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83rd INTERNATIONAL SCIENTIFIC CONFERENCE ON MEDICINE AND HEALTH SCIENCES OF THE UNIVERSITY OF LATVIA: BASIC MEDICAL SCIENCE AND PHARMACY

On 25 April 2025, the House of Science of the University of Latvia in Rīga became a hub of dynamic intellectual exchange, hosting the International Scientific Conference on Medicine organised within the frame of the 83rd International Scientific Conference of the University of Latvia. For the first time, the conference's abstract collection is being published in *Proceedings of the Latvian Academy of Sciences, Section B* — a significant milestone that reflects both the increasing scientific contributions of this annual event and its expanding international reach.

The annual conference in medicine has become an important tradition of the University of Latvia bringing together achievements in medicine, pharmacy, and other related fields. It allows the participants to get acquainted with the research performed by their colleagues. Either students or experienced researchers are welcome to present the results of their recent achievements. During the recent decade, the conference has become truly international being held in English and welcoming international participation. Networking within the meeting is expected to facilitate new research plans and foster collaboration.

Also, this year conference provided a fertile ground for the exchange of findings, and inspiration of new approaches. Researchers and clinicians presented work ranging from molecular insights in pharmacology and immunology to innovative diagnostic models in cardiology, neurology, and oncology. Other contributions addressed pressing issues in public and mental health, explored the social and psychological dimensions of healthcare, and demonstrated the integration of digital technologies into clinical decision-making. This breadth of topics exemplifies the inherently multidisciplinary nature of modern medical science, and the conference served as a reflection to this complexity — bringing together perspectives from basic sciences, clinical practice, public health, pharmacy, and beyond.

Of particular note was the strong representation of emerging investigators, alongside established scholars, many of whom engaged in cross-disciplinary dialogue that promises to evolve into long-term collaborations. International participation added further depth, with scientists and students from across Europe and Central Asia sharing insights that are regionally grounded yet globally relevant. The research highlighted a common aim of enhancing health by collaborating and conducting thorough studies, whether through the use of data to predict chronic diseases, exploring maternal health, or creating new methods to deliver medications.

By presenting this collection of abstracts, we aim not only to preserve the scientific contributions of this year's conference but also to invite the broader scientific community to engage with the questions and challenges explored herein. The research included in these proceedings affirms that medicine today is not confined to the laboratory or the clinic – it is a collaborative endeavour that transcends disciplines, borders, and generations. We anticipate that the collaborations initiated in Rīga will continue to develop and inspire future breakthroughs in the years to come.

The section "Basic Medical Science and Pharmacy" presents a collection of abstracts reflecting studies covering diverse but interrelated topics and present innovative research methods and findings from pharmacology, immunology, biochemistry, and pharmaceutical technology.

The abstracts, among other topics, examine in depth the antioxidant properties of natural compounds, emphasising their potential neuroprotective effects and influence on oxidative stress markers in mice. These studies contribute to understanding the therapeutic applications of plant-based extracts in mitigating damage caused by environmental toxins or oxidative stress.

Another focus is the immunological analysis of alcohol-induced liver injury. Researchers investigate zone-specific macrophage activation and inflammation, providing insights into the progression of liver disease and its underlying immune mechanisms. These findings underline the importance of targeted approaches to manage chronic alcohol-related liver conditions.

The publication also includes work in genomic studies, such as HLA allele frequency analysis in the Latvian population. This research not only aids in improving transplantation outcomes, but also enhances our understanding of genetic predispositions to diseases within specific populations.

Lastly, advancements in pharmaceutical manufacturing are showcased through comparative analyses between traditional and 3D-printed tablets. This innovative approach highlights the evolving landscape of drug delivery systems, particularly for paediatric and geriatric patients.

Together, these abstracts represent a dynamic intersection of basic medical science and pharmacy, offering valuable contributions to scientific knowledge and practical applications in healthcare. The studies underscore the importance of interdisciplinary collaboration in addressing global health challenges while paving the way for future research endeavours.

Mārcis Leja

Ilmārs Stonāns

Basic Medical Science

EXTRACTION AND PURIFICATION OF OMEGA-3 FATTY ACIDS FROM MICROALGAE BIOMASS

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Background. Microalgae have emerged as a sustainable and efficient source of bioactive compounds, including long-chain polyunsaturated omega-3 fatty acids, an alternative source of fatty acids obtained from fish. As the global demand for omega-3 fatty acids continues to rise, microalgae offer an environmentally friendly, scalable, and contaminant-free solution.

Aim. This study focuses on microalgae cultivation in closed photobioreactor systems (PBR's), followed by drying and the supercritical CO_2 extraction ($scCO_2$). The goal is to develop high concentration extracts rich in eicosapentaenoic acid (EPA, C20:5n3), utilising a solvent-free and sustainable extraction process.

Methods. *Phaeodactylum tricornutum* and Nannochloropsis sp. were cultivated in a bubble-column photobioreactor PBR (3 × 50 l) with a daily harvest of 25%. Biomass was then separated from the growth medium and concentrated with a centrifuge and FeCl3. Afterwards, the biomass

was freeze-dried. Supercritical CO_2 extraction was employed to isolate fatty acid rich extracts from the microalgae biomass. In addition, the raw extract was further purified using ethanol (1:20 ratio), and the mixture was filtered to remove impurities. After filtration, the ethanol was evaporated, yielding a high-concentration extract. The final extract was analysed for fatty acid profile using gas chromatography (GC).

Results. The cultivation of microalgae yielded a steady biomass production with consistent compound profiles. Freeze-drying reduced the moisture content to 1–3%, making the biomass suitable for extraction. Supercritical CO^2 extraction and further treatment efficiently increased EPA concentration. GC analysis confirmed that the final extract had a noticeable EPA concentration.

Table 1 demonstrates an increase in EPA concentrations after extraction. The total EPA was 443.0 mg/g, which further increased to 701.9 mg/g in the purified extract. The extra purification process with ethanol and filtration enhanced the quality of the extract.

Conclusion. This study demonstrates the ability to obtain a high concentration of polyunsaturated fatty acids from different microalgae species using supercritical CO2 extraction and an extra purification step. Microalgae derived polyunsaturated fatty acids can be used as a substitute for fish oil from an environmentally friendly and sustainable source.

Table 1. Concentration of EPA before and after extraction (*Nannochloropsis* sp.)

Composition	Biomass (before extraction)	Extract (scCO2)	Purified extract
Eicosapentaenoic acid (EPA) in glycerolipids (mg/g)	173.1	271.0	396.3
Eicosapentaenoic acid (EPA) in free fatty acids (mg/g)	89.1	172.0	306.6
TOTAL EPA (mg/g)	262.2	443.0	701.9

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Contracting agency. The study is conducted by JSC SISTĒMU INOVĀCIJAS with support from the European Recovery Funding within the framework of the Smart Material Competence Centre.

APOPTOSIS IN KIDNEYS EXPOSED TO T-2 MYCOTOXIN: IMMUNOLOCALISATION OF P53 AND P21

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Background. T-2 toxin, a carcinogenic mycotoxin which belongs to the group of Fusarium mycotoxins in trichothecene family, is amongst most toxic mycotoxins affecting several organs, including kidneys. It is known that T-2 mycotoxin causes oxidative stress, leading to cellular injury, apoptosis, and disrupted cell cycle regulation. Although tumour suppressor protein p53 and cyclin-dependent kinase inhibitor p21 play pivotal roles in these processes, up to now little is known about immunolocalisation of p53 and p21 in kidneys after application of T-2 mycotoxin.

Aim. The study aimed to investigate immunolocalisation of p53 and p21 in kidneys exposed to T-2 mycotoxin.

Methods. Kidney material was collected from seven 7-day-old female Ross broilers (*Gallus gallus domesticus*) with T-2 mycotoxicosis. For chicken, starting from the fourth day after hatching, application of T-2 mycotoxin in dose of 0.250 mg/day/bird was compulsive for three consecutive days. Sacrifice of chicks was 24 hours after last toxin application, thereafter specimen were fixed with 10% formalin, embedded into paraffin, slices 7 μm thick were cut followed by immunohistochemical staining with polyclonal primary antibodies Rabbit anti-p53 and Rabbit anti-p21, according to the manufacturers' quidelines (IHC kit, Abcam, UK). Biotinylated secondary antibody contained in IHC kit was used for detection, using DAB as chromogen. Negative controls contained antibody diluent (Dako, S0809) instead of primary antibodies. Nuclei were counterstained with Harris Hematoxylin. Photos of slides were taken using a PreciPoint M8 digital microscope (PreciPoint, Munich, Germany, 2024).

Results. Strong expression of p21 and p53 was noticed in epithelial cells of renal proximal tubules. Staining of distal tubules and glomerular cells was noted to be weak or absent. Staining for p21 and p53 was noticed to immunolocalise particularily in nuclei of tubular epithelial cells. Additionally, mild cytoplasmic staining of p21 was observed in cells of the renal proximal tubules.

Conclusion. The study revealed nuclear localisation of p53 and p21 in renal tubular epithelial cells, particularly in proximal convoluted tubules. Nuclear accumulation of p53 and strong expression of p21 after T-2 mycotoxin application suggest that these proteins are actively involved in regulating cellular response to oxidative stress and DNA damage.

Mild cytoplasmic staining in parallel to the strong nuclear staining of p21 may show potential role in apoptotic signalling under conditions of T-2 mycotoxicosiemphasised texts.

Acknowledgements. Authors wish to thank Mrs. Mare Tamm for laboratory assistance.

THE ROLE OF CASE MANAGERS IN LITHUANIAN PRIMARY HEALTHCARE: ASSOCIATIONS BETWEEN HEALTHCARE VISITS AND EMOTIONAL WELL-BEING OF MULTIMORBID PATIENTS

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Background. The evolution of primary healthcare teams in Lithuania has been significant through the last ten years. Recently, the case managers' specialty was included in the family physicians' team. Their aim is to identify the patient's need for complex services, to organise and manage such services, with the recommendations given by other team members and medical specialists and specific needs of the patient and (or) the patient's family members.

Aim. The aim of this study was to assess the associations of a case manager's role in primary healthcare focusing on the associations between healthcare visits frequency and emotional well-being of the patients with multimorbidity.

Methods. The study was conducted in seven Lithuanian primary health care centers (five urban, two rural) within the framework of the TELELISPA project "Improved Healthcare Quality for Patients with Multimorbidity in Lithuania" (project number 08.4.2-ESFA-K-616-01-0003). The study used a case management approach with the support of a multidisciplinary team. Participants were aged 40 to 85 years and had two or more long-term health conditions. General Anxiety Disorder-7 (GAD) scale and Patient Health Questionnaire-9 (PHQ-9) were used to estimate the emotional wellbeing of patients over the 15-month study period. Chi square test for homogeneity implemented in the statistical software package SPSS for Windows 29.0 was used for data analysis. p values < 0.05 indicate statistically significant differences.

Results. There were two groups of patients in the study: a case manager-supervised study group: n = 388 (51.3%) and a control group: n = 369 (48.7%). Both groups were homogeneous with respect to gender, age, living place and number of diseases. Statistically significantly more patients from study group compared to control group had visits to medical specialist (86.6% vs 76.1%, $p < 0.001$), the study group had more than two consultations with nurses or case managers (84.9% vs 35.6%, $p < 0.001$), and indicated that their depression symptoms (45.7% vs 31.7%, $p < 0.001$) and anxiety level (47.8% vs 29.0%, $p < 0.001$) decreased over the study period.

Conclusion. Research data shows that the inclusion of a case manager in the family physician team improved the quality of patient care and the patient's emotional well-being.

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ASSESSMENT OF SUITABILITY OF NATURAL HARD TISSUE EDUCATIONAL PREPARATIONS BY ADVANCED ESTIMATES OF THEIR OPTICAL PROPERTIES

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Background. Educational simulation using synthetic organ phantoms becomes very popular in training of wide range medical professionals. However, in some cases only specially processed natural hard tissue preparations could help to gain particular valuable skills for future practices. One of major features of such preparations is translucency enabling comprehensive control of the educational process, e.g., instrument manipulations.

Aim. The aim of this study was to elaborate a method for evaluation of optical properties of the preparations enabling assessment of its suitability and optimisation of the preparation process.

Methods. This particular study was designed to compare optical properties of natural teeth preparations made using two chemical methods – a) the traditional, using methyl ester of salicylic acid, giving satisfactory results, but toxic, vs b) using benzyl benzoate and benzyl alcohol, from the first impression giving also satisfactory results, but being much less toxic. Translucent light images of 32 teeth were captured using a collimated white light source and a digital camera (C-P8, Optika, Italy), with the teeth positioned on a dental glass slab. A small piece of prosthodontic impression material (A-silicone Elite Transparent, Zhermack, Italy) was placed nearby to normalise exposure and white balance across all images. The optical properties of tooth translucency were analysed using custom-made image processing algorithms in the MatLab environment. The algorithm segmented the apical part of each tooth using superpixel technique. Optical properties of segmented apical part of each tooth were estimated in HSV colour scheme.

Results. We did not find any statistical difference in optical properties of apical parts prepared by both methods. Statistical analysis (Mann–Whitney U test, $p > 0.2$) showed no significant difference in hue, saturation, nor in value between the two groups.

Conclusions. The optical properties of the translucent teeth prepared by both methods were similar, so we advise to use less toxic method using benzyl benzoate and benzyl alcohol. The principle of evaluation of optical properties could be used also for assessment of other tissues and other processing methods.

DETERMINATION OF ANTIOXIDANT ACTIVITY OF *PRUNUS PADUS* L. FLOWERS

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Background. *Prunus padus* L., also known as bird cherry, flowers are rich in minerals, vitamins and phenolic compounds [1]. It has been confirmed that consumption of plants which are rich in polyphenols can have a health-promoting effect [2]. And studies confirm favourable effects of *P. padus* for prevention of cardiovascular diseases, as well as antioxidant, anti-inflammatory, antimicrobial, antidiabetic effects [3].

Aim. The objective of this study was to determine antioxidant activity in flowers of *Prunus padus* L. from various regions of Lithuania.

Methods. *P. padus* flowers were collected during mass flowering in late spring of 2024. Flowers were air dried at room temperature and grinded to fine powder. Extracts were prepared with 60% methanol as extractant in an ultrasonic bath. The antioxidative potential of flowers was determined using spectrophotometric ABTS (2,2'azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing antioxidant power) methods.

Results. Average antiradical activity in samples of *P. padus* flowers from various locations in Lithuania was 145.27 ± 7.05 μmol/g based on ABTS method. Antioxidant activity in extracts varies from 68.80 ± 38.98 μmol/g (Babtai, Kaunas district), 81.16 ± 16.13 μmol/g (Kaunas) to 212.82 ± 17.06 μmol/g (Kyburiai, Utena district), 221.32 ± 17.80 μmol/g (Liaušiai, Ukmergė district).

Based on FRAP method, average reducing activity in samples of *P. padus* flowers was 34.39 ± 1.32 μmol/g. Antioxi-

dant activity in extracts varies from 17.67 ± 0.85 μmol/g (Kaunas), 23.56 ± 3,61 μmol/g (Babtai, Kaunas district) to 50.62 ± 4.15 μmol/g (Kemetiškiai, Molėtai district), 52.58 ± 18.68 μmol/g (Padumblė, Molėtai district).

Correlation between antiradical activity results from two different methods is statistically significant.

Conclusion. Results confirm that *Prunus padus* L. flowers from various locations in Lithuania possess antioxidant activity. Environmental conditions, age of plant and other conditions have a significant influence on the antioxidant effect of *P. padus* flower extracts. Therefore, in order to predict biological effects, analysis of the produced extracts of raw plant materials should be performed.

References

1. Telichowska, A., Kobus-Cisowska, J., Stuper-Szablewska, K., Ligaj, M., Tichoniuk, M., Szymanowska, D., Szulc, P. Exploring antimicrobial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L. *International Journal of Food Properties*, 2020, **23** (1), 2097–2109.
2. Telichowska, A, Kobus-Cisowska, J, Ligaj, M, Stuper-Szablewska, K., Szymanowska-Powałowska, D., Mariusz, T., Szulc, P. Polyphenol content and antioxidant activities of *Prunus padus* L. and *Prunus serotina* L. leaves:Electrochemical and spectrophotometric approach and their antimicrobial properties. *Open Chemistry*, 2020, **18**, 1125–1135. 10.1515/chem-2020-0121.
3. Telichowska, A., Kobus-Cisowska, J., Szulc, P. Phytopharmacological possibilities of bird cherry *Prunus padus* L. and *Prunus serotina* L. species and their bioactive phytochemicals. *Nutrients*, 2020, **12** (7), 1966.

IMPACT OF VDR GENE POLYMORPHISMS ON MULTIPLE SCLEROSIS THERAPY OUTCOMES IN THE LATVIAN COHORT

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Background. The treatment for multiple sclerosis (MS) generally focuses on expediting recovery from attacks, diminishing relapses, decelerating disease development, and alleviating MS symptoms. Numerous studies have indicated an association between the risk of developing multiple sclerosis and serum vitamin D levels. Vitamin D is a potent immunomodulator that can suppress inflammatory signals and influence the immune system by binding to the nuclear vitamin D receptor (VDR). Genetic variations in the VDR gene are potential molecular markers of MS in different populations.

Aim. To identify the possible association of four significant genetic variations in the VDR gene with the outcome of multiple sclerosis therapy in a Latvian disease cohort.

Methods. In the Latvian MS cohort, 230 of 296 patients received 342 distinct pharmacological treatments. Data was gathered regarding alterations in EDSS (Expanded Disability Status Scale) scores and NEDA (No evidence of disease activity) within two years following the initiation of therapy. Four SNPs of the VDR gene were genotyped by the restriction enzyme site polymorphism method: rs2228570, rs1544410, rs7975232, and rs731236.

Results. Statistical analysis revealed a significant association with EDSS change after starting therapy in the first and second year with VDR gene polymorphism genotypes. Homozygous genotypes of common alleles CC (rs2228570) and TT (rs731236), as well as the heterozygous form of polymorphism rs1544410 in GA are a risk form in the case of Glatiramer acetate therapy, or an association with a higher increase in EDSS than in other forms of therapy has been found. At the same time, patients with the heterozygous form of polymorphism rs1544410 in GA have a worse response to IFN therapy in the second year of therapy after EDSS change.

Conclusions. We present evidence that the VDR gene polymorphisms may contribute to the therapy of multiple sclerosis in the Latvian disease cohort.

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ASSOCIATION OF PROTEASOME 20S SUBUNIT BETA GENES' POLYMORPHISMS WITH MULTIPLE SCLEROSIS TREATMENT RESULTS

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Background. Multicatalytic protease complexes, or proteasomes, are one of the protein degradation pathways that occurs through an ATP/ubiquitin-dependent process, or the ubiquitin-proteasome system. Dysregulation or modulation of the proteasome system can affect various changes in the body, including altered responses to drug therapy. In most cells due to various conditions, for example, induction with interferon, in oxidative stress, in 20S proteasome occurs replacement of three catalytic subunits: PSMB5, PSMB6 and PSMB1, by other PSMB8 (LMP7), PSMB9 (LMP2) and PSMB10 (MECL-1), respectively, and forms immunoproteasome. Immune system cells, especially antigen-presenting cells, express a higher basal level of immunoproteasomes. The proteolytic activities of proteasomes are reduced in brain tissue of multiple sclerosis (MS) patients.

Aim. To identify the possible association of SNP of PSMB8 (LMP7) and PSMB9 (LMP2) with the outcome of MS therapy in a Latvian disease cohort.

Methods. In the Latvian MS cohort, 230 of 296 patients received 342 distinct pharmacological treatments. Data was gathered regarding alterations in EDSS (Expanded Disability Status Scale) scores and NEDA (No evidence of disease activity) within two years following the initiation of therapy. Information on proteasome gene polymorphisms was obtained from previous studies.

Results. Statistical analysis revealed a significant association with EDSS change after starting therapy in the first year for all analysed SNPs in two proteasomal genes. Rear allele genotypes CT+TT of PSMB8 rs2071543 ($P = 2.83 \times 10^{-2}$), common allele homozygote CC of PSMB8 rs9357155 ($P = 2.10 \times 10^{-2}$) and common allele homozygote GG of PSMB9 rs17587 ($P = 3.65 \times 10^{-2}$) are risk forms for GA therapy in the 1st year of therapy compared to other therapies. At the same time, in the 2nd year of therapy, with GA medicaments genotype of rear alleles (AA + GA) of PSMB9 rs17587 is risk form ($P = 2.80 \times 10^{-2}$) compared to common allele homozygote genotype of SNP.

Conclusion. We provide evidence that PSMB8 and PSMB9 gene SNPs may contribute to MS patients' response to therapy; further studies of the polymorphism are warranted.

Acknowledgments. The study was funded by UL project No.1.1.1.2/VIAA/4/20/718, "The role of vitamin D and its receptor gene polymorphisms in the modulation of intestinal inflammation in patients with relapsing and progressive forms of multiple sclerosis", and European Regional Development Fund project 1.1.1.1/16/A/016.

MICROBIOLOGICAL FINDINGS FOR SMARTWATCH WEARERS

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Background. Currently, among various smart electronic devices in the world, the smartwatch has become the most popular smart device according to 2023 statistical data [1]. Human skin is in close contact with the smartwatch, so it is important to find out what microorganisms are found on them and whether the microorganisms found are capable of posing a threat to human health. Studies have shown that when cultures were taken from smartwatches, not only microorganisms of normal skin microflora were found on them, but also pathogens. Multi-drug resistant microorganisms, which are dangerous pathogens in healthcare facilities, were also found on smartwatches [2].

Aim. The aim of the current study was to determine the microbiological findings of smartwatch wearers.

Methods. The study involved seven individuals, and a total of 14 swabs were obtained, swabs were taken from the participant's smartwatch and skin where the smartwatch was worn. The material was inoculated on blood Miller-Hinton agar. Culture isolates were stained using the Gram stain method and microscopically examined, and identification was performed using VITEK MS and MALDI-TOF analysers.

Results. In total, ten different microorganism species were isolated from the 14 tested samples. Some of these were found in the normal skin microflora, while others are not part of the normal skin microflora and were acquired through contact with the external environment. The least frequently encountered microorganisms in the samples were *Acinetobacter ursingii*, *Stenotrophomonas maltophilia*, and

Acinetobacter ursingii, *Stenotrophomonas maltophilia*, and *Escherichia coli*. Moderately frequent microorganisms were *Acinetobacter lwofii, Staphylococcus capitis, Pseudomonas oryzihabitans*, and *Moraxella osloensis.* Meanwhile, the most frequently encountered microorganisms were *Staphylococcus epidermidis, Staphylococcus hominis,* and *Micrococcus luteus.*

Conclusion. The skin of the hands is mainly colonised by microorganisms that are part of the normal skin microflora, but on smartwatches there are also microorganisms that are not found on the hands of the smartwatch wearer. More microorganisms accumulate on smartwatches than on hands; hence opportunistic pathogenic microorganisms are washed off the hands, but they accumulate on smartwatches. Based on the results obtained, smartwatch wearers should pay more attention not only to hand disinfection itself, but also pay special attention to disinfecting smartwatches to reduce the risk of potential spread of infectious agents.

Acknowledgements. The study was conducted in accordance with the Ethics Committee.

References

1. Laricchia, F. Smartwatches — statistics & facts. 2024. https://www.statista.com/topics/4762/smartwatches/#topicOverview.
2. Naveen, K., *et al*. Bacterial Contamination of the Wristwatches Among Clinical and Preclinical Undergraduate Medical Students. *Infect. Dis. Clin. Pract*., 2022, **31** (1), e1168. DOI: 10.1097/IPC.0000000000001168.

OVERVIEW OF GENETIC CAUSES OF PATIENTS WITH CONGENITAL ICHTHYOSIS: SINGLE CENTRE EXPERIENCE

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Background. Congenital ichthyosis is a heterogeneous group of keratinisation disorders characterised primarily by abnormal skin scaling over the whole body. These disorders are limited to skin, but patients can have systemic symptoms. There are variable inheritance patterns and several genetic causes.

Aim. The aim of the current study was to analyse genetically tested patients, and their genetic causes.

Methods. A total of 15 patients having symptoms of ichthyosis were consulted by dermatologist and clinical geneticist in the Hospital of Lithuanian University of Health Sciences, Kauno Klinikos, in the period from 2020 to 2024. Demographic data, genotypes, phenotypes from electronical medical records were analysed. Genetic testing was performed using whole exome sequencing with virtual genetic skin disorders panel. Large gene deletions confirmed with molecular karyotyping.

Results. Genetic diagnosis of ichthyosis was confirmed to all 15 patients, 8 (53.33%) male patients. Variants in STS, ALOX12B, ABCA12, KRT10, KRT2, ZMPSTE24 genes were detected. Seven (43.75%) patients had a variant in STS gene, inherited as X linked recessive manner. Six patients had hemizygous deletion Xp22.31, and one patient had a frame shift variant. Clinically dry skin with mild scaling was noted. Two patients also had mild erythema. Two (18.75%) patients had a variant in ALOX12B gene. Two patients had compound heterozygous variant. One of them had hypopigmentation and hyperkeratosis, and another had erythroderma, painful palmoplantar keratoderma with fissures. The 3rd patient with two potentially pathogenic variants was born as a collodion baby. One patient had a compound heterozygous variant in ABCA12 gene. The phenotype was found of scaling skin with moderate dandruffs, poor nail growth, pruritus. One patient had a heterozygous variant in KRT10 gene. It lead to autosomal dominant congenital bullous ichthyosiform erythroderma which presented with hyperkeratotic lichenification, erosions with yellow crusts on skin. One patient had a heterozygous variant in KRT2 gene that caused superficial epidermolytic ichthyosis. Hypertrichosis, hyperkeratotic brown-yellow like skin was noted. One (12.50%) patient had a homozygous variant in ZMPSTE24 gene. It caused restrictive dermopathy (ORPHA:1662). Limb contractures, pulmonary hypoplasia and skin cracks were noted. Both patients died during the first weeks of life.

Conclusion. Causes of congenital ichthyoses was genetically heterogenous, predominantly with STS gene deletion. Hence, genetic testing is crucial for prognosis and classification, as ichthyosis manifests differently due to affected gene.

Acknowledgements. There were no conflicts of interest.

DETECTION OF MULTIDRUG RESISTANCE AND ENCODING GENES IN MICROORGANISMS ISOLATED FROM SURGICAL MATERIALS

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Background. Bacterial resistance to antimicrobial agents is increasing worldwide and in Latvia every year. Multidrug resistance (MDR) complicates the possibility of effective treatment and can lead to severe complications. Detection of bacterial resistance and encoding genes in microorganisms helps understand the spread of resistance and develop targeted strategies for infection prevention, diagnosis, and treatment, thereby improving patient safety and reducing the burden of resistance on the healthcare system.

Aim. To determine the antimicrobial resistance of microorganisms isolated from surgical materials and detect the genes responsible for this resistance.

Methods. It is a retrospective study that includes bacterial isolates from clinical materials of Traumatology and Orthopaedics Hospital patients. Data were obtained for the year 2024. Antibiotic susceptibility testing of isolates was performed by Bauer-KirbyTM disc diffusion method and ETM-test. Multiple Antibiotic Resistance index (MAR) was calculated. Resistance genes were determined using multiplex PCR (BioFire system).

Data were processed using MS Excel 2024 and RStudio, v.2024.12.0.

Results. The study identified 548 isolates. Gram-positive bacteria (GPB) were detected in 67.7% (n = 371) of samples, while gram-negative bacteria (GNB) accounted for 32.3% (n = 177). Wound swabs were the most common source: 45.3% (n = 168) for GPB and 52.5% (n = 93) for GNB. The highest MDR prevalence was observed in CoNS (48.8%, n = 42), *Klebsiella pneumoniae* (62.5%, n = 15), *Proteus mirabilis* (60.0%, n = 18), *Acinetobacter baumannii* (44.8%, n = 13), *Escherichia coli* (35.7%, n = 5) and *Pseudomonas aeruginosa* (25.0%, n = 8). In GPB, the highest resistance was to tetracycline (45.0%), ampicillin (28.1%), erythromycin (27.3%), cefoxitin (20.9%), and clindamycin (20.1%). In GNB isolates, the highest resistance was to cefazolin (85.3%), ampicillin (76.6%), amoxicillin-clavulanic acid (60.0%). Using Pearson's Chi-square test a statistically significant correlation was found between the isolated microorganism and its antibacterial susceptibility results ($p < 0.05$). The MAR index was calculated for each multiresistant isolate. The average value of the MAR index was 0.6, with a range from 0.27 to 1.0.

Conclusion. The study identified significant MDR in both GPB and GNB isolates from surgical materials. Coagulase-negative staphylococci showed the highest rate of MDR among gram-positive bacteria. In GNB, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Acinetobacter baumannii* showed the highest levels of resistance. Resistance genes were found in all MDR bacterial isolates. MAR index value greater than 0.2 indicates a high-risk source of contamination where antibiotics are frequently used.

Acknowledgements. The authors declare the absence of a conflict of interest.

IMPACT OF GC GENE POLYMORPHISMS (RS7041 AND RS4588) ON MULTIPLE SCLEROSIS TREATMENT OUTCOMES IN THE LATVIAN COHORT

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Background. The primary goal of multiple sclerosis (MS) treatment is to accelerate recovery from attacks, reduce relapse frequency, slow disease progression, and manage symptoms. Numerous studies have established a link between MS risk and both serum vitamin D levels and its metabolic pathways. The vitamin D binding protein (VDBP) gene, also known as the GC gene, plays a key role in vitamin D transport, bioavailability, and activation, which are critical for immune regulation. Genetic variations in this gene, particularly SNPs rs7041 and rs4588, have been associated with vitamin D deficiency and are considered potential molecular markers for MS susceptibility and treatment response.

Aim. To identify the potential association of GC gene genetic variations with multiple sclerosis therapy results in the Latvian disease cohort.

Methods. In the Latvian population MS group, 230 out of 296 patients were prescribed 342 different drug therapies.

Information was collected on changes in EDSS (Expanded Disability Status Scale) scores and NEDA (No evidence of disease activity) over two years after the start of treatment — the restriction enzyme site polymorphism method genotyped GC (rs7041 and rs4588).

Results. Statistical analysis revealed a significant association for NEDA positivity after initiating therapy with GC gene rs7041 and rs4588, respectively, in the 1st or 2nd year of therapy. The homozygous form of the common allele of the polymorphism rs7041 GG is a risk factor for a negative response to Mitoxantrone as an active site therapy or a positive NEDA score in the 1st year. In contrast, the homozygous form of the common allele of the polymorphism rs4588 CC is a risk factor for treatment response in the 2nd year. No statistically significant association was found for GC gene polymorphisms with EDSS changes after the start of therapy.

Conclusions. We present evidence that the GC (rs7041 and rs4588) may contribute to multiple sclerosis therapy in the Latvian disease cohort.

Acknowledgments. The study was funded by UL project No.1.1.1.2/VIAA/4/20/718, "The role of vitamin D and its receptor gene polymorphisms in the modulation of intestinal inflammation in patients with relapsing and progressive forms of multiple sclerosis", and European Regional Development Fund project 1.1.1.1/16/A/016.

ANTIMICROBIAL RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* CLINICAL ISOLATES

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Background. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the main causes of nosocomial infections. The rapid increase in resistance of these pathogens has become one of the most pressing health care problems worldwide in recent years.

Aim. To analyse the phenotypic diversity of antibacterial susceptibility of *P. aeruginosa* and *A. baumannii* in strains isolated from surgical material.

Methods. Microorganisms were determined from surgical samples by BBLTM CrystalTM system, antibiotic susceptibility test of isolates was performed by Bauer-KirbyTM disc diffusion test and ETM-test. Isolated bacterial stains were typed according to antibiograms.

Results. The study identified 47 *P. aeruginosa* and 26 *A. baumannii* isolates. *P. aeruginosa* isolates had 100% absolute susceptibility to colistin (n = 47) in 2023. Resistance to ciprofloxacin, imipenem, levofloxacin, and piperacillin/ tazobactam was detected in 31.9% (n = 15). In 2023, *A. baumannii* isolates were most often susceptible to amikacin — 69.3% (n = 18), while resistance to ciprofloxacin was determined in 50% (n = 13). Four MDR and one XDR *P. aeruginosa* phenotype were found. Combined resistance was found in 22.72% of cases (n = 10). One MDR and four XDR *A. baumannii* phenotypes were found. Combined resistance was found in 42.3% (n = 11) of cases. *P. aeruginosa* MAR index > 0.2 was determined for 26 (55.31%) isolates. *A. baumannii* MAR index > 0.2 was determined for 21 (44.69%) isolates. Comparing the changes in resistance between 2019 and 2023, an increase in the level of *P. aeruginosa* resistance was found to amikacin (by 10.8%; $p = 0.033$), imipenem (by 23.3%, $p = 0.001$), meropenem (by 13.8%; $p = 0.016$) and piperacillin/ tazobactam (by 25.2%; $p < 0.001$), combined resistance has increased by 13.39%. The resistance level of *A. baumannii* did not change ($p > 0.05$).

Conclusions. Resistance rates of *P. aeruginosa* to amikacin, imipenem, meropenem and piperacillin/ tazobactam have increased over a five-year period. The resistance level of *A. baumannii* remains at the same high level. XDR and MDR phenotypes of *P. aeruginosa* and *A. baumannii* were found, this means that the combined resistance of these microorganisms is an actual problem in Latvia. A high MAR index of *P. aeruginosa* and *A. baumannii* indicates that the bacteria have acquired multi-resistance, probably intra-hospital.

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THE MOST FREQUENT HLA ALLELES IN POPULATION OF LATVIA: A PILOT STUDY

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Background. The major histocompatibility complex (MHC) genes encode human leukocyte antigens (HLA) which play a critical role in regulating immune responses. There are two main classes of HLA: Class I encompassing HLA-A, -B and -C, and Class II, including HLA-DRB1, -DQA1, -DQB1, -DPA1, and -DPB1, which differ in molecular structure and function. HLA matching is essential in solid organ and allogeneic hematopoietic stem cell transplantation to prevent transplant rejection. Additionally, HLA typing contributes significantly to understanding disease associations, including infectious diseases and cancers.

Despite its importance, previous studies on HLA allele frequencies in Latvia have been fragmented, and comprehensive data on HLA frequencies in population of Latvia remain absent from international databases.

Aim. The aim of the study was to analyse the most frequent HLA alleles present in Latvian population, using the Latvian National Biobank – Genome Database data.

Methods. Differences between humans at the level of HLA proteins (up to the 3rd field of resolution: synonymous DNA substitution within the coding region) of 29 HLA genes were analysed using the HLA-HD software (https://w3.genome.med.kyoto-u.ac.jp/HLA-HD/).
The allele data of the 270 samples was collected. The allele frequencies were calculated and compared within different fields of resolution. R was used to calculate the difference between population of Latvia and other populations (obtained from the Allele Frequency Net Database (https://www.allelefrequencies.net/default.asp) with HLA frequency data in the literature and determine statistical significance (denoted as *p*-value).

Results. The HLA data from 270 random individuals of the population of Latvia show a highly diverse HLA landscape: 56, 80, and 79 distinct alleles were detected for Class I HLA genes A, B and C, respectively. The most frequent Class I alleles are HLA-A02:01:01 (24.6%) and HLA-A03:01:01 (15.2%). One HLA-B allele showed above 10% population frequency: HLA-B07:02:01 (14.3%). Three alleles (HLA-C07:02:01, HLA-C06:02:01 and HLA-C07:01:01) together comprise almost 40% of all HLA-C alleles, each showing an even distribution with a share of 11–15%. The remaining 60% is made up of 76 different HLA-C alleles. Most frequent of each HLA class II alleles are DRB107:01:01 (15.0%), DQA105:05:01 (18.0%), DQB103:01:01 (20.6%), DPA101:03:01 (89.4%) and DPB1*04:01:01 (54.6%).

Conclusions. Preliminary HLA typing data show that HLA class I and II the most frequent allele distribution is similar to European region, with some exceptions. HLA typing of Latvian population is paramount for organ and cell transplantation, and data submission to international HLA databases.

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DATA-DRIVEN LEARNING APPROACH USED FOR SEGMENTATION OF HISTOLOGICAL IMAGES

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Background. Microscopic colitis (MC) is an inflammation that affects the inner lining of the colon. This could lead to chronic and watery diarrhea, abdominal pains, faecal incontinence, and weight loss. The symptoms of the disease affect overall quality of life for the patient. Diagnosis of MC is done via biopsies examination by pathologist who checks the tissue under a microscope and looks for signs of the disease (increased inflammatory infiltrate in the lamina propria without significant crypt architectural distortion and intraepithelial lymphocytosis). The diagnosis is subjective and could lead to diagnostic discrepancies between evaluators and treatment plan failures. This study focuses on the development of an algorithm for pathologist to assist them in MC diagnosis.

Aim. The aim of the current study was to develop an algorithm for MC localisation from microscopic images.

Methods. Six hundred histological microscopic images (10 patients) were used to train the elaborated algorithm. Image pre-processing was done by dividing each image into small image elements (superpixels), using simple linear iterative clustering algorithm. After this procedure we applied machine learning approach to classify the pre-processed elements to disease and healthy tissues groups. Machine learning algorithm was pretrained using images, annotated by three pathologists. The proposed algorithm together with image pre-processing was implemented in MatLab development environment.

Results. The model was tested on additional microscopic images from five patients (300 images). It showed accuracy of correct classification of analysed microscopic images equal to 0.81, sensitivity — 0.8 and specificity — 0.81.

Conclusion. The proposed algorithm can assist pathologists in MC diagnosis and make it more objective.

EXPLORING THE ROLE OF HLA LOCUS POLYMORPHISMS IN MULTIPLE SCLEROSIS TREATMENT EFFICACY

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Background. Multiple sclerosis (MS) is a chronic disease with no known cure. Current treatments focus on reducing the frequency and severity of relapses, mitigating their impact, and slowing disease progression. The human leukocyte antigen (HLA) genes play a critical role in immune regulation, as they encode proteins essential for immune system function. Variations in the intergenic regions of the HLA locus can disrupt the regulatory mechanisms of these genes, potentially influencing immune-related diseases. In this study, we investigated SNP rs9275596 (chr6: 32713854; GRCh38.p12), located between the HLA-DQB1 and HLA-DQA2 genes, which has previously been associated with multiple sclerosis in the Latvian population. Our aim was to explore its potential role in MS treatment outcomes.

Aim. To identify the possible association of SNP rs9275596 located in the human leukocyte antigen locus with the outcome of multiple sclerosis therapy in a Latvian disease cohort.

Methods. In the Latvian MS cohort, 230 out of 296 patients underwent 342 distinct pharmacological treatments. Data on changes in Expanded Disability Status Scale (EDSS) scores and No Evidence of Disease Activity (NEDA) status were collected and analysed over a two-year period following the initiation of therapy.

Results. The homozygous genotype TT of the rare allele of the SNP rs9275596 in the HLA locus contributes to reducing relapses (with average ΔEDSS in 1st year —0.30 ± 0.75 points) less frequently used medications. In the same medication group, the mean change in EDSS in patients with the homozygous genotype of the most common allele, CC, was 0.20 ± 0.26 points ($P = 3.83 \times 10^{-2}$). Still, in the case of GA therapy, the TT genotype is a risk factor for an increase in EDSS (with average ΔEDSS in the 1st year 0.41 ± 0.80 points) compared to other therapies ($P = 2.36 \times 10^{-2}$; η = 0.36). No difference in EDSS change was found in patients in the second year of therapy within the specific SNP genotype groups.

Conclusions. We provide evidence that the intergenic SNP rs9275596 of the HLA locus may contribute to the response of multiple sclerosis patients to therapy and thus, further studies of the polymorphism are warranted.

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STUDY OF PROTEASOME GENE POLYMORPHISMS AS PREDICTORS OF TREATMENT OUTCOMES IN LATVIAN PATIENTS WITH MULTIPLE SCLEROSIS

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Background. Proteasomes, the multycatalytic protease complexes, play a critical role in the degradation of proteins via ATP/ubiquitin-dependent process or ubiquitin proteasome system, which plays a crucial role in immunity and its disregulation and/or modulation may influence the development and progression of different diseases, including altered responses to drug therapy. Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system. Inflammation damages myelin, which surrounds and insulates the nerve fibres, the nerve fibres themselves, and the specialised cells that make myelin, thus leading to neurodegeneration and disabilities. The proteolytic activities of proteasomes are reduced in brain tissue of MS patients. The 20S proteasome had been identified as a target of the humoral autoreactive immune response and a major autoantigen in MS patients.

Aim. To identify the possible association of SNP of proteasomal genes located in the human 14 chromosome with the outcome of multiple sclerosis therapy in a Latvian disease cohort.

Methods. In the Latvian MS cohort, 230 of 296 patients received 342 distinct pharmacological treatments. Data was gathered regarding alterations in EDSS (Expanded Disability Status Scale) scores and NEDA (No evidence of disease activity) within two years following the initiation of therapy. Information from Information on proteasome gene polymorphisms was obtained from previous studies.

Results. Homozygous genotypes of common alleles CC in PSMB5 rs11543947, in PSMA6 rs2277460 and rs1048990 and rear allele genotypes GG/TT +AG/CT in PSMC6 two SNPs rs2295826/rs2295827 and AA +GA in PSMA3 rs2348071 are a risk form in the case of Glatiramer acetate (GA) therapy, or an association with a higher increase in EDSS in the first year of GA therapy than in other forms of therapy has been found. In the same time rear allele genotypes GG+CG in PSMA6 rs1048990 are better for EDSS change in second year period in case of other group medications ($P = 4.41 \times 10^{-2}$).

Conclusion. We present evidence that SNPs in the proteasomal gene on chromosome 14 may influence the therapeutic response in multiple sclerosis patients, indicating that further investigation of this polymorphism is necessary.

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EXPERIMENTAL ACUTE MESENTERIC ISCHEMIA IN RATS INDUCES OXIDATIVE STRESS IN KIDNEYS

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Background. Acute mesenteric ischemia (AMI) compromises the intestinal mucosal barrier enabling bacteria, endotoxins, and other substances to enter the circulatory system. This triggers the generation of reactive oxygen and nitrogen species that disrupt antioxidant defences. Oxidative stress causes distal tissue and organ damage, often leading to multiple organ dysfunction syndrome with following morbidity and mortality. Organs frequently affected include the liver, heart, kidneys, and lungs [1].

Aim. To assess several oxidative stress markers in kidney homogenates of rats subjected to an AMI model.

Methods. Experimental AMI protocols were approved (Permission No. 120/2020, Food and Veterinary service of Latvia). Wistar rats were divided into six groups: sham-operated control and groups with different ischemia durations of 15, 30, 60, 90, and 120 minutes. Surgery and tissue homogenate preparations were conducted previously by others.

Aliquots of the tissue homogenates were stored at –80 °C until use. Total antioxidant capacity (TAC) was measured using Abbexa kit (Catalog No. abx295022). Malondialdehyde (MDA) levels were assessed with the Sigma-Aldrich Lipid peroxidation assay kit (Catalog No. MAK085). Superoxide Dismutase (SOD activity was evaluated using the Sigma-Aldrich Superoxide Dismutase Activity Assay kit (Catalog No. 19160). Reduced glutathione (GSH) levels were measured using the BioAssay Systems kit (Catalog No. DIGT-250). Protein concentrations were determined by the Pierce™ BCA Protein Assay Kit (TermoScientific, Catalog No, 23225). Statistical analyses and graphs were performed with GraphPad Prism (Version 8.0; GraphPad Software, Inc., USA). One-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison test was applied. Samples were tested in triplicates. Number of rats per group were 6–9.

Results. MDA levels characterising lipid peroxidation increased from 30 min of ischemia, while TAC, SOD activity, and GSH levels decreased significantly after 60, 90, and 120 min compared to the sham-operated control group.

Conclusion. Experimental AMI induces oxidative stress in rat kidneys. Endogenous antioxidant defences effectively manage ischemia up to 15 min but are overwhelmed after 30 min, resulting in oxidative stress escalation after 60 min.

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References

1. Chen, R., Zeng, Zi., Zhang, Y. *et al*. Ischemic postconditioning attenuates acute kidney injury following intestinal ischemia-reperfusion through Nrf2-regulated autophagy, anti-oxidation, and anti-inflammation in mice. *The FASEB Journal*, 2020, **34**, 8887–8901. doi.org/10.1096/fj.202000274R.

IMMUNOHISTOCHEMICAL SIGNATURES OF ZONE-SPECIFICITY IN ALCOHOL-INDUCED LIVER DAMAGE

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Background. Alcohol-induced liver damage is linked to immune response activation and inflammation triggered by hepatocyte injury, exhibiting zone-specificity within liver lobules. Macrophages play a central role in regulating these responses, contributing to tissue repair and inflammation. The dual role of CD163-positive macrophages in tissue regeneration and fibrosis depends on the inflammatory environment, while the NF κB signalling pathway mediates ongoing inflammation in alcohol-related liver disease. Together, macrophage activation and NF-κB signalling lead to a vicious cycle resulting in the progression of alcohol-induced liver tissue damage.

Aim. The aim of this research was to investigate the immunoexpression of CD163 and NF-κB in liver tissue from young healthy adults, age-matched alcohol users, and chronic alcohol users to evaluate zone-specific macrophage activation and inflammation in the progression of alcohol induced liver damage.

Methods. Fifty-four liver tissue specimens were obtained and organised into three groups — controls (n = 11), age-matched alcohol users (n = 15), and chronic alcohol users (n = 28). Tissue specimens were immunolabelled using anti-NFźB and anti-CD163 antibodies. NF-κB expression was evaluated semi-quantitatively by grading intensity and distribution on a scale from 0 to 3, whereas CD163 expression was determined quantitatively by counting CD163 positive cells in fifteen visual fields. Statistical analysis was performed using SPSS 28.0.

Results. Analysis of NF-κB expression revealed that the intensity of expression in the lobular area was significantly increased in the age-matched group ($p = 0.05$), while both intensity and distribution in the chronic alcohol user group ($p < 0.001$, $p = 0.02$) were higher compared to the controls. Strong correlations between intensity and distribution in the lobular area ($r = 0.816$, $p < 0.001$) and portal area ($r = 0.984$, $p < 0.001$) were also observed.

CD163-positive cell counts were higher in the lobular area of age-matched alcohol users (mean 135 ± SEM 43) and chronic alcohol users (mean 226 ± SEM 38) compared to controls (mean 59 ± SEM 14), following a similar trend in the portal and central vein areas. Cumulative CD163-positive cell count was significantly increased in the chronic alcohol user group, compared to the controls ($p = 0.012$).

Conclusion. Chronic alcohol consumption leads to a significant increase in NF-κB expression, indicating persistent inflammation, while the elevation of CD163-positive macrophages suggests a shift towards M2-like polarisation, which likely contributes to fibrosis rather than regeneration. These findings highlight that alcohol abuse induces an early and sustained immune response with macrophage activation and inflammation driving the progression of liver damage, particularly in the lobular areas.

PATHOGENS OF PERIPROSTHETIC JOINT INFECTIONS, THEIR VIRULENCE FACTORS, AND SUSCEPTIBILITY TO ANTIBACTERIAL AGENTS

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Background. Periprosthetic joint infections (PJIs) are significant challenges in clinical practice, leading to increased morbidity and healthcare costs. Identifying causative pathogens and antimicrobial resistance (AMR) profiles is crucial for effective treatment. Delayed antimicrobial therapy increases the risk of severe complications, including treatment failure and mortality.

Aim. To evaluate the diversity of pathogens causing periprosthetic joint infections, evaluate their antimicrobial resistance patterns, and compare epidemiological results over the five-year period from 2020 to 2024.

Methods. Microorganisms were isolated by the BBL™ Crystal™ system & Vitek 2 Compact system. Antibiotic susceptibility testing was performed using the Bauer-Kirby™ disc diffusion test and E™-test, extended-spectrum beta-lactamase (ESBL) production was confirmed using the combined disk method. Data were obtained from the laboratory information system, and bacterial strains were typed according to antibiograms. Data were processed using MS Excel 2024 software.

Results. From 2020 to 2024, 248 PJI cases were confirmed. Gram-positive bacteria (GPB) accounted for 76% of the identified pathogens, and 48 gram-negative bacteria (GNB) accounted for 17%. By 2024, the most isolated PJI-causing pathogens were *Staphylococcus aureus* (n = 12), coagulase-negative staphylococci (n = 11), and *Streptococcus epidermidis* (n = 6), reflecting evolving trends.

A total of 56 multidrug-resistant (MDR) phenotypes were identified. Methicillin-resistant *Staphylococcus epidermidis* (MRSE) was the most prevalent, comprising 32.1% (n = 18) of MDR phenotypes. ESBL producing bacteria represented 28.6% (n = 16).

Over the five-year period, GPB showed the highest resistance to gentamicin 28.8% (n = 49) and cefoxitin 24.1% (n = 34), while GNB were mostly resistant to ampicillin 86% (n = 25) and cefazolin 76.5% (n = 26).

Conclusion

1. In this study, 248 confirmed cases of PJIs were identified. The cohort consisted of 126 males and 122 females, the average age of the affected individuals was 67.9 years, indicating a significant prevalence in the aging population.

2. GPB exhibited resistance to gentamicin 28.8% (n = 49) and cefoxitin 24.1% (n = 34).

3. GNB exhibited resistance to ampicillin 86% (n = 25) and cefazolin 76.5% (n = 26).

4. Of all gram-negative isolates, 33.3% (n = 16) demonstrated multidrug resistance, mainly characterised by ESBL producing phenotype.

5. Of all gram-positive isolates, 17.0% (n = 34) were multidrug-resistant, with methicillin-resistant *Staphylococcus epidermidis* (MRSE) being the most common.

6. The results emphasise that local monitoring of antimicrobial resistance is an important tool for improving empirical treatment and clinical decision-making.

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Pharmacy

APTAMER SELECTION TOWARDS PLASMODIUM VIVAX LACTATE DEHYDROGENASE AND VALIDATION OF SEQUENCES

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Background. Malaria, caused by Plasmodium parasites, is a life-threatening illness. The WHO aims to eliminate malaria through widespread screening, but current diagnostic tools are either too complex or lack sensitivity. There is a need for cost-effective, stable, and sensitive solutions. Aptamers, short DNA or RNA sequences that bind specific targets like monoclonal antibodies, offer benefits such as smaller size, lower cost, chemical stability, and longer shelf-life, making them ideal candidates for bioselective layer for developing point-of-care biosensors for malaria detection.

Aim. The aim of the current study was to characterise the binding affinity and selectivity of full-length and truncated aptamers, selected against Plasmodium vivax lactate dehydrogenase (Pv-LDH).

Methods. Protein-Systematic Evolution of Ligands by Exponential Enrichment (SELEX) was performed to select single stranded DNA aptamers towards Pv-LDH. Next generation sequencing was used to identify sequences enriched over SELEX cycles. Six most abundant sequences were selected to further analysis and named Pv1, Pv2, Pv3, PV4, Pv5, and Pv6, accordingly. The binding affinity of aptamers selected against PvLDH was assessed by enzyme linked oligonucleotide assay (ELONA). Next, full length and truncated aptamer affinity and selectivity towards Pv-LDH, *P. ovale*, *P. falciparum*, *P. malariae* LDH and human LDH isoforms A and B was determined by ELONA. Aptamer stability at 37 °C was tested in human serum and lysed human whole blood using gel electrophoresis.

Results. Aptamers Pv1 and Pv2 showed selectivity towards all four tested Plasmodium spp. LDHs. Remaining four aptamers showed no binding to Pv-LDH. Truncated forms of Pv1t and Pv2t showed lower affinity than full length aptamers and were not tested further. Cross-reactivity studies demonstrated that aptamers Pv1 and Pv2 exerted high affinity towards Pv-LDH, Pf-LDH, Po-LDH and lower affinity to Pm-LDH with Kd values 3.87 ± 0.6, 4.58 ± 1.8, 22.8 ± 4.3, 510.8 for Pv1, respectively and 0.63 ± 0.14, 1.75 ± 0.25, 7.46 ± 0.15, 432.9 for Pv2, respectively. Importantly, neither aptamer showed any binding to human LDH isoforms A or B.

Conclusion. Two of six selected aptamers Pv1 and Pv2 bind Pv-LDH with high affinity and show cross reactivity to *P. falciparum, P. malariae* and *P. ovale* LDHs. The selected unique aptamers could serve as sensitive and selective synthetic receptors to develop a cost-efficient point-of-care biosensor for malaria detection.

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ETHNOPHARMACY AND THE THERAPEUTIC POTENTIAL OF CLAYS

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Ethnopharmacy is an interdisciplinary field investigating traditional medicine knowledge, natural compounds' pharmacological properties, and their applications in modern medicine. The largely unexplored *De Materia Medica* by Dioscorides could be expanded with new substances to enhance therapeutic efficacy. Historically, natural materials were tested through trial and error, with only a few retaining relevance alongside modern synthetic alternatives. Clay is one such material, highly valued in Ancient Egypt and utilised by Hippocrates, Celsus, and Galen for its adsorption, antibacterial, and gastrointestinal properties. Avicenna's *Canon Medicinae*, widely used in medieval Europe, documented numerous therapeutic applications of clays, emphasising their significance in traditional medicine.

Modern research confirms the biological activity of kaolinite ($Al_2Si_2O_5(OH)_4$) and montmorillonite ($(Na,Ca)_0.33(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$), highlighting their plasticity and efficacy. Montmorillonite's high cation exchange capacity allows modifications enhancing its physicochemical properties. Studies indicate that montmorillonite, when treated with alkali or acid and combined with iron ferrocyanide, significantly improves radionuclide-binding capacity. The resulting composite demonstrates high efficiency in the multisorption of Cesium (96–98%), Cobalt (79–95%) and Strontium (78–91%).

These novel sorbents have great potential in environmental pollution control and medicine, such as radionuclide-adsorbing wound dressings. They offer dual functionality — binding radioactive contaminants and protecting wounds — which makes them valuable in military medicine and disaster response. Montmorillonite's application in oncology, where it immobilises potent yet costly cancerostatic agents, represents a promising direction in personalised medicine. When introduced near tumours, nanoparticles selectively accumulate in cancer cells, ensuring prolonged therapeutic effects while reducing the required active substance dose.

Additionally, peloids (medicinal muds) are promising therapeutic agents, promoting wound healing, blood coagulation, and skin regeneration. However, studies require an interdisciplinary approach, integrating mineralogy, biochemistry, and clinical medicine. Further research and expertise are needed to advance this field. Given ethnopharmacy's role in developing new therapeutic approaches, universities should introduce specialised courses on natural compounds' therapeutic potential, physicochemical properties, and efficacy validation. Such education fosters sustainable and scientifically robust pharmaceutical solutions by integrating traditional knowledge with modern advancements.

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DISPOSAL PRACTICES FOR UNUSED AND EXPIRED MEDICATIONS AMONG PHARMACY CUSTOMERS

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Background. Improper disposal of unused and expired medications poses a significant environmental and public health risk. Pharmaceutical waste in household garbage and wastewater contributes to contamination of water bodies and increases the likelihood of accidental poisoning and antibiotic resistance. Previous studies suggest that public awareness and accessible disposal programmes in pharmacies play a crucial role in mitigating these risks. However, research on pharmacy customers' disposal habits remains limited in Latvia.

Aim. This research aimed to identify pharmacy customers' disposal practices for unused and expired medications and key factors influencing these behaviours.

Methods. A quantitative cross-sectional study was conducted using an anonymous structured questionnaire to collect data on pharmacy customers' disposal habits, awareness, and motivations. A total of 105 respondents were surveyed in a pharmacy in Rīga between December 2024 and February 2025. Inclusion criteria required participants to be adults who had purchased medication within the past year. Exclusion criteria included medical professionals to avoid response bias. Data were analysed using descriptive statistics, including frequency distribution, percentage analysis, and cross-tabulation of key factors influencing disposal behaviours.

Results. Findings indicate that 34.3% of respondents dispose of unused medications via household waste or wastewater, with 20% flushing them and 14.3% discarding them with regular trash. In contrast, almost 60% return expired medications to a pharmacy. Despite this, 6.6% lacked knowledge about proper disposal. Older respondents (60+) were more likely (67.2%) to return medications, whereas younger individuals (under 30) had a higher tendency (45.8%) for improper disposal. Pharmacist guidance increased proper disposal likelihood by 2.5 times. Additionally, 48.6% wanted clearer disposal instructions, and 38.1% would use pharmacy disposal services if more accessible. Single-person households had higher improper disposal rates (42.7%) than larger households (28.5%).

Conclusions. Pharmacy customers' disposal practices for unused and expired medications vary, with a majority using official take-back programmes, while a significant portion disposes of them improperly, showing gaps in awareness and accessibility. Key factors influencing these behaviours include age, with older individuals more likely to follow proper disposal methods, pharmacist guidance significantly increasing participation in take-back programmes, and household size affecting disposal habits, showing the need for targeted education and improved accessibility to disposal services.

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EVALUATION OF CHEMOTHERAPY DRUG EFFICACY IN A PDAC ORGAN-ON-A-CHIP MODEL

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Background. Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive and deadly malignancies, with a median five-year survival rate below 5%. PDAC's complex genetic mutations, dense stroma, and immunosuppressive microenvironment contribute to therapy resistance, making treatment particularly challenging. Surgical resection and chemotherapy, including gemcitabine and a combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin, have helped improve survival rates for patients with early-stage pancreatic cancer. One of the models used to test drug efficacy in PDAC patient organoids is the organ-on-a-chip (OOC) system, which offers a more physiologically relevant alternative to conventional two-dimensional (2D) cell cultures. It enables continuous nutrient and drug flow, mimicking blood circulation.

Aim. To test two most used chemotherapy drugs for PDAC in OOC system and determine their efficacy on tumour patient derived cells.

Methods. Primary human PDAC organoids and human umbilical vein endothelial cells (HUVEC) were cultured in a stacked microfluidic chip composed of cyclo-olefin copolymer (COC) and a porous PET membrane. HUVECs were seeded in the bottom channel and cultured for one week before PDAC organoids were seeded into the upper channel. The media flow rate was maintained at 4 µl/min. PDAC organoids and HUVECs were also cultured under 2D conditions. 10 µM Gemcitabine and the irinotecan metabolite 10 nM SN-38 were applied to the endothelial channel in the chip and directly to wells in 2D cultures for 72 hours. Outflows from both chip channels and supernatants from 2D cell plates were collected daily for cell viability analysis and biomarker detection using multiplex and ELISA assays. OOC membrane sections were fixed, stained, and analysed cell apoptosis (Casp3+) using fluorescence microscopy.

Results. PDAC organoids can be maintained on the chip for over 50 days, enabling continuous monitoring of cell viability through tumour and endothelial channel outflows. Gemcitabine enhances apoptosis in the PDAC organoid channel by more than twofold (untreated = 20 ± 8, n = 3; Gemc. = 57 ± 16, n = 3, $p < 0.05$; SN-38 = 18 ± 14, n = 3 (%)), while in the HUVEC microfluidic channel, it leads to a fivefold increase in $Casp3^+$ cell count (Untreated = 4 ± 0.6, n = 3; Gemc. = 22 ± 13, $p < 0.05$, n = 3; SN-38 = 12 ± 9, n = 3 (%)). In contrast, SN-38 does not produce a statistically significant effect.

Conclusion. HUVEC cells exhibit sensitivity to both chemotherapy agents, indicating vascular toxicity. PDAC organoids are more susceptible to gemcitabine therapy. OOC system provides more accurate drug response predictions than 2D cell cultures, enhancing preclinical testing reliability.

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EXPANDED ANALYSIS OF THE ANTIVIRAL EFFECTS OF *SAMBUCUS NIGRA* L. EXTRACTS AGAINST SARS-COV-2 IN CALU3 AND VERO E6 CELLS

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Background. The growing need for alternative antiviral therapies has increased interest in plant-derived compounds. Elderberry (*Sambucus nigra* L.) extract, traditionally used for treating cold symptoms, has demonstrated promising antiviral properties. Our previous study showed that elderberry extract inhibits SARS-CoV2-induced cytopathic effect (CPE) in Calu3 cells, supporting its potential as an antiviral agent.

Aim. The aim of the study was to expand our analysis to a broader dataset to evaluate the consistency and reproducibility of these findings across two cell lines and multiple sample sources.

Methods. Samples were collected from ten locations, with extracts derived from six independent trees per site. Calu3 and Vero E6 cells were cultured to monolayers in 96-well plates and infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.3. Immediately after infection, elderberry extracts (5–9 mg/ml) were added to the wells. After 72 hours, virus-induced cytopathic effect (CPE) inhibition was evaluated using 0.25% Crystal Violet staining. Rem-

desivir was included as a positive control. Statistical significance was determined by one-way ANOVA or by T-test.

Results. Elderberry extracts from all ten locations demonstrated inhibition of virus-induced CPE. While the two cell lines exhibited different susceptibilities to SARS-CoV-2 infection, virus-induced CPE inhibition was observed in both, with an average of 44.12% (p = 0.0001) in Calu3 cells and 39.5% (p = 0,0001) in Vero E6 cells. Minor variations in CPE inhibition were noted between different locations. The highest inhibition rates were observed in extracts from Pūre (51.70%), Kalna Krogs (48.92%), Brocēni (39.34%), and Iecava (44.52%), while lower inhibition was recorded in Embūte (25.56%). These no statistically significant differences between samples from different locations indicate the reproducibility and robustness of the observed antiviral effects.

Conclusion. This expanded study confirms the inhibitory potential of elderberry extract against SARS-CoV2. The consistency of results across multiple sample sources and two distinct cell lines highlights its potential as a natural antiviral agent. Variations between cell lines suggest that cell-type-specific factors may influence antiviral activity. Further research, including mechanistic studies and clinical trials, is needed to explore its therapeutic applications against COVID-19 and other viral infections.

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THE EFFECT OF HYPEROSIDE ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN MICE BRAIN AND LIVER

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Background. Superoxide dismutase is a key antioxidant enzyme that protects cells by converting harmful superoxide radicals into hydrogen peroxide and oxygen. Research indicates that hyperoside exhibits antioxidant effects by scavenging reactive oxygen species (ROS) and enhancing antioxidant enzyme activity. It has been shown to protect against hydrogen peroxide (H2O2) — induced cell damage by inhibiting apoptosis [1].

Aim. The aim of the current study was to evaluate the effect of hyperoside on the SOD activity in mice liver and brain after aluminium consumption.

Methods. Experiments were conducted on 4–6 weeks old mice (20–25 g), n = 8. Mice were divided into four groups: control, hyperoside-treated, aluminum-treated, and aluminum-hyperoside-treated. Aluminum (Al^{3+}) was injected into the abdominal cavity. SOD activity was determined spectrophotometrically. Results were expressed as the mean ± SD.

Results. It was found that hyperoside, aluminum, and their combination significantly affected SOD activity in the liver ($p < 0.05$), with both aluminum and hyperoside increasing activity. However, administering hyperoside after aluminum-induced oxidative stress showed no significant difference compared to aluminum alone, despite further increasing SOD activity. In the brain, aluminum had no significant effect on SOD activity ($p > 0.05$), and hyperoside (50 mg/kg for 21 days) slightly reduced activity without significance.

Conclusion. Despite the antioxidant properties of hyperoside, the results show that its 50 mg/kg dose does not significantly affect SOD activity in the mice brain. Meanwhile hyperoside increased SOD activity in mice liver, but it was not significantly, so its effectiveness may depend on dose, timing, warranting further research.

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References

1. Piao, M. J., Kang, K. A., Zhang, R., Ko, D. O., Wang, Z. H., You, H. J., Kim, H. S., Kim, J. S., Kang, S. S., Hyun, J. W. Hyperoside prevents oxidative damage induced by hydrogen peroxide in lung fibroblast cells via an antioxidant effect. *Biochimica et biophysicaacta,* 2008, **1780** (12), 1448–1457.

COMPARATIVE ANALYSIS OF CHARACTERISTICS BETWEEN TRADITIONAL AND 3D-PRINTED GUMMY TABLETS

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Background. Gummy tablets are widely recognised as an oral pharmaceutical dosage form, particularly suited for paediatric patients due to their organoleptically appealing properties. However, they are also well suited for adult and geriatric patients, offering prolonged release profiles. Recent advancements in pharmaceutical manufacturing have enabled the production of gummy tablets using innovative technologies, such as 3D printing via the semi-solid extrusion method.

Aim. To compare the characteristics of conventionally manufactured gummy tablets and 3D-printed gummy tablets formulated with *Artemisia annua* L. extract and various excipients.

Methods. The *Artemisia annua* L. extract was prepared using an ultrasound-assisted extraction method. Three formulations with different excipient combinations — gelatin-HPMC, gelatin-pectin, and HPMC-pectin — were prepared by mechanical stirring at 60 ^{o}C. Gummies were produced using two methods: pouring the hot mixture into moulds to cool at room temperature and 3D printing via semi-solid extrusion. The printed mixtures were loaded into a 20 ml syringe and printed at 55 ^{o}C, with a printing speed of 100 mm/s, a flow rate of 120 mm/s, 50% infill density, and a layer height of 0.6 mm. Viscosity, hardness, stickiness, and moisture content of the tablets were analysed, with each test performed in triplicate and averages calculated.

Results. To ensure precise comparisons, both regular and 3D-printed gummy tablets were cubic-shaped, with identical weights (0.85 ± 0.04 g) and dimensions (8 × 8 × 8 mm). The hardness of regular tablets ranged from 3.169 ± 0.158 to 14.380 ± 0.719 N, while 3D-printed tablets ranged from 1,.265 ± 0.063 to 23.863 ± 1.193 N. The gelatin-pectin combination exhibited the highest hardness in both regular and 3D-printed tablets. Stickiness was lowest in HPMC-pectin gummies (regular: –0.0050 ± –0,0003 N; 3D-printed: –0.0130 ± 0.0007 N) and highest in gelatin-pectin gummies (regular: –0.112 ± –0.006 N; 3D-printed: –0.185 ± –0.009 N). Moisture content was highest in regular HPMC-gelatin tablets (70.00 ± 3.50 %) and lowest in 3D-printed gelatin-pectin tablets (37.00 ± 1.85 %). Viscosity analysis of the excipient mixtures showed gelatin-pectin as the least viscous (5194.6 ± 259.7 mPa·s), followed by HPMC-gelatin (5283.5 ± 264,2 mPa·s), with HPMC-pectin exhibiting the highest viscosity (5487.8 ± 274.4 mPa·s).

Conclusion. 3D-printed gummy tablets with a gelatin-pectin mixture exhibited the most favourable characteristics, including the lowest moisture content and the highest hardness.

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THE EFFECTS OF OREGONIN AND NONSTEROIDAL ANTI-INFLAMMATORY DRUG NIMESULIDE ON THE β-AMYLOID FRAGMENT AGGREGATION *IN VITRO*

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Background. Alzheimer's disease (AD) is a multifactorial neurodegenerative disease that causes a progressive cognitive impairment affecting memory, thinking and behaviour. Extracellular accumulation of β-amyloid plaques and neuroinflammation are dominant AD pathophysiology theories. Nimesulide is a potent non-selective COX enzyme inhibitor with good anti-inflammatory properties. Oregonin is a promising naturally occurring diarylheptanoid compound that is primarily found in various species of the Alder genus. Oregonin has gathered interest due to its antioxidant, anti-inflammatory and anti-neoplastic properties. Inhibition of β-secretase and suppression of β-amyloid peptides aggregation are potential mechanisms for delaying AD progression. Drug repurposing as well as search for new compounds with amyloid pathway inhibiting properties is topical.

Aim. The aim of the current study was to evaluate the effects of a natural compound oregonin and a nonsteroidal anti-inflammatory agent nimesulide on the formation of β-amyloid peptides aggregates involved in Alzheimer's disease pathogenesis in *in vitro* assays.

Methods. β-secretase inhibitor screening: a β-secretase activity detection kit based on amyloid precursor protein

cleavage was used. Fluorescent assay buffer, substrate (50 μM), enzyme (0.3 unit/μl), and tested substances (25, 50, 100 μM) were incubated at 37 ^{o}C in a kinetic mode for 2 days. β-amyloid precursor fragmentation intensity was measured by fluorescence using an Infinite 200 PRO plate reader and i-control software (Tecan Trading AG, Switzerland). A second assay assessed β-amyloid aggregate formation. β-amyloid 1-42 (150 μM), methylglyoxal (MGO) (3 mM), tested substances (25, 50, 100 μM), and thioflavin (50 μM) were incubated at 37 ^{o}C for three days. An identical assay without MGO was also conducted. Amyloid aggregate formation intensity was measured by fluorescence at 450 nm.

Results. Nimesulide and oregonin similarly inhibited β-secretase enzyme thus substantially preventing β-amyloid fragmentation at all concentrations tested. Most potent activity of both substances was seen at 24–28 hour window. In the amyloid peptide 1-42 aggregation assay with MGO as an inducer oregonin substantially prevented the formation of amyloid fibrils in dose-dependent manner reaching its highest potential after 72 hours. Nimesulide showed noticeably weaker anti-amyloid effects and in the reactions without MGO had no inhibiting effect at all.

Conclusion. Both oregonin and nimesulide possess amyloid pathway inhibiting properties. Further studies are necessary for a more practical use of oregonin and nimesulide for treatment and prevention of AD.

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EVALUATION OF THE EFFICACY OF SOMATOTROPIN-CONTAINING PRESERVATIVE SOLUTION IN PROTECTING ISOLATED PORCINE KIDNEYS FOR TRANSPLANTATION

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Background. Preservation fluids are medical products dedicated to the preservation of organs for transplantation. They are designed to maintain the vital functions of the graft, limit damage induced during transplantation, and promote regeneration processes. Our team is conducting advanced research into the effectiveness of hormones as components of preservation fluids. Somatotropin (STH) is a hormone with potential protective effects on the kidney. It has been found to be involved in regenerative processes, stimulate the synthesis of nucleic acids, and indirectly affect kidney growth.

Aim. The aim of this study was to analyse the effect of somatotropin, as a component of the Biolasol model fluid, on the vital functions of isolated porcine kidneys.

Methods. A pilot study was conducted using an isolated porcine kidney model Polish "Large White", with the permission of the II Local Ethics Committee Krakow; number 1046/2013. Biolasol fluid (FZNP "Biochefa", Poland) was modified with the addition of STH at a dose of 0.01 μg/l. Grafts were allocated to two groups: control group A (Biolasol, n = 10) and experimental group B (Biolasol + STH, n = 10). The kidneys were washed with the developed perfusion solutions, then stored in simple hypothermia (4 ^{o}C) for 24 hours and washed again. Renal function was assessed by biochemical parameters determined in the collected perfusates: AST, ALT, creatinine, urea, and electrolytes.

Results. Biolasol + STH vs Biolasol fluid reduced ALT and AST activity after 24 h of renal storage by 61% and 45%, respectively. The addition of somatotropin also reduced urea levels (by 92%). Creatinine levels did not change. The [Na+] concentration increased by 34%, while the [K+] concentration decreased by 31%. The results were statistically significant at $p < 0.05$.

Conclusion. Based on the results obtained, it can be concluded that modifying the composition of Biolasol fluid with the addition of somatotropin at a dose of 0.01 μg/l had a beneficial effect on renal function.

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NETTLE EXTRACT IMPACT ON MITOCHONDRIAL OXIDATION IN NAFLD HEPG2 MODEL

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Background. Mitochondria play a crucial role in lipid metabolism, and its dysfunction is central to the development of oxidative stress in fatty liver disease [1]. Nettle has been associated with beneficial effects on liver function, such as lipid profile improvement and hepatoprotection [2]; however, no studies have yet investigated the significance of mitochondria-related respiration effects in the mechanism of nettle's hepatoprotective properties.

Aim. To determine the effects of nettle leaf water extract on mitochondrial respiration in permeabilised HepG2 cells using an *in vitro* model of hepatic steatosis.

Methods. Lipid overload was induced by adding a 2:1 mix of oleic acid and palmitic acid to HepG2 cell media. There were four experimental groups: Control, Control + Nettle, Lipid, and Lipid + Nettle. Each sample contained a concentration of 5 million cells/ml. For the nettle groups, nettle extract (6.25 mg/ml recalculated on dry leaf powder) was added for 24 hours. High-resolution respirometry was performed on permeabilised cells to assess oxygen consumption in OXPHOS states using the modified SUIT-036 protocol. Electron-transfer pathway capacities were calculated by subtracting O2 flux before and after substrate addition and expressed as percentages of control cells. Statistical analysis was performed using One-way ANOVA and post hoc Tukey's test in GraphPad Prism 7 (Version 7.0; GraphPad Software, Inc., USA).

Results. The high-resolution respirometry data revealed that the addition of nettle extract to HepG2 cells resulted in increased oxygen consumption in the FN-OXPHOS state. In contrast, lipid mixture exposure led to decreased oxygen consumption in the FN-OXPHOS state. The co-treatment of nettle extract with the lipid mixture resulted in a significant enhancement of complex I activity, restoring oxygen flux levels to those observed in the control group of HepG2 cells.

Conclusions. The results suggest that nettle leaf water extract can mitigate lipid-induced mitochondrial dysfunction in *in vitro* hepatic steatosis model via improvement of fatty acid metabolism in mitochondria.

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References

Bailey, S. M., Cunningham, C. C. Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. *Alcohol, Oxidative Stress and Cell Injury. Free Radical Biology and Medicine*, 2002, **32** (1), 11–16. https://doi.org/10.1016/s0891-5849(01)00769-9.

Anies, A. M., Saad, S., Ibraheim, S. Protective effect of nettle and olive leaves on hyperlipidemia in experimental rats. *Bulletin of the National Nutrition Institute of the Arab Republic of Egypt*, 2020, **56** (2), 69–98. https://doi.org/10.21608/bnni.2020.190313

THE EFFECT OF LICORICE (*GLYCYRRHIZA GLABRA* L.) EXTRACT ON GLUTATHIONE IN MICE LIVER AND BRAIN

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Background. *Glycyrrhiza glabra* L. is a member of the Fabaceae family and one of the most valuable plants in the world. It contains various phytocomponents such as glycyrrhizin, 18β-glycyrrhizinic acid, glabrin A and B, and isoflavones, which have a wide range of pharmacological activities. Glycyrrhizin is a major triterpene saponin with antiviral, anti-inflammatory, and antioxidant properties [1].

Aim. The aim of the current study was to evaluate the possible antioxidant effect of *Glycyrrhiza glabra* L. root extract on glutathione (GSH) concentrations in livers and brains of BALB/c laboratory mice after aluminium exposure.

Methods. The study was performed on six-week-old BALB/c mice (n = 32). Mice were divided into four groups. AlCl3 solution was used to induce oxidative stress. Concentration of reduced glutathione (GSH) in the liver and brain of mice were determined spectrophotometrically after 21 days of administration of *Glycyrrhiza glabra* L. extract.

Results. The results showed that aluminium statistically significantly reduced GSH concentration in the liver by 11.95% ($p < 0.05$) and by 10.48% in brain of mice ($p > 0.05$) compared to control group. *Glycyrrhiza glabra* L. root extract, after AlCl3 exposure, decreased GSH concentration by 10.18% in liver ($p > 0.05$) and statistically significantly

increased GSH concentration by 55.24% in brain of mice ($p < 0.05$) compared to control group. Results demonstrated that *Glycyrrhiza glabra* L. extract after AlCl3 exposure increased GSH concentration in liver of mice by 2.01% ($p > 0.05$) and statistically significantly increased the concentration by 73.40% in brain ($p < 0.05$) compared to the aluminium-treated group.

Conclusion. Administration of *Glycyrrhiza glabra* L. extract resulted in a statistically significant increase in GSH concentration in the brain of mice but did not show any statistically significant increase in the liver after aluminium exposure. Therefore, the extract has limited antioxidant effect. However, the ability of *Glycyrrhiza glabra* L. extract to increase GSH concentration in the brain of mice suggest that it may has neuroprotective action.

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References.

1. Semenescu, I., Avram, S., Similie, D., Minda, D., Diaconeasa, Z., Muntean, D., Lazar, A. E., Gurgus, D., Danciu, C. Phytochemical, antioxidant, antimicrobial and safety profile of *Glycyrrhiza glabra* L. extract obtained from Romania. *Plants* (Basel), 2024, **13** (23), 3265. DOI: 10.3390/plants13233265. PMID: 39683057.

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