dependent, since the obtained results showed that all used concentrations of both essential oils exhibited antiproliferative effects on HCT-116 and MDA-MB-231 cells. The stronger antitumor effects have been shown in MDA-MB-231 cells after short-term treatment, especially at the highest applied concentration (200 μg/mL), where the percentage of viability was reduced for over 40%. The results also showed decreased concentrations of superoxide anion radical in treated cells, indicating their significant antioxidative role. Long-term treatments showed mild recovery effects on cell viability in both cell lines, probably caused by balancing of redox homeostasis. Elevated levels of nitrites indicate high levels of NO production and suggest its higher bioavailability due to antioxidative environment. These results suggest considerable antitumor activity of both essential oils with potential therapeutic applications.

## Acknowledgements

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreement nos. 451-03-68/2022-14/200122).

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# R-Phycocyanin from red algae *Porphyra* spp: Binding of selected heavy metal ions

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Phycobiliproteins are major photosynthetic accessory pigments in cyanobacteria and red algae. Their vivid colours arise from covalently attached tetrapyrrole chromophores. The exciting characteristic of tetrapyrrole chromophores is the ability to bind metal ions. Heavy metals are among the most abundant and most dangerous environmental pollutants, and their removal from the environment is a crucial challenge. Therefore, utilizing PBPs-metal binding properties could be helpful in heavy metal detection and/or removal. The main aim of this study is to characterize the binding of selected heavy metal ions ($Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$) to R-phycocyanin (R-PC) isolated and purified from red algae *Porphyra* spp.The protein fluorescence quenching approach revealed the strong binding affinity of R-PC to $Hg^{2+}$ ($K_d$~0.1 μM), while protein binding to $Pb^{2+}$ and $Cd^{2+}$ is lower ($K_d$~3 μM) but still in the high to moderate range. Circular dichroism spectroscopy demonstrated the ability of $Hg^{2+}$, $Pb^{2+}$ and $Cd^{2+}$ to slightly decrease the ordered secondary structures (α-helical content) in R-PC. Our results indicatethat R-PC could beexploited as a potential biosensor for heavy metal ions detection (especially $Hg^{2+}$) in aquatic systems as well as in their removal from the environment (e.g. waste-water management).

## Acknowledgements

This work was supported by: ANSO, Project No. ANSO-CR-PP-2021-0,1 and the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-68/2022-14/200288.

# Antioxidant defense, obesity, and type 2 diabetes in students

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The aim of this study was to gain an insight into student's health, nutrition habits and general lifestyle with conducting the survey, specific anthropometric measurements, analyses of antioxidative enzymes, to established novel targets for the prevention of obesity and type 2 diabetes. The study included 360 students, of both sexes, and age matched. According to the body-mass index (BMI) $\leq$ 25 kg/m$^2$ and waist circumference (WC) $\leq$ 94 cm (80 cm for females) two groups were formed : the control group with 193 students and the risk group with 167 students and examinations of the antioxidant protection and general biochemical tests for assessing the risks of diabetes were performed. The activities of the antioxidant enzymes were significantly lower among students in the high risk group -obese students compared with the control group. Significantly negative correlations were obtained between antioxidant and anthropometric parameters in risk students, and significantly positive correlations were obtained between BMI, WC and positive family anamensis and fasting glucose, postprandial glicemia, HbA1c status,microalbumin in urine. The results showed significantly positive correlation between physical activity and glutatione peroxidase (GSH-Px) and total antioxidative status (TAS) ($p< 0.05$ ) and negative correlation for smoking and activity of GR, SOD-1, GSH-Px and TA ($p< 0.01$ ).Activity of TAS and SOD showed significantly positive correlation of weekly consumation of fish and drinking red wine ($p<0,05$) and as well as suplementation of omega -3-fatty acids in the risk student population. Fasting glucose, postprandial glicemia, HbA1c status and microalbumin in urine and hsCRP were significantly higher in the high risk group. In risk group we discovered 2.33% students with type 2 diabetes with average values of glucose 9.36 ±1.38 mmol/l, postprandial glicemia 10.87±1.28 mmol/L, HbA1c status 7.49 ±0.71% vs. 57±7.71 mmol/mol, microalbumin in urine 26.50 ±5.68mg/L and hsCRP 1,23±0.71mg/dL. These data can provide a good basis for taking the primordial and primary prevention through the changes and promotion of a healthy lifestyle ("eat less and exercise more" and the modifications of the risk factors for obesity and type 2 diabetes.

# Production and application of pectinases in the liquefaction of apricot and blueberry juice

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Pectinases are widely used in the fruit juice industry for clarification, liquefaction and stabilization of juices[1]. One of the biggest problems in the production of fruit juices is the turbidity of the juice, which is mainly caused by the presence of pectin polysaccharides. Therefore, pectinase is used in juice clarification, which breaks down the pectin structure and reduces unwanted cloudiness and sediment[2]. In this work, the production of pectinases was optimized by solid state fermentation using *Aspergillus tubingensis* strain, which proved to be an efficient producer of these enzymes. Statistical method Design of Experiment was used to optimize the medium and conditions for enzyme production. The total pectinase activity obtained was determined by the DNS method (47 U/mL). Endo-pectinases activity is determined by reduction of viscosity of pectin solutions. The resulting complex of pectinase enzymes was used for the liquefaction of apricot and blueberry pulp, with a juice yield of 72% and 81%, respectively. Also, apricot juice treated with enzymes was clarified by 77% compared to juice that was not treated with enzymes. Blueberry juice obtained after treatment with pectinase enzymes has a higher antioxidant activity than the untreated juice, as determined by the DPPH assay.

## Acknowledgements

This work was financially supported by The Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract numbers: 451-03-68/2022-14/200168, 451-03-68/2022-14/200026, 451-03-9/2022-14/200017 and 451-03-68/2022-14/200288.

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# Nonlinear imaging of the hybrid layer after modifying the dentinal substrate by using collagen cross-linkers

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Recently, collagen crosslinkers have been giving promising results in improving the quality of the dentin-adhesive hybrid layer. Modified by collagen crosslinkers, the hybrid layer has higher tensile strength, is more able to resist endogenous proteases, and is shown to be more durable over time[1]. The previous research focused mostly on mechanical improvements of the hybrid layer modified by cross-linkers. However, microscopical imaging of such a substrate has not been investigated in detail, by far. Dentinal samples were randomly allocated to one of two experimental groups –1. etch-and-rinse (E&R) and 2. self-etch protocols (SE), both modified by a collagen crosslinker. Standard bonding protocols were modified by using the 0.3M EDC (1-ethyl-3(3-dimethylaminopropyl)carbodiimide) for 1 min, in both groups. Control groups included the standard procedures for E&R and SE techniques, without EDC addition. The adhesive was marked by an aqueous solution containing 0.5% eosin-Y in order to be visible microscopically. Prepared in the listed manner, the samples were cut into longitudinal slices and imaged by the Nonlinear Laser Scanning Microscopy, custom-built in the Institute of Physics Belgrade. The hybrid layer modified by EDC turned out to be wider and more homogeneous containing significantly longer resin tags, more uniformly distributed than in the control group. Compared to the control group, cohesion and adhesion fractures of the EDC-modified hybrid layer were observed less frequently.

## Acknowledgments

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract No. NIO 200114).

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# Albumin: antioxidant or pro-oxidant in patients on peritoneal dialysis?

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Albumin (HSA) is a multifunctional serum protein. Free Cys34 thiol group makes HSA a major source of free thiols in the circulation. HSA is also a dominant carrier of exchangeable copper pool with a high affinity binding site for this redox-active ion, located at the N-terminus. Combination of these two properties highlights HSA as a powerful circulatory antioxidant. The presence of free thiol groups in HSA and its copper binding capacity have been shown to be interconnected and essential for the binding and sequestering of copper ions, thus preventing metal-catalysed production of reactive oxygen species. End stage kidney disease (ESRD) is a condition accompanied by oxidative stress. The aim of this study was to examine changes in the antioxidative capacity of HSA and Cu(II) binding affinity in patients on peritoneal dialysis (PD), and relate it to the Cys34 thiol group content and structural changes of this molecule. HSA molecules are modified in ESRD patients on PD, having lower content of bound copper ions and thiol groups, reduced copper binding affinity and overall antioxidant capacity. An increased content of advanced glycation end-products and altered conformation was detected in HSA from PD patients. Function of HSA as a sequestrator of free Cu(II) ions, is impaired. A significant portion of the high-affinity metal-binding site was unable to interact with Cu(II) ions, as shown by EPR. Similar results on copper binding were obtained in subgroups of patients with and without diabetes mellitus, suggesting that ESRD and constant exposure to high glucose concentration during dialysis influenced protein modification. Since the concentration of Cu(II) in the circulation of these patients is much higher than in healthy persons and that Cu(II) binding capacity of albumin from patients is significantly lower, the albumin may be considered to turn from antioxidant to pro-oxidant activity.

## Acknowledgements

This research was funded the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract numbers: 451-03-68/2020-14/200019 and 451- 03-9/2021-14/200053.

# Metal ions and their binding proteins in early COVID-19 infection

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Metal ions seem to be involved in the pathogenesis of the novel coronavirus disease of 2019 (Covid-19) and are investigated as potential prognostic markers and supplements for the treatment. We examined the relationship between major trace elements (iron, zinc and copper) and their principal binding proteins (haemoglobin, transferrin, ferritin, albumin, alpha-2-macroglobulin and ceruloplasminin) in the circulation of patients in the early stage of infection. Infected persons exhibited similar status in respect to iron, copper and their major binding proteins in the circulation as healthy individuals, except for hemoglobin whose levels were lower, but still within reference limits. The concentrations of iron and ferritin were strongly correlated only in infected persons. The concentration of zinc ions was higher in infected than in healthy persons (in 20/60 above the upper reference limit), as well as the ratios zinc/albumin and zinc/alpha-2-macroglobulin. Increased zinc levels could be attributed to cellular redistribution of zinc ions or to zinc supplementation. Immunoblot analysis demonstrated the presence of greater quantities of proteinase-bound alpha-2-macroglobulin tetramer and albumin monomer in infected than in healthy individuals, which were correlated with the concentration of zinc ions ($r = 0.42$ and 0.55, respectively) only in healthy individuals. These correlations were lost in infected persons, most likely due to a very high zinc concentration in some participants which was not proportionally followed by changes in the distribution of protein species. Although there is still no firm confirmation on the mechanisms of zinc involvement in a defense against Covid-19 (some reports connect zinc levels with the survival rate, but others deny the relation), this ion possibly contributes to the existence of circulating protein forms which are the most optimal. Alpha-2-macroglobulin is a universal proteinase inhibitor and a mediator of the innate immune response. It can bind cytokines, growth factors and misfolded proteins, but with sensitivity and selectivity which is different for the native tetramer form, proteinase-bound or a dimer. One can hypothesize that inflammation, the predominant pathway in the immune response, and zinc status affect distribution of molecular forms of alpha-2-macroglobulin influencing its capacity to preferentially bind proteinases or cytokines, although not in a simple mathematical correlation.

# Anti-oxidative characterisation of human serum albumin from patients with kidney diseases

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Oxidative stress (OS) is defined as disrupted balance between pro-oxidants and anti-oxidants activity. Anti–oxidative capacity of organism is crucial for the prevention and/or later onset of the disease progression. One of the key elements of circulatory anti-ox defense is human serum albumin (HSA). Single free thiol group positioned at Cys34 in HSA reperesnts its main anti-ox feature. Changes in the HSA structural properties affect its capacity to sequester pro-oxidants and hence reduce its ability to fight OS. Chronic kidney disease (CKD) represents the state of impaired kidney function which last in continuity for more than three monts. It is characterized with increased proteinuria and decreased glomerular filtration rate. End stage renal disease (ESRD) is a state where kidney function is (almost) completely lost and pateints require renal replacement therapy (dialysis or transplantation). This pathological condition is markedly followed by increased OS. Impaired anti-ox mechanisms could be one of the reasons for the progression of the disease. Minding that HSA represents main regulator of oncotic preasure, and its anti-ox capacity we wanted to analyse the structural changes of this molecule in three groups of pateints with kidney disease. HSA from CKD5 and peritoneal (PD)- vs. hemodialysis (HD) patients was analysed and compared with the HSA from the control group. Methods of determining free thiol grups concentration, immunoblot, UV/VIS spectrophotometry and spectrofluorimetry were used to assess differences in the HSA anti-ox capacity and structural differences between groups of samples. Significantly lower presence of free thiols in CKD samples compared to control group indicated increased OS with the higest level present in HD samples. PD and HD samples showed presence of homodimer form of HSA, which was absent in CKD5 and control samples. Spectral analysis of HSA indicated changes in structure of PD and HD derived HSA due to increased OS. These results indicate lowered anti-ox capacity of HSA in more progressive stages of the disease and an increased need for additional anti oxidative therapy.

# Hamster fibrosarcoma volume kinetics due to metformin and nitroglycerin

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We investigated the effect of metformin and nitroglycerin combination on fibrosarcoma volume kinetics in hamsters. Syrian golden hamsters of both sexes, weighing approximately 60 g, were randomly allocated to control and experimental groups, with 8 animals per group. In all groups, $2\times10^6$ BHK-21/C13 cells in 1 ml were injected subcutaneously into the animals' backs. Peroral treatment was carried out with metformin 500 mg/kg daily, or with nitroglycerin 25 mg/kg daily, or with a combination of metformin 500 mg/kg and nitroglycerin 25 mg/kg daily. The tumor diameters and the tumor burdens were evaluated daily using calipers and the ellipsoid volume formula: volume = $4\pi abc/3$, where a, b and c are half-diameters. When the tumors were approximately 2-3 cm in the control group, all animals were sacrificed, blood samples collected for hematological and biochemical analyses and main organs toxicologically analyzed. The tumors were excised and weighed, their diameters were exactly measured, and the exact tumor volume was determined using the standard water volume displacement method. The tumor samples were pathohistologically and immunohistochemically assessed. Regarding tumor volume kinetics, control group exhibited rather rapid tumor growth and group treated with metformin and nitroglycerin combination exhibited slow growth. The combination of metformin and nitroglycerin significantly inhibited fibrosarcoma growth in hamsters without toxicity, compared to monotherapy or control.

## Acknowledgements

This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research, grants nos. 142-451-2498/2021-03 (DP), 142-451-2676/2021 (JM), 142-451-2626/2021 (DL) and Republic of Serbia, Ministry of Education, Science and Technological Development, grant no. 451-03-68/2022-14/200114.

# Diclofenac with metformin can slow hamster fibrosarcoma development

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Diclofenac and metformin separately exhibit limited anticancer potential. We examined whether combinig these two drugs in doses equivalent to human doses would synergize their anticancer activity on fibrosarcoma inoculated to hamsters. BHK-21/C13 cell culture was subcutaneously inoculated to Syrian golden hamsters of both sexes (~ 70 g), which were randomly allocated to control and experimental groups (6 animals per group). The hamsters were treated in control group 1 with physiological saline, in group 2 with diclofenac 120 mg/kg, in group 3 with metformin 1000 mg/kg, in group 4 with combination of diclofenac 60 mg/kg and metformin 500 mg/kg, via a gastric probe daily after cancer cell inoculation. The animals were sacrificed 18 days post tumor cells inoculation. Tumor growth kinetics, biophysical, pathological, histological and immunohistochemical characteristics of excised tumors and hamster organs and biochemical blood and hematological tests were compared among the groups. Only the co-treatment with diclofenac and metformin simultaneously significantly ($P < 0.05$) inhibited tumor growth.The pathohistological and immunohistochemical evaluation confirmed these biophysical findings. Neither single diclofenac, nor single metformin treatments, regardless of doubling doses used in the combined treatment, exhibited significant anticancer effect in comparison to control. Metformin exhibited significant synergistic inhibitory effect with diclofenac on all parameters of tumor growth, without toxicity and influence on biochemical blood and hematological tests.

## Acknowledgements

This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research, grants nos. 142-451-2498/2021-03 (DP), 142-451-2676/2021 (JM), 142-451-2626/2021 (DL) and Republic of Serbia, Ministry of Education, Science and Technological Development, grant no. 451-03-68/2022-14/200114.

# Disulfiram with metformin inhibit hamster fibrosarcoma growth

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We investigated the anticancer effect of disulfiram and metformin combination on fibrosarcoma in hamsters. Syrian golden hamsters of both sexes (~70 g) were randomly allocated to control and experimental groups (8 animals per group). In all groups, $2 \times 10^6$ BHK-21/C13 cells in 1 ml were injected subcutaneously into the animals' backs. Peroral treatments were carried out with disulfiram 50 mg/kg daily, or with metformin 500 mg/kg daily, or with their combination. Validation grups were treated by double doses of the single therapy, via a gastric probe after tumor inoculation. In the course of experiment, changes in tumor growth of each animal were recorded daily. To obtain volume estimates, we used calipers to measure the tumor diameters.The animals were sacrificed 19 days post inoculation. Blood samples were collected for hematological and biochemical analyses, and the main organs were toxicologically tested. The tumors were excised and weighed, and their diameters and volumes were measured. The tumor samples were pathohistologically and immunohistochemically assessed (Ki-67, PCNA, CD34, CD31, COX4, Cytochrome C, GLUT1, iNOS). The combination of disulfiram and metformin significantly inhibited fibrosarcoma growth in hamsters without toxicity, compared to monotherapy or control. The single treatments did not show significant antisarcoma effect. Administration of disulfiram with metformin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment and relapse prevention antitumor therapy.

## Acknowledgements

This study was supported by the Republic of Serbia, Ministry of Education, Science and Technological Development, grant no. 451-03-68/2022-14/200114 (KP) and by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research, grants nos. 142-451-2498/2021-03 (DP), 142-451-2676/2021 (JM), 142-451-2626/2021 (DL).

# Modulation of THP-1 derived macrophages by Bet v 1l-$BL_{H84T}$ and $BL_{H84T}$-Bet v 1l chimeras

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Allergen-specific immunotherapy (AIT) is the only therapeutic approach that has a long-term effect in curing allergies, acting like a therapeutic vaccine that utilizes the body's own immune system[1]. The improvement in AIT efficacy could be made by combining hypoallergenic (reduced risk of IgE-mediated side effects and preserved T cell epitopes)[1] with immunomodulatory agents[2]. Banana Lectin ($BL_{wt}$) is mannose binding lectin that modulates murine peritoneal macrophages by binding to their oligossacharide structures on TLR2. Reduced $BL_{wt}$ mitogenic property with preserved immunomodulatory effect was achieved by inducing single mutation of histidine at position 84 with threonine ($BL_{H84T}$)[3]. The aim of this study was to explore the immunomodulatory potential of the chimeras composed of a hypoallergenic isoform of the major birch pollen allergen Bet v 1l and $BL_{H84T}$ and to compare them with $BL_{wt}$, Bet v 1A and Bet v 1A-$BL_{wt}$ ($C_{wt}$). The chimeras Bet v 1l-$BL_{H84T}$ (C1), and $BL_{H84T}$-Bet v 1l (C2), Bet v1l, and $BL_{H84T}$ were produced by recombinant DNA technology. The immunomodulatory potential of all antigens was tested on THP-1 differentiated macrophages by measuring the level of gene expression for proinflammatory cytokines (TNFα, IL-1b, IL-6), and anti-inflammatory cytokines (IL-10 and TGFβ). The cell co-culture of epithelial cell lines and THP-1 differentiated macrophages will be employed for further assessment of the immunomodulatory potential of chimeras and their potential application in AIT.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of Republic of Serbia Contract number: 451-03-68/2022-14/200168.

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# Development and comparison of Western blot, dot blot and ELISA for mussel tropomyosin quantification

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Tropomyosin (TPM) is considered a major allergen among different shellfish species. Developing sensitive, specific, and reliable methods for quantifying TPM in food products is crucial for persons allergic to shellfish. We have previously developed a highly sensitive sandwich ELISA method for quantifying shrimp TPM. Despite high amino acid sequence homology between shrimp and mussels TPM, the method has not been reliable for quantifying TPM from mussels, underestimating its concentration up to three orders of magnitude. Therefore, this work aimed to develop alternative immunological methods for mussel TPM quantification. Western blot, dot blot, and indirect ELISA using monoclonal anti-TPM antibody and alkaline phosphatase-labeled secondary antibody were developed and compared in terms of their sensitivity. Tropomyosin in mussels extracts was quantified using highly purified natural shrimp tropomyosin as standard. The linear range for TPM quantification using dot blot was between 5 and 50 μg/mL, while Western blot has slightly increased sensitivity, with a linear range between 1.25 and 12.5 μg/mL. Indirect ELISA has further improved the sensitivity of TPM quantification, with a 0.04-0.4 μg/mL linear range. Additional work will be performed to enhance the sensitivity of the presented methods, with the final aim of reducing risks of inadvertent food contamination.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number: 451-03-68/2022-14/200168, the Science Fund of the Republic of Serbia, Program DIASPORA, #6504499, ShellPCR, and IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173).

# Di-(2-ethylhexyl) phtalate induces transcriptional changes in human granulosa cells after long-term exposure

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Di(2-ethylhexyl) phthalate (DEHP) is the most widely used plasticizer found in a variety of products[1]. Animal models have shown that DEHP has an adverse effect on ovarian granulosa cells steroidogenesis[2,3]. However, the effects of long-term exposure to low doses of DEHP on human granulosa cells have not been evaluated. The aim of this study was to investigate the *in vitro* effects of long-term exposure to low doses of DEHP on transcriptomic profile in human granulosa cells, HGrC1. The cells were exposed in the culture flasks to 50 nM and 250 nM DEHP for 14 days. Part of DEHP-exposed cells were stimulated with forskolin (FOR) for 48 h. RNA sequencing results showed that 50 nM DEHP deregulated 10 genes in basal and 18 genes in FOR-stimulated conditions. 250 nM DEHP deregulated 19 genes in basal and 21 genes in FOR-stimulated conditions. Gene ontology enrichment analysis showed that catecholamine metabolic process and regulation of apoptotic cell clearance could be DEHP targets after 2 weeks of exposure. The most pronounced effect of long-term DEHP exposure on HGrC1 cells relates to the changes in the expression of *SULT1A3* and *SULT1A4*, involved in catecholamine metabolic process. These data, for the first time, showed that low level of DEHP can alter the *SULT1A3* and *4* expression in HGrC1 after 14 days of exposure.

## Acknowledgements

This study was supported by Science Fund of the Republic of Serbia, program PROMIS, project DETOX, grant number 6062573.

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# Evaluation of new steroid derivatives as potential ligands for androgen and estrogen receptors

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The 1,3,4-thiadiazoline nucleus is one of the most studied heterocycles, and its biological activity has been demonstrated in drugs that are currently in clinical use: such as cefazolin, megazol or acetazolamide. Introduction of a thiadiazoline ring into the steroid structure could enhance bioactivity, improve selectivity and reduce the side effects of potential drugs for cancer therapy. Therefore, in this work six new thiosemicarbazone and 1,3,4-thiadiazoline androstane derivatives in 17a-homo lactone and 17α-picolyl series were tested in order to evaluate their relative binding affinity for the ligand-binding domain (LBD) of estrogen receptor α (ERα), estrogen receptor β (ERβ) or androgen receptor (AR), using a fluorescent cell assay in yeast[1,2]. Two of the tested compounds exhibited moderate binding affinity for ERα isoform, with ~1.2 fold fluorescence enhancements *vs*. controls. For ERα ligands identified in this study, we also observed a complete absence of binding for ERβ isoform. Design of compounds that specifically bind to ERα subtype, which is responsible for proliferation of breast cancer cells, is of great importance. None of the tested compounds displayed affinity for the AR.

## Acknowledgements

This study was supported by the Ministry of Education , Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125) and Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: New steroid derivatives - potential chemotherapeutics, No. 142-451-2667/2021].

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# Antioxidant activity of extracts from *Tagetes* spp. (marigold) flowers

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The present study aimed to determine and compare the antioxidant activity of ethanolic and hexane extracts isolated from *Tagetes* spp. (marigold) flowers. The plant material was cultivated in the gardens in the village of Manastirište (municipality of Vlasotince, southeastern Serbia). The extracts were isolated from fresh red and orange flower petals collected at the end of August (orange) and at the beginning of September 2021 (the red ones) by Soxhlet extraction using a solvomodule of 1/10 m/V. Their antioxidative activity was determined spectrophotometrically by using the DPPH assay. According to the results obtained, ethanolic extracts showed better antioxidant activity in comparison to the hexane extracts ($EC_{50}$ values of 0.004 mg/mL and 0.008 mg/mL *vs*. 0.512 mg/mL and 0.226 mg/mL for red and orange flower petals, respectively). The ethanolic extract isolated from the red flower petals showed remarkable antioxidant activity, equal to the antioxidant activity of L-ascorbic acid (0.004 mg/mL), indicating that it could be used as a natural alternative to synthetic antioxidants.

## Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the Program of financing scientific research work, number 451-03-68/2022-14/ 200133. Nataša Simonović is the Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

# Noncovalent and covalent binding of phycocyanobilin to S protein of SARS-CoV-2 and its receptor-binding domain

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The emergence of the coronavirus SARS-CoV-2 has attracted attention of the whole scientific community,. The SARS-CoV-2 spike (S) protein plays the most important role in viral attachment to host receptor angiotensin-converting enzyme 2 (ACE2), via the receptor-binding domain (RBD), fusion and entry into the host, and it serves as a target for the development of antibodies, entry inhibitors and vaccines. It has been demonstrated that phycocyanobilin (PCB), a bioactive open-chain tetrapyrrole chromophore of phycocyanin (PC), chromoprotein derived from the cyanobacterium *Arthrospira platensis*, can bind a plethora of different proteins, both in a noncovalent and covalent manner. This study aimed to investigate interactions of PCB with S protein and RBD respectively. Electrophoretic techniques, fluorescence spectroscopy, and inhibition of S–PCB and RBD–PCB covalent adduct formation using iodoacetamide and N-ethylmaleimide, were employed to examine interactions of PCB with S protein and RBD, while the effects of PCB binding on RBD structure were studied by CD spectroscopy. SDS-PAGE with $Zn^{2+}$ staining has revealed that PCB covalently binds to both S protein and RBD, via free cysteine residues. Binding constants determined by the fluorescence quenching method were: $2.1\times10^{7}$ $M^{-1}$ for PCB and S protein and $8.4\times10^{4}$ $M^{-1}$ for PCB and RBD. Far-UV circular dichroism spectra showed that the binding of PCB influences RBD structure by decreasing the disordered structure content. Due to moderately strong noncovalent interactions of PCB with S protein and RBD, as well as covalent adducts formation, it may exert one of its many bioactive effects via impact on S protein binding to ACE2 receptor.

## Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 810752.

# Biological potential of new molecular hybrids of thiohydantoin and zingerone derivatives

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Molecular hybridization is one of the most valuable structure modification tools currently used in drug construction. The concept of hybridization is usually achieved via chemical connection of two or more biologically active pharmacophoric moieties to form a new molecular hybrid with potentially higher activity and efficacy compared to the parent moieties. In general, a symmetrical hybrid connects two identical fragments and is expected to produce more potent and/or selective pharmacological effects compared to single molecules, whereas the nonsymmetrical hybrid drug is perceived to show both pharmacological activities resulting from the individual pharmacophores (dual action) with additional synergic effect. Zingerone is a natural compound present in significant amounth in ginger. Both natural and synthetic zingerone derivatives exhibit different biological and pharmacological activities such as anti-inflammatory, antimicrobial, anti-cancer, and hepatoprotective. On the other hand, many synthesized thiohydantoin derivatives exhibit a wide range of biological and pharmacological potentials, such as antimicrobial, anti-convulsive, anti-proliferative, anti-metastatic, anti-diabetic, and anti-HIV. In this study, a short series of new zingerone-thiohydantoin molecular hybrids were synthesized from *O*-alkyl zingerone derivatives for evaluation of their potential biological activity. Obtained new potentially bioactive compounds were tested for their antimicrobial and *in vitro* anticancer activities. These compounds showed low to moderate antimicrobial activity. The difference in the cytotoxic activity of the hybrid compounds depends on the nature of the *O*-alkyl substituent of the benzene ring. Among the tested compounds, zingerone-thiohydantoin hybrid with an *O*-buthyl substituent exerted the significant cytotoxic activity on colon HCT-116 cancer cells without toxicity on healthy MRC-5 cells.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200378).

# Redox component in the adaptation of the microalga *Chlorella sorokiniana* to Ni(II) excess

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Nickel is utilized by microalgae as a co-factor of urease. On the other hand, this transition metal represents an important pollutant of aquatic ecosystems. The effects of Ni(II) excess on microalgae and the mechanisms of adaptation are poorly understood. Redox processes represent an important component of the mechanisms of interaction of microalgae with transition metals[1]. Pertinent to this, we analyzed redox changes in *Chlorella sorokiniana* culture that are induced by high levels of Ni(II). The intracellular level of reactive oxygen species (ROS) showed a rapid two-phase increase that took place prior to Ni accumulation in the cell. This was accompanied by oxidation of thiols and drastic deglutathyonilation of proteins. PAM fluorimetry showed that Ni excess induced an increase in the efficiency of photosystem II and promoted electron flow in chloroplasts, which is most likely responsible for ROS rise. In addition, a rising trend in the chlorophyll level was observed. On the other hand, the level of lipid peroxidation and activities of key antioxidative enzymes were not increased, which implies that oxidative stress is not an important player in Ni adaptation/toxicity. After prolonged exposure the efficiency of photosystem II drops, nickel is accumulated in the cells, and new redox balance is established. Our results imply that redox signalling is involved in Ni-induced metabolic activation and that key changes take place in photosynthetic machinery.

## Acknowledgements

This study was supported by the NATO Science for Peace and Security Programme, Project number G5320, and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/200053).

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# Evaluation of the anticancer activity of disulfiram in breast cancer

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As the most prevalent disease diagnosed globally, breast cancer has now surpassed lung cancer. In 2020, there were over 2 million new diagnoses and nearly 700.00 deaths of breast cancer in women worldwide[1]. Significant progress has been made in the primary treatment choices for breast cancer, which include surgery, chemotherapy, radiation therapy, and other. However, breast cancer still has high morbidity and mortality rates, so the search for new, more effective therapeutic models continues. Instead of developing completely novel medicine, repurposing drugs that have already been used for treating other conditions can be a much better solution, less expensive and time-saving. Disulfiram (DSF; Esperal®) is approved by the FDA for the treatment in patients with alcohol dependence. DSF has proven to be a safe and effective drug for short-term and long-term therapy. Recent studies have shown that DSF participates in proteasome inhibition, DNA demethylation, induction of ROS production, and alteration of MAPK or MMP pathways. Metabolites of DSF induce p53, mediating apoptosis and cell death[2]. DSF can have a primary or adjuvant role in solving the problem of drug resistance by inhibiting the ABC drug transport protein. Evidence shows that DSF has the potential to target tumor cells which makes it a promising candidate for the treatment of malignant diseases. In our study, we have examined the anticancer effect of DSF on one normal lung epithelial (BEAS-2B), two cancer epithelial cancer (MDA-MB-231), and breast adenocarcinoma (MDA-MB-361) human cell lines. Our results showed that DSF exerts its anticancer potential on breast cancer cells, with MDA-MB-231 cells being the most sensitive when exposed to DSF. This cell line was used in additional experiments which showed that DSF induces cell apoptosis, a decrease in mitochondrial membrane potential and changes in intracellular ROS levels. This anticancer potential of DSF and its mechanisms should be further investigated in order to develop more effective breast cancer therapy.

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This study was supported by Ministry of Education, Science and Technology of Serbia (451-03-68/2022-14/200043).

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# LC/MS phenolic characterization and cytotoxic activity of *Pulmonaria officinalis* L. methanolic extract

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This study aimed to investigate the phenolic composition of the aerial part methanolic extract of *Pulmonaria officinalis* L., as well as to evaluate its cytotoxic activity against normal and cancerogenic cells. Using UHPLC-$MS^4$ Orbitrap analysis, 57 different phenolic compounds, including 38 phenolic acids and 19 flavonoids were identified. Among them, a total of 19 were quantified by UHPLC-DAD/(-)HESI-MS/MS analysis. The most prevalent components in the extract were *p*-hydroxybenzoic acid, caffeic acid, rutting, quercetin 3-*O*-glucoside, rosmarinic acid, salvianolic acid B, salvianolic acid A, and apigenin. Rosmarinic acid was the most dominant compound in the extract with a concentration of 4.51 mg/g. Based on MTT reduction assay[1], the selective cytotoxicity of the tested extract was noticed. The extract in the concentration of 200 µg/mL reduced the viability of the cancer cells (human epidermoid carcinoma A431 and murine transformed SVT2 fibroblasts) by 50%, while lower cytotoxic effects of the extract on healthy cells (HaCaT keratinocytes and BALB/c-3T3 fibroblasts) was observed. *P. officinalis* extract has potential for further research considering the high content of rosmarinic acid and selective cytotoxic effect with pronounced effect against some cancer cell lines.

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This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreements No. 451-03-68/2022-14/200122 and 451-03-68/2022-14/ 200007).

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# Transcriptional changes induced by long-term low-level bisphenol A exposure in human endothelial cells

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Bisphenol A (BPA) is a plasticizer used in the production of polycarbonate plastics and epoxy resins and can be found in a variety of products, such as food and beverage containers, medical devices, and children's toys. Because of its widespread and frequent use human exposure to BPA is inevitable. BPA is detected in different body fluids, such us urine, follicular and amniotic fluid, cord blood and placenta, blood and liver of human fetuses, in the serum of pregnant women, and breastmilk. We have previously demonstrated that environmentally relevant concentrations of BPA affect several functions of human endothelial cell line EA.hy926 after long-term *in vitro* exposure[1]. Here, we analzyed the total mRNA profile of EA.hy926 cells after 14-weeks-long exposure to three environmentally relevant concentrations of BPA ($10^{-9}$, $10^{-8}$, and $10^{-7}$ M). Gene expression profile was analyzed using the DNBSEQ platform and differentially expressed genes (DEGs) were considered when FC≥[1] and FDR≤0.001. The results show that all three concentrations of BPA affected a unique set of DEGs with $10^{-9}$ M group having the most DEGs ($10^{-9}$ M BPA = 104 DEGs, $10^{-8}$ M BPA = 16 DEGs, $10^{-7}$ M BPA = 17 DEGs). Only three DEGs were shared between all three concentrations of BPA. The most enriched biological processes were cytokine-mediated signaling and apoptotic process in the $10^{-9}$ M group, negative regulation of peptide secretion in the $10^{-8}$ M group, and immune system processes and innate immune response in the $10^{-7}$ BPA group. These results demonstrate that long-term low-level BPA exposure causes concentration-specific changes in gene expression profile in EA.hy926 cells.

## Acknowledgements

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

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# Proteomic profiling of the response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer using data-independent acquisition mass spectrometry

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The implementation and validation of the neoadjuvant chemoradiotherapy (nCRT) approach have greatly improved the survival of patients with locally advanced rectal cancer (LARC). However, the response to nCRT varies and only 20-30% of patients achieve a complete clinical or pathological response, while some patients do not respond to therapy at all. Understanding the molecular features associated with response to nCRT and using them as predictive markers is an unmet clinical need in LARC. The aim of the study was to apply for the first time a high-sensitivity proteomic approach on LARC biopsies using data-independent acquisition mass spectrometry (DIA-MS), followed by *in silico* analysis for in-depth characterization of the response. The use of DIA-MS allowed the identification of 915 differentially expressed proteins (DEPs) (215 in responders and 700 in non-responders) in a group of 20 samples (9 responders (Tumor Regression Grade, TRG 1-2) and 11 non-responders (TRG 3-5) according to Mandard scale). Shortlisting of potential biomarkers was performed using the ROCplotter software (www.rocplot.org), an online database which links gene expression and response to therapy using cancer transcriptome-level data. Out of 915 DEPs, 25 genes met all criteria and were classified as a promising biomarker. The identified proteins are currently prospectively evaluated in a new LARC cohort obtained from a European consortium of LARC samples. Selection of patients who might benefit from enrollment in a watch and wait approach rather then the current standard-of-care surgery after nCRT is expected to maximize the therapeutic effect

of nCRT, increase the quality of life of LARC patients and reduce the overall treatment and medical care cost.

## Acknowledgements

This study was supported by the Ministry of Education and Science of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200043). This study is based upon work from COST Action CA17118, supported by COST (European Cooperation in Science and Technology); www.cost.eu. We acknowledge the support by the project "The Greek Research Infrastructure for Personalised Medicine (pMED-GR)" (MIS 5002802) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund). MC is supported by the Science Fund of the Republic of Serbia (PROMIS TRACEPIGEN project No. 6060876).

# The effect of a gallic acid derivative on encephalitogenic cells

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This study aimed to evaluate the effects of a synthetic gallic acid (GA) derivative in the central nervous system (CNS) autoimmunity, *i.e.* in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. EAE was induced in DA rats by injection of autologous spinal cord homogenate, with a gallic acid derivative being applied subcutaneously (20 mg/kg, day 7-22 post-immunization). GA derivative ameliorated EAE. Cells from lymph nodes draining the site of immunization (DLNC), isolated in the inductive phase of the disease, and spinal cord immune cells (SCIC), isolated at the peak of disease, were exposed to GA derivative *in vitro*. Encephalitogenic cytokines, interferon (IFN)-γ and interleukin (IL)-17, were decreased in SCIC and DLNC under the influence of GA derivative. The proportion of IL-17-producing $CD4^+$ T cells was reduced in SCIC (flow cytometry). Treatment of microglial BV2 cells with GA derivative led to inhibition of NO, IL-6, and tumor necrosis factor release. These results imply that the synthesized GA derivative is a potent immunomodulator, able to ameliorate EAE. Its effects on the CNS autoimmunity are related to the inhibition of encephalitogenic T cells and macrophage/microglia activity in our study.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological development of the Republic of Serbia No. 451-03-9/2021-14/200007; Hellenic Foundation for Research and Innovation "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (No. 991).

# Exoinulinase gene expression in *Aspergillus welwitschiae* FAW1 induced by different carbon sources

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Fungal inulinases have wide application in industrial biotechnology, and it is presumed that their expression is regulated at the transcriptional level via promoter[1]. It is also known that different sugars have an inducing effect on gene expression in fungal genome, including inulinases[1,2]. Aim of this work was to determine which of the sugars used in growth medium, as the only carbon source, induce the extracellular exoinulinase gene *inuE* expression in *Aspergillus welwitschiae* FAW1. Inulin, rafinose, sucrose, glucose and fructose were used as carbon sources, and expression of *inuE* was monitored during 72 h of cultivation (tested after 24, 36, 48 and 72 h). Both, presence of mRNA in the mycelia and extracellular enzyme activity in the growth media were monitored. Interestingly, obtained results showed that *inuE* was induced by fructose, sucrose and rafinose and not by inulin. In all cases, the highest mRNA was detected after 24 h of cultivation, while extracellular exoinulinase activity increased from 24 h with a peak in 72 h. Further experiments are necessary for a comprehensive understanding of the regulation mechanisms of *AweinuE* promoter for its more purposeful application in biotechnology.

## Acknowledgements

This study was supported by a grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract numbers 451-03-68/2022-14/200026, 451-03-68/2022-14/200168 and 451-03-68/2022-14/ 200214)

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# Correlation of salivary and serum cytokines in HIV-positive patients with diagnosed periodontitis

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Periodontitis is an inflammatory disease of supporting dental tissues caused by dental biofilm microorganisms. In the immunopathogenesis of this disease inflammatory mediators, cytokines, have a main role. During active periodontitis, there is an increase in pro-inflammatory cytokines such as IL-1β and TNFα in local tissues and fluids, which can be followed by their increased level in circulation, as well. They can cause a systemic inflammatory response and synthesis of CRP, which is one of the basic markers of systemic inflammation1. Various co-infections, including periodontitis, can contribute to the increase circulating levels of inflammatory markers in HIV-positive patients. The aim of this research is to determine whether there is a correlation between the concentration of IL-1β and TNFα in saliva and serum, as well as their association with serum CRP values in HIV-positive patients with periodontitis. In this research participated 34 HIV-positive patients with diagnosed periodontitis. Samples of unstimulated saliva were centrifuged, supernatant was poured off and frozen at -70ºC. Blood from cubital vein was centrifuged and serum was stored at -18ºC until laboratory analysis. After thawing, the concentrations of IL-1β and TNFα were determined in saliva and serum. High-sensitivity commercial ELISA tests were used for the analysis The values were read spectrophotometrically. Results showed that there is no significant correlation between the values of salivary and serum IL-1β, as well as salivary and serum TNFα. There was also no significant corretaion of salivary and serum cytokines with serum CRP values. Concentrations of IL-1β and TNFα in the saliva in HIV-positive patients with periodontitis are not related to the values of these parameters in the serum.

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# Chemical composition of sage (*Salvia officinalis* L.) essential oil and its anthelmintic properties against sheep gastrointestinal nematodes

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The development and spread of anthelmintic resistance to commercial drugs represent a major problem in veterinary medicine, which justifies the search for alternatives. Within that context, sage (*Salvia officinalis* L.) is a well-known medicinal plant that has been cultivated for centuries due to its healing properties. The aim of this study was to determine the chemical composition of the sage essential oil and to evaluate its anthelmintic properties against sheep gastrointestinal nematodes. Chemical characterization was done by GC-MS analyses, and the anthelmintic potential of tested samples was evaluated by *in vitro* egg hatch test in a concentration range of 0.0125-50 mg/mL. The most represented identified compounds were α-thujone (38.76%), camphor (19.75%) and 1,8-cineole (8.40%), camphene (5.36%) and α-humulene (4.15%) out of a total of 27 identified compounds. The ovicidal activity of sage essential oil, reflected in the inhibition of egg hatchability of the eggs of sheep gastrointestinal nematodes, varied from 15.0-89.0% depending on the used concentration, with a calculated $IC_{50}$ value of 0.53 mg/mL. The obtained results indicate that the compounds from sage essential oil posess high anthelmintic properties and should be tested in further *in vivo* studies. Sage essential oil could find application in veterinary medicine in the context of the development of new natural anthelmintic agents for the control of sheep gastrointestinal nematodes, which could have high significance from economic point of view.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Rescript no. 451-03-1183/2021-14).

# Expression of antimicrobial peptides during diapause in heat stress conditions in *Ostrinia nubilalis* (Hbn.)

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Many insect species overcome unfavorable 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, cell cycle arrest and expression of specific genes. In order to better understand the immune response occurring at the transcriptional level during diapause of the European corn borer *Ostrinia nubilalis*, a cold-hardy insect species, the expression of genes encoding selected antimicrobial peptides (AMPs) was measured. The chosen AMPs attacin (*att*) and gloverin (*glv*) can be used as markers of immune activity. Total RNA was isolated from whole-body homogenates of non-diapausing and diapausing 5$^{th}$ instar larvae acclimated to different temperature conditions (above-average laboratory and average winter field temperatures), in order to monitor immune response to high temperatures during diapause in this species. After cDNA synthesis, Q-PCR was performed and relative gene expression was determined using actin as the reference gene and the non-diapausing (ND) group as control. Relative expression of *att* and *glv* was higher in the ND group compared to diapausing larvae during the first months of diapause (from November to January), as well as higher in larvae acclimated to field conditions, compared to those acclimated to laboratory conditions where above-average temperatures prevail. Larvae acclimated to field temperatures showed a steady increase of both *att* and *glv* expression during the time course of diapause. The deeper hypometabolic state in the first months of diapause corresponds with general suppression of transcription, while the subsequent increase in expression of AMPs in the latter part of diapause can be correlated with higher ambient temperatures that favor pathogen development which provokes immune response in larvae. Diapausing larvae acclimated to warmer conditions showed a significant peak in expression during March, which can be explained by increased immune activity as a consequence of pathological processes in larvae that precede their subsequent high mortality in April. The results of this study are of great potential for future research of global warming effects on general wellbeing of cold adapted insect species.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant no. 142-451-2386/2022-01.

# Positive kidney effects of sweet basil extract in acetaminophen-induced hepatotoxicity in rats

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Biochemical parameters in the serum, indicators of kidney function, are important in diagnosing and assessing the risk of disease. The aqueous and ethanolic extract of *Ocimum basilicum* had a protective effect against kidney damage. The aim of this study was to evaluate the *in vivo* effects of basil extract pre-treatment on kidney function in acetaminophen-induced liver injury. Experiments were carried out for 7 days on 24 Wistar rats. Animals were randomly divided into 4 groups of 6 animals. Effects of basil extract on biochemical markers of kidney injury were determined in an *in vivo* model of acetaminophen-induced liver injury. To estimate the extent of kidney damage after acetaminophen administration, the following parameters were determined in serum: urea, creatinine and uric acid. Statistical processing of the obtained results was performed using the program IBM SPSS Statistics 20.0. A decrease in urea and creatinine concentrations were observed after a seven-day treatment with basil extract compared to saline, but without statistical significance. The level of uric acid was statistically significantly lower in the acetaminophen group treated with basil extract compared to the control group treated with the extract, $p<0.05$. Although the experiments that studied the antioxidant capacity of basil had different methodology, all of them showed a positive pharmacological effect of this plant. The present study demonstrated the significant protective potential of aqueous basil extract in a model of acetaminophen induced liver injury. Possitive effects were apparent through the decreasing serum activity of biochemical kidney parameters.

# Cloning and characterization of new raw starch digestion α-amylase from thermophilic *Anoxybacillus* sp.

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One of the most abundant natural polymers with multidimensional and multifaceted application is starch. Due to energy fuel sustainability concern, the world is focusing on renewable energy including energy from renewable biological materials like starch[1]. The importance of the enzymatic hydrolysis of granular starch below the temperature of gelatinization has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing[2]. A new α-amylase gene (*Amy35*) was cloned from newly isolated thermophilic *Anoxybacillus* sp. ST4 and expressed in *Escherichia coli*. The purified recombinant α-amylase had an wide pH optimum range from 4.5 to 8.5 and optimum temperature of 75°C. The enzyme retained 95% of its activity after 3h of incubation at 50 and 60°C. Hydrolysis rates of potato, horseradish and corn starches, at 1% concentration were 20, 70 and 65%, respectively, in a period of 16 h. Analysis of the enzyme properties proved its high efficacy for the digestion of diverse raw starches below gelatinization temperature and, therefore, its potential commercial value for use as an industrial enzyme.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract numbers: 451-03-68/2022-14/200168, 451-03-68/2022-14/300026, 451-03-68/2022-14/200288 and 451-03-68/2022-14/200042.

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# Barley β-glucans: a new method for extraction and purification

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Barley β-glucans are non-starchy polysaccharides that are composed of linear chains of β-D-(1→3) and β-D-(1→4) linked glucose residues. They comprise three (cellotriosyl) or four (cellotetraosyl) consecutive β-D-(1→4) bonded glucose units mutually connected *via* β-D-(1→3) glycosidic bonds. These polysaccharides can be found in the aleurone layer of the barley grain endosperm [1]. It has been shown that β-glucans reduce blood cholesterol and influence postprandial glucose levels which makes them health beneficial. Also, they have been used as stabilizing and thickening agents in the food indutry[1,2]. Due to the presence of β-D-(1→3) glycosidic bond, β-glucans are water soluble. The extraction procedure of these polysaccharides involves several steps that include the inactivation of endogenous enzymes found in the grain, extraction with water or alkali solutions, implementation of hydrolytic enzymes so that starch and protein removal is possible and precipitation of β-glucans from the purified solution with alcohol[3]. We have developed a new protocol that enables the extraction of β-glucans without the coextraction of starch and the implementation of hydrolytic enzymes for starch removal, which makes the process of purification shorter and less expensive. The extracted and purified glucans were further characterized by FTIR spectroscopy and microanalysis.

## Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract numbers: 451-03-68/2022-14/200168 and 451-03-68/2022-14/300026

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# Facile synthesis and potential application of trypsin nanoflowers

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Enzyme immobilization is an essential technology for commercializing biocatalysis, encompassing their stabilization and reusability for an efficient application. In recent years development of nanomaterials has paved the way to the design of nanobiocatalysts. Eversince their discovery in 2012, organic-inorganic protein hybrid nanoflowers have attracted attention of the scientific community as a novel class of nanobiocatalysts with versatile applications [1,2]. In this study a protocol was devised for preparation of trypsin nanoflowers (Tryp-NF) and their potential application was discussed. Trypsin from porcine pancreas, purchased from Sigma-Aldrich (T4799), was used in experimental work. Optimum conditions for nanoflower synthesis were established by varying metal component, concentration of trypsin, metal and phosphate buffered saline, reaction temperature and incubation time. Tryp-NF were efficient in 8 consecutive cycles of Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride hydrolysis and their proteolytic activity was confirmed in gelatin zymogram, indicating that upon immobilization native structure of trypsin is preserved. These preliminary results provide a good base for further development of nanoflower-based systems for efficient protein hydrolysis and biosensor development.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (Contract numbers: 451-03-68/2022-14/200288 and 451-03-68/2022-14/200168).

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# Sucrose-rich diet does not promote fibrosis in rat pancreas

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Pancreatic stellate cells (PSCs) belong to the group of resident cells in pancreatic tissue. PSCs can be found in two biological phenotypes: quiescent and activated[1]. When activated, PSCs contribute to fibrosis through the synthesis and secretion of extracellular matrix components in the conditions like chronic pancreatitis, pancreatic cancer and islet dysfunction. One of the typical features of PSC activation is an expression of α-smooth muscle actin (α-SMA)[1]. The aim of this study was to investigate weather a chronic sucrose-rich diet, as seen in humans of the western world, is a harmful stress condition which could activate PSCs. Male Wistar rats were divided into two groups (n=6) in this 3-week study: the sucrose (S) group had an access to 10% sucrose solution in drinking water, while the other group was intact control (C). Immunohistochemistry was used on pancreas tissue sections to demonstrate the presence of α-SMA (ab5694, Abcam, 1:400). Stereology was employed on AZAN stained sections to determine the volume density ($V_v$) of connective tissue. Statistical analysis did not reveal significantly different $V_v$ between groups (mean±sem): 0.0534±0.0050 $mm^0$ in C and 0.0565±0.0048 $mm^0$ in the S group. These results are in line with not observable α-SMA positivity, apart from that detected in smooth muscles of the vascular wall. Since activated PSCs are responsible for the promotion and maintenance of fibrosis, which can lead to β-cell failure[2] and diminished insulin secretion, and given the fact that 3-week sucrose-rich diet did not alter serum insulin concentration in rats[3], it can be concluded that in this experimental setting rat pancreas can maintain the homeostasis, both on the histological and biochemical levels.

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# Methimazole-induced hypothyroidism has no impact on pancreatic β-cell nuclear diameter

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Pancreatic β-cells produce and secrete insulin. In order to function properly pancreatic and duodenal homeobox 1 (PDX1) expression is required in β-cells since this transcription factor is essential for insulin gene transcription[1]. Given the fact that PDX1 is regulated by triiodothyronine[2], deficiency of this hormone usually results in impaired insulin biosynthesis and/or secretion. The goal of this study was to investigate the effect of systemic hypothyroidism on the size of β-cell nuclei, as a possible sign of altered transcription activity within the nuclei. In this 3-week study male Wistar rats were divided into two groups (n=6). Methimazole (M) group had free access to a 0.02% M solution in drinking water, while the control (C) group was given tap water. After routine processing for light microscopy, immunohistochemistry was employed on pancreas sections to label insulin-producing β-cells. Morphometry was performed using ImageJ software. In both groups, nuclear diameter is in the range of 6-10.5 μm, with the most frequent diameter of 7.5 μm (C 42% and M 47%). Data are reported as mean±SD and are as follows: 7.65±0.283 μm in C and 7.70±0.216 μm in M group. Student's t-test did not show statistical significance between groups regarding the size of β-cell nuclei. As previously reported, insulin immunostaining was stronger in hypothyroid animals[3]. At the same time, fewer functional PDX1-positive β-cells were detected in the islets of Langerhans[3]. These features are indicative of impaired insulin secretion and synthesis, respectively. However, present study shows that reduced insulin synthesis in M group was not accompanied by a decrease in nuclear size as a typical sign of diminished transcriptional activity. It is possible that changes in chromatin remodelling in this experimental setting are subtle, and not observable by light microscopy.

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# Sugar-mediated thermal stabilisation of C-phycocyanin from *Arthrospira platensis*

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C-phycocyanin (C-PC), the major protein of cyanobacteria *Arthrospira platensis*, is a blue pigment that primarily transfers energy during photosynthesis.It has diverse biotechnological applications since it can be used in nutraceutical, cosmetics, pharmaceutical industries, and biomedical research.Its intensive blue colour and strong antioxidant activity give C-PC significant potential to replace synthetic colourants in the food industry. However, thermal treatment of food has detrimental effects on C-PC colour due to sensitivity to higher temperatures; therefore,the application of this natural colourant in food and other products is limited.Hence, improving C-PC stability is the major challenge for successful application in food and beverages colouring. In the light of this,we aim to investigate the thermal stability of C-PC in the presence of selected sugars (glucose, fructose and sucrose), commonly used in the food industry. *Ex-situ*absorption spectrophotometryshowed that 18% solution (w/v) of glucose, sucrose and fructose at pH 7,upon incubation at 65°C, exhibit 91.4, 52.9 and 52.5% of colour preservation, respectively.*In situ* fluorescence measurements revealed that free C-PC has a melting point of 55.4°C, while the presence of glucose and sucrose increases the melting point of C-PC to 64.4 and 61.4°C, respectively. On the other hand, fructose does not significantly influence the C-PC melting point. These results show that the thermal stability ofthe C-PCsolution is substantially increased in the presence of sugars, while the type of sugar significantly determines the extent of the stabilisation effect. Overall, our study provides the strategy for enhancing the application potential of C-PC as natural food colourant, providing a food product with vivid blue colour and substantial antioxidant activities.

## Acknowledgements

This work was supported by ANSO, Project No. ANSO-CR-PP-2021-01, and Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 451-03-68/2022-14/200288.

# Is vitamin D deficiency related to pro-atherogenic lipid profile?

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The association between vitamin D deficiency and atherosclerotic cardiovascular disease (ASCVD) has been documented in a number of recent observational studies[1]. On the other hand, dyslipidemia is traditionally regarded as a major risk factor for ASCVD development[1]. Since vitamin D represents a liposoluble cholesterol-derived molecule with multiple biological roles, the associations between lipid metabolism alterations and vitamin D status are rather intriguing. The serum concentration of 25-hydroxy vitamin D (25(OH)D) is a reliable vitamin D status determinant while 25(OH)D metabolite, 24,25-dihydroxy vitamin D (24,25(OH)2D), is an attractive potential biomarker of vitamin D catabolism[1]. The aim of this study was to analyze the association of 25(OH)D3 and 24,25(OH)2D3 serum concentrations with pro-atherogenic lipid status parameters. The study group comprised 54 vitamin D-deficient and 35 vitamin D-sufficient healthy subjects. Vitamin D metabolites, routine lipid status parameters, and relative proportion of small, dense LDL particles (sdLDL) were analyzed.Subjects were clustered into quartiles according to 25(OH)D3 serum concentrations. We compared lipid status parameters between the 1st and the 4th quartile and found statistically significant differences in the relative abundance of sdLDL (P=0.047). Additonally, both 25(OH)D and 24,25(OH)2D were inversely associated with a relative proportion of sdLDL (B:-0.410; SE:0.154; P=0.010; and B:-2.041; SE:0.969; P=0.039, respectively). Our study proved an association between low vitamin D status and the presence of pro-atherogenic lipoproteins indicating that vitamin D deficiency could be implicated in ASCVD development.

## Acknowledgements

This work was supported by a grant of the Ministry of Education, Science and Technological Development, Republic of Serbia (grant no. 451-03-68/2022-14/200161).

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# Analysis and comparison of diagnostic performance between RT-qPCR kits for SARS-CoV-2 detection

**Ana Banko[1*], Marija Vukčević[2], Gordana Petrović[2], Danijela Miljanović[1], Ana Lončar[2], Dragana Despot[2], Andja Ćirković[3]**

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Coronavirus disease 2019 or COVID-19 is one of the greatest pandemic in modern history caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Due to huge demands to specific, fast and sensitive laboratory diagnosis, real-time reverse transcription polymerase chain reaction (RT-qPCR) is established as the "gold standard" diagnostic method for the diagnosis of SARS-CoV-2 infection. The aim of this study was to compare and analyze the detection performance of three commercially available SARS-CoV-2 nucleic acid detection kits: Sansure Biotech, GeneFinder™, and TaqPath™. The study was performed on 354 randomly selected nasopharyngeal swabs from hospitalized COVID-19 patients and all PCR reactions were performed using the same RNA isolates and same real-time PCR machine. The final result of the Sansure Biotech RT-qPCR method was significantly more often positive then the GeneFinder™ and TaqPath™ RT-qPCR methods ($p < 0.001$ and $p < 0.001$, respectively), and the GeneFinder™ RT-qPCR had significantly more positive final results than the TaqPath™ RT-qPCR method. Although the number of positive samples differed among RT-qPCR kits, Cohen's d Kappa coefficient confirmed the agreement between three RT-qPCR methods. Comparison of the assessment of SARS-CoV-2 positivity according to the Sansure Biotech and the GeneFinder™ produced $\kappa = 0.914$, which suggests a strong strength of agreement between the two methods. Also, a strong strength of agreement was shown between the Sansure Biotech and the TaqPath™ methods with $\kappa = 0.830$, $p < 0.001$, and the GeneFinder™ and TaqPath™ methods with $\kappa = 0.904$. The specificity was the highest for the Sansure Biotech compared to the GeneFinder™ and the lowest for the Sansure Biotech in compared to the TaqPath™. The likelihood ratio for a positive test (LR+) for the Sansure Biotech was higher than the GeneFinder™, and the GeneFinder™ had greater LR+ compared to the TaqPath™ method. Our finding show a high similarity in the analytical sensitivities for SARS-CoV-2 detection, which indicates that the diagnostic accuracy of the three assays is comparable and that all three RT-qPCR methods have high diagnostic accuracy for detecting SARS-CoV-2 positive patients with great inter-rater reliability.

# ROS-mediated proapoptotic antitumor effects of Ru(II) complex on pancreatic cancer cells

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Existing therapies for the treatment of pancreatic cancer are insufficiently effective and accompanied by a large number of side effects. Ruthenium complexes have shown promising antitumor properties in the previous studies[1,2]. Thus, in this investigation, anticancer effects of cis-dichlorobis (2,2'-bipyridyl-4,4'dicarboxylic acid)ruthenium(II) (Ru(II) complex) were evaluated using human pancreatic carcinoma cell lines MIA PaCa-2 and PANC-1 *in vitro*. Cell viability estimated with SRB assay showed significant antitumor activity of Ru(II) complex on MIA PaCa-2 (~55% of control) 48 and 72 h after treatment. On the other hand, PANC-1 cell viability was decreased only 72 h after treatment with the highest concentration of Ru(II) complex (~70% of control). Seven days after the treatment, analysis of cell survival using clonogenic assay showed a significant decrease in cell growth in both cell lines. Ru(II) complex also caused $G_1$ cell cycle arrest of ~13% in both cell lines. The highest percentage of apoptotic MIA PaCa-2 cells was obtained 48 h after treatment. In addition, the intracellular level of reactive oxygen species (ROS) was significantly increased, whereas cell migration was reduced in both cell lines. Summarized, Ru(II)complex demonstrates antitumor properties mediated by increased oxidative stress and also implies the antimetastatic potential, which deserves further study.

## Acknowledgements

This study was supported by the Ministry of Education, Sciences and Technology Development of the Republic of Serbia (Grant No: 451-03-68/2022-14/200017).

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# Chemical composition and antioxidant activity of flowers of *Hibiscus* genera

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Many human diseases, like neurodegenerative, cardiovascular, and diabetes, correlate with oxidative stress. For that reason, scientists are very interested in finding potent antioxidants. Plants contain numerous phenolic compounds that possess antioxidant activity, and thus they are interesting source of antioxidants. Hibiscus species, belonging to the genus *Hibiscus,* are cultivated worldwide. They possess antihipertensive, anti-inflammatory, antipyretic, hepatoprotective, anti-diarrhoeic, anti-tumor, antioxidant, antidiabetic, anticonvulsant and many other activities[1]. In this study two samples of *Hibiscus rosa-sinensis* flowers, obtained during 2018 and 2021 years from a private garden, and one sample of *Hibiscus* spp. flower obtained from healthy food shop, were examined. Chemical composition was examined by estimation of total phenolic, total flavonoid and total anthocyanins contents and by LC-MS/MS analysis, while antioxidant activity was examined by DPPH$^{\cdot}$ and FRAP assays[2-3]. Based on our results examined flowers of *Hibiscus* genera are a rich source of phenolics and have good antioxidant activity.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

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# Prevalence of somatic *BRCA1/2* gene methylation in patients with ovarian cancer

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Ovarian cancer has the highest mortality rate amongst all gynecological cancers with the incidence of 6.6 per 100000. Early diagnostics, screening and targeted therapy, are still the greatest need in the fight against this disease. The main focus of research for several years now is usage of poly (adenosine diohosfhate-ribose) polymerase (PARP) inhibitor therapy in treatment of patients with ovarian cancer, as well as new biomarkers that coud be used as diagnostic and prognostic markers. There is a growing need for testing methylation of *BRCA1/2* genes because positive result could provide an indication for treatment with PARP inhibitors, and also, the level of DNA methylation coresponds to the stage of the disease and tumor progression. We were investigating the prevalence of promoter methyilation of *BRCA1* and *BRCA2* genes in pathogenesis of sporadic ovarian cancer. Starting material for this study was FFPE tissue of 68 women with diagnosed advanced serous ovarian cancer, previously screened for *BRCA1/2* mutations by NGS using AmpliSeq for Illumina BRCA Panel and sequenced on a MiSeq platform. 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. PCR products were visualised on Agilent Tehnologies Bioanalyzer instrument using Agilent DNA 1000 Kit as well as 2% agarose gel. We determined the level of methylation in the promoter region of the *BRCA1* and *BRCA2* genes as well as the prevalence of somatic *BRCA1/2* gene mutation in this group of patients. The prevalence of *BRCA1* somatic mutation was higher than *BRCA2* mutation prevalence, 8.8% and 2.9% respectively. These results are comparable with those in literature. We calculated the percentage of patients who had a completely methylated, intermediately methylated, or unmethylated promoter region of the *BRCA1* and *BRCA2* genes based on the findings. Complete promoter methylation of *BRCA1* gene was detected in 7.4% of patients, and of *BRCA2* gene in 1.5% of patients. Using Fisher's exact test, we examined the association between the totaly methylated promoter of the *BRCA1* and *BRCA2* genes with the somatic mutational status of these genes. We found a significant association between *BRCA1* promoter methylation and somatic mutations in *BRCA1* and *BRCA2* genes. More precisely methylation of *BRCA1* promoter and *BRCA2* somatic mutations were mutually exclusive events.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technology of Serbia (451-03-9/2021-14/200043).

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# Desaturase enzymatic activities and adiposity indices among testicular germ-cell tumor survivors

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Due to exposure to platinum-based chemotherapeutic agents and potential hypogonadism testicular germ cell tumor survivors (TGCTS) may exert an unfavorable cardiovascular phenotype and an increased risk of metabolic disturbances. Erythrocyte membrane fatty acid (FA) composition, reflecting the intricate interplay between genetic makeup, metabolic factors, and long-term dietary pattern, serves as comprehensive lipid homeostasis and cardiometabolic risk-assessment biomarker. The activity of FA desaturases is a major determinant of qualitative and quantitative FA profile. A cross-sectional study was conducted among TGCTS attending post-curative follow-up care at the Clinic of Urology, University Clinical Center of Serbia with an aim to evaluate the relationship between the desaturase enzymatic activities and the cluster of adiposity indexes including waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), waist-to-hip-to-height ratio (WHHR), body mass index (BMI), and body shape index (ABSI). Extracted and esterified red blood cell FAs were analyzed by gas chromatography. Surrogate estimates of desaturase activities were computed as product-to-precursor ratios of involved FAs: stearoyl–CoA desaturase-18 (SCD18) [18:1n9/18:0], delta-6-desaturase (D6D) [20:3n6/18:2n6], and delta-5-desaturase (D5D) [ 20:4n6/20:3n6]. The study cohort comprised 52 patients (age $\bar{x}$ =36.44±8.48 years), and, based on BMI ($\bar{x}$=26.98±4.18), 59.62% men were overweight or obese (BMI≥25.0kg/m2). The estimated activities of SCD18 and D6D were directly associated with WHR, WHHR (r=0.440 and r=0.478; $p<0.05$) and ABSI (r=0.399; $p<0.01$). Conversely, correlation analysis revealed a statistically significant inverse relationship between the D5D and obesity indicators ABSI and WHtR (r=0.356 and r=0.421; $p<0.05$). Furthermore, overweight individuals had higher D6D, and decreased D5D activity estimates in comparison with normal-weight study participants ($p<0.01$). Altered desaturase activities may be involved in abdominal adiposity pathophysiology, but clinical significance in this patient population and the underlying mechanisms warrant further investigation.

# Pro-inflammatory effect of kiwifruit allergen on THP-1 derived macrophagesand its inhibition

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Act d 1 is a major kiwifruit allergen that is previously shown to disrupt tight junctions in the epithelial monolayer and thus increase monolayer permeability. As a consequence, the allergen is able to come in contact with the antigen-presenting cells in the mucosa. The aim of this study was to explore the effect of Act d 1 on antigen-presenting cells like the THP-1-derived macrophages. Treatment time and concentration of all molecules were optimized in order to obtain the most sensitive and best responding model system. Macrophages were differentiated from THP-1 monocytes and treated with Act d 1 and a positive control stimulus, LPS. After Act d 1 treatment expression of all pro-inflammatory cytokines was increased similarly to the LPS positive control group. Cell groups that were treated with the small selected molecules prior to the Act d 1 and LPS treatment showed significantly decreased expression of the pro-inflammatory cytokines. The anti-inflammatory effect of the small molecules proved to be concentration-dependent. Many inflammatory events start in the gastrointestinal tract as a result of the effect of different food components like food allergens. Small anti-inflammatory molecules that can also be found in different foods can enable better regulation of the inflammation process in many diseases and prevent unwanted side effects.

# Addendum

# Insights into the effect of microplastics on gastric digestion: Interaction of pepsin with polystyrene

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The contamination of several food types by microplastics has been confirmed by numerous studies worldwide[1]. Aside from the risk it presents to food safety, the risk to human health is another concern that emerges because of ingestion of microplastics. Despite the extensive documentation of microplastics in food, there is limited evidence regarding its influence on the digestion of food components, particularly proteins. In this study, we investigate the effect/s of 10 μm and 100 μm polystyrene (PS) microplastics on pepsin, the main protease in human gastric digestion, by analyzing its adsorption behavior at pH 7 and pH 3, and specific enzyme activity after exposure to different quantities of PS- low (142 particles), moderate (1420 particles), and high (14200 particles). Based on the Langmuir adsorption model, the adsorption constant ($K$) of pepsin is higher at pH 3 ($K > 0.99$) compared to pH 7($K < 0.70$), suggesting preferential adsorption at acidic pH. The maximum amount of adsorbed pepsin ($qmax$) was greater in 10 μm PS because of its higher surface. Exposure to low, moderate, and high quantities of 100 μm PS at pH 3 did not significantly change the specific activity of pepsin. On the other hand, high level of 10 μm PS notably reduced pepsin activity from 2957 ± 310 U/mg to 1674 ± 270 U/mg. These findings suggest that gastric digestion could potentially be affected by 10 μm PS microplastics due to the adsorption of pepsin and/or reduction of enzyme activity.

## Acknowledgements

Supported by Ghent University Global Campus; Special Research Fund (BOF) of Ghent University (01N01718) and IMPTOX EU's Horizon 2020 research and innovation program (965173).

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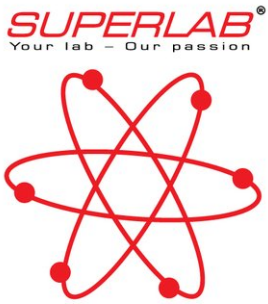
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**SERBIAN Biochemical Society. Scientific meeting of an international character (11 ; 2022 ; Novi Sad)**
"Amazing Biochemistry" : [proceedings] / Serbian Biochemical Society, Eleventh Conference, Scientific meeting of an international character, September 22nd and 23rd, 2022, Novi Sad, Serbia ; [editor Ivan Spasojević]. - Belgrade : Faculty of Chemistry : Serbian Biochemical Society, 2022 (Belgrade : Colorgrafx). - 165 str. ; 23 cm

Tiraž 150. - Str. 19: Foreword / Ivan Spasojević. - Bibliografija uz većinu radova.

ISBN 978-86-7220-124-6 (FOC)

a) Биохемија -- Апстракти

COBISS.SR-ID 73285385