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2025
Conference

The 3rd International Online Conference on Toxins

10–12 September 2025 | Online

Program and Abstract Book

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The 3rd International Online Conference on Toxins

10–12 September 2025 | Online



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Prof. Dr. Jay W. Fox

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Dr. Panagiota Katikou
Dr. Saji George
Prof. Dr. Joseph Barbieri

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Welcome from the Chair

I am excited to announce the launch of the **3rd International Online Conference on Toxins (IOCT 2025)**. The conference, organized by the MDPI open-access journal *Toxins* (ISSN: 2072-6651; Impact Factor: 4.0), will be held **online from 10 to 12 September 2025**.

Building on the success of our previous online conferences, we will continue this year but with a new addition to our program. In addition to the Best Oral Presentation Award and the Best Poster Award, the IOCT 2025 conference is proud to introduce the **Young Investigator Award**. This award is designed to honor and encourage the outstanding achievements of young researchers in toxins research. We warmly welcome submissions from young investigators for this special category.

There will be six areas of focus at IOCT 2025, providing ample opportunities for the written and oral presentation of new, exciting studies in toxinology. The main topics and sessions of the conference are as follows:

1. Plant, Animal, Insect and Microbial Toxins: New Developments;
2. Novel Insights on The Mechanism of Action and/or Pathophysiology of Toxins;
3. Use of Toxins as Tools for Research, Drug Discovery, and Therapeutics;
4. Impact of Toxins on Public Health;
5. Impact of Toxins on Agriculture;
6. Foodborne Toxins.

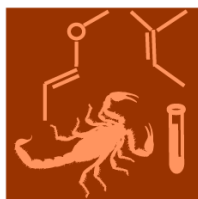
I hope that you will consider submitting your recent research findings and insights with us at the conference. I believe that your contributions would greatly enrich our scientific exchange and foster collaborative discussions among attendees.

Kind regards,



Prof. Dr. Jay W. Fox
Conference Chair

Department of Microbiology,
University of Virginia,
Charlottesville, VA, USA



toxins

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Toxins (ISSN 2072-6651) is an international, peer-reviewed open access journal which provides an advanced forum for studies related to toxins and toxicology. It publishes reviews, regular research papers and short communications. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the maximum length of the papers. The full experimental details must be provided so that the results can be reproduced. There are, in addition, unique features of this journal:

- manuscripts regarding research proposals and research ideas will be particularly welcomed
- electronic files or software regarding the full details of the calculation and experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material
- we also accept manuscripts communicating to a broader audience with regard to research projects financed with public funds

Impact Factor: 4.0 (2024); 5-Year Impact Factor: 4.5 (2024)

Session Chairs



Prof. Dr. Jay W. Fox
Department of Microbiology,
University of Virginia,
Charlottesville, VA, USA



Prof. Dr. Joseph Barbieri
Department of Microbiology
and Immunology, Medical
College of Wisconsin,
Milwaukee, WI, USA



Prof. Dr. Nilgun E. Tumer
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Dr. Panagiota Katikou
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Thessaloniki, Greece



Dr. Saji George
Department of Food Science
and Agricultural Chemistry,
McGill University, Montreal,
QC, Canada



**Prof. Dr. Juan Luis
Jurat-Fuentes**
University of Tennessee at
Knoxville, Entomology and
Plant Pathology, Knoxville, TN,
USA



Dr. Antonio Moretti
Institute of Science of Food
Production (ISPA), National
Research Council (CNR), Bari,
Italy

Keynote Speakers



Dr. Ashleigh Paparella
The University of Sydney
School of Medical Sciences,
Camperdown, Australia



Dr. Michael J. Rudolph
New York Structural Biology
Center, New York, NY, USA



Dr. Andreas Rummel
Hannover Medical
School|MHH·Institute for
Toxicology, Hannover,
Germany



Prof. Dr. Colin Berry
Cardiff School of
Biosciences, Cardiff
University Park Place, Wales,
UK



Dr. Mònica Campàs
Institut de Recerca i
Tecnologia Agroalimentàries
(IRTA), La Ràpita, Spain

Invited Speakers



Assoc. Prof. Shahzad Zafar Iqbal

Postdoctoral Fellow (UPM), Visiting
Scientist (Rutgers, New Brunswick,
NJ, USA),
Department of Applied Chemistry,
Government College University,
Faisalabad, Faisalabad, Pakistan

Program at a Glance

10th September - Morning	11th September - Morning	12th September - Morning
Session 4. Impact of Toxins on Public Health Session 5. Impact of Toxins on Agriculture	Flash Poster Session	
10th September - Afternoon	11th September - Afternoon	12th September - Afternoon
Session 6. Foodborne Toxins Session 1. Plant, Animal, Insect and Microbial Toxins: New Developments	Session 3. Use of Toxins as Tools for Research, Drug Discovery, and Therapeutics	Session 2. Novel Insights on the Mechanism of Action and/or Pathophysiology of Toxins

IOCT 2025 Program

10 September 2025 (Wednesday)

Morning Session

Session 4: Impact of Toxins on Public Health

Session 5: Impact of Toxins on Agriculture

Time: 9:00 (CEST, Basel) | 3:00 (EDT, New York) | 15:00 (CST Asia, Beijing)

CEST	Speaker	Title
9:00–9:05	Prof. Dr. Jay W. Fox Event Chair	<i>Opening Speech by the Event Chair</i>
9:05–9:10	Dr. Panagiota Katikou Session Chair	<i>Welcome from the Session Chair</i>
9:10–9:40	Dr. Mònica Campàs Keynote Speaker	Tracking Invisible Threats with Bioanalytical Strategies: Challenges and Innovations in the Detection of Marine Toxins.
9:40–9:55	Jaume Reverté Selected Speaker	Combined Detection of Tetrodotoxin and Saxitoxin in Seafood with a Multiplexed Electrochemical Biosensor
9:55–10:10	Victor Hugo Gonçalves Pinto Selected Speaker	Development and deployment of a multiplex qPCR assay for seb, stx1, and stx2 detection into the Brazilian Army biodefense system.
10:10–10:25	Wageha Awad Selected Speaker	Differential effects of deoxynivalenol and deepoxy-deoxynivalenol on Campylobacter jejuni growth and gene expression
10:25–10:30	Prof. Dr. Juan Luis Jurat-Fuentes Dr. Antonio Moretti Session Chairs	<i>Welcome from the Session Chair</i>
10:30–11:00	Prof. Dr. Collin Berry Keynote Speaker	Bacillus Thuringiensis Toxins for the Control of Pests of Agriculture
11:00–11:15	Laura Fiorella Cadenillas Sueldo Selected Speaker	Spray Drying Microencapsulation Preserves Anti-Aflatoxin B1 Activity of Plant Aqueous Extracts
11:15–11:30	Sophie Cowne Selected Speaker	The Burden of Snakebite Envenomation in Livestock: Evidence from Rural Communities in Tamil Nadu
11:30–11:45	Heltan M. Mwalugha Selected Speaker	Physiological and biochemical effects of toxigenic Aspergillus flavus on maize (Zea mays)
11:45–14:00	Break	

10 September 2025 (Wednesday)

Afternoon Session

Session 6: Foodborne Toxins

Session 1: Plant, Animal, Insect and Microbial Toxins: New Developments

Time: 14:00 (CEST, Basel) | 8:00 (EDT, New York) | 20:00 (CST Asia, Beijing)

CEST	Speaker	Title
14:00–14:05	Dr. Panagiota Katikou Dr. Saji George Session Chairs	<i>Welcome from the Session Chairs</i>
14:05–14:25	Assoc. Prof. Shahzad Zafar Iqbal Invited Speaker	The Uninvited Guests at Our Table: Understanding Foodborne Toxins
14:25–14:40	Reinhard Zeleny Selected Speaker	Towards a certified reference material (CRM) for the emetic toxin cereulide in cooked rice: a characterisation study
14:40–14:55	Paula María Poquet-Blat Selected Speaker	Detection of Ciguatoxins in Fish Species from two hotspots Coastal Areas of Gran Canaria (Spain): A Two-Year Study
14:55–15:10	Adrián Fuente-Ballesteros Selected Speaker	Natural Toxins in the Food Chain: A Study on Alkaloid Residues in Honey
15:10–15:15	Prof. Dr. Joseph Barbieri Session Chair	<i>Welcome from the Session Chair</i>
15:15–15:45	Dr. Andreas Rummel Keynote Speaker	Two recipes to food-borne botulism

15:45–16:00	Paulo Vale Selected Speaker	Transition metal doping for improving the fluorescence response of paralytic shellfish poisoning toxins upon pre-chromatographic oxidation
16:00–16:15	Federico Camicia Selected Speaker	Snake venom toxicity in <i>Caenorhabditis elegans</i>
16:15–16:30	Fazlurrahman Khan Selected Speaker	Attenuation of Extracellular Toxin Production in <i>Pseudomonas Aeruginosa</i> by Green-synthesized Nanoparticles
16:30–16:45	Akanksha Mishra Selected Speaker	Smart Detection: Application of Artificial Intelligence to Uncovering Mycotoxin Contamination in Foods

11 September 2025 (Thursday)

Morning Session
Flash Poster Session

Time: 9:00 (CEST, Basel) | 3:00 (EDT, New York) | 15:00 (CST Asia, Beijing)

CEST	Speaker	Title
9:00–9:05	Ana Vulić Poster Presenter	Prevalence of Fusarium Mycotoxins in Cereals Harvested in Croatia
9:05–9:10	Yuri Utkin Poster Presenter	Effects of Heterodimeric Phospholipase A2 from the Venom of <i>Vipera Nikolskii</i> on Rat Cardiomyocytes
9:10–9:15	Cristina Plata-Calzado Poster Presenter	Identification of potential metabolites of Cylindrospermopsin and Microcystin-LR and Effects on Brain Oxidative Stress Biomarkers in Rats after Oral Exposure to their Mixture
09:15–09:20	Salvina Panebianco Poster Presenter	Discrimination of Geographical Origin and Detection of Aflatoxins in Pistachio Seeds Using FT-IR Spectroscopy
09:20–09:25	Lourdes Barreiro Crespo Poster Presenter	Evaluation of Cyclodextrins Polymer as a Possible Clean-up Method for Detecting CTX-like Compounds in <i>Gambierdiscus</i> Cultures
09:25–09:30	Ewelina Kowalczyk Poster Presenter	Determination of Cannabinoids in Hemp Seeds, Oil, and Tea Samples Using LC-MS/MS
09:30–09:35	Thales Alves de Melo Fernandes Poster Presenter	Anti-Toxoplasma Gondii Mechanisms of a Lys49-PLA2 from Bothrops Moojeni Snake Venom
09:35–09:40	Barbara Streit Poster Presenter	From Research to Application—Proving the Efficacy of ZENZyme® in Swine
09:40–09:45	Tina Lešić Poster Presenter	Dietary Exposure to Aflatoxin B1 and Its Precursor Sterigmatocystin from Traditional Meat Products in Croatia
09:45–09:50	Zuzanna Tomkielska Poster Presenter	Discovery of Novel Ion Channel Modulators from <i>Physalia physalis</i>
9:50–09:55	Iara Aimê Cardoso Poster Presenter	Production and Functional Analysis of <i>Echis coloratus</i> Disintegrins in Platelet Aggregation
09:55–10:00	Alex Gardner Poster Presenter	Intracellular Trafficking Botulinum Neurotoxin Light Chain A1 to cleave of Plasma Membrane-Bound SNAP-25
10:00–10:05	Jonatan Dorca-Arévalo Poster Presenter	Epsilon Toxin from <i>Clostridium perfringens</i> Induces the Generation of Extracellular Vesicles in T-Lymphocytes
10:05–10:10	Iasmyn Costa da Silva Poster Presenter	Perinatal screening of <i>Crotalus durissus terrificus</i> venom in rats
10:10–10:15	Yefermin Darias Poster Presenter	Could ciguatoxins cause structural changes in fish tissue?

11 September 2025 (Thursday)

Afternoon Session
Session 3: Use of Toxins as Tools for Research, Drug Discovery and Therapeutic

Time: 13:00 (CEST, Basel) | 7:00 (EDT, New York) | 19:00 (CST Asia, Beijing)

CEST	Speaker	Title
13:00–13:05	Prof. Dr. Nilgun E. Tumer Session Chair	<i>Welcome from Session Chair</i>
13:05–13:35	Dr. Ashleigh Paparella Keynote Speaker	Inhibition of <i>Clostridioides difficile</i> TcdA and TcdB toxins with transition state analogues
13:35–14:05	Dr. Michael J. Rudolph Keynote Speaker	Structure-Guided Strategies Against Ricin and Shiga Toxins

14:05-14:20	David Mona Selected Speaker	Drosophila as an In Vivo Model for Screening Neurotoxicity in Physalia physalis Venom Fractions
14:20-14:35	Duarte Toubarro Selected Speaker	Dual Modulation of Hemostasis Using Physalia physalis Venom: Insights into Thrombin-Like and Fibrinolytic Activities
14:35-14:50	Gabriela Mathias de Andrade Selected Speaker	Purification and Biochemical Characterization of a Probable P-IIIId Class Metalloprotease from Bothrops atrox Venom Active in Platelet Aggregation
14:50-15:05	Carolina Meireles Bastos Rodrigues Selected Speaker	Purification and Characterization of C-type Lectin from Bothrops Leucurus Snake Venom with Anti-platelet and Anti-metastatic Potential
15:05-15:20	Laura Gomes Caldeira Selected Speaker	Antioxidant molecules in the sea anemone Anthopleura cascaia and their application in Alzheimer's disease
15:20-15:35	Nevin Jaison Selected Speaker	Toxin tools for functional nanocrystal production
15:35-15:50	Amanda Rosa Selected Speaker	Evaluation of Antiplatelet Effects of Type C Lectin from Bothrops Neuwiedi Venom
15:50-16:05	Caleb Alejandro Aguayo Selected Speaker	Modeling Neuromuscular Junction Intoxication Using Human iPSC-derived Organoids

12 September 2025 (Friday)**Afternoon Session****Session 2: Novel Insights on the Mechanism of Action and/or Pathophysiology of Toxins****Time: 14:00 (CEST, Basel) | 8:00 (EDT, New York) | 20:00 (CST Asia, Beijing)**

CEST	Speaker	Title
14:00-14:10	Prof. Dr. Jay W. Fox Prof. Dr. Joseph Barbieri Session Chairs	<i>Welcome from Session Chairs</i>
14:10-14:40	TBA Keynote Speaker	TBA
14:40-14:55	Bouchra Darkaoui Selected Speaker	Development and Comparative Evaluation of Antivenoms Targeting the Pathophysiological Effects of Scorpion Venom Toxins in North Africa
14:55-15:10	Michelle Khai Khun Yap Selected Speaker	Time- and Dose-dependent Intracellular Proteome Alterations by Cobra Venom Cytotoxin: System Toxicology and Mechanistic Perspective
15:10-15:25	Camila M. Carvalho Santos Selected Speaker	Use of Oseltamivir Phosphate for the Treatment of Thrombocytopenia Induced by Experimental Bothrops Jararaca Envenomation in a Murine Model
15:25-15:40	Mia Schezaro-Ramos Selected Speaker	In Vitro Neuromuscular Blockade Caused by Micrurus surinamensis Venom
15:40-15:55	Adrielly Viveiros Torres Selected Speaker	C-type Lectin-Like Proteins (CTLs) Present in Bothrops Jararaca Venom that Induce Thrombocytopenia: Purification and Mechanism of Action
15:55-16:10	Alejandra Bravo Selected Speaker	In Vivo High-Resolution MSSR Microscopy Reveals Cry Toxin-Induced Actin Disruption, Endocytic Activity, and Vesicle Release in Insect Midgut Cells
16:10-16:25	Stefanie Menzies Selected Speaker	The Unexpected Biochemical Repertoire of Mamba Intestinal Toxin
16:25-16:40	Bálint Üveges Selected Speaker	No Gain from Pain: Lack of Nociceptor Activation Suggests No Selection for defence in the Evolution of European Viper Venoms
16:40-16:55	Noah Souza Selected Speaker	Using Omics to Characterize the Role of ArtAB Toxin in Salmonella Pathogenesis

Session 1. Plant, Animal, Insect and Microbial Toxins: New Developments

sciforum-125430: *Boletus satanas* Lenz, a toxic wild mushroom from the northwest Algerian coast: morphological identification and mycochemical screening

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⁷ Pharmacy Department, Faculty of Medicine, University of Abou Bekr Belkaïd Tlemcen, 13000 Tlemcen, Algeria

⁸ Medicine Department, Faculty of Medicine, University of Abou Bekr Belkaïd, Tlemcen, 13000, Algeria

Toxic organisms are capable of producing harmful substances that can adversely affect other living organisms, including humans. Some mushrooms produce powerful toxins that can cause serious poisoning. *Boletus satanas*, with its impressive size and colour, is also renowned for its toxicity. It causes severe gastrointestinal disorders, with violent symptoms. It deserves particular attention due to its resemblance to certain edible boletus. This survey was carried out near the coastal region of Ghazaouet, in the Traras mountains, Tlemcen wilaya. The aim of the present study is to characterize the morphological features of this species and subsequently investigate the myco-chemical screening of the hydro-methanolic extract for the purpose of determining the predominant chemical families of its secondary metabolites using simple methods and techniques that can be rapidly applied. The results of mycochemical screening performed on the extract revealed the presence of substances belonging to classes of active compounds, including alkaloids, coumarins, free quinones, flavonoids, reducing compounds, terpenoids, and saponins. Tannins, anthraquinones and anthocyanins were absent. The morphological determination of this mushroom is based on a series of macroscopic and microscopic criteria. This mushroom is an ectomycorrhizal basidiomycete that grows mainly on calcareous soils, in symbiosis with some broadleaved trees. Its distinctive features include a massive morphology and contrasting colors. It can be recognized by its large pale cap, its red pores, its pot-bellied yellow stem with a red network, and its flesh, which turns blue when cut. Under the effect of Melzer's solution, the stipe undergoes an inamyloid reaction. The spore is olive brown to brown in colour. The spores measure 10-15 x 5.5-6.5 µm and are elliptical to sub-fusiform. These preliminary results encourage further research to assess and evaluate their biological activities.



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sciforum-125859: Identification of receptor-binding domains of Bacteroidales antibacterial pore-forming toxins

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Bacteroidales are abundant Gram-negative bacteria that are present in the gut microbiota of most animals, including humans, where they carry out functions that are vital to the health of their host. To thrive in this competitive environment, Bacteroidales use sophisticated weapons to outmatch their competitors. Among these, BSAPs (Bacteroidales Secreted Antimicrobial Proteins) represent a novel class of bactericidal pore-forming toxins that are highly specific to their receptor, typically targeting only a single membrane protein or lipopolysaccharide. The molecular determinants conferring this high selectivity remain unknown. In this study, we therefore investigated the model protein BSAP-1 and determined which of its domains is involved in providing receptor specificity. We demonstrate that receptor recognition is entirely driven by the C-terminal domain (CTD) of BSAP-1 using a combination of in vivo competition assays, in vitro protein-binding studies, and structural analysis. Specifically, we show that deletion of the CTD abrogates BSAP-1 bactericidal activity by preventing receptor binding, while grafting the CTD to unrelated carrier proteins enables CTD-driven interaction with the BSAP-1 receptor. Building upon this discovery, we show that BSAPs can be categorized according to the structure of their CTD and that BSAPs within the same cluster are likely to target the same type of receptor. Additionally, we show that the CTD of BSAP-1 can be repurposed to generate probes for fluorescent labelling of membrane proteins in live cells. In summary, our research demonstrates that BSAP receptor recognition is driven by their CTD and that these can be engineered to develop novel tools for the investigation of Bacteroidales biology.



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sciforum-125805: Smart Detection: Application of Artificial Intelligence to Uncovering Mycotoxin Contamination in Foods

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Globally, mycotoxin contamination of food poses a severe hazard to public health and food safety. One of the most serious hazards to human health is the contamination of agricultural products with mycotoxins, which are toxic secondary metabolites generated by fungi. Chromatographic separation is a popular approach to detecting mycotoxins, often used in conjunction with mass spectrometry, which is an accurate method but requires specialized staff and a lengthy sample preparation process. Artificial intelligence (AI) is a highly precise and reliable technology for identifying mycotoxins in food. This unique method shows how multiple AI systems can be merged. Neural networks, machine learning approaches, and deep learning models were utilized to analyze complex datasets from various analytical platforms. Furthermore, we have emphasized the need for AI in conjunction with smart sensing technologies or other unconventional methods, such as spectroscopy, biosensors, and imaging methodologies, to identify mycotoxins more quickly and safely. Among other vital challenges in this area, we question the importance of employing large and diverse datasets to train AI models, debate the need to standardize the analytical approaches, and explore strategies for obtaining regulatory approval for AI-based procedures. Furthermore, this study provides some intriguing use cases and real-world business applications in which AI outperformed the more traditional methodologies in terms of its sensitivity and specificity and the time required by incorporating the most recent research findings and emphasizing the value of interdisciplinary collaboration among food scientists, engineers, and computer scientists for future paths in AI-enabled mycotoxin detection. Ultimately, AI has the potential to transform mycotoxin monitoring systems, enhancing food safety and public health globally.



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sciforum-125800: A further elucidation of the venom proteome of Australian Elapid *Pseudechis australis*

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The *Pseudechis* genus (black snakes) occurs throughout mainland Australia and Papua New Guinea, with the most widespread species being *P. australis* (Mulga snake). Black snakes in Australia are known to have a significant number of phospholipase A2 (PLA2) toxins, with ten previously being identified. *P. colletti* (Collett's snake) and *P. papuanus* (Papuan black snake) both have PLA2-dominant venoms, with more than 90% of the whole venom being PLA2. This is consistent with in vitro studies demonstrating high PLA2 activity in the genus. We aimed to characterise the venom proteome of *P. australis* with a two-dimensional fractionation method involving reverse-phase high-performance liquid chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis alongside bottom-up proteomics. After matching the venom peptides to the transcriptome, we characterised 98% of the venom and found that 89.8% was PLA2, and the remainder of the venom consisted of only three other protein families, snake venom metalloprotease (SVMP), L-amino acid oxidase (LAAO) and triosephosphate isomerase, which made up 6.0%, 2.5% and 0.03% of the whole venom, respectively. LAAO usually occurs in abundances less than 1.6% in snake venoms worldwide, except in *Hoplocephalus stephensii*, another Australian elapid, making it the second highest abundance in Australian snake venoms. In addition, the presence of SVMP was unusual for an elapid, again consistent with other Australian elapids. We identified eleven different PLA2 toxins in the venom of *P. australis*, but the other three protein families all had one toxin. The study again demonstrates the unusual characteristics of the venoms of Australian snake venoms, and in particular the PLA2-dominant venom of the genus *Pseudechis*, consistent with its clinical effects of myotoxicity and anticoagulant activity.



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sciforum-128807: Attenuation of extracellular toxin production in *Pseudomonas aeruginosa* by green-synthesized nanoparticles

Fazlurrahman Khan

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Pseudomonas aeruginosa is a common nosocomial pathogen that causes significant morbidity and mortality, especially among immunocompromised individuals. Its capacity to develop antibiotic resistance presents a significant obstacle in treatment. It generates various extracellular toxins and virulence factors, including alkaline protease, elastase, and phospholipase C, which destroy tissues and encourage bacterial invasion. Other toxins include pyocyanin, a phenazine pigment that may damage membrane proteins, produce oxidative stress, and cause substantial tissue damage. To address this, alternative methods that target virulence factors and toxin production are being investigated. Nanotechnology has emerged as a viable technique for reducing the virulence of *P. aeruginosa* by decreasing the production of toxins and other pathogenic components. In this study, we manufactured gold (AuNPs), silver (AgNPs), and zinc oxide nanoparticles (ZnONPs) using lactic acid bacteria, specifically *Leuconostoc* sp. strain C2 and *Lactiplantibacillus* sp. strain C1. The nanoparticles produced were labeled as C1-AuNPs, C1-AgNPs, C2-AuNPs, and C2-ZnONPs. The nanoparticles exhibited antibacterial activity against *P. aeruginosa*, with MICs of 1024 µg/mL for C1-AuNPs, 32 µg/mL for C1-AgNPs, and >2048 µg/mL for C2-AuNPs and C2-ZnONPs. C1-AuNPs, C1-AgNPs, C2-AuNPs, and C2-ZnONPs decrease pyocyanin toxin production by 57.75% (at 512 µg/mL), 26.07% (at 16 µg/mL), 71.07% (at 1024 µg/mL), and 82.15% (at 1024 µg/mL). Furthermore, at the sub-MIC level, these nanoparticles limit protease activity in a concentration-dependent manner. The current findings demonstrated that inhibiting the LAB-inspired nanoparticles is a promising technique for reducing their virulence and pathogenicity.

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sciforum-129408: Detection of Mycotoxigenic Fungi and Their Mycotoxins in Dried Cannabis Buds Using Different Approaches

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Cannabis plants are susceptible to microbial contamination, including fungi capable of producing harmful mycotoxins. The presence of these toxins in cannabis products poses serious health risks, particularly to immunocompromised people. This study evaluated the safety of dried cannabis buds intended for medicinal use by examining microbial contamination and residual mycotoxins through culture-based techniques, PCR/qPCR, and ELISA. Irradiation significantly reduced viable fungal and bacterial colony-forming units (CFUs) and eliminated culturable bacteria but did not achieve complete sterilization. Viable spores of toxigenic genera such as *Aspergillus*, *Penicillium*, and *Fusarium* persisted. Sanger sequencing of the PCR product of the sample's DNA generated using Internal Transcribed Spacer (ITS) primers identified dominant mycotoxigenic fungi such as *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* in non-irradiated (NR) samples, while next-generation sequencing (NGS) revealed additional non-culturable species. PCR and qPCR studies detected biosynthetic genes for aflatoxins (Nor1), trichothecenes (Tri5), ochratoxins (PKS), and deoxynivalenol (DON) across all samples. While band intensity decreased post-irradiation, gene copy numbers remained comparable, suggesting DNA damage without full degradation. ELISA confirmed the presence of aflatoxin, ochratoxin, DON, and T2 toxins in both irradiated and licensed producer (LP) marketplace samples, with variable concentrations. LP samples showed lower microbial counts and mycotoxin gene abundance but still contained detectable residual DNA and toxins. These findings indicate that while irradiation lowers microbial loads, it does not eliminate mycotoxigenic fungi or their metabolites. Therefore, culture-based assays alone are insufficient for comprehensive safety assessments and must be complemented by molecular and immunological techniques. Given the persistence of toxigenic fungi and their toxins in irradiated and licensed products, we recommend stricter microbial safety standards, particularly for medicinal cannabis, which may put immunocompromised patients at higher risk. For high-risk pathogens like *Aspergillus* and *Fusarium*, which pose significant health concerns, a zero-tolerance threshold (10 CFU/g) is advised, supported by stringent decontamination and testing.



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sciforum-129926: Discrimination of geographical origin and detection of aflatoxins in pistachio seeds using FT-IR spectroscopy

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Food contamination risks from mycotoxins pose a serious threat to public health. These toxic compounds are produced by filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* under specific temperature and humidity conditions. They can affect a wide range of agricultural products (nuts, cereals and their derivatives) during both the pre- and post-harvest stages. Among these, aflatoxin B1 is especially dangerous and has been classified as carcinogenic to humans. As part of the project PRIN 2022 PNRR “P20223P48S” entitled “Implementing advanced elemental and chemical analysis for quality, safety and traceability assessment of PGI and PDO agri-food products” funded by the European Union NextGenerationEU, we evaluated the feasibility of detecting aflatoxins in pistachio seeds using Fourier-Transform Infrared (FT-IR) spectroscopy. As a rapid and non-destructive technique, FT-IR holds significant potential for identifying contaminants in food, including mycotoxins. In the framework of the above project, FT-IR spectroscopy was initially applied to investigating the responses of pistachio samples from different locations in Sicily. The results demonstrate the ability of the FT-IR technique to discriminate between pistachios of different geographical origins, highlighting the connection between terroir and the chemical composition of the crops. Further studies are underway to evaluate the method's potential to distinguish between contaminated and uncontaminated seeds by detecting the presence of aflatoxins B1, B2, G1, and G2. FT-IR analysis is being used to compare the chemical profiles of contaminated samples with those of mycotoxin-free ones.



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sciforum-121467: Effect of vomitoxin on intestinal motility in mice

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This study investigated the effect of vomitoxin (DON) on intestinal motility in 6-week-old male C57BL/6J mice. Sixty mice were randomized into control and DON-treated groups, with six replicates of five mice each. The control group was gavaged with saline and the DON-treated group was gavaged with 3 mg/kg body weight of DON for 14 days. The results showed that DON significantly decreased the feed intake and weight gain ($P < 0.001$) and inhibited the growth of mice; shortened the intestinal propulsive distance, decreased the villus height, villus height/crypt depth, and intestinal wall thickness ($P < 0.001$), and disrupted the intestinal morphology and structure; down-regulated the expression of intestinal motility-related genes and proteins ($P < 0.001$); increased the number of M1-type inflammatory macrophages and activated the TLR-MAPK signaling pathway, up-regulated pro-inflammatory cytokine expression, and down-regulated anti-inflammatory cytokine expression ($P < 0.001$); activated the TNFR1-Casp3 signaling pathway and increased the secretion of apoptotic marker proteins ($P < 0.001$); and decreased the number of ICC cells ($P < 0.001$). This study demonstrated that DON causes intestinal motility disorders and morphological and structural damage and is associated with changes in the expression of related gene proteins, activation of inflammatory signaling pathways, activation of pro-apoptotic signaling pathways, and a decrease in the number of ICC cells. This study provides a theoretical basis for understanding the mechanism of DON toxicity and developing prevention and control strategies.



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sciforum-124950: Exploring the biocidal properties of *Bacillus thuringiensis* INTA Mo1-10 through genomic and phenotypic analysis

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Bacillus thuringiensis is a Gram-positive, spore-forming bacterium widely recognized for its potent insecticidal properties. It is recognized as a key biological control agent and a more sustainable alternative to chemical pesticides. This study focuses on the INTA Mo1-10 strain, preserved in our collection and originally isolated from grain milling residues in Argentina. The objective was to elucidate the relationship between its genetic profile and biocidal activity through comprehensive genotypic and phenotypic characterization. Genomic DNA analysis, performed via Illumina sequencing, identified nine insecticidal protein genes: *cry1Aa*, *cry1Ab1*, *cry1Ca*, *cry1Da*, *cry1Ia*, *cry2Ab*, *cry9Ea*, *vip3Aa*, and *spp1Aa*. Also, the amino acid sequence identities ranged from 77% to 100%. Microscopic analyses revealed that sporulated cultures had bipyramidal crystals that were fully separated from spores, as observed using phase contrast and scanning electron microscopy. The presence of a parasporal crystal protein band of approximately 130 kDa was further confirmed via SDS-PAGE analysis. Bioassays demonstrated the strain's broad-spectrum biocidal activity, achieving over 83% efficacy against *Cydia pomonella* (Lepidoptera: Tortricidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) when incorporated into insect diets. For *Panagrellus redivivus* (Tylenchida: Panagrolaimidae), the strain was grown as a lawn on TSA agar plates before nematode inoculation, which resulted in comparably high mortality. Furthermore, the strain exhibited 45% larvicidal activity against *Aedes aegypti* (Diptera: Culicidae) when applied to water-filled containers. All bioassays utilized spore/crystal complexes at appropriately high doses to ensure effectiveness. The findings establish a clear correlation between the strain's genetic makeup and its biocidal efficacy. Notably, the insecticidal protein gene profile of INTA Mo1-10 closely aligns with that of other *B. thuringiensis* serovar *galleriae* strains. These results underscore the considerable potential of INTA Mo1-10 as an effective and versatile biocontrol agent, contributing to sustainable pest control strategies.



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sciforum-125616: Growth and Metabolic Responses of *Microcystis aeruginosa* to Titanium Dioxide Nanoparticles

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Microcystis aeruginosa is a freshwater cyanobacterium capable of forming massive blooms and producing microcystins, hepatotoxins with adverse effects on aquatic organisms and human health. Among the emerging contaminants in aquatic environments, titanium dioxide (TiO₂) in nanoparticle (NP) form has garnered increasing attention due to its widespread industrial application and growing presence in natural water bodies. Its impact on the physiology and secondary metabolism of photosynthetic microorganisms remains insufficiently understood. In this study, *M. aeruginosa* (LEGE-91096) was cultured in the presence of TiO₂ NPs (commercial P25) at concentrations of 0.1 and 1 mg/L. Control treatments included humic acid as a surface coating (1:1 ratio). The stability of the nanoparticles in the culture medium was characterized by DLS, and their growth rate was assessed using the OECD 201 protocol. Metabolite profiling was performed via UHPLC-MS/MS, and data analysis was conducted using Compound Discoverer software to identify variations in bioactive compound production. According to OECD 201 results, TiO₂ nanoparticles at 0.1 and 1 mg/L did not significantly affect *Microcystis aeruginosa* growth. Ongoing analysis will further allow us to explain the molecular mechanisms underlying the interaction between TiO₂ nanoparticles and cyanobacterial metabolic pathways, particularly those involved in microcystin and other bioactive compound biosynthesis.

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sciforum-128869: Protective effect of curcumin against zearalenone-induced liver injury in weaned piglets

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The aim of this paper is to investigate the protective effect of curcumin on zearalenone-induced liver injury in piglets and to explore its molecular mechanism to provide a theoretical basis for the alleviation of ZEN-induced liver injury in piglets by nutritional means in production. Twenty-four female weaned piglets (Duroc×Long White×Large White, 7.43±0.88 kg) were randomly divided into three groups: control (CON), ZEN (2 mg/kg), and ZEN+CUR (2 mg/kg ZEN + 300 mg/kg CUR). The trial lasted 28 days following a 3-day pre-feeding period. The results showed that ZEN significantly reduced piglets' body weight on day 28 compared to the CON group, but CUR supplementation restored body weight. ZEN also decreased average daily gain (ADG) and feed intake (ADFI), while CUR improved these parameters in the ZEN+CUR group. ZEN increased the liver index, causing hepatomegaly, but CUR treatment reduced the liver index. Histologically, ZEN caused liver cell swelling, hemorrhage, and vacuolar degeneration, while CUR improved these pathological changes. Serum analysis revealed that ZEN increased alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bile acids (TBA), total bilirubin (TBIL), and γ -glutamyltransferase (GGT) levels, but CUR effectively restored these levels. ZEN had no significant effect on serum aspartate aminotransferase (AST) or albumin (ALB) levels. Overall, CUR demonstrated a protective effect against ZEN-induced liver injury in piglets.



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sciforum-128811: Pyoverdine-Coated Silver Nanoparticles as a Targeted Approach to Inhibit *Pseudomonas aeruginosa* Toxin Production and Virulence

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Pseudomonas aeruginosa is becoming increasingly resistant to conventional antibiotics due to its production of protein toxins such as exotoxins, pyocyanin, and proteases, as well as siderophores like pyoverdine, which contribute to its pathogenicity and persistence in host environments. In this study, silver nanoparticles (AgNPs) were synthesized using *P. aeruginosa*-derived pyoverdine (PVD) as a biological corona, creating targeted nanostructures (PVD-AgNPs) capable of attenuating virulence by inhibiting the production of toxins and virulence factors. The minimum inhibitory concentration (MIC) of PVD-AgNPs against *P. aeruginosa* was determined to be 16 µg/mL. At sub-MIC levels, PVD-AgNPs inhibited 90% of initial-stage biofilm formation at 8 µg/mL. Eradication of mature biofilms reached 70.83% at 64 µg/mL (4× MIC). PVD-AgNPs also significantly suppressed the production of key virulence factors: pyocyanin production was reduced by 77.37% at 8 µg/mL, pyoverdine production was inhibited by 97.40% at 2 µg/mL, and protease activity was entirely inhibited at 8 µg/mL. Hemolytic activity was also reduced by 89.26% at 4 µg/mL. Additionally, PVD-AgNPs impaired *P. aeruginosa* motility, with swarming and swimming motility inhibited by 72.61% and 82.89%, respectively, at 8 µg/mL. These phenotypic changes were confirmed by a downregulation of virulence- and biofilm-associated gene expression. The results highlight PVD-AgNPs as an effective nanomaterial-based strategy to reduce *P. aeruginosa* pathogenicity through toxin suppression, attenuation of virulence factor production, and biofilm disruption.

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sciforum-128929: Snake venom toxicity in *Artemia salina*

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Introduction

In Argentina, accidents caused by venomous animals are a public health problem. Snake venom toxicity tests are usually performed in mice and rats, which causes pain and suffering. These tests are not only expensive but also highly regulated. To overcome these limitations, we evaluated the potential use of alternative invertebrate models for snake venom testing. *Artemia salina* is a small crustacean that lives in high-salinity environments and is commonly used by aquarists as fish food.

Methodology

A. salina cysts were incubated in a thermostatic bath at 28 °C in a 3.2% w/v NaCl solution until hatching. Ten nauplii larvae per well were pipetted into a 96-well microplate. Venoms from four snake species—*Naja kaouthia*, *Micrurus pyrrhocryptus*, *Bothrops neuwiedi*, and *Bothrops diporus*—were added to each well. Larval motility was quantified after 24 hours of incubation at 28 °C using the Wmicrotracker device in the 3.2% w/v NaCl solution. Each well was also video recorded to monitor potential morphological and/or motility changes caused by the venoms.

Results

The observed EC₅₀s values on motility inhibition suggest that the highest toxicity was observed in *N. kaouthia*, followed by *B. neuwiedi*, *M. pyrrhocryptus*, and *B. diporus*. Nauplii incubated with *N. kaouthia* and *M. pyrrhocryptus* venoms began to show signs of damage at 1.0 µg/mL. *B. diporus* and *B. neuwiedi* induced damage at higher concentrations. Structural damage was observed, including appendage destruction and cuticle detachment.

Conclusions

Venoms from four different species were tested for their effects on *A. salina* motility and morphology. All venoms caused structural damage and complete cessation of larval motility at the highest concentrations tested. These results suggest that *A. salina* is sensitive to snake venoms in a dose-dependent manner. Further studies will be necessary to test their usefulness in correlating the action of venom and its neutralization by antivenoms in the murine model.



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sciforum-129723: Snake venom toxicity in *Caenorhabditis elegans*

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The toxicity of snake venoms is mostly determined in mice, the most frequently used mammalian model. These assays are expensive and subject to strict ethical regulations. To overcome these drawbacks, we evaluated the potential use of alternative models for snake venom testing. *Caenorhabditis elegans* is a free-living nematode widely used in biomedical research.

The wild-type N2 strain was used in all experiments. Ten L4-stage worms were pipetted into each well of a 96-well microplate containing M9 buffer. Worms were then exposed to six different concentrations of snake venom (0, 200, 400, 600, 800, and 1000 µg/ml) in a M9 buffer. The venom used was from *Naja kaouthia* (monocled cobra). Plates were incubated at 20 °C for 24 hours, after which motility and viability were assessed. Worms exhibiting movement were considered alive, while those that became straight and rigid were considered dead. Motility was quantified using the Wmicrotracker device (Phylumtech).

The results show lethality of *N. kaouthia* venom in a dose-dependent manner, and the LD50 was estimated to be 520 µg/mL. Increased mortality was preceded by a decrease in motility, and this could be attributed to the neurotoxic activity of the venom.

High doses of *N. kaouthia* exhibited significant effects on the viability and motility of the free-living nematode *C. elegans*, suggesting a potential utility for studies on snake venom toxicity and their neutralization. Nevertheless, the mass of venom required for the LD50 determination of L4 mortality was high. Despite this drawback, considering the cost and difficulty of venom obtaining, a study on other early larval stages must be tested in order to determine if the doses of venom required could be lower. Nevertheless, the usefulness of testing toxic mechanisms in wild-type, transgenic, or mutant strains makes *C. elegans* a suitable model for snake venom toxicity testing.



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sciforum-123592: Transition metal doping for improving the fluorescence response of paralytic shellfish poisoning toxins upon pre-chromatographic oxidation

Paulo Vale

IPMA

Paralytic shellfish toxins can be quantified in bivalve molluscs by the AOAC-2005 method. This method is quite sensitive for some toxins, while other toxins suffer from strong matrix effects or fluorescence partitioning by multiple oxidation products, reducing their sensitivity. Metals were assessed as candidate catalysts to improve the oxidation reactions instead of the oyster matrix modifier preconized in this method. The N1-H sub-group of toxins exhibited increased fluorescence upon doping with iron sulphate. The increase was inversely proportional to the fluorescence reduction caused by matrix suppression. Iron acted as a catalyst, lowering the activation energy of the reaction, which otherwise required heating to achieve a similar boost in fluorescence yield.

The fluorescence of the N1-hydroxyl sub-group of toxins GTX1+4 and GTX6 increased with zinc but decreased with iron. When doped with a metal solution that had passed in fraction 2 of the carboxylic acid partitioning, both metals reduced the fluorescence, while the eluent of fraction 2 (NaCl 0.5 M) enhanced it. As metals are retained by COOH cartridges, sulfonic acid leachables might contribute to this reduction. The reduction observed for GTX1+4 was primarily due to the decrease in the proportion of the secondary peak over the primary oxidation peak, which can be reversed by doping with an oyster matrix. Nickel chloride was able to replace the oyster modifier effectively in all five bivalve matrices tested, while zinc chloride was not similarly effective. For dcNEO spiked in several bivalve matrices, adding an oyster modifier derived from *Magallana gigas* caused a 16-19% reduction in fluorescence. Doping with several metals (Ni, Zn, alkaline, and alkaline earth metals) could not achieve the same fluorescence as doping with 0.1 mM acetic acid. In this case, the matrix modifier preconized in the method was not adequate.



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sciforum-125964: Unveiling the Venom Complexity of *Hypnale hypnale*: Regional Variation, Transcriptomics, and Clinical Implications from the Western Ghats, India

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The genus *Hypnale*, endemic to the Western Ghats of India and Sri Lanka, comprises three valid species: *Hypnale hypnale*, *Hypnale nepa*, and *Hypnale zara*. Of these, *H. nepa* and *H. zara* are restricted to Sri Lanka, while *H. hypnale* is the only species present in India, where it forms a monophyletic lineage endemic to the Western Ghats and Sri Lanka. Despite its medical significance, the hump-nosed pit viper (*Hypnale hypnale*) remains understudied in venom research and neglected in antivenom development. Currently, India's polyvalent antivenom targets only the "big four" Indian snakes [Russell's viper (*Daboia russelii*), *Echis carinatus*, *Naja naja*, and *Bungarus caeruleus*), showing limited efficacy against *Hypnale* envenomation. Misidentification and inadequate clinical management further exacerbate the threat posed by this species, whose venom is known to induce coagulopathy, local tissue damage, acute kidney injury (AKI), and multi-organ failure. The Western Ghats is a recognized biodiversity hotspot, presenting significant potential for the discovery of cryptic or previously undocumented *Hypnale* species due to their complex topography and underexplored habitats. In this study, for the first time, we collected 35 venom samples of *H. hypnale* from diverse locations across the Western Ghats. We generated venom gland transcriptomes and undertook biochemical assays to investigate molecular diversity and assess regional venom variation. Our findings underscore the urgent need for *Hypnale*-specific antivenom formulation to improve treatment outcomes in affected regions.



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Session 2. Novel Insights on The Mechanism of Action and/or Pathophysiology of Toxins

sciforum-129918: In Vitro Neuromuscular Blockade Caused by *Micrurus surinamensis* Venom

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Coral snakes are the American representatives of the Elapidae family. *Micrurus surinamensis* stands out as the largest coral snake species found in Brazil, notable for its semiaquatic habits and a diet consisting exclusively of fish. Its venom is primarily made up of three-finger toxins (3FTx), while the presence of phospholipases A₂ (PLA₂) appears to be inconsistent across different studies.

Methods:

This study investigated the neuromuscular effects of *M. surinamensis* venom using isolated neuromuscular models: the mouse phrenic nerve–diaphragm (PND) and chick biventer cervicis (BC) models. Mouse sciatic nerve preparations were also used.

Results:

The venom-induced neuromuscular blockade occurred in both the PND and BC preparations in a concentration- and time-dependent fashion, with the BC model demonstrating greater sensitivity. In the PND model, the findings included the following: the concentration- and time-dependent inhibition of tetanic contractions, without evidence of a tetanic fade; the full recovery of neuromuscular function following washing; reduced muscle depolarization induced by carbachol; and the suppression of miniature endplate potentials. In the BC preparation, the venom abolished contractile responses to both acetylcholine and carbachol, and washing led to partial recovery from the neuromuscular blockade. In both models, the partial and temporary restoration of neuromuscular function was achieved using neostigmine or 3,4-diaminopyridine. The compound action potentials in the mouse sciatic nerve remained unaffected. Additionally, no signs of myotoxicity were detected in either the PND or BC preparations, as evidenced by a stable muscle tension baseline; a preserved response to direct muscle stimulation; an unchanged muscle resting membrane potential in the PND model; an unaltered contractile response to exogenous potassium in the BC model; and the absence of histological damage in the muscle tissue. The venom showed no PLA₂ enzymatic activity.

Conclusion:

These findings support the conclusion that the neuromuscular blockade caused by *M. surinamensis* venom is predominantly due to α -neurotoxins, probably of the 3FTx class. There was no contribution from β -neurotoxins (such as PLA₂) or significant muscle tissue injury.



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sciforum-125867: Oxidative stress and apoptosis are strongly involved in ricin-induced intestinal cell intoxication

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Introduction:

Ricin, a type 2 ribosome-inactivating protein, is a potent lethal toxin purified from castor bean seeds and has been classed as a chemical and biological weapon according to Schedule 1 by the OPCW. Ricin's cytotoxicity results from its lectin chain, which enables cell entry, and its active chain, which can depurinate different substrates, leading to multiple cell death pathways. Although the systemic toxicity of ricin has been extensively studied, its localized effects on the gastrointestinal tract remain a critical concern, particularly in cases of oral ingestion. The aim of this study is to elucidate ricin intoxication in human intestinal cell lines and determine its impact on the integrity of the epithelial barrier.

Methods:

Ricin cytotoxicity in intestinal-derived HT29 and Caco-2 cells was evaluated in dose- and time-response experiments, with cell viability determined via an MTS-reduction assay. The effect of ricin on Caco-2 cell layer integrity was monitored by Trans-Epithelial-Electrical Resistance (TEER) measurements. The involvement of oxidative stress in ricin-treated cells was indirectly investigated by pretreating cells with reactive oxygen species scavengers. Cell death was determined through cytofluorimetric analysis of AnnexinV/PI positivity.

Results:

Ricin showed high cytotoxicity toward HT29 and Caco-2 cells, with EC₅₀s ranging from 10 to 0.1 nM after 24–72 h of intoxication. Ricin strongly reduced TEER values in Caco-2 cells at 1–0.1 nM after 24 h of treatment. Ricin cytotoxicity (at 1 nM concentration) can be completely prevented by pre-incubating cells with antioxidant scavengers, demonstrating the involvement of oxidative stress in ricin cell death mechanisms. Moreover, ricin triggered only apoptosis without necrosis involvement.

Conclusions:

Ricin has a potent cytotoxic effect on human intestinal cell lines that can be prevented by antioxidant scavengers. This study could pave the way for new pharmacological strategies to tackle ricin intoxication.



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sciforum-127245: C-type Lectin-Like Proteins (CTLPs) present in *Bothrops jararaca* venom that induce thrombocytopenia: purification and mechanism of action

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Introduction

Bothrops snakes are the main cause of snakebites in Brazil, with envenomation leading to severe effects, especially hemostasis disorders. *Bothrops jararaca* venom induces inflammatory, coagulant, and hemorrhagic actions through components such as metalloproteinases, serine proteases, C-type lectin-like proteins (CTLPs), and phospholipases A2. CTLPs alter platelet aggregation and coagulation. Recent studies isolated and initially characterized two CTLPs (E6 and D4) from *B. jararaca* venom that induce thrombocytopenia. This project aims to characterize these proteins and understand how they reduce platelet counts during envenomation.

Objectives:

The aim of our work was to further purify and characterize CTLP-E6 and CTLP-D4 and elucidate which of their mechanisms affect platelets.

Methods:

In vivo experiments were approved by CEUAIB (protocol 8227240723) using C57BL/6 mice (30-35g, n=5-8/group). Human blood samples were obtained from volunteers (aged 18-45 years) in accordance with CONEP guidelines (CAAE: 52787521.0.0000.5469). Venom was subjected to anion exchange chromatography (Q-Sepharose), followed by cation exchange (SP-Sepharose). Fractions were analyzed by SDS-PAGE (15%) and tested in vivo: mice received subcutaneous injections of CTLP fractions or saline. Blood samples were collected 3 hours later to determine the platelet count and facilitate fibrinogen measurement.

Results:

Anion exchange chromatography showed most proteins of interest eluted in the flow-through. SDS-PAGE revealed the presence of CTLP-E6 and CTLP-C4 at ~30 kDa under non-reducing conditions, splitting into 16 kDa and 13 kDa bands under reducing conditions. These fractions underwent further purification by cation exchange. SDS-PAGE and optical density confirmed purification. Injected mice exhibited thrombocytopenia without fibrinogen changes, indicating successful CTLP purification.

Conclusions:

The study effectively purified CTLPs that induce thrombocytopenia. Further research will investigate their mechanisms of action.

Acknowledgments:

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sciforum-128857: Curcumin alleviates zearalenone-induced reproductive impairment by modulating the gut microbe–testis axis

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Zearalenone (ZEN), a mycotoxin commonly found in cereal crops and foods, induces testicular damage and disrupts gut microbial composition, revealing a critical connection between gut microbes and the male reproductive system. Curcumin (CUR), a bioactive compound derived from ginger, is known to enhance intestinal microecological balance and exhibit anti-inflammatory properties. This study aimed to investigate the mechanism by which CUR alleviates ZEN-induced reductions in sperm quality through the modulation of the gut microbe–testis axis. Forty-eight 6-week-old Balb/c male mice were randomly assigned to four treatment groups: control (CON), CUR (200 mg/kg body weight CUR), ZEN (40 mg/kg body weight ZEN), and ZEN + CUR (200 mg/kg CUR + 40 mg/kg ZEN). The experiment lasted 28 days. CUR was found to mitigate ZEN-induced reductions in testosterone levels, testicular structural damage, and disrupted spermatogenesis. ZEN exposure altered gut microbial composition, increasing the abundance of *Prevotella* and decreasing *Lactobacillus*. CUR counteracted these effects and reduced the ZEN-induced activation of IL-17A-TNF- α signaling pathway proteins. In conclusion, ZEN induces testicular inflammation and reduced sperm quality by lowering testosterone levels and disrupting gut microbial balance, which drives the testicular IL-17A signaling pathway. CUR alleviates ZEN-induced testicular inflammation and sperm quality reduction by restoring beneficial gut microbes and testosterone levels.



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sciforum-129138: Development and Comparative Evaluation of Antivenoms Targeting the Pathophysiological Effects of Scorpion Venom Toxins in North Africa

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Introduction:

Scorpion envenomation is a major public health concern in North Africa, with *Androctonus mauretanicus* (*Am*), *Androctonus australis hector* (*Aah*), and *Buthus occitanus* (*Bo*) causing severe and often fatal cases. *Am* is the most dangerous and endemic species in Morocco. Due to the limited availability of effective antivenoms, treatment remains largely symptomatic. Understanding the pathophysiological mechanisms of venom action and improving therapeutic strategies are essential. This study aims to address this gap by developing and evaluating antivenoms targeting the main toxins involved in North African envenomations.

Methods:

The monovalent antivenom was produced by immunizing albino rabbits with *Am* venom collected from high-risk Moroccan regions. Neutralization potency was assessed *in vivo* using Swiss mice through LD₅₀ and ED₅₀ determinations. Histological and immunohistochemical analyses were conducted on vital organs (brain, heart, liver, lungs, kidneys) following administration of sublethal doses of *Am*, *Aah*, and *Bo* venoms. Both antivenoms (monovalent and Mexican polyvalent) were tested under similar conditions.

Results:

The Moroccan monovalent antivenom demonstrated strong specificity against *Am* and effective cross-neutralization of *Aah* and *Bo* venoms. Interestingly, while it showed superior efficacy against *Am*, the Mexican polyvalent antivenom was more effective in neutralizing the tissue damage induced by *Bo* and *Aah*. Histological evaluations confirmed a reduction in venom-induced lesions, particularly in cardiac and hepatic tissues, following antivenom administration four hours post-envenomation.

Conclusion:

This study underscores the critical role of antivenom as a specific and effective therapeutic tool in managing scorpion envenomations. The development of targeted antivenoms adapted to regional venom profiles enhances treatment outcomes and offers a promising path forward in combating scorpionism in Morocco and North Africa.



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sciforum-125861: Edulitin 2, a Ribotoxin-like protein from *Boletus edulis*: Assessment of its Cytotoxic Effects on Colon Adenocarcinoma Cell Lines

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Introduction:

Edible mushrooms have many nutritional goods; they are rich in carbohydrates, proteins, minerals, and flavor compounds and are low in fat and calories. Moreover, mushrooms are rich in beneficial bioactive components with pharmacological properties, but they are also known to contain toxic substances. Recently, a new protein synthesis inhibitor enzyme from *Boletus edulis*, named edulitin 2, has been identified and characterized as a ribotoxin-like protein. The aim of this paper is to evaluate the cytotoxic profile of edulitin 2 on two colon adenocarcinoma cell lines, Caco-2 and HT29.

Methods:

Edulitin 2 cytotoxicity was evaluated on Caco-2 and HT29 cells measuring cell viability and apoptotic/necrotic cell death in dose-response and time-response experiments. Furthermore, the effect of edulitin 2 on the integrity of the cultured cell monolayer was assessed using Transepithelial Electrical Resistance (TEER) measurements. TEER was evaluated in both Caco-2 monocultures and Caco-2/HT29 co-cultures, where the latter more closely resembled the intestinal epithelium.

Results:

Edulitin 2 seems to require a quite long delay to exert its toxic effects. In fact, in our models, viability was slightly reduced after 24h of incubation with edulitin 2, but it was strongly affected after 48 and 72h. The half-maximal effective concentration (EC₅₀) values, calculated from dose-response curves at 72h, were in the 0.1 µM range. Flow cytometric analysis showed apoptotic cell death (early and/or late), but not necrosis. After 72 h, 1 µM of edulitin 2 strongly reduced TEER values in both models, thus suggesting the damage of cell monolayer integrity.

Conclusions:

Our data demonstrate that edulitin 2 exerts a cytotoxic effect on intestinal cells after long time incubations (48-72 h). No necrotic lesions were observed in intoxicated cells at any tested concentration. The discrimination between apoptosis and necrosis is important as apoptosis does not cause inflammation.



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sciforum-125182: Effects of heterodimeric phospholipase A₂ from the venom of *Vipera nikolskii* on rat cardiomyocytes

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Introduction:

Phospholipase A₂ (PLA₂) is one of the largest families of snake toxins. Snake venom PLA₂s affect various systems in prey, including the cardiovascular one; however, data on their effect on cardiomyocytes are practically absent. In this work, we investigated the influence of heterodimeric PLA₂s HDP-I and HDP-II from the venom of *Vipera nikolskii* on isolated rat cardiomyocytes.

Methods:

Cardiomyocytes were prepared from the hearts of anesthetized animals. Only rod-shaped cardiomyocytes with clear striations were used. They were stained with a fluorescent probe, Fura-2 or Fura-4. The fluorescence in cardiomyocytes was measured using the Cell Observer fluorescent station based on an AxioVert 200M motorized inverted microscope equipped with a 10x PlanApochromat objective and Orca-Flash R2 monochrome camera.

Results:

After HDP2 (10 μM) application, there was a prolonged lag phase with no changes in cell morphology or probe signal. Then, fluctuations in the cytosolic calcium level ([Ca²⁺]_i) were observed, followed by a sharp increase in [Ca²⁺]_i and the onset of hypercontracture, with subsequent disruption of the plasma membrane. In the experiments under conditions of electrical stimulation, after recording control values of the fluorescent response, HDP II was added and changes in the fluorescence were recorded until cell death. In all cases, a “pause” in contractions was observed, characterized by an increase in intracellular calcium and decrease in the amplitude of the fluorescent response. Then, the rhythm recovered, and later, hypercontracture was observed followed by cell destruction. The times to these events depended on the HDP II concentration and the efficiency of stimulation. No effects on cardiomyocytes were observed upon application of 1 μM HDP I.

Conclusions:

In isolated rat cardiomyocytes, PLA₂ HDP II from *Vipera nikolskii* venom induced an elevation in cytosolic calcium level followed by hypercontracture and cell destruction. This is the first indication for the direct effect of snake venom D49 PLA₂ on cardiomyocytes.



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sciforum-127875: Epsilon Toxin from *Clostridium perfringens* Induces the Generation of Extracellular Vesicles in T-Lymphocytes.

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Epsilon toxin (ETX) is a pore-forming toxin (PFT) capable of crossing the blood-brain barrier and binding to myelin structures. In vitro assays have demonstrated that ETX impairs oligodendrocytes and induces demyelination. Notably, ETX has been implicated in the pathogenesis of multiple sclerosis (MS), with ETX-specific antibodies detected in sera from MS patients.

Myelin and lymphocyte protein (MAL) is widely recognized as the receptor for ETX. Its presence is essential for ETX-induced pore formation in the plasma membrane of host cells, ultimately leading to cell death. Recent findings have shown that ETX also binds to and kills primary human lymphocytes, which express elevated levels of MAL. This suggests that ETX may influence immune responses associated with MS, although its precise mechanism of action remains unclear.

To counteract the damage caused by PFTs, some host cells release extracellular vesicles (EVs) to reduce pore insertion into the plasma membrane. ETX has been shown to induce EV production in HeLa cells overexpressing MAL-GFP. Similar effects have been observed in MOLT4 cells, a T-lymphocyte line that endogenously expresses MAL protein. Our observations confirm that ETX also stimulates EV formation in MOLT4 cells. Moreover, both MAL protein and ETX oligomers are present in these EVs, making them a valuable tool for investigating ETX's mode of action and its interaction with its receptor. We speculate that these EVs may play a role in the induction of MS.



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sciforum-124819: First Insight into the Binding of Microcystin-LR and Cylindrospermopsin to Estrogen and Androgen Receptors via In Silico Molecular Docking

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The potential endocrine-disrupting properties of cyanotoxins, such as microcystin-LR (MC-LR) and cylindrospermopsin (CYN), are of concern due to their increasing occurrence, insufficient body of research, and potential impact on human health. Thus, an in silico procedure to assess the potential ability of MC-LR and CYN to form stable complexes with the estrogen receptor (ER) and the androgen receptor (AR) was performed for the first time. For that purpose, the molecular docking of both toxins was carried out. Ligand structures were generated in ChemDraw and subsequently docked against four distinct crystal structures of the ER and additional crystal structures of the AR. AutoDock was employed for docking to predict the potential agonist or antagonist activity of the toxins. The molecular docking of MC-LR and CYN with ER conformations revealed potential binding interactions. MC-LR exhibited a stronger affinity for the agonist conformations (1ERE: -7.1 kcal/mol; 3ERD: -8.2 kcal/mol) compared to the antagonist conformations (1ERR: -6.4 kcal/mol; 3ERT: -7.8 kcal/mol). Specific interactions in the agonist conformations involved arginine nitrogen with ASP-321 and GLU-323. Similarly, CYN displayed a preference for agonist conformations (1ERE: -6.7 kcal/mol; 3ERD: -7.0 kcal/mol) over antagonist conformations (1ERR: -6.6 kcal/mol; 3ERT: -6.1 kcal/mol). Key interactions in 1ERE involved the CYN guanidine group with SER-305, ALA-307, and ASP-369. In the case of AR, MC-LR showed a binding energy of -7.2 kcal/mol, with the leucine nitrogen interacting with PRO-682. CYN exhibited a higher affinity (-8.2 kcal/mol), with interactions involving the guanidine group with GLN-798. Taking this into account, computational docking analyses indicated possible binding interactions between these toxins and both receptors.

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sciforum-124642: Identification of potential metabolites of Cylindrospermopsin and Microcystin-LR and effects on brain oxidative stress biomarkers in rats after oral exposure to their mixture

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Cylindrospermopsin (CYN) and microcystin-LR (MC-LR) are cyanotoxins of significant concern due to their widespread occurrence and toxic potential. Among exposure routes, ingestion through contaminated water and food is particularly relevant. Although CYN and MC-LR are primarily classified as cytotoxin and hepatotoxin, respectively, both cyanotoxins have shown potential neurotoxic effects. In addition, a recent study has reported CYN metabolites in the brain of orally exposed rats. However, to our knowledge, the combined impact of CYN and MC-LR on the brain remains unexplored. This study aimed to identify CYN, MC-LR, and their potential metabolites in the brain of Wistar rats after oral exposure to dose combinations of pure CYN and MC-LR (7.5+7.5, 23.7+23.7, and 75+750 µg/kg body weight) by ultra-high performance liquid chromatography coupled to a tandem mass spectrometry system (UHPLC-MS/MS). Furthermore, several oxidative stress biomarkers were assessed, including lipid peroxidation (LPO) levels and superoxide dismutase (SOD) and catalase (CAT) activities. Although CYN and MC-LR were not detected in brain tissue, up to nine potential CYN metabolites and eight MC-LR metabolites produced by different metabolic pathways were identified. Moreover, sex-dependent differences were observed in oxidative stress biomarkers. In males, exposure to the intermediate dose (23.7 + 23.7 µg/kg) significantly increased LPO levels (1.3-fold) and SOD (1.3-fold) and CAT (1.6-fold) activities compared to controls, while in females only changes in LPO levels were observed, where a significant increase was observed at all doses tested. These findings suggest that oral exposure to CYN+MC-LR mixtures may cause neurotoxic effects in rats and highlight the importance of considering sex as a biological variable in cyanotoxins toxicological assessment.

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sciforum-127703: In Vivo High-Resolution MSSR Microscopy Reveals Cry Toxin-Induced Actin Disruption, Endocytic Activity, and Vesicle Release in Insect Midgut Cells

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Introduction:

Pore-forming toxins (PFTs) play a pivotal role in bacterial pathogenicity, yet the cellular responses they elicit remain incompletely understood.

Methods:

Using in vivo Mean Shift Super-Resolution (MSSR) microscopy, we analyzed mosquito larval midgut cells exposed to *Bacillus thuringiensis* Cry11Aa toxin, achieving unprecedented nanoscale visualization of the intoxication process. Transmission electron microscopy was also used in this study.

Results:

Our observations uncovered a sequential cascade beginning with toxin binding to brush border microvilli (BBM), followed by membrane pore formation that disrupts actin cytoskeletal integrity and stimulates massive endocytosis. An additional observed response was the release of large (5–8 µm) membrane-enclosed vesicles into the midgut lumen. These vesicles, segmented by endoplasmic reticulum (ER) membranes and actin filaments, carried damaged organelles—such as mitochondria, lysosomes, and endosomes—yet lacked nuclear material. Notably, the toxin was found in close association with ER membranes within these vesicles, suggesting this process may represent a toxin-clearing defense mechanism. Transmission electron microscopy confirmed vesicle morphology and contents. A comparison with a non-toxic Cry11E97A mutant, defective in oligomerization and membrane insertion, confirmed that pore formation is essential for triggering the observed cellular events. Vesicles of similar morphology were rarely detected in untreated controls, implying that their production may reflect an amplified version of a natural epithelial renewal process.

Conclusions:

These findings shed light on a previously unrecognized aspect of midgut epithelial defense against Cry toxins, emphasizing the complexity of the insect cellular response to PFTs. Understanding this pathway offers new perspectives for enhancing the effectiveness of Bt-based pest control and managing resistance in insect populations.



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sciforum-129938: No gain from pain: lack of nociceptor activation suggests no selection for defence in the evolution of European viper venoms

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Venoms have evolved at least 100 times in the animal kingdom, with their main biological roles being prey acquisition and self-defence. Snake venoms show extreme variability in composition at all taxonomic levels, both within and between species. The selective pressures underlying this variation have long been debated. Since snake venoms are used for both foraging and defence, we hypothesise that venom composition may represent a trade-off between lethal/immobilising predatory toxins and pain-inducing defensive toxins. Here, we use the Eurasian viper genus *Vipera* to test the hypothesis that snakes feeding on weakly defended prey, such as insects, should have venoms more adapted for defence than snakes feeding on well-defended prey such as rodents. We tested the nociceptor-activating (pain-inducing) capacity of the venoms of populations of the largely insect-eating *Vipera ursinii* complex and of the primarily mammal-eating *V. ammodytes*, *V. aspis* and *V. berus*. We used high-throughput Ca^{2+} imaging and deconvolution microscopy on F11 cells and native dorsal root ganglia cells, respectively, to test the algescic activity of the venoms of European vipers. Contrary to our expectations, none of the venoms resulted in nociceptor activation, irrespective of diet. We conclude that selection for defence is unlikely to have played a significant role in driving the evolution of venom composition in this genus.



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sciforum-129504: The Unexpected Biochemical Repertoire of Mamba Intestinal Toxin

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Mamba intestinal toxin (MIT) is a minor toxin component of *Dendroaspis polylepis* venom and has been shown to have potent effects on intestinal smooth muscle. Since the publication of these findings more than 25 years ago, there has been little further investigation into the toxin. We used chromatography to fractionate the venoms of all *Dendroaspis* species and identified fractions containing MIT in *D. polylepis* and *D. angusticeps* venoms, both of which were confirmed by mass spectrometry. We next profiled the activity of purified MITs using in vitro assays relevant to the neurotoxic effects of these venoms. Both DaMIT and DpMIT showed moderate inhibition of the human muscle-type nicotinic acetylcholine receptor (nAChR) and weak binding to a chimera of the human alpha-7 nAChR and acetylcholine-binding protein. No other venom fractions from *D. angusticeps* showed inhibitory activity on the human muscle-type nAChR, which suggests that MIT may be responsible for the post-synaptic neurotoxicity previously observed for this venom. DaMIT and DpMIT both demonstrated moderate inhibitory activity of human voltage-gated potassium channels, and surprisingly, both were capable of fully neutralising the enzymatic activity of human acetylcholinesterase. Our work demonstrates that MIT may target several proteins at the neuromuscular junction to contribute to the neurotoxic effects of *D. polylepis* and *D. angusticeps* venoms.



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sciforum-124978: Time- and Dose-dependent Intracellular Proteome Alterations by Cobra Venom Cytotoxin: System Toxicology and Mechanistic Perspectives

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Naja sumatrana venom cytotoxin (CTX) is a three-finger toxin which has been reported to exhibit caspase-dependent and mitochondrial-mediated apoptosis without transitioning into necrosis in human breast cancer MCF-7 cells. It has also been found to cause membrane permeabilization, leading to secretory phenotype changes. This study investigates the time- and dose-dependent alterations of the intracellular proteome by the cytotoxin from system toxicology and mechanistic perspectives. The cytotoxicity of CTX was determined at 8 and 24 h. MCF-7 cells were treated with IC₂₀, IC₅₀, and IC₈₀ of CTX at 8 h and 24 h. Label-free quantitative (LFQ) proteomics was performed to determine the intracellular proteome changes caused by CTX. Bioinformatic analyses were then carried out to identify the significantly distinguished proteins and pathway enrichment for protein-protein interaction networks. The LFQ proteomics revealed distinctive protein families significantly distinguished at 8 and 24 h. At 8 h, CTX targeted the cell cycle checkpoints and estrogen signalling, Notch, WNT/ β -catenin, and Rho GTPase pathways. On the other hand, at 24 h, proteins related to oxidative stress and MAPK-mediated apoptosis pathways were detected. There was also a concentration-dependent substantial elevation in different protein families associated with chromatin organization, DNA damage checkpoints, and DNA fragmentation during apoptosis. The protein-protein interaction (PPI) networks demonstrated several potential clusters related to toxin internalization, which could lead to intracellular accumulation that triggers cytotoxicity. Nevertheless, the exposure to CTX did not have notable effects on cellular metabolism. In conclusion, CTX induced significant time- and dose-dependent alterations of the intracellular proteome, revealing the involvement of apoptosis-related pathways and the suppression of proliferation in MCF-7. Integrative proteomics and system toxicology uncovered the mechanistic insights of CTX's progressive actions in causing cell death.



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sciforum-127533: Use of oseltamivir phosphate for the treatment of thrombocytopenia induced by experimental *Bothrops jararaca* envenomation in a murine model

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Snakebite envenomation is a neglected tropical disease that affects between 4.5 and 5.4 million people annually. In South America, snakes of the *Bothrops* genus are the main medically relevant species involved in envenomations. *Bothrops* venom, rich in hemotoxic enzymes and toxins, frequently induces thrombocytopenia. Although it is known that venom toxins activate and remove platelets from circulation, the underlying mechanisms remain unclear. One proposed mechanism is platelet desialylation, induced by neuraminidases, which remove sialic acid from the platelet surface, promoting their clearance from circulation. Oseltamivir phosphate (Tamiflu®), an antiviral used against Influenza, inhibits viral neuraminidases and has been suggested as an adjuvant to block endogenous neuraminidase activity, potentially extending platelet lifespan.

This study investigated the effects of oseltamivir phosphate on thrombocytopenia induced by *Bothrops jararaca* venom (BjV) in Swiss mice (IACUC Instituto Butantan, protocol no. 7416200723). Animals were divided into four groups, receiving intraperitoneal injections of saline (SAL) or oseltamivir phosphate (OSELT), followed by subcutaneous administration of SAL or BjV. Blood samples were collected 3 and 6 hours after envenomation, and hematological parameters were analyzed using an automated cell counter. Data was assessed using one-way ANOVA and post hoc tests.

Envenomed groups exhibited significant thrombocytopenia, which was not reversed by oseltamivir. In healthy animals, the drug did not alter platelet levels. Lymphocytopenia was observed in BjV-treated groups, along with an increase in leukocyte count, including a mild elevation in the OSELT+SAL group. Red blood cell counts and hematocrit levels remained stable.

In conclusion, exposure to BjV led to thrombocytopenia, lymphocytopenia, and leukocytosis—all of which were not reversed by Oseltamivir treatment. While the drug appeared neutral in healthy animals, it was not effective in preventing the acute hematological effects of the venom when administered in isolation.



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sciforum-129921: Using omics to characterize the role of ArtAB toxin in *Salmonella* pathogenesis

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Salmonella is a major cause of foodborne illness, hospitalization, and death worldwide. This high-priority pathogen produces multiple virulence factors that enable it to evade host immune defenses. Among these are AB₅ toxins, a family of multi-subunit secreted proteins known to be virulence factors in other Gram-negative bacteria. AB₅ toxins have been identified in both typhoidal and non-typhoidal *Salmonella* (NTS) serovars; however, their molecular role in pathogenesis in NTS remains poorly understood. We have previously purified an AB₅-like toxin, ArtAB, and its B₅ subunit (ArtB) from *Salmonella* Typhimurium. In this study, we treated Chinese hamster ovary (CHO) cells with ArtAB, ArtB, and other AB₅ toxins to examine cellular morphology, such as cell elongation or clustering. We also performed a cytotoxicity assay with 2-fold dilutions of ArtAB and ArtB on CHO cells. Based on these results, we treated CHO cells with 10 µg/mL of ArtAB and 7.25 µg/mL of ArtB (equimolar concentrations) for 4 hours, followed by mRNA sequencing and whole-cell proteomics to investigate differential gene and protein expression. Preliminary mRNA-seq results indicate that ArtAB significantly alters the expression of genes involved with transcription factors, cell signaling, intracellular transport of biomolecules, metabolism, and extracellular matrix remodeling. In contrast, ArtB behaves similarly to the buffer control (1X PBS + 5% glycerol) and does not significantly modulate gene expression. Proteomics analysis thus far supports these findings. Uniquely, enterotoxin B₅ subunits, such as cholera toxin B (CTB), are known to serve as both vaccine antigens and protein adjuvants. Further defining the cellular signaling pathways induced by these toxins is essential to understanding how they contribute to *Salmonella* pathogenesis and may lead to the development of more effective therapeutics and vaccines.



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Session 3. Use of Toxins as Tools for Research, Drug Discovery, and Therapeutics

sciforum-126853: *Bothrops jararaca* snake venom: a reappraisal of its coagulant activity

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Introduction:

Bothrops jararaca snake venom (BjV) contains toxins that activate and inhibit blood coagulation, causing consumption coagulopathy, thrombocytopenia, endothelial dysfunction, and secondary fibrinolysis in human and animal victims of snakebites.

Objectives:

The objective of this work was to study the in vitro coagulant activity of BjV in human, mouse, and rat plasma.

Methods:

The coagulant activity of BjV was assessed in normal and coagulation-factor-deficient plasma from humans, rats, and mice (C57BL/6, *F8*^{-/-}, *F9*^{-/-}, *Vwf*^{-/-}, and pearl mice (*Ap3b1*^{-/-})). The roles of calcium ions, phospholipids, and blood cells in the coagulant activity of BjV were evaluated. Pharmacological inhibitors were used to analyze the generation of factor Xa and/or thrombin and their contribution to the coagulant activity of BjV.

Results:

Human plasma showed greater sensitivity to BjV than rat and mouse plasma. Calcium ions and phospholipids influenced its coagulant activity. Pre-incubation with rivaraxoban and/or dabigatran showed that the coagulant activity of BjV in rodent plasmas primarily depends on prothrombin activation, whereas in human plasma, it involves both thrombin-like-enzymes and prothrombin activators. **Conclusions:** This study highlighted important differences in the coagulant activity of BjV in human and rodent plasmas, underscoring the need to consider these disparities in comparative envenomation studies using animal models.



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***sciforum-129674: Crotalus molossus* Venom-Loaded PLGA Nanoparticles as potential Drug Delivery Systems for Breast Carcinoma Cells**

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Introduction

Nanomedicine has helped in the development of novel therapeutics. For example, the encapsulation of bioactive components such as peptides/proteins into polymeric nanoparticles can lead to increased bioavailability, passive targeting and sustained release. Snake venoms have proven to be rather interesting for the R&D of new medicines, as they can present antifungal, antiparasitic, antibacterial or antiproliferative activity. In the present study, we encapsulated venom from *C. molossus* in PLGA NPs and evaluated them for hemolytic activity, kinetic release profile and cytotoxic activity against T47D breast carcinoma cells.

Methods

PLGA NPs were produced by a double emulsion-solvent evaporation method. Briefly, PLGA was dissolved in dichloromethane, into which a small volume of snake venom resuspended in water was added. This solution was then emulsified by ultrasonication, forming the organic phase. Later, the organic phase was added to a stirring aqueous phase containing 2.5% PVA, and then emulsified again by ultrasonication. NPs were washed three times by ultracentrifugation, freeze-dried and stored at -20 °C until use.

Conclusions

PLGA NPs serve as an effective encapsulation system for bioactive components such as snake venom toxins. We obtained NPs with sizes of ~250 nm, PDI of 0.10, a zeta-potential of -26 mV and an EE% of ~85%. PLGA-Venom NPs were not hemolytic unlike *C. molossus* venom and presented a sustained release profile. Finally, they were cytotoxic against T47D cells, providing helpful insights into the development of these novel drug delivery systems. More studies are required to evaluate the feasibility of using these agents alongside nanoparticle systems.



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sciforum-135340: Drosophila as an In Vivo Model for Screening Neurotoxicity in Physalia physalis Venom Fractions

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Physalia physalis, or Portuguese Man O' War, is one of the most venomous cnidarians. Despite its known toxicity, detailed studies on its venom components remain limited, specifically concerning their neuroactive effects. This study seeks to explore the effects of total/crude venom (CV), as well as high-molecular-weight (HMW) and low-molecular-weight (LMW) venom fractions, using *Drosophila melanogaster* as an in vivo model. Oral administration enabled the assessment of fly mortality and locomotor behavior. CV and LMW venom fractions induced similar levels of mortality, while HMW and control groups showed comparably lower rates. The CV-treated group displayed acute locomotor impairment, with an acute toxicity pattern in climbing and moderate symptom variability. In contrast, the HMW-treated group showed minimal impairment, characterized by a predominantly straight trailing climbing pattern and reduced symptom variability. The LMW-treated group exhibited pronounced locomotor dysfunction, displaying a variability of abnormal climbing behaviors and symptoms. Real-time monitoring revealed an initial phase of hyperactivity followed by prolonged paralysis, resulting in reduced locomotor activity and altered circadian rhythm. The results indicate that neurotoxicity resides primarily in LMW components, demonstrating the utility of *Drosophila* for venom screening and neurodegenerative-related research. This work supports future proteomic and transcriptomic studies identifying novel bioactive compounds with potential therapeutic applications.



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sciforum-137818: Exploring the venom gland transcriptome of the Ecuadorian scorpion *Teuthraustes atramentarius*

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Introduction:

Scorpion venoms are complex mixtures of bioactive molecules, including neurotoxins, cytolytic peptides, enzymes, and other components that target ion channels, membrane receptors, and various physiological pathways. Although many medically important species have been extensively studied, little is known about the molecular composition of venoms in rare or endemic taxa. The scorpion genus *Teuthraustes* Simon, 1878 (family Chactidae) comprises 27 described species, with 15 recorded from Ecuador. These species exhibit a remarkable concentration in the Andean highlands and Amazonian regions, although the genus also occurs in Colombia, Venezuela, Peru, and Brazil. Specifically, *Teuthraustes atramentarius*, the type species, was originally described from Ecuador and remains endemic to the country's central highlands. Despite its early description, no omics-based studies have investigated its venom composition. Here, we present the first exploration of the venom gland transcriptome of *T. atramentarius*.

Methods:

RNA from dissected venom glands of three specimens, following venom induction by electrostimulation, was sequenced using Illumina RNA-Seq. The assembled transcriptome was analyzed with the DeTox pipeline to identify toxin candidates. Top candidates with signal peptides, cysteine patterns, and similarity to known toxins were manually inspected based on best-hit alignments and preliminary phylogenetic analysis.

Results:

Among 40,083 predicted ORFs, 288 matched ToxProt entries, with seven retained as strong toxin candidates. These included one homologous to Phi-liotoxin-Lw1a, a ryanodine receptor modulator; one Cathepsin D-like aspartic peptidase; two invertebrate defensins; two CAP superfamily cysteine-rich venom proteins (CRVPs); and one Hge-scorpine, a multifunctional peptide with antimicrobial and ion channel-blocking properties.

Remarks:

To our knowledge, this study constitutes the first omics-based approach to investigating the venom composition of *T. atramentarius*, revealing seven strong toxin candidates related to known scorpion and animal toxins. However, further research is needed, particularly proteomic validation in venom samples, broader specimen sampling, and integration with proteotranscriptomic and evolutionary approaches.



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sciforum-129813: Antioxidant Molecules in the Sea Anemone *Anthopleura Cascaia* and Their Application in Alzheimer's Disease

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Introduction:

One of the main causes of Alzheimer's disease (AD) is the accumulation of β -amyloid peptide in brain, leading to several consequences for neurons, including oxidative stress. To counteract oxidative stress, new antioxidants have been studied; however, few are able to cross the blood-brain barrier or demonstrate effectiveness. This study aimed to obtain new antioxidants from the sea anemone *Anthopleura cascaia*, whose venom has the potential to inhibit neuronal death caused by A β 42.

Methods:

A. cascaia was collected in São Sebastião/SP/Brazil, and its venom was extracted through chemical stimulation. The venom was subsequently fractionated based on its antioxidant activity and assessed by DPPH and PFRAP tests. The first fractionation step was a solid-phase extraction (SPE, C18 cartridge), with elution at 0,25, 50, 75 and 100% acetonitrile/0.1% trifluoroacetic acid (TFA). A second fractionation was conducted using HPLC with a C18 column and a 0-100% acetonitrile/0.1% TFA gradient, followed by a third fractionation using an HILIC column. The purified active molecule was analyzed by mass spectrometry and incubated with differentiated-SH-SY5Y neurons, pre-exposed to A β 42 for 48 h. Cell viability was assessed using the LDH test. Results: The 0% SPE fraction and the first peak of C18-HPLC exhibited antioxidant activity in both tests. Due to peak impurities, a third fractionation step was performed, yielding six fractions, and one of them demonstrated 40% and 50% antioxidant activity in the DPPH and PFRAP tests, respectively. Mass spectrometry analysis confirmed high purity and an ion at 232.943 m/z, with no matches in natural products databases. Furthermore, this molecule successfully inhibited neuronal death induced by A β 42, with LDH values comparable to the control.

Conclusion:

A novel antioxidant molecule with neuroprotective potential was successfully isolated from a sea anemone. This discovery could contribute to the development of a prototype for a new AD treatment by targeting a key mechanism associated with the disease.



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sciforum-125991: Intracellular Trafficking Botulinum Neurotoxin Light Chain A1 to cleave of Plasma Membrane-Bound SNAP-25

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Botulinum neurotoxins (BoNTs) are among the most potent protein toxins known to humans, yet the intracellular trafficking of BoNT-Light Chain A1 (LC/A1) to its substrate, Synaptosome Associated Protein of 25-kDa (SNAP-25), remains poorly understood. A mouse neuroblastoma-2A (N2A) cell-based assay was used to track the intracellular trafficking of cytosolic EGFP-tagged LC/A1. The results revealed that LC/A1 associates with intracellular vesicles as a function of the LC/A1-N terminus, while plasma membrane binding is facilitated by an internal region of LC/A1 that can target LC/A1 to the plasma membrane from the cytosol. An internal LC/A domain, termed the Membrane Localization Domain (MLD), is responsible for the movement of the LC/A1 to the plasma membrane, where the association may be stable or reversible depending on the LC/A subtype. Recent studies have detected the physical trafficking of EGFP-LC/A1 from the cytosol to the intracellular plasma membrane. Thus, in addition to known steps such as host receptor binding and catalysis, the intracellular trafficking of LC to the target substrate may be a critical determinant of BoNT potency. Mechanistic insights into BoNT intracellular trafficking will clarify fundamental aspects of toxin action towards the development of novel therapeutic strategies to mitigate BoNT toxicity.



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sciforum-125883: Structural Analysis of Saxitoxin and its Analogs: a Computational Study

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Saxitoxin (STX) is a potent neurotoxin produced by certain species of seaweed (dinoflagellates) and cyanobacteria. Saxitoxin has more than 50 analogs, but in this work, we studied only saxitoxin and its analogs GTX5, GTX2/3, and C1/2, which are mollusk paralyzing toxins (PSTs). Because it is highly lethal and its mechanism of action has not yet been fully elucidated, it can be used as a biological weapon. This project addresses an in silico study on the interactions of STX and its analogs selected with potential targets in order to understand its mechanism of action. Initially, the 3D structure selected in the PDB database was the Saxiphilin protein, identified by the PDB code ID 8D6M. The results obtained in this project through molecular docking performed by AMDockVina show us that some residues are present in some interactions of Saxiphilin with STX and its analogs, and the residues that appear repeatedly are aspartic acid, glutamic acid, and glycine. Furthermore, we performed a search against the BLAST database for human sequences homologous to Saxiphilin. The sequence that presented the greatest identity of 41.22% and coverage of 84% was the Lactoferrin sequence. The next will step will be to perform new docking simulations with STX and its analogs using the 3D structure of Lactoferrin.



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sciforum-124877: Structural Analysis of Saxitoxin and Neosaxitoxin Toxins with Potential Therapeutic Targets

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Throughout history, there have been several instances of toxins being used as biological weapons, including marine toxins produced by dinoflagellates and other filter-feeding organisms, such as cyanobacteria. Furthermore, they may be associated with food seafood poisoning. Despite being potentially toxic, marine toxins present a vast chemical and biological diversity, making them an exceptional reservoir for discovering new medicines. Saxitoxin (STX) is a powerful paralyzing marine toxin from dinoflagellates and cyanobacteria. Aiming at exploring the value of marine toxins and other associated bioactive molecules as a source of potential therapeutic agents for biotechnological applications, the objective of this study is to evaluate the binding mode of STX to different targets and the in vitro consequences of their interaction. These results will help us propose paths of therapeutic activities for STX. We used the molecular docking methodology for in silico assays. The structures of SXT and neosaxitoxin, obtained from the Protein Data Bank (PDB) database, were used as ligands. To identify new therapeutic targets in humans, we used the SwissTargetPrediction server, which reported 18 possible targets. We then performed molecular docking simulations with the AutoDock Vina 1.1.2 program with default parameters. Of all the targets, the best pose was in serotonin-6 receptor reported by AutoDock Vina, which presented a score of -5.5 and -5.7, and hydrogen bond interactions with Ser111, Leu183, Val107, Asn288, Thr196, Tyr310, and Leu183 residues, to saxitoxin and neosaxitoxin. The second-best pose in the carbonic anhydrase XII receptor reported presented a score of -8.06 and -6.03 and hydrogen bond interactions with Ala129, Ser130, Thr199, and Pro201 residues to saxitoxin and neosaxitoxin. From this perspective, molecular dynamics simulations will be performed for the most promising complexes, and atomic force microscopy tests will be performed to identify the interaction of STX and neosaxitoxin with their targets.



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sciforum-129697: A genetically detoxified version of Sphingomyelinase D engineered in insect biofactories for the production of antivenoms against loxoscelism

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Introduction

Systemic loxoscelism, caused by the venom of the *Loxosceles laeta* spider, can be life-threatening. Current specific treatment relies on the administration of antivenom produced from the plasma of horses that have been hyperimmunized with spider venom. However, limited venom availability is the major production bottleneck. This study evaluates whether a genetically detoxified version of Sphingomyelinase D (SphD), the main toxin responsible for severe loxoscelism, could improve antivenom production.

Methods

Two versions of SphD were engineered using the baculovirus-insect larvae (BIL) platform: r(wtSphD), retaining its toxic functions, and r(dSphD), a detoxified variant (D259G mutation). Two horses were immunized over three cycles with r(dSphD), and their specific serum antibody responses were evaluated using an indirect ELISA. After each cycle, plasma was collected to produce pilot-scale Active Pharmaceutical Ingredients (APIs) of antivenom. Neutralizing activities of sera and APIs were tested against r(wtSphD) and native *L. laeta* venom using rabbit dermonecrotic assays and direct human red blood cell hemolysis test.

Results

A specific immune humoral response was developed after each immunization cycle. Higher levels of antibodies were detected in Horse 1. Dermonecrotic injuries of native *L. laeta* venom were partially neutralized by the sera and APIs from both animals. Moreover, sera and APIs from Horse 1 at cycles 2 and 3 effectively neutralized the r(wtSphD)'s dermonecrotic and hemolytic activities, while Horse 2 only showed similar neutralization capacity at cycle 3.

Conclusions

These promising results demonstrate that the BIL platform can be used to produce a genetically detoxified version of SphD, capable of inducing an immune-effective neutralization response of its homologous toxic form. Further ongoing research is being conducted to evaluate other detoxified versions of SphD isoforms and achieve complete protection against whole spider venom. This strategy offers a scalable, affordable, and safer alternative for venom complement or replacement in antivenom production.



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sciforum-125110: Anti-*Toxoplasma gondii* mechanisms of a Lys49-PLA₂ from *Bothrops moojeni* snake venom

Samuel Cota Teixeira ¹, Thales Alves de Melo Fernandes ^{2,*}, Guilherme de Souza ¹, Alessandra Monteiro Rosini ¹, Aryani Felix Fajardo Martínez ¹, Angelica Oliveira Gomes ³, Rosiane Nascimento Alves ⁴, Daiana Silva Lopes ⁵, Maria Vitoria da Silva ⁶, Emidio Beraldo-Neto ⁶, Patrícia Bianca Clissa ⁷, Bellisa Freitas Barbosa ¹, Veridiana de Melo Rodrigues Ávila ⁸ and Eloisa Amália Vieira Ferro ¹

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Introduction:

Toxoplasmosis is an alarming public health problem that affects more than one-third of the global population. We investigated the antiparasitic effects of MjTX-II, a Lys-49-PLA₂ from *Bothrops moojeni*, in human trophoblast cells and villous explants.

Methods:

MjTX-II was purified using two ion-exchange chromatographic steps and characterized through biochemical and proteomic analyses. The BeWo cells' viability was assessed using an MTT assay. The impact of MjTX-II on *T. gondii* was evaluated via trypan blue staining and scanning electron microscopy. The effect on the parasite's adhesion and invasion was assessed via differential antibody staining. The antiproliferative effect was evaluated using a β -galactosidase assay. Cytokine and VEGF levels were measured using ELISA and a cytometric bead array. ROS concentrations were quantified according to the intracellular peroxide-dependent oxidation. The involvement of VEGFR2 and ICAM-1 in the antiparasitic mechanism was investigated through docking simulations.

Results:

MjTX-II exhibited a high degree of purity in the biochemical and proteomic analyses. The MTT assay showed that MjTX-II did not reduce the viability of the BeWo cells. MjTX-II affected the surface of the parasites, decreased the number of intracellular parasites, and inhibited their intracellular proliferation. MjTX-II modulated the cellular environment by increasing the levels of IL-6, MIF, and ROS and reducing the levels of VEGF. The anti-*T. gondii* activity involved the modulation of the ROS and VEGF levels, as the addition of the ROS scavenger NAC or rVEGF reduced the antiprotozoal effect. Bioinformatic analyses revealed that MjTX-II is able to interact with the extracellular domain of VEGFR2 in the VEGF binding site, as well as ICAM-1, which was corroborated by the immunofluorescence analysis.

Conclusions:

Non-toxic concentrations of MjTX-II impaired *T. gondii* infection by increasing the ROS and VEGF levels in the host cells and may have involved interactions with ICAM-1 and VEGFR2, highlighting MjTX-II as prominent tool for the development of effective molecules against toxoplasmosis.



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sciforum-128375: Bee Venom as a Source of Anti-Angiogenic Agents: A Computational Study

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Angiogenesis, the formation of new blood vessels, is a fundamental process in tumor development, progression, and metastasis, making it a critical target in modern cancer therapy. Among natural bioactive compounds, animal venoms have emerged as a rich source of pharmacologically active peptides with potential anti-angiogenic effects. In this *in silico* study, we explored the therapeutic potential of bee venom peptides, focusing particularly on melittin and tertiapin, as inhibitors of tumor-induced angiogenesis. Using molecular docking techniques, these peptides were evaluated for their binding affinity toward key angiogenesis-related receptors, including platelet-derived growth factor receptor alpha (PDGFR- α), vascular endothelial growth factor receptors (VEGFRs), fibroblast growth factor receptors (FGFRs), and integrin $\alpha\beta3$. Both melittin and tertiapin exhibited strong and specific binding affinities, with melittin showing notable interaction with PDGFR- α and tertiapin demonstrating a high affinity for integrin $\alpha\beta3$. Molecular dynamics simulations further confirmed the stability and integrity of these peptide-receptor complexes over time, suggesting the potential for sustained biological activity. Additionally, functional enrichment analysis using the Reactome database revealed significant modulation of angiogenesis-related pathways, particularly those involved in cell migration and vascular remodeling. These findings highlight the potential of bee venom peptides as natural, targeted modulators of angiogenesis and support their further development as candidates for anti-cancer drug discovery.



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sciforum-125481: Bee Venom Proteins Modulate the Ability of Phospholipid Membranes of the Myelin Sheath and Endoplasmic Reticulum to Absorb Protons from Bulk Water: Pharmacological Relevance

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Introduction:

Experimental evidence has shown that the rate of ATP synthesis correlates more strongly with proton movement along the surface of the inner mitochondrial membrane (IMM) than with the transmembrane proton concentration gradient in the bulk aqueous phase. This has prompted a revision of the classical chemiosmotic theory of mitochondrial ATP production. It has been proposed that lateral proton transport along the IMM surface generates an electric potential that drives ATP synthase activity. Moreover, recent studies have reported proton accumulation and storage on the membranes of the myelin sheath and endoplasmic reticulum (ER), suggesting that surface-based energy storage via absorbed protons may be a fundamental principle of cellular bioenergetics. In this study, we examined whether membranes composed of different types of phospholipids and those enriched with either acidic or basic bee venom proteins differ in their capacity to enhance proton absorption at the membrane surface.

Methods:

Five types of phospholipids commonly found in myelin sheath and ER membranes were purified using immunoaffinity chromatography. Anionic and basic protein isoforms were isolated from bee venom via CM Sephadex C-50 chromatography. Liposomal membranes were prepared through ultrasonic frequency dispersion of phospholipid suspensions. Proton absorption at the membrane surface was assessed by measuring pH differences between liposome suspensions and pure water.

Results:

Membranes of liposomes enriched with anionic proteins showed enhanced proton absorption compared to protein-free liposomal membranes. Among all tested conditions, phosphatidylethanolamine membranes enriched with basic proteins exhibited the highest proton absorption, while other basic protein-enriched membranes showed minimal absorption.

Conclusions:

These findings support a revised model of membrane-associated energy storage, which holds potential pharmacological implications. A deeper understanding of membrane-based energy accumulation mechanisms may facilitate the development of novel therapies targeting age-related and pathological impairments in cellular bioenergetics.



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sciforum-124711: Cobra Venom Cytotoxin as a Tool for Probing Polymorphic Transitions, Proton Absorption and Permeability of Membranes Made of Phosphatidylethanolamine or Phosphatidylserine

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Introduction:

Survivin, a protein regulating mitosis, is overexpressed in all cancers. Unlike its expression during G2 and mitosis in healthy cells, survivin in cancer cells is active throughout the interphase, localizing in the nucleus, cytosol, and mitochondria. Mitochondrial survivin has been shown to enhance phosphatidylserine (PS) decarboxylase activity, increasing the concentration of non-bilayer phosphatidylethanolamine (PE), a feature phospholipid in the inner mitochondrial membrane (IMM) that facilitates oxidative phosphorylation by promoting polymorphic transitions and proton absorption to facilitate mitochondrial ATP synthesis. By increasing PE through decarboxylation of PS, survivin may remodel IMM architecture, stimulating cancer development. To investigate the interplay of PE and PS in polymorphic transitions in IMM, this study uses cobra venom cytotoxin II (CTII), which, at low concentrations, promotes lipid polymorphism. By examining lipid polymorphism, membrane permeability and proton absorptivity, we aim to shed light on PE and PS roles in IMM functionality in cancer.

Methods:

Phospholipid liposomes made of either PE or PS were prepared by ultrasonic radiation of phospholipid aqueous dispersions. The effects of CTII interaction with liposomes on membrane polymorphism and permeability were assessed using the ¹H-NMR spectroscopy and the [Cu(H₂O)₂(NH₃)₄]²⁺ complex ion spectrophotometry. Proton absorption by membranes in the absence and presence of CTII was quantified by measuring pH differences in an aqueous buffer with and without liposomes.

Results:

CTII induced the formation of non-bilayer structures in both PE and PS membranes. In PS membranes, non-bilayer structures increased membrane permeability, whereas the inverted lipid micelles induced by CTII in PE membranes did not affect membrane permeability. Furthermore, CTII-treated PE membranes demonstrated superior proton absorption compared to CTII-treated PS membranes.

Conclusions:

Superior proton absorption and the inverted micelles formation triggered by CTII in PE membranes—without compromising membrane permeability—may represent key features of the IMM that enhance ATP production, thereby supporting the accelerated proliferation of cancer cells.



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sciforum-126444: Computational Predictions of Mycotoxin–Protein Interactions: A Veterinary Perspective

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Mycotoxins are naturally occurring toxic metabolites produced by fungi that contaminate animal feed, posing significant risks to livestock health and food safety. The molecular interactions of these toxins with key proteins are critical for elucidating toxicity mechanisms and developing preventive strategies. This study explores the application of computational methods, particularly molecular docking and molecular dynamics (MD) simulations, to predicting and validating the interactions between major mycotoxins and biologically relevant target proteins in food-producing animals.

Molecular docking was first employed to estimate the binding affinity of representative mycotoxins, including aflatoxin B1 and ochratoxin A, with proteins directly implicated in toxin transport, metabolism, and bioavailability and the interaction profiles. These included bovine serum albumin (BSA), a primary carrier protein mediating systemic distribution; porcine organic anion transporter 1 (OAT1), which facilitates renal elimination; and cytochrome P450 enzymes, responsible for metabolic activation. The resulting complexes were subsequently evaluated using MD simulations to assess the stability, flexibility, and dynamic behaviour of the ligand–protein interactions under physiological conditions. Parameters including the root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), hydrogen bond occupancy, and molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) energy calculations were analysed to validate the binding stability and interaction persistence over time.

The findings underscore the potential of *in silico* approaches in veterinary toxicology for identifying high-risk feed contaminants and elucidating their molecular mechanisms of action through interactions with biologically relevant proteins. This framework not only advances our understanding of mycotoxin toxicity but also supports evidence-based risk assessments, regulatory monitoring, and the rational design of protective feed additives. Importantly, characterising these protein–mycotoxin interactions provides mechanistic insights into toxicokinetics; informs the development of targeted mitigation strategies to reduce mycotoxin exposure in livestock; and contributes to safeguarding public health by limiting the transfer of residues into the human food chain. The integration of computational toxicology into veterinary research aligns with One Health principles, offering scalable tools for protecting animal health and ensuring food safety.



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sciforum-125449: Development of Broad-Spectrum Antiviral and Antitumor Therapeutics from Cobra Toxin (NT3) for Veterinary and Human Use

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Introduction:

CYGNOS BioTech is pioneering the development of broad-spectrum antiviral (PVX) and antitumor (MMX) biologic products for both veterinary and human applications. Our innovative technology has led to the issuance of nine patents covering composition, process, and usage. The US FDA has granted two Investigational New Animal Drug Applications for 1) a non-specific antiviral drug for companion animals and 2) an antitumor drug for stage II and III canine oral malignant melanoma.

Methods:

PVX and MMX are derived from cobra venom toxin (NT3) through fractionation followed by proprietary chemical processes to achieve detoxification and specific biologic activities.

Results:

Preliminary data suggest that PVX's antiviral activity targets host-cell mechanisms rather than the virus. PVX inhibits Protein Kinase activities, potentially affecting cellular pathways related to transcription and translation. Clinical studies in dogs, cats, and horses demonstrate significant antiviral and antitumor effects without adverse events. PVX appears effective for most viral infections, including enveloped RNA and DNA viruses such as canine distemper virus (paramyxovirus), Human and Feline Herpes viruses, and Feline Corona virus causing Feline Infectious Peritonitis (FIP).

Conclusions:

The global community faces increasing threats from emerging zoonotic diseases and bio-terrorism activities. The potential for a new pandemic or the release of genetically engineered viruses is a real and devastating concern. CYGNOS BioTech's technology serves as a potential platform to treat infectious diseases, applicable across veterinary, public health, and military sectors. The availability of a drug capable of addressing emerging and genetically engineered viruses as a first line of defense could have a profound impact.



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sciforum-129816: Discovery of Novel Ion Channel Modulators from *Physalia physalis*

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Animal venoms are increasingly recognized as valuable molecular toolboxes for the discovery of novel compounds with therapeutic potential. Among the most promising therapeutic targets for venom-derived molecules are voltage-gated ion channels, which are involved in multiple physiological processes and implicated in various diseases. Here, we report the discovery of novel peptides from the venom of *Physalia physalis*, predicted to target vertebrate Kv channels.

A proteomic analysis of *P. physalis* tentacles led to the identification of 30 ShK-like peptides belonging to the ShK family, based on their conserved primary structure and cysteine-rich scaffold. Some of them showed potential as therapeutic leads due to their ability to selectively target specific Kv channel subtypes. Their 3D structures were predicted using the deep-learning-based tool AlphaFold and subjected to molecular docking against a panel of human potassium channels. Several candidates exhibited low binding energies and high predicted specificity for the Kv1.3 channel. A high-affinity peptide was produced in recombinant form and functionally validated through whole-cell patch-clamp electrophysiology, confirming its inhibitory activity against Kv1.3 at nanomolar concentrations. Additionally, a radioligand competition assay with α -dendrotoxin in human brain tissue revealed low competition for Kv1.1, Kv1.2, and Kv1.6, supporting the peptide's selectivity.

These findings underscore the potential of *P. physalis* venom components as a source of new and selective ion channel modulators. Given the role of the Kv1.3 channel in a range of human diseases—including pain, autoimmune disorders, and neurological conditions—these molecules hold great promise for the development of new therapeutic drugs.



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sciforum-129919: Dual Modulation of Hemostasis Using *Physalia physalis* Venom: Insights into Thrombin-Like and Fibrinolytic Activities

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Physalia physalis, commonly known as the Portuguese man o' war, is a venomous cnidarian frequently observed in the Atlantic Ocean. Contact with its tentacles may result in local and systemic symptoms, including interference with the human hemostatic system. Despite increasing clinical reports, the molecular basis of these effects remains poorly understood. In this study, we characterized the enzymatic properties of *P. physalis* venom obtained through electrostimulation-induced cnidocyte discharge, a method that enables the selective release of venom with minimal structural contamination.

The venom demonstrated a dual modulatory effect on hemostasis. One fraction exhibited potent fibrinolytic and fibrinogenolytic activity, capable of degrading fibrin clots and completely hydrolyzing the α , β , and γ chains of human fibrinogen. The second fraction promoted rapid clot formation through the generation of ~23 kDa fibrin fragments, a hallmark of thrombin-like activity. Enzymatic assays and inhibitor profiling revealed that the fibrinolytic effect was primarily associated with a metalloprotease, whereas the thrombin-like activity was attributed to a serine protease. These enzymes act on different components of the coagulation cascade, suggesting distinct biochemical mechanisms within the same venom source.

The discovery of both fibrinolytic and thrombin-like functions within *P. physalis* venom represents a novel insight into cnidarian envenomation. It also emphasizes the biochemical diversity of marine venoms and their potential application in therapeutic development. These results not only improve our understanding of venom-induced coagulopathies but also support the exploration of marine-derived enzymes as lead candidates for new anticoagulant or procoagulant drugs.



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sciforum-125090: Epilepsy-associated mutation T226R in Kv1.1 subunit: properties of heterotetrameric Kv1.1(T226R)-Kv1.2 channel

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Introduction

Co-expression of the Kv1.1 and Kv1.2 subunits in the cells of the central nervous system results in the predominant formation of heterotetrameric voltage-gated potassium Kv1.1/Kv1.2 channels, which regulate neuronal excitability. An inherited mutation of the Kv1.1 channel, T226R, which is associated with episodic ataxia and epilepsy, disrupts the Kv1.1 channel's activity. The influence of this mutation on the properties of the heterotetrameric Kv1.1/Kv1.2 channel needs to be investigated.

Methods

Expression plasmids encoding fluorescently labeled mKate2-Kv1.1(T226R) and tandem dimers mKate2-Kv1.1-Kv1.2 and mKate2-Kv1.1(T226R)-Kv1.2 were constructed and used to transfect Neuro-2a cells. Recombinant peptide toxins and the fluorescent ligand HgTx-GFP were produced. Confocal microscopy was used to study the ligand binding and distribution of the channels in the cells. The electrical activity of the heterotetrameric channels formed by the tandem dimers was studied using a whole-cell patch-clamp technique.

Results

The expression of mKate2-Kv1.1(T226R) and mKate2-Kv1.1(T226R)-Kv1.2 in the plasma membrane and the ability of the dimers to form heterotetrameric channels with a preserved pore structure were studied by binding the fluorescent ligand HgTx-GFP. The binding affinities of peptide blockers for the wild type and the mutant heteromers were studied in a competitive assay. The activation and deactivation constants, as well as the voltage dependence, were measured for the heteromeric channels formed by the Kv1.1(T226R)-Kv1.2 and Kv1.1-Kv1.2 dimers. The essential differences that were identified in these studies are discussed.

Conclusions

The data obtained provide insights into the molecular mechanisms of epilepsy associated with the T226R mutation in the Kv1.1 subunit of the Kv1 channels.

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sciforum-128476: Epitope-based discovery of aptamer targeting cobra venom cytotoxin

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Cytotoxin (CTX) is a three-finger toxin found predominantly in cobra venoms, responsible for dermonecrosis. CTX is generally low in immunogenicity, resulting in the ineffectiveness of existing antibody-based antivenoms in attenuating the local symptoms of dermonecrosis. Aptamers emerge as promising new antivenoms for affordability, manufacturing feasibility and low immunogenicity. We found four epitopes within its functional loops: 'KLVPLFYK', 'AGKNL', 'MFMVSTPKVPV', and 'DVCPKNSLL'. This study aimed to discover aptamers that can specifically recognise these functional epitopes. The binding affinity, neutralisation efficacy, and mechanisms were determined for the aptamer candidates. Our results show that four different aptamer candidates exhibited different degrees of binding to functional epitopes 1, 2, 3, and 4. These aptamers were Apt15, Apt33, Apt26, and Apt31, respectively. Both functional epitopes 1 and 4 demonstrated the highest synergistic cytotoxicity in human skin keratinocytes, with elevated levels of receptor-interacting protein kinase-3 (RIPK3), implying that necroptosis associated with the cytotoxicity. Based on the enzyme-linked aptamer assay (ELAA), Apt31 had the strongest binding affinity towards functional epitope 4, with a K_D value of 187.7 nM. In the in vitro neutralisation assay, Apt31 conferred neutralisation potency with an ED_{50} of 0.03 nM. However, Apt31 did not rescue human skin keratinocytes in experimental post-envenomed conditions, although increasing concentrations of Apt31 reduced the RIPK3 levels in the post-envenomed keratinocytes. The results conclude that Apt31 potentially neutralised the in vitro cytotoxicity caused by functional epitopes but not the post-envenomed conditions.



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sciforum-129682: Evaluation of Antiplatelet Effects of Type C Lectin From *Bothrops neuwiedi* Venom

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Lectins are non-enzymatic proteins that bind reversibly and specifically to carbohydrates. In snake venoms, lectins are grouped into classical lectins, which bind Ca²⁺ and sugars (CTLs), and non-sugar-binding snake venom C-type lectin-related proteins (CLRPs). These proteins target distinct molecules, such as coagulation factors and receptors, on platelet surfaces and endothelial or immune cells, which are involved in hemostasis, inflammation and metastasis. Because of their specificity and versatility, C-type lectins are used as models in biotechnological studies and applications. Toward this goal, the current study aims to determine the molecular and biochemical properties of the C-type lectin isolated from *Bothrops neuwiedi* venom (CTL-Bn). The protein was isolated by D-galactose column affinity chromatography and analyzed by SDS-PAGE and mass spectrometry. We demonstrate that CTL-Bn forms oligomers whose monomers have a molecular mass of approximately 15 kDa. CTL-Bn induced hemagglutination at a minimum dose of 2.5 µg, which was inhibited by galactose, lactose and EDTA. The protein is stable at a wide pH range and shows optimal activity at 20 °C. Furthermore, CTL-Bn inhibits platelet aggregation induced by convulxin and binds to fibrinogen and glycoprotein GPVI (the main collagen receptor), which probably explains its inhibitory effect on convulxin-induced platelet aggregation. Based on docking molecular studies, we proposed that CTL-Bn binds to GPVI in a region formed by hydrophobic residues with polar and basic residues around the periphery. To evaluate the role of CTL-Bn in the metastatic process, we tested CTL-Bn on platelet aggregation induced by MDA-MB-231 tumor cells. CTL-Bn inhibited this aggregation; however, in the MTT assay, the protein did not affect the viability of the tumor cells. Altogether, our results provided a comprehensive biochemical, biological and structural characterization of CTL-Bn.



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sciforum-129851: Identifying and optimizing small molecule and peptide inhibitors targeting ricin and Shiga toxins: Type II Ribosome-Inactivating Proteins (RIPs)

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Ricin, one of the most potent toxins known, and the Shiga toxins (Stxs) produced by *Escherichia coli* (STEC) bind to the C-termini of ribosomal P-stalk proteins to depurinate the sarcin/ricin loop at a different location on the large subunit of the ribosome. The ribosome binding sites of ricin and Stxs have not yet been targeted using small molecules. We established a fluorescence anisotropy-based competition assay to evaluate small molecule inhibitors against RTA. A unique feature of this assay is its ability to identify small molecule inhibitors that bind to the ribosome binding site of RTA and allosterically inhibit its catalytic activity. The FA assay was used to measure the binding constants of small molecule inhibitors that blocked the interaction between a BODIPY-TMR labeled peptide probe mimicking the conserved C-terminal end of the P-stalk proteins (P11) and the A subunit of ricin toxin (RTA). Initially, we used the Biacore SPR to screen the Maybridge Ro3 Core library of compounds and identified CC10501 as the lead molecule. Then, we improved CC10501 using a structure-based design, and using the fluorescence anisotropy-based competition assay, we identified RU-NT-206 and RU-NT-192 as the lead molecules, which showed over 50- and 30-fold improved affinity. The K_i values measured using the FA assay showed a positive correlation with the results of a Vero cell protection assay. These results validated the P-stalk pocket as a target for inhibition and identified RU-NT-206 and RU-NT-192 as lead molecules for further optimization.



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sciforum-126273: Modeling Neuromuscular Junction Intoxication Using Human iPSC-derived Organoids

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The human neuromuscular junction (NMJ) is a specialized synapse responsible for modulating neurological influences on muscle contraction and enabling precise motor neuron-to-muscle cell communication. Compared to rodent NMJs, human neurophysiology remains poorly characterized, highlighting the need for more translatable disease models.

To study the NMJ's development and organization in humans, we employed a previously established system of neuromuscular organoids derived from induced pluripotent stem cells. This 3D co-culture system supports the concurrent development of motor neurons and skeletal muscle, exhibiting key molecular and structural hallmarks of NMJ formation, including characteristic synaptic architectures, acetylcholine receptor clustering, and spontaneous contractile activity.

To validate the physiological relevance of this model, we evaluated its response to known neurotoxins that disrupt neuromuscular transmission: botulinum neurotoxin A (BoNT/A), tetrodotoxin (TTX), and curare. Exposure to these agents resulted in reproducible impairments in synaptic communication and contractility, consistent with their known mechanisms of action. These findings demonstrate the model's sensitivity to both presynaptic and postsynaptic disruption and support its utility for studying NMJ pathology under controlled conditions.

This system enables detailed investigations into the mechanisms underlying NMJ dysfunction and offers a manipulatable platform for screening therapeutic interventions. It advances our ability to replicate human NMJ biology and disease, including disruptions to neurotransmitter release and synaptic signaling, and provides a promising tool for identifying molecular targets in neuromuscular and motor neuron disorders.



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sciforum-129130: Molecules from marine invertebrate venoms recover cathepsin D activity, impaired by oligomerized A β 42, in a neuronal cell model of Alzheimer's disease

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Introduction:

Alzheimer's disease (AD) is characterized by the accumulation of the amyloid- β (A β) peptide in its oligomeric and fibrillar conformations, which disrupts several neuronal functions, including the autophagy-lysosomal pathway, essential for eliminating misfolded, aggregated, or mutated proteins and maintaining proteostasis. Cathepsin D, the most abundant lysosomal peptidase, is dysfunctional in AD, and in this study, we evaluated the effects of molecules from marine invertebrates on restoring its activity.

Methods:

Differentiated SH-SY5Y cells were pre-treated with oA β 42 (5 μ M) for 48 hours, followed by treatment with venom from six Brazilian marine invertebrate species (1 mg/mL) for 24 hours. After the treatment, cell lysates were obtained, and the cathepsin D activity was measured using a specific substrate (GKPILFFRLK(Dnp)-D-R-NH₂-MCA) and the fluorescence intensity monitored every five minutes (λ_{ex} = 405 nm, λ_{em} : 495 nm). The secretions were fractionated through solid-phase extraction in a C18 cartridge, eluted using acetonitrile/0.1% trifluoroacetic acid, and tested again.

Results:

oA β 42 decreased the velocity of cathepsin D's enzymatic reactions by 40% compared to those in the control (cells without treatment). Venom from the corals *Renilla reniformis* and *Tubastraea tagusensis*, the sea anemone *Anthopleura cascaia*; and the sea urchin *Echinometra lucunter* restored the cathepsin D activity, bringing it back to the control levels or even exceeding them. The venom from *A. cascaia*, which exhibited the most intense activity, was fractionated further and analyzed, and an active fraction rich in peptides and small molecules was identified.

Conclusion:

Marine-derived molecules could restore the activity of the main lysosomal enzyme, cathepsin D, important for the elimination of the amyloid peptide and reductions in neuron death, which could contribute significantly to new therapies for the treatment of AD.



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sciforum-124018: New analytical cellular systems: a study of Kv1 channels, their fluorescent ligands, and peptide blockers

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Introduction:

Voltage-gated potassium channels Kv1.1 and Kv1.2 are key players of neuronal excitability. The Kv1.3 channel mediates the immune response of T lymphocytes. Many peptide toxins are blockers of Kv1 channels and help to study channel functioning under normal and pathological conditions. New pharmacology-relevant techniques are needed to search for advanced blockers and to characterize their properties for potential drug development.

Methods:

Plasmids encoding Kv1 subunits fused with the fluorescent protein mKate2 and those encoding hongotoxin 1 (HgTx1) and agitoxin 2 (AgTx2) fused with GFP were obtained. Recombinant fluorescently labeled and unlabeled peptide blockers including Ce-peptides from *Centruroides elegans* scorpion venom were produced. Interactions between blockers and Kv1 channels expressed in mammalian cells were investigated with confocal microscopy and the whole-cell patch clamp technique.

Results:

The concept of creating analytical cellular systems (ACSs) for the study of Kv1.1, Kv1.2, and Kv1.3 channels, their fluorescent ligands, and peptide blockers using confocal microscopy has been successfully implemented. Point mutations that enhanced the membrane expression of fully functional channels were introduced.

High-affinity fluorescent ligands (synthetic Atto488-HgTx1 and recombinant HgTx1-GFP and AgTx2-GFP) were developed. Using competitive binding of labeled and unlabeled ligands, the apparent dissociation constants of complexes between unlabeled blockers and Kv1 channels were determined. Peptides Ce1 and Ce4 were found to be the most selective and potent peptide blockers of the Kv1.2 channel compared to channels Kv1.1 and Kv1.3.

Conclusions:

The proposed concept of ACSs is universal and can be applied to various potassium channels. Peptides Ce1 and Ce4 can be useful in the study of Kv1.2-mediated currents in neurons, and they can also be used to construct fluorescent ligands selective for Kv1.2.

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sciforum-126859: Oral Rutin Reduces the Hemostatic Impairment Induced by *Bothrops jararaca* Venom in Experimental Models

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Introduction:

Bothrops jararaca (Bj) snakebites cause hemostatic and inflammatory disturbances in victims. Bj venom contains various proteins that induce local and systemic bleeding, thrombocytopenia, and consumption coagulopathy. Parenteral administration of rutin, a bioflavonoid, has shown potential in mitigating venom effects by reducing bleeding time, delaying coagulopathy, and decreasing local hemorrhage (doi: 10.3389/fphar.2022.828269), and thereby may accelerate the recovery of patients treated with specific antivenom therapy (SAB).

Aims:

To evaluate the potential use of orally administered nano-rutin in reducing the hemorrhagic effects and hemostatic disturbances caused by Bj envenomation. Additionally, this study aimed to explore the role of cfDNA in the pathophysiology of envenomation and assess the levels of inflammatory markers in animals treated with a combination of nano-rutin and SAB.

Methods:

The use of male C57BL/6 mice was approved by IACUC (4491070319). Mice were divided into experimental groups (n=10/group) and evaluated at 6 and 12h after s.c. administration of saline or Bj venom. Nano-rutin (v.o.) and/or SAB therapy (i.v.) were administered following venom administration.

Results:

Marked hypofibrinogenemia, increased IL-6 levels, and intense local hemorrhage were observed in envenomed mice. The association of nano-rutin with SAB restored fibrinogen and IL-6 levels faster than SAB or rutin alone. This association also blocked the development of local hemorrhage. Nano-rutin alone increased NO levels. The levels of cfDNA were not significantly different from those of the controls, but were higher with this association at 6h.

Conclusions:

The association of the administration of nano-rutin followed by SAB enhanced the recovery of inflammatory and hemostatic markers after Bj envenomation, representing a promising strategy for snakebite treatment.

Acknowledgements:

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sciforum-129590: Production and Functional Analysis of *Echis coloratus* Disintegrins in Platelet Aggregation

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Snake venom disintegrins are a group of small, non-enzymatic proteins that act by binding to integrins on platelet surfaces and other cells, preventing the interactions required for cell adhesion and platelet aggregation. We expressed and purified three disintegrins from *Echis coloratus*, containing different integrin-binding motifs: Dis_VGD (VGD motif), Dis_KGD (KGD motif), and Dis_RGD (RGD motif). The expression was carried out using the *E. coli* expression system, and the purification steps included immobilised metal affinity, anion exchange, and size exclusion chromatography. The ability to inhibit platelet aggregation was evaluated by plate-based aggregometry, using 2-MeS-ADP and CRP-XL as agonists. Dis_VGD presented no effect on platelet aggregation under the tested conditions, while Dis_RGD and Dis_KGD were capable of inhibiting platelet aggregation in a non-competitive and dose-dependent manner. When the platelet aggregation was induced by 2-MeS-ADP, Dis_RGD presented almost complete inhibition at 250 nM, while Dis_KGD presented only 20% of inhibition at the same concentration. The effect on platelet aggregation induced by CRP-XL was less pronounced, with Dis-RGD and Dis-KGD reaching 70% and 10% of inhibition at 500 nM, respectively. The VGD motif binds to integrin $\alpha 5 \beta 1$, a fibronectin receptor involved in platelet adhesion and spreading, but not in aggregation. In contrast, the RDG and KGD motifs interact with the $\alpha \text{IIb} \beta 3$ integrin, a key mediator of platelet aggregation, and are therefore expected to inhibit this process. Our study confirmed that the expressed *E. coloratus* disintegrins are functional and present the expected effect on platelet aggregation. Further analyses are underway to evaluate their impact on cell adhesion and to gain a more comprehensive understanding of their broader activity.



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sciforum-129912: Purification and Biochemical Characterization of a Probable P-III_d Class Metalloprotease from *Bothrops atrox* Venom Active in Platelet Aggregation

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Introduction:

Snake venom represents a relevant source of bioactive molecules with therapeutical potential, particularly for modulating complex pathways such as the coagulation cascade. Snake venom metalloproteinases (SVMPs) are enzymatic proteins classified according to their structural domains: (1) class P-I SVMPs, which exclusively contain the metalloproteinase catalytic domain; (2) class P-II SVMPs, which possess, in addition to the catalytic domain, a disintegrin domain; and (3) class P-III SVMPs, which contain metalloproteinase, disintegrin-like, and cysteine-rich domains. Class P-III SVMPs may also associate with C-type lectin-like domains, which are then classified as P-III_d. Snake venom C-type lectin-like proteins (snaclecs) are non-enzymatic proteins that are structurally organized as heterodimers (α and β subunits). They play a fundamental role in hemostasis through receptor-mediated interactions despite lacking carbohydrate-binding properties. In previous work from our group, a proteolytically active fraction from *Bothrops atrox* venom showed reactivity with anti-C-type lectin (CTL) antibodies. The present study aimed to characterize the biochemical and functional properties.

Methods:

The identified fraction from *B. atrox* venom was evaluated using SDS-PAGE, plasminogen activation assays, hemagglutinating-activity assays, platelet aggregation assays using washed platelets and platelet-rich plasma (PRP), Western blot analysis for reactivity with anti-SVMP-P-I and -P-III antibodies, and proteolytic-activity assays on extracellular matrix (ECM) components.

Results:

The selected fraction induced aggregation in PRP, but only weakly in washed platelets. However, it strongly promoted the aggregation of washed platelets in the presence of human fibrinogen. Western blot analysis revealed a ~26 kDa band recognized by anti-CTL and anti-SVMP-P-I antibodies, while the anti-SVMP-P-III antibody identified bands of ~26 kDa and between 43 and 72 kDa. The fraction showed no hemagglutinating activity, did not activate plasminogen, but effectively degraded ECM components.

Conclusion:

These results suggest the presence of a P-III_d in *B. atrox* venom. Further studies are necessary to fully elucidate its structure and biological activities.



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sciforum-129671: Purification and Characterization of C-Type Lectin from *Bothrops leucurus* Snake Venom with Anti-Platelet and Anti-Metastatic Potential

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Introduction:

Snake venom lectins are categorized into classical sugar-binding C-type lectins and non-sugar binding C-type-like lectins. C-type lectins are typically homodimeric proteins with affinity for beta-galactoside residues and are known to exhibit diverse biological activities, including the inhibition of platelet aggregation. Platelets play a crucial role in several physiological and pathological processes, such as thrombosis and metastasis. This study aimed to elucidate the biochemical and functional properties of leucetecin, a C-type lectin isolated from *Bothrops leucurus* venom, with a particular focus on its effects on platelet function.

Methods:

Leucetecin was purified via affinity chromatography using D-galactose gel resin. Its effects on platelet function were assessed using washed platelets and platelet-rich plasma. The potential anti-metastatic activity of leucetecin was evaluated using MDA-MB-231 breast cancer cells. Platelet-tumor cell interactions were quantified using a plate reader-based assay.

Results:

Leucetecin displayed a molecular mass of approximately 15 kDa under reducing conditions and 30 kDa under non-reducing conditions. Functionally, leucetecin inhibits platelet aggregation induced by convulxin (CVX) and the von Willebrand factor (vWF). Western Blot analysis demonstrated that leucetecin binds to key platelet receptors—GPVI, GPIb, α V integrin, and CLEC-2—correlating with its inhibitory effects on platelet aggregation. Notably, leucetecin also inhibited platelet aggregation induced by MDA-MB-231 and reduced the adhesion of these cells at non-cytotoxic concentrations. Furthermore, leucetecin disrupted platelet-tumor cell interaction.

Conclusion:

These findings suggest that leucetecin has biotechnological potential as a novel antiplatelet and/or anti-metastatic agent, due to its ability to inhibit platelet aggregation and platelet-tumor cell interactions.



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sciforum-129672: Rattling Bugs Away: The potential Antibacterial Properties of *Crotalus Mictlantecuhtli* against *S. aureus* and *E. coli*

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Introduction

Mexico is the country with the highest diversity of rattlesnakes worldwide, and their venom represents a valuable source of molecules with biomedical potential. Documented bioactivities include antiproliferative, analgesic and microbicide properties. Following that, the World Health Organization considers antibiotic resistance as a global threat. Therefore, studying alternatives to these drugs is imperative. The Veracruz neotropical rattlesnake (*Crotalus mictlantecuhtli*) is an endemic species from Central Mexico whose venom has been characterized before, but with no reports of its antibacterial properties, to the best of our knowledge.

Methods

Four protein fractions from *C. mictlantecuhtli* venom were obtained by size exclusion chromatography (SE-LC). The fractions were lyophilized and stored at -20°C until analysis. Protein quantification was performed using a Bicinchoninic Acid (BCA) Protein Assay, with bovine serum albumin (BSA) as a standard. All fractions were adjusted by protein content, diluted with saline solution (NaCl 0.9%) and added to 96-well microplates with Müller-Hinton (MH) in concentrations ranging from 10 to 0.001 µg/µL. To evaluate the antimicrobial activity, the Minimal Inhibitory Concentration (MIC) was determined via the microdilution method, which was performed by inoculating each well with ATCC strains *E. coli* (25922) or *S. aureus* (25923) standardized in brain-heart infusion to 0.5 McFarland. The results were corroborated using the agar well diffusion method in MH. Additionally, blood samples were drawn and anticoagulated with calcium-balanced lithium heparin to evaluate indirect hemolysis in 5% blood agar. The data was analyzed by two-way ANOVA and direct observation.

Results

Venom fractions 1, 3 and 4 did not differ significantly from the gentamicin control below 1 µg/µL for *E. coli* and *S. aureus* ($P > 0.05$). Fraction 1 exhibited hemolytic halos with diameters of 0.7 mm and 14 mm, respectively.

Conclusions

C. mictlantecuhtli venom contains proteins of medical interest, showing antimicrobial potential; hence, it is imperative to continue studying it.



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sciforum-129707: Refolding of recombinant Tityus toxins improves antigen quality for their use as immunogens in Antivenom production

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Introduction

Approximately 80% of the 8,000 annual envenomation cases reported in Argentina are attributed to scorpion stings, with *Tityus carrilloi* being the most medically significant species. Antivenom is the only specific treatment for severe cases. It is produced from the plasma of horses hyperimmunized with *T. carrilloi* venom. However, the venom supply represents a bottleneck in antivenom production. In *Tityus serrulatus*, a related species, recombinant toxins have been investigated as potential replacements or complements for native venom. A similar approach could be applied to *T. carrilloi* by identifying key toxin candidates and optimizing expression systems to improve antigenicity. Sodium channel-targeting toxins, which drive the most severe symptoms, have complex structures stabilized by four disulfide bonds. This study evaluated how antigenicity is influenced by refolding conditions that promote native-like conformations.

Methods

Fusion proteins 6xHis_MBP_TsNTxP and 6xHis_MBP_Tt1G were expressed in *E. coli* Shuffle® cells and purified using immobilized metal affinity chromatography under denaturing conditions. Protein expression, molecular weight, and purity were confirmed via SDS-PAGE and Western blotting. Antigenic recognition was assessed via ELISA using six independent antivenom batches against reduced/alkylated, refolded, and non-refolded protein versions.

Results

Soluble recombinant TsNTxP and Tt1G fused to MBP were successfully expressed in *E. coli*. All six antivenom batches showed stronger recognition of the refolded proteins, confirming the relevance of conformational epitopes. Moreover, TsNTxP exhibited stronger reactivity than Tt1G, supporting its potential as a complementary immunogen to *T. carrilloi* venom in antivenom production.

Conclusions

Our findings demonstrate that refolding significantly increased recognition by *T. carrilloi* antivenom for both TsNTxP (from *T. serrulatus*) and Tt1G (from *T. carrilloi*), highlighting the role of conformational epitopes in immune recognition.



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sciforum-128403: Selective Anticancer Activity of Moroccan *Naja haje* Venom and Purified Cytotoxins Against Hepatocellular Carcinoma in 2D and 3D Tumor Models

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Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, often diagnosed at advanced stages when curative options are limited and conventional therapies like sorafenib show modest efficacy and significant toxicity. This highlights the urgent need for innovative, targeted treatments with improved selectivity and safety. Snake venoms are a rich source of bioactive molecules with therapeutic potential, particularly in the field of oncology. This study explores the anticancer properties of Moroccan *Naja haje* venom and its purified fractions against HCC using both conventional monolayer (2D) cultures and physiologically relevant multicellular tumor spheroid (3D) models. Crude venom was fractionated by gel filtration and RP-HPLC, and the resulting components were tested for cytotoxicity on HepG2 and Huh7.5 liver cancer cell lines. Their efficacy was further evaluated in 3D spheroids co-culturing cancer cells with human fibroblasts (WI38), hepatic stellate cells (LX2), and endothelial cells (HUVECs). Normal hepatocytes (Fa2N-4) were used to assess off-target toxicity. Several fractions, particularly Fraction 7 and its subfraction 7d, significantly inhibited cancer cell proliferation and reduced spheroid size, with minimal toxicity to normal liver cells, demonstrating greater selectivity than sorafenib. Proteomic analysis identified cytotoxins 2, 5, and 10 as key components responsible for this activity. These findings underscore the potential of *Naja haje* venom-derived peptides as promising candidates for selective HCC therapy, warranting further investigation into their mechanisms of action and therapeutic development.



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sciforum-129706: Toxin tools for functional nanocrystal production

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Nanocrystals are unique crystalline particles with a highly ordered atomic structure and distinctive physicochemical properties which arise from their high surface-area-to-volume ratios. These characteristics have made nanocrystalline systems increasingly attractive for biomedical applications, particularly in biomanufacturing, drug delivery and therapeutic protein stabilisation. A biologically derived example of such nanocrystals is found in the parasporal inclusions of *Bacillus thuringiensis* (Bt), composed of Cry proteins. These crystalline protein structures have traditionally been employed as bioinsecticides due to their specificity and toxicity toward target insect species. However, studies have demonstrated their non-toxicity in humans and non-target insects, thereby highlighting their potential for safe biotechnological applications. The inherent crystallinity and high stability of Cry proteins have prompted exploration into their use as platforms for recombinant protein encapsulation.

This study investigates the feasibility of utilising Cry protein nanocrystals as stable scaffolds for the display of functional peptide tags. Specifically, the SpyTag peptide sequence was genetically fused to Cry proteins, and the resulting recombinant nanocrystals were produced in *E. coli*, purified, and characterised. The structural analysis via electron microscopy revealed irregularly shaped particles ranging from 700 to 800 nm in size. Functional validation was performed by incubating the SpyTag-functionalised crystals with SpyCatcher, confirming covalent complex formation through SDS-PAGE. These findings demonstrate that Cry-based nanocrystals can preserve the functionality of fused peptide tags, supporting their use as versatile platforms for recombinant protein stabilisation. This approach offers promising implications for the easy production and stable formulation of therapeutically relevant proteins with poor intrinsic stability.



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sciforum-129934: Trichothecenes as Chemical Probes: Impact on Photosynthesis, Chloroplast Morphology, and Plant Stress Response

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Introduction:

Deoxynivalenol (DON), a trichothecene mycotoxin produced by *Fusarium graminearum*, is a potent virulence factor and a critical determinant of Fusarium Head Blight (FHB) in small grain cereals like wheat and barley. Previous research, utilizing a pooled chemical-genetic screen of *Chlamydomonas reinhardtii* mutants, identified that DON activates the chloroplast unfolded protein response (cpUPR), a chloroplast retrograde signaling pathway. This demonstrated that trichothecenes induce chloroplast stress. The present research leveraged trichothecenes as chemical probes to elucidate the chloroplast-to-nucleus communication during stress. Translating insights from algal systems to higher plants offers crucial clues to ways of mitigating crop diseases like FHB.

Methods:

We investigated the impact of trichothecenes on chloroplasts in *Chlamydomonas*, *Arabidopsis*, wheat, and barley. The chloroplast damage was assessed via changes in the autofluorescence and general chloroplast morphology. Using a miniaturized Fluorescence Induction and Relaxation (FIRe) system developed by Max Gorbunov at Rutgers University, we measured the photosynthetic rates and photooxidation in *Arabidopsis* leaf tissues exposed to vacuum-infused trichothecenes.

Results:

Trichothecenes consistently caused significant chloroplast damage in higher plants. The exposure of *Arabidopsis* leaves to 50 μ M DON for 1 hour reduced the quantum efficiency of PSII (Fv/Fm) by approximately 20%. DON exposure also significantly reduced the chloroplasts' autofluorescence. In *Chlamydomonas*, the disruption of Mars1, a key cpUPR kinase, inhibited the induction of proteotoxic stress proteins, including heat shock protein CrHSP22F. Testing orthologous cpUPR-related nuclear genes in wheat showed the robust induction of TaHSP22E. The screen identified additional mycotoxin-sensitive *Chlamydomonas* mutants exhibiting defective cpUPR activation, providing further insights for higher plant research.

Conclusions:

Our findings underscore that trichothecenes are valuable tools for investigating the chloroplast retrograde signaling pathway and identifying novel players, including heat shock proteins, in the cpUPR. These comprehensive analyses, from detailed chloroplast damage assessments to genetic screens, have deepened our understanding of plant stress responses and the mechanisms by which these toxins contribute to causing cereal crop diseases.



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sciforum-129811: Trigonelline, a jellyfish alkaloid, in the restoration of cathepsin B and D activity in the substantia nigra and caudate putamen in a rat model of Parkinson's disease

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Introduction:

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the nigrostriatal pathway, primarily due to misfolded α -synuclein. Lysosomes play a crucial role in degrading these proteins through cathepsins, a mechanism impaired in PD. Trigonelline (TGN), an alkaloid found in plants and identified in jellyfish by our research group, has been shown to inhibit caspase-1. Its analog, nicotinic acid, has demonstrated beneficial effects in PD models. This study investigates the modulation of cathepsin D (CTSD) and cathepsin B (CTSB) activity using TGN in the substantia nigra (SN) and caudate putamen (CPu) of PD-induced rats.

Methods:

Male Wistar rats were divided into three groups: Control (striatal saline injection and oral vehicle), PD (striatal 6-OHDA and oral vehicle), and PD and TGN (striatal 6-OHDA and oral TGN treatment). Fourteen days after striatal injections, animals were treated with TGN (50 mg/Kg; once daily for five consecutive days). One hour after the final treatment, the animals were euthanized, and the SN and CPu were collected for enzymatic activity analysis using specific substrates. Fluorescence was measured every minutes over a 50-minute period.

Results:

Preliminary findings indicate that in the PD model, CTSD activity in the right SN was reduced (Control = 1.65 AUF/min; PD = 0.14 AUF/min), as was CTSB activity (Control = 2.37 AUF/min; PD = 1.37 AUF/min). TGN treatment enhanced the activity of both enzymes (CTSD = 0.79 AUF/min; CTSB = 3.07 AUF/min). In the CPu, CTSD activity remained similar among groups, whereas CTSB activity was reduced in PD but was not restored by TGN.

Conclusion:

TGN enhances the activity of key lysosomal enzymes in the SN, which is associated with the removal of protein aggregates. These findings suggest a potential role for TGN in maintaining lysosomal functionality in this critical brain region affected by PD.



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Session 4. Impact of Toxins on Public Health

sciforum-125155: Combined Detection of Tetrodotoxin and Saxitoxin in Seafood with a Multiplexed Electrochemical Biosensor

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The occurrence of marine toxins such as tetrodotoxin (TTX) and saxitoxin (STX) in seafood represents a serious threat to food safety due to their high neurotoxicity, which can result in severe poisoning incidents and, in some cases, fatalities. Most conventional detection techniques are tailored to identify toxins from a single family, making them less effective when both toxins are present simultaneously. Given recent reports of co-occurrence of TTX and STX in seafood, there is a clear demand for advanced bioanalytical multiplexed platforms capable of detecting both toxins in a single assay. This study introduces a novel electrochemical immunosensor designed to detect concurrent TTX and STX. The biosensor features a four-electrode array: two electrodes dedicated to identifying the presence of the toxins in seafood samples, and two control electrodes serving as reference points aligned with established regulatory safety thresholds. By integrating the output signals from the biosensor, the system enables not only toxin identification but also rapid assessment of whether concentrations exceed acceptable safety levels. This multiplexed detection strategy provides a user-friendly, time-efficient tool for screening seafood, contributing to enhanced consumer safety. Moreover, it represents a promising step toward the development of comprehensive biosensing platforms for multi-toxin detection in marine environments.



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sciforum-123122: Dietary Exposure to Aflatoxin B₁ and Its Precursor Sterigmatocystin from Traditional Meat Products in Croatia

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The assessment of human dietary exposure to mycotoxins is a crucial component of food safety strategies. This study aimed to evaluate the exposure of the Croatian population to aflatoxin B₁ (AFB₁) and its precursor sterigmatocystin (STC)—an emerging mycotoxin—through the consumption of traditional meat products (TMPs). These products are not thermally processed, and their surfaces are colonized by moulds during the ripening process, which can produce mycotoxins. Additional contamination sources include spices used in their production or a carry-over effect in meat from animals fed with contaminated feed. A total of 280 TMP samples available on the Croatian market were analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS), with detection limits of 0.03 µg/kg for AFB₁ and 0.02 µg/kg for STC. AFB₁, a known human carcinogen, was not detected in any sample, indicating that exposure to AFB₁ through TMPs is negligible. STC, classified as a possible human carcinogen, was detected in eight sausage samples and four samples of dry-cured TMPs. Dietary exposure assessment was conducted using national consumption data and average STC concentrations, applying lower bound (LB), middle bound (MB), and upper bound (UB) scenarios for values below the detection limit. The maximum estimated daily exposure was 0.292 ng/kg body weight (bw), which is well below the European Food Safety Authority's (EFSA) threshold of toxicological concern (TTC) for potentially genotoxic compounds (2.5 ng/kg bw/day). Although the prevalence of mycotoxins in TMPs was low, continuous monitoring is recommended due to the variability of contamination sources and influencing factors, in order to track long-term exposure trends in the population.



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sciforum-129571: Differential effects of deoxynivalenol and deepoxy-deoxynivalenol on *Campylobacter jejuni* growth and gene expression

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Deoxynivalenol (DON) is the most prevalent trichothecene mycotoxin in poultry feed, and *Campylobacter jejuni* is a major zoonotic pathogen in broilers. In a recent study, we demonstrated that DON not only increased *C. jejuni* colonization in the jejunum and cecum of infected birds but also elevated bacterial counts in internal organs. In contrast, its less toxic D metabolite, deepoxy-deoxynivalenol (DOM-1) significantly reduced the *Campylobacter* levels in vivo. These findings suggest that DON and DOM-1 may exert direct effects on *C. jejuni* growth dynamics.

The present study aimed to further explore this interaction in vitro through co-incubation and RNA-sequencing. Two *C. jejuni* strains were investigated: the reference strain NCTC 12744 and a field isolate (strain 1303) obtained from a broiler flock. Both strains (10⁵ CFU/ml) were cultured in 96-well plates and incubated with either DON or DOM-1 (5 and 20 µg/mL) or without additives (as a control), under microaerophilic conditions at 41.5 °C. Bacterial counts were assessed at 6 h intervals from 24 to 48 h (24 h, 30 h, 36 h, 42 h, and 48 h).

Results showed that DON significantly promoted *C. jejuni* growth beginning at 30 h of incubation. Conversely, DOM-1 markedly reduced *C. jejuni* CFU counts, confirming the in vivo evidence of its potential to lower *C. jejuni* colonization in chickens. Additionally, transcriptomic profiling revealed that DON or DOM-1 modulate specific metabolic and regulatory pathways in *C. jejuni*. In particular, DON exposure upregulated genes related to energy metabolism and stress adaptation, while DOM-1 exposure led to the upregulation of pathways associated with growth inhibition.

These data are the first to demonstrate a direct, opposing influence of DON and its metabolite DOM-1 on *C. jejuni* growth. Our findings suggest that DON contamination in feed could promote *Campylobacter* proliferation in poultry, whereas DOM-1 may mitigate it. Understanding these interactions provides novel insights into how feed-derived toxins influence foodborne pathogens and could inform strategies to mitigate *Campylobacter* risk.



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sciforum-126921: Evaluation of cyclodextrins polymer as a possible clean-up method for detecting CTX-like compounds in *Gambierdiscus* cultures

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Introduction

Ciguatoxins (CTXs) are marine toxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa* that cause harmful algal blooms. These blooms are a threat to food safety due to the consumption of fish or shellfish containing CTXs. Liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) allows the identification of CTXs congeners. Instrument sensitivity can be affected by sample matrix effects; however, sample purification could prevent erroneous results.

Methods

Different samples of *Gambierdiscus* have been analyzed by LC-MS/MS after suspension and column-cleaning protocols were tested with seven resins (Florisil, C18, Diaion® γ -CD-HDI, γ -CD-EPI, β -CD-HDI and β -CD-EPI). Firstly, solid-phase extraction (SPE) was performed on suspension and columns using established standards to test the efficacy of each resin. Secondly, SPE was performed on the column using the four most effective resins from the first phase for one *Gambierdiscus* sample. Thirdly, all *Gambierdiscus* samples were subjected to the four resins used in the second phase.

Results and conclusion

The performances of all resins and two SPE formats were evaluated using gambierone and 44-methylgambierone standards at different concentrations (2.5 ng/mL, 12.5 ng/mL and 200 ng/mL). The recovery rates obtained with 12.5 ng/mL of C18, Diaion, and γ -CD-EPI in the column were very good. Florisil was associated with the lowest number of recoveries, while β -CD-HDI and β -CD-EPI did not perform well. The clean-up in suspension worked well, but the recovery rates were lower.

The performance of C18, Diaion, γ -CD-EPI and γ -CD-HDI was evaluated by SPE in column with the addition of gambierone, 44-methylgambierone and CTX1B to a *G. excentricus* extract. Recoveries were similar for C18, Diaion and γ -CD-EPI. Gambierone had a greater recovery with C18, while 44-methylgambierone and CTX1B achieved greater recovery with diaion. Finally, all *Gambierdiscus* samples were passed through the four resins and analyzed. To compare the matrix effect and establish a toxin profile, these samples were also analyzed without SPE.



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sciforum-127653: Perinatal screening of *Crotalus durissus terrificus* venom in rats

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Instituto Butantan

The high rate of accidents involving venomous animals, particularly in developing countries, represents a serious public health problem. In Brazil, crotalic snakes are the most lethal and have the highest accident incidence rates. Serotherapy is the most appropriate method of treating crotalic envenomation. Despite the high incidence and severity of accidents, there have been very few studies on the effect of venom on the fetus when pregnant women are involved. This project aims to study and elucidate the perinatal effects of crotalic envenomation.

Pregnant Wistar rats were treated with either saline (1.0 ml/kg sc, n=8) or crude venom from *Crotalus durissus terrificus* (2.5 mg/kg sc, n=8) on either the 5th (V5) or 10th (V10) gestational day (GD). On the 21st GD, they underwent a laparotomy, and maternal reproductive performance; the weights of the embryos and placentas; fetal deaths; and visceral and skeletal alterations and/or malformations were analyzed.

The data obtained was analyzed using Student's t-test and Fisher's exact test, with the results considered significant at $p > 0.05$. In the V5 experimental group, an increase in the average weight of the male and female fetuses, as well as in the weight of the kidneys and livers of the female fetuses, was observed. Some fetuses exhibited incomplete skull ossification. In the V10 experimental group, pregnant females experienced reductions in weight and in their consumption of food and water between GD10 and GD16. The average placental weight of the male fetuses increased, and the average weight of males and females decreased. Female fetuses showed reduced weights in their kidneys, livers, and lungs.

This study demonstrated the embryotoxic potential of *Crotalus durissus terrificus* venom during embryogenesis and organogenesis. Future experiments will evaluate the visceral effects of the venom in order to investigate the presence of malformations and other alterations.

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sciforum-129436: Public Health Impact of Scorpion Venom in Children: Epidemiological, Clinical, Biological and Evolutionary Profile of Pediatric Envenomation in the Souss Massa Region of Morocco

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Introduction

Scorpion envenomation remains a significant public health issue in North Africa, particularly in Morocco, where it accounts for high morbidity and mortality, especially among children. As biological toxins, scorpion venoms can cause severe multisystemic complications, necessitating urgent medical attention. Understanding the clinical and biological impact of these envenomations is essential to improve health responses in affected regions.

Methods

This retrospective study analyzed 383 cases of pediatric scorpion stings recorded at the Regional Hospital Hassan II in Agadir (Souss Massa region) over a period of 9 years and 10 months, from January 2013 to October 2022. The patients were categorized into three age groups: under 1 year, 1–5 years, and over 5 years. Clinical, biological, and evolutionary data were collected and assessed to evaluate the severity and systemic effects of envenomation.

Results

Children under the age of 1 year exhibited the most severe clinical forms and the highest mortality rate. Clinical manifestations showed extensive toxic effects on the cardiovascular, neurological, and pulmonary systems, particularly in grade 2 and 3 cases. Alterations in vital signs (temperature, oxygen saturation, blood pressure, heart, and respiratory rates), biochemical markers (ASAT, ALAT, urea, creatinine, blood glucose), and hematological parameters revealed significant dysfunction in vital organs. A positive correlation was found between clinical evolution and factors such as age, symptom severity, and hemodynamic status.

Conclusions

This study highlights the serious health threat posed by scorpion venom in pediatric populations, especially infants. It underscores the need to strengthen prevention strategies, improve early medical management, and ensure the availability of effective immunotherapy. Scorpion envenomation represents a critical public health challenge in endemic areas and calls for targeted interventions to reduce toxin-related morbidity and mortality.



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sciforum-125976: The development of a multiplex qPCR assay for *seb*, *stx1*, and *stx2* detection and its deployment into the Brazilian Army's biodefense system

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Staphylococcal Enterotoxin B (SEB) and Shiga Toxins (STX1 and STX2) pose significant threats to food and water safety and are associated with bacterial strains that are considered to be potential bioterrorism agents (BA). Given their public health relevance and misuse potential, the rapid and accurate detection of toxin genes is essential for effective surveillance and response. The Brazilian Army plays a crucial role in biodefense efforts, including BA identification methods. This study focused on developing a multiplex qPCR assay for *seb*, *stx1*, *stx2*, and *16S rRNA* gene targets. The design of the *Stx2* primer was performed using PrimerQuest Tool; its specificity was evaluated in Primer-BLAST, and its secondary structure was analyzed using the OligoAnalyzer Tool. *Staphylococcus aureus* ATCC 25923, *S. aureus* NCTC 12493, *Escherichia coli* O157:H7, and *E. coli* ATCC 25922 strains were used for validation tests. Bacteria were grown in BHI media and incubated at 37 °C for 24 h. DNA extraction was performed using the MPTA016 pathogen kit in the Extracta32 system, and amplification was carried out on the Amplio96™ equipment. The probe concentration and DNA mass were 250 nM and 10 ng, respectively. The optimal temperatures revealed were 58.4 °C, 60.2 °C, and 58.4 °C for *stx1* (Cq=22.49), *seb* (Cq=26.10), and *16S rRNA* (Cq=14.91), respectively, supporting their inclusion in a multiplex setup. Therefore, 58.4 °C was selected for the assay. The *16S rRNA* primer concentration showed no significant difference between 250 nM and 350 nM, allowing for the selection of 250 nM. For *stx1*, 350 nM provided a superior performance. No amplification was observed in non-toxin-producing strains, confirming the *stx1* and *seb* primers' specificity. The *stx2* primer and probe exhibited melting temperatures of 62 °C and 67 °C, respectively. Only heterodimer formation was observed (−13.29 kcal/mol). Our results showed that the primers were specific, allowing for their application to identifying the target genes proposed. This protocol was integrated into the Brazilian Army's biodefense system workflows to enhance its national and military biosurveillance.



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Session 5. Impact of Toxins on Agriculture

sciforum-124902: Physiological and biochemical effects of toxigenic *Aspergillus flavus* on maize (*Zea mays*)

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Aspergillus flavus is a major fungal pathogen in maize, significantly impacting kernel quality and food safety through mycotoxin contamination. This study examined the physiological and biochemical effects of *A. flavus* inoculation on maize, assessing kernel production, fungal proliferation, mycotoxin accumulation, and key biochemical parameters. A controlled field experiment was conducted, comparing inoculated (IN) and control (CT) maize. Inoculated maize exhibited a significant reduction in kernel number per ear length (20.14 ± 0.43 vs. 21.25 ± 0.33 ; $p < 0.05$), indicating compromised reproductive success. Mold count was significantly higher in IN maize (6.32 ± 0.13 log CFU/g) compared to CT (5.55 ± 0.17 log CFU/g; $p < 0.01$), confirming enhanced fungal colonization. The concentration of Aflatoxin B1 (AFB1) increased drastically in IN maize (139.46 ± 38.64 µg/kg) compared to CT (0.11 ± 0.07 µg/kg; $p < 0.01$), posing serious food safety concerns. However, no significant differences were observed in fumonisin B1 (FB1), deoxynivalenol (DON), zearalenone (ZEA), starch, protein, or total polyphenols between treatments, suggesting that short-term fungal infection primarily affects kernel formation and mycotoxin accumulation rather than biochemical composition. These findings emphasize the critical role of *A. flavus* in maize contamination and highlight the necessity of mitigation strategies to reduce fungal colonization and aflatoxin accumulation, ensuring maize safety and quality.

Acknowledgement

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sciforum-123443: Prevalence of *Fusarium* mycotoxins in cereals harvested in Croatia

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Fusarium fungi are among the most common moulds that can produce mycotoxins. Data on the occurrence of these mycotoxins, as well as their conjugated forms in cereals grown in Croatia, are still lacking, although several published studies have shown that *Fusarium graminearum* and (as a ZEN producent) and *Fusarium culmorum* (as a trichothecene producer) are the most widespread molds in Croatian crops. The aim of this study was to determine the prevalence of 16 *Fusarium* mycotoxins as well as their conjugated forms in maize (n=66), wheat (n=30), barley (n=17), triticale (n=12), and oats (n=12) harvested in three Croatian regions (East, North, and Central) which differ in climate during a one-year period. All samples were taken immediately after harvest and stored at -20 °C until liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The results revealed different contamination patterns between the analyzed cereals, except for wheat and triticale, which exhibited the same pattern. The most prevalent mycotoxin in wheat and triticale samples was deoxynivalenol (DON), at 73.3% and 91.7%, respectively, followed by deoxynivalenol-3-glucoside (DON-3G) and HT-2 toxin. In barley samples, the prevalence of DON and DON-3G was 55.6% and 44.4%, respectively; however, in contrast to wheat and triticale samples, ZEN-14 sulfate was detected as the third most prevalent mycotoxin. The analysis of maize samples indicated that this grain is more susceptible to fumonisin contamination, as fumonisin B1 and B2 were the most frequently occurring mycotoxins in these samples, with prevalence rates of 85.1% and 34.3%, respectively. The data obtained in this trial are pointing that the contamination patterns of wheat, triticale, and barley are similar but differ from the contamination pattern of maize, although further research is necessary to confirm these findings on a representative number of samples. These results could be used to establish a link between climatic conditions and the occurrence of mycotoxins.



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sciforum-129621: Spray Drying Microencapsulation Preserves Anti-Aflatoxin B1 Activity of Plant Aqueous Extracts

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Aflatoxin B1 (AFB1) is a major global health concern due to its carcinogenic effects in humans and animals. Because AFB1 is highly stable once produced, the most effective strategy is to prevent its synthesis by toxigenic fungi. Currently, plant extracts have been increasingly studied for this purpose. These extracts often contain polyphenols as active compounds against AFB1, which are effective but unstable over time. Therefore, it is necessary to develop new formulations that enhance their preservation, storage, and applicability.

Microencapsulation through spray drying is a promising approach. This technique converts a liquid into a dry powder by spraying it into a hot air chamber, embedding the bioactive compounds in a protective matrix. Spray drying offers several advantages, including high encapsulation efficiency, stability, and low production costs, making it a practical solution for preserving plant-derived antifungal agents targeting AFB1.

This study aimed to evaluate the potential of spray drying encapsulation to preserve the anti-aflatoxin activity of different plant aqueous extracts able to inhibit AFB1 synthesis. A total of 11 coating materials were screened based on their impact on *Aspergillus flavus* growth and AFB1 inhibition. Pectin, maize starch, and maltodextrin were identified as the most suitable, showing no effect on fungal development and AFB1 synthesis. Maltodextrin was selected to encapsulate aqueous extracts of *Mimosa tenuiflora* and *Aloysia citrodora*. These extracts inhibited AFB1 by 66% and 76 % at the concentration of 0.3 mg of dry matter /mL and displayed IC₅₀_{AFB1} of 0.15 and 0.11 mg/mL, respectively. After encapsulation, the extracts inhibited AFB1 by 60% (IC₅₀_{AFB1}= 0.29 mg/mL) and 65% (IC₅₀_{AFB1}= 0.26 mg/mL) in a dose-dependent manner.

Different core-to-wall ratios were tested, with 1:2 being the most effective, preserving 92% of anti-AFB1 activity. After 1 year of storage, the encapsulated extract preserved 90% of its activity, which is better than freezing (61%). Spray drying proved to be an efficient, low-cost method for preserving anti-aflatoxin properties of plant-based extracts.



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sciforum-125595: The Burden of Snakebite Envenomation in Livestock: Evidence from Rural Communities in Tamil Nadu

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Snakebite envenomation (SBE)—the injection of venom through the bite of a venomous snake—in domestic animals is a neglected and under-reported problem. Whilst SBE is a major public health issue in humans, its impact on livestock remains poorly understood. This pilot study explored the clinical features and socioeconomic impacts of SBE in livestock in Tamil Nadu, India—a country with the world's highest snakebite incidence. Between September and October 2024, 23 farmers from Coimbatore and Pollachi districts were interviewed using a structured questionnaire, reporting 21 cattle and 4 goat SBE cases.

Reported mortality rates were 52% in cattle and 100% in goats. Common clinical signs included local swelling, hypersalivation, and bleeding, with bites occurring mainly on the limbs or face. Bites predominantly occurred during the monsoon season, and in fields or sheds. Surviving cattle recovered in an average of four days, and farmers reported impacts on milk yield, fertility, and carcass weight.

Treatment was provided to 81% of cattle and 50% of goats. Financial losses were reported by 72% of farmers, averaging INR 42,000 per cow and INR 10,750 per goat, and were particularly impactful given the small herd sizes. These findings highlight the need for further research into the incidence and impact of livestock SBE, alongside the development of prevention strategies and improved access to treatment. Preventing livestock SBE is especially critical given limited antivenom availability and supports a One Health approach to snakebite control.



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sciforum-129051: Zearalenone exposure exacerbates osteoporosis in aged laying hens by inhibiting bone formation

Qiugang Ma

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Zearalenone (ZEA) is a naturally occurring foodborne mycotoxin and is of widespread interest because of its estrogen-like effects. However, the impact of the myco-estrogen ZEA on bone health remains incompletely understood. This study investigates the mechanisms underlying the effect of ZEA on bone health by establishing osteoporosis and osteoblast models in aged laying hens. ZEA exhibited deleterious effects on hens' bone health by reducing femoral bone weight and bone mineral density (BMD), decreasing serum calcium (Ca) and phosphorus (P), elevating levels of alkaline phosphatase (ALP) and parathyroid hormone (PTH), diminishing the medullary bone micro-structural parameters (utilizing Micro-CT) and the proportion of trabeculae bone, and down-regulating the expression of osteogenic factors in bone. Interestingly, we found that hBMSCs differentiated towards adipocytes rather than osteoblasts in the ZEA micro-environment, which may be one of the reasons why ZEA causes bone damage. Subsequent experiments involving primary osteoblasts and hFob1.19 cell lines demonstrated that ZEA induced osteoblast apoptosis and impeded osteoblast proliferation. Transcriptome analysis revealed that ZEA primarily hindered osteoblast proliferation by activating the MAPK signaling pathway. Western blot identified that ZEA significantly increased ERK and P38 phosphorylation, with further confirmation through ERK and P38 phosphorylation antagonists. ZEA primarily targets the estrogen receptor (ER), specifically ER α , to activate the ERK/P38 MAPK signaling pathway. In summary, ZEA activates the ERK/P38 MAPK signaling pathway by targeting ER α , thereby inhibiting osteoblast proliferation and promoting osteoclast apoptosis, ultimately leading to decreased bone formation and increased osteoporosis.



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Session 6. Foodborne Toxins

sciforum-125897: Evaluation of Ciguatoxin-like Activity in Non-Native Fish Species from the Canary Islands

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Marine biotoxins are natural chemical contaminants produced by a wide variety of microorganisms, such as bacteria and algae, among which ciguatoxins (CTXs) stand out.

CTXs enter the human food chain through the consumption of contaminated fish and other marine organisms, resulting in Ciguatera poisoning (CP), which is one of the most prevalent foodborne intoxications worldwide.

Although CP was historically confined to tropical and subtropical regions, the ongoing process of global warming is contributing to its geographical expansion. This occurs not only through the enhancement of environmental conditions favorable to CP-associated dinoflagellates but also via the introduction of non-native species. These invasive organisms may disrupt local ecosystems, establish new toxin reservoirs, and facilitate the persistence and spread of CTXs in previously unaffected marine environments.

This study focuses on the Canary Islands, a recognized hotspot for CTXs and one of the few regions with an established official CTX monitoring program. Seven specimens from six different species [(*Caranx crysos*, n=1), (*Acanthurus monroviae*, n=2), (*Synaphobranchus* sp., n=1), (*Elagatis bipinnulata*, n=1), (*Cephalopholis taeniops*, n=1), and (*Lutjanus endecacanthus*, n=1)] were analyzed. CTX-like activity was assessed in muscle, liver, and gonadal tissues (when available) using the Neuro-2a MTT cell-based assay.

CTX-like toxicity was detected in six of the seven fish analyzed. Liver and gonad samples showed higher levels of toxicity compared to muscle tissue. No significant differences were observed between cranial and caudal samples in terms of toxic potential.

In conclusion, the detection of CTX-like toxicity in the majority of the specimens analyzed underscores the importance of continued surveillance of and research on non-native fish species that might act as new vectors and reservoirs for the toxin. The early detection of CTXs in exotic marine organisms is essential, as it may pose an emerging risk to food safety, public health, and the environment.



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sciforum-129742: From research to application—proving the efficacy of ZENzyme® in swine

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Mycotoxins are secondary metabolites from filamentous fungi contaminating food and feed. One major mycotoxin produced by some *Fusarium* species is zearalenone (ZEN). Due to its estrogen-like structure, ZEN shows strong estrogenic and anabolic effects in addition to immunotoxic and hepatotoxic effects in humans and animals. One pillar of mycotoxin risk management includes effective strategies to eliminate ZEN. The zearalenone hydrolase ZenA (ZENzyme®) can be used for the enzymatic detoxification of ZEN in animal feed to mitigate the negative effects of ZEN on farm animals. ZENzyme® cleaves the lactone ring of ZEN to form the non-estrogenic metabolite hydrolyzed zearalenone (HZEN).

For product registration, the efficacy of ZENzyme® has to be proven to authorities in the form of the reduced systemic absorption of ZEN in the presence of ZENzyme®. Therefore, in a feeding trial, 72 weaned piglets were exposed to feed contaminated with 200 µg/kg ZEN for 42 days. In one group, the feed was additionally supplemented with 10 Units/kg ZENzyme®. For assessment of ZENzyme®'s efficacy, exposure-based biomarkers were measured in plasma, urine, and feces at different time points of the feeding trial by LC-MS/MS. Applied methods did not only cover ZEN, but also the metabolites α -zearalenol (α -ZEL) and β -zearalenol (β -ZEL), as well as the enzymatic degradation products HZEN and decarboxylated hydrolyzed zearalenone (DHZEN). A significant reduction of ZEN in the presence of ZENzyme® was observed in all tested matrices. HZEN levels in feces increased significantly, if ZENzyme® was present in animal feed. DHZEN concentrations above the limit of quantification were scarcely found and most prevalent in urine samples.

These data conclusively showed both the reduction of systemic absorption of ZEN from feed in the gastro-intestinal tract of piglets and the biotransformation of ZEN to HZEN.



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sciforum-125856: Assessment of aflatoxin contamination in marketed samples of sweet potatoes, and estimation of risk assessment in local consumers

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This study aimed to assess the occurrence of aflatoxin B₁ (AFB₁) and the total amount of aflatoxins (AFs) in commercial sweet potato and ready-to-eat sweet potato samples, collected from Punjab, Pakistan. A total of 480 commercial sweet potato and 365 ready-to-eat sweet potato samples were analyzed using HPLC. The results showed that 28.5% and 19.7% of commercial sweet potato and ready-to-eat sweet potato samples were found to be contaminated with AFs. The highest mean level of total AFs was 28.5 ± 4.2 µg/kg in commercial sweet potato samples from Daska (city), and the highest mean level in ready-to-eat samples of sweet potato was 14.50 ± 2.50 µg/kg, from the same city. The results showed that 40% of samples have total AF levels in the range of 1-10 µg/kg in commercial sweet potato samples (Jamki), and 13.3% of samples have total AF levels above 20 µg/kg from the city (Kila Soba Singh). The highest mean dietary intake levels of 3.16 µg/kg body weight/day were found in female volunteers in the age group 21-30 years. The results emphasized the need to implement effective storage practices that could be beneficial in minimizing the exposure of AFs to crops. Furthermore, the results would help implement strict laws for aflatoxins in food and food products.



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sciforum-125907: Could ciguatoxins cause structural changes in fish tissue?

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Ciguatera poisoning (CP) is a foodborne illness of significant public health concern, which is caused by the consumption of fish contaminated with ciguatoxins (CTXs), lipophilic neurotoxins that bioaccumulate throughout the marine food web. Despite its global relevance, the specific morphological and ultrastructural effects of CTX exposure on fish tissues remain poorly understood. This study aimed to characterize histological and ultrastructural alterations induced by dietary CTX exposure and how toxins accumulate in tissues of the dusky grouper (*Epinephelus marginatus*).

Captive-born dusky groupers were fed an experimental diet composed of CTX-contaminated amberjack and dusky grouper tissue, with an average concentration of $(0.109 \pm 0.003 \text{ ng Eq. CTX1B/g of flesh})$. Tissue samples were collected at 24, 60, and 108 days. CTX estimation was performed using a cell-based assay in the liver, muscle and gonads, and histological examinations of liver, kidney, gonads, muscle, and intestinal tissues were conducted. Additionally, hepatic samples were analyzed by light and transmission electron microscopy.

Histopathological findings included cytoplasmic vacuolization, localized inflammation, and increased connective tissue development. Ultrastructural analysis showed signs of hepatocyte necrosis, nuclear condensation, and displacement of cellular organelles in CTX-exposed individuals. Despite toxin exposure, tissue alterations were mild and predominantly localized to the liver, with no significant histological changes detected in the intestine, gonads, or muscle. CTX accumulation was first detected in the liver and gonads, with hepatic toxin levels peaking at 60 days $(0.868 \pm 0.121 \text{ ng Eq. CTX1B/g})$ and subsequently declining, while gonads exhibited a delayed increase in toxin concentration.

These results demonstrate that dietary exposure to CTXs induces significant hepatic structural damage in *E. marginatus* and that toxin distribution across tissues evolves over time. This study provides experimental evidence supporting the role of sublethal CTX exposure in the onset of tissue-specific damage in adult reef fish under toxin concentrations relevant to natural environmental conditions.



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sciforum-125532: Determination of Cannabinoids in Hemp Seeds, Oil, and Tea Samples Using LC-MS/MS

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Introduction:

Recently, hemp products—seeds, oil, and tea—have been gaining popularity as food due to their high content of essential micro- and macro-nutrients. While hemp products offer significant nutritional benefits, they might pose a risk to consumers due to natural toxins such as cannabinoids. Cannabinoids include both psychoactive substances, such as Δ^9 -tetrahydrocannabinol (Δ^9 -THC), and non-psychoactive ones like cannabidiol (CBD). Even though hemp typically contains low levels of Δ^9 -THC, even trace amounts in food products can be concerning. Because of this, Δ^9 -THC content in products for human consumption has been regulated, with the maximum limits introduced under Commission Regulation (EU) 2023/915.

Methods:

Nine cannabinoids— Δ^9 -tetrahydrocannabinol, Δ^8 -tetrahydrocannabinol, cannabinol, cannabidiol, Δ^9 -tetrahydrocannabivarin, cannabigerol, Δ^9 -tetrahydrocannabinolic acid (Δ^9 -THCA), cannabidiolic acid (CBDA), and cannabigerolic acid—were determined in 25 samples, including hemp seeds, oil, and teas. Samples were extracted with acetonitrile and diluted before analysis. Measurements were conducted using LC-MS/MS, operating in both positive and negative electrospray ionization (ESI+/-).

Results:

Cannabinoid content varied with the tested materials. The highest concentrations of Δ^9 -THC and Δ^9 -THCA were found in tea samples (37.6–168.0 mg/kg and 28.2–69.7 mg/kg). Three of five oils contained significantly more (up to 398.3 mg/kg) Δ^9 -THC and Δ^9 -THCA than allowed. Only one hemp seed sample had Δ^9 -THC equivalents exceeding the maximum limit. Among all compounds, CBD and CBDA were found in the highest concentrations across all samples.

Conclusions:

While hemp products offer health benefits, the risks from cannabinoids must be managed. Regulatory oversight, standardized testing, and public awareness are crucial for ensuring the safety of hemp-based foods. Although hemp contains low Δ^9 -THC levels, some tested foods exceeded the allowed concentrations, posing a risk to consumers.



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sciforum-125231: Natural Toxins in the Food Chain: A Study on Alkaloid Residues in Honey

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Pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) are natural toxins which can be found in honey and are recognized for their hepatotoxic and neurotoxic properties. Given honey's widespread consumption as part of the human diet, ensuring its safety is mandatory [1]. Therefore, an accredited method [2] was employed to detect 33 PAs and 21 TAs in honey, based on ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) with a quadrupole linear ion trap (Q-TRAP) system. Honey samples were extracted using a solvent mixture of methanol and water acidified with formic acid. In a comprehensive analysis of 33 Czech and Spanish honey samples from diverse botanical origins, it was found that among the 21 Czech samples, 6 tested positive for PAs and TAs. However, only two of them exhibited quantifiable residues of echimidine (1.4 µg/kg), lycopsamine (197 µg/kg), and intermedine (140 µg/kg). Among the 12 analyzed Spanish honeys, 5 tested positive for nine PAs (intermedine, lycopsamine, heliotrine, europine, seneciphylline, senecionine, senecivernine, echimidine and lasiocarpine). Total PA concentrations in Spanish honeys ranged from 0.4 to 65.1 µg/kg. Contrastingly, none of the monitored TAs were detected in these analyzed samples. The new Commission Regulation 2023/915 does not currently include maximum residue limits for these compounds in honey. Instead, it outlines limits solely for pollen-based food supplements. In light of the demonstrable potential health hazards associated with these toxins, it is expected that accurate analytical methods and more extensive investigations for PAs and TAs in honey-based products will be developed to enhance their safety for potential consumers.



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sciforum-125904: The Detection of Ciguatoxins in Fish Species from two hotspot Coastal Areas of Gran Canaria (Spain): A Two-Year Study

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Introduction

Ciguatoxins (CTXs), produced by certain marine microalgae, can bioaccumulate and biomagnify through the marine food web, ultimately posing a health risk to humans. Previous studies have identified a high prevalence of CTX-producing microalgae in the coastal areas of Risco Verde and Sardina del Norte (Gran Canaria, Spain). These findings prompted a focused investigation of fish species from these regions over two consecutive years, 2023 and 2024.

Methodology

Fish specimens representing various trophic levels were collected from both areas, a total of 72 fish in 2023 (35 from Risco Verde and 37 from Sardina del Norte) and 82 in 2024 (41 from each area). Liver and muscle tissues were selected for CTX extraction and analysed using a neuro-2a MTT cell-based assay.

Results and conclusion

In 2023, 35 specimens from Risco Verde comprised 10 species, with 8 testing positive for CTXs. All of them showed liver toxicity, and only 3 of them also showed muscle toxicity. From Sardina del Norte, 37 specimens representing 11 species were analysed, of which 6 species were CTX-positive. All of them showed liver toxicity, and only 3 of them also showed muscle toxicity.

In 2024, 41 specimens from Risco Verde included 18 species, with 10 testing positive. Similarly, the 41 specimens from Sardina del Norte represented 15 species, of which 9 tested positive. At both locations, the Parrotfish tested positive for CTXs. Redporgy showed toxicity to CTXs in the liver at both sites as well.

The results demonstrate the widespread presence of CTXs across various fish species and trophic levels in both locations. These findings underscore the importance of ongoing monitoring to assess the risk of ciguatera poisoning in the Canary Islands.



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sciforum-116014: Towards a certified reference material (CRM) for the emetic toxin cereulide in cooked rice: a characterisation study

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Introduction:

Cereulide toxin, a cyclic dodecadepsipeptide, is produced by *Bacillus cereus* and is a causative agent in food-borne outbreaks (FBOs). More than 470 FBOs related to *B.c.* toxins have been reported in 2023 (European Union One Health Zoonoses Report, EFSA and ECDC). Insufficient hygiene practices are the main reasons for intoxications. Cereulide affects several foods, including starch-rich foods such as pasta and rice. Along with validated methods and proficiency testing schemes, certified reference materials (CRMs) are key to ensure reliable measurements. The JRC aimed to produce a rice CRM containing cereulide at a low ng/g concentration.

Methods:

Highly pure cereulide (Chiralix; Nijmegen, NL) was used to spike cooked rice. Thereafter, a rice powder candidate CRM was processed, which exhibits appropriate homogeneity and short-term stability. A characterisation exercise with ten expert laboratories using LC-MS/MS methods mostly based on ISO 18465 (Microbiology of the food chain – quantitative determination of emetic toxin (cereulide) using LC-MS/MS) was carried out. Each laboratory was requested to analyse nine independent sample replicates together with two QC samples (blank rice powder and rice powder contaminated at a very low cereulide concentration) under intermediate precision conditions.

Results:

First, the measurement results were technically scrutinised. Some data sets had to be excluded from further evaluation (e.g. working range of method inadequate, QC results incorrect, and non-adherence to the provided instructions). After statistical evaluation, it could be concluded that the laboratory mean results were about normally distributed. No mean and variance outliers were detected. The mean of laboratory means amounted to 11.25 µg cereulide per kg rice, with an uncertainty related to characterisation of 0.23 µg/kg (2.04 %).

Conclusion:

Once long-term stability data is available, a full uncertainty budget will be drawn up. The CRM will be used for method validation and quality control; therefore, it will enable laboratories to set up and safeguard reliable cereulide measurements in food and facilitate effective consumer protection.



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