The 5th Student Conference

“Zebrafish as an animal model”

18 of April 2026

ABSTRACT BOOK

Organizer: The Polish Zebrafish Society

Organizing committee:

Dr hab. Krzysztof Rakus, prof. UJ (Jagiellonian University, Krakow)

Dr. Katerina Makarova (Medical University of Warsaw)

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AWARD FOR THE BEST PRESENTATION

Student with the best presentation will be awarded a fellowship to attend 8th Workshop of the Polish Zebrafish Society (Lublin, September 2026)

This award is funded by the Polish Zebrafish Society

09:00-09:05 – Opening remarks

Prof. UJ dr hab. Krzysztof Rakus,
Department of Evolutionary Immunology, Jagiellonian University, Krakow

Invited lecture: 09:05-09:40

Seeing Thoughts: Zebrafish in Modern Neuroactive Drug Discovery
Prof. dr hab. Piotr Podlasz

Department of Pathophysiology, Forensic Veterinary Medicine and Administration,
Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn

Development and disease biology		
1 09:40-09:50	Kinga Padała, Medical University of Lublin	Functional Studies of *EHHADH* Gene Mutation During Craniofacial Development Using Zebrafish Model
2 09:50-10:00	Sylwia Drzewiecka, Medical University of Lublin	Functional Characterization of *TPM2* Splice Mutation causing Skeletal Muscle Defects using Zebrafish
3 10:00-10:10	Kinga Czachor, Medical University of Lublin	Investigating Novel *TPM2* Variant-Induced Muscle Dysfunction Using Zebrafish Model
4 10:10-10:20	Alicja Głowacka, University of Wrocław	Age-related muscle glycogen loss in zebrafish (*Danio rerio*) model
5 10:20-10:30	Małgorzata Rasińska, University of Warmia and Mazury in Olsztyn	The effect of exposure of zebrafish (Danio rerio) embryos to aspartame at an early stage of development on their further development and anxiety
6 10:30-10:40	Weronika Pielak, Adam Mickiewicz University, Poznań	Zebrafish larvae as a model to study behavior
Break 10:40-11:00		
Toxicology and environmental interactions		
7 11:00-11:10	Magdalena Piotrowicz, Medical University of Warsaw	Effects of Microplastics on Zebrafish Embryonic Development
8 11:10-11:20	Jan Pruszyński, The Kielanowski Institute of Animal Physiology and Nutrition, Jabłonna	Does zebrafish ovaries suffer from nanoparticles exposure?
9 11:20-11:30	Gabriela Golec, Medical University of Lublin	Evaluation of Isopropyl U-47700 toxicity in zebrafish model
10 11:30-11:40	Klaudia Brzozowska, Medical University of Warsaw	Interaction toxicity of ibuprofen and ethanol in zebrafish model
11 11:40-11:50	Natalia Łępa, Medical University of Lublin	Toxicity, bioaccumulation, and antitumor evaluation of organometallic compounds in *Danio rerio* larvae

Drug screening and mechanisms		
12 11:50-12:00	Oliwia Ozga, Medical University of Lublin	Assessment of the antiseizure effect of *Carlina acaulis* extracts in Pentylenetetrazole-Induced seizure model in *Danio rerio* larvae.
13 12:00-12:10	Elżbieta Kot, Medical University of Lublin	Zebrafish as a Model for Evaluating the Anticancer Activity and Toxicity of Artemisinin
Break 12:10-12:30		
14 12:30-12:40	Anna Krauze, Medical University of Warsaw	Zebrafish-Based Screening of Pumpkin Extracts for Modulation of Oxidative Stress Responses
15 12:40-12:50	Artem Wojciechowski, Jagiellonian University, Krakow	The role of 25-hydroxycholesterol in the antiviral response of zebrafish (*Danio rerio*)
16 12:50-13:00	Paulina Jędrzejczyk, Jagiellonian University, Krakow	Bacterial-derived inhibitor miropin protects against systemic infection with oral pathogens in a zebrafish larval model
17 13:00-13:10	Jagoda Szponar, Medical University of Lublin	From Plant to Model: Assessing the Antidiabetic Potential of Angelica archangelica Root Extracts in Zebrafish Larvae
18 13:10-13:20	Sandra Budziak, Medical University of Bialystok	Anti-obesity effects of innovative food formulations in a zebrafish diet-induced obesity model
19 13:20-13:30	Przemysław Pawliszyn, Jagiellonian University, Krakow	Broken hearts and leaky vessels: The off-target cardiovascular effects of Ibrutinib
13:30-13:45 - Concluding remarks, Prize for the best presentation		

Functional Studies of *EHHADH* Gene Mutation During Craniofacial Development Using Zebrafish Model

Kinga Padała[1], Paulina Swatowska[1], Przemko Tylzanowski [1,2]

[1]Department of Biomedical Sciences, Laboratory of Molecular Genetics, Medical University of Lublin, Poland, [2]KU Leuven, Laboratory for Developmental and Stem Cell Biology, Belgium

Presenting author: Kinga Padała (email: k.padala17@gmail.com)

Lips and palate are formed during craniofacial development, a complex process involving migration of the neural crest cells (NCC) and their mesenchymal derivatives. NCC migration is controlled by multiple signalling pathways, including Sonic Hedgehog (Shh), Bone Morphogenic Protein (BMP) and Wnt which regulate NCC proliferation and migration Cleft lip with or without cleft palate (CL/P) is one of the most common congenital craniofacial defects, with a complex and incompletely explored aetiology involving genetic and environmental factors. We identified a Polish family with non-syndromic cleft lip linked to novel *EHHADH* gene mutation. *EHHADH* gene is associated with the peroxisomal fatty acid β-oxidation pathway. To explore the genotype-phenotype interaction we used *in vivo* zebrafish model. We performed microinjections at the single-cell stage using wild-type (WT) and mutant (MT) mRNA of the *EHHADH* gene and collected data from embryos to larvae over 5 days post-fertilisation (dpf). The injections resulted in detectable craniofacial deformities including cleft lip. To further investigate the phenotypes, zebrafish after separate WT and MT injections are subjected to 4 types of analysis: DAPI nuclear staining was used to visualise craniofacial morphology at the cellular level. Acridine Orange staining allowed us to assess neural crest cell migration and apoptosis. Pigmentation analysis was performed to evaluate NCC-derived melanophore development. Additionally, the Tg(*fli1*:EGFP) transgenic line was used to examine vascular development and possible disruptions to the craniofacial vasculature. Together, these approaches provide a broader functional characterisation of the *EHHADH* mutation and its role in craniofacial development**.**

Functional Characterization of *TPM2* Splice Mutation causing Skeletal Muscle Defects using Zebrafish

Sylwia Drzewiecka[1], Akshaya Ramanujam[1], Ewa Hordyjewska-Kowalczyk[2], Przemko Tylżanowski[3]

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Congenital clubfoot is among the most common congenital musculoskeletal malformations. Our research focuses on a novel *TPM2* splice-site mutation identified in a patient's family with congenital myopathy and contractural features. The mutation results in an in-frame deletion of 91 amino acids. It affects the structural integrity of *β-tropomyosin*, which is a key thin filament component in slow-twitch muscle fibers that regulates muscle contraction and is essential for normal musculoskeletal development. To understand better how this splice variant affects muscle function and development, we performed gain-of-function experiments via mRNA overexpression in zebrafish embryos. Wild-type or variant-encoding mRNA was injected at the single-cell stage, and embryos were evaluated at 3 and 5 days post-injection by visual assessment, followed by Alcian Blue and Calcein staining, and birefringence analysis. Visual assessment revealed pronounced anterior-posterior axis shortening, pericardial edema, cyclopia, and generalized muscle defects. Calcein and Alcian blue staining revealed abnormal cartilage development, supporting muscle-skeletal developmental axis perturbation compared to the embryos injected with wild-type mRNA. Birefringence analysis, used to assess the structural organization of muscle tissue, confirmed significant defects in sarcomere organization and myofibril alignment. Together, these complementary approaches aim to elucidate the mechanisms by which our *TPM2* splice variant contributes to musculoskeletal pathology.

Investigating Novel *TPM2* Variant-Induced Muscle Dysfunction Using Zebrafish Model

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Clubfoot is a congenital musculoskeletal deformity caused by abnormal development of bone, muscle, and connective tissue. Mutations reported in the *TPM2* gene, encoding β-tropomyosin, have been implicated in congenital myopathies and limb malformations, however, their precise functional consequences have yet to be clarified. This study investigates the pathogenic impact of the *TPM2* R101W novel mutation identified in a Polish family with clubfoot using zebrafish as a vertebrate model. Single-cell embryos were injected with wild-type or mutant *tpm2* mRNA, and developmental and muscular phenotypes were assessed through morphological, molecular, and imaging analyses. Phalloidin staining and Evans Blue Dye uptake evaluated myofibrillar organization, membrane integrity, and somite structure, while neuromuscular function was analyzed after mitochondrial inhibition with sodium azide. α-Bungarotoxin and SV2 labeling were used to visualize pre- and postsynaptic components of the neuromuscular junction (NMJ). Mutant larvae exhibited axial shortening, craniofacial defects, and poor somite boundary definition. Confocal microscopy images revealed disorganized and fragmented myofibrils with reduced density, while Evans Blue Dye indicated increased membrane permeability. Quantitative NMJ imaging showed decreased fluorescence intensity and disrupted structural organization, particularly under metabolic stress. These findings link mitochondrial dysfunction to neuromuscular pathology and demonstrate that *TPM2* R101W impairs both muscle development and NMJ formation. Overall, the data confirm *TPM2* R101W as a pathogenic variant responsible for defective muscle architecture and neuromuscular function, contributing to congenital myopathies such as distal arthrogryposis and clubfoot.

Age-related muscle glycogen loss in zebrafish (*Danio rerio*) model

Alicja Głowacka[1], dr hab. Magda Dubińska Magiera[1], prof. UWr

[1]*Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wrocław*

Presenting author: Alicja Głowacka (e-mail: 363919@uwr.edu.pl)

Danio rerio is an excellent model organism in vertebrate development research. Its genetic similarity to humans helps understand various metabolic processes occuring in the body. Glycogen is a polysaccharide in which the energy from consumed sugar is stored, mainly in the liver and skeletal muscles. Muscle glycogen specifically serves as a critical energy reserve for locomotor activity. While much is known about general glycogen metabolism, the correlation between age and glycogen levels in muscles remains under-explored. In this study, we compared skeletal muscle tissue from groups of fish of different ages. Quantitative analysis of glycogen was performer using the anthrone reagent. Our data indicate that aged zebrafish exhibit a reduction in glycogen levels compared to younger fish. Statistical analysis confirmed a strong downhill (negative) linear relationship between age and glycogen content in skeletal muscle of Danio rerio. This study suggests that aging in zebrafish is associated with significant quantitative changes in muscle glycogen. While current results show a decrease in glycogen levels with age, our research is ongoing and we want to expand our data to include additional age groups.

The effect of exposure of zebrafish (*Danio rerio*) embryos to aspartame at an early stage of development on their further development and anxiety

Małgorzata Rasińska[1]

[1]*Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn*

The consumption of artificial sweeteners such as aspartame still raises many safety questions. During the prenatal period, the developing organism is particularly sensitive to environmental and dietary factors, and some compounds can affect brain development and behavior in offspring. The aim of this study was to investigate the effects of aspartame on the development and anxiety behavior of the model organism zebrafish (Danio rerio) during human development. Approximately 360 zebrafish embryos were used in the study. They were exposed to aspartame (at concentrations of 10 mg/l, 100 mg/l, and 1000 mg/l) for the entire study period—5 days, starting 3 hours after fertilization. Observations were made using a stereomicroscope. On day 5 post-fertilization (dpf), their behavior was assessed using an automated system to track and analyze behavior, movement, and activity during exposure to a stressor called a rapid on/off light cycle. Changes in behavior were evident at all concentrations. Increased anxiety-like behavior was observed. No changes in morphology were observed. Aspartame caused delayed hatching compared to the control group. Aspartame exposure clearly alters zebrafish behavior, resulting in behavioral changes and increased anxiety-like behavior.

Keywords: (aspartame, danio rerio, anxiety)

Zebrafish larvae as a model to study behavior

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Although stress responses are essential for maintaining homeostasis, individuals differ in their behavioral and physiological reactions to environmental challenges. Zebrafish (*Danio rerio*) larvae are a well-established model for studying stress-related behaviors due to their relatively small size, robust behavioral responses, and evolutionarily conserved stress-related genes. The aim of this study was to establish an in-house experimental setup for imaging and quantifying zebrafish larvae behavior. To perturb zebrafish behavior, larvae were exposed to several environmental conditions, including salt, acute heat, and mechanical forces. Control and treated zebrafish larvae were recorded using a smartphone camera, and the images were analyzed using the open-source Fiji/ImageJ TrackMate plugin. Among the tested conditions, only NaCl treatment altered swimming behavior, and further experiments are underway to test the activation of the stress axis and/or brain responses. The imaging setup and open-source image analysis methodology provide affordable approach for studying zebrafish larval behavior.

Effects of Microplastics on Zebrafish Embryonic Development

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The continuously increasing production of plastic packaging and other everyday items made from synthetic polymers has a significant impact on the condition of the natural enviroment. Due to improper management of such waste, it is important to investigate the effects of microplastics on aquatic ecosystems, including the use of model organisms such as *Danio rerio*. The aim of this study was to evaluate the impact of microplastic particles on the embryonic development of *Danio rerio*. For this purpose, microplastic suspensions were prepared from everyday items such as dishwashing sponges and plastic cutting boards, and embryos were exposed to the tested factor. Embryonic development and the occurance of potential abnormalities were assessed based on microscopic observations. No statistically significant developmental abnormalities were observed in comparison to the control group. However, literature data indicate that the toxicity of microplastics may depend on particle characteristics, and morphological changes under enviromental conditions may be limited. The obtained results higlight the importance of further research on the diversity of microplastic particles and their potential toxicity to aquatic organisms, particularly during early developmental stages and in the context of aquatic ecosystem protection.

Key words: microplastics, zebrafish, model organism, toxicity

Does zebrafish ovaries suffer from nanoparticles exposure?

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Presenting author: Jan Pruszyński

The study aimed to assess the impact of silver nanoparticles (AgNPs) on the morphology, growth, and ovarian development of female zebrafish (Danio rerio) from 30 days post-hatching over a 100-day period. Fish were exposed to AgNP concentrations of 0 (control), 0.001, 0.01, and 1 mg/dm³. At the end of the experiment, all individuals were euthanized, measured for length and weight, and subjected to whole-body histological analysis. Mortality rates differed slightly between groups, with the highest mortality (15.46%) recorded in the 0.01 mg/dm³ group. Fish exposed to AgNPs showed reduced body size and weight compared to the control. The smallest individuals were observed in the 0.001 mg/dm³ group (mean total length: 18.81 ± 4.46 mm), while the lowest average body weight (0.10 ± 0.05 g) was found in the 1 mg/dm³ group. Exposure to the lowest concentration (0.001 mg/dm³) accelerated sexual maturation relative to the control, whereas higher concentrations (0.01 and 1 mg/dm³) delayed this process. This pattern was reflected in the distribution of oocyte developmental stages. In the control group, oogonia accounted for 51.42% of ovarian cells, and previtellogenic oocytes for 48.58%. In contrast, in the 0.01 and 1 mg/dm³ groups, the proportion of oogonia increased to 67.31% and 65.76%, respectively, accompanied by a decrease in previtellogenic oocytes (32.69% and 34.24%). Meanwhile, the 0.001 mg/dm³ group showed more advanced ovarian development, with 58.67% previtellogenic oocytes and 41.33% oogonia. Overall, the results indicate that prolonged exposure to silver nanoparticles in aquatic environments can negatively affect zebrafish growth and may disrupt ovarian development and sexual maturation.

Evaluation of Isopropyl U-47700 toxicity in zebrafish model

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The opioid overdose crisis is known to be a significant issue worldwide. Recently, the wave of synthetic opioids (SOs) abuse has occurred resulting in countless fatalities and therefore SOs are currently considered a threat to public health and challenge for law enforcement. For that reason, SOs being a diverse group of high potency opioids, can be divided into fentanyl analogs and non-fentanyl compounds. One of the non-fentanyl related drugs that has risen popularity on illicit drug markets is Isopropyl U-47700. In this study the Fish Embryo Acute Toxicity was performed for six U-47700 concentrations and E3 as a negative control group. Behavioral analysis and heart rate assessment were carried out for zebrafish larvae at 96 hours post fertilization (hpf). Additionally, the tail coiling assay (TCA) was conducted on zebrafish larvae at 20-24 hpf for four concentrations of U-47700. Finally, light/dark motor response test with thigmotaxis was executed for three concentrations of U-47700 and for negative control group E3. Evaluation of behavioral assay resulted in dose-dependent reduction in total distance travelled in the dark and significant compound-specific reduction in heart rate. Moreover, TCA showed concentration-dependent increase in burst activity and burst count per minute at 10 μM. These results present toxic effects of U-47700 administration in zebrafish model. Nevertheless, further research and analyses are crucial to assess the toxicity of U-47700 in zebrafish model.

Interaction toxicity of ibuprofen and ethanol in zebrafish model.

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Ibuprofen is a commonly used drug with anti-inflammatory, analgesic and antipyretic characteristics. Despite its wide availability there are not many studies focusing on its effect on embryonic stages of development in organisms. The aim of this study was to investigate the toxicity of ibuprofen on Danio rerio embryos. Additionally, the interaction between ibuprofen and ethanol was studied on the model. Fish Embryo Acute Toxicity test was performed following Test Guideline No 236. Zebrafish embryos were observed for developmental changes; heart rate was measured at 48 hpf and hatching rate was determined. This study showed that high concentrations of ibuprofen caused developmental changes such as heart oedema and changes in yolk size, and affected body measurements. Furthermore, high concentrations of the drug caused a delay in hatching.

Toxicity, bioaccumulation, and antitumor evaluation of organometallic compounds in *Danio rerio* larvae

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Cancers are considered a major problem in 21st-century medicine, owing to their complexity and the difficulty of effective treatment. The rising cancer-related mortality, the side effects of current chemotherapy, and the development of resistance to cancer drugs emphasize the urgent need for novel anticancer agents with unique mechanisms of action. Promising alternatives to traditional chemotherapeutics include metallocompounds (e.g., with Ru, Ir, Fe and Os) that may potentially induce ferroptosis, a type of programmed cell death. Recently, we have synthesized and characterized novel anticancer heteronuclear Ru(II)–Cu(II) complexes with phosphine–fluoroquinolone conjugates, with one being encapsulated in bilosomes*. However, their *in vivo* properties remain largely unexplored. These studies aim to examine the toxicity and bioaccumulation of the selected compounds and to assess their potential effects on prostate cancer cells using the zebrafish larvae (*Danio rerio*) model. We performed the Fish Embryo Acute Toxicity (FET) test, in which *Danio rerio* larvae were exposed to the tested substances at different concentrations, and the effects were evaluated at 24, 48, 72, and 96 hours after exposure. Further, Inductively Coupled Plasma (ICP) analysis was conducted to determine the accumulation of metals. Additionally, we performed zebrafish xenografts of prostate cancer cells into the larvae to examine the effect of the selected encapsulated compound on tumor growth. Ongoing results will be presented.

This research was funded by the National Science Centre (NCN), Poland, under the project no. UMO-2023/51/B/ST4/00355. Natalia Łępa additionally received funding from the Medical University of Lublin for the execution of her bachelor's thesis project.

*Kozieł S., et al., (2025). Bilosomal encapsulation of binuclear phosphino Ru (II)–Cu (II) compounds enhances their selectivity and activity toward lung and prostate cancers. *Journal of Medicinal Chemistry*, 68(14), 14442-14464.

Assessment of the antiseizure effect of *Carlina acaulis* extracts in Pentylenetetrazole-Induced seizure model in *Danio rerio* larvae.

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Epilepsy is one of the most common chronic neurological disorder that affects around 50 million people around the world. The disorder is characterized by the occurrence of recurrent, unprovoked seizures. These seizures are the clinical manifestation of abnormal, excessive, and synchronous electrical activity of neurons. Pharmacotherapy plays a crucial role in the suppression and control of epileptic seizures, the main targets being sodium channels or GABA-A receptors. Although a broad range of antiseizure medications has been developed, approximately 30% of patients develop drug-resistant epilepsy, in which pharmacological treatment fails to achieve adequate seizure control. Therefore, there is a continuous need to search for new substances with antiseizure activity. Natural compounds of plant origin have repeatedly demonstrated beneficial effects in the attenuation of epileptic seizures, potentially due to their diverse biological activities, including anti-inflammatory and neuroprotective properties. In the present study, we evaluated the antiseizure activity of extracts of the leaves and roots of *Carlina acaulis*. To assess their antiseizure potential, we employed the pentylenetetrazole (PTZ)-induced seizure model in *Danio rerio* larvae up to 5 days post-fertilization (dpf), a well-established experimental paradigm based on chemically evoked hyperlocomotion resulting from GABA-A receptor antagonism. Based on the conducted experiments, we demonstrated that the leaf extract of *Carlina acaulis* exhibits antiseizure activity in the applied experimental model.

Key words: epilepsy, *Carlina acaulis*, pentylenetetrazole, *Danio rerio*

Zebrafish as a Model for Evaluating the Anticancer Activity and Toxicity of Artemisinin

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Artemisinin, a well-known antimalarial compound derived from sweet wormwood (*Artemisia annua*), has recently attracted attention for its possible anticancer properties. This study aimed to assess its therapeutic potential using both *in vitro* cancer cell cultures and zebrafish (*Danio rerio*) as an innovative *in vivo* model system. The effects of artemisinin were compared with those of the conventional chemotherapeutic agent 5-fluorouracil (5-FU) across several cancer cell lines, including colon (HCT116), liver (HepG2), and breast (CAL51) cancer cells, as well as healthy CHO-K1 cells. Artemisinin demonstrated selective cytotoxicity, with higher IC_{50} values in normal cells compared to cancer lines. In HepG2 cells, treatment with artemisinin led to a marked increase in intracellular iron levels, suggesting ferroptosis as a potential mechanism of action, whereas 5-FU did not affect iron homeostasis. Zebrafish embryos were then used to evaluate developmental toxicity and antitumour efficacy *in vivo*. Artemisinin exhibited dose-dependent toxicity, with an LD_{50} of approximately 20 µg/mL, and caused significant bradycardia at higher concentrations. In zebrafish xenograft models bearing HCT116 or HepG2 tumours, artemisinin effectively reduced tumour growth at concentrations of 10–20 µg/mL, with minimal toxicity observed in HepG2 grafts. These findings highlight the usefulness of the zebrafish model in simultaneously assessing both the safety and anticancer activity of natural compounds. Overall, this study supports further exploration of artemisinin as a promising candidate for ferroptosis-based cancer therapy.

Zebrafish-Based Screening of Pumpkin Extracts for Modulation of Oxidative Stress Responses

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Elevated generation of reactive oxygen species (ROS) induces molecular alterations in living organisms, including disruption of metabolic pathways, impaired DNA synthesis, and accumulation of mutations. These processes can lead to developmental abnormalities, morphological defects, and activation of programmed cell death (apoptosis). Natural compounds with antioxidant properties, particularly plant-derived extracts, are increasingly investigated as potential protective agents against oxidative stress. Pumpkin (Cucurbita spp.) is a rich source of carotenoids and polyphenols, making it a promising candidate for such studies. The aim of this study was to evaluate the effects of pumpkin-derived extracts on zebrafish embryos (Danio rerio) under oxidative stress conditions. Oxidative stress was induced using hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl). H_2O_2 at a concentration of 10 mM resulted in 100% mortality, regardless of the solvent used, whereas 0.5 mM completely inhibited hatching without increasing mortality. In contrast, NaOCl at tested concentrations (30 and 300 μg/L) did not significantly affect larval survival or morphology compared to the E3 control. Pumpkin extracts derived from flesh, rind, and peel were generally well tolerated; however, an increased incidence of oedema was observed (20–42%) compared to the control (15%). The solvents used (DMSO and rapeseed oil) did not exhibit detectable toxicity. Overall, pumpkin extracts appear to be toxicologically safe under the tested conditions. However, no clear protective effect against oxidative stress was demonstrated, and their potential antioxidant role in vivo requires further investigation.

The role of 25-hydroxycholesterol in the antiviral response of zebrafish (*Danio rerio*)

Artem Wojciechowski[1], Justyna Starzyk[1], Maria Zawisza[1],

Magdalena Chadzińska[1], Mikołaj Adamek[2], Krzysztof Rakus[1].

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Presenting author: Artem Wojciechowski (e-mail: artem.wojciechowski@student.uj.edu.pl)

25-hydroxycholesterol (25HC) is a member of the family of cholesterol oxidation derivatives called oxysterols. Its synthesis may be catalysed either by reactive oxygen species (ROS) or by 25-cholesterol hydroxylase (ch25h). 25HC is known to exert biological activity against the range of enveloped and non-enveloped viruses, however, knowledge about its role in the antiviral response in fish is very limited. In our project we have studied the role of 25HC in viral replication using zebrafish model. Zebrafish larvae (2 dpf, AB-TL line) were intrapericardially injected with 25HC or vehicle and 2 hour later infected with tilapia lake virus (TiLV, enveloped), spring viraemia of carp virus (SVCV, enveloped) and nervous necrosis virus (NNV, non-enveloped). The fish injected with 25HC exhibited significantly lower viral load upon infection with all the viruses studied. Moreover, we studied the impact of 25HC on the expression of genes encoding proteins involved in type I interferon pathway, proinflammatory cytokines and *ch25hb* during infection with studied viruses. We demonstrated that 25HC had almost no effect on the expression of studied genes. We have also derived zebrafish *ch25hb*$^{-/-}$ mutant line with 5-bp-long deletion using CRISPR/Cas9 method. Upon TiLV infection, viral load was significantly higher in *ch25hb*$^{-/-}$ zebrafish larvae compared to *ch25hb*$^{+/+}$ controls, which suggests that knock-out of *ch25hb* compromises the host's capacity to control TiLV replication. Our work clearly demonstrated that 25HC influences the viral replication in zebrafish larvae.

Bacterial-derived inhibitor miropin protects against systemic infection with oral pathogens in a zebrafish larval model

Paulina Jędrzejczyk[1], Kinga Rarata[1,2], Jan Potempa[3,4], Magdalena Widziołek[1]

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Periodontitis (PD) is a chronic inflammatory disease of the tooth supporting tissues, driven by a dysbiosis of the oral microbiome. It is often associated with systemic conditions such as cardiovascular diseases. Porphyromonas gingivalis (Pg), a key-stone pathogen in PD, produces cysteine proteases known as gingipains that degrade host proteins and manipulate immune responses. Pathogens in dental plaque developed adaptive mechanisms allowing them to persist in the same ecological niche, e.g. Tanerella forsythia (Tf) produces miropins, a unique serine protease inhibitors. Although miropins have been reported to inhibit gingipains, their broader role in neutralizing virulence factors of coexisting pathogens remains poorly understood. The aim of this study was to investigate the safe dose, delivery route and potential therapeutic effect of miropins against systemic infection with Pg and Fusobacterium nucleatum (Fn) using zebrafish larvae. We employed engineered miropin variants: mirRVK (inhibiting gingipains Kgp and Rgp), mirVKT (inhibiting Kgp), mirVAT (potentially inhibiting host proteases but not gingipains), or mirGGGG (inactive control). Pre-injection of zebrafish larvae with mirRVK prior to Pg infection significantly improved survival and reduced disease symptoms. In addition, this variant, but no others, significantly downregulated the expression of pro-inflammatory genes. These findings indicate that simultaneous inhibition of all major gingipains is essential to counteract pathogenicity of Pg. Our results also suggests that mirRVK improves larval survival following infection with other highly inflammagenic oral pathobiont Fn. Together, our findings demonstrate that miropins can attenuate Pg and Fn infection in vivo, highlighting their potential as novel therapeutics in PD and PD-associated systemic complications.

From Plant to Model: Assessing the Antidiabetic Potential of *Angelica archangelica* Root Extracts in Zebrafish Larvae

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Diabetes mellitus remains one of the most pressing global health challenges, with current pharmacological therapies frequently limited by side effects and insufficient glycemic control in a significant proportion of patients. Growing interest in phytotherapy has prompted investigation into plant-derived bioactive compounds with antidiabetic potential. *Angelica archangelica*, rich in coumarins, terpenoids, and flavonoids, has demonstrated promising antioxidant, anti-inflammatory, and metabolic properties. Among its constituents, imperatorin has been shown to stimulate GLP-1 secretion and inhibit key carbohydrate-metabolizing enzymes, suggesting meaningful antidiabetic activity.

Our study evaluated the safety and compound profile, as well as the effect of *Angelica archangelica* root extracts on glucose metabolism, using a zebrafish larval model.

The methodology covered three sequential phases. First, embryotoxicity was assessed by exposing 3–4 hpf embryos to five concentrations of 100% and 30% methanol-based *Angelica archangelica* extracts, evaluating coagulation and heartbeat rate. Second, following establishment of the optimal extract concentration, 3 dpf larvae were incubated in that concentration for 1 hour, then stained with 2-NBDG for 3 hours to assess glucose uptake. Third, larvae were anesthetized and imaged using fluorescence microscopy, with images analyzed in Fiji to quantify fluorescence intensity within the yolk sac region.

Results are expected to provide new insights into the antidiabetic potential of *Angelica archangelica* and support the development of phytotherapy-pharmacotherapy strategies for improved glycemic control and reduced complication risk in diabetic patients.

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Anti-obesity effects of innovative food formulations in a zebrafish diet-induced obesity model

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Zebrafish is known for its genetic and physiological similarities to humans and serves as an important model organism in biomedical research, including studies on metabolic disorders such as obesity, which is a chronic, complex disease affecting over one billion people worldwide. Diet-induced obesity in zebrafish leads to alterations similar to those observed in humans, and is associated with comparable pathophysiological mechanisms. Obese individuals are characterized by enlarged visceral fat and metabolic abnormalities. The aim of this research was to investigate the effects of novel food formulations in the diet-induced obesity (DIO) zebrafish model and to determine their potential in the obesity management. The safety of the products was assessed using toxicity evaluation and zebrafish microinjection. Then, the zebrafish obesogenic test was conducted, using three foodstuffs (formulation F1, F2 or F3). Visceral fat tissue was measured using Nile Red staining. To evaluate the effects of the products in the DIO model, standard food was enriched with 10% of the F1, F2 or F3 formulation. After 3 weeks, body weight, glycemia and lipid profile were measured. The food products in the F1, F2 and F3 formulations contributed to a significant reduction in body weight and the levels of glucose, triglycerides and cholesterol, compared to the obese control group. Additionally, the treatment resulted in a visible reduction in visceral fat tissue content. The results indicate that the tested products have beneficial effects on glycemia and lipid profile, and effectively prevent the development of obesity.

Broken hearts and leaky vessels: The off-target cardiovascular effects of Ibrutinib

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Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, has revolutionized the treatment of hematological malignancies. However, its clinical application is increasingly complicated by severe cardiovascular adverse events. Understanding the mechanisms underlying these toxicities requires robust *in vivo* models. The transparent larval zebrafish (*Danio rerio*) offers a non-invasive real-time imaging of both vascular dynamics and cardiac function. This study aimed to investigate the toxic effects of ibrutinib in zebrafish larvae. We used transgenic reporter line *Tg(kdrl:mTurquoise)* combined with lightsheet fluorescence microscopy (LSFM) to visualize the cardiovascular system. To assess vascular dysfunction, we performed FITC-dextran microangiography to determine potential vascular leakage and vasoconstriction. We also analyzed heart function by measuring ventricular fractional shortening and area change. Additionally, RT-qPCR was performed to analyze endothelial, cardiac, inflammatory and oxidative stress gene markers. Exposure to ibrutinib caused significant cardiovascular damage. LSFM imaging showed severe vascular dysfunction, including enhanced permeability and vasoconstriction in the dorsal aorta. Cardiac function collapsed, evidenced by a decline in ventricular fractional shortening and area change, leading to severe hemodynamic compromise. These physiological defects were confirmed at the molecular level by the upregulation of the stress markers. The exact molecular mechanisms driving these off-target effects are currently under investigation in our laboratory. Our results suggest that combining lightsheet imaging with gene expression analysis in zebrafish provides a highly effective model to study the toxicity of targeted therapies and screen potential protective drugs.