V 1H-TOXRUN
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PORTUGUESE
PHARMACEUTICAL
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International Joint Meeting on
ONE HEALTH 2026

FRONTIERS OF GLOBAL TOXICOLOGY:

Environments, Technologies, and Misinformation in the One Health Perspective

23-24 April 2026 | Porto, Portugal

SCIENTIFIC LETTERS SL

BOOK OF ABSTRACTS

Volume 1 Supplement 1 (2026)

ISSN 2795-5117

IUCS-CESPU Publishing

https://publicacoes.cespu.pt/index.php/sl

EDITORIAL OFFICE

IUCS-CESPU Publishing
Rua Central de Gandra, 1317
4585-116, Gandra PRD
Portugal

ADMINISTRATIVE EDITOR

Rui Azevedo

DOI: https://doi.org/10.48797/sl.2026.510

For citation purposes, cite each abstract independently as indicated on the abstract page at https://publicacoes.cespu.pt/index.php/sl/article/view/510.

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FRONTIERS OF GLOBAL TOXICOLOGY:
Environments, Technologies, and Misinformation in the One Health Perspective

CESPU INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE
UNIVERSITY INSTITUTE OF HEALTH SCIENCES

UCIBIO Applied Molecular Biosciences Unit

ORDEM DOS FARMACÊUTICOS

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EDITORIAL

EDITORIAL

Frontiers of Global Toxicology in a Changing World: Emerging Contaminants, Persistent Risks, and One Health Imperative

Ricardo Jorge Dinis-Oliveira [1,2,3,4,*], and

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DOI: https://doi.org/10.48797/sl.2026.474

Toxicology, as the central science devoted to understanding the adverse effects of chemical, physical, biological, and psychosocial stressors on living organisms, is currently at a pivotal turning point. In a world shaped by rapid technological, environmental, and societal transformations, it is increasingly evident that traditional risk assessment paradigms are no longer sufficient to address the complexity of modern exposure scenarios. Within this context, the **One Health** framework emerges not merely as a conceptual approach but as a scientific and strategic necessity for integrating human, animal, and environmental health into a unified, interdependent vision. In other words, it is time to redefine toxicology as the science of the harmful exposome, encompassing all external and internal stressors — chemical, environmental, technological, and societal — that disrupt biological balance across individuals, populations, and ecosystems, within an interconnected and evolving planetary system [1,2]. Indeed, Toxicology is no longer merely the study of poisons, but the science of systemic disruption, exploring how diverse stressors interact within complex living networks to shape health, disease, and resilience across the biosphere [3].

The 2026 Congress, under the theme *"Frontiers of Global Toxicology: Environments, Technologies and Misinformation in the One Health Perspective"*, reflects this evolution. Indeed, modern toxicology is increasingly challenged by new classes of contaminants, whose ubiquity, persistence, and still poorly understood effects raise significant concerns for science, regulation, and public health.

Among these, pharmaceutical residues have emerged as critical environmental contaminants due to their widespread use and incomplete removal in wastewater treatment systems [4]. These bioactive compounds can disrupt endocrine systems, alter microbiomes, and impact aquatic ecosystems, contributing to phenomena such as antimicrobial resistance and unintended biological effects in non-target organisms.

In parallel, electronic waste (e-waste) is among the most complex sources of exposure to hazardous chemical mixtures, including heavy metals, flame retardants, and persistent organic pollutants. In parallel, electronic waste (e-waste) constitutes one of the most complex sources of exposure to hazardous chemical mixtures, including heavy metals, flame retardants, and persistent organic pollutants [5,6]. The accelerating global digitalization intensifies this issue, particularly in regions with inadequate waste management, thereby exacerbating environmental and health inequalities.

A growing number of emerging contaminants are also attracting increasing attention due to their persistence, bioaccumulation potential, and largely unknown long-term effects. Among these, per- and polyfluoroalkyl substances (PFAS), often referred to as "forever chemicals", stand out due to their extreme environmental persistence and widespread use in industrial applications and consumer products. Indeed, PFAS contamination has been detected in water, soil, wildlife, and human biological samples worldwide, raising concerns about endocrine disruption, immunotoxicity, and carcinogenicity.

An additional rapidly expanding area of concern relates to microplastics and nanoplastics, now detected across multiple environmental and biological matrices. Their ability to act as vectors for toxic substances, combined with

potential inflammatory and genotoxic effects, poses critical questions that remain under active investigation.

Furthermore, the expansion of human activity into new domains, such as space, introduces emerging concerns regarding space-related contaminants, including advanced materials, propellants, and technological residues, whose toxicological impacts remain largely unexplored.

Light pollution, noise pollution, and other non-chemical stressors are also increasingly recognized as contributors to toxicological burden within the broader exposome concept [7]. These factors can interact with chemical exposures, influencing circadian rhythms, stress responses, and overall susceptibility to disease, underscoring the need for integrated, multidisciplinary approaches.

While engineered nanomaterials offer significant innovation potential, such as in medicine, cosmetics, food packaging, and advanced technologies, their small size and unique physicochemical properties enable them to cross biological barriers and interact with cellular systems in unpredictable ways. The toxicological implications of chronic exposure to nanoparticles remain insufficiently characterized, particularly regarding neurotoxicity, oxidative stress, and long-term accumulation [8].

In parallel, the increasing use of biocides and disinfectants, intensified during and after the COVID-19 pandemic, has led to their accumulation in aquatic and terrestrial environments. These substances may contribute to antimicrobial resistance and exert toxic effects on non-target organisms, including disruptions in microbial ecosystems that are essential for environmental and human health.

Armed conflicts and war-related activities represent a re-emergent, often underrecognized, but highly significant source of environmental contamination and toxic exposure [9,10]. War residues, including heavy metals from ammunition (such as lead and depleted uranium), explosive compounds, combustion by-products, and damaged industrial infrastructures, can persist in soils, water systems, and air long after hostilities cease. These contaminants not only pose immediate risks to civilian populations and military personnel but also contribute to long-term ecological degradation and chronic health effects, including cancer, neurological disorders, and reproductive toxicity. Moreover, conflicts frequently disrupt regulatory systems, waste management infrastructures, and environmental monitoring, exacerbating uncontrolled exposure scenarios. Within the One Health framework, the toxicological consequences of war highlight the urgent need for integrated surveillance, remediation strategies, and international cooperation to mitigate both the direct and indirect health impacts of environmental contamination in conflict and post-conflict settings.

Finally, climate change acts as a risk multiplier, altering the distribution, transformation, and toxicity of environmental contaminants. Rising temperatures, extreme weather events, and changes in ecosystems can enhance the mobility of pollutants, increase human exposure, and modify toxicokinetic and toxicodynamic processes. This dynamic interplay between environmental change and chemical risk underscores the urgency of adopting adaptive and forward-looking toxicological frameworks [11].

These few examples illustrate an unavoidable reality: toxicology is now confronted with a scenario of chronic, global, and multi-source exposure, increasingly influenced by climate change, where emerging contaminants coexist with persistent pollutants, demanding integrated, predictive, and sustainable approaches. Indeed, the issue of combined exposure to chemical mixtures, often referred to as the "cocktail effect," is a major challenge for modern toxicology since real-world exposures rarely occur in isolation; instead, individuals and ecosystems are exposed to complex mixtures of pollutants that may interact synergistically, antagonistically, or cumulatively. Unfortunately, current regulatory frameworks are still largely based on single-substance assessments, highlighting a critical gap between scientific knowledge and policy implementation. Moreover, there is a significant future for toxicology as one of the foundational sciences of human knowledge, emerging from the earliest need to differentiate between harmful and safe substances and influencing the evolution of health and environmental sciences. In this evolutionary vision, while artificial intelligence represents a powerful transformative tool for toxicology, it is equally critical to acknowledge that technological innovation must be accompanied by robust scientific literacy. The spread of misinformation, particularly in areas related to health and the environment, represents an additional and growing risk that undermines public trust, policy effectiveness, and risk perception. Thus, 21st-century toxicology must position itself not only as an experimental and predictive science, but also as a science of communication, education, and societal engagement. The promotion of resilient communities, the transition toward a circular economy, and the implementation of sustainable practices depend on effective integration between science, policy, and society.

The **four scientific sessions** of this congress reflect these priorities. From emerging threats to global health to pharmaceuticals and their environmental impact, the challenges of intensive agriculture and illicit production, and the implementation of One Health in Portugal, the program promotes essential interdisciplinary reflection. The 2026 Congress, therefore, plays a crucial role as a platform for dialogue and knowledge construction. Oral and poster presentations will highlight scientific advances, foster collaboration, and reinforce toxicology's identity as an integrative discipline aligned with the Sustainable Development Goals. In an increasingly complex world

shaped by interconnected chemical pressures, the path forward lies in a more holistic, anticipatory, and prevention-oriented toxicology. The challenges are significant, but so too are the opportunities to redefine the role of this science in protecting life in all its dimensions. This congress is proudly held as an **International Joint Meeting on One Health**, in a partnership of 1H-TOXRUN with the **Portuguese Pharmaceutical Society**, highlighting the strong institutional commitment of this professional body to advancing interdisciplinary collaboration, scientific excellence, and the implementation of One Health principles at both national and global levels. Therefore, a particularly noteworthy moment of the congress will be the high-level roundtable entitled "One Health: A Shared Mission Across Health Professions", which brings together leading representatives from a wide range of Portuguese professional orders and associations in the health sector. This session embodies the very essence of the One Health paradigm, highlighting that the complex challenges posed by emerging and persistent toxic threats cannot be addressed in isolation by a single discipline. By convening experts from medicine, biology, pharmacy, nutrition, veterinary sciences, physiotherapy, psychology, nursing, and dentistry, this round table fosters a unique platform for interprofessional dialogue, strategic alignment, and collective responsibility. This discussion is expected to generate critical insights into how cross-sector collaboration can enhance prevention strategies, improve risk communication, and strengthen the implementation of One Health principles in Portugal, ultimately contributing to more resilient health systems and better protection against current and future toxicological challenges.

Maintaining a fragmented approach is increasingly misaligned with current scientific understanding and may pose challenges for effectively safeguarding public health, food safety, and ecological sustainability. It can limit the effectiveness of our responses to complex, interconnected risks. Therefore, there is a clear opportunity for policymakers to:

- Formally recognize the One Health approach as a national strategic priority, integrating it into public health, environmental, agricultural, and educational policies.
- Establish national and regional One Health coordination committees. These committees should play an active role in policy development, emergency response, and the promotion of transdisciplinary research. They must include experts from human and veterinary medicine, environmental sciences, microbiology, epidemiology, ecology, social sciences, and, crucially, toxicologists, as One Health and toxicology are inherently interconnected.
- Integrate the One Health approach into national strategic plans, including the National Health Plan, the National Biodiversity Strategy, the Antimicrobial Resistance Action Plan, and agricultural and climate policies.
- Promote awareness and training campaigns targeting professionals, students, and the general public to enhance literacy regarding this integrated vision of health.
- Invest in integrated and interoperable surveillance systems that enable early detection of emerging threats, with effective data sharing across sectors and regions.
- Promote curricular reforms to incorporate One Health strategies into higher education programs in health-related fields such as Medicine, Nursing, Biomedical Sciences, Pharmaceutical Sciences, among others.
- Provide financial support for projects and collaborative networks that foster sustainable, evidence-based solutions with a tangible impact on disease prevention and global health promotion.

Ultimately, the future of toxicology will depend on our collective ability to move beyond disciplinary silos and embrace a truly integrated vision of health, environment, and society. The challenges ahead are complex, global, and evolving, but so too is the capacity of science to anticipate, understand, and prevent harm. By fostering collaboration across sectors, strengthening the science–policy interface, and empowering the next generation of scientists and professionals, we can transform toxicology into a proactive force for safeguarding life on Earth. This congress represents not only a forum for scientific exchange but also a call to action: to rethink, connect, and lead. The responsibility is shared, and the time to act is now.

Cordial greetings

Ricardo Jorge Dinis-Oliveira
Félix Carvalho

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SCIENTIFIC PROGRAMME

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APRIL 23

09h00 **Opening Session |** Ricardo Jorge Dinis-Oliveira (UCIBIO; 1H-TOXRUN, IUCS-CESPU), Félix Carvalho (UCIBIO; FFUP), Ana R. Freitas (UCIBIO; 1H-TOXRUN, IUCS-CESPU)

09h30 **Opening Lecture |** A Warming Planet, A Shared Vulnerability: Climate Change Through the One Health Lens. | Félix Carvalho (UCIBIO; FFUP)

10h15 **Coffee Break**

SESSION I — Emerging Toxic Threats to One Health

CHAIRS: Eduarda Silva (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Joana Prata (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Rui Azevedo (UCIBIO, 1H-TOXRUN, IUCS-CESPU)

10h45 Microplastics and One Health Concepts in Agriculture. | Tony Walker, Dalhousie University, Canada

11h15 Natural Lithium Exposure and Suicide Risk in the Overall Population. | Agostinho Almeida, FFUP

11h45 **Presentation of Selected Oral Communications**

OC 01 | The Social Support "Staircase": Decoding Neurocognitive Phenotypes in Acute Coronary Syndrome via Explainable Machine Learning. Bruno Peixoto, UCIBIO; 1H-TOXRUN, IUCS-CESPU

OC 02 | Co-Exposure to Microplastics and Parabens: Implications for *Chlorella vulgaris* Bioremediation Efficiency. Paulo de Sousa, FEUP

12h30 **Lunch & Poster Viewing**

SESSION II — Pharmaceuticals and Their Environmental Impact

CHAIRS: Félix Carvalho (UCIBIO; FFUP), Cláudia Ribeiro (UCIBIO; 1H-TOXRUN, IUCS-CESPU), Vítor Seabra (UCIBIO; 1H-TOXRUN, IUCS-CESPU)

14h00 Education for Sustainability: Integrating Green Toxicology and Environmental Stewardship into Pharmaceutical Training. | Cristina Almeida, FF-UL

14h30 Enviromental Risk Assessment of Medicinal Products: Beyond the Guidelines. | Bruno Nunes, UA; CESAM

15h00 The Paradox of Pharmaceuticals: from Medicine to Pollutant. | Ana Rita Lado, FE-UP; LSRE-LCM, ALiCE

15h30 **Coffee Break & Poster Viewing**

16h30 **Presentation of Selected Oral Communications**

OC 03 | Cattle as Reservoirs of Clinically Relevant Enterococcus: A One Health Genomic Perspective. Inês Ribeiro, IUCS-CESPU

OC 04 | Optimized Liposomal Delivery of *Actinidia arguta* Antioxidants for Topical Skin Applications. Filipa Teixeira, REQUIMTE/LAQV - ISEP; ICBAS

OC 05 | Smart Delivery of Polyphenols: Alginate Microencapsulation meets Advanced Skin Models. Mariana Marques, UCIBIO, FFUP

OC 06 | *In vitro* Toxicometabolomic Evaluation of Hepatic Cell Response to “Forever Chemicals”. Filipa Amaro, UCIBIO, FFUP

OC 07 | Metformin in a Warming World: The Hidden Danger to Freshwater Ecosystems. Ana Marta Cordeiro, ICBAS

OC 08 | Linking Cancer Therapy to Cardiac Injury: Anthracycline Toxicity in Human Heart Cells. Beatriz Moreira, FFUP

OC 09 | *In vitro* Neuronal Characterization of the Entheogenic Plant *Tagetes lucida* Cav. Maria Rita Garcia, REQUIMTE/LAQV; FFUP

18h30 **Closing of the First Day**

APRIL 24

SESSION III — Global Challenges of Intensive Agriculture and Illicit Production

CHAIRS: Bruno Peixoto (UCIBIO, 1H-TOXRUN IUCS-CESPU), Joana Barbosa (UCIBIO, 1H-TOXRUN IUCS-CESPU), Luís Fernandes (UCIBIO, 1H-TOXRUN IUCS-CESPU)

09h30 Natural Toxins and Agriculture: Sources, Risks, and the Implications for Human, Animal and Environmental Health. | Vítor Vasconcelos, FC-UP; CIIMAR

10h00 Development of Biopesticides for a More Sustainable Agriculture. | Cristina Azevedo, InnovPlantProtect

10h30 From Poppy Fields to Party Pills: Clandestine Drug Production in Europe and its Impacts. | Rita Jorge, EUDA

11h00 **Coffee Break & Poster Viewing**

11h30 **Presentation of Selected Oral Communications**

OC 10 | Changes in DNA Methylation and Histone H3 Acetylation Induced by Distinct Psychotropic Drugs on Dopaminergic SH-SY5Y Human Neuroblastoma Cells. Ema Rocha, UCIBIO, FFUP

OC 11 | Chiral Separation of Methylone and Pentedrone and Synthesis of Key Metabolites: Toward a Comprehensive Understanding of Synthetic Cathinone Toxicity. Ana Sofia Almeida, FFUP

OC 12 | Enantioseparation and Ecotoxicological Studies of a Cathinone in *D. magna*. Ivan Langa, UCIBIO; 1H-TOXRUN, IUCS-CESPU

OC 13 | Decoding Hidden Toxic Interactions of Paraquat and 2,4-Dichlorophenoxyacetic Acid in *C. elegans*. Rita Azevedo, 1H-TOXRUN, IUCS-CESPU

OC 14 | A Multiparametric Microbial and Biochemical Model for Predicting Postmortem Interval. Maria João Teixeira, 1H-TOXRUN, IUCS-CESPU

OC 15 | How Accurate is Artificial Intelligence for Estimating the Legal Majority? Silvina Moura, FMUP

13h00 **Lunch & Poster Viewing**

SESSION IV — The Compromise to Implement One Health in Portugal

CHAIRS: Carla Batista Pinto (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Daniel Barbosa (UCIBIO, 1H-TOXRUN, IUCS-CESPU), João Carrola (CITAB, UTAD)

14h30 From Prescription to Prevention: the Role of Adequate Nutrition in Reducing Medication Use within a One Health Framework. | Nuno Borges, FCNA-UP

15h00 Rehabilitation of Contaminated Eco-Systems as a Sustainable Path for Global Health. | Teresa Tavares, UM; Env2B

15h30 Multifaceted Intervention to Improve Antibiotic use in Portugal: a "One Health" Approach - Preliminary Results. | Paula Oliveira, UTAD; CITAB

16h00 **ONE HEALTH: A SHARED MISSION ACROSS HEALTH PROFESSIONS**

CHAIRS: Ricardo Jorge Dinis-Oliveira (UCIBIO, 1H-TOXRUN, IUCS-CESPU)

Round Table Discussion with the Presence of:

Agostinho Antunes Pereira | President of the Northern Regional Council of the Portuguese Biologists' Association (on behalf of President of the Portuguese Biologists Association)

António Lopes | President of the Portuguese Physiotherapists Association

Célia Carneiro | Vice-President of the Board of General Council of the Portuguese Dental Association (on behalf of President of the Portuguese Dental Association)

Félix Carvalho | President of the Northern Regional Council of the Portuguese Pharmaceutical Society (on behalf of President of the Portuguese Pharmaceutical Society)

Gaspar Ferreira | Chair of the Northern Regional Office of the Portuguese Psychologists' Association (on behalf of President of the Portuguese Psychologists Association)

João Paulo Carvalho | Vice-President of the Board of Directors of the Order of Nurses (representing the President of the Portuguese Order of Nurses)

Liliana Sousa | President of the Portuguese Order of Nutritionists

Lúcio Meneses de Almeida | President of the National Council for Health Promotion and Environmental Sustainability of the Portuguese Medical Association (on behalf of President of the Portuguese Medical Association)

Pedro Fabrica | President of the Portuguese Veterinary Medical Association

Closing Session

17h00 **Awards Presentation & Closing Ceremony** | Áurea Carvalho, Inês Caldas, Ricardo Jorge Dinis-Oliveira (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Félix Carvalho (UCIBIO; FF-UP)

17h30 **Closing cocktail**

INVITED SPEAKERS

OPENING LECTURE

FÉLIX CARVALHO | UCIBIO, FFUP

A Warming Planet, A Shared Vulnerability: Climate Change Through the One Health Lens.

SESSION I

Emerging Toxic Threats to One Health

TONY WALKER | DALHOUSIE UNIVERSITY

Microplastics and One Health Concepts in Agriculture

AGOSTINHO ALMEIDA | FFUP

Natural Lithium Exposure and Suicide Risk in the Overall Population

SESSION II

Pharmaceuticals and Their Environmental Impact

CRISTINA ALMEIDA | FFUL

Education for Sustainability: Integrating Green Toxicology and Environmental Stewardship into Pharmaceutical Training

BRUNO NUNES | U.AVEIRO, CESAM

Enviromental Risk Assessment of Medicinal Products: Beyond the Guidelines.

ANA RITA LADO | FEUP, LSRE-LCM, ALiCE

The Paradox of Pharmaceuticals: from Medicine to Pollutant.

SESSION III

Global Challenges of Intensive Agriculture and Illicit Production

SESSION IV

The Compromise to Implement One Health in Portugal

NUNO BORGES | FCNAUP

From Prescription to Prevention: the Role of Adequate Nutrition in Reducing Medication Use within a One Health Framework

TERESA TAVARES | U.Minho, Env2B

Rehabilitation of Contaminated Eco-Systems as a Sustainable Path for Global Health

PAULA OLIVEIRA | UTAD, CITAB

Multifaceted intervention to improve antibiotic use in Portugal: a "One Health" approach — preliminary results

ROUND TABLE

One Health: A Shared Mission Across Health Professions

ORAL COMMUNICATIONS

OC01

The Social Support "Staircase": Decoding Neurocognitive Phenotypes in Acute Coronary Syndrome Via Explainable Machine Learning

Ana Bastos [1], Dulce Sousa [2], Afonso Rocha [3,4], Diana Gonçalves [1], Guilherme Aroso [1], Miguel Peixoto [5,6] and Bruno Peixoto [1,7,8,*]

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DOI: https://doi.org/10.48797/sl.2026.377

ABSTRACT

Background: Despite the high prevalence of neurocognitive impairment following Acute Coronary Syndrome (ACS) [1], clinical management often overlooks the heterogeneous nature of these deficits [2]. Identifying distinct neurocognitive phenotypes is essential for personalized rehabilitation [3]. **Objective:** To organize post-ACS neurocognitive profiles using a data-driven pipeline and determine the non-linear predictors of severe impairment. **Methods:** We applied a two-stage machine learning framework to an ACS cohort. First, an unsupervised phase (K-means clustering) was used to discover latent phenotypes based on cognitive performance. Second, a supervised phase compared seven machine learning algorithms to predict phenotype membership. Explainable AI (XAI) tools, including Partial Dependence Plots (PDPs) and probability heat maps, were used to visualize variable interactions. **Results:** Two phenotypes emerged: "Mild/Moderate" (n=231) and "Severe Impairment" (n = 100). XGBoost outperformed all other models (AUC = 0.959; Sensitivity = 99.1%). A robust algorithmic consensus was achieved, with six of the seven models identifying Social Support (ESSS) as the primary predictor. XAI analysis revealed a critical "staircase effect": neurocognitive risk remains high and stagnant until a social support threshold of 35–38 points is reached. Furthermore, high social support was found to exert a "buffering effect", significantly neutralizing the cognitive impact of high depressive symptoms. **Conclusions:** Neurocognitive health post-ACS is not a linear function of clinical severity but a complex interplay of psychosocial resources. The identification of a specific social support threshold (ESSS < 35) provides a concrete clinical marker for identifying patients at risk of severe decline, necessitating a shift toward socially integrated cardiac rehabilitation.

Keywords: acute coronary syndrome; neurocognition; machine learning; social support; depression; phenotyping; *XGBoost*

Acknowledgments/Funding: This research received no external funding.

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OC02

Co-Exposure to Microplastics and Parabens: Implications for *Chlorella vulgaris* Bioremediation Efficiency

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DOI: https://doi.org/10.48797/sl.2026.415

ABSTRACT

Background: Microplastics (MPs) are pervasive contaminants in aquatic systems and wastewater (WW), with polystyrene microplastics (PS-MPs) posing significant ecotoxicological risks [1]. MPs can also act as carriers for emerging contaminants [2], such as parabens—widely used preservatives frequently detected in WW [3]. Their co-occurrence raises concerns about combined effects on treatment efficiency and ecosystem health. **Objective:** This study evaluated the individual and combined effects of PS-MPs and methylparaben (MetP) on the physiological responses and bioremediation performance of *Chlorella vulgaris* in synthetic WW. **Methods:** *C. vulgaris* was exposed to 100 mg PS-MPs/L [4] and MetP (0.796 mg/L, [3]), individually and in co-exposure, under controlled growth conditions for 168 h. Microalgal growth, metabolic activity, nutrient removal, and contaminant fate were assessed. Adsorption assays were also performed to evaluate interactions between PS-MPs and MetP. **Results:** Short-term exposure (72 h) impaired metabolic activity and increased intracellular reactive oxygen species production. However, after 168 h, *C. vulgaris* recovered metabolic function, indicating potential activation of adaptive defense mechanisms. PS-MPs caused moderate growth inhibition (14%), while MetP alone or combined with PS-MPs did not significantly affect microalgal growth. Despite physiological stress, nutrient removal remained high, with nitrogen removal up to 80% and phosphorus removal between 63–70%. Also, *C. vulgaris* removed 21.34 ± 1.12% of MetP, which increased to 26.20 ± 4.44% in the presence of PS-MPs, suggesting a vector effect. The adsorption assays showed that PS-MPs retained 0.61 ± 0.05 mg MetP per g, enhancing its bioavailability, while no significant PS-MPs degradation occurred over 168 h, by the microalga. **Conclusions:** Overall, *C. vulgaris* demonstrated resilience under combined contaminant exposure, maintaining WW treatment performance and partially removing organic micropollutants. The interaction between PS-MPs and MetP enhanced contaminant uptake, highlighting the role of MPs as vectors for ECs. These findings support the potential of microalgal systems as eco-efficient solutions for treating WW contaminated with MPs and co-occurring pollutants.

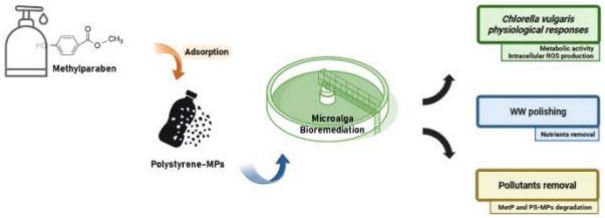

Figure 1. Physiological responses and bioremediation efficiency of *C. vulgaris* under individual and combined exposure to 100 mg PS-MPs/L and 0.796 mg MetP/L, under WW-mimicking conditions.

Keywords: microalgae-based systems; emerging contaminants; wastewater bioremediation

Acknowledgments/Funding: This work was supported by national funds through FCT/MECI: LEPABE, UID/00511/202(https://doi.org/10.54499/UID/00511/2025) and UID/PRR/00511/2025 (https://doi.org/10.54499/UID/PRR/00511/2025), and ALiCE, LA/P/0045/2020 (https://doi.org/10.54499/LA/P/0045/2020); the grant attributed by Portuguese Foundation for Science and Technology, I.P. – FCT – to Paulo Sousa by project reference 2022.14159.BD and DOI:10.54499/2022.14159.BD.

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OC03

Cattle as Reservoirs of Clinically Relevant Enterococcus: A One Health Genomic Perspective

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DOI: https://doi.org/10.48797/sl.2026.401

ABSTRACT

Background: *Enterococcus* spp. are opportunistic pathogens and One Health indicators of antimicrobial resistance (AMR) [1]. Cattle remain understudied reservoirs of multidrug-resistant (MDR) enterococci [2]. This study assessed the occurrence of clinically relevant antibiotic-resistant *Enterococcus* in cattle farms in Northern Portugal, comparing intensive dairy systems and native breeds under extensive conditions. **Methods:** A total of 120 fecal swabs were collected from Holstein-Friesian (n=60) and autochthonous breeds (n=60) across 20 farms (65 calves, 55 adults; 2023). Samples were pre-enriched with or without antibiotics (ampicillin/vancomycin/florfenicol) and plated on Slanetz–Bartley-agar, without/with those antibiotics. Identification (MALDI-TOF MS), susceptibility testing (9 antibiotics; EUCAST/CLSI) and whole-genome sequencing (Illumina) for 13 MDR isolates were performed. Prevalence was calculated per sample [3]. **Results:** *Enterococcus* were detected in 62% of samples, with frequent species co-occurrence, mainly *E. faecium* (Efm-67%), *E. hirae* (52%), *E. faecalis* (45%). Resistance to tetracycline (32%), erythromycin (30%), high-level-streptomycin (28%), ampicillin/ciprofloxacin (22% each), chloramphenicol (18%), linezolid (9%), and high-level-gentamicin (6%) was observed. MDR occurred in 26% of samples, mainly in calves and intensive farms. Linezolid resistance (LinR) genes (*optrA, poxtA, cfr*) were detected across species, mainly in intensive farms, with variable phenotypes (MIC 2-8mg/L). Ampicillin resistance was confined to *E. faecium*, including ST18 and ST80 lineages associated with hospital settings. One ST80 isolate carried *vanHAX* despite phenotypic susceptibility, suggesting a vancomycin-variable genotype. AmpR-ST80 CT9962 was restricted to one farm, whereas LinR-ST18-CT9964 spanned farms/cities (Fig.1). Efm showed mixed hospital/community virulence profiles, with clade A1 lineages (ST18/ST80/ST1693) harboring hospital-associated markers (*ptsD/orf1481*/IS*16*/full pili-clusters). Bacteriocin genes (0–6) were diverse; ST18 carried *bac*AS5 linked to hospital strains. Plasmid replicases (mostly Inc18) included hospital-associated plasmids in ST18 (rep_pNB2354p1) and ST80 (rep_pRUM). **Conclusions:** Cattle may act as reservoirs of enterococci resistant to critically important antibiotics. The detection of hospital-adapted *E. faecium* clones in intensive farming systems highlight potential transmission, supporting the urgent need for integrated One Health surveillance.

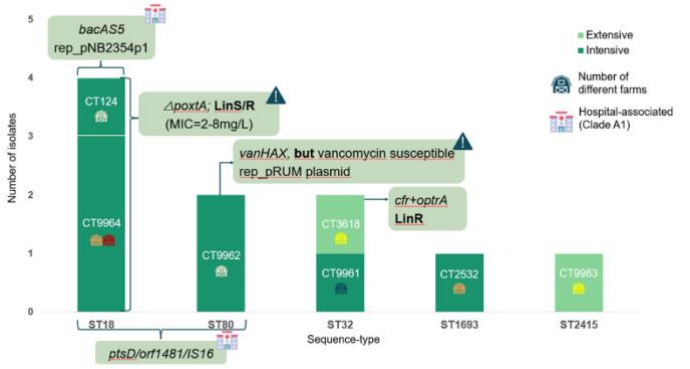

Figure 1. Distribution of *E. faecium* clones and hospital-associated markers across different farms based on whole-genome sequencing (WGS). Bars represent the number of isolates per sequence type (ST) and core-genome type (CT), colored by production system (intensive *vs* extensive). Icons indicate the number of farms where each clone was detected. Hospital-associated markers (e.g., *ptsD*, *orf1481*, IS*16*, pili clusters) were identified in specific lineages, particularly within clade A1. LinR= linezolid-resistant; LinS = linezolid-susceptible.

Keywords: antimicrobial resistance; genomic epidemiology; Enterococci

Acknowledgments/Funding: This research was funded by FCT (Fundação para a Ciência e a Tecnologia, I.P.) through projects UIDP/04378/2020,UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB) and by CESPU through the DYNAMIC-E-GI2-CESPU-2025 project.

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OC04

Optimized Liposomal Delivery of Actinidia arguta Antioxidants for Topical Skin Applications

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DOI: https://doi.org/10.48797/sl.2026.402

ABSTRACT

Background: Natural bioactive compounds offer therapeutic and cosmetic benefits but are often limited by low stability and poor skin penetration. Liposomal nanocarriers represent a promising strategy to overcome these barriers [1]. **Objective:** Optimize liposomes encapsulating an extract from *Actinidia arguta* fruit for topical use, with potential relevance in antitumor skin therapies. **Methods:** The extract was obtained by ultrasound-assisted extraction [2] and incorporated into phospholipid liposomes via probe sonication [3]. A central composite design was applied to optimize lecithin, extract concentration, and sonication amplitude, targeting minimal vesicle size (VS) and polydispersity index (PDI), and maximal encapsulation efficiency (EE). The optimized liposomes were characterized regarding phytochemical composition (LC-DAD-MS), structural integrity (TEM, FTIR, DSC) and stability. Biocompatibility was investigated by measuring cell viability of HDFa, HaCaT, and A375 cells after 24 h of exposure using the MTT assay. **Results:** The optimized liposomes consisted of 167.9 mg/mL lecithin, 42.5 mg/mL extract, and 28% amplitude (R^2 = 0.995), yielding nanosized vesicles (107.2±2.2 nm) with uniform distribution (PDI of 0.173±0.011), a strongly negative zeta potential (ZP; −47.8±1.6 mV), and an EE of 50.7±2.5%. LC-DAD-MS confirmed the presence of chlorogenic and neochlorogenic acids, catechin, kaempferol, quercetin, and sugar derivatives. Over 90 days, liposomes remained stable (VS<150 nm; PDI<0.2; ZP −50 mV). TEM, FTIR and DSC (Figure 1A, B, C) indicated preserved lipid structure and extract integrity, with evident interactions between the bioactives and phospholipids. Biocompatibility studies (Figure 1D) showed >70% viability in keratinocytes and fibroblasts, with mild stimulation at low doses. A375 melanoma cells exhibited slight, dose-dependent reductions in viability only at high concentrations, suggesting nonspecific metabolic effects related to the lipid vesicles or the sugars present on the extract. **Conclusions:** The optimized liposomal system efficiently encapsulates *A. arguta* fruit antioxidants, while maintaining stability and safety, supporting its suitability for topical delivery and further evaluation in melanoma models.

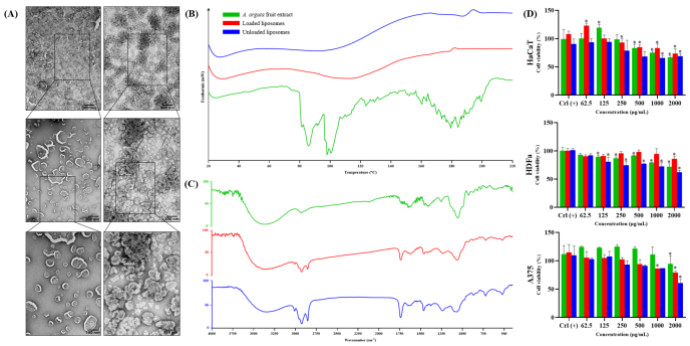

Figure 1. Characterization of optimized liposomes: (A) TEM images of loaded (left) and unloaded liposomes (right), (B) DSC thermograms, (C) FTIR spectra and (D) HaCaT, HDFa and A375 cell viability after 24h of exposure (n = 3); *p < 0.05.

Keywords: liposomes; *Actinidia arguta* fruit; topical application

Acknowledgments/Funding: This work was supported by FCT/MCTES (Portugal) through: FireSkin (2024.07134.CBM), UID/50006, UID/04378/2025, UID/PRR/04378/2025, LA/P/0140/2020, and individual grants (2024.01202.BD, 02/C06-i06/2024, 2023.14718.TENURE.016).

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OC05

Smart Delivery of Polyphenols: Alginate Microencapsulation Meets Advanced Skin Models

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DOI: https://doi.org/10.48797/sl.2026.403

ABSTRACT

Background: Polyphenols are promising cosmetic active ingredients due to their antioxidant and skin-protective properties. However, their instability and poor solubility limit their cosmetical application and effectiveness [1,2]. Microencapsulation in biocompatible polymers, such as alginate, arises as an excellent option to overcome these limitations by enhancing stability and enabling prolonged release [3]. **Objective:** This study aimed to evaluate the stability, bioactivity, and permeation of catechin, epicatechin, chlorogenic acid (CGA), neochlorogenic acid (NCGA), and their mixture in pure and encapsulated forms, using advanced 3D *in vitro* and *ex vivo* skin models, according to the European Regulation n.º 1223/2009. **Methods:** Polyphenols were encapsulated in sodium alginate by spray-drying (115/70 °C) and assessed by scanning electron microscopy (SEM), Differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). Antioxidant and antiradical activities were evaluated by Ferric Reducing Antioxidant Power (FRAP) and radical scavenging assays. Cytocompatibility was evaluated in keratinocytes (HaCaT) and fibroblasts (HDF). A 3D co-culture skin model was constructed and permeation results compared with *ex vivo* human explants coupled to Franz diffusion cells and a reconstructed human epidermis model (SkinEthic™). **Results:** Encapsulation yielded stable microparticles (37-53%) with improved thermal stability. Antioxidant/antiradical activities significantly increased, particularly for catechin, epicatechin, and CGA, demonstrating synergistic effects in the mixture. Encapsulated polyphenols presented higher cell viabilities (> 60% in HaCaT and > 80% in HDF), in comparison with the pure forms. Permeation studies revealed reduced compound diffusion and sustained release, particularly for catechin and epicatechin. *Ex vivo* human skin explants revealed minimal permeation of flavan-3-ols, while CGA, NCGA and the mixture surpassed 50% permeation after 24 hours of exposure. Furthermore, enhanced viability was observed following polyphenol applications in the constructed 3D and SkinEthic™ models, while tissue integrity was preserved (**Figure 1**). **Conclusions:** Alginate microencapsulation enhanced the stability, bioactivity, and safety of polyphenols, while enabling controlled skin delivery. The combined use of advanced 3D and *ex vivo* models provides relevant insight into polyphenols' permeation and supports their use as innovative cosmetic ingredients.

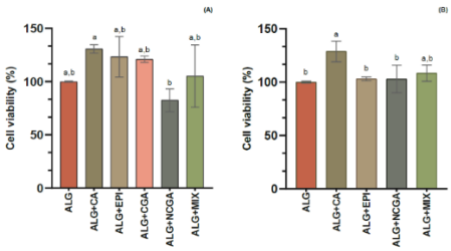

Figure 1. MTT assay with (A) 3D co-culture model and (B) SkinEthic™ RHE model after exposure to alginate microparticles. Results are expressed as mean ± standard deviation (SD) (n=3). Different letters (a,b) indicate significant differences between the compounds (p<0.05).

Keywords: 3D skin model; permeation studies; polyphenols

Acknowledgments/Funding: This work was supported by FCT/MCTES (Portugal) through: FireSkin (2024.07134.CBM), UID/50006, UID/04378/2025, UID/PRR/04378/2025, LA/P/0140/2020, and individual grants (2024.01202.BD, 2023.14718.TENURE.016).

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OC06

In vitro Toxicometabolomic Evaluation of Hepatic Cell Response to "Forever Chemicals"

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DOI: https://doi.org/10.48797/sl.2026.404

ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals widely used in industrial and consumer products [1]. Their chemical stability and resistance to biodegradation lead to environmental persistence and bioaccumulation, giving them the designation "forever chemicals" [2]. Despite growing research efforts, significant knowledge gaps remain regarding the consequences of human exposure. In this context, metabolomics emerged as a promising tool to characterize and enable early detection of PFAS-biological effects [3]. **Objective:** This study aimed to investigate *in vitro* metabolic alterations induced by perfluorooctanoic acid (PFOA) and its short-chain substitute, perfluorobutanoic acid (PFBA), in human hepatic cells applying an untargeted metabolomics approach. **Methods:** Proliferating HepaRG cells were exposed to increasing concentrations of PFOA and PFBA for 24 hours, and concentrations inducing at maximum 40% cytotoxicity were selected based on MTT viability assays (PFOA: 37.5, 75 and 150 µg/mL; PFBA: 75, 150 and 300 µg/mL). Intracellular metabolites (endometabolome) and extracellular culture medium metabolites (exometabolome) were analysed by GC-MS and NMR, enabling the detection of amino acids, organic acids, sugars, lipids, and nucleotides. Data were processed, statistically analysed, and biologically interpreted. **Results:** Multivariate analysis revealed discrimination of the HepaRG endometabolome after exposure to high PFOA, intermediate and high PFBA concentrations. These changes were characterised by a significant intracellular decrease in metabolites associated with antioxidant defence (pyroglutamate, glutamate, threonate and malate), osmotic regulation (taurine) and energy metabolism (malate). PFOA further disrupted intracellular amino acid homeostasis and altered phospholipid metabolism. Exometabolome analysis revealed discrimination across all PFAS concentrations. A dose-dependent increase in niacin excretion was observed in PFAS-exposed cells, suggesting enhanced NAD^+ biosynthesis. Additional extracellular changes were detected, but PFOA specifically affected amino acid levels. **Conclusions:** PFAS *in vitro* exposure induces a common pattern of metabolic alterations. Although these effects were more pronounced following PFOA exposure, the substitute PFBA also elicited metabolomic changes of potential toxicological relevance. These findings may serve as early metabolic indicators of PFAS-related hepatotoxicity and highlight the value of metabolomics in detecting *in vitro* chemical-induced metabolic alterations.

Keywords: per- and polyfluoroalkyl substances (PFAS); environmental exposure; metabolomics; hepatic cells

Acknowledgments/Funding: This research was funded by the EU-FORA Fellowship Program, which supported the work of Filipa Amaro (EUBA-EFSA-2023-ENREL-01).

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OC07

Metformin in a Warming World: The Hidden Danger to Freshwater Ecosystems

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DOI: https://doi.org/10.48797/sl.2026.405

ABSTRACT

Background: Metformin (MET), a widely prescribed drug for type-II diabetes mellitus, is increasingly detected in aquatic environments due to its high consumption and inefficient removal during wastewater treatment [1]**.** Concomitantly, freshwater ecosystems are experiencing intensifying thermal stress driven by climate change, which may modulate contaminant toxicity and challenge ecological risk predictions [2]**. Objective:** This study evaluated how thermal stress modulates the long-term ecotoxicological effects of MET in *Daphnia magna,* combining life-history traits with sub-individual physiological responses. **Methods:** Standard *D. magna* reproduction tests were conducted following OECD 211 guidelines [3], exposing organisms to 0-100 µg MET/L for 21 days at two temperatures: 20 °C (standard conditions) and 24 °C (warming scenario reflecting the + 4 °C increase projected by the IPCC until 2100). To evaluate potential impairments in feeding behavior after chronic exposure, post-exposure feeding inhibition assays [4] were performed after a 24h depuration period in clean medium, followed by a 4h feeding phase. Biomarkers related to oxidative stress, detoxification and neurotoxicity were quantified. **Results:** Temperature strongly influenced the reproductive responses of *D. magna*. At 20 °C, MET exposure induced concentration-dependent responses in key reproductive parameters, including fertility, reproductive output, and first brood fecundity. Under the warming scenario (24 ºC), *D. magna* exhibited an overall enhancement of reproductive performance, with earlier maturation, increased offspring production and fecundity, higher reproductive output, and greater population growth rates. While MET exposure did not affect *D. manga* feeding behavior at 20 ºC, a significant decline in feeding rate was observed at all MET concentrations at 24 ºC. Preliminary biomarker analysis further suggests that MET-induced physiological responses are temperature-dependent. **Conclusions:** Thermal stress appears to reshape the ecotoxicological responses to MET in *D. magna*, influencing both organismal performance and physiological status. By integrating life-history traits with mechanistic biomarkers, this study provides a multilevel understanding of MET effects under climate-driven warming. These findings highlight the importance of incorporating multi-stressor and multi-level approaches into future ecological risk assessment frameworks.

Keywords: pharmaceuticals; climate change; model organisms

Acknowledgments/Funding: Marta Rojais received financial support from Fundação Amadeu Dias under the BYTplus program of CIIMAR – 2025/2026 edition.

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OC08

Linking Cancer Therapy to Cardiac Injury: Anthracycline Toxicity in Human Heart Cells

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DOI: https://doi.org/10.48797/sl.2026.407

ABSTRACT

Background: Cancer patients are at increased risk of developing cardiotoxicity, largely due to the use of potent anticancer therapies that can damage cardiac tissue [1,2]. Although anthracyclines are widely used and highly effective chemotherapeutic agents, their cardiotoxic effects are frequent and may ultimately progress to heart failure. **Objective:** This study aimed to compare the cytotoxic profiles of two anthracyclines (doxorubicin and daunorubicin) in the human cardiomyocyte cell line AC16 and to investigate whether caspase inhibition can attenuate or reverse drug-induced toxicity. **Methods:** AC16 cells were seeded and maintained in medium supplemented with 12.5% foetal bovine serum and subsequently differentiated for 24 h using 2% horse serum [3]. Differentiated cardiomyocytes were then exposed to doxorubicin and daunorubicin at concentrations ranging from 0.05 to 20 µM. Cytotoxicity was assessed at 24 h and 48 h using the neutral red (NR) uptake and MTT reduction assays. Morphological changes were evaluated by phase-contrast microscopy. To examine the potential prevention of cytotoxicity, a caspase-9 inhibitor was applied to cells treated with 1 and 2.5 µM of each anthracycline. **Results:** Both anthracyclines induced time- and concentration-dependent cytotoxicity, with effects being more pronounced in the MTT reduction assay. At 48 h, the greatest cytotoxic effects were observed with 2.5 µM doxorubicin and with 2.5, 10, and 20 µM daunorubicin in both assays. In contrast, 0.05 µM doxorubicin and 0.1 µM daunorubicin did not produce detectable toxicity at either time point in any of the assays performed. Cellular density and morphology were altered at concentrations ranging from 2.5 to 20 µM after 24 h of exposure and from 1 to 20 µM after 48 h, indicating progressive structural damage over time and with increasing concentrations, with daunorubicin inducing earlier and more pronounced morphological alterations. Treatment with a caspase-9 inhibitor did not attenuate the cytotoxic effects induced by either anthracycline. **Conclusions:** These findings indicate that both doxorubicin and daunorubicin exert cytotoxic effects in AC16 cardiomyocytes, although daunorubicin appears to induce a more pronounced toxic response under the tested conditions.

Keywords: doxorubicin; daunorubicin; AC16 cells; cardio-oncology

Acknowledgments/Funding: This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. This work was carried out within the scope of the project "CD-CORE: Cancer Diagnosis, Therapies and Lifestyle – Strategies to Overcome Chemotherapy Resistance and Long-Term Adverse Effects" (Operation No. NORTE2030-FEDER-02707400), co-funded by the European Regional Development Fund (ERDF) through the Northern Regional Programme 2021–2027 [NORTE2030].

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OC09

In vitro Neuronal Characterization of the Entheogenic Plant *Tagetes lucida* Cav.

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DOI: https://doi.org/10.48797/sl.2026.406

ABSTRACT

Background: Holding a relevant ritualistic and ethnomedicinal value among the Mesoamerican tribes, *Tagetes lucida* Cav. has recently seen its uses being translated into Western communities, primarily for recreational ends, given its psychoactivity. Despite the new popularity resurgence of entheogens, the available data concerning this plant's pharmacological and safety profile remain scarce [1-3]. **Objective:** This study aimed to elucidate the bioactive compounds found in *T. lucida* aerial parts and assess their neuropharmacological and toxicological properties. **Methods:** Chemical characterization was performed by means of chromatographic hyphenated techniques. Cytotoxic potential was assessed using human neuroblastoma SH-SY5Y cells, through the evaluation of the mitochondrial and lysosomal performance, and cellular integrity [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, mitochondrial membrane potential, neutral red, and lactate dehydrogenase (LDH) assays; up to 1000 µg mL^{-1}]. Neuronal excitotoxic potential was also explored, following a direct glutamate stimulus, while the inhibition of acetylcholinesterase (AChE) and monoamine oxidase A (MAO-A) was attained in a cell-free model to assess neuromodulatory effects. **Results:** Within the aqueous extract of the aerial parts, the majority of the detected secondary metabolites corresponded to phenolic acid derivatives, followed by coumarins and flavonoids, with scoparone and herniarin being identified as the main compounds. While lysosomal integrity was preserved under normal conditions, mitochondrial depolarization was noted from 250 µg mL^{-1} onwards, while impairment of metabolic competence and loss of membrane integrity with LDH release were verified at the highest tested concentration. When co-exposed to glutamate, neuroprotection was observed from 62.5 to 250 µg mL^{-1}, counteracting its excitotoxic effects. Additionally, concentration-dependent neuromodulation was achieved via AChE and MAO-A inhibition, starting at 31.25 and 15.625 µg mL^{-1}, respectively. **Conclusions:** Considering the neurotoxic effects, indicated by loss of cell metabolic competence and integrity, and the neuroprotective potential, upon glutamate exacerbation, our findings highlight the paradoxical effects promoted by *T. lucida*. Moreover, the inhibition of both neurotransmitter-degrading enzymes suggests apparent pharmacological properties. Nonetheless, these results call for further investigation to unravel the underlying mechanisms.

Keywords: oneirogenic; recreative settings; excitotoxicity; Mexican tarragon; ethnomedicine

Acknowledgments/Funding: This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Maria Rita Garcia thanks FCT for her PhD grant (Ref. 2024.06435.BD). Nelson G. M. Gomes thanks FCT for funding through the Scientific Employment Stimulus Individual Call (Ref. 2022.07375.CEECIND).

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OC10

Changes in DNA Methylation and Histone H3 Acetylation Induced by Distinct Psychotropic Drugs on Dopaminergic SH-SY5Y Human Heuroblastoma Cells

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DOI: https://doi.org/10.48797/sl.2026.408

ABSTRACT

Background: Misuse of psychotropic drugs raises concern due to their neurotoxic and addictive potential [1, 2]. Growing evidence correlates epigenetic effects with neurotoxicity and abuse potential, foreseeing epigenetic marks as predictive markers of drug-related neurotoxicity [3]. **Objective:** Identify a common epigenetic signature (based on global DNA methylation and histone H3 acetylation changes) induced by pharmacologically distinct psychotropic drugs. **Methods:** Dopaminergic-differentiated SH-SY5Y cells were exposed for 24h to 1 µM nicotine, 10 µM methamphetamine (METH), 10 µM 4-CMC (synthetic cathinone), 10 µM tapentadol, 1 µM heroin, and morphine (opioids), and 1 µM JWH-122 and THJ-2201 (synthetic cannabinoids), based on previously obtained cytotoxicity data, and considering physiological relevance. Cells were also exposed to two non-neurotoxic substances, 10 µM mannitol and 10 µM riluzole. Genomic DNA and histones were extracted, and global DNA methylation and histone H3 total acetylation were measured using commercially available colorimetric and fluorometric kits, respectively. Data were obtained from three independent experiments run in duplicate, expressed as % 5-methylcytosine (5-mC) and % H3 acetylation, compared to the untreated control. **Results:** Only METH, morphine, and THJ-2201 increased global DNA methylation (128%, 122%, 126%, respectively), a modification associated with gene transcription silencing. Decreased histone acetylation also leads to gene transcription repression, and was observed for heroin, morphine, and THJ-2201 (68%, 73%, and 82%, respectively). On the other hand, METH, 4-CMC, nicotine, tapentadol and JWH-122 increased histone H3 total acetylation (191%, 200%, 194 %, 244% and 165%, respectively), possibly promoting gene transcription. Non-neurotoxic riluzole reduced H3 histone total acetylation (43%), while mannitol was similar to control. **Conclusions:** Our preliminary data suggest that stimulants seem to increase histone H3 acetylation, while opioids and synthetic cannabinoids can be associated with either increased or decreased histone H3 acetylation. DNA methylation results were variable across drug classes. Large variability suggests limitations of the 24h exposure period and possible instability of the dopaminergic phenotype in SH-SY5Y cells. Future assays using hiPSC cells and longer exposure periods are needed for a more sensitive neurotoxicological epigenetic assessment.

Keywords: epigenetics; psychotropic drugs; neurotoxicity; abuse potential

Acknowledgments/Funding: The work was supported by Fundação para a Ciência e Tecnologia, I.P. (FCT), in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB; and 2024.17684.PEX of EpigenDrugTox: Epigenetic-based prediction of the neurotoxicity of drugs of abuse. ER, CPT and JPS also acknowledge FCT for PhD grants 2024.05454.BD, 2022.13857.BD and research contract (under Scientific Employment Stimulus) 2021.01789.CEECIND/CP1662/CT0014, respectively.

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OC11

Chiral Separation of Methylone and Pentedrone and Synthesis of Key Metabolites: Toward a Comprehensive Understanding of Synthetic Cathinone Toxicity

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DOI: https://doi.org/10.48797/sl.2026.409

ABSTRACT

Background: Synthetic cathinones, such as methylone and pentedrone, are new psychoactive substances that pose a significant health threat due to their widespread accessibility and limited toxicological data available [1]. For a deeper understanding of their toxicological effects, it is essential to consider synthetic cathinones as a whole, including their stereochemistry and structural modifications resulting from biotransformation. Being chiral compounds, their enantiomers constitute individual distinct chemical entities that can exhibit different properties, as demonstrated in previous studies [2,3]. Likewise, metabolites should be considered alongside the parent compounds, as they may also contribute to overall activity or toxicity [4]. Despite the metabolism of synthetic cathinones being well reported, involving pathways such as β-keto reduction and *N*-demethylation [5,6], the specific effects of their metabolites remain largely underexplored. **Objective:** The primary aim of this study is to investigate the influence of chirality and metabolism on the toxicological profile of methylone and pentedrone. Specifically, this work seeks to isolate both single enantiomers and to synthesize key metabolites. **Methods:** Semi-preparative enantioseparation and evaluation of enantiomeric purity were conducted by chiral liquid chromatography (cLC) using amylose-based columns. Dihydro-metabolites were synthesized by β-keto reduction of parent drugs, while nor-metabolites by 5 synthetic steps starting from benzaldehyde derivatives. Structure elucidation was performed by spectroscopic methods (^{1}H- and ^{13}C-NMR and IR). **Results:** Optimized cLC conditions provided Rs and α values above 1.5 and 1.2, respectively. Enantiomers were obtained with enantiomeric ratios over 95% and recovery rates over 60%. Metabolites and intermediates were synthesized with yields of 48-94%. **Conclusions:** Both enantiomers of methylone and pentedrone were isolated with high enantiomeric purity. Dihydro- and nor-metabolites were successfully synthesized, with good yields. Isolated enantiomers and synthesized metabolites will serve as key tools in future metabolomic studies to evaluate the enantioselective effects and the role of metabolites toward a comprehensive understanding of synthetic cathinones toxicity.

Keywords: enantioselectivity; enantioseparation; metabolism; metabolites; synthetic cathinones

Acknowledgments/Funding: This research was supported by national funds by FCT (UIDB/04423/2020, UIDB/MULTI/04378/2020, UIDP/04423/2020 LA/P/0140/2020, PTDC/CTA-AMB/6686/2020 projects). Ana Sofia Almeida acknowledges her PhD grant provided by FCT (project reference 2023.00262.BD and DOI identifier https://doi.org/10.54499/2023.00262.BD).

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OC12

Enantioseparation and Ecotoxicological Studies of a Cathinone in *D. magna*

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DOI: https://doi.org/10.48797/sl.2026.410

ABSTRACT

Background: The synthetic cathinone 3-chloromethcathinone (3-CMC) is among the most prevalent new psychoactive substances (NPS), accounting for 46% of NPS seized in Europe in 2023 [1]. Moreover, like most synthetic cathinones that are chiral, 3-CMC enantiomers may exhibit distinct toxicological effects [2,3]. Therefore, enantioselective studies are essential for the comprehensive characterization of their associated environmental risks. **Objective:** This study aimed to optimize a chromatographic method for the semi-preparative enantioresolution of 3-CMC and assess its ecotoxicity in *D. magna*. **Methods:** The enantioresolution of 3-CMC was performed by liquid chromatography (LC) coupled with a UV/Vis detector, using a semi-preparative CHIRALPAK® AD-H column. For the ecotoxicity assessment, neonates aged ≤ 24 h were exposed to 3-CMC at concentrations ranging from 3.13 to 50 mg L^{-1} in a 48-h acute test, and to 260, 325, and 520 µg L^{-1} in a 9-day subchronic test. Various key endpoints were evaluated, including behavioural, morphophysiological, reproductive, and biochemical parameters. **Results:** The optimized method enabled the isolation of the enantiomers of 3-CMC with high purity (> 97.78%). Acute exposure assays to 3-CMC yielded an EC_{50} (48 h) of 26.14 mg L^{-1}. The racemic mixture of 3-CMC induced significant alterations in behavioural and morphophysiological parameters, while no effects were observed on reproduction. Biochemical analyses revealed a significant increase in thiobarbituric acid reactive substances (TBARS) levels across all exposed groups. However, due to the low stereochemical stability of the isolated enantiomers, enantioselective toxicity assessment was not feasible. **Conclusions:** These findings demonstrate that 3-CMC can induce toxicity in aquatic organisms even under short-term exposure, emphasizing the need for routine monitoring of this contaminant in surface waters. Moreover, the racemization of the isolated 3-CMC enantiomers in the culture medium prevented the assessment of enantioselective toxicity, highlighting the importance of evaluating stereochemical stability to ensure accurate toxicity risk assessment.

Keywords: new psychoactive substances; 3-chloromethcathinone; ecotoxicity; enantioseparation

Acknowledgments/Funding: This research was funded by FCT-Fundação para a Ciência e Tecnologia, through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX). This research was also partially supported by national funds by FCT within the scope of Base Funding UIDB/04423/2020 and UIDP/04423/2020, UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry-CIIMAR). Additional support was provided by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. Ana Rita Carvalho acknowledges the FCT PhD grant (2024.00409.BD). Ivan Langa acknowledges a Ph.D. research grant provided by the FCT-Fundação para a Ciência e Tecnologia, I.P., by project reference 2022.12874.BD and DOI identifier https://doi.org/10.54499/2022.12874.BD.

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OC13

Decoding Hidden toxic interactions of paraquat and 2,4-dichlorophenoxyacetic acid in *C. elegans*

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DOI: https://doi.org/10.48797/sl.2026.411

ABSTRACT

Background: The widespread use of herbicides in modern agriculture, particularly paraquat (PQ) and 2,4-dichlorophenoxyacetic acid (2,4-D), raises significant concerns for human health and environmental safety [1]. Although the toxicological profiles of these compounds are well-documented individually, real-world exposures typically involve mixtures, making it essential to assess their combined effects, which may differ substantially from single-compound toxicity [1]. To address this knowledge gap, this study aimed to investigate the toxicological interactions between PQ and 2,4-D and to evaluate the potential protective role of *N*-acetylcysteine (NAC) using *Caenorhabditis elegans* as a discovery platform. **Methods:** Synchronized L1-stage animals of the DC19 strain [*bus-5(br19)*] (~200/condition) were exposed, in liquid medium, to increasing concentrations of PQ (0-10 mM) and 2,4-D (0-40 mM), either individually or in combination, for 72 h [2]. The survival rate was assessed by counting the number of live and dead worms after the exposure period. Using a sublethal concentration (0.5 mM), we further explored the influence of herbicides, either individually or in combination, on (1) animal development, lifespan and reproductive fitness [3]. **Results:** PQ and 2,4-D reduced animal survival in a concentration-dependent manner, with significant effects observed at concentrations ≥1 mM for PQ and ≥10 mM for 2,4-D ($p < 0.001$). Notably, NAC prevented the reduction in animal survival caused by both herbicides, showing a more pronounced protective effect against PQ-induced toxicity. In co-exposure experiments, the effects of PQ (5 mM) on animal survival were partially restored in the presence of 2,4-D (0,5 mM), and conversely, the effects of 2,4-D (25 mM) were also ameliorated by PQ (0.5 mM). No significant changes in animal development progression were observed for PQ or 2,4-D, either individually or in combination. Nevertheless, animal lifespan was significantly reduced across all exposed groups. **Conclusions:** These findings indicate that co-exposure to PQ and 2,4-D elicits significant toxicological interactions, emphasizing the importance of assessing pesticide mixtures under environmentally relevant exposure conditions. The protective effects of NAC on animal survival further support the involvement of oxidative stress-mediated mechanisms. Additionally, the observed reduction in animal lifespan highlights the potential long-term detrimental effects of these herbicides.

Keywords: paraquat; 2,4-dichorophenoxyacetic acid; *C. elegans*

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy – i4HB, and by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "DeTTeCaK-GI2-CESPU-2025".

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OC14

A Multiparametric Microbial and Biochemical Model for Predicting Postmortem Interval

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DOI: https://doi.org/10.48797/sl.2026.412

ABSTRACT

Background: *Postmortem* interval (PMI) estimation remains a major challenge in forensic science due to the influence of multiple factors. Traditional methods are often limited to early *postmortem* stages and lack precision in advanced decomposition [1,2]. Emerging approaches based on *postmortem* microbiology (thanatomicrobiome) and biochemical alterations (thanatochemistry) show promise [3,4], although their combined application remains underexplored. **Objective:** This study aimed to perform a combined microbial and biochemical analysis of different organs of mice over time since death to develop an accurate mathematical model for PMI estimation. **Methods:** Organs (lungs, heart, kidneys, liver, and brain) from male C57BL/6J specific pathogen-free mice were collected at six PMI (0, 12, 24, 48, 72, and 96 h; n=3 animals/timepoint) under aseptic conditions. For microbial analysis, organs were homogenized in buffered peptone water and cultured aerobically on Blood Agar, MacConkey, or Slanetz–Bartley media to quantify [colony-forming units/g of tissue] total bacteria, *Escherichia coli*, and *Enterococcus faecalis*, respectively. For biochemical analysis, tissue homogenates in phosphate buffer (pH 7.4) were analyzed for urea, lactate, uric acid, glucose, total proteins, magnesium, ethanol, and iron using the Accent MC240 autoanalyzer (Cormay®). For PMI model development, markers were normalized to magnesium, and those with consistent temporal trends and $R^2 > 0.900$ were selected. **Results:** Microbial dynamics were strongly tissue- and time-dependent, with total bacterial loads peaking at 72 h in most organs and later in the brain (96 h). *E. coli* was absent in the liver, heart, and brain, while *E. faecalis* showed consistent colonization in the kidneys, lungs, and the brain, particularly from 24 h onwards. Biochemical markers also exhibited distinct temporal patterns: lactate increased early, whereas uric acid and urea correlated with later PMI stages. Iron showed a progressive tissue-dependent increase. Based on these results, a mathematical model for PMI estimation was developed (Figure 1). **Conclusions:** The integration of culture-based microbiological data with biochemical markers provides a robust multiparametric framework for PMI estimation. In particular, *E. faecalis* colonization and the temporal dynamics of lactate, uric acid, urea, and iron emerged as reliable indicators of *postmortem* progression. Further validation in complex forensic scenarios will be required.

A

$$PMI = \frac{(10.04 - |\bar{Y}x|)}{0.19} \pm 2.78 \times \left[4.89 \times \sqrt{0.19 + \frac{(\bar{Y}x + 17.95)^2}{242.39}}\right]$$

B

$$S_x = \frac{S_y}{m}\sqrt{\frac{1}{K} + \frac{1}{n} + \frac{(\bar{Y}x - \bar{y})^2}{m^2 \sum xx}}$$

Figure 1. Global model for PMI estimation. Equation A allows for the calculation of the PMI from the mean value of the following magnesium-normalized parameters: uric acid and iron in the kidneys; urea in the liver; glucose and urea in the lungs; glucose, lactate, uric acid, and iron in the heart; and lactate, uric acid, and urea in the brain. Equation B estimates the error.

Keywords: thanatomicrobiology; thanatochemistry; *postmortem* interval

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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OC15

How Accurate is Artificial Intelligence for Estimating the Legal Majority?

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DOI: https://doi.org/10.48797/sl.2026.413

ABSTRACT

Background: Age estimation plays a key role in forensic medicine. From adolescence through early adulthood, doubts may arise regarding the actual age, which can have critical medical, legal, and social implications [1]. Age assessment in this age group is closely related to the development of the third molars, when present [2]. The technological innovation is overcoming the inherent subjective errors of traditional methods and enabling the application of a wider variety of combinations of regions of interest by developing automatic approaches and high-performance algorithmic modeling [3, 4]. Currently, the focus is on the study of imaging applied to deep learning platforms, both for training on as broad a population as possible, and for increasing the robustness of such age assessment models [5]. **Objective:** Application of dental images to an automatic platform to evaluate the method's practicality and the accuracy of age estimation in the Portuguese population. **Methods:** A selection of 70 orthopantomograms from the Faculty of Dental Medicine of the University of Porto was submitted to Panacea® software to perform automatic age assessment. The inclusion criteria were participants aged 14 to 22, Portuguese nationality, and the presence of healthy lower third molars. The exclusion criteria were the presence of dental disorders, third molars rotation and/or overlap, and magnification, noise, and/or artifacts. Statistical analysis was performed using the Statistical Package for Social Sciences® software. **Results:** The sample presented a heterogeneous distribution by sex: 23 males and 47 females. The mean chronological age was 18.39 years old (+/- 1.50) for males and 17.98 years old (+/- 1.57) for females. The estimated mean age was 19.54 years old for males (+/- 1.40) and 19.38 years old for females (+/- 1.19). Comparison of the means revealed statistically significant differences between chronological and estimated ages in both cases ($p < 0.001$), and such differences were greater in females than in males (mean differences of -1.40 and -1.15, respectively). **Conclusions:** The combination of multiple relevant features is becoming increasingly diverse in the refinement of machine learning models [3]. The size and heterogeneity of the study sample likely contributed to the overestimation of age. Therefore, the potential for improving the parameters of convolutional neural networks for the Portuguese population should be considered. Alternatively, there may have occurred overfitting.

Keywords: adulthood; age assessment; machine learning; third molars

Acknowledgments/Funding: This research received no external funding.

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POSTER COMMUNICATIONS

PC01

From *In Silico* Screening to Biofilm Disruption: Insect-Derived Peptides Against *Candida spp.*

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DOI: https://doi.org/10.48797/sl.2026.414

ABSTRACT

Background: The global increase in antifungal resistance and spread of *Candida* spp. infections represent a growing clinical challenge and highlight the urgent need for alternative therapeutic strategies [1]. Natural compounds, such as insect-derived peptides, have been widely explored as potential antimicrobial agents, representing one of the most promising bioactive compounds [3,4]. **Objective:** In this perspective, our study aims to evaluate the antifungal potential of insect-derived peptides against *Candida spp*. using a combined *in silico* and *in vitro* experimental approach [2]. **Methods:** A total of 37 insect-derived peptides were screened through molecular docking analysis to evaluate their interactions with *Candida albicans* enzyme targets, including lanosterol 14-demethylase (LDM), secreted aspartic proteinase-5 (Sap-5), *N*-myristoyltransferase (NMT), and dihydrofolate reductase (DHFR). *In vitro* assays included Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) in planktonic cells and biofilm structures of ATCC strains of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. Additionally, the three-dimensional structures of each biofilm were analyzed after treatment, via Confocal Laser Scanning Microscopy (CLSM). **Results:** In *in silico* assays, Blap-6 and Gomesin demonstrated to be good candidates for drug development. Following, in *in vitro* studies, Gomesin achieved complete biofilm eradication in three out of four *Candida* species, while Blap-6 showed moderate but consistent reduction across all species. However, *C. tropicalis* demonstrated resistance to complete eradication by both peptides [2]. In three-dimensional analysis, *C. albicans* demonstrated the highest loss of integrity in the biofilm matrix with absence of intact cells. *C. glabrata* showed some resistance in biofilm extracellular matrix disruption, without compromising cell disturbance [2]. **Conclusions:** These findings highlight insect-derived peptides as promising antifungal candidates and support their potential development as alternative therapeutic agents or templates for the design of novel peptide-based antifungal drugs targeting *Candida* infections.

Keywords: *Candida* spp.; insect peptides; antifungal activity; biofilm

Acknowledgments/Funding: This research received internal funding - PhytoCandd_GI2-CESPU-2023 and ToxiBug4Ca-GI2-CESPU-2025. External funding was also received through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 and UIDP/00511/2020 and ALiCE, LA/P/0045/2020, e.Biofilm project (Grant agreement ID: 101087568).
The authors would like to thank FCT for the CEEC IND5ed (ref. 2022.05712.CEECIND/CP1733/CT0015) and PepMic, Co. Ltd. for kindly providing the peptides.

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PC02

Antifungal Activity of Rockrose (*Cistus ladanifer*) Decoction Extracts against Planktonic Cells and Biofilms of *Candida spp.*: An in vitro Study

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DOI: https://doi.org/10.48797/sl.2026.400

ABSTRACT

Background: *Candida* infections remain a significant clinical concern due to their increasing tolerance and resistance to antifungal agents, as well as the limited efficacy of current treatments. Beyond planktonic growth, *Candida* spp. can form highly structured biofilms embedded in an extracellular matrix, which markedly increases their persistence and antifungal tolerance [1]. Species such as *Candida albicans*, *C. glabrata, C. tropicalis* and *C. parapsilosis* are frequently implicated in human disease, and novel bioactive compounds capable of targeting both planktonic cells and biofilms are urgently needed. *Cistus ladanifer*, a Mediterranean plant rich in phenolic and terpenoid compounds, has been reported to exhibit antimicrobial activity, making it a promising natural alternative. **Objective:** This study aimed to evaluate the antifungal potential of *C. ladanifer* decoction extracts against planktonic and biofilm-associated *Candida* spp [1-3]. **Methods:** Minimum Inhibitory Concentration (MIC) assays were performed according to EUCAST guidelines (50–1500 mg/L). Minimum Fungicidal Concentration (MFC) was determined by CFU quantification after serial dilutions and SDA plating. To assess biofilm susceptibility, Minimum Biofilm Eradication Concentration (MBEC) assays were conducted using 24h pre-formed biofilms in 96-well plates. Biofilms were exposed to fresh RPMI-1640 containing extract concentrations higher than those used for MIC and MFC. The plates were incubated for 24 h at 37 °C. Viability was quantified by CFU enumeration (Log_{10} CFU/cm²). Total biomass was assessed by Crystal Violet staining, with absorbance measured at 570 nm. **Results:** The decoction extracts exhibited antifungal activity against all *Candida* species tested, with MIC values matching MFC. Susceptibility varied among species, confirming species-dependent sensitivity to *C. ladanifer* extracts. MBEC and biomass analyses revealed reductions in viable biofilm cells and total biofilm mass following exposure to the extracts, indicating potential anti-biofilm effects. **Conclusion:** *Cistus ladanifer* decoction extracts demonstrate measurable antifungal activity, affecting both planktonic cells and biofilms in a species-dependent manner. These findings support the potential of plant-derived compounds as complementary strategies for controlling *Candida* infections.

Keywords: *Candida* spp.; antimicrobial resistance; *Cistus ladanifer*

Acknowledgments/Funding: This research was funded by Pfizer® the Global Medical Grants—Reference 60395063, by 1H-TOXRUN—CESPU PhytoCandd_GI2-CESPU-2023 and ToxiBug4Ca-GI2-CESPU-2024 (CESPU internal funds), 2024.13432.PEX (national FCT funds), LA/P/0045/2020 (ALiCE), UIDB/00511/2020, and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC)

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PC03

Untreated Water as a Reservoir of Antibiotic-Resistant *Escherichia coli*: Essessing Potential Public Health Risks

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DOI: https://doi.org/10.48797/sl.2026.416

ABSTRACT

Background: In rural and peri-urban settings, untreated water sources such as wells, boreholes, springs, and public fountains are frequently used for domestic and agricultural purposes [1]. The presence of *Escherichia coli* is a key indicator of fecal contamination and may reflect environmental reservoirs of antimicrobial resistance (AMR), posing potential public health risks [2]. **Objective:** To evaluate antimicrobial susceptibility patterns of *E. coli* isolated from untreated water sources and explore associated environmental and exposure-related factors. **Methods:** Within the WATER project (CESPU, WATER-GI2-CESPU-2025), 24 *E. coli* isolates obtained from 40 untreated water samples (wells, boreholes, fountains, and one spring) collected across four parishes in the municipality of Chaves (Northern Portugal) were tested against 10 antibiotics using the disk diffusion method (EUCAST/CLSI guidelines). Screening for extended-spectrum beta-lactamase (ESBL) production was performed using the double-disk synergy test [3]. Data on water use, the surrounding environment, and household characteristics were also collected. **Results:** Isolates exhibited high susceptibility, with no resistance detected to third-generation cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, or trimethoprim-sulfamethoxazole. Resistance was most frequently observed for ampicillin (37.5%) and tetracycline (29.2%), while resistance to amoxicillin-clavulanic acid was low (4.2%). Overall, 62.5% of isolates were resistant to at least one antibiotic, whereas 37.5% were fully susceptible. No ESBL-producing or multidrug-resistant (MDR, resistance to ≥3 antimicrobial classes) isolates were identified. Environmental data indicated that positive water sources were commonly associated with small-scale agriculture and animal presence, and were used by households, including older individuals (>65 years). **Conclusions:** Untreated water sources may act as environmental reservoirs of *E. coli* with resistance to commonly used antibiotics, particularly ampicillin and tetracycline. The detection of resistant isolates in water sources used for human consumption, including by potentially vulnerable populations, highlights a possible route of exposure. These findings support the need for integrated monitoring of water quality, antimicrobial resistance, and environmental factors to better assess and mitigate public health risks.

Keywords: antimicrobial resistance; untreated water sources; One Health

Acknowledgments/Funding: This research was funded by CESPU, under the WATER-GI2-CESPU-2025 project. This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). Work supported by National Funds through FCT - Fundação para a Ciência e a Tecnologia in the scope of the project UID/50027-Rede de Investigação em Biodiversidade e Biologia Evolutiva.

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PC04

Multidrug-Resistant *Enterococcus spp.* in Cattle Farm Environments: a One Health Perspective

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DOI: https://doi.org/10.48797/sl.2026.417

ABSTRACT

Background: *Enterococcus* spp. are commensals of humans and animals but also important opportunistic pathogens and reservoirs of antimicrobial resistance (AMR) [1]. Data on antibiotic-resistant enterococci in cattle remain limited in Europe, including Portugal [2,3], and the contribution of farm environments to AMR dissemination is largely unknown. **Objective:** To assess the occurrence and AMR profiles of *Enterococcus* spp. across cattle farm environments in Northern Portugal, including facilities, surrounding areas, and humans and animals in close contact. **Methods:** Thirty samples were collected from three cattle farms in two cities, including fomites [feed trough, feed floor (n=2), medication room (n=2) and fridge surfaces, shoe soles (n=2; veterinarian and farmer), milking robot teat cups (n=3) and surfaces, milk storage and milking parlour surfaces, toilet surfaces (n=3)], environmental matrices [bedding sawdust, dog feces (n=2), pigeon feces, soil near the stable], feed (n=4), and milk [individual cow, robot-collected (n=2), and bulk tank milk]. Samples were pre-enriched in BHI with or without antibiotics (ampicillin/vancomycin/florfenicol) and plated on Slanetz-Bartley agar with or without antibiotics. Identification was performed by MALDI-TOF MS and susceptibility testing (EUCAST/CLSI). Prevalence was calculated per sample. **Results:** *Enterococcus* spp. were detected in 20/30 samples (67%) across all sample types except milking parlour surfaces. Resistance was most frequent to tetracycline (TET, 75%), erythromycin (ERY, 67%), and high-level streptomycin (STR, 42%), followed by ciprofloxacin (CIP) and chloramphenicol (CLO, 25% each), high-level gentamicin (CN, 17%), ampicillin (AMP) or linezolid (LIN, 8% each). Resistance to vancomycin was not observed. Multidrug-resistant enterococci (MDR; ≥3 classes) were identified in 25% of positive samples, namely from dog feces, feed (n=2) and shoe soles (n=2), particularly after antibiotic enrichment. MDR isolates included *E. faecium* (AMP+CIP+ERY+TET+STR) from dog feces and *E. faecalis* (LIN+CIP+ERY+TET+STR+CN+CLO) from a farmer's shoe sole. **Conclusions:** Cattle farm environments are reservoirs of MDR *Enterococcus* spp., including strains resistant to critically important antibiotics such as linezolid. Although the sources of resistance, whether originating from cattle or other environmental sources, are unknown, results highlight potential environmental exposure and underscore the need for strengthened One Health AMR surveillance (**Figure 1**).

Figure 1. Schematic representation of the sampling sites within and around a cattle farm, encompassing animal-, human-, and environment-associated samples. Sites are numbered as follows: Fomites: 1, feed trough; 2, feed floor; 3, medication room surface; 4, medication fridge surface; 5, shoe soles (veterinarian and farmer); 6, milking robot teat cups; 7, milking robot surface; 8, milk storage room surface; 9, milking parlour surface; 10, toilet surfaces; Environmental matrices: 11, bedding sawdust; 12, dog feces; 13, pigeon feces; 14, soil near the stable; Feed - 15; Milk: 16, including individual cow, robot-collected, and bulk tank milk.

Keywords: Enterococci; cattle farm environment; linezolid resistance

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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PC05

Single Exposure to Gadolinium or Gadoteric Acid – Influence on Metal Elements Levels in Blood, Kidney and Brain

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DOI: https://doi.org/10.48797/sl.2026.418

ABSTRACT

Background: In gadolinium-based contrast agents (GBCA), used in magnetic resonance imaging, gadolinium [Gd (III)] is chelated to prevent its release and toxicity [1]. Gadoteric acid (Gd-DOTA), a macrocyclic GBCA, presents one of the most stable structures [1]. Nevertheless, endogenous metal cations compete with Gd (III) for the ligand and may replace it; when this transmetallation occurs, Gd (III) is released from its chelate and can either be excreted by the kidneys or be deposited in several organs, such as the kidney and brain [1–3]. Both transmetallation and active cell membrane metal transporters appear to contribute to tissue Gd (III) deposition [3]. **Objective:** To evaluate the short- and long-term effects of a single exposure to Gd (III) or Gd-DOTA on metal elements homeostasis, by evaluating their values on the blood, kidney and brain. **Methods:** Male Wistar rats (ORBEA #7-2022; 28-Nov-2022) were divided in 3 groups *(n*=10 each) and exposed to a single dose (0.1 mmol/kg) of Gd (III), Gd-DOTA or vehicle (control); 2 days and 20 weeks after exposure, metal elements were evaluated on blood, brain and kidney by inductively coupled plasma-mass spectrometry (ICP-MS). **Results:** In blood, 2 days after exposure, Gd (III) group showed significantly higher manganese (Mn) than the control group, and increased copper (Cu) values compared to Gd-DOTA and controls; 20 weeks after, significantly higher zinc (Zn) levels were found in the Gd (III) group compared to the Gd-DOTA group. In the brain, 2 days after exposure, Gd-DOTA showed lower Mn values than the control group. In the kidney, 20 weeks after exposure, for the Gd (III) group, the levels of Cu, Zn and molybdenum (Mo) were significantly higher than for controls; no statistically significant differences were observed in short-term effects. **Conclusions:** Exposure to a single dose of Gd (III) interferes with metal homeostasis, as shown by the significant variations in blood levels of some metals, both at short- (Cu and Mn) and long-term (Zn) effects; and in the kidney, by metal alterations in the long-term effects (Cu, Zn and Mo). Exposure to a single dose of Gd-DOTA did not show a significant impact on blood, kidney and brain values of the metal elements studied, except for the short-term effect on the brain's Mn, which was significantly decreased. Further studies are warranted to evaluate its true safety, especially on its effect on the brain, and in cases of repeated exposures to this GBCA and/or in case of pre-existing renal function impairment.

Keywords: tissue deposition; transmetallation; zinc; copper; manganese; molybdenum

Acknowledgments/ Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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PC06

Gadolinium and Gadoteric Acid Single Exposure – Long-Term Impact on the Kidney's Gene Expression

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DOI: https://doi.org/10.48797/sl.2026.419

ABSTRACT

Background: In gadolinium-based contrast agents (GBCAs), gadolinium [Gd (III)] is chelated to prevent its toxicity. Gadoteric acid (Gd-DOTA), a macrocyclic GBCA with a more stable structure, is commonly used in magnetic resonance imaging [1]. The kidney is one of the major targets of Gd (III), since renal excretion is the main elimination route for most GBCAs [2]. In healthy rats exposed to a single dose of Gd (III) or Gd-DOTA, the kidney transcriptome, compared to controls, showed different gene expression patterns [3]. **Objective:** This study aims to evaluate the long-term effects of Gd (III) or Gd-DOTA single exposure on kidney gene expression. **Methods:** Male Wistar rats were divided into 3 groups *(n*=10 each) and exposed to a single dose (0.1 mmol/kg) of Gd (III), Gd-DOTA or vehicle (control); 20 weeks after exposure, renal tissue was collected to evaluate differential gene expression of its transcriptome (RNASeq), followed by Gene Set Enrichment Analysis (GSEA) of all the identified differentially expressed genes. **Results:** Compared to controls, the Gd (III) group showed an up-regulation of *Ly6al* (Lymphocyte Antigen 6 Complex, Locus A-like), *Snap91* (Synaptosome Associated Protein 91) and *Fosfb (*FosB proto-oncogene, AP-1 transcription factor subunit) genes; and Gd-DOTA group showed an upregulation of *Ly6al*, *Snap91* and *Ugt2b7* (UDP-glucuronosyltransferase family 2 member B7) genes, and a down-regulation of *Cyp26b1* (Cytochrome P450, family 26, subfamily b, polypeptide 1) gene. Gd-DOTA, compared to the Gd (III) group, showed an upregulation of *Ly6al* and *Ugt2b7* genes, and downregulation of *Cyp26b1* and *Fosfb* genes. GSEA analysis for Gd-DOTA showed values of reasonable enrichment (>2) in all the studied genes, while for Gd (III), only *Cyp26b1* and *Fosfb* were altered. **Conclusions:** Single exposure to free Gd (III) or Gd-DOTA induced distinct transcriptional responses in the kidney, showing that Gd-DOTA had a unique profile of gene expression, compared to free Gd (III). Moreover, the GSEA results imply a possible alternative biological pathway(s) activation for each compound. Gd (III) or Gd-DOTA exposure induced long-term disturbances in the expression of genes associated with immunity and xenobiotic metabolism. Further confirmatory studies (qPCR assays) are necessary to validate our gene expression results, allowing the exploration of our data for new insights about Gd (III) impact on kidney function, and Gd-DOTA safety, as *Fosfb* appears to be involved in acute kidney injury [4].

Keywords: nephrotoxicity; kidney transcriptome; next-generation sequencing analysis; RNAseq

Acknowledgments/ Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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PC07

Diode Laser as a Promising Non-Invasive Technique in Oral Medicine Treatments: Quality of Life in Focus

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DOI: https://doi.org/10.48797/sl.2026.420

ABSTRACT

Background: Oral lesions can be treated using minimally invasive techniques that optimise tissue repair and reduce post-operative morbidity [1–3]. **Objective:** The objective was to evaluate the efficacy and visible clinical changes of the diode laser as a non-invasive therapeutic method, as an alternative to conventional therapies, in the management of various oral pathologies. **Methods:** Authorisation to conduct the study was obtained from the Ethics Committee of Fernando Pessoa University. Clinical follow-up was carried out on a sample of 31 participants of different age groups, in accordance with the ethical principles of the Declaration of Helsinki. Participants were selected regardless of gender and age, based on predefined inclusion and exclusion criteria. Firstly, patients received detailed information about the study, and their medical history was recorded. In addition, the OHIP-14 questionnaire was administered to assess the impact of oral health on quality of life, with photographs taken before and after laser treatment. In the second phase, 8 days later, the patients were re-evaluated, with a new photographic record of the treated area, as well as the administration of the OHIP-14 questionnaire and the distribution of a questionnaire to collect subjective data, such as pain, comfort during treatment, and adverse effects. **Results:** The results showed that laser application had a positive impact on oral health-related quality of life, reflected by a reduction in the OHIP-14 score, which fell from 2.29 in the first phase to 0.12 in the second phase ($p<0.001$). The most marked reductions occurred in physical pain and psychological distress; for the remaining parameters assessed, an improvement in the OHIP-14 score was also observed, albeit to a lesser extent. Analysis of the questionnaire collecting subjective data reinforces the previous findings, showing a reduced need for analgesics, an increased perception of well-being, and an increased perception of overall satisfaction [4]. **Conclusions:** Through the analysis of the results, we found that laser application proved to be an effective alternative in treating the observed conditions, having improved objective parameters and healing. Furthermore, improvements were observed in patients' individual experiences, reducing pain, anxiety, and discomfort in the post-operative period.

Keywords: diode laser; photobiomodulation; Oral Medicine; health quality; OHIP-14; LLLT

Acknowledgments/Funding: This research received no external funding.

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PC08

Aurora B, Eg5, and MPS1 as Potential Biomarkers in Ovarian Carcinoma

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DOI: https://doi.org/10.48797/sl.2026.421

ABSTRACT

Background: Ovarian carcinoma remains one of the most lethal gynecological malignancies, largely due to late diagnosis, high recurrence rates, and the development of resistance to conventional therapies [1]. Mitotic regulators, such as Aurora B, Eg5, and MPS1, play critical roles in cell division and chromosomal stability and are frequently dysregulated in cancer. Their aberrant expression may contribute to tumor progression and therapeutic resistance, highlighting their potential as clinically relevant biomarkers [2,3,4]. **Methods:** This study aimed to evaluate the expression of the mitotic proteins Aurora B, Eg5, and MPS1 by immunohistochemistry in ovarian carcinoma cell models, comparing parental OVCAR8 cells with OVCAR8 cells exhibiting dual resistance to carboplatin and paclitaxel. Caspase 3, an apoptosis marker, and Ki-67, a proliferation marker, were also included. For result interpretation, both the percentage of stained cells and staining intensity were considered. **Results:** Immunohistochemical analysis revealed distinct expression patterns of the mitotic proteins Aurora B, Eg5, and MPS1 between parental OVCAR8 cells and their carboplatin- and paclitaxel-resistant counterparts. Aurora B showed a clear increase in the percentage of positively stained cells in resistant cells compared to parental cells, indicating a more pronounced expression in the resistant phenotype. Eg5 and MPS1 were detected in both sensitive and resistant cell lines, with no marked differences in the proportion of positive cells; however, subtle variations in staining intensity were observed, with some resistant cells displaying increased intensity. In addition, Ki-67 expression was higher in resistant cells, consistent with an enhanced proliferative profile. In contrast, caspase-3 staining showed no substantial differences between parental and resistant cells under the conditions evaluated. **Conclusions:** Our findings suggest an association between enhanced expression of mitotic regulators and the acquisition of chemoresistance in ovarian carcinoma cells, particularly in the case of Aurora B. The increased Ki-67 expression further supports a more proliferative phenotype in resistant cells, while the absence of marked differences in caspase-3 indicates that apoptotic activity is not substantially altered under these conditions.

Keywords: ovarian carcinoma; mitosis; Aurora B; Eg5; MPS1

Acknowledgments/Funding: This research was funded by the CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. FlavCanAct-GI2-CESPU-2025, TargetMito-GI2-CESPU-2025 and TARGET4OC-GI2-CESPU-2025).

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PC09

The Gut Microbiota-Intestinal Barrier-Endotoxemia Axis in Insulin Resistance: A Systematic Review

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DOI: https://doi.org/10.48797/sl.2026.422

ABSTRACT

Background: Insulin resistance is a key feature of metabolic disorders such as obesity and type 2 diabetes [1]. Increasing evidence supports a key role of the gut microbiota–intestinal permeability–metabolic endotoxemia axis in metabolic dysfunction, linking microbial dysbiosis with systemic inflammation and impaired glucose homeostasis [2,3]. **Objective:** To understand the relationship between the gut microbiome, the intestinal permeability and metabolic endotoxemia in the development of obesity and insulin resistance. **Methods:** This systematic review followed PRISMA 2020 guidelines. A literature search was conducted in PubMed and Embase using keywords related to gut microbiota, intestinal permeability, endotoxemia, and insulin resistance. Studies involving adult humans that assessed these exposures and insulin resistance outcomes were included, while animal studies, reviews, and articles lacking quantitative data were excluded. After screening 1,511 records, 19 studies were included in the qualitative synthesis. **Results:** Alterations in gut microbiota composition were consistently associated with insulin resistance and metabolic dysfunction. Dysbiosis was characterized by reduced microbial diversity, depletion of butyrate-producing taxa (e.g., *Faecalibacterium*, *Roseburia*), and expansion of Pseudomonadota, contributing to increased lipopolysaccharide-mediated inflammation. Functional changes in microbial metabolic pathways, especially those related to carbohydrate metabolism, were also linked to dysregulated host metabolism. Increased intestinal permeability was associated with translocation of bacterial components, notably lipopolysaccharide, contributing to metabolic endotoxemia and chronic low-grade inflammation. These processes interfere with insulin signaling pathways and promote metabolic dysfunction. Interventions targeting gut microbiota show potential metabolic benefits, although evidence remains limited. Interventional studies targeting the microbiota, including fecal microbiota transplantation and symbiotic supplementation, showed improvements in metabolic parameters, although evidence remains limited. **Conclusions:** The available evidence supports an integrated role of the gut microbiota–intestinal barrier–metabolic endotoxemia axis in the development of insulin resistance. While causal relationships require further clarification, modulation of the gut microbiome represents a promising target for therapeutic strategies aimed at improving metabolic health.

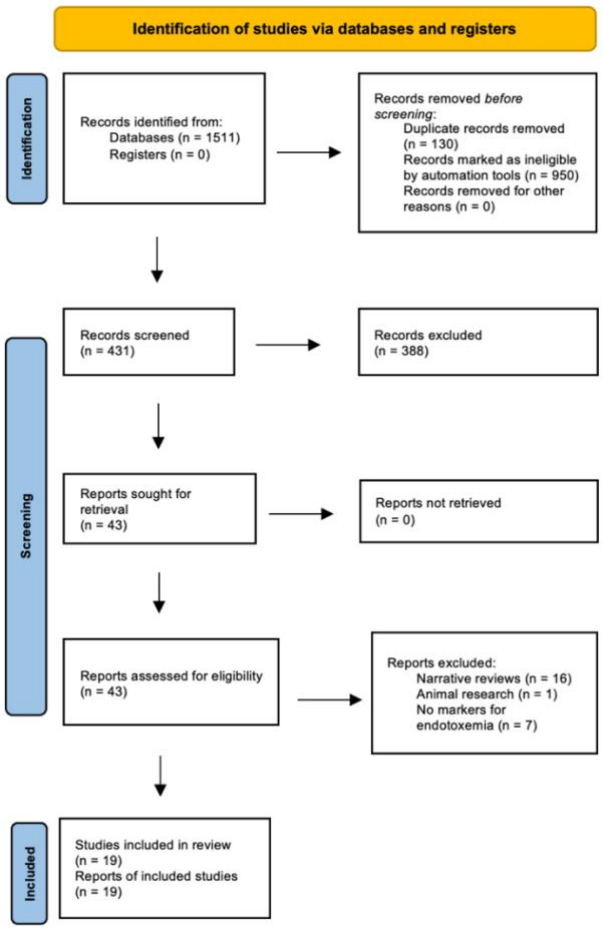

Figure 1. PRISMA 2020 flow diagram illustrating the study selection process for the systematic review, including database searching (PubMed and Embase), screening, eligibility assessment, and final inclusion of studies (n=19).

Keywords: gut microbiota; intestinal permeability; metabolic endotoxemia; insulin resistance

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and through annual funding from the 1H-TOXRUN Research Unit of the University Institute of Health Sciences (IUCS-CESPU).

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PC10

Hemoglobin Adducts as Biomarkers of Alcohol Abuse

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DOI: https://doi.org/10.48797/sl.2026.423

ABSTRACT

Background: Hemoglobin can form covalent adducts with small reactive molecules such as acetaldehyde, an ethanol metabolite [1,2]. These hemoglobin adducts persist throughout the erythrocyte lifespan (~120 days), making them potential long-term biomarkers of alcohol exposure. However, conventional analytical methods, including high-performance liquid chromatography-mass spectrometry and enzyme-linked immunosorbent assay, often lack sensitivity, specificity, or detailed structural information, highlighting the need for alternative detection strategies [3]. **Objective:** This study aimed to evaluate the applicability of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, particularly the saturation transfer difference (STD)-NMR technique, for detecting and characterizing hemoglobin-acetaldehyde adducts as potential markers of ethanol consumption. **Methods:** Human hemoglobin solutions were incubated with acetaldehyde (16 µM–20 mM) at 4 °C for 30 min. Ultraviolet-visible spectroscopy was used to assess hemoglobin concentration and oxidation state. Structural analysis was performed using 1D and 2D NMR experiments (^{1}H-^{1}H TOCSY and ^{1}H-^{13}C HSQC), while STD-NMR (saturation times 0.25–5 s) evaluated ligand–protein interaction dynamics [4]. **Results:** A novel resonance at 1.47 ppm (doublet) was identified, consistent with acetaldehyde binding to hemoglobin. The STD-NMR response showed a concentration-dependent signal increase, linear between 80 µM and 6 mM ($R^2 = 0.994$), followed by a plateau at 20 mM, indicating saturation of binding sites and confirming interaction. **Conclusions:** NMR spectroscopy enables sensitive, non-destructive detection and structural characterization of hemoglobin-acetaldehyde adducts. This approach shows strong potential for developing reliable biomarkers of chronic alcohol exposure. Further validation in complex biological matrices is ongoing to assess its analytical robustness and clinical relevance.

Keywords: hemoglobin adducts; ethanol; acetaldehyde; nuclear magnetic resonance spectroscopy

Acknowledgements/Funding: This work was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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PC11

The Role of Gene-Environment Interactions Between Smoking and DNA Repair SNPs in Oral Cancer risk: a Systematic Review

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DOI: https://doi.org/10.48797/sl.2026.424

ABSTRACT

Background: Oral cancer is the sixth most predominant cancer worldwide, with a poor prognosis and low survival rate. Aetiology is multifactorial, involving both genetic and environmental factors such as smoking, and it is likely that the interplay between these factors contributes significantly to the development of oral cancer [1]. **Objective:** The aim of this work was to identify gene-environment interactions (GxE) involving smoking and Single Nucleotide Polymorphisms (SNPs) in DNA repair genes, previously associated with oral cancer risk, through a systematic review of the scientific literature [2,3]. **Methods:** The systematic review was registered in the PROSPERO database and conducted according to PRISMA guidelines and PICO criteria. PubMed, Scopus and Web of Science were searched using an expression combining both MeSH and common language terms. Rayyan was used to remove duplicates and select the studies according to the predefined inclusion and exclusion criteria. All relevant data from selected articles were extracted into an Excel datasheet to be further analysed. **Results:** Most GxE interactions with smoking involved SNPs in nucleotide excision repair (NER) pathway genes (46% of significant findings), namely, *ERCC2* rs13181, *XPA* rs10817938 and *ERCC6* rs2228528. Isolated significant findings were observed in additional SNPs, but without replication from different studies. **Conclusions:** Smoking interacts with DNA repair SNPs, mainly across the NER pathway, to increase oral cancer risk. However, most of the results are scarce and unreplicated. Further studies in larger populations of different origins should be performed to confirm these findings.

Keywords: smoking; nucleotide excision repair SNPs; oral cancer risk

Acknowledgments/Funding: This work is supported by National Funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UID/04279/2025 and DOI identifier https://doi.org/10.54499/UID/04279/2025 - Centro de Investigação Interdisciplinar em Saúde.

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PC12

Interactions Between Alcohol Consumption and Xenobiotic Metabolism SNPs in HNSCC Risk: Insights from a Systematic Review

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DOI: https://doi.org/10.48797/sl.2026.425

ABSTRACT

Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most predominant cancer worldwide, with a poor prognosis and low survival rate. Alcohol consumption is a well-established environmental risk factor, despite other environmental and genetic risk factors having also been implicated [1,2,3]. Considering the role that xenobiotic metabolizing enzymes play in alcohol metabolism, it is likely that the interplay between these factors contributes significantly to the development of HNSCC. **Objective:** The aim of this work was to identify gene-environment interactions (GxE) involving alcohol consumption and Single Nucleotide Polymorphisms (SNPs) in xenobiotic metabolism genes, previously associated with HNSCC risk, through a systematic review of the scientific literature. **Methods:** The systematic review was registered in the PROSPERO database and conducted according to PRISMA guidelines and PICO criteria. PubMed, Scopus and Web of Science were searched using an expression combining both MeSH and common language terms. Rayyan was used to remove duplicates and select the studies according to the predefined inclusion and exclusion criteria. All relevant data from selected articles were extracted into an Excel datasheet to be further analyzed. **Results:** Most GxE interactions with alcohol consumption involved SNPs in Cytochrome P450 genes SNPs (mainly *CYP1A1*, *CYP1B1* and *CYP2E1*), where replicated results were observed among different studies. SNPs in other phase I enzymes, such as *ADH1B*, *ADH1C* and *ALDH2*, and phase II enzymes, such as *GSTM1*, were also strongly implicated. Isolated significant findings were observed in additional SNPs, but with less conclusive evidence. **Conclusions:** Alcohol consumption interacts with xenobiotic metabolism SNPs, mainly through Phase I enzymes, to increase HNSCC risk. Further studies in larger populations of different origins are needed in order to confirm these findings and expand current knowledge on this important subject.

Keywords: alcohol consumption; xenobiotic metabolism SNPs; HNSCC risk

Acknowledgments/Funding: This work is supported by National Funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UID/04279/2025 and DOI identifier https://doi.org/10.54499/UID/04279/2025 - Centro de Investigação Interdisciplinar em Saúde.

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PC13

Enantioselectivity in Antitumor Activity of Flavone Derivatives

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DOI: https://doi.org/10.48797/sl.2026.460

ABSTRACT

Background: Nature represents a rich source of bioactive compounds, with flavones standing out as privileged scaffolds owing to their multiple biological activities [1]. Their potential as antitumor agents arises from their ability to act on multiple cellular pathways implicated in cancer progression and to attenuate multidrug resistance (MDR) [2]. However, to overcome inherent pharmacokinetic drawbacks and target selectivity, flavones are commonly subjected to structural modifications [3]. The incorporation of chiral moieties, such as amino acids, is used to improve the pharmacokinetic parameters and selectivity [4]. **Objective:** This study screened a library of thirty-two new chiral flavone derivatives (CDFs) to identify promising chemotherapeutic candidates. Beyond cytotoxicity, their effects on tumor metabolism, cell death mechanisms, and their potential as P-glycoprotein (P-gp) inhibitors to overcome multidrug resistance were evaluated. **Methods:** Cell viability assays were performed against a panel of four human cancer cell lines: A375-C5 (melanoma), MCF-7 (breast), NCI-H460 (lung), and HCT-15 (colorectal). The most promising CDFs were further characterized with respect to their impact on metabolic profiles through the quantification of extracellular glucose and lactate levels. Additionally, apoptosis induction was evaluated by Annexin V/PI double staining and flow cytometry, while the Rhodamine 123 exclusion assay was conducted in HCT-15 cells to assess the compounds' potential as P-gp inhibitors. **Results:** Derivatives 6HF-DTrp and 7HF-DTrp were the most potent candidates (GI_{50} < 25 μM), with 7HF-DTrp displaying high specificity (SI up to 7.94) across all tumor lines. While metabolic and apoptotic responses varied by cell type, both compounds were confirmed as P-gp inhibitors through *in vitro* and *in silico* assays. **Conclusions:** These findings identify 6HF-DTrp and 7HF-DTrp as potent, selective, and P-gp inhibitory leads, highlighting their potential as candidates for both monotherapy and combination strategies in cancer treatment.

Keywords: chiral flavones; antitumor activity; multidrug resistance

Acknowledgments/Funding: This research was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of the projects UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025) and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020), the projects UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025) and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025) of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy – i4HB. Additional support was provided by the "FlavScreen-GI2-CESPU-2023" and "FLV4cancer-GI2-CESPU-2025" projects funded by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário Crl. Flávia Barbosa acknowledges financial support from CESPU through grant BD/DCB/CESPU/01/2024. Cláudia Pinto acknowledges financial support from FCT through grant 2022.12419.BD.

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PC14

Targeting the Warburg Effect in Breast Cancer with Chiral Derivatives of Flavonoids

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DOI: https://doi.org/10.48797/sl.2026.461

ABSTRACT

Background: Triple-negative breast cancer (TNBC) is the breast cancer subtype with the worst prognosis due to a lack of expression of human epidermal growth factor receptor 2 or hormone receptors [1]. Furthermore, TNBC cells exhibit metabolic reprogramming, prioritizing glycolysis even when oxygen is available. This metabolic shift, known as the Warburg effect, not only enhances tumor resistance to cytotoxic agents but also fuels its invasive behavior [2]. Flavonoids are examples of bioactive compounds with demonstrated antitumor activity, and their conjugation with amino acids has been reported to increase their cytotoxic effects [3]. Our studies further demonstrate that chiral derivatives of flavonoids (CDFs) showed promising anticancer potential. **Objective:** This study aims to investigate the cytotoxicity and mechanistic profile of a specific enantiomeric pair of CDFs (TriCe-LTrp and TriCe-DTrp) on two TNBC cell lines (MDA-MB-231 and Hs578T) and a non-tumor control cell line (MCF-10A). **Methods:** Cell viability was assessed to determine the selectivity index of the selected CDFs. The metabolic profile, evaluated by quantifying glucose and lactate, and the induction of apoptosis, measured by Annexin V flow cytometry, were analyzed following treatment. Additionally, cell cycle distribution was characterized using propidium iodide flow cytometry to further evaluate the effects of the compounds on the tumor cell lines. **Results:** Both TriCe-LTrp and TriCe-DTrp exhibited low GI_{50} values in MDA-MB-231 and Hs578T cell lines. TriCe-LTrp demonstrated high selectivity on both tumor lines, with high selectivity indices, whereas TriCe-DTrp showed no selectivity, highlighting the critical role of chirality. Regarding metabolism, TriCe-DTrp significantly decreased lactate production in both lines and glucose consumption in Hs578T cells. Conversely, TriCe-LTrp reduced glucose consumption in both cell lines, but only significantly in Hs578T cells, though its impact on lactate was not significant. Flow cytometry analysis using Annexin V and propidium iodide revealed that TriCe-LTrp significantly induced apoptosis in MDA-MB-231 cells, while similar trends for TriCe-LTrp in Hs578T and for TriCe-DTrp in both lines did not reach significance. **Conclusions:** These findings identify TriCe-LTrp as a highly selective and potent lead compound for triple-negative breast cancer therapy due to its dual ability to modulate tumor metabolism and induce apoptosis.

Keywords: breast cancer; chiral flavones; antitumor activity

Acknowledgments/Funding: This research was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P. and by the European Commission's Recovery and Resilience Facility, within the scope of the projects UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025) and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020), the projects UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025) and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy – i4HB. Additional support was provided by the "FLV4cancer-GI2-CESPU-2025" project funded by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário Crl. Flávia Barbosa acknowledges financial support from CESPU through grant BD/DCB/CESPU/01/2024. Cláudia Pinto acknowledges financial support from FCT through grant 2022.12419.BD.

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PC15

Uncovering Ketamine Enantioselective Toxicity Using *Caenorhabditis elegans* as a Discovery Platform

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DOI: https://doi.org/10.48797/sl.2026.462

ABSTRACT

Background: The increasing recreational use of ketamine represents a growing public health concern due to the limited understanding of its long-term and enantioselective toxicological effects, as the two ketamine enantiomers are thought to exhibit distinct pharmacological and toxicological profiles [1, 2]. Understanding these differences is essential to better characterize the biological impact of this psychoactive substance. **Objective:** This study aims to investigate the potential enantioselective toxicity of ketamine using *Caenorhabditis elegans* as a discovery platform. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200/condition) were exposed, in liquid medium, to increasing concentrations of ketamine (racemic mixture and (*S*)-ketamine enantiomer; 0 - 10 mM) [3]. Following a 72-h incubation in M9 buffer containing OP50 bacteria as a food source, the animal survival rate was determined by counting the number of live and dead worms after the exposure period. Using sublethal concentrations, we plan to further explore the influence of ketamine on (1) animal development, (2) lifespan, and (3) reproductive behavior. Additional experiments will explore the hatching rate of unexposed F1 embryos laid by exposed animals, as well as the growth of larvae derived from these embryos, to investigate putative heritable toxicological signatures. **Results:** Exposure of synchronized L1-stage animals to ketamine (racemic mixture) at concentrations ≥ 5.0 mM for 72 h significantly decreased survival in a concentration-dependent manner. In contrast, the (*S*)-ketamine enantiomer significantly reduced survival at concentrations ≥ 2.5 mM. Observational evidence indicates that sublethal concentrations of ketamine (both racemic and *S*-ketamine enantiomer) delay animal development, highlighting optimistic perspectives regarding the impact of different ketamine forms on organism-level endpoints. **Conclusions:** Overall, these findings suggest that ketamine induces time-dependent effects in *C. elegans*, with distinct response patterns between the racemic mixture and (*S*)-ketamine, suggesting the presence of enantioselective toxicity. Ongoing analyses will further clarify the impact of these exposures on organismal development, lifespan and reproductive behavior, and potential heritable toxicological signatures, contributing to a deeper understanding of ketamine's toxicological profile.

Keywords: ketamine; enantioselective toxicity; *C. elegans*

Acknowledgments/Funding: This work was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the Research Unit on Applied Molecular Biosciences–UCIBIO (projects UIDP/04378/2025 and UIDB/ 04378/2025), the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2025), the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos, and by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "DeTTeCaK-GI2-CESPU-2025".

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PC16

Assessing the Cytotoxic and Metabolic Impact of Cebranopadol in a Panel of Cell Lines

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DOI: https://doi.org/10.48797/sl.2026.463

ABSTRACT

Background: Cebranopadol is a novel, first-in-class analgesic with a unique pharmacological profile. It acts as a full agonist at the μ-opioid (MOP) and nociceptin/orphanin FQ peptide (NOP) receptors [1]. This dual mechanism shows potential for preserving analgesic potency while reducing adverse effects typical of traditional opioids [2]. Currently, cebranopadol is in phase III clinical trials and has received FDA fast-track designation, reflecting its promise as a next-generation analgesic [3]. Nonetheless, further large-scale studies are necessary to fully establish its safety, long-term efficacy, and comparative advantages over conventional and other emerging opioid analgesics [4]. **Objective:** The present study aimed to assess the metabolic impact of cebranopadol exposure in three cell lines: BV-2 (murine microglia), HepG2 (human hepatocellular carcinoma) and SH-SY5Y (human neuroblastoma). **Methods:** Following 48 hours of exposure to half the respective IC_{50} concentrations, as previously determined through the sulforhodamine B (SRB) assay, BV-2, HepG2, and SH-SY5Y culture medium samples were collected for spectrophotometric quantification of glucose and lactate levels. **Results:** In BV-2 cells, extracellular glucose levels showed a slight decrease, whereas they remained unchanged in SH-SY5Y and HepG2 cells. Lactate levels increased in culture media samples from cebranopadol-treated BV-2 and SH-SY5Y cells compared to those of the controls, while a decrease was observed in HepG2 cells. These results suggest an opioid-induced shift towards anaerobic metabolism in BV-2 and SH-SY5Y cells, whereas fermentative processes may be hindered in HepG2 cells. **Conclusions:** While supratherapeutic concentrations of cebranopadol show low cytotoxicity in BV-2, HepG2, and SH-SY5Y cells, they may affect metabolic processes differently across the three cell lines by interfering with aerobic and anaerobic metabolic fluxes. Further investigation of the molecular and cellular mechanisms behind these metabolic alterations is warranted to fully characterize the safety profile of cebranopadol.

Keywords: cebranopadol; cytotoxicity; cell metabolism

Acknowledgments/Funding: This research was funded by Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), project/grant CebraTox-GI2-CESPU-2025.

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PC17

In silico Investigation of the Interaction Between Paclitaxel and Ergosterol in P-glycoprotein

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DOI: https://doi.org/10.48797/sl.2026.465

ABSTRACT

Background: Multidrug resistance (MDR) mediated by the overexpression of P-glycoprotein (P-gp) is one of the main obstacles in cancer treatment, as this transporter reduces the intracellular concentration of chemotherapeutic agents such as paclitaxel (PTX) [1]. Natural compounds have shown promise as P-gp modulators for reversing MDR [2]. Ergosterol (ERG), a sterol commonly found in fungi, has shown potential as a modulator of this transporter [3]. However, the molecular details of its interaction and its combination with PTX are not yet fully understood. **Objective:** To investigate the molecular interactions between PTX and ERG at the P-gp binding site using single molecular and sequential docking simulations to assess possible competitive effects. **Methods:** The human P-gp structure (PDB: 7A6E) and the AutoDock VINA algorithm were used. Individual docking studies were performed to characterize the isolated affinity of each ligand. Subsequently, a sequential docking strategy was applied, in which one ligand was held fixed in the best conformation, and the second was docked again at the same site, simulating prior occupation of the transporter. **Results:** In the individual binding assays, both PTX and ERG exhibited high affinity, with binding energies of –10.77 kcal/mol (Kd ≈ 12.60 nM) and –10.58 kcal/mol (Kd ≈ 17.30 nM), respectively. In sequential docking, the prior presence of ERG significantly reduces the affinity of PTX (-8.04 kcal/mol), resulting in an increased dissociation constant in the micromolar range (12.70 µM). Conversely, ERG maintained a favorable affinity (-10.19 kcal/mol) even in the presence of PTX. Ligand efficiency (LE) analysis revealed that ERG (LE = 0.365) exhibits superior energy utilization to PTX (LE = 0.174) relative to its molecular size. **Conclusions:** Structural analysis reveals that ergosterol exerts steric interference at the P-gp binding site, resulting in partial competition with paclitaxel. Due to its higher binding affinity, ergosterol partially obstructs the transporter's binding pocket, which may impair the binding stability and transport efficiency of the chemotherapeutic agent without preventing its initial interaction with the active site.

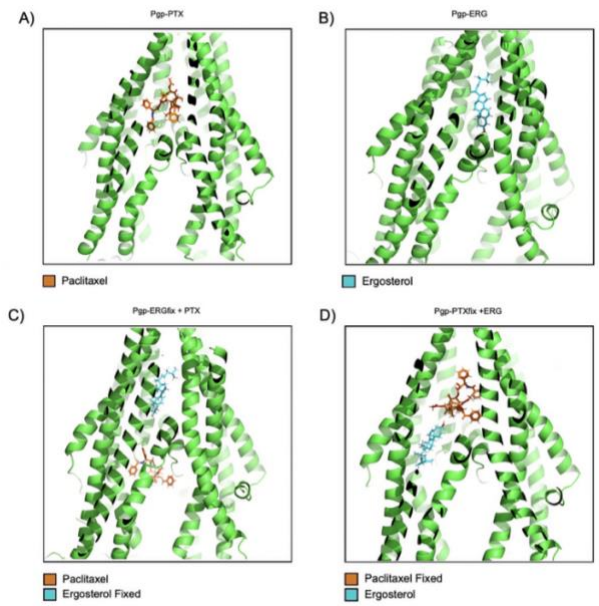

Figure 1. *In silico* representation of the co-occupation and structural overlap of paclitaxel and ergosterol in the binding pocket of human P-gp (PDB: 7A6E).

Keywords: ergosterol; paclitaxel; P-glycoprotein; multidrug resistance

Acknowledgments/Funding: The authors would like to thank CESPU—Cooperativa de Ensino Superior Politécnico e Universitário—for the PhD scholarship BD/DCB/CESPU/01/2023 awarded to Jéssica Fonseca)
This work is funded by national funds from FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project/support UID/06157/2025 https://doi.org/10.54499/UID/06157/2025

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PC18

Combined Effects of Paclitaxel and Dichloroacetate on Oral Cancer Metabolism

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DOI: https://doi.org/10.48797/sl.2026.464

ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity [1]. Despite therapeutic advances, including the use of paclitaxel (PTX), treatment efficacy remains limited due to drug resistance and toxicity. The Warburg effect, characterized by enhanced aerobic glycolysis and acidification of the tumor microenvironment, contributes to resistance to anticancer therapies [2,3]. Dichloroacetate (DCA), a modulator of mitochondrial metabolism, represents a promising strategy to restore oxidative phosphorylation and promote apoptosis in cancer cells [4,5]. **Objective:** This study aimed to evaluate the effects of DCA, alone or in combination with PTX, on tumor metabolism and cell viability in OSCC cell lines (SCC25 and SCC09), compared to non-tumor oral keratinocytes (HOK). **Methods:** The cytotoxicity of DCA and PTX was evaluated using the sulforhodamine B (SRB) assay to determine the GI_{50} values in SCC09, SCC25, and HOK cells. The Combenefit platform was used to analyze DCA/PTX combinations and identify synergistic interactions in SCC25 cells. **Results:** Both DCA and PTX reduced the viability of OSCC cells after 24 hours. PTX exhibited higher cytotoxicity than DCA, affecting both tumor and normal cells, indicating limited tumor selectivity. In contrast, DCA displayed moderate cytotoxicity with variable sensitivity among cancer cell lines and no detectable GI_{50} in HOK cells under the tested conditions. **Conclusions:** PTX showed potent cytotoxic effects but lacked selectivity for tumor cells, whereas DCA exhibited moderate cytotoxicity and minimal impact on normal cells. Notably, combination analysis revealed multiple synergistic interaction points between DCA and PTX in OSCC cells, supporting their complementary mechanisms of action. These findings suggest that DCA may enhance the therapeutic efficacy of PTX while potentially allowing dose reduction and improved tumor selectivity.

Keywords: oral cancer; dichloroacetate; paclitaxel; tumor metabolism

Acknowledgments/Funding: This research was funded by the CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl, under the grants "RECAP-DCA-GI2-CESPU-2025", and "TargetMito-GI2-CESPU-2025".

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PC19

Evaluation of the Antitumor Effect of Auranofin in Ovarian Cancer Cell Lines

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DOI: https://doi.org/10.48797/sl.2026.466

ABSTRACT

Background: Ovarian cancer is one of the deadliest gynecologic malignancies, largely due to late-stage diagnosis and the frequent emergence of resistance to standard chemotherapy. First-line treatment typically combines platinum-based agents, such as carboplatin, with taxanes, such as paclitaxel; however, many patients eventually develop chemoresistance, limiting therapeutic options and worsening outcomes [1]. Drug repurposing has emerged as a promising strategy to identify new anticancer therapies among compounds with established safety profiles [2]. Auranofin, an FDA-approved drug initially used to treat rheumatoid arthritis, has recently gained attention for its anticancer potential. Its main mechanism involves inhibition of thioredoxin reductase, leading to redox imbalance and oxidative stress in cancer cells [3]. **Methods:** In this study, we evaluated the effect of auranofin on cell viability in ovarian cancer models using the OVCAR8 cell line, which presents resistance to carboplatin, and the OVCAR8 PTX RC cell line, which is resistant to both carboplatin and paclitaxel [1]. Cells were cultured under 2D conditions and exposed to increasing concentrations of auranofin. Cell viability was assessed using the Presto Blue metabolic assay, which measures cellular metabolic activity as an indicator of viable cells. **Results:** Auranofin treatment resulted in a pronounced dose-dependent reduction in cell viability in both cell lines. The estimated IC_{50} values were 1.06 ± 0.08 µM for OVCAR8 and 1.42 ± 1.16 µM for OVCAR8 PTX RC, indicating a high sensitivity to this compound across both models. Importantly, both cell lines exhibited a steep decline in viability at concentrations around 1–3 µM, highlighting a narrow transition window and potent biological effect. **Conclusions:** In conclusion, these findings highlight the potential of auranofin as a repurposed therapeutic agent for ovarian cancer, particularly in the context of platino-resistance. The observed efficacy in two carboplatin-resistant cellular models, including one also resistant to paclitaxel, supports further investigation of this compound. Future studies, including the use of three-dimensional models, patient-derived systems, and combination approaches with standard chemotherapeutic agents, will be important to better elucidate its mechanisms of action and evaluate its translational potential.

Keywords: ovarian cancer; auranofin; drug repurposing

Acknowledgments/Funding: This work was funded by CESPU—Cooperativa de Ensino Superior Politécnico e Universitário CRL, under the grant "TARGET4OC-GI2-CESPU-2025". Also, this work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDB/04378/2020 (DOI: 10.54499/UIDB/04378/2020) of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI: 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. A.E.C.L acknowledges Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Inovação (MCTES), and União Europeia (UE) for financial support through a PhD fellowship (2025.01464.BD) that was co-sponsored by Fundo Social Europeu (FSE) through Programa Operacional Regional Norte (Norte 2030).

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PC20

Evaluation of Novel Xanthene Derivatives as Potential Neuroprotective Agents for Alzheimer's Disease

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DOI: https://doi.org/10.48797/sl.2026.467

ABSTRACT

Background: Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by the accumulation of amyloid-beta (Aβ) plaques, hyperphosphorylated Tau tangles, and increased oxidative stress, among other pathological features. Despite being the leading cause of dementia, AD remains inadequately treated, as current therapies offer limited efficacy [1, 2]. With global cases projected to more than triple by mid-century, there is a pressing need for next-generation disease-modifying therapies targeting these fundamental pathological mechanisms. Xanthenes, oxygen-containing tricyclic heterocycles with a dibenzo[b,e]pyran scaffold, have emerged as promising neuroprotective agents. Their ability to modulate neurodegenerative processes highlights their therapeutic potential in AD [2, 3]. **Objective:** This study aimed to synthesize xanthene derivatives incorporating dopamine and levodopa moieties (Figure 1), containing the catechol group, a pharmacophoric feature associated with neuroprotection [4], and to evaluate their cytotoxicity and neuroprotective effects. **Methods:** Eight xanthene derivatives incorporating dopamine and levodopa moieties were synthesized via condensation reactions with carboxylic acids (structures confirmed by nuclear magnetic resonance spectroscopy). Their cytotoxicity (0–25 mM) was evaluated in SH-SY5Y cells differentiated into a cholinergic phenotype, using the neutral red (NR) uptake and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assays. Neuroprotective potential was assessed against *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative stress, using a reactive oxygen/nitrogen species (ROS/RNS)-sensitive fluorescent probe and the NR uptake assay. **Results:** All synthesized xanthene derivatives were successfully obtained, structurally characterized, and were non-cytotoxic at concentrations up to 10 mM. All compounds markedly attenuated *t*-BHP-induced oxidative stress (reduced ROS/RNS generation and/or significant decrease in *t*-BHP-induced cell death), and their protective effects were not reversed by the Nrf2 inhibitor ML385, indicating an Nrf2-independent mechanism. **Conclusions:** These findings establish xanthene derivatives as potent oxidative stress modulators, although additional studies are needed to evaluate their efficacy against other AD hallmarks. Elucidating these broader mechanistic targets will be essential to validate their potential as multitarget drug candidates for AD therapy.

Keywords: Alzheimer's disease; xanthene derivatives; neuroprotection

Acknowledgments/Funding: This work was supported by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the Research Unit on Applied Molecular Biosciences–UCIBIO (projects UIDP/04378/2025 and UIDB/ 04378/2025), the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2025), and the project UID/50006/2025 (DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos). Inês Costa is supported by a PhD fellowship from FCT (2023.04980.BD).

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PC21

Evaluation of Cannabinoid Extraction from Cannabis Flowers Using Ohmic Heating

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DOI: https://doi.org/10.48797/sl.2026.468

ABSTRACT

Background: *Cannabis sativa* has significant therapeutic potential, particularly for neurological disorders such as epilepsy, Alzheimer's disease, and Parkinson's disease, as well as for chronic pain and inflammation [1]. Despite its medicinal relevance, it is also consumed recreationally due to the psychoactive properties of D^9-tetrahydrocannabinol (D^9-THC) [1]. The extraction and analysis of cannabinoids is essential to support research and ensure the safety and regulation of cannabis-based products. Although several extraction methods have been explored, ohmic heating (OH) has recently emerged as an innovative and potentially more sustainable technique for extracting phytochemicals from plant matrices [2,3]. **Objective:** This study aims to develop an extraction protocol for cannabinoids using ohmic heating by quantifying D^9-THC, tetrahydrocannabinolic acid (D^9-THCA), and total D^9-THC. **Methods:** Dried cannabis flowers were pulverized in a Retsch Mixer Mill MM 400 equipped with steel balls (25 Hz, 12 cycles ´ 15 seconds). The OH extraction was performed using 80% ethanol and 20% water with added NaCl for 10 minutes. For the quantification, an Agilent 1260 Infinity II HPLC-DAD system was used, equipped with an InfinityLab Poroshell 120 EC-C18 (3.0 ´ 150 mm, 2.7 µm) column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column. The gradient elution was performed using methanol with 0.05% formic acid and deionized water with 0.1% formic acid mixtures, with a flow rate of 0.5 mL/min, run time of 30 min, and injection volume of 5 µL [4]. **Results:** Several extraction parameters were studied to enhance the extraction of cannabinoids, namely the percentage of ethanol, extraction time and temperature. For an extraction period of 10 minutes, the highest total THC value was obtained using 80% ethanol (12.7%) compared to 40% (2.5 %). An increase in time and temperature (t = 20 minutes, 80 ºC) led to similar total D^9-THC extraction results (13.0%) even though the percentage of D^9-THC increased. **Conclusions:** The percentage of ethanol significantly influenced the quantity of total D^9-THC extracted. Increasing the extraction time did not affect the total D^9-THC extraction yield, although a higher degree of decarboxylation was observed.

Keywords: cannabis; metabolites; ohmic heating

Acknowledgments/Funding: This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). The authors acknowledge the funding provided by Avextra Portugal SA.

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PC22

Isoquinolinequinone *N*-Oxides as Promising Antitumor Compounds in Pancreatic Cancer Cell Lines

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DOI: https://doi.org/10.48797/sl.2026.469

ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) represents the 12th most commonly diagnosed malignancy worldwide and the sixth leading cause of cancer-related mortality, with more than 500,000 new cases and nearly 470,000 deaths recorded in 2022 [1]. Its poor prognosis is largely driven by intrinsic and acquired drug resistance, leading to mortality rates above 90%. Current treatment mainly relies on gemcitabine, either alone or in combination with paclitaxel [2,3], highlighting the urgent need for novel anticancer agents, particularly those able to overcome chemoresistance. Recently, isoquinolinequinone (IQQ) *N*-oxides have shown promising antitumor activity in pairs of sensitive and multidrug-resistant (MDR) lung and colorectal cancer cell models [4,5]. **Objective:** This study aimed to evaluate the antitumor effects of IQQ *N*-oxides in several PDAC models, including a sensitive and resistant PDAC cell line. **Methods:** MiaPaCa-2, Capan-1, BxPC-3, Panc-1, and the resistant Panc-1-CDR PDAC cells were treated with different concentrations of IQQ *N*-oxides (RK1-RK9) or gemcitabine for 48 h. Cell viability was assessed by SRB assay, while long-term clonogenic potential was determined by colony formation assay (2 or 6 days). Anti-migratory effects were evaluated using a wound healing assay over 48 h. **Results:** RK2 and RK3 emerged as the most potent derivatives, with GI_{50} values ranging from 0.80 to 2.35 µM across multiple cell lines. Both compounds remained effective in the gemcitabine-resistant Panc-1-CDR model, with GI_{50} values of 1.41 and 1.14 µM, respectively. Moreover, the clonogenic assay showed a marked concentration and time-dependent reduction in colony formation, with near-complete inhibition by day 6 when Panc-1 cells were treated with RK2 and RK3. Additionally, treatment with RK2 and RK3 significantly impaired cell migration at 1µM concentration, limiting wound closure to 60% and 51%, respectively, after 48h (compared to 80% in control cells). **Conclusions:** The IQQ *N*-oxide derivatives, RK2 and RK3, effectively reduced PDAC cell growth, including in a gemcitabine-resistant PDAC cell line, and suppressed key malignant traits such as self-renewal and migration, supporting their potential as promising therapeutic leads for PDAC.

Keywords: chemoresistance; isoquinolinequinone *N*-oxides; pancreatic cancer

Acknowledgments/Funding: This work was partially funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário CRL (grants with Ref. PLASTICAR-G12-CESPU-2025).

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PC23

Lipid Nanoparticle-Encapsulated Cannabidiol Enhances Selective Cytotoxicity in Melanoma Cells

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DOI: https://doi.org/10.48797/sl.2026.470

ABSTRACT

Background: Melanoma is the most lethal form of skin cancer, accounting for approximately 325,000 new cases and 57,000 deaths worldwide in 2020 [1], with incidence projected to exceed 500,000 cases annually by 2040 [2]. Despite therapeutic advances, treatment remains limited by drug resistance and off-target toxicity. Cannabidiol (CBD) has shown promising anticancer potential [3,4]; however, its clinical use is hindered by poor physicochemical properties. Lipid nanoparticle-based delivery systems may overcome these limitations by enhancing CBD stability, cellular uptake, and tumor selectivity [5]. **Objective:** This study aims to evaluate the effect of CBD-loaded lipid nanoparticles (NP-CBD) on cell viability in metastatic melanoma (MeWo) and normal keratinocyte (HaCaT) cell lines, focusing on their ability to enhance cytotoxic efficacy and selectivity compared to free CBD. **Methods:** MeWo and HaCaT cells were exposed to increasing concentrations (0.05–100 µM) of free CBD or NP-CBD (mean diameter of 205.6 ± 3.4 nm) for 48 h. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. **Results:** Free CBD induced a concentration-dependent reduction in cell viability in both MeWo and HaCaT cells, with comparable IC_{50} values (4.6 µM and 3.9 µM, respectively), indicating limited selectivity toward tumor cells. In contrast, NP-CBD enhanced cytotoxic effects in MeWo cells (IC_{50} 3.2 *vs* 4.6 µM, $p = 0.0001$), while significantly reducing toxicity in HaCaT keratinocytes (IC_{50} 8.6 *vs* 3.9 µM, $p < 0.0001$). This resulted in a clear shift toward increased tumor cell sensitivity and a more favorable safety profile. **Conclusions:** Lipid nanoparticle encapsulation significantly improves the biological performance of CBD by enhancing its antitumor activity and selectivity. NP-CBD represents a promising nanotechnology-based strategy to optimize CBD delivery, supporting the development of more effective and less toxic therapeutic approaches for melanoma.

Keywords: melanoma; cannabidiol; lipid nanoparticles; cytotoxicity; anticancer therapy; nanotechnology

Acknowledgments/Funding: This research was funded by PT national funds through the UID/50006 project and FCT/MCTES through the SALIVA+ project (DOI 705 10.54499/2022.08978.PTDC).

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PC24

Cumulative Fluoride Exposure: Integrating Systematic Evidence with Analytical Determination in Tea-Based Beverages

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DOI: https://doi.org/10.48797/sl.2026.471

ABSTRACT

Background: Fluoride (F^-) plays an important role in dental health, promoting remineralization and inhibiting demineralization through the formation of acid-resistant fluorapatite. Although community water fluoridation has long been considered a major public health achievement, increasing scientific and ethical scrutiny has emerged concerning its systemic use.[1,2] With fluoride now derived from multiple sources, including dental products, drinking water, and beverages, assessment of total intake has become methodologically challenging. **Objective:** This study aimed to investigate the "halo effect" of water fluoridation by combining systematic review evidence with analytical determination of fluoride levels in commonly consumed beverages. **Methods:** A systematic literature review was conducted using PubMed, ScienceDirect, and PubChem to examine fluoride's physicochemical properties, pharmacokinetics, and health outcomes. Fluoride concentrations in teas, herbal infusions (n=14), and iced tea beverages (n=9), were quantified by potentiometry using a fluoride ion-selective electrode (ISE) after standard calibration and sample preparation procedures. **Results:** The literature evidence indicates a narrow therapeutic window for fluoride: optimal intake supports dental caries prevention, whereas chronic exposure above 0.1 mg/kg/day is linked to dental and skeletal fluorosis. Recent EFSA assessments (2024–2025) also report potential neurodevelopmental risks, including reduced IQ in children exposed to water fluoride concentrations above 1.5 mg/L.[3] Analytical results showed fluoride concentrations ranging from 0.1 to 0.5 mg/L in iced tea beverages and up to 1 mg/L in teas and herbal infusions, indicating that these products may represent a relevant source of dietary fluoride exposure. **Conclusion:** F^- exposure should be assessed cumulatively, as beverages, drinking water, and dental products collectively contribute to total intake, particularly in young children, pregnant women and individuals with clinical conditions. The observed variability in fluoride content across teas and infusions underscores the influence of environmental factors, plant origin, infusion preparation methods, and water quality. These findings highlight the need for more comprehensive exposure assessments to inform safe, evidence-based public health policies and recommendations.

Keywords: fluoride exposure; beverage analysis; health risk assessment; neurotoxicity

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB and through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). CESPU for the grant ORPasTeA_GI2-CESPU-2023, BioMPreg-GI2-CESPU-2025, ONCOCARE-GI2-CESPU-2025 and TRYP-MAP-GI2-CESPU-2025.

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PC25

Microplastics in Carolino Rice from Portugal

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DOI: https://doi.org/10.48797/sl.2026.472

ABSTRACT

Background: Food contaminants are substances unintentionally present in food that can threaten human health. Microplastics are an emerging and pervasive class of contaminants with a broad environmental distribution that can also contaminate foods [1]. Moreover, food processing and packaging can further contribute to microplastic contamination of food products [2]. Rice is among the most widely consumed foods globally. However, studies examining microplastics in rice remain scarce. **Objective:** The objective of this work was to assess microplastic contamination in a Portuguese-grown rice (Carolino variety), commercially available packaged either in paper or plastic. **Methods:** Triplicates of 1 kg of rice (Carolino variety) were acquired in Portuguese markets in 2023. Density separation was achieved by mixing about 25 g of rice with 75 mL of a saturated NaCl. After mixing for 1 min, the solution was left to rest for 24 h, followed by filtration of the supernatant (glass fiber filter of 1.2 µm pore, Whatman GF/C). Then, the filter was treated with 10 mL of 30% H_2O_2 (Labkem) to remove natural organic matter and with 0.01 mg/mL of Nile Red (Sigma-Aldrich), to stain microplastics. Fluorescent particles (i.e., suspected microplastics, MPs) were counted and photographed under fluorescent microscopy (Olympus BX43) and later measured in ImageJ. The best practices in contamination control were followed. The two procedural blanks showed low cross-contamination (0 – 1 MPs/filter). Data was recorded in Excel 365 and statistical analyses were performed in IBM SPSS Statistics (v19), with α=0.05. **Results:** The median concentration of microplastics in Carolino rice was 0.12 MPs/g. Most microplastics were fibers (median circularity of 0.043) with median dimensions of 518.2 µm. No statistically significant difference was observed between paper and plastic-packaged rice for these variables (p=0.845). **Conclusions:** Concentrations of microplastics in Caroline rice are similar to those reported in the literature, such as 0.30 MP/g in Indian rice [3]. Similar concentrations found in paper and plastic-packaged rice suggest contamination prior to packaging. An estimated annual intake of 1812 MP/person is expected for the Portuguese population, based on rice consumption (15.1 kg/person). Microplastics as external contaminants of Carolino rice do not seem to pose a grave risk to human health, considering concentrations and particle sizes.

Keywords: food safety; food contaminants; microplastics

Acknowledgments/Funding: This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. The work received financial support from FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the exploratory project 2023.11600.PEX (https://doi.org/10.54499/2023.11600.PEX) and by Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), project MICROEXPOS_GI2-CESPU-2023. Sara A. M. Silva acknowledges her PhD fellowship 2024.04118.BD from FCT/MCTES through national funds.

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PC26

Sustainable Fining Strategies in Port Wine: a One Health Perspective on Non-Animal Protein Alternatives

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DOI: https://doi.org/10.48797/sl.2026.473

ABSTRACT

Background: Fining is a key step in Port wine production, traditionally relying on animal-derived proteins to improve clarity and stability. However, concerns related to sustainability, allergenicity, and environmental impact have intensified the search for alternative approaches [1-3]. Within a One Health framework, the adoption of non-animal fining agents may contribute to more sustainable practices while addressing consumer health and environmental considerations. **Objective:** To evaluate the clarification efficiency and economic feasibility of non-animal protein-based fining agents applied to different Port wine styles. **Methods:** Eleven fining treatments were assessed, including ten alternative agents of plant, yeast, and fungal origin, with gelatine as a reference. Trials were conducted in White, Ruby, and Tawny Reserve Port wines, using three dosage levels within recommended ranges. Bentonite (45 g/hL) was applied to ensure protein stability. Clarification efficiency was evaluated through spectrophotometric measurement of colour intensity. **Results:** Liquid pea protein and yeast-derived protein demonstrated the highest reductions in colour intensity across the evaluated wines. However, these alternatives were associated with substantially higher costs compared to gelatine, with increases ranging from approximately four- to twenty-four-fold depending on wine style and dosage. **Conclusions:** Non-animal fining agents can achieve clarification performance comparable to conventional gelatine, supporting their potential as more sustainable and allergen-free alternatives. Nevertheless, despite higher costs, these alternatives may remain viable considering their technological performance and sustainability advantages. From a One Health perspective, these findings highlight the need to balance technological performance, economic feasibility, and sustainability in the transition towards more responsible wine production systems.

Keywords: One Health; Port wine; sustainable fining

Acknowledgments/Funding: This work received financial support from Sogrape Vinhos and the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 – Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos. Mariana Carvalho acknowledges her Innovhealth scholarship supported by the Young STEAM Impulse Programme of the Resilient P.PORTO Programme (02/C06-i03.03/2021.PID-15), co-funded by the Next Generation EU programme funds under the Recovery and Resilience Plan (PRR).

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PC27

Vegan Fining Strategies in Port Wine: Implications for Chemical Composition and One Health Sustainability

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DOI: https://doi.org/10.48797/sl.2026.475

ABSTRACT

Background: Wine fining is a critical step for stabilisation and clarification, traditionally relying on animal-derived agents such as gelatine. However, increasing regulatory, environmental and ethical concerns have driven the search for sustainable alternatives aligned with the One Health framework, integrating human, environmental and food system health [1-3]. **Objective:** This study aimed to evaluate the impact of vegan fining agents on the physicochemical, phenolic, volatile and elemental composition of different Port wine styles. **Methods:** Winery-scale fining trials were conducted using White, Tawny and Ruby Port wines. Two non-animal-based alternatives (pea protein and yeast-derived products) were compared with reference controls. Treatments were applied in triplicate (n=3) using 40 L stainless steel tanks. Wines were analysed according to official OIV methods, including basic physicochemical composition, colour and phenolic parameters, volatile compounds and elemental composition. Data were analysed by one-way ANOVA followed by Tukey's HSD post hoc test, with significance set at $p<0.05$. **Results:** Fining effects were strongly matrix-dependent. In White wines, colour intensity and total phenolics were significantly reduced by gelatine, while vegan agents showed intermediate behaviour. In Tawny wines, most parameters remained stable, although total anthocyanins decreased significantly with protein fining, particularly for gelatine. In Ruby wines, fining treatments significantly reduced colour intensity and total phenolic index, with gelatine showing the strongest effect, while vegan treatments induced moderate changes. Volatile composition was only marginally affected, with minor variations in higher alcohols and esters, indicating limited sensory impact. Elemental composition remained largely unchanged across treatments, and cadmium showed no variability. Overall, observed differences were often statistically significant but of low oenological magnitude. **Conclusions:** Vegan fining agents demonstrated comparable performance to gelatine in maintaining wine quality while reducing reliance on animal-derived products. From a One Health perspective, these findings support the adoption of non-animal-based fining strategies as a sustainable alternative, minimising environmental impact and aligning with evolving consumer and regulatory expectations without compromising wine stability or composition.

Keywords: One Health; Port wine; sustainable fining

Acknowledgements/Funding: This work received financial support from Sogrape Vinhos and the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 – Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos. Mariana Carvalho acknowledges her Innovhealth scholarship supported by the Young STEAM Impulse Programme of the Resilient P.PORTO Programme (02/C06-i03.03/2021.PID-15), co-funded by the Next Generation EU programme funds under the Recovery and Resilience Plan (PRR).

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PC28

Mutual Conditioning Between Nutrition and Oral Health in Odontogeriatrics: Cross-Sectional Observational Study

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DOI: https://doi.org/10.48797/sl.2026.476

ABSTRACT

Background: Oral health and nutrition exhibit a bidirectional relationship, particularly in older adults [1,2]. Oral cavity alterations may limit food intake, while dietary habits — especially frequency of consumption and sugar intake — directly affect caries prevalence and dental deterioration [2,3]. **Objective:** This study aims to explore the interaction between nutritional habits and oral health in institutionalized older adults, highlighting the mutual conditioning between these dimensions. **Methods:** A cross-sectional observational study was conducted with 67 older adults attending Day Care Centers in the Greater Porto region. Clinical indicators (Decayed, Missing, and Filled Teeth Index (DMF-T), number of decayed teeth), dietary habits (number of daily meals, fruit intake, consumption of sugary foods and beverages), and Oral Health–Related Quality of Life (OHRQoL) measures — including mastication difficulties, dietary satisfaction, and food limitations — were assessed. Body Mass Index (BMI) was also recorded. **Results:** Meal frequency analysis showed that 33% of participants consumed four meals/day, 20% three meals, 10% two meals, and 2% one or two meals. Mean daily intake was 2,12 servings of fruits/vegetables and 0,94 exposures to sugary foods [3,4,5]. Sugary beverages were consumed by 25,37% of participants, with 76,47% consumed outside main meals, and only 23,53% reported brushing afterward. Mean BMI was 26,8. Regarding OHRQoL, 29,85% reported very frequent difficulty chewing due to oral conditions, 19% occasional difficulty, and 34,32% occasionally limited the type or quantity of foods [4,5]. The mean DMF-T was 24,40, with an average of 2,36 decayed teeth per individual, reflecting the relationship between sugar intake and cumulative caries experience [1,2,4,5]. **Conclusions:** Findings demonstrate a bidirectional cycle: frequent sugar exposure combined with inadequate oral hygiene promotes caries and oral deterioration, reflected in high DMF-T and decayed teeth [1,2]. Conversely, oral problems constrain dietary choices, reducing fruit and vegetable consumption and potentially contributing to overweight and suboptimal nutritional status [3]. Self-reported OHRQoL measures indicate that oral health affects mastication, dietary satisfaction, and the diversity of food intake, reinforcing the reciprocal relationship. These results underscore the importance of integrated interventions addressing both diet and oral hygiene in geriatric populations [4,5].

Keywords: oral health; nutrition; geriatric dentistry

Acknowledgments/Funding: This work received no external funding. The authors gratefully acknowledge the support of Fernando Pessoa University and the DELEQOL Research Group: health.

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PC29

Distinct Urinary Metabolomic Profiles in Cigarette vs. Next-Generation Nicotine Users

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DOI: https://doi.org/10.48797/sl.2026.479

ABSTRACT

Background: Conventional cigarette smoking remains a leading cause of preventable morbidity and mortality, primarily due to systemic exposure to toxicants generated during combustion. Meanwhile, the rising use of electronic cigarettes (EC) and heated tobacco products (HTP) is reshaping nicotine use patterns; however, their biological effects differ from those of conventional cigarettes and remain incompletely characterized beyond targeted biomarkers. Urinary metabolomics provides a sensitive, integrative readout of host metabolism and xenobiotic processing, enabling detection of early biochemical perturbations and exposure signatures relevant to toxicological assessment and harm evaluation [1,2]. **Objective:** This study aimed to compare urinary metabolomic profiles among EC users, HTP users, conventional tobacco (CT) smokers, and non-smokers, to identify product-associated biochemical perturbations relevant to systemic toxicological effects. **Methods:** Urine samples from CT smokers, EC users, HTP users, and non-smokers (n=10 per group) were analyzed by gas chromatography-mass spectrometry (GC-MS) [3]. Metabolites were putatively identified using spectral library matching and comparison with standard compounds. Semi-quantitative data were assessed using multivariate and univariate statistical analyses. **Results:** A total of seventy-five urinary metabolites were consistently detected across all participant groups, including amino acids, organic acids, sugars, and other small polar compounds. While multivariate analyses showed no clear separation among the four groups, pairwise comparisons revealed significant metabolic differences. Compared with non-smokers, CT users showed increased levels of combustion-derived compounds (quinate, furoylglycine, guaiacol) and hippurate conjugates (2-hydroxyhippurate). EC users exhibited higher levels of amino acid metabolites (β-alanine and 3-methylhistidine) compared with both non-smokers and CT users, while energy-related intermediates were reduced (citrate) relative to CT users. HTP users showed elevated hippurate conjugates (2-hydroxyhippurate) compared with CT users, and lower levels of amino acid, carbohydrate, and nucleotide metabolites (3-methylhistidine, scyllo-inositol, 3-aminoisobutyrate, and uracil) compared with EC users. **Conclusions:** Urinary metabolomics identified product-specific metabolic signatures that distinguish CT from EC/HTP use, demonstrating its sensitivity as a tool for toxicological profiling of novel nicotine products.

Keywords: electronic cigarettes; heated tobacco products; urine; metabolomics

Acknowledgements/Funding: This work was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and the project 2024.13406.PEX (DOI identifier 10.54499/2024.13406.PEX). The Cooperativa de Ensino Superior Politécnico e Universitário (CESPU) is gratefully acknowledged for the PhD grant no. BD/DCB/CESPU/02/2023 to Vânia Monteiro.

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PC30

Endocrine Response to Intermittent Fasting: A Systematic Review of Hormonal Changes

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DOI: https://doi.org/10.48797/sl.2026.480

ABSTRACT

Background: Intermittent fasting encompasses alternating periods of food intake and dietary restriction [1]. This practice has gained prevalence, motivated by cultural, religious, and health objectives [1], yet endocrine adaptations across different protocols remain incompletely characterized [1,2]. The heterogeneity of fasting protocols (short-term, alternate-day, periodic, and time-restricted feeding [2]) contributes to variable endocrine responses [3]. **Objective:** This study aimed to systematically review endocrine responses to intermittent fasting by examining appetite-regulating hormones (leptin, ghrelin), metabolic hormones (insulin), gastrointestinal hormones (GLP-1, PYY, CCK), and thyroid axis hormones across different protocols and populations [2,3]. **Methods:** A systematic review of 9 peer-reviewed articles (2020–2025) was conducted to examine hormonal responses to intermittent fasting [4,5]. Studies analyzed leptin and ghrelin secretion [4], insulin sensitivity [6,7], gastrointestinal hormones, and thyroid hormone metabolism with emphasis on hypothalamic–pituitary–thyroid axis regulation [8]. **Results:** Ghrelin increased during fasting [4,5], while leptin decreased, reflecting reduced adipose energy status [4]. Leptin reduction was associated with decreased thyroid-stimulating hormone and triiodothyronine, promoting energy conservation [8]. Insulin responses remained inconclusive, dependent on protocol, duration, meal timing, and individual characteristics [6,7,9]. Gastrointestinal hormones showed modest, context-dependent changes, varying across populations [3,10,11]. Thyroid hormone activity decreased peripherally, while the central axis remained stable, representing coordinated adaptive responses [1,8]. **Conclusions:** Intermittent fasting induces coordinated endocrine changes [1]. Ghrelin elevation and leptin reduction are consistent markers [4,5], while insulin responses show variability [6,7,9]. Heterogeneity reflects differences in fasting duration, protocols, and populations [3]. Time-restricted eating shows promise when aligned with circadian rhythms [2]. Future research with standardized methodologies is essential [1].

Keywords: intermittent fasting; endocrine hormones; appetite regulation; metabolic adaptation; leptin; ghrelin

Acknowledgments/Funding: This research received no external funding.

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PC31

Long-Term Neurotoxic Effects of Mitoxantrone in Aged Mice: Modulation of Neuroinflammatory and Apoptotic Pathways

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DOI: https://doi.org/10.48797/sl.2026.482

ABSTRACT

Background: Chemotherapy-induced cognitive dysfunction ("chemobrain") is an increasingly relevant clinical issue among cancer survivors. The neurotoxic effects of mitoxantrone (MTX), particularly in the elderly, remain poorly understood. **Objective:** This study aimed to evaluate the long-term neurotoxic effects of a clinically relevant cumulative dose of MTX in aged mice. **Methods:** Male CD-1 mice (18–20 months) received MTX (4.5 mg/kg, cumulative dose) administered biweekly via intraperitoneal injection over three weeks. Two months after the final administration, brains were collected. Immunofluorescence was used to assess proteins associated with apoptosis, inflammation, and neuronal damage in the prefrontal cortex (PFC) and the hippocampal formation (HF). Glutathione-related compounds were analysed, and whole-brain metabolomics is ongoing. Statistical comparisons were performed using an unpaired t-test with Welch's correction. **Results:** MTX induced region-specific alterations in neurotoxic pathways. In the HF, p53 levels decreased in the hilus and increased in CA3, while Bax and Bcl-2 showed a decreasing trend. In the PFC, apoptosis-inducing factor (AIF) increased significantly. TNF-α levels were elevated in the PFC, with increasing trends in hippocampal regions. Hyperphosphorylated tau levels were significantly increased in both PFC and HF. Glutathiolomic analysis revealed a tendency toward decreased cysteinylglycine levels. **Conclusions:** A clinically relevant cumulative dose of MTX induces persistent and region-specific modulation of neuroinflammatory and apoptotic pathways in the aged brain, highlighting increased susceptibility to MTX-induced neurotoxicity.

Keywords: mitoxantrone; neurotoxicity; ageing

Acknowledgements/Funding: This work is financed by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO) and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB and through the project EXPL/MEDFAR/0203/2021. A. Dias-Carvalho acknowledges FCT and UCIBIO for her PhD grant (UI/BD/151318/2021). This work was carried out within the scope of the project "CD-CORE: Cancer Diagnosis, Therapies and Lifestyle – Strategies to Overcome Chemotherapy Resistance and Long-Term Adverse Effects" (Operation No. NORTE2030-FEDER-02707400), co-funded by the European Regional Development Fund (ERDF) through the Northern Regional Programme 2021–2027 [NORTE2030]. The authors declare no conflicts of interest.

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PC32

Ketamine and the Glutamatergic System: A Systematic Review of NMDA Receptor Modulation

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DOI: https://doi.org/10.48797/sl.2026.484

ABSTRACT

Background: Ketamine was first synthesized in 1962 by Calvin Stevens as an alternative to phencyclidine. Its effects stem primarily from glutamatergic modulation, particularly from the antagonism of *N*-methyl-*D*-aspartate receptors (NMDAR) [1]. **Objective:** This study aims to systematically review the pharmacodynamics of ketamine, with particular attention to its effects on the glutamatergic system. **Methods:** The bibliographic search was conducted using books and scientific databases (PubMed and ScienceDirect), using the following terms: "ketamine", "NMDA receptors", "ketamine pharmacology". Only articles published in English from 2000 to 2025 were considered. A total of 7 articles were included. **Results:** Both enantiomers of ketamine, (*S*)-ketamine and (*R*)-ketamine, are noncompetitive antagonists of NMDARs; however, (S)-ketamine has an affinity/potency for NMDARs that is approximately four times greater [2]. In Xenopus oocytes expressing recombinant NMDAR GluN2A–D subunits, in the absence of Mg^{2+}, ketamine shows a higher affinity for NMDARs-GluN2B subtype [3]. However, the affinity depends not only on the subunits that constitute the receptor but also on Mg^{2+} levels and affinity for the receptor [4]. *D*-serine acts as a coagonist of ketamine by binding to the $glycine_B$ site of NMDARs. In PC-12 cells, ketamine influences intra- and extraneuronal levels of *D*-serine: (*S*)-ketamine increases intraneuronal levels and reduces extraneuronal levels, while (*R*)-ketamine decreases both. Furthermore, studies in rats have demonstrated that ketamine modulates the transcription of the gene encoding for serine racemase (*Srr*), increasing *Srr* mRNA levels in the striatum, hippocampus and cortex, but decreasing them in the forebrain [5]. **Conclusions:** The glutamatergic system plays a major role in short-term memory retention and consolidation, as well as in cognition [6]. Interference with this system leads to decreased memory retention, cognitive impairment, and dissociation [1]. Ketamine exerts both direct and indirect effects on the glutamatergic system, which explains the characteristic effects of its use. These effects underlie its use in recreational contexts [1] and as a facilitator drug in sexual assaults [7].

Keywords: ketamine; *N*-methyl-*D*-aspartate receptors; glutamatergic system; psychoactive effects

Acknowledgements/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "DeTTeCaK-GI2-CESPU-2025".

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PC33

Oral Health and Systemic Diseases in Older Adults: Impact on Quality of Life – A Study in Porto

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DOI: https://doi.org/10.48797/sl.2026.486

ABSTRACT

Background: Population ageing is associated with an increased prevalence of chronic diseases and oral conditions, such as caries, edentulism and periodontal disease. Oral health (OH)is an integral component of general health and directly influences quality of life (QoL), affecting dimensions such as mastication, speech, body image, and social interaction. In institutionalised older adults (OA), factors such as polypharmacy, low health literacy and difficulties in accessing care further aggravate these conditions [1,2]. **Objective:** To assess the most frequent pathologies in OA and analyse their relationship with OH and their impact on QoL. **Methods:** An observational, cross-sectional and descriptive study was conducted with 67 OA (65-98years). Data were collected through sociodemographic questionnaires, medical history, assessment of oral hygiene habits, O'Leary Plaque Index, OHIP-14, and GOHAI [3]. Descriptive and inferential statistical analyses were performed, with a significance level set at $p<0.05$. **Results:** Most participants presented systemic pathologies (62.7%) and were taking regular medication. The most frequent conditions included hypertension, diabetes, cardiovascular diseases, and respiratory disease. These conditions are often associated with changes in OH, such as increased dental plaque accumulation, xerostomia, and a higher prevalence of periodontal disease [4]. Individuals with chronic diseases showed poorer oral hygiene indicators, with a high plaque index (65%), suggesting a relationship between multimorbidity and reduced self-care capacity. Polypharmacy, which was common in this population, contributed to salivary alterations, favouring the development of dental caries and oral discomfort. The QoL questionnaires revealed a significant negative impact, including limitations in mastication, pain, psychological discomfort and difficulties in daily activities. OA with associated pathologies showed worse QoL scores on the GOHAI, reinforcing the relationship between general health, OH and QoL [3,4]. **Conclusions:** Common systemic pathologies in OA are closely related to OH, potentiating a negative impact on QoL [1,4]. The presence of chronic diseases, combined with inadequate oral hygiene habits and barriers to accessing care, contributes to the worsening of oral conditions. It is therefore essential to implement multidisciplinary strategies focused on prevention, OH education, and the integration of medical and dental care in geriatric institutions [2]. The promotion of OH is fundamental for active and healthy ageing and for improving QoL.

Keywords: oral health; older adults; quality of life; systemic pathologies

Acknowledgments/Funding: This work received no external funding. The authors gratefully acknowledge the support of Universidade Fernando Pessoa and the DELEQOL: Saúde Group.

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PC34

Antitumor and Antimitotic Activity of Aminochalcones in Human Cancer Cells with Differential P-Glycoprotein Expression

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DOI: https://doi.org/10.48797/sl.2026.488

ABSTRACT

Background: Cancer remains a leading cause of mortality, with drug resistance, particularly mediated by P-glycoprotein (P-gp), limiting therapeutic efficacy [1]. Chalcones have emerged as promising anticancer agents due to their ability to inhibit tubulin polymerization and disrupt microtubule dynamics [2]. In this context, dual-targeting strategies combining antimitotic activity with P-gp inhibition, including chalcone-based derivatives, have recently been reported as a means to overcome multidrug resistance [3]. **Objective:** This study aimed to evaluate the antitumor and antimitotic activity of eight aminochalcone derivatives in human cancer cell lines of diverse origins: melanoma (A375-C5), breast adenocarcinoma (MCF-7), non-small cell lung carcinoma (NCI-H460), ovarian carcinoma with dual resistance to paclitaxel and carboplatin (OVCAR8 PTX/CBP-R), and colon adenocarcinoma (HCT-15), each exhibiting different levels of P-glycoprotein expression, with HCT-15 as a well-established model for multidrug resistance studies. **Methods:** The antitumor activity of all compounds was assessed in five cancer cell lines using the sulforhodamine B assay to determine the GI_{50} values at 48 h (defined as the concentration required to inhibit 50% of cell growth), with doxorubicin as a control. Selectivity indices were calculated using the non-cancerous breast MCF10A cell line. Antimitotic activity was evaluated by phase-contrast microscopy and DAPI staining. **Results:** Seven of the eight compounds demonstrated potent cytotoxic activity across all tested cancer cell lines. The strongest selectivity was observed in HCT-15 cells, which highly express P-gp. All compounds exhibited antimitotic activity, although the extent varied among derivatives. **Conclusions:** These findings support the potential of aminochalcone derivatives as antimitotic and anticancer agents, particularly for targeting P-gp-mediated drug-resistant cancer cells.

Keywords: antitumor activity; antimitotic potential; aminochalcones; human cancer cell lines; Pgp-resistant cells

Acknowledgments/Funding: This research was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020). This research was also funded by the CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl, under the grants "FlavScreen_GI2-CESPU-2023", "FlavCanAct-GI2-CESPU-2025", and "TargetMito-GI2-CESPU-2025".

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PC35

Chronic Cortisol Elevation Impairs Insulin Sensitivity and Glucagon-Mediated Fasting Glycaemia

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DOI: https://doi.org/10.48797/sl.2026.490

ABSTRACT

Background: Chronic cortisol elevation, from prolonged stress, Cushing's syndrome, or therapeutic glucocorticoids, disrupts glucose homeostasis by impairing insulin signalling and enhancing hepatic glucose production, yet integrated hormonal mechanisms remain incompletely characterized [1,2]. **Objective:** This work aims to systematically review how chronic hypercortisolism affects: (I) peripheral insulin sensitivity; (II) compensatory glucagon secretion and hepatic sensitivity; and (III) fasting glycaemia maintenance in adult populations [2,3]. **Methods:** Systematic search (MEDLINE, Scopus, 2014–2025) of human studies reporting chronic cortisol/glucocorticoid exposure (endogenous or iatrogenic) and fasting metabolic outcomes. Inclusion criteria: adults (≥18 years) with measurements of fasting glycaemia, hepatic glucose production, insulin sensitivity, glucagon levels, or counterregulatory responses. Exclusion criteria: paediatric studies, pregnancy/lactation, animal/*in vitro* models, or studies lacking fasting measures or cortisol/glucagon data [4]. Thirteen peer-reviewed articles were synthesized. **Results:** Chronic excess of cortisol consistently impairs peripheral insulin sensitivity through post-receptor signalling defects and enhanced lipolysis, reducing insulin-dependent glucose uptake by skeletal muscle and adipose tissue [1,4]. Simultaneously, cortisol attenuates insulin's suppression of glucagon and increases hepatic glucagon receptor sensitivity, amplifying hepatic glucose production and gluconeogenesis [3]. These dual defects, diminished insulin efficacy combined with exaggerated glucagon actions, sustain fasting hyperglycaemia [2]. Central neural dysregulation and disrupted circadian feedback loops further destabilize basal glucose control, particularly in populations with obesity or prediabetes [3,5]. **Conclusions:** Chronic hypercortisolism triggers coordinated insulin–glucagon axis imbalance, impairing fasting glycaemia maintenance through combined peripheral resistance and enhanced hepatic glucose output [1]. Targeting hypothalamic–pituitary–adrenal (HPA) axis modulation and stress reduction may prevent glucocorticoid-related dysglycemia [2]. Standardized prospective cohort studies with detailed biomarker kinetics and longitudinal follow-up are essential to establish glucocorticoid-induced prediabetes risk profiles and evidence-based intervention strategies [4,5].

Keywords: cortisol; insulin resistance; glucagon; fasting glycaemia; glucocorticoids

Acknowledgments/Funding: This research received no external funding.

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PC36

Impact of Psilocybin and *Psilocybe cubensis* Extract on Gut Microbiota in Wistar Han Rats

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DOI: https://doi.org/10.48797/sl.2026.492

ABSTRACT

Background: Psilocybin, the main psychoactive compound found in *Psilocybe* mushrooms, has gained increasing attention due to its potential therapeutic effects in neuropsychiatric disorders [1]. Beyond its central effects, increasing evidence highlights the relevance of the gut–brain axis, suggesting that psychedelics may also influence intestinal microbiota composition. Whole mushroom extracts contain additional bioactive compounds that may modulate these effects, yet comparative preclinical data between pure psilocybin and mushroom extracts remain limited [2,3]. **Objective:** To evaluate the impact of pure psilocybin and *Psilocybe cubensis* extract on gut microbiota *in vivo*. **Methods:** Eighteen male Wistar Han rats (250–275 g; 8–9 weeks old) were randomly assigned to three groups (n=6): control (0.9% NaCl), psilocybin (3 mg·kg^{-1}), and *P. cubensis* extract (equivalent to 3 mg·kg^{-1} psilocybin/psilocin). Treatments were administered by oral gavage (0.5 mL·kg^{-1}). Fecal samples were collected at baseline (T1) and at days 7 (T7) and 14 (T14) post-exposure for microbiota analysis. Microbial profiling was performed using long-read amplicon sequencing targeting the full-length 16S rRNA gene. Libraries were prepared using SMRTbell technology and sequenced on the PacBio platform. Bioinformatic analysis enabled high-resolution taxonomic assignment and reconstruction of microbial community structure, improving species-level identification accuracy. Statistical analysis included ANOVA and multivariate analysis of beta-diversity (p<0.05). All procedures were approved by the institutional Animal Welfare Committee and DGAV, in accordance with European and national legislation. **Results:** Baseline microbiota composition (T1) was similar across all groups, clustering closely together, as expected prior to treatment. This profile remained comparable to the control group at T7 and T14. In contrast, distinct shifts in microbial community structure were observed in treated groups. Both psilocybin and *P. cubensis* extract induced separation from baseline and control profiles at T7, with further divergence at T14. This effect was more pronounced in the psilocybin group, which exhibited the greatest distance in cluster analysis, indicating a stronger impact on microbiota composition. **Conclusions:** These findings suggest a time-dependent modulation of gut microbiota induced by both treatments, with differential magnitude between the pure compound and the whole extract.

Keywords: psilocybin; gut microbiota; psychedelics

Acknowledgments/Funding: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project 2024.06933.RESTART. The 1H-TOXRUN and the projects PsiloTox_GI2-CESPU-2023; UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos; UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO, LA/P/0140/2020 and the PhD grant 2021.04999.BD are also acknowledged.

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PC37

Psilocybin and *Psilocybe cubensis* Extract Exhibit Divergent Behavioural and Toxicological Effects in Rats

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DOI: https://doi.org/10.48797/sl.2026.497

ABSTRACT

Background: Psilocybin, a key psychoactive compound found in *Psilocybe* mushrooms, has gained increasing attention due to its therapeutic potential in neuropsychiatric disorders [1]. However, comparative preclinical data between isolated psilocybin and whole mushroom extracts remain scarce, particularly regarding behavioural reinforcement and peripheral toxicity [2,3]. **Objective:** To compare the behavioural and toxicological effects of pure psilocybin and *Psilocybe cubensis* extract in Wistar Han rats. **Methods:** Male Wistar Han rats (n=18) were randomly assigned to control (0.9% NaCl), pure psilocybin (3 mg.kg^{-1}), or *P. cubensis* extract (equivalent to 3 mg·kg^{-1} psilocybin/psilocin) groups. Treatments were administered orally. Behavioural effects were assessed using a conditioned place preference (CPP) paradigm, with evaluations at 1, 7, and 14 days post-treatment. Locomotor/exploratory activity was estimated by compartment entries. Peripheral effects were evaluated through relative organ weights and lipid peroxidation (TBARS assay) in the heart, brain, liver, and kidney. Statistical analysis was performed using ANOVA ($p<0.05$). All procedures were approved by the institutional Animal Welfare Committee and DGAV, in accordance with European and national legislation. **Results:** Pure psilocybin significantly decreased CPP scores at days 1 and 7, suggesting aversive or non-reinforcing effects, while no significant preference changes were observed for the extract. The extract group showed a transient increase in exploratory behaviour at day 7, whereas psilocybin-treated animals consistently displayed reduced entries in the drug-paired compartment. At the peripheral level, psilocybin increased relative liver weight, indicating potential hepatic stress or metabolic adaptation. In contrast, the extract reduced renal lipid peroxidation, suggesting a protective or antioxidant effect likely associated with additional bioactive compounds. **Conclusions:** Pure psilocybin and *P. cubensis* extract exhibit distinct behavioural and toxicological profiles. These findings highlight the relevance of matrix effects in psychedelic research and reinforce the need to consider whole-extract formulations when assessing safety and pharmacological outcomes.

Keywords: Psilocybin; *Psilocybe cubensis*; behavioural toxicology

Acknowledgments/Funding: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project 2024.06933.RESTART. The 1H-TOXRUN and the projects PsiloTox_GI2-CESPU-2023; UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos; UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO, LA/P/0140/2020 and the PhD grant 2021.04999.BD are also acknowledged.

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PC38

Histopathological and Inflammatory Changes in Oral Mucosa Associated with Tobacco Exposure: Preliminary Findings from an Ongoing Comparative Study

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DOI: https://doi.org/10.48797/sl.2026.499

ABSTRACT

Background: Electronic cigarettes have been promoted as a safer alternative to conventional tobacco smoking. However, increasing evidence suggests that their aerosols contain nicotine and other potentially harmful substances that may induce oxidative stress, inflammation, and cellular damage in oral tissues [1]. Experimental studies have shown adverse effects on oral epithelial and connective tissue cells, including reduced cell viability, impaired wound healing, and dysregulated inflammatory responses [2]. In addition, e-cigarette exposure has been associated with tissue damage and molecular alterations potentially linked to carcinogenesis in the oral mucosa [3]. Nevertheless, histopathological and inflammatory evidence from human oral mucosal samples remains limited. **Objective:** To investigate and compare the histopathological and inflammatory profiles of oral mucosa samples obtained from individuals with different tobacco exposure backgrounds. **Methods:** An ongoing comparative cross-sectional study is being conducted across four groups: conventional tobacco smokers, e-cigarette users, non-smokers, and former smokers. Oral mucosa samples are being collected during routine dental implant procedures, in accordance with ethical approval and standardized protocols. A minimum of 10 participants per group is planned. Histopathological evaluation and immunohistochemical analysis are being performed to characterize tissue alterations and inflammatory marker expression, including CD3, CD20, CD163, MPO, and IL-6. **Results:** Preliminary qualitative immunohistochemical analysis revealed a trend toward a more prominent inflammatory profile in conventional tobacco smokers, characterized by more frequent expression of CD163, MPO, and IL-6. Control samples were predominantly negative for CD163 and MPO, with only occasional positivity for CD20 and IL-6, whereas former smokers exhibited generally low immunoreactivity. The single e-cigarette sample showed mild positivity for all evaluated markers; however, interpretation of this finding remains limited by the small sample size. **Conclusions:** Preliminary findings suggest that conventional tobacco exposure may be associated with a more prominent inflammatory profile in the oral mucosa. Given the limited number of e-cigarette samples available to date, further studies in larger and more balanced cohorts are needed to better characterize the histopathological and immunoinflammatory effects of different tobacco exposure patterns.

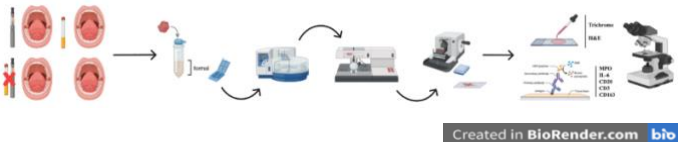

Figure 1. Workflow of histochemical and immunohistochemical procedures and optical microscopy analysis.

Keywords: smoking; electronic cigarettes; inflammation; buccal mucosa; immunohistochemistry

Acknowledgments/Funding: This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU) under grant number "VOMICS-GI2-CESPU-2025".

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PC39

Ultrastructural and Phylogenetic Study of Microsporidia Parasites Infecting the Trunk Muscle of Two Marine Fishes from the East Atlantic Ocean Water

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DOI: https://doi.org/10.48797/sl.2026.500

ABSTRACT

Background: Microsporidia are small, obligate intracellular parasites with unique cellular and molecular features infecting both invertebrates and vertebrates worldwide. Common in fish and crustaceans, they make sanitary control crucial for public health and the aquaculture industry. Nevertheless, studies focusing on aquatic organisms from Portuguese fauna remain limited [1,2]. **Objective:** This study aimed to expand current knowledge on the diversity of microsporidians infecting commercially important teleost fish species captured along the northern Atlantic coast of Portugal. **Methods:** Specimens of the three-bearded rockling *Gaidropsarus vulgaris* and pout *Tripsopterus luscus* were necropsied and infected tissue was photographed using differential interference contrast microscopy for morphological characterization. Samples were processed for histology, transmission electron microscopy and molecular analyses targeting the 18S and 28S ribosomal ribonucleic acid (rRNA) genes, including the internal transcribed spacer region. Positive polymerase chain reaction products were cloned, sequenced, and analyzed using BLAST in MEGA11 software. Phylogenetic relationships were inferred using Maximum Likelihood and Bayesian Inference methods [3]. **Results:** In both infections, no xenoma formation was observed. Instead, a generalized degradation of trunk muscle myofibrils was evident. The infections were predominantly characterized by late sporogonic stages developing in direct contact with the host cell cytoplasm. The two *Microsporidium* species identified could be distinguished based on spore size and shape, and ultrastructural characteristics, particularly the number of polar tube coils and the patterning of the spore surface. Molecular and phylogenetic analyses revealed that the obtained ribosomal deoxyribonucleic acid (rDNA) sequences showed strong affinity with members of the genera *Microgemma*, *Spraguea*, and *Tetramicra*. **Conclusions:** The obtained rDNA sequences cluster within the Marinosporidia clade, grouping with *Tetramicra brevifilum* (AF364303) and *Microgemma caulleryi* (AY033054). The ultrastructural characteristics observed during the late sporogonic stages, together with tissue tropism, genetic distances among related parasites, and phylogenetic data, indicate that the two *Microsporidium* spp. described herein are closely related. These organisms exhibit a high sequence identity, with 99.7% similarity between them and 99.4% similarity to *Tetramicra brevifilum*.

Keywords: marine fishes; parasites; microsporidia

Acknowledgments/Funding: This research was funded by National funds through FCT, project - NORTE2030-FEDER-01799200: ATLANTIDA II.

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PC40

Application of the Buccal Micronucleus Cytome Assay for Genotoxicity Detection in Dogs

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DOI: https://doi.org/10.48797/sl.2026.501

ABSTRACT

Background: In Europe, concern for animal welfare has been steadily increasing, covering both ethical considerations and health aspects [1]. Consequently, veterinary research has increasingly prioritized the search for biomarkers that can anticipate the development of serious diseases. Among the available approaches, the Buccal Micronucleus Cytome (BMCyt) assay stands out as a low-impact method, relying on cells collected from the buccal mucosa to assess genomic damage and chromosomal alterations. [2]. Higher occurrence of cellular irregularities, including the presence of micronuclei, has been closely linked to an elevated likelihood of developing conditions such as cancer, neurodegenerative diseases, and premature ageing, often linked to exposure to genotoxic and cytotoxic agents. While this method has been well established in human studies, research involving animals remains limited [3]. **Objective:** This work aims to outline an improved method for obtaining exfoliated buccal cells from dogs, while also examining a marker of genomic instability through both light and fluorescence microscopy techniques. **Methods:** Specimens were obtained from six female dogs housed in breeding facilities, including gestating females, with the purpose of evaluating chromosomal instability. Using methodologies adapted from human studies, key nuclear alterations were successfully detected and measured. **Results:** The results showed a greater proportion of micronuclei relative to findings reported in earlier studies. Methodological aspects, including the reduction of artefacts and the need for adequate personnel training, are essential for reliable analysis. **Conclusions:** This study confirmed the reliability of the BMCyt approach for collecting and analyzing canine samples, while also enhancing insight into the role of micronuclei as indicators of early pathological changes in this species.

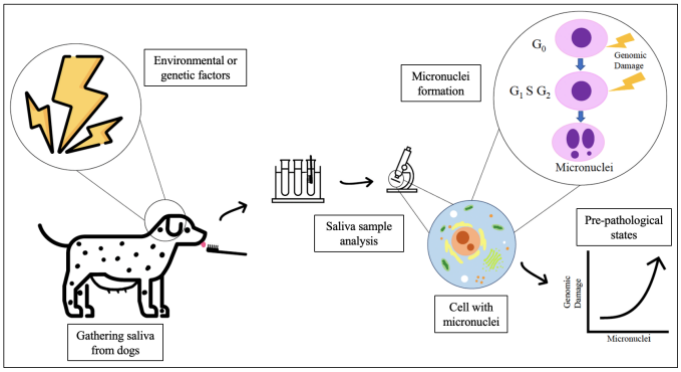

Figure 1. Key aspects involved in the collection and analysis of micronuclei as biomarkers of stress-related genotoxicity.

Keywords: genomic damage; exfoliated buccal cells; saliva; biomarker; animal welfare

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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PC41

Microbial Contamination in Saturated Saline Solution Used for Cadaver Preservation in Veterinary Anatomy Teaching

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DOI: https://doi.org/10.48797/sl.2026.502

ABSTRACT

Background: The use of animal cadavers is essential for veterinary anatomy education, requiring preservation methods that maintain tissue characteristics similar to those of living organisms. At CESPU, cadavers are preserved using a saturated saline (SS) solution, considered effective in maintaining tissue texture, color, and joint mobility [1,2]. However, its ability to control microbial growth, particularly for halotolerant strains, remains poorly studied [3]. Common bacterial species associated with animal cadavers include *Staphylococcus aureus*, *Bacillus* spp., *Enterococcus* spp., and *Escherichia coli* [4]. **Objectives:** This study aimed to detect fecal contamination indicators, namely *Enterococcus* spp. and *E. coli*, in the SS solution used for preserving animal cadavers in veterinary anatomy classes at CESPU. **Methods:** A total of 42 SS samples were collected from 7 cadavers (3 cats and 4 dogs), including samples obtained before immersion and after 7, 14, and 21 days of preservation. The SS solution was renewed weekly, with each cadaver immersed for 7 days before replacement. Samples were inoculated onto selective media: Slanetz-Bartley agar and Kanamycin Esculin Azide agar for *Enterococcus* spp., and MacConkey agar and Chromogenic Coliform agar for *E. coli*. Up to two typical colonies per sample were subcultured on brain heart infusion agar and identified using MALDI-TOF mass spectrometry. **Results:** Among the 21 SS samples collected before cadaver immersion, 1 showed bacterial growth, yielding two isolates: *Enterococcus hirae* and *E. coli*. Of the 21 samples collected after immersion, 15 showed microbial growth. Identified isolates included *Enterococcus faecalis* (n=2), *Enterococcus faecium* (n=3), *Enterococcus hirae* (n=1), *Enterococcus raffinosus* (n=1), and *Enterococcus* spp. (n=7). Enterococci were detected in samples collected at 7 (n=5), 14 (n=4), and 21 days (n=5). Additionally, two *E. coli* isolates were recovered from a single sample collected at 14 days. **Conclusion:** These findings indicate that the SS solution does not fully inhibit microbial growth during cadaver preservation. The detection of *Enterococcus* spp. and *E. coli*, particularly after immersion, suggests possible fecal contamination and highlights the need for regular microbiological monitoring and enhanced biosafety measures to reduce occupational exposure and ensure a safer learning environment.

Keywords: veterinary microbiology; biosafety; cadaver preservation

Acknowledgments/Funding: This work received financial support from the projects BioSafeVet-GI2-CESPU-2025 - BioSafeVet - Microbial Risk Assessment of Saline-Preserved Cadavers to Promote Safer Veterinary Anatomy Teaching by the CESPU-Cooperativa de Ensino Superior Politécnico e Universitário, the 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU), 16024 - COMPETE2030-FEDER-00716600 - NEUROBIOTECH - Combining New Cell-based Therapies and Biodegradable Polymers to Promote Peripheral Nerve Regeneration, and from the national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025, UIDB/50006/2020 DOI

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PC42

Influence of Microplastic Exposure on Nutrient Uptake and Growth Performance of *Chlorella vulgaris*

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DOI: https://doi.org/10.48797/sl.2026.503

ABSTRACT

Background: Microplastics (MPs) are persistent emerging contaminants frequently detected in wastewater (WW), where conventional treatment systems often fail to remove them, posing environmental and Human health risks [1]. Microalgae-based systems have emerged as sustainable alternatives for WW remediation due to their capacity for contaminants and nutrient removal [2]. However, the effects of MPs on microalgal performance and treatment efficiency under different WW operational conditions remain poorly understood. **Objective:** This study evaluated the physiological responses and bioremediation performance of *Chlorella vulgaris* exposed to different types of MPs under varying WW conditions. **Methods:** *C. vulgaris* was exposed to 100 mg/L [3] of five commonly detected MPs: polypropylene (PP), polystyrene (PS), polyamide (PA), low-density polyethylene (LDPE), and high-density polyethylene (HDPE). Experiments were conducted under different WW conditions: variations in nitrogen (N) availability, organic carbon concentration, and photoperiod regimes (12:12 h light/dark cycle versus continuous light). Microalgal growth, metabolic activity, and bioremediation efficiency were assessed. **Results:** MPs induced heterogeneous metabolic responses depending on the MPs' type and environmental conditions. HDPE and LDPE consistently reduced esterase activity, whereas PS increased esterase activity under N-limited conditions. LDPE also induced intracellular oxidative stress specifically under N limitation. Despite these effects, *C. vulgaris* maintained growth and biomass production in most scenarios. Growth inhibition (13-27%) occurred only under combined nutrient starvation and a 12:12 h photoperiod. Heterotrophic metabolism partially compensated for reduced photosynthetic activity during dark phases. Under N-limited conditions, *C. vulgaris* achieved high bioremediation efficiency, removing up to 94 % of N and >97.5 % of glucose even in the presence of MPs. In contrast, limited organic carbon impaired nutrient removal due to energy constraints. **Conclusions:** Overall, WW conditions strongly modulated the physiological stress induced by MPs. Nutrient limitation and light/dark cycles intensified metabolic disturbances, whereas N-limited environments promoted adaptive responses that supported microalgal resilience. *C. vulgaris* maintained high bioremediation capacity in most conditions, highlighting its potential as a robust and eco-friendly tool for polishing MP-contaminated wastewater.

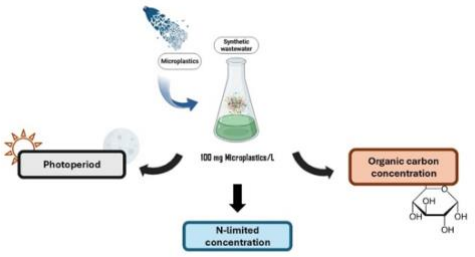

Figure 1. Wastewater bioremediation performance by exposing *Chlorella vulgaris* to different types of MPs, at 100 mg/L, under varying wastewater operational conditions.

Keywords: microalgae based-systems; microplastics; wastewater bioremediation

Acknowledgments/Funding: This work was supported by the national funds through FCT/MECI: LEPABE, UID/00511/2025 (https://doi.org/10.54499/UID/00511/2025) and UID/PRR/00511/2025 (https://doi.org/10.54499/UID/PRR/00511/2025), FCT/MCTES (PIDDAC); and ALiCE, LA/P/0045/2020 (https://doi.org/10.54499/LA/P/0045/2020); the grant attributed by Portuguese Foundation for Science and Technology, I.P. – FCT – to Paulo Sousa by project reference 2022.14159.BD and DOI:10.54499/2022.14159.BD.

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PC43

Crossing Cellular Boundaries: Functionalized Nanoplastics and their Impact on Human Neuroblastoma Cells

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DOI: https://doi.org/10.48797/sl.2026.504

ABSTRACT

Background: The detection of nanoplastics (NPs) in food, water, and air has intensified global concern regarding their environmental and health impacts [1,2]. Human exposure occurs unintentionally through ingestion, inhalation, or skin contact, raising questions about their interactions with biological systems, especially the nervous system [3–5]. Although accumulating data confirms the biological activity of NPs, the pathways underlying their neurotoxic potential — especially those shaped by surface functionalization — are still largely unresolved. **Objective:** This study investigates the neurotoxic effects of four polystyrene nanoplastics (PS-NPs): unmodified 50 and 100 nm PS-NPs and 100 nm amine- and carboxyl-functionalized PS-NPs, using the human SH-SY5Y neuronal cell line. **Methods:** SH-SY5Y cells were incubated with varying NP concentrations (1–500 µg/mL) for 24 or 48 hours. Before initiating cytotoxicity assays, the physicochemical features and medium stability of the particles were verified. The analysis focused on metabolic activity, ROS/RNS generation, nanoparticle uptake, and cellular or subcellular structural alterations. **Results:** Functionalized PS-NPs, notably amine-modified particles, induced higher toxicity than non-functionalized ones. Cell viability declined in a concentration- and time-dependent manner, with significant reductions observed at 200–500 µg/mL. Elevated ROS/RNS levels occurred for plain 100 nm and amine-functionalized NPs, with oxidative stress intensifying over time. Electron microscopy revealed marked subcellular damage — endoplasmic reticulum dilation, mitochondrial impairment, and Golgi disorganization — correlated with NP size, concentration, and surface chemistry. Surface-modified NPs exhibited enhanced internalization efficiency, with amine-functionalized variants demonstrating the highest accumulation within neuronal cells. Mechanistic analyses indicated activation of apoptosis, autophagy, and lysosomal dysfunction, strongest in cells exposed to functionalized PS-NPs. **Conclusions:** NP surface functionalization critically influences neurotoxicity, raising significant concerns about the long-term impact of NP exposure and its potential involvement in neurodegenerative disease processes.

Keywords: polystyrene nanoplastics; SH-SY5Y cell line; neurotoxicity; surface functionalization

Acknowledgments/Funding: This research was funded by PT national funds through the UID/50006 project and FCT/MCTES through the SALIVA+ project (DOI 705 10.54499/2022.08978.PTDC).

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PC44

Behavioral Risk Factors and Health: Assessing Alcohol and Tobacco Use in a Diabetic Population

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DOI: https://doi.org/10.48797/sl.2026.477

ABSTRACT

Background: Diabetes mellitus (DM) represents a major public health challenge worldwide and in Portugal, where its prevalence continues to increase. Alcohol and tobacco consumption are recognised behavioural risk factors that may exacerbate both microvascular and macrovascular complications associated with diabetes. Understanding the patterns of these behaviours in local diabetic populations is essential for developing targeted prevention and health promotion strategies. **Objective:** To assess the levels and patterns of alcohol and tobacco consumption among adults with diabetes residing in Ponte de Lima and to explore potential health implications associated with these behaviours. **Materials and Methods:** A descriptive cross-sectional exploratory study was conducted with 30 adults diagnosed with diabetes mellitus, recruited through non-probabilistic snowball sampling. Data were collected using three instruments: a sociodemographic questionnaire, the Alcohol Use Disorders Identification Test (AUDIT), and the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), both developed and validated by the World Health Organization. Descriptive statistical analysis was performed to characterise the sample and consumption patterns. **Results:** Participants were predominantly female (70%) with a mean age of 54.7 years. Most participants had Type 2 diabetes (73.3%) and were treated primarily with oral antidiabetic medication (86.7%). Alcohol consumption was generally low, with 33.3% reporting consumption once a month or less and 93.3% reporting one to two drinks on a typical drinking occasion. AUDIT results indicated no evidence of hazardous or harmful alcohol use. Regarding tobacco use, 66.7% of participants reported never having smoked, and 80% reported no tobacco consumption in the previous three months. ASSIST scores suggested minimal risk, with no significant reported health, social, or financial consequences related to substance use. **Conclusions:** Alcohol and tobacco consumption in this sample of adults with diabetes was generally low and largely non-problematic. Although limited by the small non-probabilistic sample, the findings suggest a potentially favourable behavioural profile within this community. Continued health education and preventive strategies remain important for supporting metabolic control and reducing the risk of diabetes-related complications.

Keywords: diabetes mellitus; alcohol consumption; tobacco use; health behaviour

Acknowledgments/Funding: This research received no external funding.

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PC45

A Sustainable Approach to Analyzing Neutral Cannabinoids Using HPLC-DAD

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DOI: https://doi.org/10.48797/sl.2026.505

ABSTRACT

Background: Gałuszka *et al.* [1] proposed 12 principles of green analytical chemistry that underscore the importance of minimizing environmental impact and enhancing analytical efficiency in scientific practices, all the while safeguarding operator safety. Researchers are encouraged to utilize HPLC techniques that facilitate quicker and more efficient separation of cannabinoids. In the literature, this separation is primarily accomplished using reversed-phase HPLC methods, with acetonitrile (ACN) and methanol (MeOH) consistently identified as the preferred organic modifiers [2]. However, their toxic and flammable characteristics present significant risks. ACN can be metabolized in the liver into harmful substances, and chronic exposure can lead to negative health effects. Furthermore, even slight exposure to MeOH, whether through inhalation or skin contact, can negatively impact the nervous system, liver, and kidneys [3]. Therefore, it is crucial to explore alternative organic solvents for the mobile phase to achieve a more environmentally friendly LC separation of cannabinoids [4]. **Objective:** This study aims to develop an HPLC-DAD analytical method for the separation of six neutral cannabinoids, using ethanol (EtOH) as the organic mobile phase as an alternative to MeOH and ACN. **Methods:** The chromatographic separation of cannabinoids was performed on an Agilent 1260 Infinity II HPLC-DAD system, utilizing an InfinityLab Poroshell 120 EC-C18 column (3.0 x 150 mm, 2.7 µm) that was protected by a Poroshell 120 EC-C18 3.0 mm guard column. A gradient elution was carried out using a mixture of ethanol and deionized water, both containing formic acid, at a flow rate of 0.5 mL/min over a duration of 18 minutes. The method developed demonstrated great selectivity, linearity, precision, and accuracy. **Results:** The optimized method was achieved by modifying chromatographic conditions, including gradient, flow rate, run time, and column temperature. Diode array analysis was conducted to evaluate specificity, while UV quantification was carried out at 230 nm. To ensure that the analytical method meets its intended purpose, parameters such as linearity. accuracy, precision, and lower range limits were established in accordance with regulatory guidelines, including ICH Q2 and M10, and AOAC appendix F. **Conclusions:** A greener analytical method using EtOH as organic mobile phase was successfully developed for the quantification of six neutral cannabinoids.

Keywords: cannabis; CBD; Δ^9-THC; HPLC-DAD; green analytical chemistry

Acknowledgments/Funding: This work was founded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC46

Exploring Cannabinoid Profile Changes During Cannabis Flower Decarboxylation, Extraction, and Purification

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DOI: https://doi.org/10.48797/sl.2026.506

ABSTRACT

Background: *Cannabis sativa* L. has become widely cultivated. The inflorescences, resins, and oils derived from this plant are employed for both medicinal and recreational purposes, primarily due to the effects associated with cannabinoids such as cannabidiol (CBD) and the psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC). However, cannabis plants biosynthesize these compounds in their acidic forms, necessitating a decarboxylation process during the extraction phase [1]. Moreover, depending on the final formulation, the extracts may undergo further purification before being incorporated into the final product. These steps enhance the quality of the end product and increase its attractiveness to consumers [2]. **Objective:** This research aims to understand the variations in Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-THC, and cannabinol (CBN) during the sample processing of the THC-rich cultivar Z-Face. The processing steps include the decarboxylation of acidic cannabinoids, Soxhlet extraction, and purification of extracts through winterization and activated charcoal treatment. **Methods:** Decarboxylation was performed at 120 ºC for 1 hour. This was followed by a 2-hour Soxhlet extraction with 96% ethanol to extract cannabinoids. The solution underwent winterization at -80 ºC for 24 hours to remove waxes and lipids. Finally, 50% (w/w) activated charcoal was added and mixed for 1 hour to remove chlorophyll and other pigments. Cannabinoid quantification was conducted using an Agilent 1260 Infinity II HPLC-DAD system, equipped with an InfinityLab Poroshell 120 EC-C18 column (3.0 x 150 mm, 2.7 µm) [3]. **Results:** Each step was refined by: (i) Conducting decarboxylation studies at varying temperatures and durations to effectively convert Δ^9-THCA into Δ^9-THC, while achieving minimal CBN formation; (ii) Optimizing Soxhlet extraction time and number of cycles; (iii) Determining the appropriate winterization temperature and duration; and (iv) Examining the percentage of activated charcoal used, along with the temperature and duration of treatment. **Conclusions:** The extraction and processing of THC-rich Z-Face flowers, initially containing 16.5% Δ^9-THCA (15.6% total Δ^9-THC), resulted in final extracts with 58.9% Δ^9-THC, corresponding to an 8.9% enrichment during the purification steps.

Keywords: cannabis; Δ^9-THC; Soxhlet extraction; THCA decarboxylation.

Acknowledgments/Funding: This work was founded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC47

Investigating Psychedelic Tryptamines: Extraction and Quantification from *Psilocybe* Mushrooms

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DOI: https://doi.org/10.48797/sl.2026.498

ABSTRACT

Background: *Psilocybe cubensis* mushrooms contain the tryptamines psilocybin and psilocin, which are recognized for their therapeutic potential in mental health [1]. Additionally, due to their psychedelic effects, these mushrooms are also used for religious and recreational purposes. The increasing use of these tryptamines and other psychoactive substances has led to regulatory measures, particularly Portuguese Decree-Law No. 15/93 of January 22, which aims to regulate the production, distribution, possession, and consumption of these substances. Psilocybin is generally more prevalent, with concentrations in dried mushrooms ranging from 0.5% to 1.5% [2]. **Objective:** The goal was to develop an efficient method for extracting and quantifying psilocin and psilocybin, as well as a fast and simple technique for identifying tryptamines. **Methods:** The mushrooms were finely pulverized using a cold porcelain mortar and pestle and extracted twice through kinetic maceration on a magnetic stirrer plate, with cold methanol containing 10% water serving as the extraction solvent (0.1 mL/mg of mushroom). An Agilent 1260 Infinity II HPLC-DAD system with a Poroshell 120 EC-C18 3.0 x 150 mm, 2.7 µm column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column was used for psilocin and psilocybin quantification [3]. **Results:** In order to optimize the extraction method, various parameters were taken into account, including solvent, extraction time, the number of extractions, agitation rate, temperature, and the solvent-to-dry material ratio. The concentrations of psilocybin and psilocin in the mushroom were found to be 1.98% and 0.10%, respectively. A Thin Layer Chromatography (TLC) method was developed for the rapid identification of psilocybin and psilocin, utilizing Ehrlich's reagent as the detection solution. **Conclusions:** An optimized extraction protocol was successfully established to maximize the recovery of target compounds. Additionally, a fast TLC identification method was developed for application in forensic sciences.

Keywords: psilocybin; psilocin; HPLC-DAD; mushrooms

Acknowledgments/Funding: This work was founded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC48

Chiral HPLC Method Optimization for Enantioseparation of 2-Methylmethcathinone

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DOI: https://doi.org/10.48797/sl.2026.496

ABSTRACT

Background: 2-Methylmethcathinone (2-MMC) is a chiral new psychoactive substance whose enantiomers may differ in pharmacological and toxicological behavior. Reliable enantioselective analysis is therefore required for forensic and environmental applications. Recent work shows that polysaccharide-based chiral stationary phases (CSPs) are broadly effective for cathinones [1]. **Objective:** This study aims to develop and optimize a rapid, robust high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method achieving baseline separation [resolution (Rs) ≥ 1.5] of 2-MMC enantiomers. **Methods:** A two-stage workflow was used. First, a screening compared a Lux AMP 3 µm (150 × 4.6 mm) under ammonium bicarbonate (pH 11) with methanol or acetonitrile (isocratic/gradient) against a Lux Amylose-1 3 µm (150 × 4.6 mm) operated in normal-phase (*n*-hexane/isopropanol). Ultraviolet detection was set at 254 nm. Performance criteria included Rs and run time. **Results:** The Lux AMP configuration yielded limited enantioresolution across tested conditions (maximum Rs ≈ 1.11). In contrast, Lux Amylose-1, with n-hexane/isopropanol as mobile phase, produced baseline separation of 2-MMC enantiomers (Rs > 1.5) with short analysis times and consistent retention, providing suitable peak shape and repeatability. These outcomes align with literature showing high success rates of amylose/cellulose CSPs for cathinones under normal-phase and polar-organic modes [2,3]. **Conclusions:** The screening-to-optimization strategy delivered a fast enantioselective HPLC-UV method for 2-MMC. Beyond analytical separation, the method provides a robust platform to develop and adapt enantioselective procedures for biological samples (*e.g.,* oral fluid, blood, urine), enabling enantiomer-resolved quantification in clinical and forensic toxicology.

Keywords: 2-methylmethcathinone; chiral HPLC; enantioseparation

Acknowledgments/Funding: This research was funded by FCT-Portuguese Foundation for Science and Technology, through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX; and supported by 1H-TOXRUN from Instituto Universitário de Ciências da Saúde (IUCS-CESPU)") and by FCT within the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), the project UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. Ivan Langa acknowledges a Ph.D. research grant provided by the FCT with reference 2022.12874.BD and DOI identifier https://doi.org/10.54499/2022.12874.BD.

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PC49

Gorilla Glue Cannabis Extracts: Cannabinoid Profiling and CBD Hydrolysis Investigation

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DOI: https://doi.org/10.48797/sl.2026.495

ABSTRACT

Background: Medicinal plants are a significant source of bioactive compounds with pharmacological relevance, leading to increased interest in species such as *Cannabis sativa L.* This plant is particularly rich in cannabinoids, a class of biologically active compounds with potential therapeutic benefits. To date, more than one hundred cannabinoids have been identified; however, only a few, such as cannabidiol (CBD), have been the focus of extensive research [1]. In fact, Epidiolex® is an oral solution that contains 100 mg of CBD per 1 mL and has already been approved for the treatment of epilepsy symptoms [2]. The decarboxylated Gorilla Glue (GG) cultivar extract used in this study was chosen for its high CBD content. A decarboxylation step was essential for converting cannabidiolic acid (CBDA) present in the flower into its biologically active neutral form, CBD. This cannabinoid is the most commonly found in cannabis-related products; consequently, it has the potential to become an environmental contaminant, highlighting the critical need to assess its stability in different conditions. **Objective:** This study aims to evaluate the cannabinoid profile of decarboxylated GG extract and the stability of CBD within the extract under different pH conditions (4, 7, and 9) at 50 ºC over a 9-day period. **Methods:** Cannabis flowers were finely ground using a mixer mill (Retsch MM400) equipped with stainless steel grinding balls. The decarboxylation step was carried out in an oven at 130 °C for 2 h. Finally, dynamic maceration extraction was conducted in the same ball mill using 96% (v/v) ethanol for 10 minutes. The resulting extracts were filtered, diluted, and analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity II system equipped with an InfinityLab Poroshell 120 EC-C18 column [3]. Hydrolysis assays were then conducted by incubating the extract under sterile conditions at different pH levels. **Results:** The cannabinoid profile of the GG decarboxylated extract, expressed as % (w/w), was found to be: 49.34% CBD, 2.43% CBC, 1.82% Δ^9-THC, 0.89% CBDA, and 0.35% CBN. After nine days under hydrolysis conditions, CBD remained present at 81%, 60%, and 61% at pH levels of 4, 7, and 9, respectively. **Conclusions:** CBD constitutes 49.34% of the decarboxylated GG extracts, and preliminary hydrolysis studies indicated that it is more stable under acidic conditions.

Keywords: cannabis; CBD; decarboxylation; hydrolysis

Acknowledgments/Funding: This work was founded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC50

Cannabinoid Profiling of Z-Face Cannabis Cultivar Extracts

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DOI: https://doi.org/10.48797/sl.2026.493

ABSTRACT

Background: Cannabis is a versatile plant that has been used for thousands of years for its medicinal, nutritional, cosmetic, and agricultural applications. To date, 566 chemical compounds have been identified in *Cannabis sativa*, including 125 cannabinoids [1]. The Z-Face cultivar under investigation is notably rich in Δ⁹-THC, the only cannabinoid with well-established psychotropic effects. It interacts with CB1 receptors in the central nervous system, inhibiting the release of neurotransmitters such as GABA and glutamate. As a result of its psychoactive properties, Δ⁹-THC is highly regulated and represents the most widely used drug of abuse worldwide. Nevertheless, these same properties offer significant therapeutic potential, particularly for their analgesic, antiemetic, and appetite-stimulating effects [2,3]. Decarboxylation is essential to convert acidic cannabinoids found in the plant into their pharmacologically active neutral forms [3]. **Objectives**: The aim of this study was to determine the cannabinoid profile of extracts from the Z-Face cultivar in both decarboxylated and non-decarboxylated forms. **Methods**: The inflorescences were ground using a Retsch 400 ball mill and extracted with 96% (v/v) ethanol. The extract was filtered under reduced pressure, and the solvent was evaporated using a speedvac. For the decarboxylated samples, heating at 120 °C for 1 hour was performed prior to extraction. Cannabinoid quantification was carried out using a high-performance liquid chromatography with diode array detection (HPLC-DAD) system, employing an optimized method for determining 14 cannabinoids [4]. **Results**: In the non-decarboxylated extract, nine cannabinoids were quantified. The major cannabinoids, expressed as a percentage (w/w), were THCA 49.13% and Δ^9-THC 8.11%. Other cannabinoids included CBGA 0.87%, CBCA 0.60%, THCVA 0.26%, CBG 0.24%, CBNA 0.19%, CBC 0.13%, and CBN 0.10%. The composition of the decarboxylated extract was predominantly Δ^9-THC (56.48%), with only trace amounts of THCA (0.23%). Additional cannabinoids quantified included CBG 1.44%, CBN 0.94%, CBC 0.76%, THCV 0.34%, and CBGA 0.27%. **Conclusion**: The primary cannabinoid identified in the Z-Face cultivar extract is THCA (49.13%), while the decarboxylated extract contains Δ^9-THC, as expected.

Keywords: cannabinoids; cannabis; Δ⁹-THC; HPLC-DAD

Acknowledgments/Funding: This work was funded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC51

GC-MS Terpene Profiling in Cannabis Extracts: Method Optimization and Analytical Validation

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DOI: https://doi.org/10.48797/sl.2026.491

ABSTRACT

Background: The importance of Cannabis sp. in modern medicine stems from its complex phytochemical profile, where terpenes may play a role through the entourage effect. This entourage effect proposes that Δ^9-tetrahydrocannabinol (Δ^9-THC) interacts with other compounds in the cannabis plant in ways that meaningfully modify its effects. For instance, Spindle et al. reported that D-limonene reduced the acute anxiogenic effects of Δ^9-THC [1]. To ensure the standardization of cannabis-based products, GC-MS is essential by enabling accurate quantification of these compounds in complex plant matrices [2]. **Objective:** This study aims to optimize and validate a GC-MS method for the characterization of major terpenes in cannabis leaves and flowers. **Methods:** Two cultivars of cannabis flowers were used: one rich in CBD (Blue Cheese) and the other rich in Δ^9-THC (Z-Face). In addition, leaves from a Δ^9-THC-rich cannabis cultivar provided by Avextra were also analyzed. Samples were pulverized and extracted with ethyl acetate for 10 minutes using a Retsch 400 ball mill. GC-MS analysis was performed using an SH-Rxi-5ms column under a temperature gradient. Terpenes were identified in SIM mode based on characteristic ions [3]. The method was validated according to ICH guidelines. **Results:** The method showed high precision and accuracy, demonstrating linearity ($R^2 > 0.999$) for all ten analyzed terpenes. The three most abundant terpenes identified in the leaves were β-caryophyllene (0.014%), α-bisabolol (0.011%), and α-humulene (0.09%), expressed as percentage (*w*/*w*). In the Blue Cheese cultivar, the dominant terpenes were α-bisabolol (0.174%), β-caryophyllene (0.108%), and α-pinene (0.086%). The predominant terpenes in the Z-Face cultivar included β-caryophyllene (0.445%), α-humulene (0.252%), and limonene (0.227%). Terpinolene was the only terpene not detected in all three samples. **Conclusion:** A validated GC-MS method was established for terpene characterization in cannabis extracts. Higher terpene levels were found in flowers compared to leaves, with distinct profiles observed between the CBD- and THC-rich varieties.

Keywords: cannabis; GC-MS; terpenes

Acknowledgments/Funding: This work was founded by Avextra Portugal SA. The authors also acknowledge financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC52

Occurrence of Pyrrolizidine Alkaloids in Portuguese Teas and Herbal Infusions: a Toxicological Assessment using *C. elegans*

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DOI: https://doi.org/10.48797/sl.2026.487

ABSTRACT

Background: Pyrrolizidine alkaloids (PAs) and their *N*-oxide (PANOs) are plant secondary metabolites with recognized hepatotoxic and genotoxic potential, particularly the 1,2-unsaturated forms [1]. Tea and herbal infusions (THI), widely consumed for their known health benefits, are one of the main dietary sources of human exposure to these contaminants [2,3]. **Objective:** This study aimed to detect and quantify PAs/PANOs in THI available in the Portuguese market using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method and to assess the toxicity of selected extracts. **Methods:** Twenty-six samples (chamomile, lemon balm, lemon verbena, green, and black tea) were acquired from the Portuguese market. Samples were extracted by solid-liquid extraction with acidified water at 80°C, followed by solid-phase extraction and analyzed using a Luna® 3µm PFP (2) 100 Å column in the LC-MS/MS system. Based on LC-MS/MS results for PAs occurrence, 4 herbal infusions with total PA concentrations ≥ 20 ng/g were selected for assessing their safety at human-relevant exposure levels. Extracts were prepared using the same infusion procedure and then lyophilized. The *C. elegans* strain DC19 [*bus-5(br19)*] was used to evaluate the effects of the extracts on animal survival and lifespan. Synchronized L1 larvae were exposed to increasing extract concentrations and analyzed after 72 h. Survival was determined by counting live and dead worms, while lifespan was monitored every 2 days by recording mortality. **Results:** Among the 26 samples, 13 contained detectable PAs, of which 5 had total concentrations ≥ 20 ng/g. Herbal infusions showed the highest PA concentrations, ranging from 25.4 to 68.8 ng/g. None of the extracts reduced survival at any tested concentration corresponding to the equivalent consumption of 1–6 cups ($p > 0.05$) [% of surviving animals in all conditions ≥ 95%]. The highest concentration was used to assess lifespan; no significant differences were detected ($p > 0.05$). **Conclusions:** Although PAs/PANOs concentrations were low and no significant effects on *C. elegans* survival or lifespan were observed, the variability among samples highlights the need for monitoring PAs/PANOs in THI. Moreover, the absence of phenotypic alterations at the organismal level does not exclude the possibility of subtle cellular or molecular effects that may not be immediately detectable. Thus, additional cellular, molecular, or biological endpoints are needed to support a more comprehensive risk assessment.

Keywords: tea and herbal infusions; pyrrolizidine alkaloids; LC-MS/MS; *C. elegans*; food safety

Acknowledgments/Funding: This work was supported by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant SPAinT-GI2-CESPU-2022 and CESPU – Cooperativa de Ensino Superior Politécnico e Universitário for the grant ORPasTeA_GI2-CESPU-2023;and FCT - Fundação para a Ciência e Tecnologia, I.P./MECI, in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. DP and ARLR acknowledge the financial support from FCT, I.P./MECI through national funds: LSRE-LCM, UID/50020/2025 (https://doi.org/10.54499/UID/50020/2025); and ALiCE, LA/P/0045/2020 (https://doi.org/10.54499/LA/P/0045/2020).ARLR acknowledges FCT funding under the Scientific Employment Stimulus - Individual Call (https://doi.org/10.54499/2022.00184.CEECIND/CP1733/CT0001).

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PC53

Unraveling the Role of Telomeres and Telomerases in the Response to Neurotoxicants

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ABSTRACT

Background: Telomeres are repetitive DNA sequences that protect chromosome ends and whose length is maintained by telomerase activity [1]. While telomere dynamics are well characterized in proliferating cells, their role in post-mitotic cells such as neurons remains poorly explored [2]. Particularly, the impact of telomere attrition or telomerase dysfunction on the cellular response to neurotoxicants is largely unknown. **Objective:** We aimed to investigate whether telomere shortening increases the susceptibility of neuronal cells to the toxic effects of common environmental neurotoxicants (i.e., HgCl2, acetaldehyde). **Methods:** The effects of $HgCl_2$ (0-100mM) and acetaldehyde (0-10mM) on the metabolic activity (MTT reduction assay) and lysosomal integrity (Neutral Red uptake) of SH-SY5Y human neuroblastoma cells were assessed by determining IC10 and IC50 for each neurotoxicant. These assessments were performed either 24h after exposure to the neurotoxicants or following a 72h pre-treatment with 1mM XAV939, a tankyrase-1 inhibitor that limits telomerase access to telomeres, promoting telomere shortening [3]. Relative telomere length in XAV939-treated treated alone and in combination with biologically relevant, subtoxic concentrations of $HgCl_2$ (10 and 25 mM) or acetaldehyde (0.1 and 5 mM) was measured using quantitative real-time PCR. **Results:** Our findings revealed that $HgCl_2$ and acetaldehyde reduced the metabolic activity and lysosomal integrity of SH-SY5Y cells in a concentration-dependent manner. Also, $HgCl_2$ shifted the IC10 from 26 to 15 mM for metabolic activity and from 21 to 6 µM for lysosomal integrity in XAV939-treated cells, compared to cells not exposed to XAV939, suggesting that shortened telomeres may have increased the cells' susceptibility to $HgCl_2$. In turn, XAV939 pretreatment did not alter the impact of acetaldehyde on those parameters. Notably, XAV939-induced telomere shortening was confirmed by qPCR analysis. Interestingly, our data showed that 100µM acetaldehyde and 10 µM $HgCl_2$ reduced the cells' telomere length to 45% and 77% of control levels, respectively, at the same time point. **Conclusions:** Our preliminary findings suggest that telomere shortening increases SH-SY5Y cells vulnerability to the toxic effects of $HgCl_2$, evidencing a possible involvement of telomere-related mechanisms in the response to neurotoxicants. However, further research is required to confirm the importance of such mechanisms in neurotoxicity-related responses.

Keywords: telomere shortening; neurotoxicity; telomerase inhibition

Acknowledgments/Funding: This work was funded within the scope of the project ALCOHOLAGEING (Operation No. 15705; COMPETE2030-FEDER-00680600), funded by the European Regional Development Fund (ERDF) through the Innovation and Digital Transition Programme (COMPETE 2030) and by National Funds from Fundação para a Ciência e Tecnologia, I.P. (FCT) (FCT) under Call No. MPr-2023-12. The work was further supported by FCT, in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. JPS also acknowledges FCT for the researcher contract (under Scientific Employment Stimulus) 2021.01789.CEECIND/CP1662/CT0014, respectively.

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PC54

Heroin and Tapentadol Promote Accelerated Senescence of SH-SY5Y Human Neuroblastoma Cells at Subtoxic Concentrations

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DOI: https://doi.org/10.48797/sl.2026.485

ABSTRACT

Background: Opioids are widely used in clinical practice due to their analgesic efficacy. However, their potential for abuse and deleterious effects represents a major global health concern [1]. Importantly, there is growing evidence suggesting that long-term opioid use may promote inflammatory responses and oxidative stress, altering molecular pathways involved in cell senescence, a feature often associated with accelerated ageing and cellular functional decline [2,3]. **Objective:** This work aimed to elucidate the impact of both recreationally and therapeutically used opioids (i.e., heroin and tapentadol, respectively) on neuronal cell ageing. **Methods:** SH-SY5Y human neuroblastoma cells were exposed to 1 nM and 1 µM of either heroin or tapentadol for 72h, and cell senescence was evaluated by measuring β-galactosidase activity (a widely used marker of senescent cells) using a commercially available kit (Abcam, USA). At the same time point, reactive oxygen species (ROS) levels were assessed using dichlorofluorescein (DCFH-DA). Notably, we have previously shown these opioids' concentrations to be below toxicity thresholds. 25 nM doxorubicin was used as a positive control. In parallel, SH-SY5Y cells were chronically exposed, every 2-3 days, for a total of 30 days (between passages 21 and 25), to the same opioid concentrations. Genomic DNA was collected every other passage, and relative telomere length was measured through quantitative real-time PCR. **Results:** We observed increased β-galactosidase activity in opioid-exposed cells compared with untreated controls, in a concentration-dependent manner. Specifically, heroin increased this enzyme's activity by 1.25- and 1.33-fold (for 1 nM and 1 µM, respectively), while tapentadol increased it by 1.19- and 1.29-fold at 1 nM and 1 µM, respectively. None of the opioids, at the tested concentrations, altered ROS levels after 72h exposure. From passages 21 to 25, telomere length decreased by 6% in control cells. However, opioid treatment exacerbated progressive telomere shortening, with reductions of 15.0 and 28.5% for tapentadol, and 10.0 and 27.1% for heroin at 1 nM and 1 µM, respectively. **Conclusions:** Overall, our preliminary data indicate that opioid use promotes early signs of cellular senescence and accelerated ageing (i.e., telomere shortening) in SH-SY5Y human neuroblastoma cells. Nonetheless, additional assays are ongoing to further characterize the effects of these opioids' chronic exposure on neuronal cells.

Keywords: opioids; cell senescence; telomere shortening

Acknowledgments/Funding: This work was funded within the scope of the project ALCOHOLAGEING (Operation No. 15705; COMPETE2030-FEDER-00680600), funded by the European Regional Development Fund (ERDF) through the Innovation and Digital Transition Programme (COMPETE 2030) and by National Funds from Fundação para a Ciência e Tecnologia, I.P. (FCT) under Call No. MPr-2023-12. The work was further supported by FCT, in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. JPS also acknowledges FCT for the researcher contract (under Scientific Employment Stimulus) 2021.01789.CEECIND/CP1662/CT0014, respectively.

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PC55

Decoding the Underexplored Biological Impact of Synthetic Cathinones using *C. elegans* as a Translational Toxicity Model

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DOI: https://doi.org/10.48797/sl.2026.483

ABSTRACT

Background: The recreational use of New Psychoactive Substances (NPSs) represents a significant and growing public health concern. Among these, synthetic cathinones are increasingly consumed, yet their biotoxicological effects remain poorly characterized [1, 2]. In this context, *in vivo* models such as *Caenorhabditis elegans*, a suitable model for high-throughput toxicity screening that exhibits a high degree of conservation of molecular pathways with humans [3], are essential to elucidate the systemic and long-term effects of these substances on critical biological processes, which remain poorly understood. **Objective:** Using *C. elegans* as a discovery platform, this study aimed to evaluate the toxic effects of methylone and pentedrone on animal development, reproduction, and lifespan. **Methods:** Synchronized L1-stage animals of the DC19 strain [*bus-5(br19)*] (~200 animals/condition) were exposed, in liquid medium, to increasing concentrations of synthetic cathinones (0–10 mM) for 24–72 h. Survival rates were assessed by counting the number of live and dead worms. Using sublethal to low-lethal concentrations (0–2.5 mM), we further explored the impact of synthetic cathinones on (1) animal development, by measuring the body length using Fiji software; (2) reproductive behavior, by counting the total number of embryos laid by individual F0-exposed animals within a 24-h time window; and (3) lifespan, by monitoring exposed animals every two days throughout their lifespan. **Results:** Short-term exposure (24 h) of *C. elegans* to methylone or pentedrone did not affect animal survival rates at concentrations ≤1.0 mM, whereas higher concentrations (≥5.0 mM) significantly reduced viability. In contrast, prolonged exposure (72 h) significantly reduced animal survival rates at concentrations ≥1.0 mM, indicating enhanced toxicity over time. Sublethal concentrations of both compounds impaired animal development in a reversible manner and significantly reduced reproductive output, while progeny viability remained unaffected. Additionally, no significant effects on animal lifespan were observed, suggesting selective disruption of developmental and reproductive processes. **Conclusions:** These findings indicate that synthetic cathinones exhibit pronounced time-dependent toxicity in *C. elegans*. This highlights previously underexplored systemic risks and reinforces the value of *C. elegans* as a powerful *in vivo* platform to uncover the biological impact of psychoactive substances.

Keywords: synthetic cathinones; systemic toxicity; C. *elegans*

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "DeTTeCaK-GI2-CESPU-2025".

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PC56

Cellular Mechanisms of 1,3-DMAA-Induced Neurotoxicity in SH-SY5Y Cells

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DOI: https://doi.org/10.48797/sl.2026.481

ABSTRACT

Background: 1,3-Dimethylamylamine (1,3-DMAA) is a chiral sympathomimetic amine commonly added to dietary supplements marketed for weight loss, performance enhancement, and recreational purposes [2]. Despite regulatory bans, 1,3-DMAA continues to be detected in doping controls and dietary supplements, raising toxicological concerns. However, the cellular mechanisms underlying its neurotoxic potential remain incompletely characterized [3]. **Objective**: This study aimed to investigate the cytotoxic mechanisms induced by 1,3-DMAA in a human neuronal cell model (SH-SY5Y), contributing to a better understanding of its toxicodynamics. **Methods**: SH-SY5Y cells were exposed for 48 h to 1,3-DMAA (1.3×10^{-4} to 1.5×10^{1} mM; n=5); mitochondrial metabolic activity was assessed using the MTT assay and the lysosomal integrity through the neutral red uptake (NR) assay. Based on the MTT results, cells were subsequently exposed to the EC_{20} (4.21 mM), EC_{40} (4.91 mM), and EC_{60} (5.59 mM), and changes in intracellular reactive oxygen species (ROS) production and mitochondrial membrane potential ($\Delta\Psi m$) were assessed using fluorometric probes. Autophagic features were evaluated using acridine orange (AO) staining to detect acidic vesicular organelles. **Results**: 1,3-DMAA induced concentration-dependent cytotoxicity, with a greater impact on mitochondrial function than lysosomal integrity, as evidenced by lower EC_{50} values in the MTT assay compared to the NR assay (5.24 mM versus 6.36 mM, respectively). 1,3-DMAA induced a concentration-dependent increase in intracellular ROS levels from EC20 (236.67%; $p<0.001$) and EC40 (211.87%; $p<0.01$) and peaking at EC_{60} (272.05%; $p<0.0001$). In contrast, $\Delta\Psi m$ remained unchanged at lower concentrations, with a significant increase observed at EC_{60} (317.32%; $p<0.0001$). AO staining showed increased acidic vesicular organelles at higher concentrations. **Conclusions**: The concomitant increase in ROS and mitochondrial hyperpolarization of $\Delta\Psi m$ indicates a pro-oxidant state. The increase in acidic vesicular organelles suggests activation of autophagic processes and/or progression to apoptosis. These findings provide mechanistic insight into 1,3-DMAA-induced neurotoxicity and establish a foundation for further toxicological investigations.

Keywords: neurotoxicity; *in vitro* assay; SH-SY5Y cells

Acknowledgments/Funding: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 – Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos; UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO, LA/P/0140/2020, and the PhD grant 2021.04999.BD are also acknowledged. Maria Almeida acknowledges her Innovhealth scholarship supported by the Young STEAM Impulse Programme of the Resilient P.PORTO Programme (02/C06-i03.03/2021.PID-15), co-funded by the Next Generation EU programme funds under the Recovery and Resilience Plan (PRR).

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PC57

Lisdexamfetamine Induces Concentration-Dependent Toxicity and Developmental Effects in *Caenorhabditis elegans*

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DOI: https://doi.org/10.48797/sl.2026.478

ABSTRACT

Background: Lisdexamfetamine dimesylate (LDX) is a prodrug of *d*-amphetamine, used for the treatment of attention-deficit/hyperactivity disorder (ADHD) [1, 2]. Despite its clinical use, some knowledge gaps remain regarding its toxicity, including its potential to induce heritable toxicological signatures. **Objective:** To address these gaps, this study aims to use *Caenorhabditis elegans*, a rapid, tractable model with a short lifespan, transparent body, and well-characterized development, as an exploratory discovery platform for efficient toxicological assessment [3]. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200/condition) were exposed, in liquid medium, to increasing concentrations of LDX (0 - 10 mM). Following a 72-h incubation in M9 buffer containing OP50 bacteria as a food source, distinct organism-level phenotypes were assessed. Survival rate was determined by counting the number of live and dead worms after the exposure period. Using sublethal concentrations (0.5, 1.0, and 2.0 mM), we further explored the influence of LDX on (1) animal development, by measuring the body length using Fiji software; lifespan, by monitoring exposed animals every two days throughout their lifespan; and (3) reproductive behavior, by counting the total number of embryos laid by individual F0 exposed animals within a 24-h time window. Further experiments explored the hatching rate of unexposed F1 embryos laid by exposed animals, as well as the growth of larvae derived from these embryos, to investigate putative heritable toxicological signatures. **Results:** LDX reduced animal survival in a concentration-dependent manner at 3.0-10.0 mM. Concentrations of 0.5-1.0 mM showed no significant effects on F0 development, lifespan, reproduction, or F1 outcomes. At 2.0 mM, directly exposed animals exhibited delayed development, with similar growth retardation observed in F1 progeny at 48-72h post-exposure. Lifespan analysis and reproduction assays at 2.0 mM LDX are currently ongoing. **Conclusions:** LDX demonstrates concentration-dependent toxicity in *C. elegans*. While lower sublethal doses appear well-tolerated, 2.0 mM delays development in exposed animals and in their F1 progeny, warranting further heritable toxicity investigation.

Keywords: lisdexamfetamine dimesylate; heritable toxicological signatures; *C. elegans*

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and by CESPU, under the grant "DeTTeCaK-GI2-CESPU-2025".

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PC58

Lisdexamfetamine: From Pharmacology to Forensic Implications

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DOI: https://doi.org/10.48797/sl.2026.426

ABSTRACT

Background: Lisdexamfetamine dimesylate (LDX) is a prodrug of *d*-amphetamine used in the treatment of neuropsychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD) [1]. **Objective:** This systematic review aims to provide a comprehensive overview of LDX pharmacokinetics, pharmacodynamics, clinical efficacy, safety profile, and forensic considerations. **Methods:** A literature search was conducted in PubMed without a limitation period using the keywords "lisdexamfetamine", "lisdexamfetamine dimesylate", "LDX", "ADHD", "pharmacokinetics", "pharmacodynamics", "forensic implications", "abuse", "clinical applications", "clinical efficacy", and "adverse effects", either individually or in combination. All types of articles were included. A total of 113 articles were selected. **Results:** LDX undergoes rapid absorption via peptide transporter 1 (PepT1) in the small intestine, achieving C_{max} within 1–2h [2-5], and is hydrolyzed in erythrocytes, by an unidentified aminopeptidase, into *d*-amphetamine and *l*-lysine [5, 6]. It is primarily eliminated in the urine (96.4%), with minimal fecal elimination (0.3%) [2]. LDX does not significantly alter the activity of CYP1A2, CYP2D6, and CYP3A4 (7), suggesting low potential for drug-drug interactions. Its stimulant activity results from trace amine-associated receptor 1 activation, monoamine oxidase inhibition, and reverse transport of the vesicular monoamine transporter 2, dopamine transporter, noradrenaline transporter, and serotonin transporter, increasing neurotransmitter levels in the synaptic cleft [1, 8]. Common adverse effects of LDX include dizziness, somnolence, appetite suppression, headache, nausea, and fatigue [9]. Concerns regarding growth suppression arise mainly in the first year of treatment and diminishing thereafter [9, 10]. Although LDX exhibits a lower reinforcing potential than *d*-amphetamine [11], supra-therapeutic doses may induce similar abuse liability and toxicity. However, its higher lethal dose threshold (five times that of amphetamines) reduces overdose risk [12]. **Conclusions:** LDX demonstrates established therapeutic benefits in ADHD, often yielding superior outcomes compared with other stimulant medications. In forensic settings, distinguishing between prescribed use and illicit intake remains a significant challenge. Considering its potential applications beyond ADHD, further large-scale investigations are needed to fully define LDX's pharmacological, toxicological, and clinical profile.

Keywords: lisdexamfetamine dimesylate; *d*-amphetamine; attention-deficit/hyperactivity disorder

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and by CESPU, under the grant "DeTTeCaK-GI2-CESPU-2025".

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PC59

Using Zebrafish to Assess the Impact of 3-CMC on Embryonic and Neural Development

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DOI: https://doi.org/10.48797/sl.2026.427

ABSTRACT

Background: Synthetic cathinones are a class of new psychoactive substances (NPS) increasingly detected in the environment due to their widespread human consumption. 3-Chloromethcathinone (3-CMC) is a halogenated and N-alkylated derivative of cathinone with a chiral centre [1]. It shares structural similarities with methcathinone and 4-chloromethcathinone (4-CMC, clephedrone). Like other cathinones, 3-CMC interacts with monoamine transporters, exerting psychostimulant effects by promoting the release of dopamine, norepinephrine, and serotonin [2]. The growing presence of NPS in wastewater and surface waters highlights the urgent need to investigate their potential toxic effects on aquatic organisms [3]. **Objective:** This study aimed to evaluate the effects of 3-CMC on embryonic development, neurotransmitter levels (dopamine, serotonin, and their metabolites) and apoptosis in zebrafish (*Danio rerio*). **Methods:** Embryos, approximately 3 h post-fertilisation, were exposed for 96 h to five concentrations of 3-CMC (0.02 to 200 µg/L) in triplicate. Mortality, spontaneous movements and heart rate were assessed during the exposure period. At 96 hpf, samples for apoptosis levels measurement were homogenised after exposure to acridine orange (10 µg/mL) for 15 min before measuring fluorescence (excitation/emission: 535/590 nm). Serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite), and 5-hydroxyindolacetic acid (5-HIAA, a serotonin metabolite) were quantified by liquid chromatography coupled to a UV detector at 210 nm, while dopamine was assessed at 280 nm. **Results:** The results showed no significant effects on mortality, spontaneous movements or heart rate of zebrafish embryos. Likewise, no significant alterations were detected in neurotransmitter levels or apoptosis in exposed larvae compared to the control. **Conclusions:** These findings suggest that, under the tested concentrations, 3-CMC does not induce detectable developmental, neurochemical or apoptotic responses in zebrafish early life stages. Nevertheless, these results are preliminary. Given the increasing occurrence of NPS in aquatic environments, further research is needed to understand their impact (including 3-CMC) on other endpoints and the long-term effects of 3-CMC to improve environmental risk assessment and support the development of appropriate mitigation strategies.

Keywords: embryonic development; *Danio rerio*; synthetic cathinones

Acknowledgments/Funding: This work is supported by National Funds by Portuguese FCT, under the projects UID/04033/2025: CITAB and LA/P/0126/2020 (https://doi.org/10.54499/LA/P/0126/2020), the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and the project PTDC/CTA-AMB/6686/2020 - ENANTIOTOX, with DOI 10.54499/PTDC/CTA-AMB/6686/2020 (http://doi.org/10.54499/PTDC/CTA-AMB/6686/2020). Ondina Ribeiro acknowledges FCT for the PhD Grant 2022.12242.BD.

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PC60

Oral Methylphenidate Induces Sex-Related Differences in Brain Plasticity Proteins in Juvenile Wistar-Kyoto Rats

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DOI: https://doi.org/10.48797/sl.2026.428

ABSTRACT

Background: Methylphenidate (MPH) is widely prescribed as the first-line pharmacological intervention for Attention Deficit/Hyperactivity Disorder (ADHD) among children. However, the increasing cases of misdiagnosis and misuse raise concerns about the neurodevelopmental consequences of exposing children and adolescents to MPH [1,2], given the high brain plasticity during these critical phases [3]. **Objective:** This study aimed to evaluate the impact of repeated exposure to clinically relevant oral doses of MPH on pre- and post-synaptic protein markers involved in synaptic plasticity, and neuronal development, with a focus on sex-related differences. **Methods:** 37 healthy Wistar-Kyoto (WKY) rats (18 males and 19 females), aged 15 days (equivalent to human infancy), were randomly distributed into two groups. The MPH-group received daily oral MPH (5 mg/kg in 5% sucrose) via gavage, while the control group received an equivalent volume of 5% sucrose solution [4]. Treatment continued for 15 consecutive days, with doses adjusted individually based on each animal's weight. On PND 30, the animals were sacrificed and their brains dissected. GAP43 and PSD95 proteins were assessed in brain regions, including the prefrontal cortex (PFC), striatum, hippocampus, and cerebellum, by Western blot. Meanwhile, MAP2 and synaptophysin were evaluated in sections of the PFC, motor cortex, striatum, and hippocampus (CA1, CA3, hippocampal hilum, and dentate gyrus) by immunohistochemistry. **Results:** MPH exposure revealed region- and sex-specific alterations in synaptic plasticity proteins. MPH-treated males showed reduced levels of synaptophysin and GAP43 in the hippocampal CA1 region and cerebellum, respectively, along with increased PSD-95 levels in the striatum. In MPH-treated females, a reduction in PSD-95 levels was observed in the PFC. Additionally, control males showed higher MAP2 levels in the striatum compared to females. **Conclusions:** Early-life exposure to therapeutic doses of MPH can induce changes in neuronal development and synaptic plasticity in both sexes. These findings highlight the importance of considering sex in MPH's brain plasticity research, particularly given the underrepresentation of females in studies conducted on laboratory animals. Also, they emphasize the need to investigate the safety of non-clinical exposure to psychoactive drugs during early development and its potential long-term neurotoxic effects.

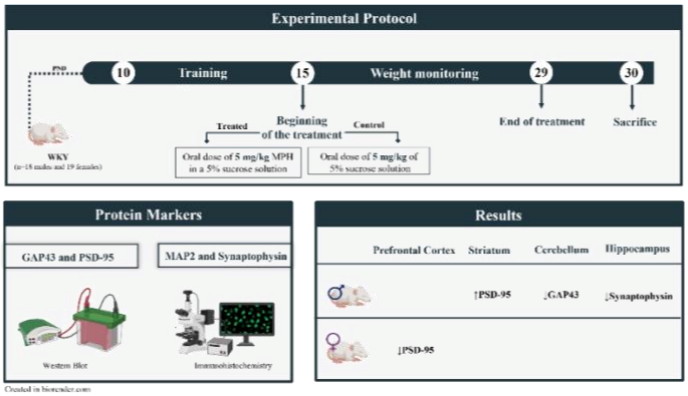

Figure 1. Graphical abstract of the experimental design and main findings.

Keywords: attention deficit hyperactivity disorder (ADHD); methylphenidate (MPH); neuroplasticity; Wistar-Kyoto

Acknowledgments/Funding: This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO) and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB. Also, supported by national funds through Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project "EXPL/MED FAR/0203/2021".

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PC61

Uncovering 4-Cl-α-PPP Toxicity and Mitigation Strategies Using *Caenorhabditis elegans* as a Translational Model

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DOI: https://doi.org/10.48797/sl.2026.429

ABSTRACT

Background: Synthetic cathinones, commonly referred to as "bath salts", are a class of New Psychoactive Substances designed to mimic the effects of traditional drugs of abuse. These compounds exert amphetamine-like effects by interacting with dopamine, serotonin, and noradrenaline transporters, leading to a range of adverse outcomes, including neurotoxicity [1, 2]. However, for several emerging synthetic cathinones, such as 4'-chloro-alpha-pyrrolidinopropiophenone (4-Cl-α-PPP), their toxicological profile remains poorly characterized, highlighting the need for further investigation. Additionally, exploring mitigation strategies to counteract their harmful effects may represent a promising approach. **Objective:** Using *C. elegans* as a discovery platform, this study aims to characterize the effects of 4-Cl-α-PPP on animal development, lifespan, reproductive behavior, and potential heritable toxicological signatures, as well as to identify novel strategies to mitigate its toxicity. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200 per condition) were exposed in liquid medium to increasing concentrations of 4-Cl-α-PPP. After 72 h of incubation in M9 buffer supplemented with OP50 bacteria as a food source [3], survival was assessed by counting live and dead worms [2]. Further experiments were conducted to evaluate the protective effect of *N*-acetyl-cysteine (NAC; 1 mM) against 4-Cl-α-PPP-induced reductions in survival. **Results:** Exposure to increasing concentrations of 4-Cl-α-PPP resulted in a concentration-dependent decrease in animal survival. While no significant effects were observed at lower concentrations (≤0.05 mM), a marked reduction in survival was detected at 0.75 mM [survival percentage (mean ± standard deviation): control (0 mM) = 98.47 ± 1.66; 0.75 mM = 18.78 ± 17.97, $p<0.01$], becoming more pronounced at higher concentrations (≥1.0 mM), with complete lethality observed at 2.5 mM [survival percentage (mean ± standard deviation): 1.0 mM = 0.29 ± 0.87, $p<0.0001$; 2.5 mM = 0.00 ± 0.00, $p<0.0001$]. Notably, co-incubation with NAC (1 mM) attenuated the decrease in survival induced by 4-Cl-α-PPP. Additional studies addressing developmental, lifespan, reproductive, and heritable effects are currently ongoing. **Conclusions:** These findings show that 4-Cl-α-PPP causes a marked, concentration-dependent decrease in animal survival, partially prevented by NAC, indicating that oxidative stress plays a major role in 4-Cl-α-PPP toxicity.

Keywords: 4'-chloro-alpha-pyrrolidinopropiophenone; synthetic cathinones; *C. elegans*

Acknowledgments/Funding: This work was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the Research Unit on Applied Molecular Biosciences–UCIBIO (projects UIDP/04378/2025 and UIDB/ 04378/2025), the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2025), the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos, and by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant "DeTTeCaK-GI2-CESPU-2025".

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Antibiotic Toxicity Under Climate-Change Stressors in Fish – A Liver Histopathology Assessment

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DOI: https://doi.org/10.48797/sl.2026.430

ABSTRACT

Background: Antibiotics in aquatic systems, combined with temperature and pH changes, threaten non-target organisms. Histopathological analysis provides integrative evidence of sublethal toxicity, reflecting cumulative physiological disruptions of several stressors on fish health [1]. **Objective:** This study evaluated liver histopathological alterations in *Danio rerio* after chronic exposure to environmentally relevant concentrations of sulfamethoxazole (150µg SMX/L), trimethoprim (30µg TRIM/L), and their mixture (MIX: 150µg SMX/L + 30µg TRIM/L) under different environmental conditions [2]. **Methods:** Three independent assays were conducted to assess the effects of temperature (26, 28, and 32 °C), pH (6.5, 7.5, and 9.0), and a combined climate-change scenario (28 °C + pH 9.0) in zebrafish liver. Liver alterations were analyzed using qualitative and semi-quantitative methods, and a total liver histopathological index (LI) was calculated. An Independent Action (IA) model was applied to integrate the effects of temperature and pH with antibiotic exposure and to evaluate the interactive impacts on liver histopathology [3]. **Results:** Qualitative analysis revealed circulatory (e.g., sinusoidal dilation), regressive (e.g., necrosis and hepatocellular degeneration), and progressive (e.g., hepatocyte nuclear hypertrophy) alterations across all tested scenarios. Semi-quantitative analysis showed that increasing temperature intensified the LI, even in the absence of antibiotics. At 32 °C, SMX induced severe lesions (e.g., hepatocellular degeneration, necrosis), indicating a temperature-dependent increase in LI. LI values increased for all antibiotic treatments, at pH 7.5 and for TRIM and MIX at pH 9.0. Although no significant alterations in LI values were detected under combined scenario, IA model revealed synergistic interactions for SMX and TRIM under combined temperature and pH, with liver damage exceeding predicted effects, whereas MIX exhibited antagonistic interactions, resulting in lower-than-expected damage. In general, histological lesions were observed across all antibiotic treatments and scenarios, indicating persistent adverse effects. **Conclusions:** Overall, these results highlight liver histopathology as a sensitive biomarker of toxicity and show that environmental stressors strongly modulate antibiotic effects. This underscores the need to adopt multi-stressor approaches in ecological risk assessment, particularly under future climate-change scenarios.

Keywords: sulfamethoxazole; trimethoprim; mixtures; temperature; pH; zebrafish; histology

Acknowledgments/Funding: This research was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020). Bárbara S. Diogo was supported by a FCT Ph.D. grant (ref. 2022.10505.BD).

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PC63

Danio rerio Responses to 3,4-Dichloroaniline Under Different Thermal Regimes

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DOI: https://doi.org/10.48797/sl.2026.431

ABSTRACT

Background: The aromatic amine 3,4-dichloroaniline (3,4-DCA) is a degradation product of several phenylurea herbicides, including diuron and linuron, and is frequently detected in freshwater environments [1,2]. It is currently being considered for potential inclusion in the 5th Watch List of the Water Framework Directive, mainly due to uncertainties regarding its predicted no-effect concentration (PNEC). 3,4-DCA has been linked to adverse effects in non-target aquatic organisms [2]. Rising temperatures may modulate contaminant toxicity by altering organismal physiology, metabolic rates, and chemical interactions, highlighting the importance of assessing their combined effects [3]. **Objective:** This study aimed to evaluate how elevated temperature, aligned with IPCC climate projections, affects the chronic sub-individual toxicity of 3,4-DCA in *Danio rerio*. **Methods:** Juvenile *D. rerio* were exposed for 28 days to environmentally relevant concentrations of 3,4-DCA (≤ 30 µg/L) under two temperature regimes representing standard testing conditions and a projected warming scenario (25 and 30 ºC). Sub-individual responses were assessed using a multi-biomarker approach targeting antioxidant and detoxification pathways, energy metabolism, neurotoxicity, and genotoxicity. **Results:** Temperature strongly modulated 3,4-DCA toxicity. Combined exposure disrupted antioxidant capacity increased oxidative stress and altered energy metabolism. Elevated temperature also affected antioxidant defenses, detoxification activity, and cellular energy allocation. At 30 ºC, significant effects of 3,4-DCA were observed, including oxidative stress (5.93–8.89 µg/L), energy imbalance (5.93 µg/L), and genotoxicity (≥ 30 µg/L). **Conclusions:** Chronic exposure to 3,4-DCA disrupts cellular defense mechanisms and metabolic processes in zebrafish, particularly at elevated temperatures. Although organisms activated compensatory antioxidant and metabolic responses, these mechanisms were insufficient to fully counteract the oxidative and metabolic disturbances induced by the combined stressors. This suggests increased energetic demands associated with maintaining detoxification and cellular homeostasis under warm conditions. Overall, these findings suggest that warming conditions may intensify the biological stress imposed by 3,4-DCA, highlighting the importance of considering temperature as a relevant factor when evaluating the ecological risks of contaminants in freshwater systems.

Keywords: Freshwater contamination; Aromatic amine; Zebrafish; Climate change; Biomarkers

Acknowledgments/Funding: This research was funded by national funds through FCT - Fundação para a Ciência e Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020). Daniela Rebelo was supported by an FCT PhD grant (2022.13777.BD).

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PC64

Assessing the Ecotoxicity of Ofloxacin: Effects on Swimming Behaviour and Morphophysiological Endpoints in *Daphnia magna*

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DOI: https://doi.org/10.48797/sl.2026.432

ABSTRACT

Background: Ofloxacin (OFL) is a widely used fluoroquinolone antibiotic that is frequently detected in aquatic environments due to its persistence and limited removal in wastewater treatment plants [1]. Its environmental presence raises concerns about potential adverse effects on non-target organisms. **Objective:** This study aimed to evaluate the ecological risk of OFL in *Daphnia magna* as a freshwater invertebrate model, by examining swimming behaviour and morphophysiological endpoints. **Methods:** *D. magna* neonates (<24 h) were exposed to an environmentally relevant (1 $\mu g \cdot L^{-1}$) and a 100-fold higher (100 $\mu g \cdot L^{-1}$) concentration of OFL for 9 days, with 5 replicates per treatment. After exposure, swimming behaviour (swimming speed, swimming activity, and total distance) was determined by analysing 1-min video recordings. Additionally, morphophysiological parameters (body size, heart size, and area) were also evaluated. **Results:** Results showed a significant reduction in both swimming speed and activity at the highest concentration (100 $\mu g\ L^{-1}$), indicating an impairment of locomotor activity, a behavioural alteration previously documented in *Daphnia* exposed to OFL and other fluoroquinolones [2, 3]. However, no significant changes were observed in total distance travelled. Furthermore, a significant decrease in body size was registered at both tested concentrations, aligning with morphological impairments reported for similar antibiotics [2, 3]. **Conclusions:** These findings revealed that OFL can impair swimming activity and highlighted that even environmentally relevant concentrations may interfere with the normal development in *D. magna*. Further studies are currently ongoing to elucidate the mechanisms underlying these sub-lethal effects and their ecological implications.

Keywords: ofloxacin; *Daphnia magna*; sub-lethal effects

Acknowledgments/Funding: ARC acknowledges the FCT PhD grant (2024.00409.BD). This work was supported by national funds through the FCT/MCTES (PIDDAC), under the project STAR -STereoselective environmental processes in Antibiotics: role for Resistance (DOI: 10.54499/2022.02842.PTDC). Additionally, this work was financially supported by Fundação para a Ciência e a Tecnologia, I.P./MECI through national funds: LSRE-LCM, UID/50020/2025 (https://doi.org/10.54499/UID/50020/2025); ALiCE, LA/P/0045/2020 (https://doi.org/10.54499/LA/P/0045/2020), project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.CBMA (UID/04050/2025, https://doi.org/10.54499/UID/04050/2025); and ARNET (LA/P/0069/2020, https://doi.org/10.54499/LA/P/0069/2020). Also, this work received financial support through annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). ARL acknowledges the support from FCT in the form of a researcher position (http://doi.org/10.54499/2022.00184.CEECIND/CP1733/CT0001).

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PC65

Ecotoxicological Effects of the Plant Protection Product NATIVO on Non-Target Terrestrial Plants: an Integrated Toxicity Index Approach

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DOI: https://doi.org/10.48797/sl.2026.433

ABSTRACT

Background: The ecotoxicological assessment of fungicides is essential for understanding their potential impacts on non-target organisms and for supporting environmental risk assessment [1,2]. In this context, the fungicide NATIVO is widely used in agriculture to control fungal diseases in various crops. However, intensive use may promote the dispersion of active compounds into soils, posing a potential risk to non-target organisms such as terrestrial plants. **Objective**: To evaluate the phytotoxicity effects of the fungicide in four terrestrial plants (*Pennisetum glaucum*, *Triticum aestivum*, *Lactuca sativa*, and *Raphanus sativus*) using germination, development, and growth endpoints. **Methods**: Plant bioassays were performed according to the guidelines of OECD 208 and ISO 18763, for 72h under controlled conditions in the dark. For each species, 8 concentrations (1-93 µL/L) of the commercial product NATIVO were evaluated, with 6 replicates per treatment, and 10 seeds per replicate. After exposure, parameters related to germination and early seedling development were evaluated. Subsequently, for each parameter and treatment, the percentage of effect was calculated. Based on these values, the doses were classified into toxicity indices (non-toxic, moderately toxic, and toxic), following the ecotoxicological thresholds related to EC_{10} and EC_{50} [3]. Finally, a toxicity index was estimated based on the integrated response of the evaluated parameters for each species. **Results**: In general, the most pronounced effects were observed in the root and shoot growth parameters. For root growth, *L. sativa* and *T. aestivum* exhibited inhibition percentages exceeding 50%, with NATIVO classified as toxic for this parameter and species. For shoot growth, *L. sativa*, *R. sativus*, and *P. glaucum* exhibited inhibition percentages exceeding 50%, and NATIVO was also classified as toxic for this endpoint and species. The integrated analysis indicated greater sensitivity of *L. sativa*, followed by *R. sativus*, *T. aestivum*, and *P. glaucum* to NATIVO. **Conclusions**: NATIVO exposure can significantly impair early development, particularly growth-related parameters of terrestrial plants. Residual contamination in soils may therefore affect plant establishment and regeneration processes. These findings underscore the importance of considering diverse plant species in ecotoxicological studies to provide a more realistic assessment of the environmental risks associated with fungicide use.

Keywords: fungicides; terrestrial plants; environmental risk

Acknowledgments/Funding: This work is funded by national funds through the FCT – Foundation for Science and Technology, I.P., within the scope of the project Clime (Bio)Pest: The Hidden Impact of Plant Protection Products: Ecotoxicity and Climate Change Interactions (ref: 2024.15964.PEX; DOI https://doi.org/10.54499/2024.15964.PEX).

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PC66

Ecotoxicological Effects of Nadifloxacin on the Swimming Behaviour of *Daphnia magna*

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DOI: https://doi.org/10.48797/sl.2026.434

ABSTRACT

Background: Nadifloxacin (NDFX) is a chiral fluoroquinolone widely used as a topical treatment for inflammatory acne lesions [1] and is frequently detected in aquatic environments due to its persistence and inefficient removal in wastewater treatment plants [2, 3]. Its environmental presence raises concerns about potential enantioselective effects on non-target aquatic organisms. However, toxicity data for freshwater invertebrates such as *Daphnia magna* remain scarce, highlighting the need to evaluate its potential ecological risks. **Objective:** This study aimed to assess the potential sub-chronic effects of NDFX racemate and its individual enantiomers on *Daphnia magna* by evaluating swimming behaviour endpoints. **Methods:** Sub-chronic exposure assays initiated using neonates (<24 h) exposed for 9 days to 100 µg.L^{-1} of racemic NDFX or each isolated enantiomer. Each treatment consisted of 5 replicates, with 20 organisms per replicate. After the exposure period, swimming behaviour endpoints (swimming speed, swimming activity, and total distance travelled) were assessed through the analysis of 1-min video recordings. **Results:** No significant changes in swimming behaviour endpoints were observed in organisms exposed to either NDFX racemate or its isolated enantiomers. **Conclusions:** Overall, NDFX exposure did not cause significant changes in swimming behaviour in *Daphnia magna*, and no enantioselective effects were observed under the tested conditions. Further studies are required to clarify the potential mechanisms of NDFX toxicity in aquatic organisms.

Keywords: nadifloxacin; *Daphnia magna*; swimming behaviour

Acknowledgments/Funding: RV acknowledges the project 10.54499/2022.02842.PTDC for the research grant. ARC acknowledges the FCT PhD grant 2024.00409.BD. This work was financially supported by national funds through the Fundação para a Ciência e a Tecnologia FCT/MECI (PIDDAC), under the project STAR - STereoselective environmental processes in Antibiotics: role for Resistance (DOI: 10.54499/2022.02842.PTDC). Additionally, this work was financially supported by FCT, I.P./MECI through national funds: LSRE-LCM, UID/50020/2025 (https://doi.org/10.54499/UID/50020/2025); ALiCE, LA/P/0045/2020 (https://doi.org/10.54499/LA/P/0045/2020), the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB; CBMA (UID/04050/2025, https://doi.org/10.54499/UID/04050/2025) CITAB (UIDB/04033/2023); and ARNET (LA/P/0069/2020, https://doi.org/10.54499/LA/P/0069/2020). Also, this work received financial support through annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). ARLR acknowledges the support from FCT in the form of a researcher position (http://doi.org/10.54499/2022.00184.CEECIND/CP1733/CT0001).

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PC67

Volatile Chemical Fingerprinting of Alternative Tobacco Products: Insights into Electronic Cigarette and Heated Tobacco Aerosol Composition

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DOI: https://doi.org/10.48797/sl.2026.436

ABSTRACT

Background: Next-generation nicotine delivery systems, including electronic cigarettes (E-cig) and heated tobacco products (HTP), are often promoted as reduced-risk alternatives to conventional tobacco (CT). However, the extent to which they limit user exposure to harmful chemicals remains insufficiently characterized [1]. **Objective:** In this study, we compared the volatile organic compounds (VOCs) present in native materials (pre-heating) with those emitted during the heating or combustion of E-cigs, HTPs, and CTs. **Methods:** For each product category, the three most commercially popular brands in Portugal were selected. Native matrices (138 ± 3.84 mg of tobacco or e-liquid) were sealed in glass vials for analysis, while aerosols/smoke were produced under controlled puffing or smoking conditions using standardized machine-smoking protocols [2,3] and collected in dedicated flasks. VOCs from native materials and aerosol/smoke were extracted via headspace solid-phase microextraction (HS-SPME) and dichloromethane solvent extraction, followed by gas chromatography–mass spectrometry (GC–MS). **Results:** More than 100 compounds were identified across all products. The volatile profiles of unheated HTP sticks and CT shared notable similarities, with ketones, alcohols, terpenoids, and pyridine derivatives dominating. In contrast, e-liquids displayed more chemically diverse signatures enriched in alcohols, esters, pyranones, and lactones. Thermal processing substantially reshaped these profiles, generating VOCs which were absent from the native materials. HTP aerosols showed additional aldehydes and ketones, E-cig aerosols contained newly esters, and CT combustion produced benzenoid compounds and polycyclic aromatic hydrocarbons, which were not detected in E-cig or HTP aerosols. **Conclusion:** Importantly, many VOCs detected in E-cig and HTP aerosols currently lack hazard classification under the Globally Harmonized System (GHS), underscoring major gaps in toxicological knowledge. Overall, these findings demonstrate that thermal processes induce extensive chemical transformations across all product types, highlighting the need for comprehensive toxicological evaluation of both known and uncharacterized constituents to support evidence-based regulatory policies.

Keywords: electronic cigarettes; heated tobacco products; volatile chemical composition; temperature-induced compound formation

Acknowledgements/Funding: This work was funded by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - *Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos*, and the project 2024.13406.PEX (DOI identifier 10.54499/2024.13406.PEX). The Cooperativa de Ensino Superior Politécnico e Universitário (CESPU) is gratefully acknowledged for the PhD grant no. *BD/DCB/CESPU/02/2023* to Vânia Monteiro.

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PC68

Evaluation of the Enantioselective Neurotoxicity of MDPV in Zebrafish Larvae

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DOI: https://doi.org/10.48797/sl.2026.435

ABSTRACT

Background: The rising recreational use of synthetic cathinones (SC), especially among youth [1], has led to their detection in aquatic environments at ng–µg L^{-1} levels [2], posing potential risks to freshwater vertebrates [3]. As SC are designed to act on the nervous system, their presence in the environment may cause unpredictable adverse effects in non-target organisms [1]. Among these compounds, 3,4-methylenedioxypyrovalerone (MDPV) has been identified in wastewater and aquatic systems [2], but its enantioselective ecotoxicological effects remain poorly understood. **Objective:** This work aimed to evaluate the behavioral effects of racemic MDPV ((*R*,*S*)) and its enantiomers ((*R*) and (*S*)) in early life stages of zebrafish (*Danio rerio*). **Methods:** Embryos (≈ 3-hours post-fertilization (hpf)) were exposed to MDPV forms (0.18−2.8 µg L^{-1}) for 96-h at 28 °C, using 50 animals per concentration and control group (5 replicates). Larvae behavior was assessed at 120-hpf in a random subsample of 5 individuals per concentration and replicate, evaluating locomotion and avoidance responses. **Results:** (*R*,*S*)-MDPV mainly induced hyperlocomotion, increasing speed and activity, along with reduced center exploration. (*R*)-MDPV produced hypoactivity, whereas (*S*)-MDPV caused pronounced locomotor suppression, altered spatial exploration, and impaired avoidance behavior. Clear enantioselective differences were observed, with (*S*)-MDPV emerging as the most neurotoxic, while the racemate generally showed lower toxicity than the individual enantiomers. **Conclusions:** MDPV disrupts zebrafish larval neurobehavior in a concentration-dependent and enantioselective manner, with (*S*)-MDPV being the most toxic. These findings underscore the importance of considering chirality in environmental risk assessments of psychoactive contaminants, as behavioral alterations can compromise survival by reducing predator avoidance.

Keywords: chiral psychoactive drugs; 3,4-methylenedioxypyrovalerone; ecotoxicity; *Danio rerio*

Acknowledgments/Funding: Pérez-Pereira A. and Ribeiro O. acknowledge the FCT PhD grant, 2022.09843.BD and 2022.12242.BD, respectively. Funding: This work was financially supported through the FCT/MCTES by national funds (PIDDAC): ENANTIOTOX project (doi: 10.54499/PTDC/CTA-AMB/6686/2020); STAR project (doi: 10.54499/2022.00184.CEECIND/CP1733/CT0001); UID/04378/2025 and UID/PRR/04378/2025 – UCIBIO; LA/P/0140/2020 – Associate Laboratory i4HB; UID/04033/2025 and LA/P/0126/2020 – CITAB, INOV4Agro; UID/04423/2025, UID/PRR/04423/2025, and LA/P/0101/2020 – CIIMAR/CIMAR LA. Also, this work received financial support through the annual funding of 1H-TOXRUN of the IUCS-CESPU.

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PC69

3-Chloromethcathinone Abiotic Degradation Studies – Preliminary Data

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DOI: https://doi.org/10.48797/sl.2026.437

ABSTRACT

Background: New Psychoactive Substances (NPSs), such as the synthetic cathinone 3-chloromethcathinone (3-CMC), have raised concerns regarding the potential social and health risks they may pose [1,2]. 3-CMC is a chlorinated derivative of methcathinone that has gained prevalence in the illicit market following the legal control of its analogues [1]. Due to the inefficient removal by wastewater treatment plants, this NPS and/or its degradation products frequently reach the surface waters, being a potential threat to non-target organisms [3]. Although its toxicity has been documented, there is a critical knowledge gap regarding its environmental degradation/transformation and enantioselective ecotoxicity [2]. **Objective:** This study aims to evaluate the enantioselective stability and degradation kinetics of 3-CMC under controlled conditions, in accordance with Organisation for Economic Co-operation and Development (OECD) guideline 111, with a focus on the influence of pH on its degradation and the potential occurrence of enantioselective transformation. **Methods:** For the 9-day hydrolysis assay, ultrapure water buffered at pH 4, 7, and 9 was spiked with racemic 3-CMC at 26 $mg.L^{-1}$ (n = 3) and incubated at 25 °C and at room temperature under constant agitation. Aliquots were collected at intervals (days 0, 1, 2, 5, 7, 9) and analysed by high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD), using a Lux® 3 µm AMP (150 × 4.6 mm) chiral analytical column. The mobile phase consisted of methanol/5 mM ammonium bicarbonate, at a flow rate of 1.0 $mL.min^{-1}$. **Results:** Under non-buffered control conditions, both enantiomers exhibited minimal degradation rates after 9 days, with 3.29% for the first eluted enantiomer (E1) and 4.95% for the second eluted enantiomer (E2). At pH 4, 3-CMC remained highly stable, with only 2.8% and 1.8% degradation rates for E1 and E2 at day 9, respectively. In contrast, degradation increased markedly at neutral and alkaline pH. At pH 7, E1 degraded by 46.9%, while E2 underwent complete degradation. At pH 9, both enantiomers underwent further extensive degradation reaching 89.5% for E1 and 91.9% for E2. **Conclusions:** Overall, the data obtained reveal a strong pH-dependent and enantioselective hydrolysis, with E2 degrading more extensively than E1, under neutral and alkaline conditions. These results highlight the relevance of enantioselectivity for an accurate environmental risk assessment.

Keywords: new psychoactive substances; 3-chloromethcathinone; enantioselectivity; degradation kinetics

Acknowledgments/Funding: This research was funded by FCT-Portuguese Foundation for Science and Technology, through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX); and supported by 1H-TOXRUN from Instituto Universitário de Ciências da Saúde (IUCS-CESPU) and FCT in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. Ivan Langa acknowledges the PhD research grant provided by the FCT, with the project reference 2022.12874.BD and DOI identifier https://doi.org/10.54499/2022.12874.BD.

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PC70

Forensic Dating of Blood Stains: Integrated Analysis by FTIR, pH and Catalysis Activity in Different Matrices

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DOI: https://doi.org/10.48797/sl.2026.438

ABSTRACT

Background: The dating of blood stains represents one of the most relevant challenges in forensic science investigation. Estimating the time elapsed since the deposition of evidence can provide crucial information for reconstructing criminal events [1]. In recent years, spectroscopic techniques have emerged as promising tools for analyzing aged blood stains, with Fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR) standing out due to its speed, non-destructive nature, and ability to identify molecular changes over time [2, 3]. **Objective:** The present study investigates how different surfaces - wood, glass, 100% cotton tissues and metal - influence the biochemical evolution of blood stain aging for forensic dating purposes, using an analytical approach based on ATR-FTIR spectroscopy and complementary biochemical methods. **Methods:** Human blood samples (50 µL) were deposited on glass, metal, wood, and fabrics. ATR-FTIR spectroscopy, pH measurement, and catalase assays were used to monitor molecular and enzymatic changes over 30 days under controlled conditions. **Results:** Hemoglobin degradation and pH variations exhibited distinct trajectories between different surfaces, particularly on 100% cotton tissues. Substrates such as wood and fabric accelerated or altered oxidation patterns compared to glass and metal. **Conclusions:** The integration of spectroscopic and enzymatic techniques, added to pH and catalase activity studies, seem to be able to help in the development of surface-specific chronological models, increasing the precision of time-since-deposition estimates.

Keywords: blood stains; ATR-FTIR; catalysis; substrate effect; pH

Acknowledgments/Funding: This research was funded by Pfizer® the Global Medical Grants—Reference 60395063, by 1H-TOXRUN-CESPU "PhytoCandd_GI2-CESPU-2023" and "ToxiBug4Ca-GI2-CESPU-2024" (CESPU internal funds), 2024.13432.PEX (national FCT funds), LA/P/0045/2020 (ALiCE), UIDB/00511/2020, and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC).

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PC71

Alternative Osteometric Approaches for Sex and Stature Estimation Using Fragmentary Femoral Elements

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DOI: https://doi.org/10.48797/sl.2026.439

ABSTRACT

Background: In forensic contexts, human remains are frequently recovered in a fragmentary state due to taphonomic processes, trauma, disasters, or deliberate body disposal [1,2]. Under such circumstances, traditional anthropological methods that rely on complete skeletal elements can't always be applied [3]. Thus, the development of alternative approaches capable of estimating the biological profile from incomplete skeletal elements is of considerable importance in forensic anthropology. **Objective:** This study aims to evaluate the potential of several femoral measurements to estimate sex and stature when only partial femoral elements are available. **Methods:** A sample of femora with known biological profiles was analyzed using osteometric measurements of the proximal femur and diaphysis. Variables included midshaft perimeter (PM), vertical diameter of the femoral head (DVC), transverse diameter of the femoral head (DTC), femoral neck length (FNAL), and femoral neck width (FNW). Sex differences were evaluated using descriptive statistics and t-tests. The relationship between these variables and stature was assessed through Pearson correlation. **Results:** All measurements showed higher mean values in males, reflecting sexual dimorphism. A statistically significant difference between sexes was observed for the midshaft perimeter (PM), indicating greater diaphyseal robustness in males. Stature estimates ranged from 146.9 to 171.5 cm (mean = 157.5 cm). Among the variables analyzed, the vertical diameter of the femoral head (DVC) demonstrated the strongest correlation with stature (r = 0.92), followed by the transverse diameter (DTC; r = 0.87) and femoral neck length (FNAL; r = 0.84). These results indicate that proximal femoral dimensions may provide reliable proxies for stature estimation when femoral length is unavailable. **Conclusions:** The findings suggest that femoral head and neck measurements can contribute significantly to sex and stature estimation in fragmentary skeletal remains. Such alternative osteometric approaches may improve the reconstruction of the biological profile in forensic cases where complete long bones are not preserved.

Keywords: forensic anthropology; biological profile; fragmentary remains

Acknowledgments/Funding: This research received no external funding.

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PC72

Cranial Anatomical Variants: Frequency of Foramina Depicted by the Human Skull

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DOI: https://doi.org/10.48797/sl.2026.440

ABSTRACT

Background: The human skull depicts a great number of anatomical variations namely in the number and morphology of foramina, grooves, ossicles, sutures and tubercules [1]. Despite their value in clinical and surgical investigations, as well as in trauma analysis and personal identification of human remains [1,2,3,4], no uniformization exists on the data of the frequency of these traits. **Objective:** This work aims to review the reported frequency of selected cranial foramina in different populations and document their presence in the Identified Skeletal Collection of CESPU (CEIC). **Methods:** The following keywords were used to scope PubMed® with a time-limit of 26 years: "Dry human skulls" AND "mental foramen" OR "mastoid foramen" OR "nasal foramen" OR "occipital emissary foramen" OR "meningo-orbital foramen" OR "supraorbital foramen variation" OR "infraorbital foramen variations" OR "parietal foramen" OR "zygomaticofacial foramen". Reviews, case reports and studies conducted only with imaging technology were excluded. In addition, a preliminary observational screening was conducted on skulls from the CEIC to record the presence of the traits reported in literature. **Results:** A total of 52 papers were considered, concerning the accessory mental (AMF), mental (MF), meningo-orbital (MOF), supra (SOF) and infraorbital (IOF), parietal (PF), nasal (NF), mastoid emissary (MEF), occipital (OF) and zygomatic (ZF) foramina. Regarding the MF, most studies focus on the frequency of the AMF, which is overall low. Differences in the MOF were observed within the same population. One-sided absence of the SOF has been reported. The IOF was present in all studies. A relevant percentage of skulls presented no NF in the only study assessed. Different populations show, overall, a high frequency of the MEF and the ZF. The preliminary screening of skulls (n=15) from the CEIC confirmed the presence of several of the above-mentioned traits, including those affecting the frontal, parietal, orbital, maxillary, and occipital regions. These traits were observed in different frequencies within the screened sample, with higher proportions in the parietal and frontal regions and lower in the temporal and mandibular regions. **Conclusions:** The frequency of cranial foramina is variable across different countries. Preliminary observations in the CEIC confirm the presence of these variants and highlight their potential relevance as complementary non-metric traits for forensic human identification.

Keywords: non-metric traits; foramina; frequency; forensic human identification

Acknowledgments/Funding: This research received no external funding.

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PC73

Skeletal Trauma Patterns in Falls: An Illustrative Case from the CEIC Identified Skeletal Collection

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DOI: https://doi.org/10.48797/sl.2026.441

ABSTRACT

Background: Falls represent one of the most common causes of accidental trauma and death, particularly among elderly individuals (1). In forensic anthropology and forensic pathology, the identification of fracture patterns compatible with falls is essential for reconstructing the mechanism of injury and differentiating accidental trauma from other causes such as interpersonal violence (2). Documented skeletal collections provide valuable material for illustrating and studying trauma patterns under known contextual information. **Objective:** The aim of this work is to present an illustrative case from the Identified Skeletal Collection of CESPU (CEIC) demonstrating skeletal lesions consistent with fractures typically associated with falls, and to compare the observed injuries with patterns described in the forensic and clinical literature. **Methods:** A documented individual from the CEIC presenting skeletal trauma compatible with fall-related injuries was selected. A macroscopic analysis of the skeleton was performed in to identify fracture location, morphology, and distribution. The observed lesions were then compared with trauma patterns commonly described in the literature for accidental falls. **Results:** The analyzed case presented fractures affecting anatomical regions frequently involved in fall-related trauma. These included lesions in the cranial region and in skeletal elements of the upper limbs that are commonly associated with protective reactions during impact. The distribution and morphology of the fractures were consistent with blunt force trauma produced by a fall (3). When compared with previously reported patterns in the literature, the injuries observed in this individual correspond to typical skeletal manifestations described in accidental fall scenarios (4). **Conclusions:** This illustrative case highlights the potential of documented skeletal collections for demonstrating characteristic trauma patterns associated with specific mechanisms of injury. The comparison with previously reported cases supports the interpretation of the observed lesions as compatible with falls and emphasizes the relevance of skeletal trauma analysis in forensic investigations.

Keywords: falls; skeletal trauma; fracture patterns; forensic anthropology; identified skeletal collections

Acknowledgments/Funding: This research received no external funding.

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PC74

Intracellular pH Profile of Platelets as a Potential Biomarker of Postmortem Interval: Preliminary Results

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DOI: https://doi.org/10.48797/sl.2026.442

ABSTRACT

Background: One of the major challenges in forensic science is the precise determination of the postmortem interval (PMI). Several methods have been proposed to estimate PMI; however, many of them present significant limitations and large margins of error. Consequently, the development of more accurate and reliable approaches remains a major challenge in forensic science [1]. In this context, blood biomarkers have attracted increasing attention as potential tools for improving PMI estimation [2]. As platelets respond rapidly to changes in the microenvironment, leading to the production of metabolites that can modify their intracellular pH (pHi), postmortem assessment of the pHi profile of platelets promises to be a useful tool for PMI determination. **Objective**: To validate an experimental flow cytometry assay for platelet pHi determination. **Methods**: The BCECF-AM (2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester) fluorescent probe was used [3]. This probe emits green or yellow fluorescence depending on the pH value. The test was performed on whole blood (anticoagulant: sodium citrate) diluted in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. Ammonium chloride (10 mM) and sodium propionate (100 mM) were used as controls for intracellular acidification and alkalinization. **Results**: The concentration of the fluorescent probe was optimized, obtaining values between 2mM and 4mM. Subsequently, kinetic assays were performed to evaluate the effect of pHi modifying agents on blood platelets. The assays showed an increase in probe fluorescence with ammonium chloride and a decrease with sodium propionate. **Conclusions**: Flow cytometry revealed to be a suitable methodology for analyzing platelet pHi. Indeed, the experimental conditions tested made it possible to detect differences in the pHi of blood platelets induced by acidifying and alkalizing agents. These preliminary findings provide a solid foundation for further research; *in vitro* and *vivo* studies, assessing the potential ability of platelet pHi profile to estimate PMI, are needed to validate its potential applicability in forensic scenarios.

Keywords: postmortem interval, cellular biomarkers, platelet pHi; flow cytometry; technique validation

Acknowledgments/Funding: This work received no external funding.

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PC75

The Dynamics of 3D Printed Projectiles: a Systematic Review

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DOI: https://doi.org/10.48797/sl.2026.443

ABSTRACT

Background: The relative ease with which it is now possible to produce ballistic components through additive manufacturing (3D-printing) brings the need to study them to understand the impact they may have in a forensic context [1]. **Objectives:** The main goal of this review is to summarize the state of the art on dynamical studies of 3D-printed projectiles for light weapons. **Methods:** To produce this review, a search was conducted using the keywords "3D printed projectiles" and "low caliber projectiles" on the ResearchGate, Google Scholar, and Wiley Online Library databases. The Boolean operators "!" and "AND" were used with those keywords. The initial search was conducted without any date restrictions. Subsequently, the number of relevant articles was narrowed down, limiting publications to dates between 2023 and 2025. **Results:** To obtain information about comparative tests between 3D printed projectiles and conventional projectiles, three articles with similar themes were analyzed. Bisić et al. 2025 [2] and Vandenburgh 2025 [3] show that projectiles produced by additive manufacturing can be fired with firearms and maintain structural integrity. The muzzle velocity and the corresponding kinetic energy are smaller for printed projectiles than for conventional ones [2], typically characteristic of aged or poorly stored ammunition. Integrity during flight was also ascertained, mainly through observation of impact marks on [2, 3]. It was demonstrated that some types of "homemade" 3D-printed projectiles can effectively penetrate materials (that simulate human skin) and even be lethal (theoretically) depending on the energy density at the moment of impact [1]. It is also described that variations in projectile printing quality may affect flight stability, leading to a relatively high velocity dispersion and impact angles [2]. However, the results described are not sufficient to provide a thorough understanding of the external/terminal ballistics of 3D-printed projectiles. **Conclusions:** In conclusion, the external/terminal ballistics of 3D-printed projectiles should be studied further and in greater depth, especially aspects that are poorly explored in the articles mentioned, such as flight stability, velocity, and kinetic energy at muzzle and throughout the trajectory, to optimize forensic protocols.

Keywords: 3D printed low caliber projectiles; forensic protocols; energy density; kinetic energy

Acknowledgments/Funding: This research was funded through annual funding from 1H-TOXRUN at Instituto Universitário de Ciências da Saúde (IUCS-CESPU).

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PC76

Assessing Ash-Derived Contaminants in Freshwaters Following Wildfires in Northern Portugal

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DOI: https://doi.org/10.48797/sl.2026.444

ABSTRACT

Background: Wildfires are increasing in frequency and intensity worldwide, with Portugal recording the second-largest burned area during the summer of 2025 [1]. Wildfire-derived ash acts as reservoir of contaminants, releasing trace elements and other potentially toxic compounds that may alter water chemistry when mobilized through surface runoff during post-fire precipitation events [2]. Despite this threat, the impact of ash-derived pollutants on freshwater systems in Northern Portugal remains insufficiently characterized, particularly regarding environmental forensic and public health implications [3]. **Objective:** This study aimed to evaluate key physicochemical parameters (nitrate, pH, and electrical conductivity) associated with ash-derived contamination in wildfire-affected areas, comparing pre- and post-precipitation conditions. **Methods:** Surface water samples were collected from six wildfire-affected sites in Northern Portugal (Lousada, Penafiel, and Paredes) on 17 October 2025 (pre-rainfall baseline) and 11 December 2025 (post-rainfall) to assess the effects of runoff-driven contaminant mobilization. Nitrate concentrations were determined using UV–Vis spectrophotometry (Unicam UV/Vis spectrophotometer, ATI Unicam) and expressed in mg/L. pH and electrical conductivity were measured using a Crison GLP21 pH meter and a Crison GLP31 conductimeter, respectively. Data were analyzed using Jamovi (v2.6.44) and are presented as mean ± standard error of the mean (SEM). **Results:** Mean nitrate concentrations across all sites increased slightly from 0.14 ± 0.04 mg/L (pre-rainfall) to 0.16 ± 0.04 mg/L (post-rainfall), although this difference was not statistically significant ($p > 0.05$). Similarly, pH values showed a non-significant increase from 6.15 ± 0.11 to 6.48 ± 0.26 ($p > 0.05$). In contrast, electrical conductivity decreased from 173.2 ± 45.7 to 153.0 ± 45.1 µS/cm, also without statistical significance ($p > 0.05$). Site D3 exhibited the highest pre-rainfall values (nitrate: 0.29 mg/L; pH: 6.43; conductivity: 321 µS/cm), whereas post-rainfall maxima were observed at site D1 (nitrate: 0.39 mg/L; pH: 7.07; conductivity: 365 µS/cm). **Conclusions:** Post-fire rainfall induced only minor, non-significant changes in water quality, though site-specific variability highlights the need for continued monitoring.

Keywords: wildfires; ash-derived contamination; surface water quality

Acknowledgments/Funding: This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB and through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). CESPU for the grant BioMPreg-GI2-CESPU-2025 and TRYP-MAP-GI2-CESPU-2025

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PC77

mtDNA Polymorphisms for the Differentiation of Forensically Relevant *Calliphoridae* Species

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DOI: https://doi.org/10.48797/sl.2026.445

ABSTRACT

Background: Forensic entomology estimates the minimum post-mortem interval (minPMI) by identifying insect species colonising remains. Within Calliphoridae, closely related taxa (e.g., *Lucilia*, *Calliphora*, *Chrysomya*) exhibit overlapping distributions and limited morphological differentiation, particularly in immature or degraded samples, increasing the risk of misidentification and affecting minPMI estimates [1]. Molecular markers such as COI and CytB are widely used, but sequencing remains time-consuming and costly for routine casework. **Objective:** To identify polymorphic sites in COI and CytB genes across selected Calliphoridae species and select candidate SNPs for developing a rapid, cost-effective SNaPshot assay for species identification. **Methods:** More than 20 sequences per species (*L. sericata*, *L. cuprina*, *L. caesar*, *L. illustris*, *C. vicina*, *C. vomitoria*, *C. albiceps*, *C. megacephala*) were retrieved from GenBank. Sequences were aligned in Geneious and analysed phylogenetically in MEGA12. Multiple sequences per species ensured consistency. Polymorphisms were screened and filtered to retain sites conserved within species and variable between species as candidate SNPs. **Results:** COI showed consistent interspecific variation. The highest polymorphism occurred in *C. albiceps/C. megacephala* (27 sites), followed by *C. vicina/C. vomitoria* (21). Moderate variation was observed in *L. caesar/L. illustris* (5), and minimal in *L. sericata/L. cuprina* (3). These sites were species-conserved and interspecifically variable, supporting their diagnostic use. CytB showed limited variability, with informative sites only in *C. vicina/C. vomitoria* (10), and none in other pairs. **Conclusions:** COI provides robust diagnostic polymorphisms for Calliphoridae species identification, while CytB offers complementary resolution in specific taxa. These results support the development of a targeted SNP panel for rapid, accurate, and cost-effective forensic identification. Additional markers (e.g., ITS2, 28S rRNA, NAD4, NAD6) may improve resolution in closely related species [2,3]. Reliance on public sequences may underrepresent intraspecific variability, highlighting the need for validation with new data. Future work includes specimen collection, DNA sequencing, SNP validation, and design of species-specific tailed primers for SNaPshot, establishing a scalable framework to improve identification accuracy and efficiency in forensic entomology.

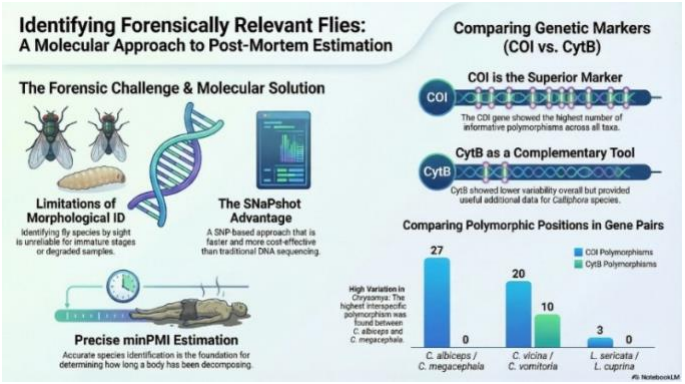

Figure 1. COI and CytB polymorphisms as markers for molecular identification of forensically relevant blowflies.

Keywords: Calliphoridae; forensic entomology; SNP

Acknowledgements/Funding: This research was funded through annual funding from 1H-TOXRUN at Instituto Universitário de Ciências da Saúde (IUCS-CESPU).

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PC78

Impact of Capecitabine on Fingerprints in Oncologic Patients: A Literature Review

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DOI: https://doi.org/10.48797/sl.2026.448

ABSTRACT

Background: Forensic fingerprint (FP) analysis is a key tool for human identification, based on the principles of permanence, variability, and mutability. However, emerging evidence suggests that certain pharmacological treatments, particularly in oncology, may induce alterations in dermal papillary ridges (DPRs), potentially compromising biometric reliability [1]. Chemotherapeutic agents, such as capecitabine (CAP), have been associated with Hand-Foot Syndrome (HFS) and acquired adermatoglyphia. CAP is an oral prodrug of 5-fluorouracil used to treat breast, colorectal, and pancreatic cancers, with HFS as its most characteristic adverse effect [2]. **Objective:** This study aims to review the literature on the impact of CAP on FP patterns in oncologic patients, focusing on DPRs alterations and underlying pathophysiological mechanisms relevant to forensic identification. **Methods:** A literature review was conducted using scientific databases, including PubMed, Scopus, and Web of Science. The search strategy included the following keywords: “capecitabine”, “fingerprints”, “dermatoglyphics”, “adermatoglyphia”, “hand-foot syndrome”, and “forensic identification”. Inclusion criteria comprised original articles and case reports, addressing dermatological toxicity and/or FP alterations associated with CAP therapy. Exclusion criteria included studies not involving capecitabine and non-human studies. **Results:** Dermatoglyphic alterations may range from reduced DPRs clarity to complete loss of FP patterns (acquired adermatoglyphia) [3]. These changes are often associated with HFS but may occur independently. Some cases appear reversible after treatment discontinuation, although persistent alterations have been reported. Evidence remains limited, particularly regarding longitudinal follow-up. CAP is frequently implicated, possibly due to local effects on keratinocyte proliferation and skin integrity [1]. **Conclusions:** CAP-related FP alterations are an emerging and clinically relevant issue, potentially affecting patients’ quality of life and challenging FP-based identification in forensic contexts [1-3]. Further research is needed to clarify mechanisms, prevalence, and reversibility.

Keywords: anticancer drugs; dermal papillary ridges; forensic sciences; lophoscopy; oncology

Acknowledgments/Funding: This research was funded by the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC79

Comparative Evaluation of Antioxidant Activity in Two Coastal Dune Plants from Vila do Conde (Portugal): Preliminary Data

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DOI: https://doi.org/10.48797/sl.2026.449

ABSTRACT

Background: Coastal dune systems host a diversity of plant species adapted to harsh environmental conditions, such as high solar radiation, nutrient-poor soils, and limited water availability. These constraints lead to specific ecological requirements, allowing the establishment of characteristic vegetation zones. Environmental conditions not only influence plant distribution but also modulate their phytochemical composition [1]. Therefore, studying their chemical and biological profiles is essential to explore potential pharmacological applications and support traditional uses [2]. **Objective:** This study aimed to evaluate the antioxidant activity of aqueous extracts from two costal plant species, *Medicago marina* and *Otanthus maritimus,* collected from sand dunes of Vila do Conde (Portugal). **Methods:** For both samples, plant material was collected and oven-dried at 40 ºC. Leaves were ground with a blender into a fine powder and subjected to aqueous extraction using two different procedures: extraction at 50 ºC (for 60 min) and extraction under boiling conditions (for 10 min), followed by filtration and liofilization. The dried extracts were reconstituted in water and the antioxidant activity was evaluated using the DPPH radical scavenging assay by measuring their ability to neutralize free radicals at different extracts concentrations and duplicate [3]. **Results:** At a concentration of 1000 µg/mL, *Medicago marina* exhibited relatively low inhibition percentages, with no differences observed between the extract obtained at 50ºC (4.89%) and by boiling (4.95%). In contrast, *Otanthus maritimus* showed a higher inhibition percentage, with a further enhancement in radical scavenging capacity observed in the extract obtained by boiling (50 ºC = 23.64%; boiling = 37.85%). Both species demonstrated antioxidant activity in a concentration-dependent manner, with *Otanthus maritimus* consistently exhibiting greater radical scavenging capacity than *Medicago marina.* **Conclusions:** The findings indicate that *Medicago marina* and, especially, *Otanthus maritimus* are promising sources of natural antioxidants. Further phytochemical characterization and biological studies are recommended to explore their potential pharmacological applications and support traditional uses.

Keywords: dune plants; antioxidant activity; coastal ecosystems

Acknowledgments/Funding: This research was funded by FCT-Portuguese Foundation for Science and Technology in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy- i4HB

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PC80

SNaPshot in Forensic Entomology: Molecular Identification of *Calliphoridae* (*Diptera*) Species

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DOI: https://doi.org/10.48797/sl.2026.450

ABSTRACT

Background: Forensic entomology enables estimation of the minimum post-mortem interval (minPMI) by analysing the sequence and age of insects colonising decomposing remains. Traditionally based on morphological/morphometric methods, insect identification is constrained when distinguishing closely related taxa. In these cases, relying solely on morphological characteristics is insufficient, making it challenging to accurately identify the insect species present. To surpass this limitation, molecular identification methods have been developed to support daily practice and identify insect species, such as cytochrome c oxidase subunit I (COI), which is considered the most successful gene sequence for insect identification. However, gene sequencing has its disadvantages, such as being time-consuming and expensive. Other techniques, such as SNaPshot - a minisequencing technique based on single nucleotide polymorphism (SNP) that allows species differentiation - have been proposed to improve the precision of identification of forensically important species [1,2]. **Objective:** This study aims to identify gaps in the use of SNaPshot-based analysis for the molecular identification of forensically relevant insect species, particularly closely related taxa and immature stages. **Methods:** A systematic literature review was conducted in accordance with PRISMA guidelines using Google Scholar, Web of Science, ScienceDirect, and PubMed databases. Studies published between 2020 and 2025 addressing the molecular identification of *Calliphora* spp., *Lucilia* spp., and *Chrysomya* spp. (Diptera: Calliphoridae) using SNaPshot. **Results:** Systematic research clarified grey areas in molecular identification using SNaPshot, highlighting its value for forensic important blowfly species (2020–2025). Only one addressed the distinction between *Calliphora* spp. and *Lucilia* spp., and there were no reports of *Chrysomya* spp. This gap is an opportunity to broaden knowledge and explore new techniques for successful identification [3]. **Conclusions:** The findings highlight a significant gap in the use of SNP-based techniques, such as SNaPshot, to identify forensically relevant blowfly species. Expanding molecular approaches may improve species-level identification, particularly for closely related taxa and immature stages, strengthening forensic entomology in medico-legal investigations.

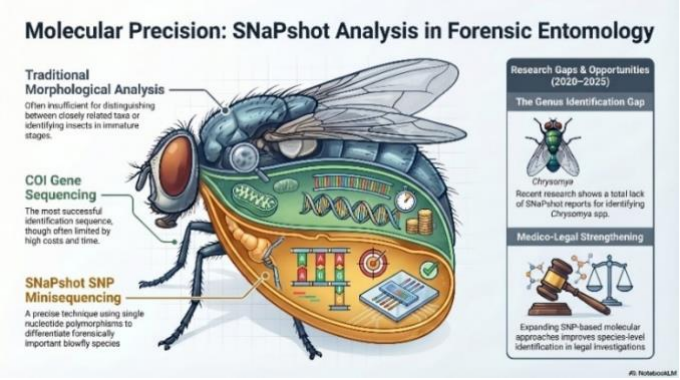

Figure 1. Systematic representation of inclusion and exclusion based on research of manuscripts across public access databases

Keywords: Calliphoridae; Diptera; molecular identification; SNaPshot; forensic entomology

Acknowledgments/Funding: This research was funded through the annual funding by 1H-TOXRUN from Instituto Universitário de Ciências da Saúde (IUCS-CESPU).

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PC81

Recovering DNA from Biological Fluids: Effects of Surface, Time and Collection Method

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DOI: https://doi.org/10.48797/sl.2026.451

ABSTRACT

Background: The forensic value of bodily fluids depends on their detection and the recovery of DNA of sufficient quality for profiling [1]. This is influenced by pre-analytical factors (e.g., substrate type, environmental exposure, time since deposition) and collection methods, which affect the persistence and interpretation of traces [2,3]. **Objective:** To review literature on DNA recovery from bodily fluids, focusing on the influence of substrate, time, environmental conditions, and sampling strategies on DNA yield and profiling success. **Methods:** A systematic review following PRISMA guidelines was conducted using PubMed, Google Scholar, and ScienceDirect. Search terms included "*bodily fluids*", "*DNA recovery*", "*DNA transfer*", "*DNA persistence*", and "*forensic analysis*". Studies published between 2021–2026, in English, full-text, and using human samples were included. After screening, 63 studies were selected for qualitative analysis. **Results:** Substrate type was a major determinant of DNA persistence and recovery. Porous materials retained DNA longer, while non-porous surfaces allowed higher initial recovery but faster loss under environmental exposure. Surface features influenced deposition and persistence; challenging materials (e.g., brass, TiO_2-coated glass) were linked to reduced recovery and poorer profiles. DNA quantity and profile completeness declined over time, with variation by fluid and conditions. Blood and semen were more stable, whereas saliva and touch DNA were more variable and technique-dependent. Environmental factors consistently drove degradation and variability. Low or negative qPCR results did not always predict STR failure. Sampling strategy strongly affected recovery: swab type, technique, wetting, and operator performance influenced yield. No single method was optimal; swabbing suited many non-porous surfaces, while tape-lifting, cutting-out, or vacuuming were better suited to specific contexts. Substrate and environmental exposure were the most consistent factors affecting recovery and profiling success. **Conclusions:** DNA recovery is context-dependent, particularly with respect to substrate, environment, time, and sampling strategy. Evidence supports adapting collection methods rather than applying uniform protocols, though further validation is needed. Variability in study design limits comparisons, highlighting the need for standardisation and controlled studies to improve forensic interpretation.

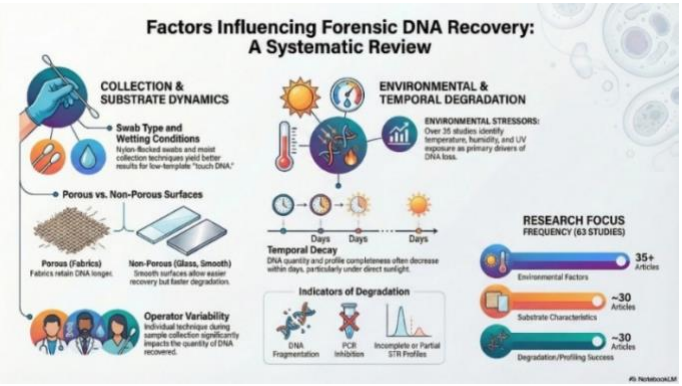

Figure 1. Overview of the main factors influencing forensic DNA recovery identified in this systematic review.

Keywords: body fluids; DNA persistence; DNA recovery; forensic genetics; surfaces

Acknowledgements/Funding: This research was funded through annual funding from 1H-TOXRUN at Instituto Universitário de Ciências da Saúde (IUCS-CESPU).

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PC82

Skin & Intestine Decomposition: *Candida albicans* and *E. coli* Contribution to Cadaveric Phenomena

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DOI: https://doi.org/10.48797/sl.2026.452

ABSTRACT

Background: The PMI determination provides valuable insights through the study of cadaveric phenomena and variations in the microbial load of decomposing tissues [1]. Microorganisms such as the fungus *Candida albicans* and the bacteria *Escherichia coli* are natural constituents of the human microbiome and can have impact on the cadaverization process. As decomposition progresses, the necromicrobiome changes, and understanding how these microbial populations evolve under different environmental conditions is important for forensic investigations [2-3]. **Objective:** This study aims to determine whether, fungal growth (*C. albicans*) on the skin tissue and bacterial proliferation (*E. coli*) in the intestine are enhanced or inhibited after death. It further investigates how environmental factors, such as heat and dryness, can influence microbial proliferation and tissue alterations. **Methods:** Cultures of *C. albicans* and *E. coli* were prepared using selective media (SDA and LBA respectively) and incubated as 37°C. Inocula were standardised to concentrations of ~1x10^8 to 10^9 cells/mL. Experimental conditions include controlled hot/dry for both and cold/humid specifically for skin. Pig skin (1cm^2) and intestinal pieces were placed in 6-well plates with RPMI-1640 medium to support microbial growth. The development was monitored and quantified through CFUs and photography at 0, 3, 24, 48 and 120 hours [2-3]. **Results:** The CFUs of *C. albicans* increased during the first 48h *postmortem*, before stabilising, suggesting that higher temperatures and humidity levels favour fungal proliferation. In contrast, regarding the *E. coli* trail, the proliferation was so extensive across all the time points that precise quantification could not be achieved, it was concluded that replication continued beyond the 120h mark. **Conclusions:** The increase in *C. albicans* skin load up to 48h appears to relate to nutrient availability, while subsequent stabilization follows nutrient reduction and the presence of toxic compounds. *E. coli* may prove useful in estimating longer PMI, although increased dilutions are required for accurate quantification. Both microorganisms may serve as tools to predict PMI, though further in-depth studies are necessary.

Keywords: *Candida albicans*; *Escherichia coli*; forensic

Acknowledgments/Funding: This research received no external funding.

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PC83

Ground Penetrating Radar Detection of Buried Explosive Devices in European Soil

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DOI: https://doi.org/10.48797/sl.2026.453

ABSTRACT

Background: The detection of buried explosive devices remains a significant challenge, particularly in the context of humanitarian demining in areas affected by armed conflict [1]. In recent years, this issue has become particularly relevant in Europe following the conflict between Russia and Ukraine, reinforcing the need to develop detection methods that are simultaneously effective, safe and operationally viable. In this context, ground-penetrating radar (GPR) has emerged as a promising technique for locating buried objects. However, its performance depends strongly on factors such as soil characteristics, object properties, and burial conditions [2]. **Objective:** Evaluate the performance of GPR in detecting explosive devices buried in sandy loam soil in European enviroment, specifically in Portugal. **Methods:** A total of 15 inert explosive devices (projectiles, grenades, mines, fuzes, and an improvised explosive device) were buried at different depths ranging from 10 to 70 cm and orientations (horizontal and vertical). Data were collected in March 2026, during the winter season, using a GPR system (Noggin SmartCart Sensors&Software Inc. 250 and 500 MHz) equipped with 250 MHz and 500 MHz antennas and processed using ReflexW software. **Results:** GPR detected most of the buried objects, with 11 of 15 targets identified using the 250 MHz antenna and 13 of 15 using the 500 MHz antenna. However, both datasets exhibited lower resolution and higher noise levels compared to data acquired in summer 2025. Non-detections observed previously persisted (60 mm mortar round by 250 MHz antenna; coastal artillery round by 500 MHz antenna), with additional targets not detected (Barcarena hand grenade, impact fuze and fuze by 250 MHz antenna; 120 mm training mortar round by 500 MHz antenna). These differences may be explained by the variability in soil moisture conditions observed in the field (1.8–5.2%), indicating spatial heterogeneity in water content. Increased moisture contributes to higher electrical losses and attenuation of the electromagnetic signal, resulting in less well-defined hyperbolas. Additionally, the heterogeneous distribution of water reduces the dielectric contrast between the targets and the surrounding medium and increases signal noise, ultimately affecting target detectability [3]. **Conclusions:** Overall, the results highlight the potential of GPR for detecting buried explosive devices in European soils, while emphasizing the influence of environmental conditions on its performance.

Keywords: buried object detection; geophysical methods; humanitarian demining; radar signal interpretation; soil properties

Acknowledgments/Funding: This research was funded by the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU). We would like to thank the GNR of Lisbon for providing the burial material and the training ground used in this study, and the GeoSmartSolution for providing the GPR equipment and the guidance team for their monitoring and scientific support throughout the work.

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PC84

The Proliferation of 3-D Printed Firearms in Latin America: An Ecosystem Analysis

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DOI: https://doi.org/10.48797/sl.2026.454

ABSTRACT

Background: The rising number of seizures of 3D-printed firearms (3DPF) in Latin America indicates a clear transition from isolated incidents to a decentralized and operational illicit production ecosystem. In Brazil, this evolution is evidenced by the proliferation of clandestine workshops, the consolidation of online distribution networks, and targeted law enforcement operations addressing both production and dissemination chains. Across the region, other countries exhibit convergent patterns of risk, indicating diffusion rather than isolated adoption [1]. The growing incorporation of 3DPF into criminal activities introduces substantial challenges for forensic identification, evidentiary standardization, and regulatory control, particularly considering the rapid pace of technological development and adaptation [2]. **Objective**: To characterize the 3DPF landscape in Latin America, focusing on Mexico, Chile, and Ecuador, while identifying Brazil as the main forensic and criminological hub. The study analyses production modes, seizure patterns, and links to organized crime. **Methods**: A qualitative content analysis was conducted using open-source data, including police reports, official press releases, regional security assessments, and peer-reviewed publications (2020–2026). Sources were screened to identify confirmed cases involving functional 3DPF, components, clandestine workshops, distribution networks, and links to organized crime. **Results and Discussion**: Brazil presents the most developed ecosystem, with widespread clandestine workshops, online commercialization, and large-scale operations. The 2026 “Shadowgun” operation dismantled a multi-state network and led to multiple arrests [1], including the leader of an extremist group linked to the Urutau platform. This hybrid 3DPF, produced in Brazil and tested in the United States, highlights the transnational dimension of this phenomenon [3]. Mexico, Chile, and Ecuador increasingly integrate 3DPF into narcotrafficking dynamics, reinforcing convergence with organized crime [4;5;6]. **Conclusions:** Latin America constitutes an uneven but interconnected 3DPF landscape. Brazil operates as the central hub, while other countries occupy different stages of adoption and control. Although Colombia and Argentina lack documented prominence, they present structural vulnerabilities and potential for future proliferation.

Keywords: 3D-printed firearms; Latin American crime; emerging technologies

Acknowledgments/Funding: This work received financial support through the annual funding of 1H-TOXRUN from the University Institute of Health Sciences (IUCS-CESPU).

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PC85

Preliminary Assessment of IED-Related Blast and Fragmentation Effects in Ballistic Gelatin

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DOI: https://doi.org/10.48797/sl.2026.455

ABSTRACT

Background: Ballistic gelatin (BG) is widely used as a soft tissue surrogate in forensic and biomedical research, particularly in studies of projectile-related trauma, enabling controlled analysis of penetration, cavitation, and energy transfer [1–3]. Although its behaviour under ballistic impact is well characterised, its application to blast- and fragmentation-related phenomena remains underexplored. Recent studies have examined fragment–gelatin interactions under controlled conditions, emphasising energy transfer and cavity formation in trauma modelling; however, approaches approximating improvised explosive device (IED) scenarios remain limited. **Objective:** To assess whether standardised BG can serve as a preliminary model for qualitative evaluation of shrapnel-related trauma under different detonation conditions, focusing on cavity morphology, penetration characteristics, fragment dispersion, and structural integrity. **Methods:** Blocks of 10% ballistic gelatin (Bloom 250A) were prepared following Jussila's protocol and validated using established calibration procedures [3,4]. Three configurations were tested: (i) exposure to fragments from a simulated defensive grenade containing metallic spheres; (ii) placement of a commercial electric detonator adjacent to the BG surface; and (iii) placement of the same detonator within the gelatin block. Post-detonation effects were documented photographically and qualitatively assessed for cavity morphology, penetration depth, fragment distribution, and matrix preservation. **Results:** The simulated grenade produced marked surface disruption and wide dispersion of metallic fragments. Surface detonations generated shallow cavities with limited fragment retention. Internal detonations resulted in deeper, more centralised cavities and greater structural disruption. Distinct and reproducible patterns of matrix alteration were observed across configurations. **Conclusions:** These preliminary assays demonstrate the feasibility of BG in blast-related experimental settings and its ability to preserve sufficient structural integrity for comparative qualitative analysis of detonation patterns. Although no quantitative measurements or statistical analysis were performed, the findings support its use as an initial model for studying IED-related blast and fragmentation effects under controlled conditions, with potential forensic and experimental applications. Further work will incorporate quantitative metrics and high-speed imaging.

Keywords: ballistic gelatin; blast injury; improvised explosive devices (IEDs); shrapnel dispersion; wound ballistics

Acknowledgments/Funding: This research was funded through annual funding from 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC86

Chemotherapy-Related Adermatoglyphia and Fingerprint Changes in Cancer Patients: a Literature Review

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DOI: https://doi.org/10.48797/sl.2026.456

ABSTRACT

Background: Human identification can rely on the biological stability of fingerprints (FP) due to their unique and permanent nature [1]. However, several oncological treatments may compromise this permanence by altering dermal papillary ridges (DPR), undermining the biometric reliability of FP-based identification and causing legal barriers [2]. Various classes of chemotherapeutic agents, including cytotoxics (antimetabolites, alkylating agents, and taxanes), targeted therapies (tyrosine kinase inhibitors), and immunotherapies, have been linked to dermatological adverse effects such as Hand-Foot Syndrome (HFS) and acquired adermatoglyphia [1]. **Objective:** Examine the effects of different chemotherapeutic classes on FP morphology, focusing on changes to DPR and the resulting challenges for forensic identification. **Methods:** A systematic search was performed across PubMed, Scopus, and Web of Science using descriptors such as "chemotherapy", "adermatoglyphia" and "forensic identification." Articles were included if they reported alterations in FP patterns due to anticancer therapy (*N*=14). Studies lacking a forensic context or unrelated to human lophoscopic morphology were excluded. **Results:** Chemotherapy-induced fingerprint alterations range from a subtle blurring of ridge definition to a complete loss of dactyloscopic patterns [3]. Although often associated with HFS from antimetabolites or multikinase inhibitors, other drug classes contribute significantly to ridge loss, such as taxanes (e.g., paclitaxel) [1]. Proposed mechanisms involve direct cytotoxic effects on keratinocyte turnover, inflammation of basal epidermal layers, and impaired skin regeneration. While recovery is often observed following treatment interruption, persistent biometric damage has been documented [1]. Data remains sparse regarding prevalence, risk factors, and long-term outcomes. **Conclusions:** Chemotherapy-induced adermatoglyphia represents a significant clinical and legal barrier that demands increased awareness among healthcare and forensic professionals [1-3]. Further investigation is essential to map the incidence, biological mechanisms, and reversibility across different chemotherapeutic classes.

Keywords: chemotherapeutic agents; dactyloscopy; dermal papillary ridges; forensic identification

Acknowledgments/Funding: This research was funded by the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC87

From Fingerprints to Spoofs: a Systematic Review of Real-Life Spoofing Cases

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DOI: https://doi.org/10.48797/sl.2026.457

ABSTRACT

Background: Fingerprints (FP) recognition has gained popularity due to its wide range of applications, from unlocking smartphones to border control systems [1,2]. However, the increasing use of FP also makes them attractive targets for malicious actors, who seek develop methods to compromise FP recognition systems. One of the main threats to these systems is spoofing attacks [1]. **Objective:** Analyse reported cases of FP spoofing, highlighting their occurrence and potential misuse. **Methods:** For this review we followed the PICO framework and PRISMA guidelines. Several studies and reports retrieved from IEEE Xplore, J-Stage, and International Journal of Computer Applications were analysed. Terms like "fingerprint" AND "spoof" OR "attack" were used. Articles older than 15 years, duplicates, editorials, and reviews were excluded. Studies that reported any real-life spoofing case were included. **Results:** Several real-world cases illustrate the practical feasibility of FP spoofing and its associated risks. In 2008, the Chaos Computer Club successfully lifted the latent FP of a German minister from a glass he used and utilized it to produce 4000 plastic spoofs [3]. In 2013, the same organisation further demonstrated the vulnerability of FP systems by recreating a FP from a high-resolution photograph and generating a functional spoof using wood glue [4]. In another case reported in 2013, a medical doctor used silicone-based spoofs to fraudulently register coworkers' attendance, thereby bypassing the biometric system of a hospital in São Paulo [1,4]. In 2014, a hacker reproduced the FP of a German politician from photographs and successfully used the fabricated FP to unlock a smartphone [2]. The analysis of these cases highlights a significant gap in the literature, namely the limited availability of detailed case reports on fingerprint spoofing, which hinders systematic research and the development of effective countermeasures. Moreover, these cases consistently demonstrate the capability of spoof to bypass existing biometric security systems. **Conclusions:** This review highlights the risks associated with the unintended exposure of FP, reinforcing the urgent need for robust protective measures, particularly in commonly used devices. Furthermore, it underscores the importance of systematic reporting of spoofing incidents, since increased documentation is essential to raise awareness, support scientific research, and guide the development of effective countermeasures.

Keywords: fingerprint recreation; illicit use; spoof attacks

Acknowledgments/Funding: This research was funded by the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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PC88

Forensic Identification from Remote Digital Sources: a Systematic Review of Fingerprint Recovery and Reliability

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DOI: https://doi.org/10.48797/sl.2026.459

ABSTRACT

Background: Fingerprint (FP) biometrics have benefited from high-resolution smartphone image sensors, allowing remote acquisition of papillary patterns without physical contact [1]. Beyond traditional scanners, social media images may contain latent biometric data recoverable for forensic investigation. Case reports confirm successful identification of suspects through FP in photographs [1, 2]. Furthermore, digital FP from uncontrolled environments or video frames can be effective for Automated Fingerprint Identification Systems (AFIS) [3]. Deep learning models further enable ridge reconstruction from low-quality samples [4]. The rising exposure of these images requires a systematic evaluation of current evidence. **Objective:** To review the literature and forensic case reports in order to assess the technical feasibility and reliability of human identification using FP from unconventional digital sources. **Methods:** This review followed the PICO framework and PRISMA guidelines. An exploratory search was conducted in PubMed, ScienceDirect, Scopus, and IEEE Xplore using the keywords: "fingerprints", "photographs", "identification", and "AFIS". Inclusion criteria were peer-reviewed forensic case reports and studies on remote capture, minutiae extraction, and AFIS integration, written in English and available in full text. Non-peer-reviewed documents, editorials, reviews, duplicates, and other biometric modalities were excluded. Analysis focused on image processing and ridge reconstruction. **Results:** Forensic case reports confirm identification via unconventional sources, such as photos of handheld objects [1] and mobile images [2], providing sufficient quality for high-confidence AFIS hits [3]. Success depends on frame selection and digital filters to preserve papillary structure despite lighting and perspective challenges [1, 5]. Advanced processing and Convolutional Neural Networks (CNNs) transform photographic fragments into valid evidence meeting law enforcement standards [3, 4]. **Conclusions:** Forensic practice is evolving with FP increasingly accessible through public digital records [1, 3]. Advances in reconstruction allow accuracy levels compatible with forensic standards [3, 5]. This highlights the need for standardized guidelines for handling biometric data from uncontrolled sources and increased awareness of privacy risks.

Keywords: biometric identification; fingerprint reconstruction; remote fingerprint capture

Acknowledgments/Funding: This research was funded by the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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ORDEM DOS FARMACÊUTICOS

Volume 1 Supplement 1 (2026)

ISSN 2795-5117

Scientific Letters

IUCS-CESPU Publishing | https://publicacoes.cespu.pt/index.php/sl/