III 1H-TOXRUN INTERNATIONAL CONGRESS 2024

No Boundaries for Toxicology: One Health, One Society, One Planet

The Big Challenges of the 21st Century

2-3 May 2024 | Porto, Portugal

SCIENTIFIC LETTERS SL

BOOK OF ABSTRACTS

Volume 1 Supplement 1 (2024)

ISSN 2795-5117

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

TOX RUN

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https://doi.org/10.48797/sl.2024.261

For citation purposes, cite each abstract independently as indicated on the abstract page at https://publicacoes.cespu.pt/index.php/sl/issue/view/8.

ONE HEALTH
TOXICOLOGY RESEARCH UNIT

III 1H-TOXRUN INTERNATIONAL CONGRESS 2024

NO BOUNDARIES FOR TOXICOLOGY: ONE HEALTH, ONE SOCIETY, ONE PLANET

THE BIG CHALLENGES OF THE 21ST CENTURY

2ND – 3RD OF MAY'24
PORTO / PORTUGAL

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ONE HEALTH TOXICOLOGY RESEARCH UNIT

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III 1H-TOXRUN International Congress 2024

NO BOUNDARIES FOR TOXICOLOGY:
ONE HEALTH, ONE SOCIETY, ONE PLANET

THE BIG CHALLENGES OF THE 21ST CENTURY

02-03 MAY, 2024 | PORTO, PORTUGAL

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Organizing Entities

- 1H-TOXRUN – One Health Toxicology Research Unit
- IUCS-CESPU – Instituto Universitário de Ciências da Saúde, CESPU

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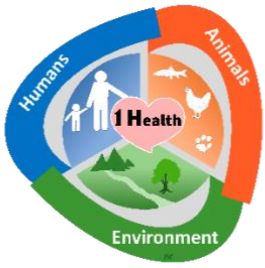

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EDITORIAL

EDITORIAL

A tribute to Robert Bilott and one of the major toxicological discoveries: "Dark Waters" and the poison truth behind the Teflon nightmare

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https://doi.org/10.48797/sl.2024.186

Welcome to the III 1H-TOXRUN INTERNATIONAL CONGRESS, 2024, entitled "No Boundaries for Toxicology: One Health, One Society, One Planet: The Big Challenges of the 21st Century". We intend this event will provide an enjoyable and successful environment for professionals working in Toxicology and all biomedical- and environmental-related areas to debate technical-scientific updates and share experiences and reflections.

Year after year, we are much convinced that One Health and Toxicology were made to be together. The Editorial of this 1H-TOXRUN International Congress aims to pay tribute to Robert Bilott and the poison truth behind a toxic chemical, perfluorooctanoic acid (PFOA), also known as C8 (Bilott, 2019). His discovery is perhaps one of the most recent proofs that Toxicology has a major impact on our lives and on society, safeguarding human and animal health, protecting the environment, and ensuring the safety of products and workplaces. In other words, it serves as a foundation for evidence-based decision-making in public health, industry, and regulatory agencies. What is odd is that it was a lawyer who uncovered one of the most tragic Toxicology scandals of the last 60 years. Since I can remember, we have been formatted by several advertising campaigns for the benefits of anti-adherent frying pans to prevent food from sticking during cooking. However, PFOA, used in the manufacturing process of many non-stick coatings, was never a major health problem of discussion. Everything began when Wilbur Tennant, a farmer from Parkersburg (West Virginia, USA), reported that his cows were dying. He believed that the DuPont® chemical company was responsible. Later, it was evident that PFOA, a synthetic chemical developed by the company as a key ingredient in the production of Teflon (i.e., a non-stick coating used in cookware and other products), was the major cause. In the decade of 1960, DuPont® began using PFOA at its plant in Parkersburg in the manufacturing process for Teflon. Very soon, several studies, promoted by the company, have linked its exposure to various health problems, including kidney and testicular cancer, birth defects, and other serious illnesses. Robert Billot's work as an environmental attorney brought significant public attention to environmental contamination and corporate accountability. He filed a federal lawsuit against DuPont® on behalf of Tennant and other thousands of affected residents. As the case progressed, Robert Bilott uncovered evidence that DuPont® had been aware of the health risks associated with C8 for decades but had concealed this information. Already in 2024, the court approved a historic $1.18 billion settlement with Dupont regarding per- and polyfluoroalkyl substance (PFAS) contamination in America's public water systems. But this is probably only the beginning since much higher compensations are expected, exceeding the $12.5 billion settlement with 3M, another company producing PFOA. Of note, 3M announced it will exit PFAS production by 2025, but "forever chemicals" like PFOA will be among us for generations. The case prompted increased scrutiny of C8 by regulatory agencies, leading to stricter regulations on its use and exposure limits. Robert Bilott's work on the C8 case was depicted in the 2019 film "Dark Waters", starring Mark Ruffalo as Bilott. The case remains a landmark example of environmental litigation and corporate accountability, and the movie deserves to be seen with the hope that we can learn from history to not repeat such kind of tragedies. The circumstances are disturbing since the toxicity of this "forever chemical" was completely covered and protected by other interests. According to a 2007 study from the USA Centers for Disease Control and Prevention, C8 is in the blood of 99.7% of Americans (Calafat et al., 2007). As a Toxicologist, this comes, to me, as a big shock and offers an additional motivation for the

1H-TOXRUN to continue its major mission. Indeed, this reality demonstrates that 1H-TOXRUN has a big challenge for the future, joining human, animal, and environmental health together with forensic implications.

This year's Congress is under the theme "THE BIG CHALLENGES OF THE 21st CENTURY". I am thankful to the prestigious personalities who accepted our invitations to share their knowledge and research in the four sessions set under the One Health umbrella.

A particularly relevant moment will be the presentation of Poster and Oral Communications on different topics of Toxicology and related areas. We intend to bring attention to the interdisciplinary perspective of modern Toxicology and disseminate advanced and novel knowledge focused on safety and health risks, on prevention and mitigation of damages from social- and environment-related pressures on health and wellbeing, and on promoting awareness and initiatives to build resilient communities and a circular economy.

I would like to acknowledge the Organizing and Scientific Committees for their outstanding collaboration and commitment to organizing this scientific congress. Thank you to all our sponsors and partners for their support and contributions to the success of the III 1H-TOXRUN International Congress. Namely, 6 grants from the Study Group for Food- and Water-borne Infections (EFWISG) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) were received and granted to 6 students. Awards for best oral and poster communications and honorable mentions will be granted by the peer-reviewed scientific journal Water Emerging Contaminants & Nanoplastics, and CESPU Formação, respectively. My last words go to the *Scientific Letters* editorial staff for their fruitful collaboration in preparing the book of abstracts.

On May 8th and 9th, 2025, we will be hosting the IV 1H-TOXRUN INTERNATIONAL CONGRESS, with the aim of bringing together Toxicology and One Health to create a societal impact. All authors with relevant work and sharing the same spirit of this holistic One Health vision for Toxicology will be welcome. We expect that, following the interdisciplinary background of different professionals, we can put this congress as the major world platform for this toxicological vision.

I wish you a pleasant congress,

Cordial greetings

Ricardo Jorge Dinis-Oliveira

President of the Organizing Committee

Further Reading

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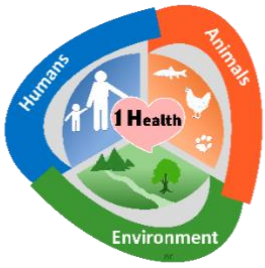

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THE BIG CHALLENGES OF THE 21ST CENTURY

02-03 MAY, 2024 | PORTO, PORTUGAL

SCIENTIFIC PROGRAMME

MAY 02

09h00 ***Opening session*** | Ricardo Jorge Dinis-Oliveira, Ana R. Freitas & Sara Ricardo, 1H-TOXRUN Management Board

09h30 ***Opening lecture*** | Nature inspired bioengineering in health and bioeconomy. | Cecília Roque, UCIBIO

10h15 ***Coffee break***

Session I
Mental Health & Well-Being

CHAIRS: Diana Dias da Silva (ESS-PPorto), Daniel Folha (UCIBIO; 1H-TOXRUN-IUCS-CESPU), Susana I. Sá (FMUP)

10h45 On the other side: stories of those who treat mental health. | Susana Almeida, IPO

11h15 Mental health and help-seeking: the role of stigma in a 2019-2021 Cohort Study at the University of Porto. | Virgínia Conceição, ISPUP

11h45 Toxicity as a trigger to neuro-inflammation. | Ana Moreira, SPMI

12h30 ***Lunch & Poster viewing***

Session II
Infectious Diseases & Antimicrobial Resistance

CHAIRS: Ângela Novais (UCIBIO-FFUP), Carolina Pereira (UCIBIO; 1H-TOXRUN-IUCS-CESPU), Paolo De Marco (UCIBIO; 1H-TOXRUN-IUCS-CESPU)

14h00 Microbial genomics: the borderless compass of the One Health concept. | João Paulo Gomes, INSA

14h30 Antimicrobial resistance: our One Health challenge. | Jorge Ferreira, FAO

15h00 Unraveling global challenges and innovations in antimicrobial resistance. | Luísa Peixe, UCIBIO-FFUP

15h30 ***Coffee break & Poster viewing***

16h30 ***Presentation of selected oral communications***

OC 01 | Ivermectin: An Ally to Reverse P-glycoprotein-associated Multidrug Resistance in Ovarian Cancer. Mariana Nunes, i3S

OC 02 | Urinary volatile metabolomic biomarkers for detection of bladder cancer. Ângela Carapito, UCIBIO-FFUP

OC 03 | Bacteriocin Dynamics in *Enterococcus faecium* and *Enterococcus lactis*: Implications for Clinical and Commensal Strain Interactions. Ana Cristina Santos, UCIBIO-FFUP

OC 04 | Exploring the Potential of Combined Natural Photosensitizers, Gentamycin, and Colistin in Antimicrobial Photodynamic Inactivation of *Pseudomonas aeruginosa* Biofilms. Ariana Gonçalves, FEUP

OC 05 | Assessing the Cytotoxic Effects of Sunitinib on AC16 Cardiac Cells and its Potential Association with Autophagy. Cláudia Oliveira, UCIBIO

OC 06 | Enantiomeric biodistribution and toxicity of 3-chloromethcathinone (3-CMC) in Wistar rats after acute exposure – preliminary data. Ivan Langa, UCIBIO;1H-TOXRUN-IUCS-CESPU

OC 07 | The effect of synthetic cannabinoid ADB-FUBINACA on primary neuronal cultures ß-galactosidase activity: preliminary findings. Rita Bravo, UCIBIO/i4HB; FFUP

OC 08 | Tramadol and its main metabolites: Toxicological effects on zebrafish embryos and larvae. Pedro Rodrigues, CIIMAR

OC 09 | Drug use among the student population of the University of Porto: Analysis of prevalence patterns. Rita Bravo, UCIBIO/i4HB; FFUP

OC 10 | The unseen ingredients: a systematic review of the prevalence of microplastics and nanoplastics in our food. Ana Carolina Rosa, LAQV/REQUIMTE; FFUP

18h30 ***Closing of the first day***

MAY 03

Session III
Environmental & Health Sustainability

CHAIRS: Cristina Couto (UCIBIO, 1H-TOXRUN-IUCS-CESPU), João Carrola (CITAB, UTAD), Vítor Seabra (UCIBIO, 1H-TOXRUN-IUCS-CESPU)

09h00 How can we achieve a sustainable nuclear fuel cycle? | Laurence Harwood, University of Reading

09h30 Lithium: a crucial non-toxic metal for the energy transition. | Alexandre Lima, FCUP

10h00 Representing the functional aspect of planet Earth in law. | Paulo Magalhães, CIJ/FDUP

10h30 ***Coffee break & Poster viewing***

11h00 ***Presentation of selected oral communications***

OC 11 | Development of enhanced buccal films with *Actinidia arguta* fruit extract for oral mucositis prevention: from in vitro buccal models to ex vivo investigations. Filipa Teixeira, REQUIMTE/LAQV; ISEP

OC 12 | Evaluation of Antitumor Activity of Xanthones Conjugated with Amino Acids. Flávia Barbosa, UNIPRO-IUCS-CESPU

OC 13 | Chemical differences between alternative and traditional tobacco products. Vânia Monteiro, UCIBIO; 1H-TOXRUN-IUCS-CESPU

OC 14 | Ecotoxicological Effects of 3,4-dichloroaniline on *Daphnia magna*: Implications for Aquatic Ecosystem Health and Management. Daniela Rebelo, ICBAS; CIIMAR, FCUP

OC 15 | Sublethal enantiotoxicity of MDMA on the development, teratogenicity, genotoxicity and swimming behaviour of zebrafish (*Danio rerio*) embryo. João Carrola, UTAD

OC 16 | Exposure to Glyphosate-based herbicide induces avoidance behavior and impairs coelomocyte viability in *Eisenia andrei* earthworms. Diovana Batista, UNIJUÍ, Brasil

OC 17 | Understanding the Ecological Consequences of Deep-Sea Mining: Cadmium's Influence on Microbial Diversity in Pacific Seamount Sediments. Saif Rahman, CIIMAR

OC 18 | Bacterial Coaggregation's Role in Water Disinfection Resistance. Ana Cristina Afonso, ALiCE-LEPABE; FEUP

OC 19 | Deciphering Zebrafish Spectral Signatures: Insights From Raman Spectroscopy. Ana Isabel Abreu, CIIMAR

OC 20 | Microplastic Occurrence and Distribution in the Ave River Estuary. Daniela Padilha, FCUP; CIIMAR

13h00 ***Lunch & Poster viewing***

Session IV
The Future is Now

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

14h30 Beyond assistance: Chatbots as pillars in education, research, and healthcare. | Ricardo Correia, FMUP

15h00 European Space Agency's SciSpace human research activities. | Melike Balk, ESA/ESTEC

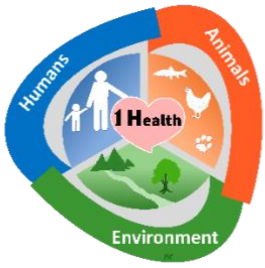
15h30 The future of sustainable food systems. | Duarte Torres, FCNAUP

16h00 Sustainable societies and industries 5.0. | Filipe Portela, AC-UM/IOTECH

16h30 ***Round table discussion*** | Moderated by Daniel Catalão, RTP

17h30 ***Awards presentation & Closing ceremony*** | Ricardo Jorge Dinis-Oliveira, Cláudia Ribeiro, Áurea Carvalho & Ana Raquel Freitas, 1H-TOXRUN Management Board

18h00 ***Closing cocktail***



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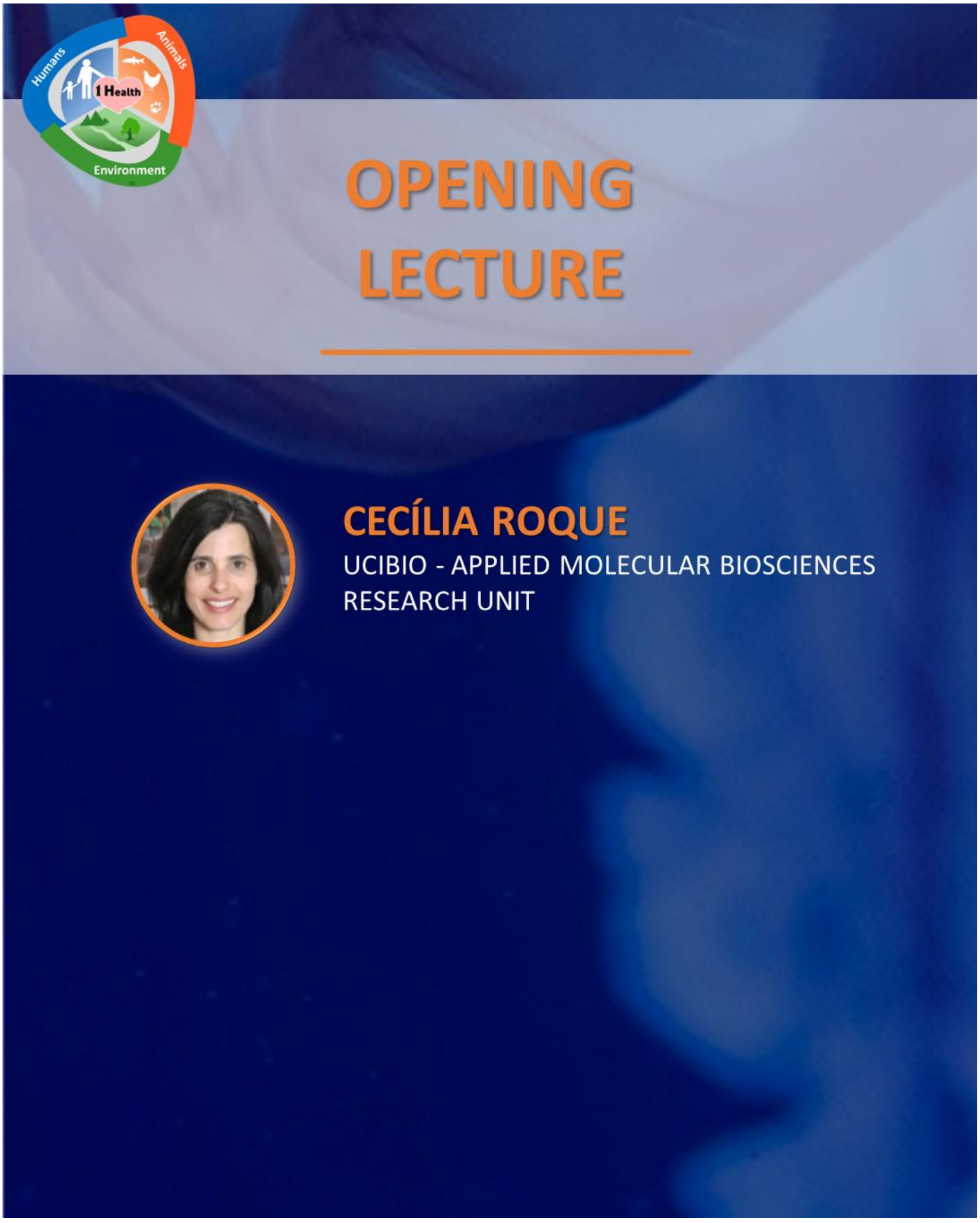
INVITED LECTURES


Humans
Animals
1 Health
Environment
OPENING
LECTURE
CECÍLIA ROQUE
UCIBIO - APPLIED MOLECULAR BIOSCIENCES
RESEARCH UNIT

Invited Lecture 01

Nature inspired bioengineering in health and bioeconomy

Cecília Roque [1,2,*]

[1] UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal
[2] i4HB – Institute for Health and Bioeconomy, Chemistry Department, School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal

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https://doi.org/10.48797/sl.2024.178

Abstract

Nature is a source of inspiration for science and technology, having generated several solutions that foster biotechnology and bioengineering, namely through new and personalized tools in Health, as well as new tools for Bioeconomy.
We take inspiration from dynamic molecular recognition events in Nature to engineer biological and chemical tools that give rise to advanced functional materials.
The first topic will address nature-inspired ligands for reversible binding to target molecules, based on the underlying principle of atom economy. While a variety of applications can be foreseen for such systems, we will focus on contributions in biomanufacturing. Rationally designed chemical combinatorial libraries support the development of robust peptidomimetics that can be easily adapted to several targets and to chromatographic and non-chromatographic matrices.
The second topic will focus on the biotechnological production of biodegradable materials, and on their application, namely in the field of artificial olfaction. Together with in-house developed electronic noses and machine learning algorithms, such materials are tuned to mimic the sense of smell and used for biosensing purposes.

Keywords: biomimetics; bioseparation; biosensing

Acknowledgments

This work has received funding from Fundação para a Ciência e Tecnologia (Portugal) for projects PTDC/BII-BIO/28878/2017, PTDC/CTM-CTM/3389/2021, Research Unit on Applied Molecular Biosciences – UCIBIO (UIDP/04378/2020 and UIDB/04378/2020) and Associate Laboratory Institute for Health and Bioeconomy – i4HB (LA/P/0140/2020), from INCD funded by FCT and FEDER under the project 01/SAICT/2016 nº 02215, from the European Research Council (ERC) under the EU Horizon 2020 research and innovation programme (SCENT-ERC-2014-STG-639123, 2015−2022 and grant agreement No. 101069405 — ENSURE — ERC-2022-POC1), and from European Union's Horizon 2020 programme under grant agreement No. 899732 (PURE Project).


Humans
Animals
1 Health
Environment
SESSION I
MENTAL HEALTH & WELL-BEING
SUSANA ALMEIDA
IPO - PORTUGUESE ONCOLOGY INSTITUTE OF PORTO
VIRGÍNIA CONCEIÇÃO
ISPUP - INSTITUTE OF PUBLIC HEALTH OF THE UNIVERSITY OF PORTO
ANA MOREIRA
SPMI - PORTUGUESE SOCIETY OF INTEGRATIVE MEDICINE

Invited Lecture 02

On the other side: stories of those who treat mental health

Susana Almeida [1,2,3,*]

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Invited Lecture 03

Mental health and help-seeking: the role of stigma in a 2019-2021 Cohort Study at the University of Porto

Virgínia Conceição [1,2,*]

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https://doi.org/10.48797/sl.2024.226

Abstract

Transitioning to university life represents a significant developmental milestone filled with increased life stressors, including academic and interpersonal challenges. This period is particularly pivotal in late adolescence (18-25 years), a stage characterised by identity evolution, sexual-affective relationship development, and future life exploration. Our observational cohort study, conducted at the University of Porto from February 2019 to March 2021, aimed to evaluate mental health trajectories and help-seeking behaviours among first-year students during this critical phase and the effects of a depression stigma reduction intervention. Results initially revealed low engagement with mental health services, largely due to prevalent stigma and limited mental health literacy. Our intervention had a significant effect in decreasing stigma and increasing help-seeking behaviours among this cohort of students. Compared to the control group, these differences remained the same during the pandemic, more than 12 months after the intervention. Finally, we also observed that students who sought help presented less relevant depressive symptoms in the following assessment, highlighting the importance of seeking adequate help in situations of vulnerability and need.
The study highlights the critical role of stigma in influencing mental health service utilisation among university students. It supports the effectiveness of targeted interventions to reduce stigma and promote mental health resilience.

Keywords: university students; mental health; help-seeking behaviours; stigma reduction; depressive symptoms

Invited Lecture 04

Toxicity as a trigger to neuro-inflammation

Ana Moreira [1]

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https://doi.org/10.48797/sl.2024.149

Abstract

The world is evolving rapidly, and humankind must continuously adapt to these emerging realities. Chronic, unresolved inflammation is now a major driver of disease [1]. Social, environmental, and lifestyle factors contribute to systemic chronic inflammation, which in turn can lead to various diseases that represent the primary causes of disability and mortality worldwide. Neuroinflammation may be triggered by several factors, including infections, traumatic brain injury, autoimmune diseases, and exposure to toxins or chemicals. Toxic substances such as heavy metals, pesticides, herbicides and insecticides, vaccine components, plasticizers, air pollutants, and certain medications can directly damage neurons or disrupt normal cellular functions in the brain. This damage can activate immune responses in the brain, leading to the activation of microglia. Activated microglia release pro-inflammatory cytokines, chemokines, and reactive oxygen species, which can harm neurons and impair normal brain function [2-4]. Chronic and excessive neuroinflammation can be detrimental and contribute to the onset and progression of neurological disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, among others [4]. Functional and Integrative Medicine, building on the principles of Environmental Medicine and Toxicology, employs laboratory diagnostics to assess patients. Subsequently, modern therapeutic detoxification methods are applied, including therapeutic apheresis, chelation with EDTA, and orthomolecular supplementation. It is urgent that we focus on this issue to assist populations in avoiding contamination and toxicity and to establish governmental policies for our protection.

Keywords: toxicity; neuroinflammation; integrative functional medicine

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SESSION II

INFECTIOUS DISEASES & ANTIMICROBIAL RESISTANCE

JOÃO PAULO GOMES
INSA - NATIONAL INSTITUTE OF HEALTH
DOCTOR RICARDO JORGE

JORGE FERREIRA
FAO - FOOD AND AGRICULTURE
ORGANIZATION OF THE UNITED NATIONS

LUÍSA PEIXE
UCIBIO – RESEARCH UNIT ON APPLIED
MOLECULAR BIOSCIENCES
FFUP – FACULTY OF PHARMACY OF THE
UNIVERSITY OF PORTO

Invited Lecture 05

Microbial genomics: the borderless compass of the One Health concept

João Paulo Gomes [1,2,*]

[1] Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal
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https://doi.org/10.48797/sl.2024.150

Abstract

It is nowadays recognized that it is fundamental not to dissociate the human, animal, and environmental aspects when talking about health. In terms of infectious diseases, it is well known that pathogenic microbial agents, whether bacteria, viruses, fungi, or parasites, and their respective drug resistance genes, often circulate in these three "host environments", causing animal or human disease. This phenomenon is exacerbated in this era of globalization and free market, marked by intense migrations, intensive production, and international trade of food products, as well as increasing proximity to wild animals. In this regard and within the framework of the One Health concept, microbial genomics shows up as the comprehensive approach to understanding the interconnectedness of human, animal, and environmental health. By leveraging high-throughput sequencing technologies, researchers can thus unravel the genetic diversity of microbial communities across these diverse ecosystems, highlighting the importance of collaborative efforts in disease surveillance and control. Through comparative genomic analyses, researchers can trace the origins and transmission dynamics of infectious diseases, elucidating pathways of cross-species transmission and spillover events. Furthermore, microbial genomics facilitates the prediction and mitigation of emerging infectious threats, guiding the development of targeted interventions and public health strategies. By integrating genomic data from diverse sources, such as clinical samples, wildlife reservoirs, and environmental samples, researchers can identify potential reservoirs of pathogens and assess the risk of zoonotic transmission. In conclusion, microbial genomics embodies the essence of the One Health concept by transcending disciplinary boundaries and fostering interdisciplinary collaboration. By elucidating the complex interplay between humans, animals, and the environment at the genetic level, microbial genomics provides valuable insights into the dynamics of infectious diseases and informs evidence-based approaches to promote global health security.

Keywords: One Health; microbial genomics; surveillance; infectious diseases

Invited Lecture 06

Antimicrobial resistance: our One Health challenge

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https://doi.org/10.48797/sl.2024.151

Abstract

Antimicrobial Resistance (AMR) is recognized as one of the current top global health issues. Often, it is simplified to the version "resistance to antibiotics", and therefore associated by many only to human health. In reality, its dimensions and implications are much broader, as all antibiotics are antimicrobials, but not all antimicrobials are antibiotics. Antimicrobials are used not only in human medicine but also in animals (companion, exotic and food production, terrestrial and aquatic) and plants/crops, with a spill over to the environment, making it a perfect illustration of a One Health issue. In particular, after the adoption of the Global Action Plan (GAP) on AMR in 2016, a multitude of policies, events and initiatives have been implemented at global, regional, national and local levels, many of them focusing on: i. raising awareness on AMR; ii. collection of, as much as possible, harmonized and integrated data and iii. responsible and prudent antimicrobial use, when needed. AT FAO, the AMR Action Plan is the overall umbrella, that covers, for example, the development of the InFARM (International AMR Monitoring System) database and the upcoming RENOFARM (Reduce the Need for Antimicrobials on Farms for Sustainable Agrifood Systems Transformation) initiative. AMR can also be foodborne, and the same can be said the other way around: some of the foodborne infections are caused by antimicrobial resistant microorganisms. However, at the moment, the exact dimensions of these phenomena are not quantifiable at global scale. Without creating an over dimensioned alarm, it is important to emphasize that AMR is also a food safety issue, that threatens the future global food security. The cornerstones to control AMR are: 1) a (always challenging) change in behaviors, that reflects the concept that antimicrobials are global common goods, that we are all responsible for, envisioning a healthy future for the upcoming generations and communities and 2) an agrifood systems transformation that ensures a sustainable food production.

Keywords: antimicrobial resistance (AMR); antimicrobial use (AMU); food safety; One Health

Invited Lecture 07

Unravelling global challenges and innovations in antimicrobial resistance

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https://doi.org/10.48797/sl.2024.152

Abstract

Antimicrobial resistance (AMR) remains a formidable adversary in global health, contributing significantly to mortality from both nosocomial and community-acquired infections [1-6]. This talk aims to dissect the multifaceted nature of AMR, exploring its roots, ongoing global challenges, and innovative countermeasures being developed to mitigate its impact. Despite decades of awareness, AMR continues to escalate, driven by liberal antibiotic usage in humans and animals. I will elucidate how such practices have not only fostered the prevalence of super-drug resistant bacteria within clinical and community settings but have also extended the problem into the environment, creating domains where intervention is particularly challenging. Several pivotal challenges will be addressed, including the minute concentrations of antibiotics capable of selecting for resistance to socio-economic, health, and environmental determinants. In response to these daunting challenges, the scientific and regulatory communities have embarked on several promising initiatives such as the EU's prohibition on certain antibiotics for animal use. The talk will emphasize the necessity of a comprehensive strategy to manage and mitigate antimicrobial resistance (AMR) effectively. This approach encompasses enhanced diagnostics, robust antimicrobial stewardship, and innovative treatment options [6]. Additionally, it will stress the importance of raising societal awareness about antibiotic use, engaging the general public through citizen science projects to foster a broader understanding and proactive behavior towards AMR.

Keywords: antimicrobial resistance; drug-resistant bacteria; global health challenges; antibiotic policy

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SESSION III

ENVIRONMENTAL & HEALTH SUSTAINABILITY

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Invited Lecture 08

How can we achieve a sustainable nuclear fuel cycle?

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https://doi.org/10.48797/sl.2024.153

Abstract

Dealing with spent nuclear fuel is key if nuclear fission is to be used more widely going forward. Nuclear power is close to carbon neutral, but spent nuclear fuel has a storage lifetime of ~300,000 years. Reprocessing spent nuclear fuel is carried out on large scale using the PUREX "Plutonium Uranium Reduction and Extraction" process. The spent nuclear fuel is reduced to 15% of its original weight and the separated uranium and plutonium reused as "Mixed Oxide Fuel". In the civil sector, this was carried out by the UK at Sellafield (now curtailed) and continues in France at La Hague. A plant in Rokashamura in Japan has been mothballed after the Fukushima accident. The residual waste must be stored for ~9,000 years with most of the remaining radiotoxicity due to traces of the minor actinides, neptunium, americium and curium, constituting just 0.1% of the original spent fuel. Separation of these minor actinides from the chemically very similar lanthanides (rare earths) in the last 15% of waste remaining after PUREX is the key step for future reprocessing. If separated, the minor actinides can be used as fuel in the next generation of nuclear reactors and converted into benign products, but lanthanides will cause the fission process to shut down if introduced into the reactor pile as they absorb neutrons efficiently. Removing the minor actinides from post PUREX waste will mean that the final residue need only be stored for 300 years. The highly challenging separation of the chemically very similar minor actinides from the lanthanides has been achieved using nitrogen-bearing organic ligands developed at Reading University. This can lead to significantly improved handling of spent nuclear fuels and means that waste nuclear fuel need not be a long-term storage liability but a source of yet more clean power.

Keywords: nuclear waste; actinides; lanthanides; partitioning

Acknowledgments

This research was funded by EPSRC (UK), University of Reading (UK), European Union (ACCEPT, SACSESS, GENIORS Consortia), Pro Nu Dec (UK), Rogers Leaske (UK), Archimedes Foundation (Estonia).

Invited Lecture 09

Lithium: a crucial non-toxic metal for the energy transition

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https://doi.org/10.48797/sl.2024.154

Abstract

Lithium is a strategic metal globally. The expanding market for lithium (Li) batteries, utilized in a diverse range of products from electric vehicles and renewable energy storage and backup to smartphones and laptops, is driving a growing demand for lithium, amplifying the risk of supply shortages and surging prices. With the ongoing energy transition, advanced geological exploration technologies are essential to uncover mineral deposits containing critical elements like Li. Currently, most of it originates from pegmatites, primarily spodumene. Spodumene concentrates can be obtained from various sources such as tailings, waste piles, quarries serving ceramic and glass industries containing spodumene in pegmatites. The majority of lithium deposits in Europe are associated with Li–Cs–Ta (LCT) pegmatites. While traditional exploration methods rely heavily on geochemical surveys and related tools, there is a pressing need to assess alternative techniques, particularly remote sensing (RS) and geophysics. Recent developments have explored the application of remote sensing data in detecting lithium (Li), focusing on identifying either pegmatite or brine deposits. The Greenpeg H2020 project that was focused in Europe is scheduled to be concluded in 2024. However, our objective extends beyond this timeline, aiming to implement exploration tools tailored for pegmatites in tropical environments. Our cutting-edge research on Critical Raw Materials (CRM) within the Community of Portuguese Language Countries (CPLP) primarily involves a comparative study of Li minerals sourced from pegmatites across CPLP regions. With studies specialized in pegmatites in Portugal, Mozambique, and Brazil, our overarching goal is to secure research that conduct an intensive analysis of each pegmatite field type and establish connections between them. By doing so, we aim to enhance exploration techniques specifically tailored for rare-element pegmatites, thereby advancing our understanding and optimizing the utilization of these valuable mineral resources. With that we hope to help to secure energy transition for Europe.

Keywords: lithium; pegmatite; energy transition

Acknowledgments

This work was funded by the European Commission's Horizon 2020 innovation programme under grant agreement no. 869274 by the project GREENPEG New Exploration Tools for European Pegmatite Green-Tech Resources.

Invited Lecture 10

Climate as nobody's thing or as common heritage?

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https://doi.org/10.48797/sl.2024.155

Abstract

When the latest ILC report states that "Atmosphere and airspace are two different concepts which must be distinguished (...)", this opens the pathway to make the "functional" dimension of the earth system autonomous, from the "static" territorial element of sovereignty. This development makes it possible to answer the question: "What is Climate from a legal point of view?". The current inability to legally portray the functional dynamics of the planet was at the origin of the non-recognition of the good Stable Climate itself as a Common Heritage of Humankind, and the choice to address the problem – climate change is a Common Concern of Humankind. This option has limited the action strategy to avoid/mitigate/neutralize emissions, preventing the internalization of benefits in the good Stable Climate, because they disappear into the global legal void. This makes it impossible to build an economy capable of actively care/restore/regenerate climate. Today, there is no incentive or compensation system for negative emissions. The tragic paradox in which we find ourselves is that the current legal status of climate change as a "concern of humankind", in fact, maintains and prolongs the condition of the climate system as a nobody's thing, the status of res nullius [1], the dumpster of humankind [2]. This status, conditions and limits all efforts to remove CO_2 from the atmosphere (through nature-based solutions or the use of new technologies), to neutralize and maintain current emissions or generating rights to make even more emissions. In other words, it does not allow the development of an economic activity to remove CO_2 already in excess and restore the functioning of the Climate System.

Keywords: climate change; climate system; stable climate; Common Heritage of Humankind; Common Concern of Humankind

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SESSION IV

THE FUTURE IS NOW

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Invited Lecture 11

Beyond assistance: chatbots as pilars in education, research, and healthcare

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Invited Lecture 12

European Space Agency (ESA)'s human research science activities

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https://doi.org/10.48797/sl.2024.156

Abstract

The mission of the European Space Agency (ESA)'s human spaceflight and robotic exploration (HRE) programme is a sustainable and international endeavour to visit new places and make novel discoveries. The ESA HRE strategy includes three destinations where humans will work with robots to gather new knowledge: low-Earth orbit on the International Space Station, the Moon and Mars. To enable human exploration beyond low Earth orbit it is crucial to reduce the risks of spaceflight in human health and performance. ESA is running a dedicated research programme that leads to the development and delivery of human health, performance, and countermeasures and risk mitigation solutions for unwanted effects from space hazards, and advanced habitability and medical support technologies. The main research topics focus on human health and performance (physiology and behavioural health), radiation, habitability and medical capabilities. Furthermore, space and space analogues also offer unique possibilities to study health problems related to diseases, ageing and immobility which then might yield benefits for terrestrial medicine. This presentation will focus on ESA's Human Exploration activities, including mainly human research across the variety of platforms and destinations within the ESA HRE portfolio. Here, the aim to outline recent highlights stemming from ESA Life Sciences research, focusing mainly on Human research activities, the research priorities for the future, and how the science community can get involved with ESA's HRE science programme.

Keywords: ESA; human physiology; Space research; International Space Station; planetary exploration

Invited Lecture 13

The future of sustainable food systems

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https://doi.org/10.48797/sl.2024.158

Abstract

For millennia, humanity has thrived during the Holocene epoch, characterised by relatively stable climatic conditions, low atmospheric concentrations of greenhouse gases, and rich biodiversity. This epoch has uniquely supported Earth's life systems as we understand them. Currently, we reside in what is proposed as the Anthropocene epoch, marked significantly by human activities that overwhelmingly influence terrestrial, atmospheric, and marine environments. The precise onset of the Anthropocene is debated, though it likely began around the late 18th century with the advent of the Industrial Revolution. This period is evidenced by increased carbon dioxide and methane concentrations in polar ice cores. However, a more pronounced shift occurred post-mid-20th century, during what is termed the "Great Acceleration", characterised by exponential growth in human population, industrial output, and resource consumption, including fossil fuels, plastics, and food, alongside significant impacts on freshwater resources, deforestation, and biodiversity loss. This transformative era poses the critical question: how long can such trajectories be sustained without irreparable harm to Earth's systems? The "Planetary Boundaries" framework, developed by a global consortium of scientists over the past decade, addresses this concern by delineating nine critical biogeophysical thresholds that must not be crossed to prevent destabilising the Earth system. These boundaries include climate change, biodiversity loss, land-use changes, freshwater use, biogeochemical flows (nitrogen and phosphorus), ocean acidification, ozone depletion, introduction of novel entities, and atmospheric aerosol loading. This framework quantifies the Earth system's resilience and sets limits to human-induced changes that, if exceeded, could lead to catastrophic environmental shifts. Presently, significant breaches have been identified in several of these boundaries. The global food system has been a primary driver of biodiversity loss, approaching what could be considered a sixth mass extinction. It has also contributed to disrupting nitrogen and phosphorus cycles through unconstrained fertiliser use, leading to widespread eutrophication of aquatic systems. It has also been a substantial source of greenhouse gas emissions, predominantly from livestock production. Looking ahead to 2050, with projected global population increases and rising per capita incomes, it is anticipated that, without intervention, greenhouse gas emissions from agriculture will double, and the demand for agricultural land and nutrients will surge dramatically. This escalation poses additional risks to water resources and could further accelerate ocean acidification. To mitigate these impacts, a multifaceted approach is necessary. This includes reducing food waste across the supply chain, leveraging technological advancements to enhance agricultural efficiency and reduce environmental footprints, and shifting dietary patterns towards less resource-intensive foods. Specifically, measures would involve improving food storage and distribution, optimising nutrient application, advancing water conservation techniques, and fostering dietary shifts away from red and processed meats towards plant-based foods. Addressing these challenges requires a robust, coordinated policy response that integrates economic incentives, educational efforts, and infrastructural improvements to foster sustainable consumption and production patterns. Ultimately, the goal is to operate within safe ecological limits to ensure the continued stability and resilience of the Earth system, safeguarding it for future generations.

Keywords: Planetary Boundaries; global food system; food waste; plant-based foods; shifting dietary patterns

Invited Lecture 14

Sustainable societies and industries 5.0

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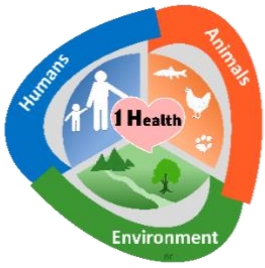
https://doi.org/10.48797/sl.2024.157

Abstract

Today's cities have transcended their historical simplicity into multifaceted ecosystems teeming with challenges and opportunities. The quest for environmentally conscious and sustainable societies has never been more pressing, led by industries that profoundly influence our urban fabric. From the spectre of CO_2 emissions to the challenge of using eco processes or from the promotion of product circulation to the task of waste management or reuse of natural resources (e.g., water) extensive usages, cities grapple with complex issues that reverberate through the lives of their inhabitants. The population remains largely unaware of the tangible repercussions of their daily actions. We must be alert to these issues, reduce our carbon footprint, and decrease natural resource usage. Unfortunately, there is no single solution to the earth's problems. Still, Information and Communication Technologies (ICT), particularly Artificial Intelligence (AI) and Gamification can help make it a better place to live. This presentation aims to draw attention to some of society's problems and show some solutions that are being developed to help create a more sustainable environment in the future. From predictive analytics to intelligent resource management systems, AI is key to unlocking unprecedented insights and efficiencies in our quest for greener, more sustainable urban environments. Gamification is a complementary key because it can help engage citizens and motivate them to perform more green actions. Raising people's awareness and endowing them with the correct technologies are the keys to an increasingly sustainable society and even more digital and resource-optimized industry.

Keywords: information and communication technologies; artificial intelligence; gamification; sustainable societies; resource-optimized industry



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SELECTED **ORAL COMMUNICATIONS**

Oral Communication 01

Ivermectin: an ally to reverse P-glycoprotein-associated multidrug resistance in ovarian cancer

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https://doi.org/10.48797/sl.2024.129

Abstract

Background: The standard treatment for ovarian cancer (OC) involves carboplatin and paclitaxel, which can be followed by PARP inhibitors (PARPi) [1]; however, resistance to therapy is common. Increased drug efflux is a well-described mechanism of resistance to paclitaxel and PARPi [2]. Prior treatment with paclitaxel may increase P-gp upregulation and indirectly induce PARPi resistance [3]. Ivermectin, a broad-spectrum antiparasitic agent, has been found to enhance the anti-cancer efficacy of chemotherapeutic drugs and, in some cases, reverse resistance [4]. Ivermectin can act as a chemosensitizer by blocking drug efflux capacity, increasing intracellular drug accumulation, and enhancing antineoplastic efficacy [5]. **Objectives:** We aim to study the therapeutic benefits of combining paclitaxel or olaparib with ivermectin. **Methods:** To achieve this, we measured the cytotoxic effects of paclitaxel and olaparib separately and also in combination with ivermectin on two tumor chemoresistant (OVCAR8 and OVCAR8 PTX R) and one non-tumoral (HOSE6.3) cell lines. To measure cellular viability, we used the Presto Blue assay. We also assessed the synergistic interactions using the SynergyFinder Plus Software. **Results:** Our findings show that OVCAR8 PTX R, a paclitaxel-resistant cell line established in our laboratory, is also resistant to olaparib. We also discovered that ivermectin can increase the sensitivity of tumor cells to antineoplastic drugs and enhance their effectiveness. When paclitaxel and olaparib were combined with ivermectin, it resulted in the highest cytotoxic effect and the strongest synergistic effect compared to drugs alone. **Conclusions:** It would be advisable to assess P-gp expression before administering PARPi to patients who failed paclitaxel therapy. Both drugs are P-gp substrates, and their active efflux from cells can compromise clinical response. The potential of combining ivermectin with paclitaxel or olaparib is promising. Our results show that ivermectin, a substrate and modulator of P-gp, has the potential to reverse chemoresistance mediated by P-gp blockage.

Keywords: chemoresistance; paclitaxel; P-glycoprotein; olaparib; ovarian cancer

Acknowledgments

This research was funded by CESPU under the grant number "OVCARTEST_GI2-CESPU_2022". Mariana Nunes acknowledges Fundação para a Ciência e a Tecnologia (FCT)/ Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) and União Europeia (UE) for financial support through a PhD fellowship (2020.04720.BD and DOI:10.54499/2020.04720.BD) co-sponsored by Fundo Social Europeu (FSE) through Programa Operacional Re-gional Norte (Norte 2020).

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Oral Communication 02

Urinary volatile metabolomic biomarkers for detection of bladder cancer

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https://doi.org/10.48797/sl.2024.130

Abstract

Background: Early detection of bladder cancer (BC) is necessary for improved outcomes, but current diagnostic methods may lack the necessary sensitivity. Metabolomics, particularly the analysis of urinary volatile organic compounds (VOCs), offers a promising, non-invasive approach to BC detection [1,2]. **Objective:** The study aimed to investigate potential urinary biomarkers for the detection and staging of BC using metabolomics approaches, in particular gas chromatography-mass spectrometry (GC-MS), and to apply machine learning algorithms to establish a biomarker panel for the accurate detection and staging of BC. **Methods:** Our study collected urine samples from 196 participants, including 98 BC patients (67 non-muscle invasive bladder cancer, and 31 muscle-invasive bladder cancer) and 98 cancer-free controls. Ethical approval was obtained, and informed consent was signed by all participants. Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used for volatile profiling in urine. Statistical analyses included principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), receiver operating characteristic (ROC) analysis, and univariate tests to identify biomarkers for BC detection and staging. **Results:** Our study found 19 urinary volatile metabolites that effectively discriminate BC patients from cancer-free controls. A panel of ten potential biomarkers was identified for the detection of BC with a sensitivity of 81%, specificity of 90% and accuracy of 85%. This biomarker panel consisted of two aromatic compounds, three ketones, three alcohols, one pyran-like compound, and one fatty acid. Compared to controls, the panel achieved 83% sensitivity, 87% specificity, and 84% accuracy for NMIBC, and 90% sensitivity, 77% specificity, and 87% accuracy for MIBC. No significant differences in urinary volatile profiles were observed between NMIBC and MIBC patients. **Conclusions:** The identified panel of 10 biomarkers showed significant potential to discriminate between BC patients and controls. While further research is warranted to differentiate NMIBC from MIBC cases, our findings highlight the value of urinary volatile biomarkers in advancing diagnostic approaches for improved patient care.

Keywords: bladder cancer; metabolomics; volatile organic compounds; gas chromatography – mass spectrometry; urinary biomarkers

Acknowledgments

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/04378/2020 and UIDB/04378/2020), and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020). Â.C. acknowledges FCT for her PhD grant (2021.05844.BD).

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Oral Communication 03

Bacteriocin dynamics in *Enterococcus faecium* and *Enterococcus lactis*: implications for clinical and commensal strain interactions

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https://doi.org/10.48797/sl.2024.131

Abstract

Background: *Enterococcus faecium*-Efm and *E.lactis*-Elts (former Efm-cladeB) colonize the human gut, with Efm also being a leading hospital-pathogen. Dynamics influencing strain dominance in competitive environments (e.g., infection/colonization) are not fully understood, but bacteriocins may provide competitive advantage to clinical Efm or commensal Elts strains. **Objective:** We explored bacteriocin content of contemporary Efm and Elts, isolated from healthy/diseased humans, and correlated it with their inhibition profiles against strains across these species. **Methods:** A collection of 129 strains [91 clinical-Efm (77 vancomycin-resistant-VRE); 35 healthy-volunteers (21-Efm;14-Elts)] from 1996-2022 were challenged against each other by a qualitative bacteriocin production/sensitivity-assay (soft-agar-overlay-technique). Eighty-eight representatives were sequenced (Illumina-NovaSeq) to establish clonality, antibiotic profiles (CGE-tool), and bacteriocins (homemade-database) [1]. **Results:** Elts (93%) and Efm (87%) carried ≥ 1 bacteriocin. Twenty-one bacteriocins were found, including 8 newly identified. Efm exhibited greater diversity (1-9; $\bar{x}$=3.6 *vs* 1-5; $\bar{x}$=2.6) and both species presented exclusive bacteriocin genes (Efm:*bac43/AS5/AS9/enxA/B/entB*; Elts:*entL50A/B/GM-1*). Bacteriocins 43/AS5/AS11/AS9/entA were significantly associated with clinical-Efm-strains (p<0.05), whereas AS8/bac32/entQ/AS4/entl50A/B/GM-1 were exclusive to commensal ones. All were susceptible to inhibition, while 53% of Elts and 65% of Efm (clinical-50%; commensal-39%) inhibited ≥ 1 strain. Those unable to inhibit others were mostly recovered < 2007 or lacked *bac43*. More bacteriocin genes correlated with less inhibition, and similar profiles resulted in comparable inhibition patterns. Among clinical isolates, ST117, ST78 and ST80 showed a higher inhibitory spectrum. ST78-related strains, particularly ST117, demonstrated activity against ST18-related strains previously dominant in Portuguese hospitals, but not vice-versa. VRE were inhibited by 26% of commensal-strains (Efm/Elts with diverse profiles/STs), while inhibiting up to 85% of them. **Conclusions:** Distinct bacteriocin profiles in clinical/commensal isolates, coupled with strain-specific and/or mutual strain inhibition dynamics, suggest a competitive landscape for Efm. Commensal strains inhibited VRE, showcasing their potential to counteract resistant strains. This delicate balance, influenced by unknown factors, underscores the valuable insights bacteriocins could provide for future eco-evo strategies combating human infections caused by Efm.

Keywords: bacteriocins; competitive interactions; vancomycin-resistant *Enterococcus faecium; Enterococcus lactis*

Acknowledgments

This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the exploratory project EXPL/SAU-INF/0261/2021 and UIDP/04378/2020, UIDB/04378/2020 and i4HB research project LA/P/0140/2020. Ana C. Almeida-Santos acknowledges her UI/BD/151317/2021 fellowship from FCT/MCTES through national funds.

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Oral Communication 04

Exploring the potential of combined natural photosensitizers, gentamycin, and colistin in antimicrobial photodynamic inactivation of *Pseudomonas aeruginosa* biofilms

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https://doi.org/10.48797/sl.2024.132

Abstract

Background: The increasing prevalence of wound infections poses a growing threat to the healthcare system [1]. Compounding this challenge, the presence of biofilms significantly impairs both the healing process and the effectiveness of the treatment of wound infections [2]. Antimicrobial photodynamic inactivation (aPDI) has shown promising results in combating this global problem. However, its potential against Gram-negative bacteria such as *Pseudomonas aeruginosa* remains a major challenge [3]. **Objective:** The aim of this study is to investigate the effect of subinhibitory concentrations of colistin (CL) in conjugation with curcumin-gentamycin (Cur-Gen) dual combinations on boosting the aPDI of *P. aeruginosa* biofilms. **Methods:** The efficacy of CL in enhancing the photodynamic activity of Cur-Gen against biofilms was investigated using the strain *P. aeruginosa* ATCC 10145. Membrane permeability after CL treatment was assessed by flow cytometry. The synergistic concentrations of the triple combination Cur-Gen-CL were determined using the checkerboard assay. The efficacy of blue light (420 nm, 30 mW/cm^2, 10 min) to photoactivate Cur-Gen-CL and thus promote its ability to prevent and control biofilms was investigated. The total mass, metabolic activity and cell culturability of the *P. aeruginosa* biofilms were quantified by crystal violet, alamar blue and colony forming units (CFU), respectively. **Results:** Subinhibitory CL concentrations (4 µg/mL) increased the membrane permeability of *P. aeruginosa* by approximately 30%. This effect enhanced the efficacy of the photoactivated Cur-Gen-CL triple combination in preventing the formation of *P. aeruginosa* biofilms compared to Cur-Gen alone. In addition, the photoactivated Cur-Gen-CL combination achieved a complete reduction in the culturability (approximately 7 log CFU/cm^2) of preformed *P. aeruginosa* biofilms and a reduction in biomass and metabolic activity by over 60 and 90%, respectively. **Conclusions:** This study has shown that the use of CL is a promising strategy to enhance the aPDI effect of the Cur-Gen combination against *P. aeruginosa* biofilms.

Keywords: antibiotic combinations; antimicrobial photodynamic inactivation; colistin; natural photosensitizers; *P. aeruginosa* biofilms

Acknowledgments

This research was funded by national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020). Ariana S.C. Gonçalves acknowledges individual PhD fellow-ships from FCT (2022.10913.BD).

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Oral Communication 05

Assessing the cytotoxic effects of sunitinib on AC16 cardiac cells and its potential association with autophagy

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https://doi.org/10.48797/sl.2024.133

Abstract

Background: Cancer survivorship rate has witnessed a notable increase, leveraged by amazing therapeutic advancements. However, these treatments have limitations and problems. Sunitinib (SUN) is a multikinase inhibitor, used in renal cell carcinoma and gastrointestinal stromal tumors. However, its use is limited due to cardiotoxicity, among other adverse effects. Therefore, it is important to understand the mechanisms associated with SUN-induced cardiotoxicity. Autophagy has been linked to SUN cytotoxicity on several models [1]. **Objective:** To investigate a possible relationship between autophagy and the cytotoxicity induced by SUN in human differentiated AC16 cardiac cells. **Methods:** AC16 cells were differentiated with horse serum for 24 hours and then exposed to clinically relevant concentrations of SUN (1-20 µM) for 24 or 48 hours, after which two cytotoxicity assays were performed: the MTT reduction and the Neutral Red uptake assays. Then, two working concentrations were chosen (0.01 and 1 µM) to address the effect of some autophagy modulators such as chloroquine (CQ), 3-methyladenine (3-MA) or rapamycin (RAP) on the observed cytotoxicity. Furthermore, the levels of p62, LC3-I e LC3-II were analyzed by western blotting, as well as cellular morphology of cells by phase contrast microscopy. Statistical analysis was performed using two-way ANOVA followed by Sidak's *post hoc* test or one-way ANOVA followed by Tukey's *post hoc* test, pending on the conditions tested. **Results:** SUN caused a time- and concentration-dependent cytotoxicity in AC16 cells. Furthermore, SUN seems to accumulate inside AC16 cells at 24-hour exposure in the higher concentration (10 µM). Furthermore, the incubation with SUN 10 µM increases total p62 levels and increases the LC3-II/LC3-I ratio. The results of modulators are ongoing. **Conclusions:** Our findings demonstrate that SUN induces substantial cytotoxicity in AC16 cells, accompanied by elevated levels of p62 and an increased ratio of LC3-II/LC3-I, indicative of autophagy activation.

Keywords: sunitinib; autophagy; cardio-oncology; cardiotoxicity

Acknowledgments

This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), 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. VMC acknowledges FCT for her grant (SFRH/BPD/110001/2015) that was funded under the Norma Transitória – DL57/2016/CP1334/CT0006.

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Oral Communication 06

Enantiomeric biodistribution and toxicity of 3-chloromethcathinone (3-CMC) in Wistar rats after acute exposure – preliminary data

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https://doi.org/10.48797/sl.2024.134

Abstract

Background: There has been a surge in global attention to New Psychoactive Substances (NPS) [1]. Synthetic cathinones stand out as a widely consumed NPS class. Notably, 3-chloromethcathinone (3-CMC) accounted for over 34% of NPS seizures in 2021 [2], which underscores concerns regarding its consumption and health effects. Of note, 3-CMC is chiral and mostly sold as a racemate. As human metabolism and pharmacological effects can be enantioselective [3], determination of the impact of enantioselectivity in toxicokinetics/toxicodynamics is essential for the assessment of 3-CMC effects. **Objective:** This work aimed to evaluate *in vivo* the enantioselective biodistribution and toxicity of racemic 3-CMC, after an acute exposure to 3-CMC. **Methods:** Ten-week-old male Wistar rats were administered intraperitoneally with saline or 3-CMC (10 or 20 mg/kg; n=6). Twenty-four hours after, animals were deeply anesthetized and nine organs (brain, liver, kidneys, lungs, heart, spleen, gut, muscle, adipose tissue), blood and urine were collected. For evaluation of the enantiomeric biodistribution, a previous *in house* established indirect method by gas chromatography [3], was adapted and validated. Some biochemical analysis was performed using an analyser, whereas TBARS, ATP, glutathione and total protein were determined by spectrophotometry. Organs were also processed for histological analysis. **Results:** After 24 h, 3-CMC was not found in most organs. Both enantiomers were detected in urine with one dominant enantiomer, suggesting enantioselectivity in metabolism. The histopathological results showed possible central chromatolysis in the brain (20 mg/kg), liver inflammation, renal lesions, lungs' haemoptysis, and alveolar haemorrhage, in most 3-CMC-exposed animals. No differences were observed in the heart. **Conclusions:** Our findings show rapid 3-CMC renal elimination, with enantioselectivity in metabolism. Although biochemical evaluations are ongoing, the results are expected to give further insights on the 3-CMC toxicity and histological abnormalities found in the brain, kidneys, liver and lungs.

Keywords: new psychoactive substances; drugs of abuse; enantioselectivity; toxicokinetics; risk assessment

Acknowledgments

This project was financed by FCT–Portuguese Foundation for Science and Technology, through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 (ENANTIOTOX) and 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). Ivan Langa acknowledges a Ph.D. research grant provided by FCT 2022.12874.BD.

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Oral Communication 07

The effect of synthetic cannabinoid ADB-FUBINACA on primary neuronal cultures ß-galactosidase activity: preliminary findings

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https://doi.org/10.48797/sl.2024.135

Abstract

Background: ADB-FUBINACA (ADB-FUB) is a synthetic cannabinoid (SC) that has gained popularity among users as a new psychoactive substance. This stems from SC's pharmacological similarity to the active principle of cannabis, Δ^9-tetrahydrocannabinol (THC). However, unlike THC, SCs demonstrate full agonism of cannabinoid receptors 1 and 2 [1]. Recent scientific developments have shown that cannabis use may aggravate ageing-related parameters [2,3]. Moreover, a study using human fibroblasts revealed that 1 µM THC (2h-long exposure, for 15 days) can increase ß-galactosidase activity [3], which serves as a first-line marker for cellular senescence. **Objective:** This study was designed to investigate whether these biologically-relevant concentrations could accelerate neuronal ageing. **Methods:** PHC were isolated from Wistar rat day 18-19 embryos and cultured for up to 21 days-in-vitro (DIV). Exposure to 1 pM, 1 nM and 1 µM ADB-FUB (concentrations previously shown to be non-cytotoxic to PHC) started either on DIV3 or DIV7 and was maintained up to 21 DIV. At that final timepoint, ß-galactosidase activity was evaluated. DMSO at 0.02% was employed as solvent control. **Results:** Under these experimental conditions, PHC exposed to 1 nM and 1 µM ADB-FUB in the DIV3-21 protocol had lower ß-galactosidase activity when compared to control conditions ($p<0.05$, 1 nM; $p<0.001$, 1 µM). No statistically significant results were registered for PHC under the DIV7-21 exposure protocol. **Conclusions:** These findings are, to the best of our knowledge, the first evidence of a potential "anti-ageing" effect of ADB-FUB. Evaluation of other senescence-related endpoints will follow. Moreover, experiments using another *in vitro* neuronal model (human neuroblastoma cell line SH-SY5Y) are underway to compare the effects of the same drug in different models and further substantiate conclusions on ADB-FUB's effect.

Keywords: synthetic cannabinoids; *in vitro* neurotoxicity; ageing

Acknowledgments

This work was supported by FCT—Fundação para a Ciência e a Tecnologia (projects UIDP/04378/2020 and UIDB/04378/2020 of the Applied Molecular Biosciences—UCIBIO), the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB, the PhD grant 2020.04493.BD (RRB) and research contract (under Scientific Employment Stimulus) 2021.01789.CEECIND/CP1662/CT0014 (JPS).

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Oral Communication 08

Tramadol and its main metabolites: toxicological effects on zebrafish embryos and larvae

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https://doi.org/10.48797/sl.2024.136

Abstract

Background: Tramadol (TRA) and its main metabolites are among the most detected pharmaceutical compounds in aquatic ecosystems, reaching concentrations in the µg/L range. In humans, TRA acts on different receptors of the noradrenergic, serotoninergic, dopaminergic and opioid systems. Also, it is metabolized into the active metabolite o-desmethyltramadol (OTRA) and inactive metabolite n-desmethyltramadol (NTRA). In fish, TRA was reported to have several toxic effects [1,2]. However, for OTRA and NTRA there is no information about their toxicity in fish. **Objective:** the main aims of this work were to investigate and compare the effects of TRA, OTRA and NTRA on zebrafish embryonic development; behavioural responses; expression profiles of genes related to the monoaminergic and detoxification systems and proteome, as well as develop and initial draft of an adverse outcome pathway (AOP) **Methods:** Zebrafish embryos were exposed (0.1−100µg/L) for 168hpf to TRA, OTRA and NTRA [3]. During the exposure, several developmental malformations were registered. Behaviour was evaluated through a sensorimotor assay. Gene expression for 32 target genes was obtained through qPCR, while shotgun proteomics protocols were employed for the proteome evaluation. **Results:** TRA and OTRA were found to cause significant increases in embryonic anomalies, namely in the yolk sac, spine, and otoliths, at the highest concentrations. Decreased sensorimotor responses were observed for exposure to 0.1 and 100µg/L of TRA and OTRA. Different gene expression profiles were found between TRA and the metabolites OTRA and NTRA, with a predominance of non-monotonic responses. Shotgun proteomics indicates that TRA and OTRA impact pathways linked to vital biological processes. An initial drat AOP was performed. **Conclusions:** The obtained results indicated that exposure to TRA, OTRA and NTRA can negatively impact aquatic non-target species at different levels of biological organization. These results also raise awareness for the inclusion of pharmaceutical in monitoring programmes.

Keywords: tramadol; metabolites; embryonic development; genomics; proteomics

Acknowledgments

Pedro Rodrigues was supported by a fellowship from FCT (SRFH/BD/134518/2017). This research was funded by the BiodivRestore ERA-NET COFUND Action (a joint programme of Biodiversa and Water JPI) project BioReset (DOI: 10.54499/DivRestore/0004/2020) and by UIDB/04423/2020 and UIDP/04423/2020 programmes, through FCT (Portuguese Foundation for the Science and Technology).

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Oral Communication 09

Drug use among the student population of the University of Porto: Analysis of prevalence patterns

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https://doi.org/10.48797/sl.2024.137

Abstract

Background: Alcohol, tobacco, and illicit drugs use by university students is a complex issue that has garnered concern and interest from public health officials, educators, and scientific community [1]. This concern is driven by various factors, including the potential to affect academic performance and mental and physical health. **Objective:** This study was designed to determine the prevalence and patterns of psychotropic drug use among students at the University of Porto (UP). **Methods:** This was an observational, transversal, and descriptive study approved by the Ethics Committee of CHUP/ICBAS (2021/CE/P006[P346/CETI/ICBAS]). Voluntary participants (n=4,052) from the student body of UP enrolled in the academic year 2021/2022 completed a web-based questionnaire assessing self-reported drug use across a range of specified psychoactive substances, between February and April of 2022. Gender, age, living situation, economic status, drug use patterns and intent were also collected and used as stratification and association variables. **Results:** The three most consumed substances were alcohol, tobacco, and cannabis and its derivatives (CAD). Notably, 23.0% of respondents declared smoking cigarettes every day, while only 1.8% of alcohol users drank every day (36.8% drank it 2-4 times per month). “Nightclubs and bars” and “other people’s homes” were the most cited locations for alcohol and cigarette use. Illicit drugs have more variety when it comes to settings depending on their usual context and motive for use. “Having fun” was reported by half of the students as the reason for drinking alcohol, while curiosity was the main drive for smoking tobacco and CAD. **Conclusions:** Students have access to and consume different (il)licit drugs. These results confirm the importance of this type of research, tracing a more defined image of the drug use prevalence patterns of this population, which in turn can aid health officials and other institutions when constructing and enforcing population-specific prevention and drug treatment measures.

Keywords: drug use prevalence; illicit drugs; alcohol; tobacco

Acknowledgments

This work was supported by FCT—Fundação para a Ciência e a Tecnologia (projects UIDP/04378/2020 and UIDB/04378/2020 of the Applied Molecular Biosciences—UCIBIO), the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HBM and the PhD grant 2020.04493.BD. The authors have no conflict of interest to declare.

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Oral Communication 10

The unseen ingredients: a systematic review of the prevalence of microplastics and nanoplastics in our food

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https://doi.org/10.48797/sl.2024.138

Abstract

Background: Plastics have revolutionized every aspect of modern life [1, 2], offering convenience but also bringing forth environmental and health hazards. Their exponential production and improper disposal have led to the global burden of microplastics (MPs) and nanoplastics (NPs) in the environment [3, 4]. These tiny particles possess the alarming ability to infiltrate the food chain, thereby posing a potential threat to human health [1-3, 5]. **Objective:** This study aims to evaluate the prevalence of MPs/NPs contamination in food and beverages, emphasizing the urgency of addressing this invisible threat and providing future directions. **Methods:** Employing a PRISMA methodology, a comprehensive literature review was conducted on PubMed, Scopus, and Web of Science until August 15, 2023. From an initial pool of 4078 potentially relevant studies, duplicates and unrelated works were removed, and 229 articles focusing on edible products were selected for analysis, resulting in 1630 data points. Systematic categorization included food types, detection techniques, particle characteristics, and polymer compositions. **Results:** Our investigation unveiled that over 95% of the examined food items were found to be contaminated with MPs/NPs. Predominant detection methods included FTIR spectroscopy and microscopy, uncovering fragments and fibers in a spectrum of colors such as blue, black, red, transparent, and white. Polypropylene, polyethylene, and PET emerged as the primary polymers present across diverse food categories. Notably, fruits and vegetables exhibited the highest contamination rates (126150 items/g), followed by sauces, beverages, and dairy products (45 to 8 items/L). Conversely, seafood, sweeteners, canned foods, salts, meats (0.7 up to 0.014 items/g), rice (56 µg/g), and soy-based products (0 µg/g) displayed comparatively lower contamination levels. **Conclusions:** This study highlights significant gaps in our knowledge regarding the extent of MPs/NPs contamination in our diet. Future research is imperative to expand our understanding of their presence, particularly in under- or unexplored foods categories such as fruits and vegetables, dairy products, meat, bakery products, baby foods, and beverages other than water. This issue cannot be underestimated, as it pertains directly to safeguarding human health in the face of an unseen but potentially pervasive threat.

Keywords: microplastics; nanoplastics; prevalence; food chain contamination

Acknowledgments

This work was funded by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) and the SALIVA+ project (2022.08978.PTDC).

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Oral Communication 11

Development of enhanced buccal films with *Actinidia arguta* fruit extract for oral mucositis prevention: from *in vitro* buccal models to *ex vivo* investigations

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https://doi.org/10.48797/sl.2024.139

Abstract

Background: Oral mucositis (OM) is a common side effect of cancer treatments such as chemotherapy [1], being characterized by disruption of the oral mucosa integrity, inflammation, and pain [1,2]. The treatment strategies to prevent and treat OM are still unsatisfactory, leading to the search of new active compounds, particularly from natural sources, such as *Actinidia arguta* fruits [1]. *A. arguta* fruit, commonly known as kiwiberry, has been associated with different therapeutic properties and pro-healthy benefits, particularly antioxidant, anti-inflammatory and anticancer effects [3,4], due to the fruit's outstanding content in phenolic compounds, vitamins, and organic acids [3,4]. **Objective:** The aim of this study was to develop buccal films with *A. arguta* fruit extract as active ingredient to prevent OM symptoms. **Methods:** The films were prepared by solvent casting employing 1% of HPMC K100 LV EP, 2.5% glycerin, and *A. arguta* extract as solvent, previously prepared by Ultrasound-Assisted Extraction [4]. **Results:** Different films parameters were assessed, namely physical features (weight: 194.8 mg; thickness: 0.37 mm; disintegration time: 15.05 min; moisture content: 10.53%; swelling capacity: 55.95%), mechanical properties (resistance to extension: 10.11 N; percent of elongation: 36.10%; Young's modulus: 0.0034 MPa) and antioxidant/antiradical activities (TPC = 6.46 mg GAE/g film; FRAP = 49.45 µmol FSE/g film; ABTS = 3.74 mg AAE/g film; DPPH = 4.90 mg TE/g film). *In vitro* cell assays attested the absence of negative effects on HSC-3 and TR146 oral cell lines. Most important, the compounds release profile was assessed through *in vitro* cell models (TR146) and *ex vivo* assay with porcine mucosa coupled to LC/DAD-ESI-MS quantification. The results revealed high permeation of rutin (88.32%), quercetin-3-*O*-glucoside (84.95%) and catechin (79.74%). **Conclusions:** Overall, these results highlight the significant potential and safety of buccal films with *A. arguta* fruit extract to prevent OM condition.

Keywords: *Actinidia arguta*; buccal *in vitro* model; porcine mucosa *ex vivo* assay; antioxidant compounds; oral diseases

Acknowledgments

This research was funded by EXPL/BAA-GR/0663/2021 – Kiwi4Health – Exploring the Eco-Innovative Re-Use of Kiwiberry and projects REQUIMTE/LAQV—UIDB/50006/2020, UIDP/50006/2020, and LA/P/0008/2020. Ana Margarida Silva (SFRH/BD/144994/2019) and Catarina Macedo (2022.12642.BD) are thankful for their Ph.D. grants financed by POPH-QREN and subsidized by the European Science Foundation and Ministério da Ciência, Tecnologia e Ensino Superior. Francisca Rodrigues (CEECIND/01886/2020) is thankful for her contract financed by FCT/MCTES—CEEC Individual Program Contract. The participation in this event is supported by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário through "GI2-CESPU/Voucher Out-23-Out24" to Filipa Teixeira.

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Oral Communication 12

Evaluation of antitumor activity of xanthones conjugated with amino acids

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https://doi.org/10.48797/sl.2024.140

Abstract

Background: Cancer is a complex disease characterized by several alterations that confer on the cells, the capacity to proliferate uncontrollably and to resist cellular death. Multiresistance to conventional chemotherapy drugs is often the cause of treatment failure; thus, the search for natural products or their derivatives with therapeutic action is essential. Chiral derivatives of xanthones (CDXs) have shown potential inhibitory activity against the growth of some human tumor cell lines and the influence of the stereochemistry [1,2]. **Objective:** This study aimed to screen a library of previously synthesized CDXs in a panel of cancer cell lines to identify the most promising compounds for further study as possible chemotherapy drugs, as well as to analyze their effect on several parameters of cancer cells and verify whether the compounds under study were substrates of P-glycoprotein (P-gp), one of the main mechanisms of resistance in cancer therapy. **Methods:** In this study, cell viability assays were performed on three tumor cell lines: MCF-7, NCI-H460, and A375-C5 using a library of previously synthesized CDXs. CDXs' effect was analysed based on several parameters of cancer cells, like extracellular levels of glucose and lactate, the mechanism of cell death, and it was also verified whether these compounds were substrates of P-gp. **Results:** The cytotoxic activity of forty-six CDXs was evaluated, and an enantiomeric pair was considered as lead compounds and selected for other studies since they presented GI_{50} values lower than 15 µM for all cell lines. The selectivity index higher than 1 against cancer cells was found for both enantiomers, indicating that these compounds are considerably more specific to cancer cells, which is desirable. No significant changes were identified in the metabolic parameters analysed and it was not possible to determine whether the compounds are P-gp substrates. The main mechanism of death triggered by these compounds was apoptosis. **Conclusions:** These results show that some CDXs present promising antitumor activity, but other mechanisms besides metabolism, should be triggered by these compounds. Evidence of enantioselectivity was found being the D enantiomer more cytotoxic, compared to L.

Keywords: derivatives of xanthones; cancer; P-glycoprotein; multidrug resistance

Acknowledgments

This research was funded by national funds through the Foundation for Science and Technology (FCT) within the scope of UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry—CIIMAR) and ERDF, as a result of the projects PTDC/CTAAMB/6686/2020 and PTDC/CTAAMB/0853/2021. This research was also funded by CESPU—Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. Flav4Tumor GI2-CESPU-2022 and XANTAAL-GI2-CESPU-2022). FB gratefully acknowledges CESPU for a PhD Grant BD/DCB/CESPU/01/2024.

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Oral Communication 13

Chemical differences between alternative and traditional tobacco products

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https://doi.org/10.48797/sl.2024.141

Abstract

Background: Electronic cigarettes (E-cigs) and heated tobacco products (HTPs) have gained popularity as alternatives to traditional tobacco products (TTPs), claiming to reduce harm. The carcinogenic properties of chemicals in the smoke of TTPs are widely recognized. However, there is still an incomplete understanding of the different chemicals in E-cigs and HTPs and their toxicity to human cells [1]. **Objective:** Thus, this study aimed at characterizing and comparing the chemical composition of three different brands of E-cigs, HTPs and TTPs. **Methods:** We selected the three top-selling brands of E-cigs, HTPs, and TTPs in Portugal, and each brand (n=9) was analyzed in triplicate. Volatile compounds present in all brands were extracted by headspace solid-phase microextraction (HS-SPME) and solvent extraction (dichloromethane). The volatile compounds in the headspace and solvent extracts were analysed by gas chromatography-mass spectrometry (GC-MS). Compound annotation was performed by comparing the mass spectrum of each chromatographic peak in the sample with a mass spectral library and standards, where available. **Results:** A total of 53 compounds were detected in E-cigs, 44 in HTPs and 41 in TTPs by HS-SPME. Solvent extraction revealed 43 compounds in E-cigs, 35 in HTPs and 22 in TTPs. Only 7 compounds were common to E-cigs, HTPs, and TTPs. Overall, the chemical classes included alcohols, aldehydes, ketones, esters, pyridines and others. The composition of HTPs and TTPs was similar (20 compounds in common), particularly in the classes of ketones, alcohols, terpenoids, and pyridines. In contrast, E-cigs contain a larger number of compounds than HTPs and TTPs, including several alcohols, esters, pyranones, and lactones. The volatile composition of HTPs and TPPs showed less variation between different brands, whereas E-cig brands showed greater variability in their composition. **Conclusions:** HTPs have a volatile chemical composition similar to that of TTPs in their original form, so their health effects will depend on the impact of the different types of combustion. E-cigs show a distinct chemical profile across all brands, with chemical classes that are potentially relevant for toxicological studies.

Keywords: electronic cigarettes; heated tobacco products; volatile chemical composition; gas chromatography-mass spectrometry

Acknowledgments

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/04378/2020 and UIDB/ 04378/2020), and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020). The Cooperativa de Ensino Superior Politécnico e Universitário (CESPU) is gratefully acknowledged for the PhD grant no. BD/DCB/CESPU/02/2023.

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Oral Communication 14

Ecotoxicological effects of 3,4-dichloroaniline on *Daphnia magna*: implications for aquatic ecosystem health and management

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https://doi.org/10.48797/sl.2024.142

Abstract

Background: Aromatic amines are extensively employed in dye, pharmaceutical, pesticide, and polymer manufacturing. These compounds exert environmental impacts, affecting aquatic ecosystems and biodiversity [1]. 3,4-dichloroaniline (3,4-DCA) is a candidate for inclusion in the 4th Watch List of Water Framework Directive due to its presence in aquatic ecosystems and known ecotoxicological effects [2, 3]. **Objective:** This study aimed to assess the individual and sub-individual chronic effects of 3,4-DCA on *Daphnia magna*, considering environmental concentrations [0.07 µg/L to 6 µg/L in wastewater treatment plant influents, superficial water (Germany), and effluents (USA), river waters (Portugal and USA) and groundwaters (Portugal) [3]] and previous studies (subchronic exposure). **Methods:** Chronic exposure (21 days) was conducted with *D. magna* exposed to a range of ecologically relevant concentrations of 3,4-DCA (≤ 6 µg/L). Different biological responses were evaluated: 1) individual - growth and reproduction; and 2) sub-individual - antioxidant defense and detoxification, energetic metabolism, neurotransmission, and genotoxicity. **Results:** Significant effects were observed in *D. magna* following 3,4-DCA exposure. Somatic growth rate increased (≥ 2 µg/L), catalase activity decreased (0.222 µg/L) followed by an increase (6 µg/L), and genetic damage index increased above 0.294 µg/L. The rise in somatic growth may signal resource allocation changes, affecting fitness and reproduction. Catalase activity fluctuation and the absence of significant results in other antioxidant defenses and lipid peroxidation suggest that this enzyme was able to neutralize oxidative stress and damage. Genotoxicity suggests future impacts on population and genetic diversity. **Conclusions:** This study provides crucial insights into the chronic ecotoxicological effects of 3,4-DCA on *D. magna*, under ecologically relevant concentrations. It underscores the importance of considering diverse biological endpoints in ecotoxicological assessments. These findings play a pivotal role in assessing the ecological risks associated with aromatic amines, enabling the identification of measures to safeguard global environmental integrity and human health.

Keywords: ecotoxicology; aromatic amine; aquatic ecosystem; growth; biomarkers

Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Rodrigues is hired through the Regulamento do Emprego Científico e Tecnológico – RJEC from the FCT program (doi: 10.54499/2020.00464.CEECIND/CP1599/CT0002). Daniela Rebelo is supported by FCT PhD grants (2022.13777.BD).

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Oral Communication 15

Sublethal enantiotoxicity of MDMA on the development, teratogenicity, genotoxicity and swimming behaviour of zebrafish (*Danio rerio*) embryo

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https://doi.org/10.48797/sl.2024.143

Abstract

Background: Chiral psychoactive substances (PAS) as amphetamine-like substances are among the main group of drugs seized in Europe. The increased attractiveness of these drugs resulted in their widespread occurrence in the environment raising concern about their possible hazardous effects to non-target organisms [1, 2]. PAS metabolism in humans and biodegradation in wastewater treatment plants is stereoselective. Enantiomers of PAS may differ in environmental fate and toxicological effects [2, 3]. Understanding their enantiomer-specific toxicity and sublethal effects using several biomarkers is crucial for an accurate risk assessment of chiral contaminants. **Objective:** Investigate the potential enantioselective effects of 3,4-methylenedioxymethamphetamine (MDMA) in zebrafish (*Danio rerio*) embryos. **Methods:** For zebrafish 96-h assay, 50 fertilized eggs (≈ 3 hpf) were exposed to different concentrations of MDMA racemate and enantiomers ranging from 0.02 to 200 µg/L. Six replicates per test concentration and control were performed and organisms were exposed for 6 days. Embryonic development, malformations, genotoxicity and larvae swimming behaviour parameters were evaluated for MDMA. The range of concentrations for both organisms was selected to include environmental reported levels and higher concentrations to get insights into potential toxicity. **Results:** Regarding zebrafish, preliminary data showed an enantioselective effect with significant increase in the percentage of developmental malformations at 96 hpf in zebrafish larvae exposed to (S)-MDMA in comparison with (R,S)-MDMA and (R)-MDMA at the lower concentrations. An increase in larvae size was observed in the organisms exposed to the racemate in comparison to the enantiomers. Also, a significant increase in genotoxicity was observed in organisms exposed to racemate. Changes and enantioselective effects were also observed in swimming behaviour, (R)-MDMA inducing a reduction in the distance to the centre of the well compared to (S)-MDMA, and a decrease in inactive time in (S)-MDMA exposed organisms. **Conclusions:** This study showed the toxicity of MDMA towards zebrafish embryo, and importance of enantiotoxicity studies for a better risk assessment.

Keywords: recreative drug; zebrafish model; biomarkers; aquatic pollution; non-target species

Acknowledgments

This study was funded by FCT–Portuguese Foundation for Science and Technology, through the FCT/MCTES (PIDDAC), under the ENANTIOTOX project (http://doi.org/10.54499/PTDC/CTA-AMB/6686/2020) and CITAB project (https://doi.org/10.54499/UIDB/04033/2020). Ondina Ribeiro thanks the FCT for the PhD research grant 2022.12242.BD.

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Oral Communication 16

Exposure to a glyphosate-based herbicide induces avoidance behavior and impairs coelomocyte viability in *Eisenia andrei* earthworms

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https://doi.org/10.48797/sl.2024.144

Abstract

Background: Glyphosate-based herbicides (GBH) are the most widely used agrochemicals [1]. Earthworms are key soil organisms used as bioindicators and alternative experimental models for studying the immune system [2,3]. **Objective:** We tested whether agronomic dosages of GBH induce avoidance behavior and alter the immunological profile of earthworms *Eisenia andrei*. **Methods:** Adult earthworms (0.318 ± 0.007 g) were divided into four groups and exposed for 48h: Control group (native soil), GBH1.5, GBH3, and GBH6 groups (native soil with GBH at concentrations equivalent to 1.5, 3.0, and 6.0 L/ha, respectively). Under these conditions, we applied the Avoidance Behavior Test (% of animals that escape from contaminated areas) and Acute Toxicity Test. We used glyphosate (Roundup®, Original DI, Monsanto, 44.5% w/v active ingredient) or water (control) in each experimental unit (n=6; 6 animals/experimental unit, 6 replicates each, in a box with 600g of soil, 95% of dystrophic red latosol:5% organic matter). The coelomocytes were collected by a non-invasive method [4]. **Results:** The highest concentration (GBH6) induced avoidance behavior in earthworms (% avoidance = GBH6 = 83.3±18.2, p=0.01) without modification in the immune profile. Furthermore, there was a reduction in cell viability of the coelomocytes obtained from the GBH6 (p=0.001) and also GBH3 (p=0.01) groups, when the animals had no option to avoid the contaminated area (CTRL: 75.7±18.9%; GBH1.5: 63.7±22%; GBH3: 56.7±29.6%; and GBH6: 56.0±21.4%). **Conclusion**: The presence of GBH in the soil at a typical agronomic dose (3.0 L/ha) or higher (6.0 L/ha) threatens the immune defense of earthworms and may lead to the loss of the ecological function of soil.

Keywords: glyphosate; behavior; immune cells; environmental pollution

Acknowledgments

This study was partially financed by the Coordination for the Improvement of Higher Education Personnel (CAPES) in Brazil, under grant 001, and by The Brazilian National Council for Scientific and Technological Development (CNPq) under grants 307926/2022-2 and 405546/2023-8 to TGH.

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Oral Communication 17

Understanding the ecological consequences of deep-sea mining: cadmium's influence on microbial diversity in Pacific seamount sediments

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https://doi.org/10.48797/sl.2024.145

Abstract

Background: Metal-rich nodules in the deep ocean contain significant quantities of high-value metals such as copper, nickel, and cobalt [1]. Mining deep-sea minerals is considered as an alternative to land mining, to reduce environmental effects and gain geopolitical advantages [1,2]. However, this action may expose deep-sea microbes to toxic concentrations of metals such as cadmium (Cd) which are shown to have an impact on metabolic and biogeochemical processes [3,4]. **Objective:** This study aims to quantify microbial diversity in deep-sea habitats, specifically Pacific Ocean seamounts, while examining the genomic responses caused by various degrees of Cadmium (Cd) exposure. **Methods:** Sediment samples from Pacific Ocean seamounts were collected, 96h pre-exposed to different concentrations of Cd, and later sequenced with 16S rRNA gene sequencing with Illumina MiSeq. Bioinformatics analysis has been conducted to investigate the taxonomic and functional diversity of microbial communities as well as their genomic responses to metal exposure. **Results:** According to the initial findings, the microbial communities in all samples were dominated by bacteria, with also the presence of archaea. Diversity in bacterial communities is higher compared to the archaeal groups. Upon completion of the study, we expect to observe a shift in the diversity of microbes found in sediment samples as Cd concentrations increase. Some microbes may be more resistant to Cd and become more abundant, whilst others may become less abundant. **Conclusions:** Deep-sea mining is expected to have impacts on the microbial communities, possibly hampering the diversity of the communities. A change in microbial diversity can have cascading impacts on the nutrient cycle and the overall health of the ecosystem. Future research should investigate the other aspects of the impacts that can come along with deep-sea mining before the approval of commercial operation.

Keywords: deep-sea mining; microbial diversity; gene sequencing; cadmium

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 101038095. This work was also supported by the project ATLANTIDA (NOR TE-01- 0145-FEDER-000040), and by the project DeepResist (2022.06475.PTDC), funded by FCT (Fundação para a Ciência e Tecnologia).

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Oral Communication 18

Bacterial coaggregation's role in water disinfection resistance

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https://doi.org/10.48797/sl.2024.146

Abstract

Background: In the realm of water treatment and public health, the interplay between microorganisms and disinfection processes represents a constant and dynamic challenge [1]. As society strives to ensure the availability of safe and clean water, understanding the factors contributing to bacterial resistance to water disinfection emerges as a critical area of research [1]. Among these factors, the phenomenon of bacterial coaggregation has gained increasing attention for its pivotal role in shaping microbial communities and influencing the effectiveness of disinfection strategies [2]. **Objective:** This work aims to elucidate the intricate relationship between bacterial coaggregation and resistance to water disinfection. **Methods:** Biofilms of the emerging drinking water (DW) pathogen *Stenotrophomonas maltophilia* [3,4] were formed on polyvinyl chloride (PVC) coupons for 7 days and studied for their resistance to sodium hypochlorite disinfection when co-cultured with two strains from the DW context: one exhibiting coaggregation behavior (*Delftia acidovorans* 005P) and another without coaggregation tendencies (*D. acidovorans* 009P). **Results:** It was observed that high doses of free chlorine (> 10x MBC) were not able to completely kill biofilm bacteria within 30 minutes of disinfection. However, biofilms that combined *S. maltophilia* with *D. acidovorans* 005P (coaggregating strain) showed greater resistance to disinfection. In addition, the biofilms that contained the coaggregating strain were also those that presented a greater content of extracellular polymeric substances (EPS), namely proteins and polysaccharides, and a greater thickness. **Conclusions:** It is known that coaggregation is mediated by protein-saccharide interactions and that in this case, they may be contributing to the biofilm's tolerance to disinfection [1,5]. Thus, understanding the role of bacterial coaggregation in disinfection resistance is pivotal for developing innovative strategies that can effectively combat the persistence of pathogenic microorganisms and safeguard public health in an increasingly complex and interconnected world.

Keywords: biofilm characterization; drinking water disinfection; drinking water safety; sodium hypochlorite; waterborne pathogen

Acknowledgments

This work was financially supported by: LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020), and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020), funded by national funds through FCT/MCTES (PIDDAC); UIDB/04469/2020 (CEB) and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020; UIDB/04033/2020 (CITAB); the FCT contract attributed to Inês B. Gomes (2022.06488.CEECIND) and the FCT PhD grant attributed to Ana Afonso (2020.04773.BD).

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Oral Communication 19

Deciphering Zebrafish spectral signatures: insights from Raman spectroscopy

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https://doi.org/10.48797/sl.2024.147

Abstract

Background: Raman spectroscopy (RS) is a sensitive technique used for gathering chemical and molecular data, resulting in a biochemical fingerprint of the sample. This method is label-free and provides rapid detection [1]. Furthermore, it has proven invaluable in the forensic, biological and medical contexts [2]. It is also suitable for investigations assessing toxic effects of some chemicals, enabling swift acquisition of extensive organism-specific information. Its application to zebrafish serves various purposes, including physiological [3] and toxicological evaluation [4]. However, no base characterization of this organism has been provided in such studies that could be useful for the planning of experiments aiming at diagnosing and follow-up environmental contamination. **Objective:** The present study aimed to characterize the developmental Raman profile of different zebrafish organs, by drawing a baseline analysis of embryos and larvae up to 168 hpf. **Methods:** Different organs or tissues were examined daily from 24 to 168 hours post-fertilization, according to their time window of emergence in the embryo, including the heart, muscle, brain, iris, swim bladder, and melanocytes. Chemometric analysis, employing partial least-squares discriminant analysis (PLS-DA), was employed to characterize the organs and ascertain the contribution of spectral bands to their discrimination. **Results:** A total of 117 spectral bands were identified, with 24 demonstrating robust, systematic representation. The bands were found in the 223 to 3431 cm^{-1} spectral range; most of them were related to amino acids and relevant macromolecules, such nucleic acids, proteins and lipids. Only three bands were found to be common to all recorded organs and tissues. PLS-DA generated distinct spectral fingerprints for each organ, illustrating variations over early development. **Conclusions:** Overall, the work developed provided a clear baseline profile of organs and tissues of zebrafish embryos and larvae, identifying Raman bands of expeditious acquisition and their expected variation over different developmental stages, before and after hatching.

Keywords: chemometric analysis; early development; Raman bands; zebrafish organs

Acknowledgments

This research was funded by the project BioReset (DOI 10.54499/DivRestore/0004/2020), ERA-NET Confund Bio-divRestore and by UIDB/04423/2020 and UIDP/04423/2020; Isabel Oliveira Abreu was supported by an FCT fel-lowship (SFRH/BD/115529/2016). Cláudia Teixeira is supported by a PhD grant from FCT (2022.10117.BD).

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Oral Communication 20

Microplastic occurrence and distribution in the Ave river estuary

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https://doi.org/10.48797/sl.2024.148

Abstract

Background: Microplastics (MPs) have been found in all types of aquatic ecosystems, posing a threat to the environment, wildlife and human health [1-3]. Although rivers act as marine litter pipelines to the ocean, estuaries can act as buffer zones preventing garbage from reaching the sea, thus, acquired data on those ecosystems contribute to a better understanding on the entry of litter into the ocean from terrestrial sources [4]. As such, knowledge of estuarine hydrodynamics is critical to understand problems such as water quality, residence time and dispersion rate of pollutants [5]. In addition, scientific data on MPs in Portugal aquatic ecosystems is scarce. **Objective:** This study aimed to: 1) evaluate and characterize MPs in the Ave Estuary water column, and 2) identify the potential sources, and analyse the main hydrodynamic patterns and the water residence time to understand the MPs transport patterns. **Methods:** Short-term campaigns were performed to measure vertical profiles of velocity, salinity, temperature and pH in the Ave Estuary. MPs particles were sampled using a planktonic horizontal trawl, processed following standardized protocols [6], identified, measured, and sorted by shape and colour [7,8]. **Results:** This study revealed a clear stratification of the Ave Estuary, as well as considerable contamination by MPs (annual average density of 63.1 ± 4.7 MPs/m³) with a significant prevalence of blue (34%) fragments (53%), measuring between 0.5 mm and 1 mm (42%). **Conclusions:** The obtained results showed a very strong seasonal pattern, and pointed to public litter, tourism and fishing activities as potential main sources of MPs in the study area and to an urgent need for adequate management of MPs and of marine and coastal ecosystems. This study represents a contribution to the pressing need of temporal and spatial monitoring on MPs pollution in estuaries and other coastal ecosystems.

Keywords: microplastics; emerging contaminants; estuarine hydrodynamics; coastal ecosystems

Acknowledgments

This research was supported by MAELSTROM, grant number 101000832, within the R&D Institution CIIMAR, supported by the EU Horizon 2020 Framework Program for Research (2014-2020), and by FCT (2022.07420.CEECIND, 2021.02308.CEECIND).

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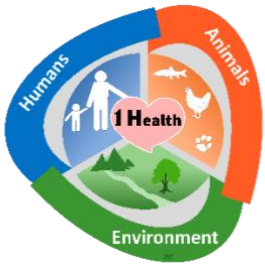

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POSTERS

Poster 01

Emergency Crisis Kit used in veterinary palliative care of a cat with suspected gastric lymphoma and other co-morbidities

Joana Maria da Costa [1,ǂ,*], **Joana Correia Prata** [1,2,3,ǂ], **Felisbina Queiroga** [4], **Hugo Parrinha** [5] **and Teresa Barroso** [2,3,6]

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https://doi.org/10.48797/sl.2024.159

Abstract

Background: Emergency Crisis Kits for palliative veterinary patients ensure adequate measures to be taken in a sudden and/or acute situation until assistance from a veterinary medical doctor can be procured [1]. **Objective:** This case study demonstrates the use of a personalized Emergency Crisis Kit on a cat with gastric lymphoma and other co-morbidities. **Results:** It was found that having an Emergency Crisis Kit helped to improve the cat's quality of life, as well as relieve the caretaker burner syndrome of owners, promoting closeness, empathy, and confidence with the veterinary team. **Conclusion:** Emergency Crisis Kits empower pet owners to be more active in the palliative management of their animals, by providing aid in moments of pain, distress and discomfort and by preventing stressful and traumatic experiences [2,3]. However, the use of Emergency Crisis Kits must be further disseminated and implemented.

Keywords: veterinary palliative care; emergency crisis kits; feline gastric lymphoma

Acknowledgments

This research received no external funding.

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Poster 02

Unlocking success: the crucial role of good clinical practices in veterinary practice for antimicrobial resistance control

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https://doi.org/10.48797/sl.2024.160

Abstract

Background: Antimicrobial resistance (AMR) poses a global health challenge, impacting both humans and animals [1]. The emergence and spread of antibiotic-resistant bacteria among pets heighten the risk of transmission to humans or animals, given their close interaction [2]. **Objective:** This study aimed to underscore the importance of implementing good clinical practices in veterinary medicine, using a clinical case of a dog exhibiting purulent rhinorrhea diagnosed with extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* (*K. pneumoniae*). **Methods:** A retrospective analysis was conducted on cases admitted to the Veterinary Hospital (UPVet) of ICBAS, University of Porto, throughout 2022. A clinical case was selected based on the isolation of a multidrug-resistant bacterial strain considered clinically relevant for public health. Antimicrobial resistance profiling and Whole Genome Sequencing (WGS) were conducted on the isolated strains [3]. **Results:** A 1-year-old dog presenting signs of vomiting and mucopurulent rhinorrhea was brought to UPVet for an emergency appointment. During the examination, a nasal sample was taken, revealing a pure culture of ESBL *K. pneumoniae*. Amikacin was chosen for treatment and the animal was promptly placed in isolation with appropriate biosafety measures. After seven days, methicillin-resistant (MRSP) *Staphylococcus pseudintermedius* was also isolated in a nasal swab. Genotypic analysis showed similarities between ESBL-producing *K. pneumoniae* strains, while the MRSP strains differed: the first MRSP exhibited resistance to aminoglycosides and the second carried the *aac(6')-aph(2")* gene, heightening its resistance to amikacin. After antibiotic treatment, neither ESBL-producing *K. pneumoniae* nor MRSP were isolated. Within 6 months post-case, no similar *K. pneumoniae* from UPVet was found. **Conclusions:** The emergence of MRSP strains may have been favored by ongoing antibiotic therapy. Nevertheless, veterinary intervention focused on identifying the potential causative agent and selecting the optimal antibiotic choice likely contributed to resolving the infectious process. The implemented biosafety measures may have played a crucial role in containing the spread of *K. pneumoniae* throughout hospital facilities. Hence, targeted therapy, alongside proper clinical practices, improves treatment success rates and mitigates antimicrobial resistance, safeguarding the health of animals, humans, and the environment.

Keywords: antimicrobial resistance; clinical practice; antibiotherapy; veterinary medicine

Acknowledgments

This research received no external funding.

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Poster 03

Colistin-resistant *Escherichia coli* in calves and adult cattle from Portuguese dairy farms

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https://doi.org/10.48797/sl.2024.161

Abstract

Background: Antimicrobial resistance poses a pressing and significant challenge, driven by the intricate interplay among animals, humans, and the environment [1]. *Escherichia coli*, a commensal within animal and human gut microbiota, stands as one of the most ubiquitous bacteria. With a multitude of resistance genes, many *E. coli* strains exhibit a multidrug-resistant phenotype, diminishing the effectiveness of available antimicrobial agents [2] and becoming a major challenge in human therapeutics. Resistance to colistin, which is currently only used in humans as a last resort against multidrug-resistant bacteria [2,3], is unknown in fecal *E. coli* from Portuguese bovine. **Objective:** To detect *E. coli* carrying mobilized-colistin resistance (*mcr*) genes isolated from Portuguese dairy cattle. **Methods:** Fecal pools divided by age groups (8 adult or 8 calf samples) were collected from Holstein-Friesian dairy cattle produced on 8 farms in the Northern region (Braga and Porto), with a history of antibiotic administration in the last 3 months. Each farm comprised 2 pools totaling 128 samples (8 farms´16 pools). Samples were plated onto MacConkey agar (with or without 3 µg/mL colistin). Typical colonies from each plate were identified by MALDI-TOF MS and screened for *mcr*-1, *mcr*-2 and *mcr*-3 genes by PCR [3]. **Results:** Representative *E. coli* (*n*=26) identified in 15/16 pools (7 adults and 8 calves) were selected for *mcr* screening. The *mcr*-1 gene was detected in 46% (*n*=12) of the *E. coli* isolates from 11 positive pools (6 isolates from 6 calf pools and 6 isolates from 5 adult animal pools). In 5 farms, both age groups analyzed were positive. No *mcr*-2 and *mcr*-3 were identified. **Conclusions:** This study represents the first detection of the *mcr*-1 colistin-resistance gene in *E. coli* from dairy cattle, including calves, in Portugal. These highlight the potential public health risk posed by livestock as a reservoir and source of *mcr*-1genes able to reach humans through the food chain or the environment.

Keywords: antimicrobial resistance; bovine; *Escherichia coli*; *mcr* genes; Holstein-Friesian

Acknowledgments

This work was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário, under the CoRECattle-GI2- CESPU-2022 project, entitled Prevalence of colistin-resistant Enterobacteriaceae on intensive and non-intensive cattle production systems. Work supported by National Funds through FCT in the scope of the UIDP/50027/2020 project, as well as UIDB/50006/2020 and UIDP/50006/2020.

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Poster 04

A challenge in antibiotic stewardship: detection of Vancomycin-Variable *Enterococcus faecium* (VVE) in human clinical and commensal samples (2009-2022)

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https://doi.org/10.48797/sl.2024.162

Abstract

Background: Vancomycin-variable-enterococci (VVE) are *van*A+ enterococci expressing a vancomycin-susceptible phenotype that can revert to a resistant phenotype (VRE) after vancomycin exposure. **Objective:** We aimed to screen and characterize VVE in a large collection of *Enterococcus faecium* (Efm) [1]. **Methods:** We performed a *van*A-PCR screening on an extensive Efm collection (2009-2022), including hospital (*n*=255) and healthy-human (*n*=161) isolates, followed by disk-diffusion susceptibility testing. Vancomycin MICs (Etest) were performed in *vanA*+ isolates with a susceptible phenotype. VVE were sequenced (Illumina-MiSeq/Eurofins-Germany) and representatives of each clonal-complex-CT were sequenced by Nanopore (Plasmidsaurus/USA). cgMLST, antimicrobial resistance and plasmid-replicases (*rep*;Resfinder/PlasmidFinder-CGE-tools) were evaluated. *van*A-transposons and plasmids were characterized and compared to references using Geneious-Prime tools, alongside NCBI blastn/blastx. **Results:** We identified seven VVE (7/416; 2%), six causing infections (3-urine, 1-pus, 1-blood from 2009 and 1-tissue from 2011) and one healthy-human (2022), indicating daily contact with non-treated water and no hospitalization in the previous 12-months. All VVE were vancomycin susceptible (MIC:1.5-4mg/ml), resistant to ampicillin, erythromycin, ciprofloxacin and identified as ST78: five CT230 (4-clinical;1-commensal) and two clinical CT330. Hybrid assemblies of two clinical isolates, CT230 and CT330, showed a homologous Tn*1546* structure with 18,211bp (*van*H-*van*A-Δ*van*X-IS*1216*-*van*Y-*van*Z-other_genes-IS*1216*-Δ*tnp*A-*tnp*B-*van*R-Δ*van*S) flanked by *IS1216*. The presence of Δ*van*X and Δ*van*S by IS*1216*-insertions might explain the lack of resistant phenotype. The healthy-human isolate apparently carries an identical Tn*1546* (nanopore-sequencing is ongoing). Closed genomes carried two different plasmids with Tn*1546*. One is a mosaic plasmid (~150kb) presenting Inc18-like-*rep*_pRE25, Rep1 (*rep*_pTT39_p3) and Rep3 (Δ*rep*_pVRE1-*Van*A). The other (~107kb) carried a Rep3-like (*rep*_pZY2). No homologous plasmids have been described. The healthy volunteer isolate had similar *rep* content to hospital isolates, possibly indicating plasmid similarities or recombined plasmids. **Conclusions:** We firstly report identical strains and Tn*1546*-VVE platforms in human clinical and commensal samples across distant years. This indicates potential colonization leading to VVE selection upon hospital admission and/or antibiotic administration. Continuous surveillance of VVE is crucial for optimizing antibiotic stewardship and ensure effective treatments.

Keywords: vancomycin-variable enterococci; *Enterococcus faecium*; *van*A; Tn*1546*

Acknowledgments

We acknowledge FCT/MCTES for the national funds from the UCIBIO projects UIDP/04378/2020 and UIDB/04378/2020 and i4HB research project LA/P/0140/2020. Ana C. Almeida-Santos also acknowledges the UI/BD/151317/2021 fellowship from FCT through national funds.

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Poster 05

Characterization of vancomycin-resistant *Enterococcus faecium* causing infections in one Portuguese hospital (2022-2024)

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https://doi.org/10.48797/sl.2024.163

Abstract

Background: Vancomycin-resistant *Enterococcus faecium* (VR*Efm*) are leading nosocomial pathogens linked to high mortality rates and costs [1,2]. VR*Efm* are also included in the WHO global priority list of antibiotic-resistant bacteria for which new antibiotics are urgently needed [3]. Their epidemiology is puzzling within Europe and VR*Efm* data in Portuguese hospitals are lacking since the 2000s. **Objective:** We aimed to characterize the antibiotic susceptibility of VR*Efm* obtained from one hospital in the Porto metropolitan area during 2022-2024. **Methods:** Thirty-seven pure cultures obtained from diverse clinical specimens were sent by the hospital in blood agar plates and inoculated onto Slanetz-Bartley agar. Colonies with different morphologies (typical of *Enterococcus* spp.) were further cultivated onto BHI agar. To specifically select VR*Efm*, PCR screening of *vanA/vanB* genes was performed along with a species-specific gene, *gluP*, to distinguish *Enterococcus faecium* and *Enterococcus lactis* (former *E. faecium* clade B) [4]. Antibiotic susceptibility was performed by disk diffusion or broth microdilution (linezolid) (EUCAST/CLSI). WGS (Illumina-NovaSeq) was performed on the linezolid-resistant isolate. **Results:** All VR*Efm* harbored the *vanA* gene and were multidrug-resistant (MDR: resistant to ≥ 3 antibiotics of different families). All isolates were resistant to ampicillin, ciprofloxacin, vancomycin and teicoplanin. Most to erythromycin (94%) and quinupristin-dalfopristin (88%), and less to tetracycline (16%), streptomycin (15%), high-level gentamicin (6%), or linezolid (3%; MIC=8mg/L). None of the isolates were resistant to chloramphenicol. The linezolid resistant isolate (sequence type 80) carried a G2576T mutation in the 23s rRNA gene. Preliminary findings indicated that three cultures (9%) exhibited colonies with different susceptibility to streptomycin (*n*=2) and quinupristin-dalfopristin (*n*=1). **Conclusions:** Contemporaneous VR*Efm* isolates are MDR, demanding dependence on last-resort alternatives, and *van*A continues to be the dominant gene in local VR*Efm*. Continuing surveillance of linezolid susceptibility and the need for different approaches investigating colony-level diversity are needed to optimize treatment, infection control and antibiotic stewardship.

Keywords: *Enterococcus faecium*; hospital infections; antimicrobial resistance; VR*Efm*; public health

Acknowledgments

We acknowledge FCT/MCTES for the national funds from the UCIBIO projects UIDP/04378/2020 and UIDB/04378/2020 and i4HB research project LA/P/0140/2020.

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Poster 06

Efficacy of selected phenolic acids in inhibiting the LasI/LasR and PQS quorum sensing pathways of *Pseudomonas aeruginosa*

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https://doi.org/10.48797/sl.2024.164

Abstract

Background: Quorum sensing (QS) is a bacterial intercellular communication mechanism mediated by extracellular signalling molecules known as autoinducers [1]. LasI/LasR and pseudomonas quinolone signalling (PQS) are fundamental components of the QS system in *Pseudomonas aeruginosa*. These systems perform a crucial role in the regulation of gene expression in response to cell density, coordination of biofilm formation, and expression of virulence factors, as well as antimicrobial resistance [2]. Therefore, the use of QS inhibitors (QSI), especially those that interact with multiple QS systems, could be a promising strategy, not only because it can increase the efficacy of bacterial infection treatment, but also because it reduces the selective pressure for the development of resistance [2]. In particular, phenolic acids are a group of plant secondary metabolites (*i.e.* phytochemicals) that exhibit a broad spectrum of antibacterial activity and have excellent properties in modulating bacterial cell-cell communication [3]. **Objective:** In this study, the efficacy of ferulic acid and sinapic acid in disrupting the LasI/LasR and PQS-QS system of *P. aeruginosa* was investigated. **Methods:** The efficacy of phenolic acids (ferulic and sinapic acids) as QSI of *P. aeruginosa* was investigated using bioreporter strains (*P. aeruginosa* PA14-wild type, PA14-R3, PAO1-wild type, and PAO1-CTX). For this purpose, the ability to inhibit the production of the autoinducers *N*-acyl-homoserine lactone (AHL) and PQS as well as the QS responses mediated by AHL were evaluated by a high-throughput QS inhibition screening system based on a co-cultivation assay. **Results:** Phenolic acids have the potential to inhibit the LasI/LasR and PQS systems by more than 85%, even at subinhibitory concentrations. In addition, reductions of 80% were observed in the production of AHL by the LasI/LasR system of *P. aeruginosa.* **Conclusions:** This study has shown that the phenolic acids tested are promising as inhibitors of LasI/LasR and PQS, allowing them to reduce the pathogenicity/virulence of *P. aeruginosa.*

Keywords: antibiofilm activity; biofilm infections; ferulic acid; sinapic acid; quorum sensing inhibition

Acknowledgments

This research was funded by national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020). Miguel Leitão acknowledges an individual PhD fellowship (2021.07145.BD) from FCT.

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Poster 07

Synergistic bactericidal combinations: far-UV-C, mechanical cleaning and chlorine against Gram-negative and -positive bacteria

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https://doi.org/10.48797/sl.2024.165

Abstract

Background: The issue of indoor contamination stands as a global health challenge, contributing to the spread of infectious diseases [1,2]. Consequently, there is an urgent need to explore new disinfection strategies that rely on reduced concentrations of conventional cleaning chemicals [3,4]. **Objective:** This study aimed to explore innovative disinfection approaches utilizing far-UV-C (222 nm) radiation along with chlorine and mechanical cleaning, offering a promising solution with minimal application doses. **Methods:** The study assessed the bactericidal efficacy of far-UV-C (222 nm) at various intensities (78.4 μW/cm^2 to 597.7 μW/cm^2 for 1 minute) against *Escherichia coli* and *Staphylococcus epidermidis* cells adhered to polystyrene microtiter plates by cellular culturability. Furthermore, combinations with mechanical cleaning (ultrasounds for 1 minute) and free chlorine (0.1, 0.5, and 1 mg/L for 5 minutes) were tested. The triple combination of mechanical cleaning + free chlorine (0.5 mg/L) + far-UV-C (54 mJ/cm^2) was also evaluated against bacteria adhered to materials commonly found in hospital settings and other public spaces: polyvinyl chloride (PVC), stainless steel (SS), and polyetheretherketone (PEEK). **Results:** Disinfection with far-UV-C (54 mJ/cm^2) and free chlorine at 0.5 mg/L for 5 minutes achieved a complete reduction of culturable *E. coli* cells and a logarithmic reduction of 2.98 ± 0.03 CFU/cm^2 for *S. epidermidis*. The triple combination resulted in a total reduction of culturable cells for both adhered bacteria. Bacterial adhesion to PVC, SS, and PEEK varied, influencing the bactericidal activity of the triple combination, with logarithmic reductions of up to 3 CFU/cm^2. **Conclusions:** The study underscores the efficiency of far-UV-C (54 mJ/cm^2) combined with chlorine (0.5 mg/L; 5 minutes) and mechanical cleaning (1 minute) as an effective disinfection strategy under mild conditions. Utilizing a combination of mechanical and chemical disinfection strategies is recommended to detect regrowth events and enhance the effectiveness of microbial growth control.

Keywords: bacterial contamination; bactericidal activity; public places disinfection; surface disinfection; UV-C irradiation

Acknowledgments

This work was supported by national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020); funded by national funds through Germirrad-POCI-01-0247-FEDER-072237, by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Mariana Sousa acknowledges the FCT grant 2023.00337.BD.

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Poster 08

Development of a hydrogel with antioxidant and antibacterial properties loaded with grape pomace extracts for topical treatment of chronic wound infections

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https://doi.org/10.48797/sl.2024.166

Abstract

Background: Chronic wound infections are an emerging issue affecting millions of people globally, with profound psychological and socio-economic consequences [1]. Nonetheless, effective treatments to promote wound healing remain scarce [2]. Natural hydrogels appear as promising alternative wound dressings due to exudate absorption capacity and inherent wound-healing properties [3]. **Objective:** This study explores grape pomace (GP), the main residue of winemaking production, as a source of added-value raw material targeted for the treatment of *Staphylococcus aureus* chronic wound infections. **Methods:** Crude GP extracts (constituted by stalks or a mixture of skin and seeds from red and white grape varieties) attained using a modified solid-liquid extraction (water, ethanol, and acetone) were evaluated for their antioxidant capacity (ABTS and DPPH assays), total phenolic and total flavonoid content (TPC and TFC assay). A white GP extract was incorporated in a hydrogel composed of a chitosan-alginate matrix cross-linked by glutaraldehyde and calcium chloride. The proposed dressing was characterized by swelling, degradation, and release properties, and bioactivity was tested (antioxidant and antimicrobial activity). **Results:** Red GP extracts showed higher levels of polyphenol and flavonoid richness, but white GP extracts demonstrated superior extraction yields and antioxidant activity. Extract incorporation in the hydrogel improved its swelling and antimicrobial properties, such as bacterial membrane disruption and culturability reduction. **Conclusions:** This study resulted in a biomaterial with notable swelling and antibacterial capacity against *S. aureus*, with the potential to promote wound healing by exudate absorption and infection control. This offers alternatives for existing ineffective, side effects-laden treatments against a pathogen of clinical concern – *S. aureus*. Additionally, it contributes to the valorization of value wine production by-products, promoting a circular economy and mitigating environmental impacts.

Keywords: chronic wounds; circular economy; grape pomace extracts; hydrogel

Acknowledgments

This work was financially supported by national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020). Anabela Borges thanks the Portuguese Foundation for Science and Technology (FCT; Lisbon, Portugal) for the financial support of her work contract through the Scientific Employment Stimulus—Individual Call-[CEECIND/00823/2021; DOI: 10.54499/2021.00823.CEECIND/CP1679/CT0014]. Lília S. Teixeira acknowledges individual PhD fellowships from FCT (2023.04774.BDANA).

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Poster 09

Epidemiology and discrimination of clinically relevant *Enterobacter cloacae* complex species in Northern Portugal

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https://doi.org/10.48797/sl.2024.167

Abstract

Background: *E. cloacae* complex species are increasingly implicated in infections caused by multidrug-resistant bacteria, but their epidemiology is scarce due to the limitations of automated methods in accurate species identification (e.g. VITEK2/ MALDI–TOF MS) [1]. FT-IR is a promising quick, simple and low-cost alternative for bacterial discrimination [2]. **Objective:** We aim to assess the epidemiology of *Enterobacter* spp. isolates causing infections in two hospitals from North of Portugal, and the potential of FT-IR to differentiate the main clinically relevant *Enterobacter* species. **Methods:** We analyzed forty-five *Enterobacter* isolates from infection (*n*=43) or colonization (*n*=2) identified between 2019-2021 by VITEK2. Species identification was confirmed by PCR and sequencing of *hsp60*, used to build a phylogenetic tree with MEGA7 software. Antibiotic susceptibility testing was performed by standard methods according to EUCAST. Spectra from the most frequent species were acquired in the ATR mode of FT-IR equipment (Spectrum Two, Perkin-Elmer) in standardized conditions (4000-400cm-1; 4cm-1 resolution), processed (SNV, Saviztky-Golay) and used to identify species discriminatory profiles using PLSDA with Clover MS Data Analysis software, as described [3]. **Results:** Only 73% of the isolates were *Enterobacter* identified as *E. hormaechei* (*n*=19), *E. kobei* (*n*=7), *E. asburiae* (*n*=3), *E. bugandensis* (*n*=2), *E. cloacae* (*n*=1) and *E. ludwigii* (*n*=1). A few isolates produced VIM-1 (*E. hormaechei*), KPC (*E. cloacae*) or ESBL (4 species) The remaining isolates were identified as *K. aerogenes* (*n*=7), *K. variicola* (*n*=3), *E. coli* (*n*=1) and *K. michiganensis* (*n*=1). By using a PLSDA model, we were able to discriminate *E. kobei* and *E. hormaechei* with 92% average correct predictions. **Conclusions:** We found that *E. hormaechei* and *E. kobei* are the most frequent species causing hospital infections and that FT-IR can accurately differentiate these species, opening the possibility for its expansion to other *E. cloacae* complex species.

Keywords: bacterial identification; *Enterobacter* spp.; FT-IR spectroscopy

Acknowledgments

This research was funded by UCIBIO and i4HB research units through FCT (Grants Nº UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020). ABG and AN are supported by FCT (2020.09440.BD and 2021.02252.CEECIND/CP1662/CT0009, respectively).

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Poster 10

Antifungal activity of *Limosilactobacillus reuteri* against *Candida albicans* and Non-*Candida albicans Candida*

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https://doi.org/10.48797/sl.2024.168

Abstract

Background: Oral candidiasis is one of the most present fungal infections, with *Candida albicans* being the foremost responsible for this infection, but in recent years the non-*Candida albicans Candida* species play a significant role in the rise of cases of oral candidiasis [1]. Phenomena of resistance to regular antifungals are rising, which makes it harder to control cases of oral candidiasis. Thus, searching for new approaches like probiotics, and their use in the oral cavity, becomes necessary and urgent [2]. Here we present the antifungal activity of newly isolated oral strain of *Limosilactobacillus reuteri,* a potential probiotic. **Objective:** Evaluation of *Limosilactobacillus reuteri* AJCR antifungal activity against reference strains of *Candida* spp.. **Methods:** *Limosilactobacillus reuteri* AJCR was isolated from the oral cavity of a healthy, caries free, volunteer, following the methodology of Rossoni *et al*. [2]. Four reference strains, *Candida albicans* SC5314, *Nakaseomyces glabrata* ATCC2001*, C. tropicalis* ATCC750, and *C. parapsilosis* ATCC22019, were used to evaluate the antifungal activity in planktonic cells. The methodology followed was adapted from the EUCAST guidelines [3]. Different amounts of *L. reuteri* (10^8-10^2 CFU/mL, final concentration) were added, to standardized suspensions of the *Candida* strains studied. **Results:** At *L. reuteri* concentration of 10^8 CFU/mL, no viable cells of *C. albicans* SC5314 and of *C. tropicalis* ATCC750 were detected. At the same concentration, *Nakaseomyces glabrata* ATCC2001 suffered a reduction in growth of around 4 Log. *Candida parapsilosis* ATCC22019 suffered a reduction of around 2 Log at concentration of 10^7 CFU/mL of the oral isolate. **Conclusions:** *Limosilactobacillus reuteri* AJCR has shown an excellent antifungal activity against planktonic cells of *C. albicans* SC5314 and *C. tropicalis* ATCC750, and a significant reduction in the growth of *Nakaseomyces glabrata* ATCC2001 and *C. parapsilosis* ATCC22019. To further characterize the antifungal activity of the isolate, studies with biofilm cultures must be performed.

Keywords: probiotics; oral candidiasis; *L. reuteri*

Acknowledgments

This research received no external funding.

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Poster 11

Association between gut microbiome composition and pediatric obesity – a systematic review

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https://doi.org/10.48797/sl.2024.169

Abstract

Background: Obesity is one main 21st-century pandemic, emerging at younger ages and presenting several health problems and increasing risks of multiple diseases involving metabolic pathways related to inflammation (e.g., cardiovascular diseases, diabetes, and cancer risk). Recent data suggest that the microbiome is connected with obesity pathophysiology. Nonetheless, scarce information is available about gut microbiome and obesity in pediatric age. So, understanding the symbiotic relationship between the human organism and the intestinal microbiome can be crucial for addressing a possible therapeutic issue from early childhood. **Objective:** Verify the association between the intestinal microbiome and obesity in pediatric age. **Methods:** This systematic review was designed according to PRISMA. The search was carried out in October 2023, using *PubMed* and *Scopus* databases, with a peer-to-peer selection of articles. The choice was carried out in two phases, initially by title and abstract analysis and later by a full reading of the selected manuscripts, based on the inclusion criteria, namely "pediatric age (from birth to 18 years of age)", "fecal microbiota or intestinal", those that were clinical, cohort, case-control and cross-sectional studies, and that included children with obesity. **Results:** So, a total of 8 clinical studies and 2 observational studies were selected and included for this analysis. The selected studies show that gut bacterial communities are directly or indirectly related to obesity. Thus, childhood may be the critical period to implement specific microbiota interventions to prevent multifactorial diseases related to obesity. Papers analysis showed that some genera tended to be associated with an obesogenic profile in children, namely: *Prevotella, Phascolarctobacterium, Paraprevotella, Bacillus, Dorea, Ruminococcus gnavus, Clostridium* sensu stricto groups, *Eubacterium halli* group and *Fusicatenibacter* [1,2]. In this review, the Firmicutes phylum was shown to be in smaller quantities in obese children, with Bacteroidetes being associated with an obesogenic profile [3]. **Conclusions:** The results from these studies reflect microbiome differences between obese and normal-weight children, however further investigation is needed to clarify the importance of microbiome composition on obesity at pediatric age. Nonetheless, is important to implement as soon as possible diverse measures to minimize this obesity pandemic in pediatric age, like the implementation of regular physical activities and exercises [4], healthier and more sustainable diets and food literacy.

Keywords: children; diet; gut microbiota; obesity; pediatric age

Acknowledgments

This work was partially funded by the Portuguese Foundation for Science and Technology (FCT) under the CITAB project (https://doi.org/10.54499/UIDB/04033/2020).

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Poster 12

Biosafety and perioral botulinum toxin application: research study

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https://doi.org/10.48797/sl.2024.170

Abstract

Background: Orofacial harmonization encompasses techniques, procedures, and products used for therapeutic, rehabilitative, or preventive purposes. Orofacial Harmonization involves the use of injectable products such as botulinum toxin type A and/or hyaluronic acid with different molecular weights and degrees of cross-linking, as well as a variety of bio-stimulators. **Objective:** To analyze the application of botulinum toxin in the perioral region from both clinical and biosafety perspectives. To discuss the importance of identifying anatomical points of relevance directly associated with biosafety. **Methods:** This research compares the results after the application of a Botulinum Toxin protocol in 4 clinical aspects: a) labial commissures, b) gummy smile, c) perioral area, and d) chin. Clinical and biosafety perspectives of patients are evaluated. A convenience sample was utilized, consisting of 10 patients for each field to be evaluated (a, b, c, d) who met the inclusion criteria: patients over 25 years old and under 60 years old; without cognitive interferences and/or psychiatric pathologies; residents in Portugal. Exclusion criteria were used such as: patients with contraindications for the application of Botulinum Toxin protocol, such as: allergy to botulinum toxin type A; allergy to human albumin and/or sucrose; generalized muscular disease; presenting infection or inflammation in the area to be treated. **Results:** The clinical evaluation demonstrated higher therapeutic efficacy in groups a) labial commissures, b) gummy smile, and d) chin. The perioral area proved to be less promising in obtaining results with the application of Botulinum Toxin. Due to respect for facial anatomical structures, 100% success was achieved in biosafety control, with no trans or post-operative complications recorded. **Conclusions:** This study allowed for finding statistically significant relationships between the variables under study and the literature, thus enabling the creation of some scientific bases. Orofacial Harmonization, through individualizing the patient's natural physical traits, facilitates the harmonization of intra-oral structures with adjacent supporting tissues and muscles.

Keywords: orofacial harmonization; botulinum toxin; perioral area; gummy smile

Acknowledgments

This work received no external funding.

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Poster 13

Genomic and phenotypic heterogeneity of *Gardnerella* genus – implications for women's urogenital health

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https://doi.org/10.48797/sl.2024.171

Abstract

Background: *Gardnerella* species have been associated with bacterial vaginosis but are also found in asymptomatic women [1]. **Objective:** Characterize *Gardnerella* urogenital isolates from asymptomatic women (HW) and women diagnosed with overactive bladder (OAB), to understand bacterial factors that may influence health outcomes. **Methods:** *Gardnerella* isolates (urine, *n*=5 HW, *n*=4 OAB) were subjected to WGS (Illumina). Virulence determinants searched included vaginolysin, a cytolysin specific to human erythrocytes, and sialidases, crucial in mucosal surface interactions. Antimicrobial resistance genes were predicted using ResFinder. Sialidase activity was confirmed using neuraminic acid fluorogenic substrate. Susceptibility to different antibiotics (benzylpenicillin, meropenem, clindamycin, metronidazole, and erythromycin) was determined by agar dilution method following EUCAST guidelines. **Results:** *G. vaginalis, G. pickettii, G. leopoldii,* and *G. greenwoodii* were identified. The presence of sialidases was variable among isolates: *nanH2* was not detected, and *nanH3* was detected in 3 isolates. Isolates with *nanH3* displayed sialidase activity, with exception of one isolate, likely due to observed nucleotide sequence alterations. Different types of vaginolysin (*vly*) were detected in 8 isolates: type 1A was detected in *G. vaginalis* and *G. greenwoodii*; type 1B, associated with vaginal symptoms was detected in *G. vaginalis, G. pickettii* and *G. greenwoodii*; and type 2, associated with odour and discharge, was identified in *G. leopoldii* (HW and OAB). Genes previously associated with macrolides and lincosamides resistance were predicted *in silico*, but further analysis showed no functional operons or low homology with the reference gene. In general, MICs to the tested antibiotics were low, with exception of c18Ua_112-*G. leopoldii* with metronidazole MIC=8mg/L. **Conclusions:** Our findings underscore the complexity of *Gardnerella*'s role in urogenital health. The majority of low MIC observed support the effectiveness of commonly prescribed antibiotics against *Gardnerella*. Vaginolysin was prevalent, displaying diverse types associated with different clinical manifestations. Further exploration of host-microbe interactions is needed to decipher the mechanisms and clinical implications of these variations.

Keywords: urogenital microbiome; virulence; antimicrobial susceptibility

Acknowledgments

This work is financed by national funds from FCT - UIDP/04378/2020; UIDB/04378/2020; LA/P/0140/2020. MS, FG and TGR were supported by FCT (2020.05038.BD, DL57/2016/CP1346/CT0034-DOI: 10.54499/DL57/2016/CP1346/CT0034; and UIDP/QUI/04378/2020, respectively).

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Poster 14

In the network of periodontitis immunomediators

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https://doi.org/10.48797/sl.2024.172

Abstract

Background: Severe periodontitis is considered the sixth most prevalent disease worldwide [1]. It is a chronic inflammatory condition affecting the tooth-supporting structures due to genetic, environmental, and microbial factors [2]. The host immune response partially contributes to the magnitude of the inflammatory process. Furthermore, this process involves a diversity of immunomediators that initiate and sustain the inflammatory response, ultimately contributing to bone destruction [3]. Classical clinical parameters are reliable measures convenient for diagnosing and monitoring periodontitis, but they are less predictable in relation to disease progression. The focus is to identify quantifiable biomarkers in saliva. Saliva is a fluid that reflects the oral cavity environment and the immune status associated with periodontitis [4]. **Objective:** Our objective is to evaluate the clinical relevance of multiple immunomediators involved in the inflammatory response of periodontitis and explore biomarkers to severity and progression. **Methods:** The study includes 68 patients, divided in 17 patients with periodontitis stage I/II, 29 patients with periodontitis stage III/IV and 22 healthy controls (HC). The research assesses an extensive array of cytokines tied to inflammation, analyzed in salivary fluid, and detected using a Legendplex-panel by flow cytometry. **Results:** A pro-inflammatory profile is observed in patients with severe disease (stage III/IV), characterized by elevated levels of IL-1β and IL-6 compared to HC (p=0.006; p=0.015). Instead, anti-inflammatory IL-10 is significant increase in periodontitis patients in stage III/IV compared to HC (p=0.013). The chemokine IP-10, important in orchestrating a proper inflammatory response, demonstrates a significant decrease in patients with periodontitis. Additionally, correlations between the inflammatory profile and the phenotype of periodontitis are observed, as characterized by clinical measurements. **Conclusions:** This study highlights that IL-1β and IL-6 are more effective in identifying disease stage than clinical grade profile. Alongside the presence of IL-10 underscores its potential as combined biomarkers.

Keywords: immunomediators; saliva; periodontitis

Acknowledgments

This research was funded by IUCS-CESPU-project-AEPIAPD-GI-GI2-CESPU-2022. The study was approved by IUCS Ethics Commission (CE/IUCS/CESPU-08/21).

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Poster 15

Omentum picture: unraveling the enigma of the immune microenvironment in appendicitis scenario

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https://doi.org/10.48797/sl.2024.173

Abstract

Background: Appendix obstruction triggers inflammation and exaggerated immune responses, leading the omentum, rich in Milky Spots (MS) housing immune cells, to play a vital role in peritoneal immunity. These MS collect antigens and pathogens, inducing immune responses like inflammation, immune tolerance, or fibrosis [1,2]. Their activities, including angiogenesis, stem cell differentiation, and immune responses, are essential for wound healing and infection containment. However, these activities may also promote pathological responses like rapid tumor growth and metastasis [1,3]. Omental MS contribute to tissue homeostasis, aiding tissue repair through the regulation of leukocyte recruitment and activation by specialized stromal fibroblasts and mesothelial cells [1,3,4]. **Objective:** This study aims to semi-quantitatively evaluate leukocyte subpopulations and extracellular matrix composition in omentum samples from three acute appendicitis patients' groups: Group I without peritoneal blockage, Group II with peritoneal blockage, and the control Group III. **Methods:** Histological omentum samples underwent staining techniques with H&E, Trichrome, Orcein, and Reticulin for optical microscopy analysis. **Results:** Observations revealed higher presence of polymorphonuclear cells and/or lymphocytes, increased fibrosis, and abundant extracellular matrix reticular fibers. The control group exhibited minimal polymorphonuclear cells, lymphocytes, or fibrosis, while groups I and II showed increased polymorphonuclear cells, lymphocytes, and reticular fibers. Collagen fibers demonstrated a similar trend, albeit at lower density, while elastic fibers were scarce. These findings indicate a consistent association among the patient groups: while the control group showed low leukocyte counts and minimal fibrosis, groups I and II, characterized by increased fibrosis, demonstrated elevated leukocyte numbers and a greater presence of reticular fibers. **Conclusions:** These findings align with literature, emphasizing the omentum's crucial role in maintaining tissue homeostasis and supporting tissue repair through the regulation of leukocyte recruitment and activation by specialized fibroblastic stromal cells and mesothelial cells [3,4]. Further studies characterizing the omentum microenvironment are essential for identifying response predictive biomarkers.

Keywords: milky spots; immune cells; peritoneal cavity; histochemical techniques

Acknowledgments

This research was supported by CESPU and 1H-TOXRUN.

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Poster 16

Evaluating the impact of high sugar diet on juvenile rats: a histomorphological study on gut epithelial barrier

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https://doi.org/10.48797/sl.2024.174

Abstract

Background: Intestinal barrier is an important structure that defends and maintains the homeostasis of the host. [1-3]. It is highly influenced by food due to the interaction between nutrients and gut microbiota [2]. A diet rich in sugar can disrupt gut homeostasis, leading to a dysfunctional barrier and causing chronic diseases [3]. The enterochromaffin cells (ECs) are dispersed throughout the intestine wall, playing a role in the regulations of barrier function, responding to changes in intestinal microenvironment [1]. **Objective:** This study aims to evaluate the effect of a high-sugar diet on intestinal morphology, specifically on the histology and the expression of ECs. **Methods:** Male Wistar rats aged between 21-23 postnatal days were divided into two groups: a control group that drank water, and a high-sugar (HS) diet group that drank a 30% sucrose solution. All animals were fed standard rat chow. Tissue samples from the duodenum, jejunum, and colon, were cut 5 µm thick and stained with Hematoxylin-eosin (HE) to evaluate the integrity of the structure of the intestinal walls, and with Fontana-Masson (FM) staining the identification of ECs. **Results:** Qualitative results were obtained from tissue sections of control and HS-diet groups. Regarding the duodenum and jejunum, there is an accumulation of lipid droplets in the mucosa of the rats with an HS diet. There was also infiltration of inflammatory cells in the HS-diet group [3]. In the colon, the HS-diet group showed a reduction in the size of the external muscular layer and fewer Lieberkühn glands, aligned with previous study [4]. The ECs were found dispersed in the colon tissue of HS-diet group, while they were less stained in the control group. **Conclusions:** This study showed that high sugar liquid diet induced some morphological alterations in the small intestine and the colon, but no differences were observed in the ECs in colon.

Keywords: high sugar diet; histological morphology; enterochromaffin cells; intestinal epithelial barrier

Acknowledgments

This work is supported by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant FoodMicrobiome_CESPU_2017.

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Poster 17

Interindividual variability in platelet responsiveness: involvement of genetic factors

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https://doi.org/10.48797/sl.2024.175

Abstract

Background: Platelets not only play a pivotal role in haemostasis and are critical mediators of thrombosis, but also intervene during infection and inflammation, promoting leukocyte modulation, angiogenesis, and fibroblast proliferation [1,2]. Platelets response is very heterogeneous, with hyper-reactive platelets exhibiting a more procoagulant activity, releasing microparticles and amplifying proinflammatory states [3]. Genetically, platelets are highly polymorphic [3], being an important aspect to investigate the mechanisms underlying platelets hyper-responsiveness as well as their potential for predict the adverse/benefit effects associated with antiplatelet therapies. **Objective:** To investigate the functional relevance of platelet polymorphisms related to platelet reactivity analysing multiple parameters of platelet activation (PA). **Methods:** Genotypes and allele frequencies of platelet polymorphisms (PP) PlA and HPA-2 were determined in 49 normal individuals from the North of Portugal. To characterize PA profile in basal conditions and in response to physiological agonists, we have applied aggregometry and flow cytometry to assess a panel of biochemical markers including calcium mobilization, pro-coagulant activity, GPIIbIIIa activated and P-selectin expression, and platelet-leukocyte interactions. **Results:** The obtained allelic frequencies for PlA and HPA-2 polymorphisms do not differ from those found in Caucasian populations. Our results are consistent with the idea that the presence of HPA-2b allele of GPIba is related with a higher basal PA and hyper-reactivity. Presence of PlA2 allele of GPIIIa also seems to be related with a platelet hyperactivity profile. **Conclusions:** PP as genetic risk factors for thrombosis must be carefully addressed, however our data support that PlA and HPA-2 variants are relevant in variability of platelet responsiveness, namely via ADP, and thus, playing a role in thrombogenesis. A comprehensive insight into these intricate biological processes, need large genetic and epidemiological studies but, understanding the functional role of PP may give us tools to develop and apply inter-individually based therapeutic strategies in antiplatelet therapy schedules.

Keywords: genetic polymorphisms; platelet reactivity; platelet activation markers

Acknowledgments

This research received no external funding.

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Poster 18

Prevalence and characteristics of emotional distress and neurocognitive impairment in people living with HIV in Huambo/Angola

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https://doi.org/10.48797/sl.2024.188

Abstract

Background: As life expectancy for people living with HIV (PLWH) increases, there's a growing acknowledgment of the crucial need to address their psychological and neurocognitive well-being [1,2]. **Objective:** This study aims to determine the prevalence of emotional distress and neurocognitive impairment in PLWH receiving care at a primary HIV center. **Methods:** 204 participants were assessed through the Hospital Anxiety and Depression Scale to evaluate emotional distress, anxiety, and depression, while the Mini-Mental State Examination was utilized to assess neurocognitive functioning. **Results:** 62.7% of the participants exhibit clinically significant levels of emotional distress, with 62.7% and 53.2% experiencing significant levels of anxiety and depression, respectively. Furthermore, 58.3% demonstrate impairment in global neurocognitive functioning. Notably, participants reporting emotional distress tend to have lower levels of education and monthly income. Factors such as gender, limited daily access to food, and potable water are associated with an increased risk of distress. Participants displaying neurocognitive impairments tend to be older, have fewer years of formal education, exhibit depressive symptoms, and have been living with HIV for an extended period. **Conclusions:** These findings underscore the high prevalence of emotional distress among PLWH and highlight the significance of factors such as education, income, and access to necessities like food and clean water. Consequently, it is imperative that public health policy makers develop and implement mental health services within HIV centers to address these critical concerns.

Keywords: psychosocial; depression; anxiety

Acknowledgments

This research received no external funding.

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Poster 19

Unveiling the neuroprotective potential of new xanthene derivatives in Parkinson's disease

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https://doi.org/10.48797/sl.2024.191

Abstract

Background: Parkinson's disease (PD) is a neurodegenerative condition marked by the premature loss of dopaminergic neurons in the substantia nigra pars compacta. Additionally, PD is linked to several neuropathological processes, such as the formation of Lewy bodies, neuroinflammation, mitochondrial dysfunction, ferroptosis and oxidative stress [1-4]. Despite the notable progress in PD research, the development of an effective, long-term disease-modifying treatment remains elusive. For that reason, xanthene derivatives have been intensely studied and demonstrated diverse biological activities [5]. **Objective:** The main objective of this work was to evaluate, in vitro, the potential neuroprotective effects of new xanthene derivatives against MPP+, a neurotoxin widely used in vitro to mimic PD by interfering with electron transport chain, impacting ATP production and leading to reactive oxygen species (ROS) generation [6]. **Methods:** Differentiated SH-SY5Y cells were used as in vitro model and compounds (0–25 µM) cytotoxicity evaluated, 24 h after exposure, by the neutral red uptake and resazurin reduction assays, to select non-cytotoxic concentrations. To evaluate the compounds' neuroprotective effects, MPP+ was used (500 and 1000 µM). The cytotoxicity of the chemical aggressor was evaluated by the NR uptake assay 24 h after exposure to the chemical insult in the presence and absence of the xanthene derivatives (10 and 25 µM, non-cytotoxic concentrations). **Results:** All the tested compounds demonstrated to be non-cytotoxic for concentrations up to 25 µM. Some xanthene derivatives significantly reduced MPP+-induced cell death. **Conclusions:** Given the neuroprotective effects of these innovative compounds against MPP+, further studies are needed to deeper elucidate the mechanism(s) underlying the observed neuroprotection, and to explore their potential against other pathological hallmarks of PD.

Keywords: neurodegenerative disease; neuroprotection; MPP+; disease-modifying drugs

Acknowledgments

This research was funded by Fundação para a Ciência e Tecnologia (FCT) in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and LA/P/0140/2020 (i4HB). IC acknowledges FCT for PhD grant 2023.04980.BD.

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Poster 20

Evaluating the antitumor activity of selonsertib in pancreatic cancer cell lines

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https://doi.org/10.48797/sl.2024.194

Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is a pathology that ranks seventh among the most common causes of cancer mortality worldwide [1]. Importantly, drug resistance is a major clinical problem for patients with PDAC. Unfortunately, few efficient therapeutic options are available for this type of resistant cancer, and the standard chemotherapy remains gemcitabine or gemcitabine combined with paclitaxel [2,3]. Our research group identified Chitinase-3-Like 1 (CHI3L1) as being involved in reducing PDAC drug response in vitro [4], suggesting that combining CHI3L1 inhibitors with conventional chemotherapy may overcome PDAC drug resistance. **Objective:** The main objective is to assess the antitumor and chemosensitising effect of selonsertib (a compound currently in phase III clinical trial for the treatment of diabetic nephropathy and kidney fibrosis) on PDAC sensitive and resistant cell lines. Methods: The effect of gemcitabine (positive control) and selonsertib in the PANC1 PDAC cell line was evaluated with the Sulforhodamine B (SRB) assay. Then, the GI50 concentrations (that inhibits 50% of cell growth) after 48 h incubation were determined from the drug response curves. The effect of selonsertib in a resistant counterpart cell line to PANC1 (PANC1-CDR resistant to gemcitabine, recently established in our laboratory, unpublished results) is being evaluated. **Results:** Our data demonstrated that gemcitabine inhibited the growth of PANC1 cells with a GI50 of 0.76±0.1 µM, which is in agreement with the literature. Interestingly, selonsertib efficiently inhibited the growth of PANC1 cells with a GI50 of 9.5±3.3 µM. The effect of selonsertib on the growth of the resistant PANC1-CDR cell line is being determined. **Conclusions:** In this work, we showed the antitumor potential of selonsertib in the PANC1 PDAC cell line (that harbors KRAS mutation and has an aggressive phenotype). Future work will evaluate the effect of selonsertib on other PDAC cell lines (with different genetic backgrounds) and on the PANC1-CDR resistant cell line. Moreover, we expect to disclose the chemosentitizing effect of selonsertib in PDAC sensitive and resistant cells.

Keywords: antitumor activity; CHI3L1; combination therapy; pancreatic Cancer; selonsertib

Acknowledgments

This research received no external funding.

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Poster 21

Exploring the biosurfactant potential of Actinobacteria isolated from *Ruta graveolens*

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https://doi.org/10.48797/sl.2024.249

Abstract

Background: Biosurfactants are surface-active compounds known for their emulsifying properties and numerous advantages, including low environmental toxicity, eco-friendliness, biodegradability and acceptability. These molecules are amphiphilic, containing both hydrophilic and hydrophobic ends, allowing them to interact at the aqueous-non-aqueous interface [1]. Bacterial biosurfactants are interesting due to their various fields of applications, including biomedicine, cosmetics, food, pharmaceutics, water treatment and oil recovery [2]. Actinobacteria are an important group of microorganisms with high potential for producing different bioactive metabolites including antimicrobial, anticancer and other pharmaceutical compounds [3]. Medicinal plants, such as *Ruta graveolens*, are a rich source of bioactive compounds, and the association of actinobacteria endophytes with such plants are an attractive source for bioprospecting for novel compounds with biomedical and industrial applications [3]. **Objective:** The purpose of this study was to explore the biosurfactant activity of actinobacterial strains previously isolated from *R. graveolens*. **Methods:** Fifteen previously isolated actinobacterial strains were inoculated into 100 mL Erlenmeyer flasks containing 30 mL of Kim's broth supplemented with 3% filtered olive oil as a hydrophobic carbon source. After two weeks, biosurfactant production was analyzed by measuring the emulsification activity. **Results:** Eight out of the 15 actinobacterial strains showed emulsification activity. All results were compared with a positive control consisting of Triton X100 (1mg/ml), and a negative control consisting in Kim's broth. Almost all strains that revealed positive activity are affiliated to the actinobacterial species *Tsukamurella tyrosinosolvens* (7/8), with one strain belonging to the species *Microbacterium gisengiterae*. Comparative analysis with the positive control (with an emulsification activity of 60%), indicated that 3/8 samples showed high emulsification activity (>40%), 1 showed moderate activity (37%) and 4/8 showed low/moderate activity (20-30%). **Conclusions:** The subsequent phases of this study will involve analyzing if the potential biosurfactant compounds can reduce the surface tension and if they can represent new molecules.

Keywords: actinobacteria; medicinal plant; *Ruta graveolens*; biosurfactant

Acknowledgments

The authors also acknowledge the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020, through national funds provided by FCT and the European Regional Development Fund (ERDF).

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Poster 22

Neurotoxicity evaluation of four structurally similar synthetic cathinones in a cholinergic neuronal model: a comparative analysis

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https://doi.org/10.48797/sl.2024.250

Abstract

Background: Synthetic cathinones are potent central nervous system (CNS) stimulants that induce diverse neurological and psychiatric effects such as delusions, hallucinations, and agitation [1]. This fast-expanding class of new psychoactive substances (NPS) represented the majority of those seized in the European Union in 2021 [2]. Despite the growing recreational use, knowledge on their neurotoxic mechanisms, namely during neuronal differentiation, remains limited. **Objective:** This study aimed to compare the neurotoxicity of four structurally-related synthetic cathinones (3-CMC, 4-CMC, 4-CEC, and Ethcathinone), differing in the presence and position of a chlorine in the aromatic ring and in the length of the N-alkyl group, using a well-characterized neuritogenesis in vitro model. **Methods:** Lysosomal integrity, analyzed by the neutral red uptake assay, and reactive oxygen/nitrogen species (ROS/RNS) production, evaluated with the DCFH-DA probe, were assessed in NG108-15 neuroblastoma x glioma cells differentiated into a cholinergic phenotype [induced by serum-starved medium (1% FBS) supplemented with forskolin (30 µM) and retinoic acid (10 µM)], following a 24 h exposure to the tested drugs (1 nM – 1 mM). Results: All synthetic cathinones tested significantly reduced lysosomal integrity and increased ROS/RNS formation. The cathinones with a chlorine in the position 4 of the aromatic ring exhibited more pronounced effects, causing cell viability loss at concentrations ≥100 µM, while for the others it was only significant for concentrations ≥500 µM. Moreover, structures with a longer N-alkyl substituent appeared to show higher cytotoxicity (probably related to their higher lipophilicity), with 4-CEC demonstrating greater lysosomal integrity loss (50%) relatively to control than 4-CMC (80%) at 100 µM. **Conclusions:** Halogen positioning within the aromatic ring, as well as their lipophilicity, influenced the cytotoxicity of synthetic cathinones, being seemingly associated with increased oxidative stress. Future research should encompass mechanistic studies, particularly focusing on specific neurogenesis-related processes, to comprehensively unravel the mechanisms underlying neurotoxicity.

Keywords: new psychoactive substances; central nervous system; NG108-15 cells; cytotoxicity; ROS production

Acknowledgments

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) in the scope of grants UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and LA/P/0140/2020 (i4HB). RFM and JPS acknowledge FCT for PhD grant 2020.07135.BD and research contract (under Scientific Employment Stimulus) 2021.01789.CEECIND/CP1662/CT0014, respectively.

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Poster 23

Comparing cytotoxic effects of synthetic cathinones and methamphetamine on cardiac AC16 cell

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https://doi.org/10.48797/sl.2024.251

Abstract

Background Synthetic cathinones are psychoactive derivatives of the natural drug cathinone. Despite being sold as safer than traditional drugs, over the years there has been a rise in fatalities associated with the cardiotoxic effects of synthetic cathinones [1]. Still, few studies address the mechanisms underlying this toxicity. **Objective:** This work aimed to study the cardiotoxicity of synthetic cathinones (mephedrone, 3,4-DMMC, ethcathinone, α-PHP, 4-CEC, 4-MEC and α-PVP) in the AC16 human cardiomyocyte cell line and compare it with the toxicity of methamphetamine. **Methods:** The human cardiomyocyte cell line AC16 was differentiated using horse serum for 24 hours [2]. After differentiation, they were exposed for 48 hours to different concentrations of methamphetamine (1-10 mM), and to the following synthetic cathinones: mephedrone (0.1-10 mM), 3,4-DMMC (0.05-5 mM), ethcathinone (1-10 mM), α-PVP (0.5-5 mM), α-PHP (0.5-5 mM), 4-CEC (0.5-5 mM) and 4-MEC (0.5-5 mM). After the exposure period, two cytotoxicity tests were performed: the MTT reduction and neutral red uptake assays. **Results:** All the cathinones, (mephedrone, 3,4-DMMC, ethcathinone, α-PHP, 4-CEC, 4-MEC and α-PVP) as well as methamphetamine presented significant cytotoxicity in differentiated AC16 cells, with their cytotoxic effects escalating proportionally with higher concentrations. In the MTT reduction assay, significant cytotoxicity was observed with 3,4-DMMC at a concentration as low as 0.1 mM, mephedrone at 0.25 mM, α-PHP and 4-CEC at 0.5 mM, α-PVP and 4-MEC at 1 mM, while methamphetamine exhibited meaningful toxicity starting at 2.5 mM, and ethcathinone at 5 mM. Over all, the results obtained in the NR uptake assay revealed that higher concentrations were needed to induce cytotoxicity. **Conclusions:** Although synthetic cathinones are sold as safer than methamphetamine, some have an equal or even greater potential to cause cytotoxicity in this cell model. However, further studies are needed to determine the impact of these cathinones on cellular processes.

Keywords: cardiotoxicity; cathinones; cytotoxicity; AC16 cells

Acknowledgments

This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the projects: UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO), LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB, and UIDB/04046/2020 and UIDP/04046/2020 of the Research Unit BioISI-Biosystems & Integrative Sciences Institute.

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Poster 24

Tramadol effects on the nucleus accumbens – insights from *in vitro* and *in vivo* studies

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https://doi.org/10.48797/sl.2024.252

Abstract

Background: Tramadol is mainly used for the treatment of moderate to severe pain. It synergistically combines two distinct mechanisms of action, being a selective agonist for m-opioid receptors (MOR) and inhibiting serotonin and noradrenaline reuptake, which improves its analgesic and safety profile [1]. However, it is not devoid of neurobehavioral toxicity potential [2], whose molecular alterations are not fully clarified. Due to its primary role in reward, motivation and drug self-administration behaviors, the nucleus accumbens (NAC) is anticipated to participate in the mechanisms of tramadol addiction, dependence and toxicity. **Objective:** The aim of this review is to summarize the main neurotoxicity biomarkers and effects of tramadol exposure on the NAC. **Methods:** A bibliographic research of neurotoxicity biomarkers and findings concerning the NAC, upon exposure to tramadol, was performed on the National Library of Medicine (PubMed), with no temporal restrictions and considering *in vitro* and *in vivo* studies. **Results:** *In vivo* studies showed increased levels of MOR, p-CREB and ΔFosB in the NAC after acute and chronic exposure to tramadol (5 and 10 mg/kg) [3]. Even if apoptosis and inflammation are major NAC findings in in vivo studies, autophagy was also upregulated in in vitro studies with PC12 cells exposed to 50 mM tramadol [4]. In addition, it has been found that tramadol enhances dopamine levels in the NAC shell and that NAC cannabinoid receptor 1 (CB1R) is involved in tramadol reinforcing effect and reinstatement [5]. **Conclusions:** In conclusion, although tramadol controls pain more effectively and with fewer adverse events than classical opioids, its neurotoxic potential is of particular concern. The nucleus accumbens has a relevant contribution to such neurobehavioral toxicity, as shown by multiple alterations in important cell death, inflammation and related signaling pathways. A personalized and cautious tramadol prescription is thus mandatory.

Keywords: tramadol; nucleus accumbens; neurobehavioral toxicity; biomarkers

Acknowledgments

This research was funded by Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), projects/grants CBToxAtOpi-GI2-CESPU-2022 and NToxAtOpi_GI2-CESPU-2023.

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Poster 25

Impact of tramadol on the hypothalamus: assessment of potential toxicity biomarkers

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https://doi.org/10.48797/sl.2024.253

Abstract

Background: Tramadol is a widely prescribed opioid for the control of moderate to severe pain. It is a synthetic opioid, with an atypical mechanism of action, acting as an agonist of μ-opioid receptors and by inhibiting the reuptake of noradrenaline and serotonin [1,2]. In spite of its analgesic effectiveness, tramadol exposure causes several adverse reactions, and despite advancements in understanding its toxicity mechanisms, its neurotoxic effects have not been fully elucidated yet [2,3], particularly its effects on the hypothalamus. **Objective:** The aim of this literature review is to summarize the main biomarkers of hypothalamic neurotoxicity resulting from tramadol exposure. Methods: A bibliographic search carried out in the National Library of Medicine (PubMed) looked at biomarkers of toxicity within the hypothalamic structure resulting from the exposure to tramadol, without temporal restrictions. **Results:** In vivo studies showed that, while tramadol increases the synthesis of prodynorphins at low doses (20 mg/Kg), this effect is not observed at doses of 80 mg/Kg, which results in a decrease in their synthesis in the hypothalamus [2]. After acute tramadol administration, an increase in pERK1/2 levels was observed in the hypothalamus [4]. In vivo tests showed an increase in the expression of IRS2 and glucokinases in the hypothalamus [2]. High reductions in α2-adrenergic receptors in the structure of the hypothalamus have also been reported [5]. **Conclusions:** In summary, while tramadol is effective in pain control, its neurotoxic potential in the hypothalamus is apparent and dependent on the dose administered. It is associated with a decrease in noradrenaline reuptake through downregulation of adrenergic receptors, which can be harmful, as well as an increase in kinase expression or even an increase in the expression of insulin signaling pathway elements. Careful administration of tramadol is imperative due to its neurotoxic potential.

Keywords: tramadol; hypothalamus; neurotoxicity; biomarkers

Acknowledgments

This research was funded by Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), projects/grants CBToxAtOpi-GI2-CESPU-2022 and NToxAtOpi_GI2-CESPU-2023.

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Poster 26

Zein/HP-β-CD nano-in-micro-particles as a platform to enhance cannabidiol oral delivery

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https://doi.org/10.48797/sl.2024.254

Abstract

Background: Nutraceuticals have been significantly advocated for preventive care, performance enhancement, and immune support. Recently, the scientific community has focused not only on the beneficial properties of nutraceuticals, but also on optimizing their delivery in the body [1]. Nanotechnology represents an effective strategy for manipulating and enhancing nutraceutical activity, tackling the technological limitations hindering their development and efficient oral delivery [2]. Cannabidiol (CBD) is an active compound that shows major anti-inflammatory effects; unfortunately, CBD application in the food and pharmaceutical sectors are limited due to low bioavailability, extensive first-pass metabolism, poor water solubility and sensitivity to oxidation [3]. **Objective:** Herein, we report the development of an oral delivery platform for CBD based on the combined use of zein, a natural prolamin extracted from the corn endosperm, with 2-hydroxypropyl-beta-cyclodextrin (HP-b-CD) and their processing through nano/microtechnologies. **Methods:** Once the experimental parameters were optimized, nanoparticles (NPs) were prepared through a liquid-liquid dispersion method and then transformed into a solid product (Nano-in-Micro-particles) through spray drying, a rapidly emerging technology able to improve the stability of the final product. All the systems were fully characterized through a large panel of techniques such as dynamic light scattering (DLS) and zeta potential (z) analysis, ultraviolet-visible (UV-Vis) spectroscopy, scanning electron microscopy (SEM), and analytical sieving. **Results:** HP-b-CD held a crucial role in the formulation process due to its ability to form host-guest inclusion complexes with zein and act as a stabilizing agent of NPs. In addition, NPs were able to encapsulate lipophilic model molecules excellently. **Conclusions:** Our final product consisted of good-quality powders that were easily handled and stable when stored. Overall, the zein/HP-b-CD platforms developed here can be considered a novel tool for the efficient delivery of lipophilic compound in the body.

Keywords: zein; 2-hydroxypropyl-beta-cyclodextrin; nanoparticles; micropowders; cannabidiol

Acknowledgments

This research received no external funding.

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Poster 27

Precision medicine, predictive models of prognosis and quality of life in oncology

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https://doi.org/10.48797/sl.2024.255

Abstract

Background: Precision medicine or personalized medicine, is an approach to healthcare that emphasizes the customization of medical treatment and interventions to individual patients based on unique genetic, environmental, and lifestyle factors. **Objective:** To present Quality of Life results in Head and Neck Cancer patients. To discuss how patient reported outcomes can contribute to precision medicine. **Methods:** In this study, 380 head and neck cancer patients were evaluated. The published and validated Portuguese version of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) was used. The QLQ-C30 (version 3) incorporates 5 functional scales (physical, role, cognitive, emotional, and social), 3 symptom scales (fatigue, pain, nausea and vomiting), the global health status/quality of life scale (QoL), and 6 single items to assess additional symptoms or problems (dyspnea, loss of appetite, insomnia, financial difficulties, constipation, and diarrhea). **Results:** The study showed that women have lower overall Quality of Life results. It also emphasizes the importance of early diagnosis, which often relates to stages with better prognosis and better Quality of Life outcomes. The study showed that tumor location has an impact on Quality of Life self-perception. Values of Health Related Quality of Life should be analyzed along with socio-demographic and clinical variables in order to better understand the epidemiology, pathogenesis, and prevention of these cancers favoring a precision medicine. **Conclusions:** Patient reported outcomes have been recognized as very useful in individual and multidisciplinary decisions. Routine provision of patient reported outcomes to oncologists positively affects the process of care in several dimensions such as oncologist–patient communication, awareness of patients' problems and discussion of symptoms during consultations. Patient reported outcomes to support precision medicine providing the assessment to crucial data to provide effective, evidence-based supportive care, rehabilitation and symptom management.

Keywords: patient reported outcomes; quality of life; head and neck cancer; precision medicine

Acknowledgments

This research received no external funding.

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Poster 28

Impact of baby-feeding practices on Portuguese children's health outcomes: preliminary results

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https://doi.org/10.48797/sl.2024.256

Abstract

Background: Baby-feeding practices, including breastfeeding and formula milk, exclusively or combined, can have protective or harmful impacts on children's health, such as respiratory infections, allergies, and other chronic diseases [1-3]. **Objective:** This study explores the association between baby-feeding practices (exclusive breastfeeding and its duration, exclusive formula milk, and combination of breastfeeding with formula milk and/or water with the timing of introduction) with the development of health conditions/symptoms. Methods: A structured survey was completed by 192 parents of children (5-10 years). In addition to baby-feeding practices information, data on children's health conditions during the first two years of life (bronchitis, asthmatic bronchitis, bronchiolitis, and pneumonia) and until date (doctor-diagnosed asthma, sneezing attacks, eczema, and otitis) were collected. Associations were explored via univariate analyses, with findings presented as odds ratios (OR) and 95% confidence intervals (CI). **Results:** Around 58% (n=110/190) of mothers exclusively breastfed their children for 9.5±10.7 months. Among other mothers, 14.3% (n=11/77) used formula milk solely, and 85.7% (n=66/77) combined both. Children exclusively breastfed had lower odds of all conditions than others, except for pneumonia (OR=1.20; CI:0.28-5.17) with no statistical differences. A month increase in breastfeeding duration was associated with 6% lower bronchitis odds and 1.05 times higher sneezing attack odds ($p>0.05$). When comparing formula milk and combination feeding, children exclusively fed formula milk had 1.50 and 2.64 times higher odds of bronchitis and bronchiolitis, respectively, but 81% lower eczema odds ($p>0.05$), while combining both feeding practices was associated with 62% lower bronchiolitis odds and 5.39 times higher eczema odds. Minimal impact was found on otitis (OR=0.99; CI:0.26-3.75), while its odds were 1.08 times higher per month increase in the age of formula milk introduction. **Conclusions:** These preliminary findings suggest an overall protective impact of exclusive breastfeeding over formula milk, with a positive influence of combining them on early-in-life conditions.

Keywords: breastfeeding; formula milk; children; bronchitis; asthma; eczema; otitis

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through the projects with references UIDB/04750/2020 (https://doi.org/10.54499/UIDB/04750/2020), LA/P/0064/2020 (https://doi.org/10.54499/LA/P/0064/2020), PTDC/CTA-AMB/3040/2021 (https://doi.org/10.54499/PTDC/CTA-AMB/3040/2021), UIDB/00511/2020 (LEPABE, https://doi.org/10.54499/UIDB/00511/2020), UIDP/00511/2020 (https://doi.org/10.54499/UIDP/00511/2020) and LA/P/0045/2020 (ALiCE, https://doi.org: 10.54499/LA/P/0045/2020). Georges Hatem also acknowledges FCT for individual funding (2022.11261.BD).

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Poster 29

Ethics and deontology, training, and clinical practice in orofacial harmonization - questionnaire design and construction

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https://doi.org/10.48797/sl.2024.257

Abstract

Background: The term "Orofacial Harmonization" is a concept based on the application of clinical and therapeutic procedures that, in synergy with various specialties and areas of Dentistry, aims to promote the aesthetic and functional harmony of the orofacial region. The journey of Orofacial Harmonization began in various areas of Biomedical and Medical Sciences, in addition to Dental Sciences. Areas such as anatomy-physiology, molecular biology, immunohistochemistry, cytogenetics, pharmacology, and medicine are interconnected with the concept of Orofacial Harmonization. **Objective:** Description of all phases for constructing a research instrument for Oral Health Professionals/Students. We present the final version of the questionnaire already submitted and approved by the Ethics Committee. **Methods:** The present investigation is based on the construction of a Research Instrument. Initially, the construct and dimensions were defined, followed by the development of a pool of items. The type of scale was chosen. Content analysis was conducted with recognized experts in this research area. The experts provided their opinions on dimensions, appropriateness of formulation, comprehension of the questions, degree of pertinence, relevance, and the use of scales. **Results:** Six stages were conducted: objectives' recognition, literature review, question development, expert review, pilot testing, and ethical approval. The final research instrument consists of 3 parts: collection of sociodemographic variables (a total of 5 questions), a specific questionnaire on training and professional practice in Orofacial Harmonization (a total of 30 questions), and a questionnaire on ethics and deontology in Orofacial Harmonization (a total of 17 questions). **Conclusions :** Orofacial Harmonization represents a growing area of interest and expansion in Dentistry, reflecting an emergent need to understand the ethical, deontological, and professional practice perspectives associated with this field of knowledge. The relevance of this questionnaire lies in its ability to capture the opinions, experiences, and expectations of professionals and future professionals.

Keywords: orofacial harmonization; research instrument construction; research instrument validation

Acknowledgments

This research received no external funding.

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Poster 30

Microplastics in eggs from cage and free-range production systems

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https://doi.org/10.48797/sl.2024.258

Abstract

Background: Microplastics (plastics ≤ 5 mm) are ubiquitous contaminants that may cause adverse human health effects [1]. However, information on human exposure to microplastics from terrestrial foods is lacking [2], including in eggs [3]. **Objective:** This work aims to compare the number and physical characteristics of microplastics present in eggs from cage and free-range production systems. Methods: Eggs identified as cage (size M, n = 6) and free-range (size M/L, n = 5) were acquired in local supermarkets, opened into glass flasks, subjected to lyophilization (7 days), stored in an oven at 30ºC, followed by sample preparation, and photographing particles in 1.2 µm glass fiber filters (GFFC, Prat Dumas) under blue light (450 nm, SPEX Forensic) in the optical microscope (Zeiss Scope A1 Axio) using a Canon 550D camera equipped with an orange filter (Slim K&F Concept) [4]. Procedures were followed to prevent contamination and using three procedural blanks. Particles were analyzed in ImageJ, statistics were conducted on IBM SPSS Statistics 26 considering $\alpha = 0.05$, and results were expressed in microplastics per egg (MP/egg) and per gram (MP/g). **Results:** There was no significant difference in the number of microplastics per egg ($p = 0.199$) nor per gram ($p = 0.462$) between free-range (17 MP/egg; 0.4 MP/g) and cage eggs (9 MP/egg; 0.2 MP/g). The largest size of microplastics was significantly different ($p = 0.003$) between free-range (84.8 µm) and cage eggs (121.7 µm). Only 2 microplastics were found in one of the three procedural blanks. **Conclusions:** Estimated daily intake is 24 and 44 MP/day for cage and free-range eggs, respectively, similar to a previous estimate of 24 MP/day [2]. Comparisons between eggs production systems must be further explored, considering differences in dietary and environmental exposure of hens.

Keywords: microplastics; food safety; human exposure

Acknowledgments

This research received no external funding.

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Poster 31

Decoding tea and infusions labels: understanding ingredient lists

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https://doi.org/10.48797/sl.2024.259

Abstract

Background: Concerns linking food and public health extend beyond nutrients to include the use of food additives [1-3]. Certain additives, like artificial sweeteners, were associated with higher risk of non-communicable diseases [3], raising doubts about their widespread use. While NutriScore is a nutritional labelling tool that addresses nutrients [2], it overlooks additive quantity, which may have "cocktail effects" on the consumer [1,3]. Tea and infusions (THI) are perceived as unprocessed foods, free of additives [1]. Our previous study showed that THI are primarily consumed by females, with choices being age-dependent [4]. Nevertheless, there are still gaps in the composition of THI products in the Portuguese market, remaining unclear whether all options are "healthy choices". **Objective:** The study aimed to evaluate the content of various commercial THI products, focusing on the types and number of additives listed on their labels. **Methods:** Information was collected from labels of 294 THI products. With first ingredient, products were categorized: tea or plant infusions (non-flavoured, flavoured), fruits, spices, and soluble products. Additive data were categorized with Codex Alimentarius and analyzed using JASP (version 0.18.43). **Results:** Herbal infusions comprise 57.8% (34.7% non-flavoured and 23.1% flavoured), 29.9% tea (10.9% non-flavoured and 19.0% flavored), 4.8% soluble, and 3.7% consisted of both fruit and spice infusions. Among THI, 43.9% had one to eight additives, including flavours, sweeteners, acidity regulators, bulking/emulsifier agents, antifoaming agent and colour. Flavours were the most prevalent additive (75.6%), appearing in one to three different flavors. Sweeteners were present in soluble THI, 57.1% having three different types. Other products containing sweeteners were spice infusions (18.2%), tea (5.4%) and herbal infusion (4.4%) both flavoured. **Conclusions:** The study showed the presence of various additives in many THI products and, some of these additives, if consumed uncontrolled, could pose a health threat, especially for the most vulnerable individuals.

Keywords: commercial THI; food labels; additive list; food safety

Acknowledgments

This work was funded by CESPU – Cooperativa de Ensino Superior Politécnico e Universitário under the grant SPAinT-GI2-CESPU-2022.

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Poster 32

Environmental contamination as a source of multi-toxics via maternal exposure and connection with childhood diseases like autism spectrum disorders – *One Health* perspective

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https://doi.org/10.48797/sl.2024.260

Abstract

Background: Environmental pollution exposes human to various toxics. Food is one main route of toxic exposure, encompass pesticides, per- and polyfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), metals and nanoplastics [1]. Maternal exposure is a concern due to rising childhood neurodevelopmental disorders like autism spectrum disorders (ASD) [1,2] and environmental contaminants are suspected to be a main potential cause of these disorders, with significant socioeconomic costs [2,3]. **Objective:** Verify the association between maternal exposure to toxics and the increase in the prevalence of ASD at pediatric ages. **Methods:** This mini-review was based on the search of review papers using the *PubMed* database and after that, a second selection was done using the title and abstract analysis to select the most relevant publications. **Results:** Women of childbearing age are exposed to complex mixtures of environmental toxicants, leading to potential maternal transfer to the fetus. Even at low-dose, pregnant women exposed to toxics through food, air, or skin pose a risk to developing fetus (4-18 weeks) [4], potentially impairing brain growth and function. Some studies indicate that pesticides, phthalates, cosmetics (e.g. fragrances, face makeups) detergents, and food flavors, pesticides, lead, methyl-mercury, aluminum, PCBs, PAHs, PBDEs, and perfluorinated compounds may have an impact on ASD emergence. Others focused on biomarkers in autistic individuals [5], namely prenatal methylmercury exposure in mother-child pairs from a population with high fish consumption. There is also evidence that advanced parental ages, genetic predisposition, drugs and pharmaceutical use during pregnancy, stressful life events, or environmental hardship can also contribute to ASD. **Conclusions:** Human exposure to multiple toxicants is nearly unavoidable, contributing to neurotoxic effects linked to brain disorders. Early-life exposures to toxicants can impair brain development and may contribute to an increase in ASD prevalence. It is important to enhance awareness among women to avoid some toxicants, particularly during pregnancy, to minimize the risk of neurodevelopmental disorders.

Keywords: environmental pollution; food toxicants; early-life exposure; cognitive diseases; autism spectrum

Acknowledgments

This work was partially funded by FCT under the project of CITAB, UIDB/04033/2020.

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Poster 33

Nutritional profile evaluation of plant-based fermented products alternative to yogurts available in the Portuguese Market

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https://doi.org/10.48797/sl.2024.248

Abstract

Background: In recent years, there has been a growing number of people following a plant-based diet, accompanied by the emergence of dairy analogs on the market, presenting themselves as plant-based alternatives. These products seek to mimic the organoleptic characteristics of dairy products, but their nutritional profiles are not always similar [1,2]. **Objective**: Classify the plant-based fermented products alternative to yogurts according to nutritional classification systems – Nutri-Score and Traffic light [3,4] - and compare their nutritional profile with yogurts. **Methods**: Market research was carried out on the websites of three Portuguese supermarket chains, along February 2024, collecting existing information on available plant-based fermented yogurts. Both nutritional classifications systems were applied to 47 products, which met the inclusion criteria "availability of the nutritional declaration and ingredient list online". Subsequently, the nutritional composition of these products was compared with that of yogurts available in the Portuguese Food Composition Table [5]. **Results**: Of the 47 considered products, 49% (n=23) obtained classification A, 32% (n=15) classification B and 19% (n=9) classification C, according to Nutri-Score, while according to Traffic light, 19% (n=9) of these products showed all parameters in green. On average, per 100 grams of product, plant-based fermented yogurts have a lower sugar content (6.5 g compared to 8.4 g in yogurts), while the values of lipids (2.4 g), saturated fatty acids (0.9 g) and salt (0.19 g) of these preparations are similar to that of yogurts (2 g, 1.2 g and 0.17 g, respectively). Most products included are fortified (57%), with calcium and vitamins D, B12 and B2 being the most frequently added micronutrients. **Conclusion**: According to Nutri-Score, the highest ratings, A and B, represent most products while the highest Traffic light rating constitutes only 19% (n=9) of all products. The macronutrient content of these products is similar to yogurts. Micronutrient fortification is not present in all plant-based fermented yogurts available on the Portuguese market.

Keywords: plant-based fermented products alternative to yogurts; nutritional composition; Nutri-Score; traffic light

Acknowledgments

This research received no external funding.

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Poster 34

Potential impact of packaging type material in beer quality

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https://doi.org/10.48797/sl.2024.247

Abstract

Background: The materials in which beverages are packed are crucial to maintain quality, nutritional properties, and safety of the final product. In what beer concerns, and even under optimal storage conditions, its quality deteriorates as the product approaches the expiration date, regardless of the container type in which it is packed [1]. Therefore, a careful selection of packaging is imperative to maintain beer´s integrity and quality, when tasted by the consumer. **Objective:** This work aims to discuss how the type of beer packaging influences the integrity and quality of this beverage during storage. **Methods:** A literature review was performed, using PubMed, b-on, and Web of Science databases. Articles related to the influence of packaging types on beer quality, in English, and published in the last 5 years were included. **Results:** Plastic bottles (PB) proved to be the least suitable type of packaging for long-term storage, as beer exhibits significant changes in volatile compounds, turbidity, color, bitterness intensity, and decrease in CO_2 content, unlike glass bottles (GB), aluminum cans (AC), and stainless steel kegs (KG), where these parameters remain constant [1-3]. Furthermore, Brown GB proved to be a more effective barrier against light compared to green GB and PB, as well as containing lower amounts of phthalates than beers in PB and AC [4,5]. AC was capable of retaining all organoleptic characteristics, showing a slight increase in bitterness after 10 months of storage [1]. However, beers stored in AC were characterized by higher aluminum contents compared to the products stored in glass bottles [6]. **Conclusions:** The beer packaging material plays a crucial role in its quality. Thus, GB, AC and KG, due to their barrier characteristics and protection against external factors, generally outperform PB in preserving the integrity and quality of beer.

Keywords: beer; packaging material; quality

Acknowledgments

This research received no external funding.

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Poster 35

Antioxidant profile of Portuguese and Spanish craft beers

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https://doi.org/10.48797/sl.2024.246

Abstract

Background: The antioxidant potential of craft beer (CB) may be due to high quality of the raw materials (water, malt, hop and yeast) and traditional techniques [1-4]. The scarcity of studies evaluating the antioxidant potential of CB highlights the relevance of this study. **Objective:** *In vitro* evaluation of the antioxidant activity of aqueous extracts of Portuguese and Spanish CB. **Methods:** Experimental study using six CB with different styles: Milk Stout (EL-MS), India Pale Ale (EL-IPA, ALM-IPA), Imperial Stout (EME-IS), Oatmeal Stout (ALM-OS), Pilsner (EL-P), Munich Dunkell (B-MD) and two industrial beers, Pilsner (S-P) and Munish Dunkell (S-MD). The pH, acidity content (AC) and total phenolic compounds (TPC) were determined. The antioxidant capacity was assessed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the hydrogen peroxide (H_2O_2) assays, expressed in concentration for 50% activity inhibition (IC_{50}). One-way ANOVA and Student's t-test were used for statistical analysis in GraphPad® Prism 8.0 software, with a significance level of 0.05. **Results:** The pH of the beers varied between 3.89±0.00-4.78±0.05 and AC between 0.13±0.01-0.44±0.01%. ALM-IPA had the highest TPC value (8.96±0.64mg gallic acid equivalents/g). IPA style presented the lower IC_{50} values in H_2O_2 (ALM-IPA with an IC_{50}=23.54±1.53µg/mL, p<0.05) and ABTS (EL-IPA with an IC_{50}=55.21±4.68µg/mL, p<0.05) assays. The industrial beers have lower TPC values compared to CB (same style, p<0.05), and lower capacity to neutralize the H_2O_2 and ABTS radicals. For Omisore *et al*. (2005), samples with IC_{50}>50µg/mL are classified as being moderately active, while samples with IC_{50}<50µg/mL have high antioxidant capacity. Also, the results for IPA style are in line with Breda *et al*. (2022), in which light-colored beers had better antioxidant profiles. However, according to Silva *et al*. (2022) the best antioxidant profiles were associated with dark beers. **Conclusions:** The samples showed antioxidant potential, but further tests should be carried out considering the complex underlying antioxidant mechanisms.

Keywords: craft beer; antioxidant activity; phenolic compounds

Acknowledgments

This research received no external funding.

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Poster 36

Looking at the potential of marine macroalgae supplementation to afford neuroprotection against the effects of inorganic mercury in fish (*Diplodus sargus*)

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https://doi.org/10.48797/sl.2024.245

Abstract

Background: Marine macroalgae have potential as a source of many natural compounds with health benefits [1], but their use to mitigate aquatic contaminants bioaccumulation in fish and resultant toxicity is an underexplored research topic. **Objective:** Asses if a marine macroalgae-enriched diet can provide neuroprotection *Diplodus sargus* when exposed to waterborne inorganic mercury (iHg), namely by reducing bioaccumulation in the brain and mitigating oxidative stress and behavioral impairments. **Methods:** Fish were fed for 3 months with a marine macroalgae enriched-diet (Ma) [total incorporation of 5%, with the species *Ulva rigida*, *Fucus vesiculosus* and *Gracilaria gracilis*], while non-supplemented fish were fed with a standard diet (S). Upon that period, both groups were exposed to inorganic Hg (iHg) (2 µg L^{-1}) for 7 days (E7) (constituting groups MaHg and SHg), followed by a post-exposure period of 14 days (PE14). Control fish (MaC and SC), unexposed to iHg, were maintained over the experiment. At those experimental times, Hg levels in the brain were assessed, together with antioxidants and lipid peroxidation. Motor behavior was also evaluated. **Results:** The brain of MaHg fish had significantly lower levels of Hg than SHg fish, both at E7 and PE14. Interestingly, fish under a macroalgae-enriched diet exhibited a significant decrease of glutathione peroxidase and glutathione reductase activities upon exposure to iHg for 7 days, as well as of total glutathione content. MaHg fish also exhibited a higher velocity in the first run when compared to unexposed fish, as well as a lower time in the first run. Fish fed with macroalgae-diet run faster in the first run than their congeners under a standard diet. **Conclusions:** Current data underpinned potential neurological advantages of macroalgae supplementation to fish, namely by decreasing Hg bioaccumulation and improving motor behavior. Moreover, a decrease of antioxidants was found in supplemented fish when exposed to Hg.

Keywords: inorganic mercury; marine macroalgae; fish brain; behavior

Acknowledgments

This research was funded by FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through National funds. Pereira P. is funded by National funds (OE) through FCT (Fundação para a Ciência e a Tecnologia, I.P.), under the Scientific Employment Stimulus–Individual Call [CEECIND/01144/2017; https://doi.org/10.54499/CEECIND/01144/2017/CP1459/CT0009].

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Poster 37

Parasitological research of myxozoans in 2 species of benthic fish from Northeast Atlantic waters

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https://doi.org/10.48797/sl.2024.244

Abstract

Background: Myxozoa Grassé, 1970 constitutes a diverse and widespread group of endoparasites that mainly use fish as intermediate hosts and are frequently associated with increased mortality [1]. The increase in the commercialization of sole species in Portugal promotes economic growth in fishing industry and there are presently efforts to produce it in aquaculture. However, despite its commercial relevance, there is a lack of information about parasitic infections in several benthic fish. **Objective:** This study aimed to investigate the diversity of myxosporeans parasitizing stocks of two sole species of high commercial value, *Solea senegalensis* and *Synaptura cadenati*, caught in Northeast Atlantic waters. **Methods:** The specimens were necropsied and a myxozoan survey was carried out in the internal and external tissues. Samples of tissue were analysed by light microscopy and, when infected, were photographed for morphological characterization, as well as prepared for histology and molecular procedures targeting 18S rDNA. Positive PCR products were sequenced, and the consensus sequences were analysed by BLAST in MEGA11 software. **Results:** The morphological data revealed the presence of two parasites in the *S. cadenati*, in the gallbladder *Ceratomyxa* sp. and in the urinary bladder *Ortholinea* sp. with a prevalence of 16.7% and 25%, respectively. In *S. senegalensis*, a parasite of the genus *Zschokkella* was identified in the gallbladder and a *Sphaerospora* sp., was found in the urinary bladder with the prevalence of 27.3% and 9.1%, respectively. Molecular analysis confirmed the identification of both gallbladder species, while the parasites infecting the urinary bladder are currently being processed. **Conclusions:** In *S. senegalensis*, a new occurrence of *Zschokkella soleae*, a species previously described in *Solea solea*, was found in the same tissue [2]. In *S. cadenati*, a new species of *Ceratomyxa* was identified, whose molecular analysis demonstrated that it is closely related to *Ceratomyxa* spp. from the same geographic area.

Keywords: marine fishes; parasites; myxozoa; 18S rDNA

Acknowledgments

National funds through FCT: project PTDC/BIA-BMA/6363/2020, employment contracts 2022.06670.CEECIND.

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Poster 38

Metabolic profiling of renal cell carcinoma tissue using gas chromatography metabolomics

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https://doi.org/10.48797/sl.2024.243

Abstract

Background: Renal cell carcinoma (RCC) is marked by dysregulation of angiogenesis, energy metabolism, and nutrient sensing pathways [1]. This diversity is an obstacle to achieving long-term responses to treatment, notwithstanding progress in targeted and immunotherapeutic drugs. **Objective:** This study aimed to characterise the metabolic dysregulations that occur in RCC tissue using a metabolomics approach. **Methods**: Tumour and non-tumour kidney tissues were collected from 18 patients who underwent nephrectomy at the Portuguese Oncology Institute of Porto (IPO-Porto). Ethical approval (238/2018) and written consent were obtained. Tissues were homogenised, and metabolites were extracted using a methanol-water technique. Metabolites were then analysed by gas chromatography-mass spectrometry (GC-MS) analysis. Statistical methods and pathway analysis were used to interpret potential dysregulations associated with RCC. **Results**: RCC tissue showed a significant reduction in amino acid levels (including alanine, asparagine, aspartate, serine, tyrosine, among others), except for β-alanine and glutamate, which exhibited significant elevated levels. Perturbations in organic acids were observed, with a significant decrease in fumarate and gluconate levels and an increase in 3-aminobutyrate, citrate, and lactate. Increased levels of glucose and maltose were also found in RCC tissue, whereas sugar derivatives such as *myo*-inositol and *scyllo*-inositol showed decreased levels. Pathway analysis suggested dysregulation in amino acid, energy (TCA cycle, pyruvate metabolism), sugar, and glutathione metabolism pathways in RCC tissue. **Conclusions**: These results reveal the metabolic reprogramming related with the development and progression of RCC. Understanding these alterations provides important insights for improving RCC treatment strategies.

Keywords: renal cell carcinoma; tissue; metabolic reprogramming; metabolomics

Acknowledgments

This research was financed by national funds through the FCT, IP, in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO and LA/P/0140/2020 of the Associated Laboratory i4HB. Filipa Amaro thanks the FCT for her PhD scholarship UI/BD/151313/2021. The authors declare no conflicts of interest.

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Poster 39

Self-perceived need to improve the environment quality of life and socio-environmental and health factors among residents of Anil, Rio de Janeiro - Cross-sectional study

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https://doi.org/10.48797/sl.2024.242

Abstract

Background: The epidemiological investigation of a health problem allows preventive measures to be taken about the health-disease process and the geographic and environmental features of a particular place. Environmental sanitation practices are essential for the quality of life (QoL) by controlling the physical surroundings with the aim of preventing diseases and ensuring greater social hygiene [1]. **Objective**: This study aims to assess the self-perception of quality of life in the Canal do Anil area, together with the risk/protective factors involved in the need to "improve" the environmental QoL. **Methods**: Cross-sectional analytical observational study, approved by the CEP/CONEP system. Non-probabilistic sampling of residents from the Canal of Anil area (n=494). Face-to-face application of a questionnaire (sociodemographic, general health, sanitation, and lifestyle in the residential area, and the WHOQOL-bref [2]). The link between "the need to improve the environmental QoL" and the relevant co-variables (bivariate analysis) were assessed by the unadjusted odds ratio (OR) and subsequently the adjusted multivariate analysis of a binary logistic regression (0.05 for the inclusion of co-variables and 0.2 for their exclusion). The assessment of the multivariable model relied on the 2loglikelihood, Cox and Snell coefficient correlations, the Nagelkerke test, and Area Under the Curve (AUC) derived from the model. **Results**: The multivariate relationship between QoL and physical health shows that the worse the self-perceived physical health, the worse the self-perceived QoL ($p<0.001$). The significant risk factors in the Environmental domain, were low economic income [earning one minimum wage (OR=10.2), earning 2-3 minimum wages (OR=6.7), not having had ascariasis/roundworm (OR=2.5), having a water tank at home (OR=3.0), drinking non-bottled water (OR=2.0), no pavement near the house (OR=2.0) and an accumulation of garbage (OR=2.6). **Conclusions**: Sociodemographic and environmental factors, as well as health conditions are paramount for people´s perceptions of the need for a better environmental QoL well-being.

Keywords: WHOQOL-Bref; QoL; health promotion; environment and health; sustainability

Acknowledgments

This research received no external funding.

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Poster 40

Anticancer drugs and their impact on chemobrain development: an *in vitro* investigation

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https://doi.org/10.48797/sl.2024.241

Abstract

Background: Cancer incidence has been increasing worldwide, over the past few years. “Chemobrain” refers to alterations in cognitive function after cancer treatment, including memory deficits and reduced attention capacity [1]. The blood-brain barrier restricts the entry of certain anticancer drugs into the brain. However, "chemobrain" can also arise from factors extending far beyond direct drug exposure to the brain [1]. **Objective:** This work aims to access how neurons are affected by different anticancer drugs, such as doxorubicin (DOX), methotrexate (MTX) and sunitinib (SUN), all known to cause clinical cognitive deficits. **Methods:** Differentiated human neuroblastoma cells (SH-SY5Y) were exposed for 24h or 48h to clinically relevant concentrations of DOX (0.1-10 µM), SUN (1-10 µM) and MTX (5 and 10 µM). Two classical cytotoxicity assays (neutral red uptake and MTT reduction) were performed at the end of the exposure times. In a different paradigm, autophagy inhibitors [3-methyladenine (3-MA) or chloroquine (CLQ)] were used to determine their effects on SUN cytotoxicity. **Results:** DOX led to concentration-dependent cytotoxicity, which was amplified in the longest exposure time in both assays. On the other hand, MTX caused significant toxicity at 5 µM and 10 µM, which was time-dependent but not concentration-dependent in the MTT reduction assay. In the NR uptake assay, toxicity was seen only on the longest incubation time. Regarding SUN, both assays revealed a time- and concentration-dependent cytotoxicity. For SUN, cells appeared with yellow inclusions and autophagy modulators were used. As for autophagy inhibitors, results were dissimilar, since for SUN 10 µM, 3-MA was partially protective, whereas CLQ significantly increased SUN’s cytotoxicity in both assays at 24h. **Conclusions:** These findings highlight DOX’s, MTX’s and SUN’s cytotoxicity in neurons, with DOX and SUN being equally potent. Additionally, autophagy inhibitors suggest dysregulation of autophagy as a possible mechanism underlying SUN´s neurotoxicity. Nonetheless, further research is needed.

Keywords: chemobrain; chemotherapy, neurotoxicity; neurons; cancer

Acknowledgments

This research was 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.

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Poster 41

Exploring quercetin's potential to counteract intestinal pro-inflammatory effects induced by silver nanoparticles

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https://doi.org/10.48797/sl.2024.240

Abstract

Background: Silver nanoparticles (AgNP) elicit an intestinal response characterized by vascular and cellular changes, alongside the release of cytokines linked to the activation of the nuclear factor-κB (NF-κB) pathway. Consequently, a search was conducted for a compound capable of counteracting the primary pro-inflammatory effects induced by these nanoparticles [1]. Given the reported anti-inflammatory properties of quercetin and its prevalence in the human diet, the use of this compound could be considered a potential strategy for safeguarding the body against the harmful effects of AgNP [2]. **Objective:** Evaluate the potential protective role of quercetin against the pro-inflammatory effects induced by 5 nm polyvinylpyrrolidone (PVP)-AgNP in C57BL/6J mice. **Methods:** Two subacute doses of 5 nm PVP-AgNP were orally administered once daily for 14 days using a novel dosing technology (HaPILLness), facilitating stress-free, precise oral dosing. Quercetin (1 mg/kg bw) was concurrently administered via intraperitoneal injection once daily for the same 14-day period. **Results:** Our findings revealed that quercetin effectively reduced the intestinal inflammatory response caused by AgNP, through a reduction of the vascular and cellular alterations and also a tight regulation of the major NF-κB inflammatory pathway, leading to a notable decrease in cytokine production. **Conclusions:** This study provides novel insights into the potential role of quercetin in alleviating the intestinal pro-inflammatory effects induced by 5 nm PVP-AgNP.

Keywords: silver nanoparticles; inflammation; quercetin, intestine

Acknowledgments

This work received financial support from FCT/MCTES (UIDB/50006/2020/DOI 10.54499/UIDB/50006/2020) through national funds. This work was also supported by the FCT/MCTES (Strategic Projects 10.54499/LA/P/0008/2020, 10.54499/UIDP/50006/2020, and LA/P/0058/2020:CIBB). AS thanks FCT and ESF for her PhD grant-SFRH/BD/150656/2020. MF acknowledges her CEEC contract-2020.04126.CEECIND/CP1596/ and LAQV-REQUIMTE for her contract under the reference LA/P/0008/2020. VMC acknowledges the funding of FCT, IP, under Norma Transitória DL57/2016/CP1334/CT0006.

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Poster 42

Toxicity of resin-matrix composites in dentistry

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https://doi.org/10.48797/sl.2024.239

Abstract

Background: Resin-matrix composites (RMC) are widely used as restorative materials due to their mechanical, optical, and aesthetic properties. However, some concerns have emerged due to polymerization shrinkage, material degradation, and the release of monomers from the organic matrix, such as the derivatives of bisphenol A (BPA), which are potentially toxic [1-3]. Therefore, it is important to understand the factors that contribute to the incomplete monomer conversion of RMC [1,4]. **Objective:** This systematic review aims to comprehensively explore the factors contributing to the toxicity associated with RMC and to establish clinical criteria that reduce the release of residual monomers. **Methods:** A systematic review was performed according to the PRISMA criteria. A PICO question was established and three databases, PubMed, Cochrane Central, and Web of Science were selected to run this research. Filters were established to retrieve articles in the last 20 years, in English. The inclusion criteria were *in vivo* studies/humans, Randomized Controlled Trial (RCT) and toxicity studies. The study characteristics of the included articles were extracted using a predefined Excel file. **Results:** A total of 1,261 articles were retrieved from the three electronic databases. Following the elimination of duplicates, a total of 1,227 articles remained for further selection by title and abstract, after which 20 articles were subjected to comprehensive reading; 13 articles were included and 7 excluded. The analysis of the selected articles indicated that low levels of monomers were detected in the participants´ saliva and urine, suggesting relatively low local and systemic toxicity. Dental operator-dependent factors were identified. Adherence to RMC placement protocols is essential. **Conclusions:** This systematic review suggests that low levels of free monomers were detected in participants with RMC and adherence to manufacturer's instructions is important. Further studies are needed to stablish causality, considering the exposure to non-dental BPA source materials.

Keywords: toxicity; resin monomers; BPA; resin-matrix composite; dentistry

Acknowledgments

This research received no external funding.

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Poster 43

Prediction of fenpyroximate affinity to the NADH-ubiquinone oxidoreductase protein from complex I, for different bee species

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https://doi.org/10.48797/sl.2024.238

Abstract

Background: Pesticide usage has several beneficial impacts on agricultural production. However, it also has negative environmental impacts on soil, water, and non-target species, which can potentially cause a decrease in biodiversity. A good example is the decline of bee populations due to pesticide use. Fenpyroximate (FEN) is a fungicide that acts by inhibiting mitochondrial complex I electron transport, focusing on the translocation of protons from NADH to ubiquinone oxidoreductase. It is among those detected in Caatinga crops and was already reported to have negative impacts on bees [1,2]. **Objective:** The main objective of this study is to understand the sensibility of different species of bees from the Caatinga biome to FEN using *in silico* analysis. **Methods:** A protein-ligand analysis was used to determine FEN's affinity and binding sites to the NADH-ubiquinone oxidoreductase complex I protein (NADH) for 26 bee species inhabiting the Caatinga biome. A phylogenetic tree was performed to determine the similarity and position of similar binding sites and pockets, and the protein-ligand docking was performed with Autodock (v4.2.6). **Results:** NADH sequences for the 26 bee species had a length between 438 and 566 bp, sharing 79% of pairwise residues for all sequences. Results show that the binding energy of FEN to NADH was between -8.39 and -10.28kcal/mol, inhibition constants between 28.98 and 713 nM, and ligand efficiency between 0.27 and 0.32. For all species, a minimum of 3 and a maximum of 10 ligand aminoacids were found. **Conclusions:** This bioinformatics analysis pipeline is a useful complementary tool to animal testing, giving important insights for determining the mechanisms underlying toxicity of pesticides in non-target bee species. Additionally, it was possible to determine a rank of species sensitivity to FEN that can be used as a policy-maker tool.

Keywords: fenperoxymate; native bees; bioinformatics; protein-ligand docking

Acknowledgments

Pedro Rodrigues was supported by a research contract (BEESNESS – DOI:10.54499/CIRCNA/BRB/0293/2019). Nuno Gonçalo Ferreira was supported by FCT through 2021.02111.CEECIND (DOI: 10.54499/2021.02111.CEECIND/CP1665/CT0003). FCT also supported this study within the scope of UIDB/04423/2020, UIDP/04423/2020 and Project BEESNESS (DOI:10.54499/CIRCNA/BRB/0293/2019).

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Poster 44

In vitro and *in silico* evaluation of 5-MeO-DMT, LSD, and mescaline's interaction with CYP450 enzymes

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https://doi.org/10.48797/sl.2024.237

Abstract

Background: 5-Methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), lysergic acid diethylamide (LSD), and mescaline are classic hallucinogens known for their recreational use, which increased in the last decades. Despite some available data on the metabolism of these drugs [1-3], a scientific gap exists regarding their possible interactions with CYP450 enzymes. Nevertheless, this information is of crucial relevance to predict drug-drug interactions and understand toxicological phenomena, in particular interindividual variability. **Objective**: This study aimed to evaluate *in vitro* and *in silico* the interaction of 5-MeO-DMT, LSD, and mescaline with the enzymes CYP2A6/2B6/2D6/2E1/3A4. **Methods:** The *in vitro* assessment of CYP450 inhibition was performed using the Vivid®CYP450 screening kits. IC_{50} was calculated using GraphPad Prism 9.3.0. For *in silico* assessment, molecular dynamics were performed using the PMEMD.cuda module in AMBER16. Calculations were made on the last 100 ns of the trajectory (stable zone) to assess the interaction mode/strength between enzyme and ligand, namely MMGBSA, per-residue decomposition energy, and hydrogen bonds. **Results:** Based on the IC_{50} (μM), LSD (0.35) and 5-MeO-DMT (3.47) present the capacity to be inhibitors of CYP2D6. Based on the MMGBSA (kcal/mol), LSD showed the highest binding affinities for all enzymes, while mescaline showed the lowest. The strong interaction of LSD with CYP2A6 is mediated by a hydrogen bond established with the protein residue Asn297. For interaction with CYP2B6, the residues Thr302 and Lys479 were important in mediating the interaction with 5-MeO-DMT and LSD. Key residues mediating the interaction of 5-MeO-DMT and LSD with CYP2D6 included Phe120, Leu213, and Phe483. For interaction with CYP2E1, residues Phe207, Phe298, and Thr303 are important; and for CYP3A4, an important hydrogen bond between LSD and Ala370 was identified. **Conclusions:** Both LSD and 5-MeO-DMT are predicted to have strong potential to be CYP2D6 inhibitors. A strong interaction was also identified *in silico* between LSD and CYP2A6.

Keywords: drug-drug interaction; hallucinogens; metabolism; pharmacokinetics; toxicology

Acknowledgments

This research was funded by i) FCT funds in the scope of the PhD grant 2021.04999.BD, the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB; ii) TOXRUN-IUCS-CESPU funds in the scope of the project PsiloPharma_PI2RL_IINFACTS_2021; and iii) EUROX Pharma funds.

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Poster 45

In vitro and *in silico* evaluation of psilocybin and psilocin's interaction with CYP450 enzymes

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https://doi.org/10.48797/sl.2024.236

Abstract

Background: Psilocybin is a hallucinogen produced by "magic mushrooms", being rapidly metabolized in the organism into psilocin [1, 2]. A scientific gap exists regarding the possible interactions between psilocybin/psilocin and CYP450 enzymes. Given their biological importance, and since the binding of drugs to CYP450 enzymes can interfere with the metabolism of other substrates leading to drug-drug interactions, this research topic is of utmost importance. **Objective**: This study aimed to evaluate *in silico* and *in vitro* the interaction of psilocybin and psilocin with the enzymes CYP2A6/2B6/2D6/2E1/3A4. **Methods:** The *in vitro* assessment of inhibition was performed using the Vivid®CYP450 screening kits. IC_{50} was calculated using GraphPad Prism 9.3.0. For *in silico* assessment, molecular dynamics were performed using the PMEMD.cuda module in AMBER16. Calculations were made on the last 100 ns of the trajectory (stable zone) to assess the interaction between enzyme and ligand, namely MMGBSA, per-residue decomposition energy, and hydrogen bonds. **Results:** Psilocin showed the capacity to be an inhibitor of CYP2A6/2B6/2D6/2E1/3A4, based on the respective IC_{50} values (μM) of 2.06, 6.17, 11.89, 6.37, and 2.36. Considering the MMGBSA, higher values were obtained for psilocin, corroborating the stronger binding affinity of this compound. The interaction of psilocybin/psilocin with CYP2A6 is mediated by a hydrogen bond established with the protein residue Asn297. Other important residues include Phe107 and Ile366. For CYP2B6, the strong binding of psilocin is mediated by interactions with Ile114, Thr302 (hydrogen bond), and Leu363. For interaction with CYP2D6, the most important residue seems to be Ser304, with which it forms a hydrogen bond; for CYP2E1, key residues include Phe207, Thr303, and Phe478. A strong hydrogen bond is formed between psilocin and CYP3A4 residue Phe304, contributing to the high binding affinity. **Conclusions:** The results suggest a potential for psilocin to inhibit all enzymes, especially CYP2A6 and CYP3A4.

Keywords: drug-drug interaction; hallucinogens; metabolism; pharmacokinetics; toxicology

Acknowledgments

This research was funded by i) FCT funds in the scope of the PhD grant 2021.04999.BD, the projects UIDP/04378/2020 and UIDB/04378/2020 of the UCIBIO, and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB; ii) TOXRUN-IUCS-CESPU funds in the scope of the project PsiloPharma_PI2RL_IINFACTS_2021; and iii) EUROX Pharma funds.

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Poster 46

Optimization of the chromatographic conditions of a multi-residue GC-MS method for trace analysis of pesticides and endocrine disruptors in drinking water

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https://doi.org/10.48797/sl.2024.235

Abstract

Background: Nowadays, the presence of pesticide residues and endocrine disruptors in sources for abstraction of drinking water has become a serious concern [1,2]. These classes negatively affect the environment, biodiversity, and public health. In this sense, monitoring these compounds in drinking water is essential to ensure its quality and safety on its use. Therefore, the development of sensitive analytical methods able of detecting the presence of these compounds even at residual concentrations is needed [1-3]. **Objective:** The aim of this study is to develop an analytical procedure by gas chromatography coupled to mass spectrometry (GC-MS) for the simultaneous quantification of different classes of pesticides and endocrine disruptors in drinking water. **Methods:** A method is being developed to analyze 14 compounds from a wide range of pesticide and endocrine disruptor classes. The latter were derivatized using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (MSTFA +1% TMCS) reagent and pyridine. Afterwards, the mixture was evaporated, reconstituted in acetate ethyl anhydrous and analyzed by GC-MS. The chromatographic conditions were: a capillary column 5% diphenyl 95% dimethyl polysiloxane (30 m x 0.25 mm x 0.25 µm), the injector temperature set at 280 °C, and a temperature ramp from 70 °C to 280 °C in a total run time of 30 minutes. **Results:** The target pesticides do not need to be derivatized for analysis, however, since endocrine disruptors require this process, all compounds were subjected to the derivatization step using the optimized conditions for endocrine disruptors, with the aim of including all compounds in the same sample preparation procedure and in a single chromatographic run. Analysis of the results allowed for the distinction and identification of all compounds. **Conclusions:** Derivatization conditions were optimized, and an analytical chromatographic method was developed allowing the separation and identification of 14 compounds belonging to various classes of pesticides and endocrine disruptors in 30 minutes.

Keywords: water quality assessment; environmental contaminants; chromatographic analysis

Acknowledgments

Diana Fernandes acknowledges the fellowship from project DropH2O (2022.08738.PTDC). Funding: This work is financially supported by national funds through the FCT/MCTES (PIDDAC), under the project 2022.08738.PTDC, PTDC with DOI 10.54499/2022.08738.PTDC (https://doi.org/10.54499/2022.08738.PTDC). ARR acknowledges the support from FCT funding under the Scientific Employment Stimulus Individual Call (2022.00184.CEECIND) and FCT/MCTES (PIDDAC): LSRE-LCM - UIDB/50020/2020 (DOI: 10.54499/UIDP/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020); and ALiCE - LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020).

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Poster 47

Phytocannabinoid profiling in cannabis extracts: derivatization and gas chromatography optimization

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https://doi.org/10.48797/sl.2024.234

Abstract

Background: Cannabis has been used for years for both medicinal and recreational purposes, having its potential therapeutic benefits attributed to cannabinoids [1]. Cannabis-based treatments are gaining popularity, with delta-9-tetrahydrocannabinol (THC) being approved by Food and Drug Administration for chemotherapy side effects and cannabidiol (CBD) for seizures [2,3]. Hence, characterization of chemical extracts of cannabis is imperative for medical purposes and to assess its environmental impact. Gas chromatography coupled with mass spectrometry (GC-MS) is the most common technique used for quantifying cannabinoids in plant extracts due to its high sensitivity [4,5]. **Objective:** Optimization of the derivatization and chromatographic procedures for cannabinoids analysis. Development of a GC-MS method for the simultaneous quantification of several cannabinoids present in different extracts of the *Cannabis* sp cultivar ZF plant, a commercially available hybrid weed strain. **Methods:** Compounds were derivatized using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS) and pyridine and heated at 60 ˚C for 30 min. Subsequently, the solution was evaporated, reconstituted in anhydrous ethyl acetate, and analyzed by GC-MS. The chromatographic conditions were established using a capillary column containing 5% diphenyl-95% dimethylpolysiloxane (30 m × 0.25 mm × 0.25 µm), an injector temperature set to 280 ºC, with a temperature ramp starting at 100 ºC and increasing up to 280 ºC at a flow rate of 1 mL/min to a total run of 20 min. **Results:** Different proportions of MSTFA + 1% TMCS and pyridine, heating temperature and time were attempted for optimization of the derivatization conditions. Established conditions allowed the identification of cannabinoids while preventing the decarboxylation of the more sensitive acidic cannabinoids. Chromatographic conditions were also optimized to allow the simultaneous separation of the compounds in the same run. **Conclusions:** Derivatizations conditions were optimized, and gas chromatographic conditions were established for the analysis of cannabinoids in cannabis extracts.

Keywords: cannabinoids; phytochemical analysis; medicinal cannabis

Acknowledgments

This research was funded by Avextra Portugal SA., by the supply of the standards used.

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Poster 48

Development and validation of an analytical method to quantify cytotoxic drugs in gauze dressings used in luer-lock connection systems for a safer preparation

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https://doi.org/10.48797/sl.2024.233

Abstract

Background: Despite patient´s benefits, healthcare professionals face occupational hazardous when handling cytotoxic drugs used for cancer treatment [1]. Several organizations outline procedures, conditions, and equipment recommended for safe handling. The International Society of Oncology Pharmacy Practice (ISOPP) standards [2] are the guidelines recommended by the European Parliament but, unlike others (e.g. Occupational Safety and Health Administration (OSHA)) [3], they omit the recommendation of using dressings at luer-lock connections to contain cytotoxic leaks. **Objective:** This study aims to develop and validate an analytical method for cyclophosphamide (CP) and 5-fluorouracil (5-FU) identification and quantification in gauze dressings for monitoring real-life occupational situations. **Methods:** Gauze dressings (20x10 cm) containing varying concentrations of CP and 5-FU were placed in 15 mL falcon tubes containing acetonitrile:methanol:water (19:13:68). After stirring, the samples were filtered through a 0.22 µm filter and analyzed using HPLC-DAD, equipped with a C18 column (Hypersil Gold ™ 150mm x 4.6mm and 5µm particle size). The mobile phases employed were acetonitrile:methanol:water (19:13:68) (CP) and 0.5% acetic acid in water (for 5-FU), with detection set at 205 nm (CP) and 260 nm (5-FU). **Results:** The mean of five calibration curves was calculated for each drug, each exhibiting $R^2 > 0.997$, confirming the linearity for both drugs. Regarding sensitivity, LOD of 0.006 µg/cm^2 (5-FU) and 0.11 µg/cm^2 (CP) and LOQs of 0.02 µg/cm^2 (5-FU) and 0.32 µg/cm^2 (CP), were obtained. Accuracy ranged between 93%-110%, while precision ranged between 91%-99%. At room temperature, gauze dressings deliberately contaminated exhibited superior stability for 5-FU compared to CP. **Conclusions:** The method has been successfully validated for analyzing CP and 5-FU residues in gauze, meeting essential validation criteria. Upon suitability verification, the method will be applicable for the evaluation of the importance of using gauze dressing when handling cytotoxic drugs, by assessing contaminations occurring during their real-life handling routine.

Keywords: cytotoxic drugs; occupational hazardous; chemotherapy; HPLC-DAD

Acknowledgments

This research 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/04378/2020 and UIDB/ 04378/2020), and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020).

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Poster 49

Efficient cannabinoid extraction using FlackTek technology

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https://doi.org/10.48797/sl.2024.232

Abstract

Background: FlackTek™ technology can enhance product quality and potency by employing a bladeless, non-invasive mixing process. This approach minimizes the requirement for excessive heat, effectively safeguarding the compounds of interest from degradation and damage that can happen during mixing and purging. By achieving uniform distribution and homogeneity, FlackTek combines pulverization and blending, ensuring uniform reduction in particle size and even dispersion across the material resulting in higher-quality products. This technology has been applied in several fields, like 3D printing, adhesives and sealants, chemicals, electronics, inks and coatings, medical devices, cosmetics, pharmaceuticals, polyurethanes, silicones [1,2]. Several methods have been employed for cannabis extraction, such as, supercritical carbon dioxide and solvent-based techniques, including Soxhlet extraction, dynamic maceration, and ultrasonic and microwave-assisted extraction [3]. **Objective:** The goal of this work was to apply FlackTek™ technology to oil cannabis extraction. **Methods:** THC-dominant cultivar: 5ng of ground flower was mixed with 20 g of MCT oil (1:4). Using FlackTek conditions: 2000 RPM, samples were collected at time points: 5, 10, 15, 20, and 30 min. Extraction following Ph Eur 11.5 cannabis flower monograph was used as reference method. Tetrahydrocannabinolic acid (THCA) and tetrahydrocannabinol (9-THC) content was analyzed in a HPLC-DAD system (Agilent 1260 Infinity II) with a C18 column (Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm) and extraction was performed using a 1200-500Vac FlackTek SpeedMixer. CBD-dominant cultivar: 5 g of ground flower was mixed with 10 g of olive oil (1:2). Using FlackTek conditions: 3500 RPM, samples were collected at time points: 5, 10, and 15 min. Cannabidiolic acid (CBDA) and cannabidiol (CBD) were quantified using a HPLC-DAD system (Thermofisher Ultimate 3000) with a C18 column (Hypersil Gold3UM 150x3 mm) and a FlackTek SpeedMixer DAC330-100 PRO was used for extraction. **Results:** THCA measurements are within %RSD < 5, indicating that there is no significative difference between the five extraction time points. To confirm that THC is as easily extracted, THCA in the ground flower was first decarboxylated to THC at 120 ºC, for 30 min in an oven, before extraction. **Conclusions:** FlackTek technology enables rapid and effective extraction of cannabinoids directly into MCT or olive oil. Specifically, THCA/ THC was successfully extracted in just 5 minutes, and CBDA/ CBD in 10 minutes.

Keywords: cannabis; extraction; THC; CBD

Acknowledgments

We wish to express our gratitude to the FlackTek team (Matt Gross, Ted Reynolds, and James Earle) and Andrea Ferrari for their generosity in sharing their time, experiences, and for providing the equipment and invaluable assistance for this research.

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Poster 50

Development and validation of an on-line SPE coupled to liquid chromatography with fluorescence detection method for the quantification of bisphenol A in human saliva

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https://doi.org/10.48797/sl.2024.231

Abstract

Background: Resin-matrix composites, commonly used in dentistry, often contain derivatives of Bisphenol A (BPA) such as Bisphenol A-Diglycidyl Methacrylate and Bisphenol A-Dimethacrylate in their organic matrix composition [1]. Although BPA is not a direct ingredient of these composites, it may be present as a contaminant or degradation product, potentially released into the oral cavity following dental restoration. BPA is an endocrine disruptor that can interfere with the body's hormone-regulating endocrine system. Thus, the development of sensitive and specific analytical methods for its detection and quantification of BPA in biological matrices is crucial due to its prevalence and potential impact on human health [2]. **Objective:** This study aimed to develop and validate an on-line solid phase extraction method coupled with liquid chromatography and fluorescence detection (SPE-HPLC-FD) for quantifying BPA in human saliva. **Methods:** Prior to on-line SPE-HPLC-FD analysis, 6 mL of acetonitrile were added to a 2 mL saliva aliquot to precipitate proteins. The supernatant was then evaporated and reconstituted in a 2 mL solution of 5% ethanol. A 500 µL aliquot was directly injected into the HPLC system. The initial step involved sample clean-up in the on-line SPE column (Restricted Access Media column; RP-18 ADS-20 mm × 4 mm; 25 mm) in the first dimension, the retained components were automatically transferred to the analytical column (Luna PFP (2) column-150 mm × 4.6 mm; 3 mm, 100 Å) for component resolution and subsequent fluorescence detection in the second dimension. **Results:** The developed method was validated according to the ICH Guidelines [3] and revealed to be linear over the dynamic range of 5.0 ng/mL and 50 ng/mL, with accuracy and precision ranging between 89.9-104.9% and 1.1-6.7%, respectively. Recoveries ranged from 91.5% to 97.1%. **Conclusions:** The developed method required low sample volume and allowed for automation, simplification, short time analysis and low solvent consumption. This method represents a significant advancement in the detection and quantification of BPA in biological matrices.

Keywords: on-line solid phase extraction (SPE); liquid chromatography; bisphenol A; dental composites; saliva

Acknowledgments

This work is financially supported by national funds through the FCT/MCTES (PIDDAC through the projects UIDB/04033/2020 and UIDP/04033/2020 (CITAB); UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry - CIIMAR).

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Poster 51

Optimization of the derivatization procedure for the separation of the stereoisomers of 1,3-dimethylamylamine (1,3-DMAA) by gas chromatography - preliminary data

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https://doi.org/10.48797/sl.2024.230

Abstract

Background: 1,3-Dimethylamylamine (1,3-DMAA), also known as methylhexanamine, is a central nervous system stimulant with structural similarities with amphetamines and therefore presenting overlapping biological and detrimental effects [1]. Despite being banned, the presence of 1,3-DMAA in doping controls and dietary supplements continues to be of significant concern. This molecule has two stereogenic centres and thus four stereoisomers [2]. It is widely recognized that enantiomers may exhibit different biological activity, including pharmacokinetics, pharmacodynamics, and toxicity. Consequently, the development of analytical methods for enantioselective separation of 1,3-DMAA is crucial for an accurate determination of the risks associated with each of these stereoisomers. **Objective:** To develop an indirect method by gas chromatography coupled to mass spectrometry (GC-MS) for the separation and identification of the stereoisomers of the 1,3-DMAA. **Methods:** 1,3-DMAA was regenerated with sodium hydroxide, extracted with 0.1% triethylamine in hexane and then derivatized using the enantiomeric pure reagent (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-MTPA-Cl). Subsequently, the sample was evaporated, reconstituted in anhydrous ethyl acetate, and analyzed by GC-MS. The chromatographic conditions were established using a capillary column containing 5% diphenyl-95% dimethylpolysiloxane (30 m × 0.25 mm × 0.25 µm), an injector temperature set to 280 ºC, with a temperature ramp starting at 140 ºC and increasing up to 215 ºC at a flow rate of 1 mL/min to a total run of 12.32 min. **Results:** As preliminary data indicate, the derivatization procedure allowed the formation of 4 diastereomers of 1,3-DMAA. The chromatographic conditions were optimised, allowing for the separation of the four diastereomers within 12 min. **Conclusions:** Derivatization and chromatographic conditions were established for enantioselective separation of 1,3-DMAA by GC-MS. Further validation of the method will be crucial for understanding the diastereomers' differential pharmacokinetics and pharmacodynamics, and consequently, the perils associated with their presence in food supplement samples.

Keywords: enantioselectivity; dietary supplements; chromatographic analysis

Acknowledgments

This research received no external funding.

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Poster 52

Short-term and long-term effects of gadolinium and gadoteric acid exposure on rat kidney and liver functions

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https://doi.org/10.48797/sl.2024.229

Abstract

Background: There are currently concerns about the safety of gadolinium-based contrast agents (GBCA), as they can release gadolinium [Gd (III)], known to be toxic. Free Gd (III) deposition at different organs, as kidney and liver, has been reported [1,2]. We found that Gd (III) promotes inflammation and fibrosis in proximal tubular cells [3]. GBCA with macrocyclic structure, as gadoteric acid (Gd-DOTA), are considered more stable. **Objective:** To evaluate the short-term and the long-term effects of Gd (III) and Gd-DOTA exposure on biomarkers of renal and hepatic functions, using an animal model. **Methods:** In both short-term (48h) and long-term (20 weeks) studies, eight weeks-old male Wistar rats were divided in 3 groups (*n*=10 each) exposed to: a single dose (0.1 mmol/kg) of Gd (III), of Gd-DOTA (0.1 mmol/kg) or vehicle (control). At the end of protocols, blood was collected and the levels of creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were evaluated using routine automated assays; cystatin C was measured by ELISA. **Results:** In the short-term study (48h), Gd (III) group presented significantly higher values of AST and ALT, and lower urea levels than the control group; Gd-DOTA group presented higher AST values, compared to the control group. Twenty-weeks after exposure, higher values of AST, ALT, and creatinine, than Gd-DOTA and control groups and, lower cystatin levels, compared to the control group, were found for the Gd (III) group. **Conclusions:** Single exposure to free Gd (III) induced short-term and long-term changes in liver biomarkers; the exposure to Gd-DOTA was associated with fewer short-term disturbances in transaminases, and with no long-term influence in their values. Exposure to Gd-DOTA had little influence in traditional kidney biomarkers. Despite the significantly safer profile for Gd-DOTA, further studies are necessary, testing other biomarkers, to clarify the short-term and the long-term impact of this GBCA.

Keywords: enantioselectivity; dietary supplements; chromatographic; nephrotoxicity; hepatotoxicity

Acknowledgments

This work received financial support by FCT through National Funds for the project 2022.08400.PTDC. This work was supported by FCT through 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.

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Poster 53

Developing robust heterotypic 3D lung cancer cultures for drug screening: preliminary results

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https://doi.org/10.48797/sl.2024.228

Abstract

Background: To propel advancements in cancer treatment, it is imperative to conduct initial drug testing using *in vitro* models. In laboratory settings, the efficacy of most compounds is rigorously evaluated through 2D cell cultures. However, many compounds showing promise in these models fail to perform similarly in preclinical or clinical trials, prolonging the timeline for developing effective drugs for human use [1,2]. Therefore, substantial research efforts are focused on creating *in vitro* models that better mimic the *in vivo* tumor microenvironment, as demonstrated by the exploration of 3D cell culture systems [3]. **Objective:** to implement a standard protocol for heterotypic 3D lung cancer cultures, aiming to provide a more effective alternative for anticancer drug screening. **Methods:** Monocytes were polarized into macrophages 72 hours prior seeding by adding phorbol-12-myristate-13-acetate. A549 lung cancer cells were then co-cultured with polarized macrophages (THP-1), lung fibroblasts (IMR-90), and lung endothelial cells (HPMEC) on ultralow attachment plates and monitored for 10 days. Spheroids were photographed on days 2, 4, and 6 post-seeding. **Results:** During the initial protocol standardization, different cell ratios were tested: i) 10,000 cells/well with a ratio of 1:3:3:10 for A549, THP-1, IMR-90, and HPMEC, respectively; ii) 8,000 cells/well with a ratio of 3:3:3:10; and iii) 10,000 cells/well with a ratio of 3:3:3:10. Surprisingly, none of the ratios consistently generated a single spheroid. To address this issue and aid spheroid compaction, a centrifugation step was introduced immediately after plating at either 1000 RPM for 10 minutes at 22°C or 4000 RPM for 10 minutes at 22°C. Notably, centrifugation at 4000 RPM for 10 minutes at 22°C proved most effective in producing single, compact, robust spheroids with an appropriate diameter (>350 nm). **Conclusions:** Our initial findings indicate successful development of single heterotypic 3D lung cancer spheroids. Standardization of histological analyses is ongoing, and further experiments will be undertaken to characterize this novel *in vitro* model of lung cancer.

Keywords: 3D heterotypic cell culture; lung cancer; drug screening; protocol standardization

Acknowledgments

B.P. gratefully acknowledges CESPU (BD/CBAS/CESPU/01/2020) and Fundação para a Ciência e a Tecnologia (FCT) (2022.09451.BD) for financial support.

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Poster 54

Gadolinium and gadoteric acid exposure induce long-term down-regulation in erythroid-related genes

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https://doi.org/10.48797/sl.2024.227

Abstract

Background: Gadolinium-based contrast agents (GBCA) differ in their potential to release gadolinium [Gd (III)], known to be toxic. Gadoteric acid (Gd-DOTA) is a macrocyclic GBCA, with a more stable structure. After GBCA exposure, Gd (III) retention in red blood cells (RBC) and kidney has been reported [1,2]. Nephrogenic systemic fibrosis, a severe condition found in renal disease patients exposed to GBCA, is associated with decreased hemoglobin (Hb) levels [3]. **Objective:** To evaluate the long-term effects of Gd (III) and Gd-DOTA exposure on erythropoietic function, using an animal model. **Methods:** In a long-term study (20 weeks after exposure), male Wistar rats were divided in 3 groups *(n*=10 each): exposure to a single dose (0.1 mmol/kg) of Gd (III), of Gd-DOTA or vehicle (control). At the end of the protocol, blood and renal tissue were collected; erythrogram was determined, and next-generation sequencing analysis was employed to evaluate differential gene expression of kidney tissue transcriptome. **Results:** Gd (III) group presented significantly lower RBC and hematocrit values, and higher mean *cell hemoglobin concentration (MCHC) and a trend towards lower Hb levels*; Gd-DOTA group presented trends to similar changes, without reaching statistical significance. In both groups, down-regulation of *HBA1* (encodes Hb subunit alpha 1), *HBB* (encodes Hb subunit beta) and *SLC4A1* (encodes band 3, a transmembrane chloride/bicarbonate anion exchanger1, found in RBC and kidney) genes was observed. **Conclusions:** Single exposure to free Gd (III) induced long-term down-regulation in erythroid-related genes that may underly erythropoietic and erythrocyte disturbances, as suggested by less RBC and increased MCHC. Although only alteration tendencies in these biomarkers were observed, exposure to Gd-DOTA showed the same genes downregulation. Further studies are necessary to confirm gene expression data through qPCR, to better understand the interplay between Gd (III) and erythropoiesis, and to evaluate Gd-DOTA safety.

Keywords: gadolinium; Gd-DOTA; RBC; gene expression

Acknowledgments

This work received financial support by FCT through National Funds for the project 2022.08400.PTDC. This work was supported by FCT through 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.

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Poster 55

Preliminary chemical profile and *in vitro* pharmacological evaluation of the hallucinogenic plant *Diplopterys cabrerana*

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https://doi.org/10.48797/sl.2024.221

Abstract

Background: For the last few years, Ayahuasca ceremonies have been gaining popularity in recreational settings in Europe and North America [1]. Similar to *Psychotria viridis*, *Diplopterys cabrerana* is also suggested to contain the psychoactive compound *N,N*-dimethyltryptamine, and is therefore used in Ayahuasca rituals for its ability to induce hallucinations, euphoria and entheogenic effects [1-3]. However, while information on the toxic profile of *D. cabrerana* remains very limited, its acquisition is easily accomplished by consumers. **Objective:** We aimed to characterize the aqueous extracts of *D. cabrerana* leaves, mimicking those typically consumed, to identify bioactives that underlie the psychoactive or toxic effects, and evaluate their impact on neuronal function, neurotransmission and radical stress. **Methods:** Chemical characterization was attained by HPLC-DAD. Impact upon neuronal viability was assessed by the MTT assay (up to 1000 µg/mL) in SH-SY5Y neuroblastoma cells. Impact on neuromodulation and neuroinflammation was evaluated through acetylcholinesterase and 5-lipoxygenase inhibition, while antiradical properties were assessed by evaluating nitric oxide (•NO) and xanthine oxidase (XO) activity. Inhibition of the α-glucosidase enzyme was also evaluated. Statistical comparisons among groups performed by one-way ANOVA followed by Dunnett post hoc test. **Results:** Preliminary characterization results revealed the presence of several catechin derivates, alongside two apigenin derivates and one tryptamine derivate. Cytotoxicity was not verified up to the highest concentration tested. Acetylcholinesterase inhibition was recorded starting at 250 µg/mL, and a concentration-dependent inhibition of 5-lipoxygenase was found (IC_{50}=79.77 µg/mL). Concentration-dependent scavenging effects upon •NO and XO inhibition were verified at concentrations higher than 1.953 µg/mL and 31.25 µg/mL, respectively. At last, inhibition of α-glucosidase occurred with concentration-dependency and an IC_{50} of 4.78 µg/mL. **Conclusions:** Although antiradical, anti-inflammatory and antidiabetic properties were verified, with no *in vitro* cytotoxicity being detected, further research is needed to elucidate the underlying mechanisms that might be involved in our preliminary results.

Keywords: new psychoactive substances; ayahuasca; hallucinogenic plants; recreational setting; neurotoxicity

Acknowledgments

This work received financial support from PT national funds through the project UIDB/50006/2020 of REQUIMTE/LAQV. The authors declare no conflict of interest.

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Poster 56

Chemical characterization and in vitro studies on the impact of the 'Dream Herb' *Calea zacatechichi* Schltdl. upon neuronal cells

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https://doi.org/10.48797/sl.2024.202

Abstract

Background: An increasing number of psychoactive plants, particularly those with long history of use on ritualistic practices, are now being progressively used in recreational context, many of which remaining unregulated [1]. *Calea zacatechichi* is traditionally used in divination rituals, due to its oneirogenic properties. Yet, considering the psychoactive effects of this 'Dream Herb' and easiness of purchase, the potential for recreational use is high, with scarce information concerning its toxicity [2-4]. **Objective:** We aimed to characterize *C. zacatechichi* aqueous extracts, mimicking those typically consumed, to identify bioactives that underlie the psychoactive or toxic effects, and evaluate their impact upon neuronal function, neurotransmission and radical stress. **Methods:** Chemical characterization was attained by HPLC-DAD-ESI (Ion Trap)/MS^n and HPLC-DAD. Impact upon SH-SY5Y and BV-2 cell viability was assessed by the MTT assay and LDH release (up to 1000 µg/mL). Impact on neuromodulation and neuroinflammation was evaluated through acetylcholinesterase and 5-lipoxygenase inhibition, while antiradical properties were approached upon nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet-}$). Statistical comparisons among groups performed by one-way ANOVA followed by Dunnett post hoc test. **Results:** Qualitative analyses enabled the identification of 28 compounds, the majority being hydroxycinnamic acid derivates, namely 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, followed by flavonoid derivates, particularly quercetin-3-*O*-rutinoside. Cytotoxic effects were verified at concentrations above 125 µg/mL with LDH leakage starting at 250 µg/mL. Acetylcholinesterase inhibition was recorded at 1000 µg/mL, and a concentration-dependent inhibition of 5-lipoxygenase was found (IC_{50} = 71.12 µg/mL). Concentration-dependent scavenging effects upon $^{\bullet}NO$ and $O_2^{\bullet-}$ were verified at concentrations higher than 62.5 µg/mL. **Conclusions:** Even though apparent antiradical and anti-inflammatory properties were attained with *C. zacatechichi*, the pronounced cytotoxic effects upon neuronal cells cannot be overlooked, requiring further investigation to elucidate the underlying mechanisms that might be involved, given the possible deleterious consequences this plant can induce among its consumers.

Keywords: new psychoactive substances; oneirogenic plants; recreational setting; phytochemical characterization; neurotoxicity

Acknowledgments

This work received financial support from PT national funds through the project UIDB/50006/2020 of REQUIMTE/LAQV. The authors declare no conflict of interest.

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Poster 57

KSP and MPS1 kinases as potential therapeutic targets for ovarian cancer

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https://doi.org/10.48797/sl.2024.203

Abstract

Background: Ovarian cancer ranks among the top causes of cancer-related deaths in women worldwide [1]. The conventional treatment for ovarian cancer involves surgery and chemotherapy, typically using a combination of paclitaxel and carboplatin [2]. However, despite the initial positive response to this treatment regimen, the development of treatment resistance has emerged as a significant challenge in managing the disease [3]. This scenario underscores the need for the discovery of new biomarkers and potential therapeutic targets and alternative therapeutic strategies for ovarian cancer. **Objective:** The main goal of this study is to explore the potential of targeting mitotic kinases KSP and MPS1 for ovarian cancer treatment. The specific objectives are to analyze the expression of KSP and MPS1 in (i) ovarian cancer cell lines, OVCAR 8 wt and OVCAR 8 R (double resistant to paclitaxel and carboplatin [4]), and (ii) using bioinformatic analyses. **Methods:** The expression of KSP and MPS1 in ovarian cancer cells was evaluated at both mRNA transcript and protein levels using qReal-Time PCR and Western Blotting, respectively. The UALCAN cancer database was used to analyze KSP and MPS1 expression and correlate it with clinicopathologic indicators. **Results:** We found that KSP and MPS1 were overexpressed both at mRNA (OVCAR 8 wt: 4.34±0.61 and 7.64±0.49, respectively; OVCAR 8 R: 4.65±0.43 and 6.69±1.03, respectively) and protein (OVCAR 8 wt: 1.74 and 1.97±0.12, respectively; OVCAR 8 R: 2.30 and 2.55±0.05, respectively) levels in ovarian cancer cell lines compared to their non-cancer cell line counterpart (HOSE 6.3, normalized as 1). Similar results were obtained from UALCAN analysis for KSP at the protein level. Regarding mRNA expression levels, we found no difference between normal and tumor tissues. **Conclusions:** Our results showed that both KSP and MPS1 are overexpressed in ovarian cancer, highlighting the potential of these kinases as therapeutic targets for ovarian cancer.

Keywords: KSP; MPS1; therapeutic targets; ovarian cancer

Acknowledgments

This research was funded by CESPU—Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. upPTXovcar-GI2-CESPU-2022 and Ref. SGA4Cancer-GI2-CESPU-2022 to P.M.A.S and H.B.). J.P.N.S. grateful-ly acknowledges CESPU (BD/CBAS/CESPU/01/2021) for financial support.

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Poster 58

KSP and Aurora B as potential biomarkers and therapeutic targets for head and neck cancer

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https://doi.org/10.48797/sl.2024.204

Abstract

Background: Head and neck cancer (HNC) is the seventh most prevalent worldwide [1]. The treatment for HNC often involves a combination of modalities such as surgery, radiation therapy, and chemotherapy. However, HNC patients' survival rate has shown little improvement [2]. Treatment failures are often attributed to local recurrence, lymph node metastases, and drug resistance [3]. Hence, the need for new biomarkers which allow personalized treatments and novel therapeutic approaches arises. Proteins involved in mitosis, like KSP and Aurora B, are potential candidates. KSP is engaged in bipolar spindle formation and chromosome segregation, while Aurora B is essential for kinetochore stability and cytokinesis regulation [4,5]. Inhibiting these proteins halts mitosis and triggers cell death [6,7]. Moreover, the targeting of these proteins can potentially be used not only as monotherapy but also to improve other therapeutic approaches. **Objective:** to analyze the potential of KSP and Aurora B as biomarkers and potential treatment targets in head and neck cancer. **Methods:** Bioinformatic tools such as UALCAN, Timer 2.0 and BioGrid were used to collect and analyze data. **Results:** Both KSP and Aurora B are overexpressed in HNC patients. Nonetheless there is no statistically significant correlation between higher expression of either protein and patient survivability even though a tendency can be observed for a higher expression of KSP and a better prognosis. Furthermore, the overexpression of both proteins seems to be correlated with the expression of prosurvival proteins, such as BCL-2 and BCL-xL, and BRD4, an epigenetic activator involved in cancer development. **Conclusions:** KSP and Aurora B are overexpressed in HNC patients, but no significant correlation was found regarding patient survivability potentially making them unsuitable as biomarker candidates. However, the overexpression of both proteins is correlated with proteins involved in cancer survival and development which opens the possibility for co-targeting strategies.

Keywords: bioinformatics; head and neck cancer; biomarkers; KSP; Aurora B

Acknowledgments

This research was funded by CESPU—Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. upPTXovcar-GI2-CESPU-2022 and Ref. SGA4Cancer-GI2-CESPU-2022 to P.M.A.S and H.B.). J.P.N.S. grateful-ly acknowledges CESPU (BD/CBAS/CESPU/01/2021) for financial support.

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Poster 59

Unraveling fiscalin derivatives' interactions with P-glycoprotein: a computational study

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https://doi.org/10.48797/sl.2024.205

Abstract

Background: P-glycoprotein (P-gp), a crucial efflux transporter located on the apical membrane of vital barrier tissues, plays a critical role in the detoxification of several endobiotics and xenobiotics [1,2]. Notably, fiscalin derivatives exhibit diverse interactions with P-gp, demonstrating inhibitory or activating effects. This interaction leads to a reduction or increase in the transported substrate levels, respectively [3]. **Objective:** The aim of this study was to elucidate, in silico, the P-gp binding sites and interactions of different fiscalin derivatives. Methods: Molecular Operating Environment (MOE) software was used to build all the energy minimized small-molecules 3D-structures, and Autodock Vina was used to perform the molecular docking to estimate the binding affinity between fiscalins and two human P-gp models [4,5] [at the drug-binding pocket (DBP) and nucleotide binding domains (NBDs) 1 and 2]. The best ranked poses were visualized and the interactions of the ligands with specific P-gp residues were analyzed using the BINANA software. **Results:** Molecular docking analysis unveiled a notable preference of fiscalins for binding to DBP, where all the ligands bind to residues located in the modulators (M)-site. Additionally, the assessment of interactions between fiscalins and P-gp residues within NBD1 and NBD2 revealed potential novel binding sites. Across these three locations, fiscalins exhibited binding to specific P-gp residues, establishing shared hydrophobic contacts and other significant interactions, including hydrogen-bonds; pi-pi, t-stacking and cation-pi interactions; and salt bridges. **Conclusions:** The present study confirmed the ability of fiscalin derivatives to bind to P-gp, especially at the M-site of the DBP, as well as at both NBDs. The identified binding interactions may potentially be involved in the fiscalins-mediated P-gp activation.

Keywords: *in silico* studies; fiscalin derivatives; molecular docking; MOE software; BINANA software

Acknowledgments

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) in the scope of grants UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), LA/P/0140/2020 (i4HB), UIDP/04567/2020 and UIDB/04567/2020 (CBIOS), and grant number 2021.06177.BD, through national funds.

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Poster 60

Binding affinity of synthetic cannabinoids to human serum albumin by high-performance affinity chromatography

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https://doi.org/10.48797/sl.2024.206

Abstract

Background: The use of new psychoactive substances (NPS) has been growing since the 2000's, being the synthetic cannabinoids one of the groups reported on a larger scale. This group of NPS primarily interacts with the endocannabinoid system, which is involved in many physiological functions. Numerous reports of severe morbidity and mortality, linked to their consumption, have been documented [1,2]. Studies of toxicodynamics and toxicokinetics of synthetic cannabinoids are pivotal to increase the knowledge of this class of NPS. To our knowledge, studies of the interaction of synthetic cannabinoids with human serum albumin (HSA), the most abundant plasma protein, are still very limited [3]. **Objective:** This work aims the evaluation of binding affinity of a series of synthetic cannabinoids by high-performance affinity chromatography (HPAC). HPAC is a widely used and efficient technique to study intermolecular interactions between HSA and drugs [4,5]. **Methods:** The interaction of synthetic cannabinoids with HSA was investigated by HPAC by zonal elution experiments for measuring the retention times of each cannabinoid on an HSA column. Mixtures of potassium phosphate buffer (67 mM, pH 7.0) and acetonitrile were used as mobile phases in reversed elution mode. **Results:** The binding percentages (%*b*) values ranged from 94 to 99%. ADB-FUBINACA and AMB-FUBINACA (MMB-FUBINACA) showed the highest binding affinity both with a %*b* of 99%. **Conclusions:** The synthetic cannabinoids bounded to HSA with high affinity, which can interfere with drugs pharmacokinetics by increasing their free fraction in blood, as result of their eventual displacement from albumin or even by saturation of this protein. Zonal displacement chromatography studies are being conducted, using competitors with known specific binding sites on HSA, such as warfarin and (*S*)-ibuprofen, to shed light on the sites where the selected cannabinoids bind to the protein.

Keywords: synthetic cannabinoids; human serum albumin; high-performance affinity chromatography; binding affinity; displacement studies

Acknowledgments

This research was supported by national funds through FCT within the scope of Base Funding UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Products and Medicinal Chemistry - CIIMAR).

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Poster 61

Synthesis and structure elucidation of dihydro-metabolites of the synthetic cathinones pentedrone and methylone

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https://doi.org/10.48797/sl.2024.207

Abstract

Background: Synthetic cathinones are new psychoactive substances widely abused due to their psychostimulant effects. Among them, pentedrone and methylone are part of the most consumed in the USA and Europe [1]. The metabolism of synthetic cathinones is well described in literature. Methylone can undergo, for instance, *N*-demethylation, *O*-demethylation, *N*-oxidation, and *β*-keto reduction to afford the metabolites nor-methylone, dihydroxymethcathinone (DHMC), *N*-hydroxymethylone and dihydromethylone, respectively [2]. For pentedrone, metabolites resulting from *N*-demethylation and *β*-keto reduction have been described resulting in the formation of nor-pentedrone and dihydropentedrone [3]. Although synthetic cathinones are widely studied, information about the biological effects of their metabolites is scarce. Moreover, to perform studies with metabolites, their synthesis is required. **Objective:** The aims of this work were the synthesis of the dihydro metabolites of pentedrone and methylone by reduction of the parent compounds with sodium borohydride and structure elucidation by spectroscopic methods [4]. **Methods:** Sodium borohydride (3 e.q.) was added to a solution of pentedrone or methylone in ethanol. The reaction was stirred in an ice bath for 15 min and then at room temperature for 2 h. The solvent was evaporated, the residue diluted with water and pH was adjusted to 12–14. An extraction was performed with ethyl acetate and the combined organic layers were dried with anhydrous sodium sulphate, filtered, and evaporated to afford the reduced metabolites. The structure elucidation was performed by IR, GC-MS, ^{1}H and ^{13}C-NMR. **Results and Conclusions:** Dihydropentedrone and dihydromethylone were successfully synthetized with good yields (64-96%) being their structure elucidated by spectroscopic methods. In future work, the synthesis of other metabolites of pentedrone and methylone will be attempted for further toxicology studies.

Keywords: metabolites; metabolism, organic synthesis; structure elucidation; synthetic cathinones

Acknowledgments

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 (2023.00262.BD).

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Poster 62

Understanding the ecotoxicological effects of Sulfamethoxazole and Trimethoprim on zebrafish embryo

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https://doi.org/10.48797/sl.2024.208

Abstract

Background: In the last 50 years the two most used antibiotics to prevent and treat bacterial infections are Sulfamethoxazole (SMX) and Trimethoprim (TRIM) [1]. These antibiotics were considered priority substances by the Water Framework Directive, being essential to determine the risk they may pose to aquatic ecosystems and human health. Different studies report the toxicity of SMX and TRIM to several aquatic organisms, namely Danio rerio, however, the existing data are quite incomplete. **Objective:** Evaluate the sub-lethal effects of SMX and TRIM in Danio rerio embryos. **Methods:** Fish embryo acute toxicity assays were performed, evaluating the percentage of survival, hatching, and morphological alterations after SMX (0.156 to 2.5 mg/L) and TRIM (25 to 400 mg/L) exposure for 96 h [2, 3]. Sub-individual parameters were also evaluated, namely oxidative stress (CAT and GSTs activities and TBARS levels) and neurotoxicity (AChE activity) biomarkers. **Results:** SMX showed to be more toxic, causing a maximum of 15 % mortality after 48 h at 0.156 mg/L. A hatching delay of the zebrafish embryos was observed after exposure to concentrations up to 30 mg SMX/L and 100 mg TRIM/L. Both antibiotics caused different morphological alterations in zebrafish, being the most common enlarged swim bladder, body curvatures, and pericardial oedema. Other abnormalities were also observed, namely hemagglutinations, yolk sac oedemas, and head and eye malformations after exposure to both antibiotics. Additionally, SMX and TRIM caused an oxidative stress scenario and neurotoxicity, however only SMX caused lipid peroxidation. **Conclusions:** These results highlight the impact of SMX and TRIM in D. rerio embryos, showing that these antibiotics can affect individually (e.g., growth and survival) and sub-individually (e.g., antioxidant defenses) this species. More studies should be conducted to better understand the toxicity of these antibiotics at ecologically relevant concentrations and in long-term effects on zebrafish life cycle.

Keywords: *Danio rerio*; antibiotics; acute toxicity; bioassays; biomarkers

Acknowledgments

The research conducted on this topic was funded by the Foundation for Science and Technology and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Sara Rodrigues is hired through the Regulamento do Emprego Científico e Tecnológico—RJEC from the FCT program (doi: 10.54499/2020.00464.CEECIND/CP1599/CT0002). Bárbara S. Diogo was supported by a FCT Ph.D. grant (2022.10505.BD).

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Poster 63

Cyanobacteria and cyanotoxins in eutrophic reservoirs: exploring challenges for aquatic sustainability

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https://doi.org/10.48797/sl.2024.209

Abstract

Background: Globally, numerous eutrophic freshwater ecosystems exhibit an overgrowth of cyanobacteria, often coupled with the generation of cyanotoxins [1, 2], with a frequency increase due to climatic changes. These toxins encompass a diverse range of harmful metabolites with various repercussions on the ecosystem. They adversely affect the resilience and integrity of the food chain, contributing to water quality deterioration, oxygen depletion, and species lethality [3]. Additionally, these compounds may lead to the degradation of ecosystem services, particularly impacting recreational activities, agriculture, and the drinking water quality [4]. **Objective:** The present work aims to identify the effects of phytoplankton and cyanotoxins on the ecosystem health, and perceive the limitations of phytoplankton as the only biological element for classifying the water quality of reservoirs, specially eutrophic reservoirs. **Methods:** Water samples were collected in Aguieira reservoir since 2018. The phytoplankton community was analysed regarding the composition and abundance, as well as to investigate the potential for biotoxin production and its potential impacts on the water body quality. **Results:** The cyanobacteria group consistently appeared in all samples, predominantly within the most representative groups. Furthermore, organisms belonging to the genus Microcystis sp. were frequently detected in the samples, and these organisms produce toxins that can exert adverse effects on the ecosystem. **Conclusions:** The increasing prevalence of cyanobacteria and cyanotoxins in the environment raises concerns due to their impact on human and animal well-being and biodiversity. Furthermore, these harmful substances possess the ability to bioaccumulate, increasing their concentration in the food chain and presenting a potential threat to individuals who ingest contaminated organisms, such as fish. It is crucial to establish surveillance systems (such as early detection of the presence of cyanobacteria and cyanotoxins), as well as to promote information/sharing to the general public to effectively adopt preventive measures in the ecosystem.

Keywords: water quality; ecosystem services; biodiversity impact; environmental health

Acknowledgments

This research was funded by Foundation for Science and Technology, and by the Strategic Program UIDB/04423/2020 and UIDP/04423/2020. Ivo Pinto was supported by FCT Ph.D. grants (2022.l0194.BD) and Luísa Azevedo is supported by FCT under the Scientific Employment Stimulus program (CEEC-INST/00007/2021/CP2775/CT0002).

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Poster 64

Evaluation of oxidative stress and apoptosis responses in zebrafish (*Danio rerio*) larvae after butylone exposure

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https://doi.org/10.48797/sl.2024.210

Abstract

Background: The emergence of new psychoactive substances (NPS) in the worldwide drug market raises huge concerns over public health, drug policy and environmental adverse effects [1]. Butylone (BTL) is a synthetic chiral cathinone [2] reported in wastewater effluents and despite its increasing presence in freshwater ecosystems, no studies were found on the BTL toxic evaluation in fish, including the well-known zebrafish (*Danio rerio*) model. Zebrafish is widely used to investigate the potential adverse effects of different toxics, including NPS, specifically during sensitive early-life stages [3]. **Objective:** The main goal of this study was to evaluate the potential toxic effects of BTL on the apoptosis response, enzymatic and non-enzymatic biomarkers levels. **Methods:** Zebrafish embryos with ≈ 3 hours post-fertilization (hpf) were exposed for 96 hours to different concentrations of (*R,S*)-BTL (0.01, 0.1, 1, 10 and 100 µg/L). After exposure, the determination of apoptosis level was carried out by measuring the fluorescence (excitation/emission 535/590 nm) of 10 larvae from each repetition/concentration, immersed in acridine orange dye. Furthermore, 30 larvae from each treatment concentration were collected to assess the effects on reactive oxygen species generation, enzymatic activities (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, lactate dehydrogenase, acetylcholinesterase, and glutathione S-transferases) and non-enzymatic biomarkers (protein carbonyls, thiobarbituric acid reactive substances, reduced glutathione, and glutathione disulphide). **Results:** No statistically significant differences were observed in any of the different biomarkers analysed. **Conclusions:** These findings suggest that the exposure to environmentally relevant concentrations of BTL (0.1 µg/L) during the early life stages of zebrafish may not alter the redox homeostasis with the induction of oxidative stress nor induce apoptosis, reflecting no adverse effects for acute exposure. However, further research is needed to investigate in more detail the impacts of BTL on other biomarkers, like genotoxicity, behavioural changes, and focus on chronic exposure and multigenerational effects, consequently improving the accuracy of environmental risk assessment.

Keywords: psychoactive substances; butylone; biochemical biomarkers; apoptosis; *Danio rerio*

Acknowledgments

Ondina Ribeiro thanks the FCT for the PhD research grant 2022.12242.BD. Funding: This study was funded by FCT– Portuguese Foundation for Science and Technology, through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 – ENANTIOTOX from FCT - Enantioselective ecotoxicity and bioaccumulation of psychoactive substances (http://doi.org/10.54499/PTDC/CTA-AMB/6686/2020) and the CITAB project UIDB/04033/2020 and UIDP/04033/2020 (https://doi.org/10.54499/UIDB/04033/2020.

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Poster 65

Butylone effects on swimming behaviour and biochemical parameters on *Daphnia magna*

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https://doi.org/10.48797/sl.2024.211

Abstract

Background: Psychoactive substances (e.g. cathinones) may be detected in environmental samples, posing risks to wildlife and human health [1, 2]. Butylone (BTL) is a chiral synthetic cathinone available as a racemate [1]. Nevertheless, both human metabolism and biodegradation at wastewater treatment plants may be enantioselective causing changes in its enantiomeric fraction (EF) [2]. Therefore, enantiomers may exhibit different toxicity effects on non-target aquatic organisms, like daphnia [3]. **Objective:** This study aimed to assess the sub-chronic effects on *Daphnia magna* exposed to BTL (racemate and single enantiomers) focusing on swimming behaviour and biochemical parameters. **Methods:** Daphnids (<24h) were exposed to concentrations of 0.10, 1.0, or 10 µg L^{-1} of *rac*-BTL or 0.10, or 1.0 µg L^{-1} of each BTL enantiomer for 9 days (5 replicates per concentration and a control). On day 5, several swimming parameters were determined and on day 9, daphnids were collected for evaluation of biochemical parameters. **Results:** No significant changes were observed in swimming parameters in organisms exposed to *rac*-BTL. However, organisms exposed to (*S*)-BTL at 0.10 µg L^{-1} showed a significant increase in total distance. Regarding oxidative stress, *rac*-BTL increased tiobarbituric acid reactive species levels at 10 µg L^{-1}, and an increase in reactive oxygen species levels was found after exposure to (*S*)-BTL at 1.0 µg L^{-1}. A decrease in catalase activity at 0.10 µg L^{-1} was observed in the organisms exposed to *rac*-BTL whereas no changes were observed for single enantiomers. The activity of acetylcholinesterase showed a significant decrease at all concentrations of *rac*-BTL whereas an increase was found after exposure to (*R*)-BTL at 0.10 µg L^{-1}. **Conclusions:** This study demonstrates that exposure to BTL may cause enantioselective toxicity effects on *D. magna* related to swimming parameters, oxidative stress, and enzymatic activity. To understand better the toxicity and mechanisms caused by BTL exposure in *D. magna*, future studies are ongoing.

Keywords: ecotoxicity; psychoactive drugs; daphnia; enantioselectivity; biomarkers

Acknowledgments

Ana Rita Carvalho thanks project PTDC/CTA-AMB/6686/2020 for the research grant. Funding: This work is finan-cially supported by national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 -ENANTIOTOX - Enantioselective ecotoxicity and bioaccumulation of psychoactive substances, with DOI 10.54499/PTDC/CTA-AMB/6686/2020 and supported by UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry), and the project of CITAB, UIDB/04033/2020 through national funds provided by FCT. ARLR acknowledges the support from LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC), and FCT funding under the Scientific Employment Stimulus - Individual Call (2022.00184.CEECIND).

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Poster 66

Assessment of the effects in mussels (*Mytilus edulis*) chronically exposed to environmental realistic concentrations of nickel nanoparticles using a biomarker approach

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https://doi.org/10.48797/sl.2024.212

Abstract

Background: The presence of metallic nanoparticles (NP) in the aquatic environment is a cause for concern as they can have adverse effects on biota [1,2]. **Objective:** The purpose of this study was to evaluate the effect on mussels (*Mytilus edulis*) chronically exposed to environmentally realistic concentrations of nickel nanoparticles using oxidative stress and lipid peroxidation biomarkers. **Methods:** In this study, 60 individuals of *Mytilus edulis* were chronically (28 days) exposed in a semi-static test (100% of water renewal each two days) to three concentrations (0.05 mg/L, 0.5 mg/L, 5.0 mg/L) of nickel (Ni-NP), including a negative control. The activity of oxidative stress enzymes (Glutathione S-transferases - GSTs and Catalase-CAT) and the damage caused by lipid peroxidation (Thiobarbituric Acid Reactive Substances - TBARS) were subsequently assessed in the gills and digestive glands. Data was analysed using One-Way ANOVA (factor: concentration) and, if needed, by Dunnett test (to compare each exposed group to control). **Results:** Statistically significant differences were observed in the activity of catalase present in the digestive glands between the control group and the other exposed groups, both for the gills and the digestive gland. The highest catalase activity was observed in both cases in the control group. No statistically significant differences were observed in GST activity, either in the gills or in the digestive glands, between the different experimental groups. Mussels exposed to the highest levels of nickel showed higher concentrations of TBARS in the gills, differing statistically from the control group. However, in the digestive glands, the highest concentration was in the non-exposed group. **Conclusions:** Ni-NP have been shown to be potentially harmful to the mussels, as in most of the concentrations tested, they negatively influenced the antioxidant response, which suggests an alteration in the mussels' homeostasis that needs further investigation.

Keywords: environmental stressors; ecotoxicology; organism test; oxidative stress enzymes; lipid peroxidation

Acknowledgments

This research was supported by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through Portuguese funds provided by FCT and by the project Ocean3R (NORTE-01-0145-FEDER-000064) supported by North Portu-gal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

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Poster 67

Exploring the swimming behavior of *Daphnia magna* exposed to a mixture of five psychoactive substances – preliminary data

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https://doi.org/10.48797/sl.2024.213

Abstract

Background: Psychoactive substances (PAS) are commonly found in freshwater ecosystems increasing concern about the potential negative impacts on non-target organisms, such as aquatic invertebrates [1]. Indeed, the environmental occurrence of amphetamine (AMP), and recreative drugs as 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and synthetic cathinones as butylone (BTL) and 3,4-dimethylmethcathionone (3,4-DMMC) has been reported in aquatic ecosystems and wastewaters ranging from ng/L to µg/L. Recent studies have revealed diverse adverse effects of single PAS exposure to aquatic organisms, like *Daphnia magna* [2, 3] however, these substances occur in the environment as complex drug mixtures which may lead to unforeseen toxicity impacts and/or induce different final adverse effects than single PAS exposure. **Objective:** This work aimed to assess the potential effects on swimming behavior induced by mixtures of PAS on the aquatic organism *D. magna*. **Methods:** Neonates from the third/fourth brood of *D. magna*, less than 24 hours old, were exposed to two levels, 0.1 and 1.0 µg/L, of a selected mixture of 5 PAS, namely MDA, MDMA, AMP, BTL and 3,4-DMMC, for 7 and 14 days. Exposure concentrations and control were conducted with 5 replicates each containing 20 organisms. On days 7 and 14, swimming behavior endpoints as active time, total distance and swimming speed were determined. **Results:** No significant changes were observed in the swimming behavior parameters such as active time, total distance and speed on the organisms exposed to both concentrations of the PAS mixture after 7 or 14 days. **Conclusions:** Although previous studies have shown significant changes in swimming behavior, morphophysiological and biochemical parameters on *D. magna* exposed to single exposure of these PAS [2,3], the exposure to this selected mixture in the same range of concentrations, 0.1 and 1.0 µg/L, did not cause changes on swimming behavior even after prolonged exposure, i.e., 14 days, including for the higher level tested. Additional parameters such as oxidative stress and antioxidant enzymes levels are ongoing to better understand the global effects of this combined PAS exposure.

Keywords: ecotoxicity; psychoactive substances; daphnids; combined exposure; swimming parameters

Acknowledgments

Ana Rita Carvalho thanks the project PTDC/CTA-AMB/6686/2020 for the research grant. Funding: This work is fi-nancially supported by national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 -ENANTIOTOX - Enantioselective ecotoxicity and bioaccumulation of psychoactive substances, with DOI 10.54499/PTDC/CTA-AMB/6686/2020 and supported by UIDB/04033/2020 (CITAB).

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Poster 68

Evidence on the relationship between airborne exposure to endocrine-disrupting chemicals among school-age children and asthma onset or exacerbation: a systematic review

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https://doi.org/10.48797/sl.2024.214

Abstract

Background: Evaluating airborne exposure to endocrine-disrupting chemicals (EDCs) in children is paramount, given their vulnerability, which can lead to enduring health impacts such as asthma-related outcomes [1]. **Objective:** This systematic review aims to identify existing scientific evidence assessing airborne exposure to EDCs among school-age children and asthma onset or exacerbation. **Methods:** This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [2] and was registered with the International Prospective Register of Systematic Reviews (CRD42023466637). It was conducted in the PubMed, Web of Science, Scopus, and Cochrane Library databases from inception to November 1st, 2023. Studies focusing on children aged 5-18 years with at least one EDC compound examined in various matrices and investigating the relationship between EDC exposure and asthma onset or exacerbation were included. Exclusion criteria encompassed the use of animal or *in vitro* models, the absence of quantitative EDC exposure data, reviews, and studies unavailable in English. The risk of bias was assessed using the Newcastle Ottawa scale [3], and the studies' characteristics were retrieved according to Rooney et al. [4]. **Results:** Overall, 63 studies were included, with the majority published in 2022 (n=8) and conducted in Asia (n=25). Most of them focused on asthma onset rather than its exacerbation (51 vs. 20), with urine (n=35) and blood (n=11) as the favored matrices. The most commonly investigated EDCs were, in descending order, phthalates, Polycyclic Aromatic Hydrocarbons (PAHs), heavy metals, bisphenols, organophosphates esters, triclosan, nitrogen peroxide, and parabens. Four studies had a high risk of bias in the selection domain, 8 in the comparability domain, and none in the outcome/exposure domain. **Conclusions:** Research primarily examined phthalates and PAHs, with limited attention on paraben and triclosan. Comprehensive studies with robust exposure assessment and asthma characterization are vital for understanding EDCs' impact on health, particularly the effects of EDC mixtures

Keywords: endocrine-disrupting chemicals; airborne exposure; asthma; children

Acknowledgments

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) through the fellowship 2022.11261.BD and the project under the reference PTDC/CTA-AMB/3040/2021 (https://doi.org/10.54499/PTDC/CTA-AMB/3040/2021).

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Poster 69

Chronic exposure to the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV) reveals enantioselective effects in *Daphnia* reproduction

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https://doi.org/10.48797/sl.2024.215

Abstract

Background: The abuse of synthetic cathinones (SC) is popular among young consumers for recreational purposes and has increased dramatically in the last years. Consequently, SC have been found in the aquatic environment at low concentrations [1]. Since SC are designed to change nervous system function, they may pose unpredictable harmful effects on non-target organisms, such as aquatic invertebrates [2]. Many SC, like 3,4-methylenedioxypyrovalerone (MDPV), are chiral, and therefore may exhibit enantioselectivity including in ecotoxicity [2,3]. Considering the limited information available on MDPV ecotoxicity and enantioselectivity, it is vital to assess its potentially enantioselective effects on aquatic organisms. **Objective:** This work aimed to assess the adverse effects of (*R,S*)-MDPV, (*R*)-MDPV, and (*S*)-MDPV on the survival, body size and reproduction of *Daphnia magna* after 21 days of chronic exposure. **Methods:** *Daphnia* neonates (< 24 hours) were individually exposed to concentrations ranging between 0.10 to 1.79 µg L^{-1} of (*R,S*)-MDPV, (*R*)-MDPV and (*S*)-MDPV for 21 days, using 10 organisms per concentration. Survival and reproduction data were recorded every day until day 21, whereas body size was determined at day 7 in a random subsample of 5 individuals per concentration (using microphotography analysis with ImageJ). **Results:** Chronic assays showed significant inhibition of the population rate of increase at 1.79 µg L^{-1} and reproductive output (number of offspring per female) at 1.00 and 1.79 µg L^{-1}, only for (*R*)-MDPV. Although (*R,S*)-MDPV and both enantiomers did not cause significant effects in mortality, it should be noted that organisms exposed to (*R*)-MDPV showed a slight decrease in survival at 1.00 and 1.79 µg L^{-1} when compared to the control. **Conclusions:** The present study demonstrated that chronic exposure to MDPV can impair *D. magna* reproduction, with (*R*)-MDPV causing adverse chronic effects at the tested concentrations, unlike the (*S*)-enantiomer and (*R,S*)-MDPV.

Keywords: chiral psychoactive drugs; synthetic cathinone; enantiotoxicity; aquatic pollution; microcrustacean

Acknowledgments

Ariana Pérez-Pereira acknowledges the Foundation for Science and Technology (FCT) for the PhD grant 2022.09843.BD and the IB-S for facilities access and collaboration. Funding: This work is financially supported by national funds through the FCT/MCTES (PIDDAC), under the project PTDC/CTA-AMB/6686/2020 -ENANTIOTOX - Enantioselective ecotoxicity and bioaccumulation of psychoactive substances, with DOI 10.54499/PTDC/CTA-AMB/6686/2020 and partially supported by FCT - through the projects UIDB/04033/2020 and UIDP/04033/2020 (CITAB); UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry - CIIMAR).

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Poster 70

Guardians of the flora: the peril of pesticides in Caatinga to native bees behaviour

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https://doi.org/10.48797/sl.2024.216

Abstract

Background: The impact of pesticides on bees and the devastating effects of pesticides evokes a sense of urgency and concern among scientists and environmentalists alike [1]. Once hailed as agricultural saviours, pesticides now threaten bee populations globally. Neonicotinoids and organophosphates harm bee behaviour, hindering navigation, foraging, and reproduction. Sublethal doses further weaken bees, impacting survival and pollination efficiency [2]. These disruptions not only affect plant reproduction but also threaten the bee-plant relationship [3]. The cumulative effects of pesticides, alongside other stressors, pose a significant challenge to bee populations and the vital ecosystem services they provide, but they are yet unknown to many compounds and bee species. **Objective:** This study investigates the potential sublethal effects of the acaricide fenpyroximate on the behaviour of native bees in the Caatinga biome. **Methods:** Bees of the species *Melipona quadrifasciata* were exposed to sublethal doses of fenpyroximate, a common fungicide employed in the Caatinga region, under controlled conditions for 24 hours: 0.028 µg/bee (2.5% recommended field application dose) and 0.56 µg/bee (50% recommended field application dose). Following exposure, bee behaviour was recorded for 5 minutes, and the following parameters were analysed: percentage of time moving, average speed, average speed moving, travelled distance, meander, and explored area. **Results:** Our findings demonstrate significant alterations in bee behaviour at 0.56 µg/bee (half of the recommended field application dose) but not at 0.028 µg/bee. The most pronounced effects were observed on the speed and explored area, while no significant changes were detected for parameters such as the percentage of time moving or meandering. **Conclusions:** This study highlights the importance of incorporating behavioural assays in bee toxicity testing. Although we observed no immediate mortality at the tested doses, fenpyroximate impacted bee behaviour. These findings support the use of behavioural tests as a valuable tool for assessing the sublethal effects of pesticides.

Keywords: biodiversity; Caatinga biome; conservation; native bees; resilience

Acknowledgments

This research was funded by FCT (Portuguese Foundation for Science and Technology) within the scope of UIDB/04423/2020, UIDP/04423/2020 and by the Project BEENESS (DOI:10.54499/2021.02111.CEECIND/CP1665/CT0003). BEESNESS also supported Isabel Oliveira Abreu and Cláudia Teixeira. Nuno G. C. Ferreira was supported by FCT through 2021.02111.CEECIND (DOI: 10.54499/2021.02111.CEECIND/CP1665/CT0003).

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Poster 71

Development of image classification models for the identification of earthworms exposed to glyphosate-based herbicide: a pilot study

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https://doi.org/10.48797/sl.2024.217

Abstract

Background: Glyphosate-based herbicides (GBH) may threaten ecosystems and human health [1]. Animal models using earthworms as environmental bioindicators have been proposed [2], but they must be practical and cheaper [3]. **Objective:** We test if machine learning models of earthworm image classification can be used to identify GBH-exposed environments. **Methods:** 144 adults *Eisenia andrei* earthworms were divided into Control (water), GBH1.5, GBH3.0, and GBH6.0 groups (Roundup® Original DI, equivalent to 1.5, 3.0, and 6.0 L/ha). After 48 hours, each worm was photographed at least two times with a mobile camera (76-88 images/group). Random images were used to train models (85%) and separated for testing (15%). Also, we generated 20 artificial images (AI) variations of each original image (OI) using data augmentation techniques using imgaug library [4], reaching >1,600 images/group. Thus, we trained models six times each in Google's Teachable Machine with 50, 20, and 10 epochs (learning rate=0.001; batch size=16) using OI with the four (OI-4G) or two groups (OI-2G, Control vs. GBH6.0), or using AI (AI-4G or AI-2G). The resulting models were tested using Python with new images, and the accuracy was compared using 2-way ANOVA, followed by Tukey's test. **Results:** The OI-2G model showed better accuracy when trained with 50 epochs (P=0.02), but the AI-2G model presented the best accuracy in all epochs tested ($P < 0.002$). In contrast, the OI-4G model presented the worst performance compared to the others ($P<0.0001$) (% Accuracy: OI-4G=52±5; OI-2G=77±5; AI-4G=79±3; AI-2G=93±3). When tested, AI models had lower accuracy when compared to OI models (%Accuracy: OI-4G=47; OI-2G=86; AI-4G=38; AI-2G=65). **Conclusions:** It is possible to detect the presence of GBH in the soil by evaluating earthworm images using machine learning models, even with small sample sizes (photos) and without images created artificially. Models need to be improved to detect the concentration of GBH.

Keywords: glyphosate; soil pollution; image model; machine learning

Acknowledgments

This study was partially supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) in Brazil, under grant 001, and by the Brazilian National Council for Scientific and Technological Development (CNPq) under grants 307926/2022-2 and 405546/2023-8 to TGH, and 309425/2023-9 to RZF.

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Poster 72

Environmental exposure to parabens affects drinking water bacterial virulence

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https://doi.org/10.48797/sl.2024.218

Abstract

Background: Drinking water distribution systems are known to harbor biofilms, constituting a source of microorganisms that may be released in drinking water (DW) through a consumer's tap [1]. The exposure of these biofilms to parabens is inevitable [2]. However, their effects on microbial virulence and their impact on DW quality and safety remain poorly understood. **Objective:** This work evaluates the changes in bacterial virulence from exposure to parabens (methylparaben - MP, butylparaben – BP, and propylparaben – PP) individually and in combination (MIX) on biofilms formed by *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* isolated from DW. **Methods:** Biofilms were grown for 26 days on polyvinyl chloride with and without parabens at 150 ng/L. Bacterial virulence was characterized in terms of bacterial motility (swimming, swarming, twitching), siderophores, protease, lipase, and gelatinase activity. Bacterial tolerance against different antibiotics was evaluated after MP exposure. **Results:** Cells from MP-exposed *A. calcoaceticus* biofilms revealed decreased motility. Regarding *S. maltophilia*, MP exposure resulted in a decrease to half of its lipase activity, as well as a decrease in its motility ($P<0.05$). Contrarily, the exposure to BP and MIX potentiated lipase activity (to double for both parabens) ($P<0.05$). BP and MIX increased the swimming ability of *S. maltophilia* by increasing the halos by 1.6 and 2.2 times, respectively in comparison to non-exposed counterparts ($P< 0.05$). Swarming motility was also potentiated by BP and MIX revealing a bacterial halo 4 and 5 times higher than non-exposed counterparts, respectively ($P<0.05$). Exposure to MP at 150 ng/L for 5 and 10 weeks increased *A. calcoaceticus* tolerance to trimethoprim-sulfamethoxazole and ceftazidime, respectively ($P<0.05$). **Conclusions:** Parabens at environmental concentrations affect bacterial virulence with potential implications for human health. MP seems to reduce bacterial motility and lipase activity, whereas BP and MIX potentiate these virulence factors. Long exposure to MP may cause bacterial tolerance to antibiotics.

Keywords: biofilms; drinking water; motility; parabens; virulence

Acknowledgments

This research was funded by FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 and UIDP/00511/2020 and ALiCE, LA/P/0045/2020; PRESAGE – Aquatic/0007/2020, funded by FCT/MCTES (PIDDAC); Ana Rita Pereira's PhD scholarship (2021.06226.BD) and Inês B. Gomes contract (2022.06488.CEECIND/CP1733/CT0008) both pro-vided by FCT.

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Poster 73

Bone and renal effects of low-level environmental exposure to cadmium in postmenopausal women: a cross-sectional study in Brazil

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https://doi.org/10.48797/sl.2024.219

Abstract

Background: It is widely recognized that exposure to high levels of the hazardous heavy metal cadmium (Cd) leads to renal and bone damage [1], but the association with low-level environmental exposure remains less clear. **Objective:** To assess the effect of environmental Cd exposure on bone and kidney function in postmenopausal women, who may already be at heightened risk of bone and kidney-related complications. **Methods:** A cross-sectional study with 380 postmenopausal women aged 50-70 years, living in the region of Cascavel, Paraná, Brazil was conducted. These participants had no history of occupational exposure to Cd. Information on demographic, clinical, and health behavior factors was obtained. Urinary Cd (UCd), a marker of lifetime exposure to this metal, was measured through inductively coupled plasma mass spectrometry. Renal tubular function was assessed by measurement of urinary beta-2-microglobulin (b2-MG). Bone mineral density (BMD) at the lumbar spine, femoral neck, and total hip was assessed by dual-energy x-ray emission. Data were analyzed using univariate and multivariate statistics. **Results:** UCd concentrations were generally low in this population, with a median concentration of 0.30 µg/g creat (P25=0.15; P75=0.55 µg/g creat). Univariate analysis showed a significant negative correlation between BMD and age at all measured sites ($p<0.001$), whereas a significant positive correlation with body mass index (BMI, $p<0.001$) was found. For both lumbar spine and femoral neck sites, we observed a trend to higher UCd levels from normal to osteoporosis groups ($p=0.110$ and $p=0.067$, respectively). A statistically higher percentage of women with UCd levels above the P95 of the Brazilian women reference level [2] was observed in osteopenia and particularly in osteoporosis groups versus normal group (7.2% and 20% vs 7.7%, $p=0.012$; 12% and 16% vs 5.3%, $p=0.024$, respectively). In multivariate analyses, after controlling for confounding variables, age and prior fractures were negatively associated with BMD at all measured sites ($p<0.001$ and $p<0.05$, respectively), whereas a positive association was found for BMI ($p<0.001$), and no significant association was found for UCd. UCd was found to be a significant predictor of renal tubular damage ($p<0.001$), with higher UCd concentrations associated with increased urinary b2-MG levels. **Conclusions:** These findings suggest a strong association of environmental Cd exposure with renal tubular dysfunction in this population. However, the association with BMD appears to be weak, suggesting nuanced effects of low-level Cd exposure on bone health.

Keywords: cadmium; bone mineral density; postmenopausal; beta-2-microglobulin; osteoporosis

Acknowledgments

This research received support from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020), through national funds.

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Poster 74

Association between Diethyl Phthalate (DEP) exposure and hypertension in pregnancy: an *ex vivo* vascular approach

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https://doi.org/10.48797/sl.2024.220

Abstract

Background: Hypertensive disorders in pregnancy are one of the leading causes of gestational morbidity and mortality [1]. Several risk factors have already been identified, such as a sedentary lifestyle, advanced age, alcohol and tobacco consumption, and familial predisposition, but a new one is gaining prominence due to its high presence in our daily lives, which is exposure to environmental contaminants. Being widely used in the plastic industry, phthalates are one of these environmental contaminants, due to their ubiquitousness and endocrine disrupting properties [2,3]. Besides, phthalates have been associated with impaired health, and a link with pregnancy hypertension has already been suggested in some epidemiological studies [4-6]. **Objective:** To analyze the connection between diethyl phthalate (DEP) exposure and hypertension in pregnancy and its vascular impacts. Methods: Human umbilical arteries (HUA) from normotensive and hypertensive pregnant women were collected, and DEP's non-genomic (within minutes) and genomic (24h exposure) effects on vascular reactivity were analyzed, through the organ bath technique. A range of DEP concentrations was analyzed over the response of three different vasocontractile agents (serotonin, histamine, and KCl) as well as the contribution of cyclic guanosine monophosphate (cGMP) and Ca^{2+} channels pathways. **Results:** The non-genomic effects show that DEP leads to an endothelium-independent vasorelaxation by interfering with serotonin and histamine receptors. After 24h exposure, the results show that the vasorelaxant effect of DEP seems to occur through the NO/sGC/cGMP/PKG signaling pathway, and to interfere with the L-type Ca^{2+} channels. **Conclusions:** The vascular effects induced by DEP in normotensive HUA are similar those from hypertensive pregnancies, suggesting that the development of hypertension in pregnancy may be a consequence of exposure to DEP.

Keywords: personal-care products; diethyl phthalate; hypertensive disorders in pregnancy; human umbilical arteries; vasorelaxation

Acknowledgments

This research was funded by Portuguese Foundation for Science and Technology, grant number 2020.07020.BD (PhD fellowship), UIDB/00709/2020 and UIDP/00709/2020 (CICS-UBI projects), UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020 (CESAM projects).

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Poster 75

Cerebrovascular mechanism of Bisphenol A exposure in stroke ischemic events

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https://doi.org/10.48797/sl.2024.201

Abstract

Background: The endocrine-disrupting compound bisphenol A (BPA) is produced in large quantities all over the world and is found in epoxy resins and polycarbonate plastics, which are used to make baby bottles and food and drink storage containers [1]. Due to its androgenic and estrogenic qualities, BPA has been shown to accumulate in brain tissue and is thus linked to negative neurological and vascular outcomes, including strokes [2]. This type of brain injury significantly impairs blood vessels and arteries, especially the middle cerebral artery (MCA), which in turn impairs smooth muscle cells (SMC) viability. To maintain cerebral homeostasis and vascular integrity, SMC is essential in controlling vascular tone [3]. **Objective:** Thus, this study aims to investigate the mechanisms via which a 24-hour exposure to BPA modifies the contractile function of smooth muscle cells of the middle cerebral arteries (SMC-MCA) of rats. **Methods:** Therefore, explants were isolated from the MCA of Wistar rats and MTT assays were carried out to test the response to BPA in the SMC-MCA. Contractility tests by Planar Cell Surface Area were performed to analyse the vasoactive response of SMC-MCA in response to the contractile agent, noradrenaline, and the relaxing agent, sodium nitroprusside. Proteins and ion channel subunits expression implicated in the MCA vasoactive response were assessed by RT-qPCR. **Results:** The incubation concentration determined the genomic effects, which resulted in modifications to the contractile response by altering the expression of the sGC protein and the α subunit of BKCa 1.1. **Conclusions:** In summary, these findings suggest that BPA exposure modifies SMC-MCA's vascular homeostasis and may, thus, be connected to the development of ischemic stroke. This underscores the pressing need to comprehend this connection and the underlying pathways, necessitating additional research.

Keywords: bisphenol A; endocrine disrupting compound; stroke; ischemia; vasoreactivity

Acknowledgments

This research was funded by CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology (FCT) / Ministry of Science, Technology, and Higher Education (MCTES). Margarida Lorigo acknowledges the PhD fellowship from FCT (Reference: 2020.06616.BD). The authors are grateful to the technical staff of "CICS-UBI Animal Facilities" (Covilhã, Portugal) for their disinterested collaboration, particularly to Maria José Pinto and Maria João Silva, for the daily treatment and care of the animals.

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Poster 76

Passive tobacco smoke exposure and children's health outcomes: a preliminary analysis

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https://doi.org/10.48797/sl.2024.200

Abstract

Background: Children's exposure to Passive Tobacco Smoke (PTS) in utero and during infancy can increase the risk of childhood respiratory diseases and infections (e.g., otitis) [1-3]. **Objective:** This study explores the association between children's exposure to PTS in utero (maternal, frequent smoking family member during pregnancy), up to one year, and current maternal smoke exposure with, to date, and the first two years of life health conditions. **Methods:** Data were collected from 192 parents of children (5-10 years) using a structured survey. Apart from PTS exposure data, it collected children's early health outcomes (developed in the first two years of life) including bronchitis, asthmatic bronchitis, and bronchiolitis, while to date conditions included doctor-diagnosed asthma, eczema, and otitis. Descriptive and univariate analyses were performed using SPSS v.29, with results presented through odds ratios and 95% confidence intervals. **Results:** In utero, 12.3% of children were exposed to maternal tobacco and 18.8% to PTS from another family member. Twenty-two percent were exposed to PTS during their first year of life, and 17.5% had currently smoking mothers. Among others, PTS exposure in utero from maternal smoking was associated with 1.32 and 1.10 times higher odds of bronchitis and otitis, respectively with no statistical significance. Children exposed to another smoking family member had 6.53 times significantly higher odds of asthmatic bronchitis than others ($p<0.05$) and 1.49 times higher odds of asthma ($p>0.05$). PTS exposure during the first year of life was associated with 1.80 and 2.14 times higher odds of bronchiolitis and eczema, while children with currently smoking mothers had 1.72 and 1.21 times greater odds of bronchitis and eczema, respectively ($p>0.05$). **Conclusions:** Although further investigations with a larger sample size are imperative to validate these associations, these preliminary findings reinforce the harmful effects of children's PTS exposure on their health.

Keywords: tobacco smoke exposure; passive smoke exposure; children; bronchitis; asthma; eczema; otitis

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through the projects with references UIDB/04750/2020 (EPIUnit, https://doi.org/10.54499/UIDB/04750/2020), LA/P/0064/2020 (ITR; https://doi.org/10.54499/LA/P/0064/2020), PTDC/CTA-AMB/3040/2021 (EDC(Mind)2; https://doi.org/10.54499/PTDC/CTA-AMB/3040/2021), UIDB/00511/2020 (LEPABE, https://doi.org/10.54499/UIDB/00511/2020), UIDP/00511/2020 (https://doi.org/10.54499/UIDP/00511/2020) and LA/P/0045/2020 (ALiCE, https://doi.org: 10.54499/LA/P/0045/2020). Georges Hatem also acknowledges FCT for individual funding (2022.11261.BD).

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Poster 77

Active shooter live exercise: an important diagnose tool

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https://doi.org/10.48797/sl.2024.199

Abstract

Background: Active-threat incidents are increasingly frequent, even within the European Union (EU). Multi-casualty incidents (MCI) are occurring in previously unaffected locations, amplifying the challenge for law enforcement and emergency services readiness. Violence in confined spaces, notably educational institutions, presents substantial security risks to public safety [1,2,3]. A common factor among active shooter scenarios is the selection of densely populated public venues. The Guarda Nacional Republicana (GNR) possesses territorial policing authority covering approximately 95% of the national territory, including the IUCS-CESPU Campus. **Objective:** The main objective of this study was to identify challenges faced by various institutions in responding to active threat situations with casualties on an Academic Campus. An integrated response is crucial for saving lives during active threat events. Coordinating law enforcement, emergency medical services, and academia necessitates joint training to optimise survival rates and mitigate post-event consequences. **Methods:** A simulation assessed the performance of an integrated Rescue Task Force (RTF), comprising the first Police Patrol, the Intervention Unit, and the Special Team from INEM, in response to an active shooter incident. The simulation replicated a tactical incident, an MCI scenario of an active shooter on the IUCS-CESPU Campus. Multiple GNR teams, including Patrol and Intervention Units, were deployed to identify, search, and neutralise the threat. An emergency service team also participated in the final simulation. **Results:** Internal evaluations confirmed that all operators met the minimum mission standards. The average mission duration was 10 minutes. **Conclusions:** While completion of the simulation was not a definitive indicator of operational readiness, participant perception of its effectiveness as a training platform significantly improved post-exercise.

Keywords: active-shooter; risk-assessment; threat-response; simulation

Acknowledgments

This research received no external funding.

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Poster 78

Unveiling the impact of parabens on human neutrophils oxidative burst under hyperglycaemia conditions

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https://doi.org/10.48797/sl.2024.197

Abstract

Background: Parabens, also known as a family of alkyl esters of 4-hydroxybenzoic acid (4-HB), are natural or synthetic compounds that have been used as powerful antimicrobial agents across multiple industries such as cosmetic, pharmaceutical and food industries, since the 1920s [1]. Their widespread use stems from their low cost, broad-spectrum antimicrobial activity, chemical stability, and low risk of triggering allergic reactions [2]. However, concerns have arisen regarding their potential to disrupt the endocrine system and promote chronic inflammation, classifying them as endocrine-disrupting chemicals. Chronic tissue inflammation is implicated in the pathogenesis of numerous diseases, including Diabetes mellitus (DM), often involving the activation of neutrophils [3]. **Objective:** This study aims to unveil the effect of commonly used parabens and their metabolite on human neutrophils in physiological and hyperglycemia conditions, a common feature of DM. **Methods:** Isolated human neutrophils were exposed to four parabens (methyl-, ethyl-, propyl- and butyl paraben) and their metabolite, 4-HB. The production of reactive species, a marker of neutrophils' oxidative burst, was detected through a fluorescent probe, dihydrorhodamine 123. To disclose the mechanism involved in the production of reactive species by parabens and their metabolite, an inhibitor of NADPH oxidase, an inhibitor of myeloperoxidase (MPO), an inhibitor of protein kinase C (PKC) and an inhibitor of phospholipase C (PLC) were tested. **Results:** In general, it was demonstrated that parabens induced neutrophils' oxidative burst similarly under both physiological and hyperglycemic conditions. The mechanism of action of parabens involves the activation of PLC, followed by the subsequent activation of PKC, ultimately leading to the activation of NADPH oxidase and MPO. No effects were observed with the exposure of neutrophils to 4-HB. **Conclusions:** This study provides insights into the mechanisms by which parabens modulate neutrophil function under physiological and hyperglycemic conditions, potentially contributing to our understanding of their role in DM.

Keywords: parabens; neutrophils; inflammation; diabetes

Acknowledgments

This work received financial support from FCT/MCTES (UIDB/50006/2020/DOI 10.54499/UIDB/50006/2020) through national funds. This work was also supported by the FCT/MCTES through the Strategic Projects LA/P/0008/2020 (DOI: 10.54499/LA/P/0008/2020) and UIDP/50006/2020 (DOI: 10.54499/UIDP/50006/2020. Ana Ramalho thanks FCT and ESF (European Social Fund) for her PhD grant ref. 2023.00361.BD. MF acknowledges her CEEC contract-2020.04126.CEECIND/CP1596/ and LAQV-REQUIMTE for her contract under the reference LA/P/0008/2020.

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Poster 79

Subcellular distribution of some purine catabolism enzymes in brown trout (*Salmo trutta*) liver

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https://doi.org/10.48797/sl.2024.196

Abstract

Background: Subcellular location of purine catabolism enzymes is of great interest in comparative studies due to the significant variability among species [1]. This metabolic pathway reflects in large extent a phylogenetic evolution with the loss of several enzymes in certain species, resulting in differences in the end products that are excreted [2]. Particularly in fishes, some of those enzymes are described as cytosolic for some species and as peroxisomal for others. In relation to purine catabolism enzymes in brown trout liver, published data points that urate oxidase is a peroxisomal enzyme, consistent with findings in other vertebrates [1,2]. **Objective:** This study aims to identify the subcellular location of some purine catabolism enzymes in crude cell fractions of brown trout liver. **Methods:** A centrifugal fractionation technique was applied to obtain crude cell fractions, followed by spectrophotometric measurement of enzyme activities. Assays included xanthine oxidase/xanthine dehydrogenase, allantoinase, and allantoicase, alongside organelle marker enzymes, such as succinate dehydrogenase (mitochondria), arylsulphatase (lysosomes), catalase and D-aminoacid oxidase (peroxisomes) and NADPH cytochrome c reductase (microsomes). **Results:** Xanthine oxidase and allantoicase activities were undetectable in brown trout liver fractions. This observation is consistent with the notion that certain organisms possess the xanthine oxidoreductase enzyme primarily in the xanthine dehydrogenase form or exclusively in this form. Over 85% of xanthine dehydrogenase and allantoinase activities were recovered in the supernatant fraction. **Conclusions:** The paucity of organelle marker enzymes in the cytosolic fraction of brown trout liver suggests that both xanthine dehydrogenase and allantoinase are cytosolic enzymes, with high recovered activities in the supernatant fraction. The absence of allantoicase activity in brown trout supports allantoic acid as the final purine catabolism product in salmonids [2].

Keywords: xanthine oxidase; allantoinase; allantoicase

Acknowledgments

This research was funded by Fundação para a Ciência e Tecnologia (FCT) for funds to CIIMAR (UIDB/04423/2020 and UIDP/04423/2020).

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Poster 80

Oral methylphenidate effects on GAP43 and PSD95 expression in the developing brains of Wistar Kyoto rats

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https://doi.org/10.48797/sl.2024.195

Abstract

Background: Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent neuropsychiatric disorder in childhood and adolescence [1]. Differences in brain structure, function, and connectivity were seen between ADHD and healthy individuals [2,3] but the underlying cellular and neurobiological mechanisms of ADHD are not fully understood [3,4]. **Objective:** To evaluate how clinically relevant oral doses of methylphenidate (MPH), a first-line ADHD treatment, affect body growth and the expression of brain proteins involved in synaptic plasticity, integrity, and neuronal growth. **Methods:** Wistar-Kyoto rats (18 males and 19 females) were randomly assigned to two groups. The treated group received a daily dose of MPH (5mg/kg in a 5% sucrose solution) by gavage, while the control group received an equivalent volume of 5% sucrose solution [5]. Administration began on postnatal day 15 (equivalent to childhood in humans) and lasted for 15 days, with the doses adjusted individually based on the animal's weight (25g per 100μL of drug or vehicle). The animal's weight was monitored throughout the experiment. On postnatal day 30, the animals were sacrificed, the brain was dissected in separate relevant areas. Liver, heart, and kidneys were also collected. Subsequently, the brain areas were processed by Western Blotting. **Results:** No statistically significant changes in body and peripheral organ weight were noticed among the control and treated groups in either males or female rats. Furthermore, MPH did not affect the expression of GAP43 and PSD95 proteins in the diencephalon and prefrontal cortex. Moreover, no notable sex differences were observed for these same parameters. **Conclusions:** Although MPH induced no significant changes in the analyzed parameters in males, females and even no sex related changes, further investigation into new brain areas, markers, and mainly ADHD models is crucial to understand the role of MPH on development.

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

Acknowledgments

This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., 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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Poster 81

Exploring the complex web of body measurements: what is the relationship between human height and cranial and facial measurements?

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https://doi.org/10.48797/sl.2024.193

Abstract

Background: Some studies focusing on craniometric analysis have been conducted, and not all of them seem to agree with their conclusions. For instance, Sarangi et al. (1981) and Introna et al. (1993) [1]: the former argues that there is no significant correlation between stature and cranial measurements, while the latter believes in such a relationship, developing a regression formula for stature estimation from the skull. In fact, subsequent works, such as those by Chiba and Terazawa (1998) and Patil and Mody (2005) [2-3], have supported Introna et al.'s conclusions, endorsing the establishment of the mentioned correlation. Nevertheless, for the Portuguese population, there are still few methods correlating the stature estimated by mathematical methods with cranial dimensions. **Objective:** To assess the existence of a relationship between the estimated stature from the femur and humerus with cranial and facial measurements in a sample of Portuguese skeletons. **Methods:** Twenty skeletons were studied, of which 14 (70%) were male. The following measurements were taken: total length of the humerus, maximum length of the femur, physiological length of the femur, maximum length of the skull, maximum width of the skull, maximum height of the skull, and upper facial height. Stature was calculated by applying the mathematical models by Mendonça (2000) [4], using the physiological length of the left femur and the maximum length of the left humerus. The chi-square test was used to assess any potential association between the cranial measurements under consideration and the estimated stature, while Spearman's correlation was applied to establish correlations between cranial measurements and estimated stature. **Results:** No significant associations were found between the cranial measurements taken and the estimated stature using any of the models, in both sexes ($p>0.05$). However, in terms of correlation, in males, the maximum height of the skull showed a moderate and statistically significant correlation with stature (0.658, $p=0.011$ for stature estimated with the humerus, and 0.633, $p=0.015$ for stature estimated using the femur). In females, the highest correlation value with estimated stature was -0.725, with the maximum width of the skull, followed by the maximum length of the skull (-0.464), both values lacking statistical significance ($p>0.05$). **Conclusions:** The obtained results show a correlation between the maximum height of the skull and stature in men, whereas this correlation is not observed in women; it is acknowledged that these differences may be related to sampling issues, particularly sample size. Despite being a preliminary study with a small sample size, it became evident that the relationship between estimated human stature using the femur and humerus, and cranial and facial measurements is worth exploring.

Keywords: biological profile; stature estimation; cranial measurements

Acknowledgments

This research received no external funding.

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Poster 82

Forensic Sciences towards sustainability, step by step

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https://doi.org/10.48797/sl.2024.192

Abstract

Background: "Tackling P.L.A.N.E.T." ("Promoting Living Affordably and Nurturing Environmental Transformation") is a pioneering sustainability initiative that was launched in September 2023 at University Institute of Health Sciences (IUCS) Campus. This initiative embodies an intentional, inclusive, transformative, and proactive Action Plan aimed at cultivating healthy and sustainable behaviors throughout the campus. It is intended to create an internal model that will help identify gaps and opportunities, understand where and how behaviors can be improved, aligning with the United Nations Sustainable Development Goals (SDGs). **Objective:** Higher education institutions play a fundamental role in the intellectual and social development of students, but they also have a significant impact on the environment. They generate a considerable amount of waste, the management of which is essential to minimize the environmental impact. The knowledge of which, how much, when and why are reagents used in the lab classes is absolutely fundamental to a sustainable change and is the main objective of this work [1]. **Methods:** Laboratory protocols carried out in the curricular units throughout IUCS Forensics Science Degree in the last three years were collected and analyzed. Information about chemicals and quantities used were collected. **Results:** Preliminary results show that about 50% of the curricular units (19 out of 39) have lab work with more than 200 different chemical reagents. From the 611 hours of classes, 182 are dedicated to lab work. Strong acids and bases are used frequently, as well as very strong oxidants. Additionally, organic solvents as chloroform, dichloromethane and ether are used in large quantities for extraction procedures. **Conclusions:** Preliminary data show that a lot of waste is produced during laboratory experimental works. It is important to adjust protocols to substitute more hazardous substances, reduce and recycle organic solvents. By collecting this information, we will be able to propose guidelines to reduce lab waste and contribute to a more sustainable form of teaching and learning.

Keywords: forensic sciences; sustainability; lab experimental work

Acknowledgments

This research received no external funding.

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Poster 83

Mapping of key bacterial species for *Postmortem* interval calculation

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https://doi.org/10.48797/sl.2024.190

Abstract

Background: Estimation of the *Postmortem* Interval (PMI), the time elapsed since death, is one of the most challenging issues in forensic sciences [1]. Most studies focus on extensive bacterial sequencing, but culture-based experiments for higher taxonomic resolution remain scarce [2]. **Objective:** We aimed to analyze total bacterial counts and map *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* in different organs and timepoints. **Methods:** Male C57BL/6J SPF mice underwent three independent assays during 11 *postmortem* timepoints. Feces and organs (n=10: intestine/stomach/skeletical muscle/liver/spleen/kidney/bladder/lungs/ brain/heart) were collected and resuspended in buffered peptone water, then plated onto enriched nonselective and selective culture media (n=4). Following routine aerobic incubation, Colony Forming Units (CFU) per gram/tissue or per mL/sample were quantified for total/individual bacterial loads. Species were identified by MALDI-TOF MS and statistics were done in GraphPad-Prism v.10.0.1. **Results:** Species (n=44) from 13 families and 3 phyla were identified, with notable consistency in the presence of *Staphylococcus xylosus*, *E. faecalis*, and *E. coli* across all experiments. Particular families were consistently identified across all organs, including Enterococcaceae and Enterobacteriaceae mostly in the later stages of decomposition, and Bacillaceae resisting often until the last timepoint, whereas Staphylococcaceae was variably detected. The early and substantial contamination observed in skeletal muscle, stomach, and intestine, makes them unsuitable for PMI calculations. *E. faecalis* appeared promising as a potential biomarker for kidney, liver, and, possibly, brain invasion at later timepoints, whereas *E. faecalis* and *E. coli* in the bladder, and *E. coli* in the spleen and heart, warrant further investigation for similar biomarker potential. **Conclusions:** This is one of the first quantitative cultural studies assessing how time elapsing postmortem affects the growth/evolution of key bacterial species, with *E. faecalis* and *E. coli* emerging as promising traceable biomarkers in real postmortem contexts. While recognizing the limitations of not considering the complex microbiota network, our pilot study brings an easy species-specific approach and offers a baseline for future human-oriented investigations.

Keywords: forensic sciences; microbiome; bacteria; taxonomy; *postmortem* interval

Acknowledgments

This research received no external funding.

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Poster 84

Freshwater and estuarine diatom composition and seasonal variation: influence of environmental factors and its forensic application

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https://doi.org/10.48797/sl.2024.189

Abstract

Background: Diatoms are unicellular microalgae common in aquatic systems. Different species and communities are characteristic of the ecosystems where they are found, allowing temporal and local associations to be made. Additionally, they do not occur naturally in the human body. Therefore, diatoms can provide relevant information in forensic investigation situations, i.e., determining the cause of death by drowning [1,2], or be used to establish associative indices between places, individuals, and/or objects [3,4]. Nevertheless, the diatom composition of an aquatic ecosystem depends on diverse factors such as seasonality and physiochemical and hydrological conditions of the water body. **Objective:** This study focused on analyzing the composition of diatoms in three types of aquatic environments (stream, estuary, and wells) throughout 4 seasons and exploring the influence of the physicochemical parameters of the water in diatom composition and variation. Methods: Samples were collected from two sampling locations in the stream and estuary, and from two wells. Afterward samples were processed and analyzed by optical microscopy for diatom identification. Physicochemical parameters (temperature, pH, conductivity, turbidity, dissolved oxygen and nutrients) were measured. **Results:** Differences in the composition and abundance of diatoms between the three aquatic systems were found, demonstrating the influence of hydrological and anthropogenic characteristics in diatom composition. The stream and estuary showed the higher diversity and abundance of diatoms in summer, whereas samples from the wells showed the lowest and even absence of diatoms in one well. Seasonal and spatial variations were evident and association of diatom composition with physicochemical parameters were found for some species. **Conclusions:** This preliminary study highlights the importance of investigate diatom composition in different aquatic systems and its relationship with seasonal and physicochemical factors. This study provides a basis for future research that could develop a useful database for forensic investigations.

Keywords: aquatic systems; seasonality; diatoms; forensic sciences

Acknowledgments

This research received no external funding.

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Poster 85

Behavior of teeth and restorative materials when exposed to different temperatures: A systematic review

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https://doi.org/10.48797/sl.2024.187

Abstract

Background: Teeth are recognized as the most indestructible elements of the human body, being considered one of the most resistant tissues, providing morphological, macroscopic or radiological analysis, allowing human identification, and having good resistance to environmental action. When exposed to different temperatures, restorative materials generally retain their original properties and respond in a predictable way, allowing for the possibility of accurate and legally acceptable identification. **Objective:** Answer the following research question: what is the impact of different temperature ranges on dental pieces and restorative materials such as amalgam, composite resin, glass ionomer and metal-ceramic crowns? **Methods:** Search in the PubMed, B-On and ScienceDirect databases. The research was subject to duly identified inclusion and exclusion criteria and the studies were selected following the criteria of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram. The assessment of the methodological quality of the articles was carried out according to the Quality Assessment Tool For In Vitro Studies (QUIN) for in vitro studies. **Results:** Of a total of 46,639 potentially eligible articles, only 8 in vitro studies were included after applying the established inclusion and exclusion criteria. These studies suggest that restorative materials demonstrate the ability to resist elevated temperatures and present specific patterns of macroscopic and microscopic changes in different temperature ranges. **Conclusions:** Analysis of in vitro studies suggests that restorations performed with amalgam, ceramic/metal-ceramic crowns and endodontic procedures experience fewer changes, both in terms of chemical composition and in macroscopic or microscopic aspects, when compared to other restorative materials such as composite. These materials provide a source of forensic evidence following exposure to temperatures up to 1000°C, which can be used for comparison and positive identification of victims following exposure to high temperatures.

Keywords: high temperature; forensic dentistry; dental restoration; teeth

Acknowledgments

This research received no external funding.

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Poster 86

Study of dental records archive: Global perspective

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https://doi.org/10.48797/sl.2024.185

Abstract

Background: Forensic medicine is a field that applies medical knowledge to legal issues focusing on human identification. Dental records are of great importance in this area due to the valuable information they can provide. **Objective:** The aim of this research is to examine the worldwide participation and dedication to the preservation of dental records, while also noting variations in this practice among different nations. **Methods:** An email was sent to the representative dentistry associations in several countries to inquire about their awareness of the importance of preserving dental records. The addresses were collected from the official website of the International Dental Federation. International SOS has determined the risk category and classified it into five levels, considering variables such as military forces, state of war, government and legal control, among others. These levels are high risk, medium risk, low risk and insignificant risk. **Results:** The Portuguese Dental Association suggests that dental records should be kept for at least 20 years after the last treatment. Several countries, including Israel, Russia, Finland, Iceland, Norway, and South Africa, which are classified as medium or high risk, attach great importance to such procedures, with a minimum retention period of 75 years. However, it may be advisable for Guinea-Bissau and Brazil to consider implementing regulations or extending their minimum periods (currently at a minimum of 4 years) to mitigate potential risks. **Conclusions:** At a global perspective, it was found that the countries surveyed did not always have an ideal correlation between the level of risk and awareness of the importance of complete and updated dental records. It is recommended that all nations enhance the implementation of dental records, ensuring they are stored effectively and are accessible. With the world constantly changing, it is important to constantly update these elements.

Keywords: forensic dentistry; risk countries; dental records

Acknowledgments

This research received no external funding.

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Poster 87

Dental fitness system adopted by NATO

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https://doi.org/10.48797/sl.2024.184

Abstract

Background: The Portuguese Armed Forces play a crucial role in international missions, as they are committed to the North Atlantic Treaty Organization (NATO), the European Union (EU), and the United Nations (UN). It is worth noting that oral health is a significant factor in the military selection process, which underscores the importance of preventive care and minimizing dental emergencies. **Objective:** This text outlines the dental requirements for military personnel in NATO countries, as well as the dental fitness classification system. The database contains information on NATO's classification for selection purposes. **Methods:** The search terms *Dental Fitness*, *Military, Dental Record*, and *Dental Condition* were used to conduct a thorough search of articles on the official NATO website, PubMed, and ScienceDirect databases. Subsequently, inclusion and exclusion criteria were applied. **Results:** According to the NATO guide, military personnel are classified into two low-risk dental fitness classes: Dental Fitness Class 1, which does not require any treatment or appointments, and Dental Fitness Class 2, which includes pre-existing dental conditions that seldom result in emergencies within 12 months. The guide does not specify the high-risk classes. Dental Fitness Class 3 is typically assigned when there is a possibility of an oral emergency within the next 12 months or if the condition is left untreated. Dental Fitness Class 4 is assigned to military personnel who require an annual examination, have undetermined dental status or incomplete records. Ideally, Class 1 and 2 military personnel are selected, although definitions and assessments may change. It is worth noting that some countries accept Class 3 conditions, while others prioritize the highest classification in case of doubt. **Conclusions:** These classifications help to standardize and enable each country to select the most suitable soldiers for missions, thereby reducing associated risks.

Keywords: dental fitness; military; dental record; dental condition

Acknowledgments

This research received no external funding.

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Poster 88

Skin decomposition: *Candida albicans* contribution to cadaveric phenomena

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https://doi.org/10.48797/sl.2024.183

Abstract

Background: In corpses, yeasts grow in the dead skin, and with the help of bacteria and other microorganisms they work together to dissolve the dead into the soil. The fungi of the necromycobiome change as the human body decays. For example, the bloat phase has the greatest diversity of fungi found in the body [1]. There is a need to better understand how microbial populations evolve in different environments following death, particularly considering the implications for forensic investigations, since microorganisms can play a crucial role in determining the *Postmortem* Interval (PMI) [2]. Fungi species, such as Candida albicans, are part of the human microbiome and can influence the cadaverization process. Dependently on the place and yeast load, the presence of *C. albicans* can indicate fungal infection [3]. Studying these microorganisms in human tissues and under controlled conditions, mimicking real-world scenarios, aids in understanding forensic PMI. The growth patterns of *C. albicans*, can shed light on the interplay between cadaveric decomposition and microbial development, thus contributing to the advancement of forensic science. **Objective:** This study aims to determine whether, after death, there is a propensity or inhibition of fungal growth on the skin, which can provide a comprehensive insight into the interactions between cadaveric decomposition and microbial development in different forensic contexts. **Methods:** *C. albicans* was cultured on SDA (Sabouraud Dextrose Agar) and incubated for 24 hours at 37°C. Afterwards, an inoculum of ~$1x10^8$ cells/mL was prepared and the impact in two different controlled environments, hot/dry (37°C/5% relative humidity) and cold/humid (4°C/60% relative humidity), was assessed. *C. albicans* inoculum was placed in conditions mimicking reality, with 1 cm^2 of pig skin, in 6-well plates with RPMI-1640, allowing growth over 24 hours. Results were analyzed through colony-forming units (CFUs) and photographed to verify tissue structure at 0, 5, 24, 48, and 120 (5 days) hours. **Results:** *C. albicans* CFUs increased in the first 48 hours *postmortem*, followed by a stabilization as expected, it seems that higher temperatures and increased humidity are enhanced microbial growth. **Conclusions:** After 48 hours, the increase of the skin load of *C. albicans* seems to be related to the presence of nutrients in the skin, while the subsequent stabilization/decrease follows the probable tissue nutrients reduction and presence of compounds that are toxic to yeast cells. This scenario shows that the necromycobiome, particularly related to *Candida* may be used as another tool to predict PMI. Nonetheless, further, and deeper studies are still needed.

Keywords: *C. albicans*; cadaveric; skin

Acknowledgments

This research received no external funding.

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Poster 89

Moral harassment in the security forces: Is it worrying?

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https://doi.org/10.48797/sl.2024.182

Abstract

Background: Moral Harassment is a set of continuous violent acts that cause psychological and/or physical harm to victims [1]. In Portugal, it was only in the 1990s that was considered, and it currently has a major impact on society, in public and private institutions [2, 3], which led to legislation, protecting victims and penalizing aggressors [4]. **Objective:** Obtain knowledge to identify, prevent, and reduce moral harassment within security forces. **Methods:** A questionnaire survey was created, having 3 parts: i) sociodemographic data of the sample; ii) Leymann Inventory of Psychological Terrorization (LIPT-60) scale; iii) questions about moral harassment. The questionnaire was implemented in LimeSurvey and distributed by email to all members of the Associação dos Profissionais da Guarda and the Associação Sindical dos Profissionais da Polícia. Data was analyzed using descriptive statistics and IBM SPSS Statistics 29.0. **Results:** We obtained 302 complete answers (62 females; 240 males). The negative behaviors with the highest percentage (77.82%) are related to preventing self-expression, and the dimensional indicators with the highest average are the Job Demeaning Index (0.95) and the Express Intimidation Index (1.04). This instrument presents a very good reliability (Cronbach's alpha=0.9817). The characterization of the sample revealed that 96% had heard of moral harassment, 30.8% had been/were victims of the phenomenon, of which the majority were male (74.2%). 19.2% of the total participants considered that the violence they had suffered disrupted their work and performance. The main symptoms were difficulty falling asleep and insomnia, anxiety, anguish and sadness, and nervousness, and 17.2% of victims even mentioned ideas of homicide and/or suicide. **Conclusions:** A significant level of moral harassment is lived within the security forces, with laboral discredit and intimidation being the most prevalent forms. This is a worrying and emerging situation, and preventive measures need to be taken to avoid more severe consequences such as suicide.

Keywords: moral harassment; physical and psychological damage; work environment

Acknowledgments

This research received no external funding.

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Poster 90

INNOMINATE – Digital catalog and tools of the identified skeleton collection of IUCS-CESPU

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https://doi.org/10.48797/sl.2024.180

Abstract

Background: The University Institute of Health Sciences (IUCS) - CESPU houses the XXI CEIC - XXI Century Identified Skeletal Collection; it holds over a hundred human remains undergoing or awaiting full processing – cleaning, documentation, and proper storage. All data are documented in printed templates during processing, including information on osteological material presence, absence, preservation status, measurements, and biological profile estimations [1]. The absence of a well-structured digital repository hinders interested parties from fully leveraging the collection's pedagogical/scientific potential. **Objective:** The aim of this work is to build a web catalogue for the XXI CEIC, allowing students, professors, and experts in related fields easy access to data and tools suitable for their purposes [2]. **Methods:** A review of other online osteological collections in Portugal or abroad was conducted. We extracted all data fields to be included from existing records and templates and structured them in a relational database for easy querying in SQL. Extensive user interface models/mockups were created, considering functionality and appearance, with support images/icons generated by artificial intelligence, hand-drawn, or open source. The collection will be available at https://ceic.iucs.cespu.pt, with access permissions pending Ethics Committee review. **Results:** In Europe, there are 151 osteological collections, 43 being contemporary [3]. Portugal, alongside the XXI CEIC, holds 9 of these, for which no web databases were found [1]. In the United States of America, among 288 catalogued forensic databases, only four comprised human osteological remains [4]. Our web catalogue will feature anonymous personal information, such as dates and places of exhumation; description of bones condition (presence/absence); cranial measurements; data required for determining the preservation state, and biological profile estimation with associated calculation tools. **Conclusions:** The XXI CEIC aims to be a research and pedagogical tool in Health and Forensic Sciences, and a web platform to interact with it will be key to reaching its full potential.

Keywords: osteology; identification; anthropology; computing; forensic sciences

Acknowledgments

This research received no external funding.

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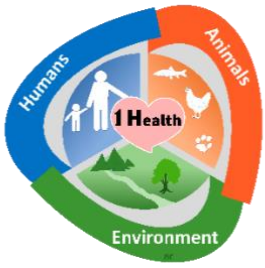

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SCIENTIFIC LETTERS SL

Volume 1 Supplement 1 (2024)

ISSN 2795-5117

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