BOOK OF ABSTRACTS

NETWORKING

ADVANCED ALTERNATIVE MODELS AND ANALYTICAL TOOLS FOR RISK ASESSMENT STUDIES

Univeristy of Valencia
Research group (GIUV2021-513)

WORKSHOP

December 2nd, 2022
Faculty of Pharmacy
University of Valencia

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Coordinador por: Dra. María José Ruiz, Dr. Yelko Rodríguez-Carrasco

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SCIENTIFIC PROGRAMME

Workshop: NETWORKING ON ADVANCED ALTERNATIVE MODELS AND ANALYTICAL TOOLS FOR RISK ASESSMENT STUDIES

DATE: Friday, December 2nd, 2022

VENUE: Faculty of Pharmacy, Burjassot, Valencia, Spain

LUNCHEON: 13:30-14:45 (Bar of the Faculty).

Workshop: 10:00 to 17:30 (saló d'actes)

10:00-10:30 Registration

10:30-10:45 Welcome/Opening of the Workshop

10:45-12:25 Chairpersons: Prof. María José Ruiz, Prof. Mónica Fernández

10:45-11:10 Title: "*In vitro* models and its application in cytotoxicity of mycotoxin mixtures"

Prof. María José Ruiz, Universitat de València (Spain)

11:10-11:35 Title: "Use of bioimaging in drug discovery of new antimicrobials"

Prof. Carlos García-Estrada, Universidad de León (Spain)

11:35-12:00 Title: "Microscale technologies and microfluidic platforms for biomedical studies"

Prof. Elisa Cimetta, Universita degli Studi de Padova (Padova, Italy)

12:00-12:25 Title: "Beneficial effects of bioactive compounds obtained by natural waste products on oxidative stress associated diseases"

Prof. Luca Vanella, Universita degli Studi de Catania (Sicily, Italy)

12:25-13:30 Posters viewing

13:30-14:45 Invited members Luncheon

SCIENTIFIC PROGRAMME

14:45-16:25 Chairpersons: Prof. Houda Berrada, Prof. Yelko Rodríguez-Carrasco

14:45-15:10 Title: “Computational tools for the prediction of toxicological properties”
Dr. José Luis Vallés Pardo, ProtoQsar, Valencia (Spain)

15:10-15:35 Title: “Impact of Pulsed Eletric Fields and High-Pressure Processing on mycotoxins recoveries from contaminated foodstuffs”
Prof. Houda Berrada, Universitat de València (Spain)

15:35-16:00 Title: “Volumetric absorptive microsampling as an alternative tool for human biomonitoring of mycotoxin s in blood”
Dr. Roger Pero-Gascon, Centre of Excellence in Mycotoxicology and Public Health, Department of Bioanalysis (Ghent, Belgium)

16:00-16:25 Title: “Trends on human biomonitoring and risk assessment of mycotoxins”
Prof. Maria João Silva, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisbon (Portugal)

16:25-17:30 Roundtable. Chairpersons: Prof. María José Ruiz, Prof. Houda Berrada

17:30 Closing

21:00 Workshop dinner/Social event

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In vitro models and its application in cytotoxicity of mycotoxin mixtures

Ruiz, María José*; Juan-García, Ana; Rodríguez-Carrasco, Yelko; Tolosa, Josefa; Taroncher, Mercedes; Fernández-Franzón, Mónica

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Abstract

Since humans are exposed to different mycotoxins through daily intake. There is increasing concern about the toxic effects to consumers, depending on the concentrations in food, interactions between them and the time of exposure. The toxicity of mycotoxins has been extensively performed by in vitro toxicity methods taken individually. But, their toxicity may be enhanced when they are present simultaneously or are present with their metabolites as a result of metabolism. However, data on combined toxic effects of mycotoxins are limited and the health risk assessments are usually based on one single mycotoxin exposure. Considering the number of possible mixtures to occur, it is considered necessary to develop strategies to assess the toxicity produced by combinations of mycotoxins, and other contaminants, in order to make a correct and real risk assessment. The aim of this study was the selection of different mixtures, including different mycotoxins produced by similar or different fungi species, and the identification of the type of interaction between the mixtures was performed using the isobologram analysis. The results demonstrated that the isobologram method provides an accurate prediction of the interaction between mycotoxins in combination. However, it cannot elucidate the mechanisms by which these types of interactions are produced. Although, the main trend shows an increase in toxicity when mycotoxins are combined, the results obtained do not permit to conclude a similar behavior of cells. For the combinations studied, synergistic, antagonistic and addictive effects were obtained from the analyses performed. So, predictive models based on combined data could help to better understand the interaction between mycotoxins and their implications in food safety assessment. However, a further analysis of the molecular mechanism underlying these interactive effects is required.

Keywords: mycotoxins, interaction, cell culture, proliferation assays

Acknowledgements: This work was funded by the Spanish Ministry of Science and Innovation (PID2020-115871RB-100).

Use of bioimaging in drug discovery of new antimicrobials

García-Estrada, Carlos; Melcón-Fernández, Estela; Pérez-Pertejo, María Yolanda; Balaña-Fouce, Rafael; Reguera, Rosa M

Area of Toxicology, Department of Biomedical Sciences, Faculty of Veterinary Medicine, University of León, Spain

Abstract

Pharmaceutical research and development require a systematic interrogation of a candidate molecule through preclinical and clinical studies. To ensure that resources are spent on only the most promising molecules, early studies must understand fundamental attributes of the drug candidate. The use of non-invasive molecular bioimaging has been increasingly applied to improve and accelerate certain steps in drug development, including the identification of appropriate therapeutic targets, evaluation of on-target and off-target effects of candidate therapies, assessment of dose response, and the evaluation of drug or biological biodistribution and pharmacodynamics. To this aim, molecular bioimaging has been applied in early clinical trials, particularly in phase 0 studies, to demonstrate proof-of-concept or to explain variation in treatment effect. Many of these applications have involved the retasking of technologies that were originally intended as clinical diagnostics. Based on the wide experience and recognition of the rich information provided by in vivo molecular imaging, these molecular bioimaging techniques are increasingly being used to address the enormous time and costs associated with bringing a new drug to clinical launch. Focusing on preclinical in vitro and in vivo bioimaging, we show how genetic engineering of pathogens, such as the generation of fluorescent cell lines of Leishmania parasites, can ease the drug screening process for the detection of hit compounds, in addition to allow visualization of the infection process and assessment of the efficacy of candidate compounds in animal models.

Keywords: drug discovery; bioimaging; leishmaniasis; antimicrobials

Acknowledgements: This research was partially funded by AEI PID2020-119031RB-I00 to RMR. EMF is contracted with EU PRIMA (PCI2022-132925) Project.

Microscale technologies and microfluidic platforms for biomedical studies

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Abstract

In our bodies cells reside in a complex milieu, the microenvironment (μEnv), regulating their fate and function. Most of this complexity is lacking in standard laboratory models, leading to readouts poorly predicting the in vivo situation. This is particularly felt in cancer research, as tumors are extremely heterogeneous and capable of conditioning both the local μEnv and distant organs. We hypothesize that the development of microbioreactors (μBRs) exploiting classical engineering principles and reconstructing biologically sound niches will solve the limitations of existing classical culture models. We thus propose to develop platforms and to test their edge over classical approaches in answering biological questions. The focus of our research is on decoding the role of extracellular vesicles (EVs) in determining Neuroblastoma (NB) aggressiveness. Our μBRs generate time and space-resolved concentration gradients, support fast dynamic changes, and reconstruct precise interactions between cells and tissues while performing multifactorial and parallelized experiments. We will here present our recent results highlighting the effect of μEnv conditions (ie oxygen tension) on the cargo and function of NB EVs, and on their pro-metastatic traits. Each device is designed to guarantee control over key parameters and enable to address specific questions in the complex processes involved in tumor metastatic dissemination. We expect that our technologies, paired with strong mathematical approaches to analyze and interpret the data, will bridge the gap between in vitro techniques and in vivo biological phenomena leading to significant and novel results, shedding light on previously unexplored scenarios.

Keywords: microbioreactors; microenvironment; extracellular vesicles; metastasis

Beneficial effects of bioactive compounds obtained by natural waste products on oxidative stress associated diseases

Vanella, Luca

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Abstract

The awareness of the large amount of waste produced along the food chain starting from the agricultural sector, from industrial transformation to the domestic context, has in recent years aroused strong concern also in public opinion, which wonders about the possible consequences that this could have on environmental sustainability, resource waste and human health. By-products generated by fruits and vegetables-processing industries such as, represent a rich source of bioactive compounds useful to counteract metabolic syndrome. The link between obesity, adipose tissue expansion, and inflammation has been intensively studied over the past several years. A wealth of experimental evidence has highlighted the role of adipose tissue in the development of systemic inflammation through the release of various adipokines such as IL-6, which promote inflammation. Interestingly, our data demonstrated that olive leaves extract, pomegranate waste extract and bioactive compounds from lemon extract can affect lipid accumulation, adipocyte differentiation and overcome TNF-alpha induced insulin resistance. The ectopic deposition of triglycerides is associated to higher risk of diabetes and liver disorders. Our *in vitro* results showed that "*pastazzo*", which derives from the processing of red oranges, and the olive leaves, deriving from olive tree pruning, showed antioxidant activity, reduced the accumulation of free fatty acids in hepatic cells and could act as cholesterol-lowering agents. The results of individual quantification of anthocyanins in "*pastazzo*" showed that cyanidin 3-glucoside is the predominant anthocyanin. An interesting *in vivo* study carried out in our laboratory demonstrated that cyanidin 3-glucoside was able to reduce lipid peroxidation and to protect animals against ochratoxin-induced oxidative damage. Based on obtained results, preparation of natural extracts that derives from waste products can be useful for preventing, counteracting or delaying the onset of the complications of diseases associated to oxidative stress such as obesity, diabetes, hepatic steatosis, intestinal bowel disease and cancer.

Keywords: nutraceuticals; oxidative stress; metabolic syndrome; inflammation

Computational tools for the prediction of toxicological properties

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ProtoQSAR SL, Centro Europeo de Empresas Innovadoras (CEEI), Paterna (Spain)

Abstract

Before their commercialization, chemicals of any industrial area (drug, cosmetic, food-related, etc.) need to comply with national and international norms such as the EU REACH, in order to guarantee their low or null impact in the human health and/or the environment (e.g., the contamination of air or aquatic sources). This represents a risk assessment process that involves a number of toxicological and ecotoxicological tests, and some of them involve animals as test subjects, which the consequent economical and ethical concerns. Thus, the computational methods become a very powerful and competitive tool in the development of new compounds. Amongst them we can find trend-analysis, read-across, SAR and QSAR, being the last one the most robust and powerful. In this methodology we use experimental information for different toxicological parameters and the structural features of existing compounds in order to create mathematical models that correlate the toxicity with the structure of the molecules. The regulatory entities (e.g., the ECHA) recognize and promote the QSAR models -under some specific conditions- as valid toxicology in-silico tests, and they can be included in the risk assessment reports, decreasing considerably the timings and the monetary investment in the research of new compounds. A brief review of computational methods for risk assessment will be provided in this presentation, and some case studies will be shown as examples.

Keywords: QSAR; genotoxicity; ecotoxicity; in-silico

Impact of Pulsed Electric Fields and High Pressure Processing on mycotoxins recoveries from contaminated foodstuffs

Pallarés, Noelia; Sebastià, Albert; Castagnini, Juan Manuel; Ferrer, Emilia; Barba, Francisco J; Berrada, Houda

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Abstract

Food security along with excellent nutritional and sensory food properties are the main claims of consumers nowadays. The food processing industry is looking for technologies that can remove hazardous chemicals, such as mycotoxins which occurrence has been widely reported in cereals, dried fruits, spices, coffee, fruits and their by-products. Pulsed electric field (PEF) technology allows the application of electrical treatments of different electric field strength for short periods of time to a selected food placed between two electrodes in static batches, or under continuous flow with pulse repetition adapted to the food flow rate to enhance pore formation and increase permeability of biological membranes. High hydrostatic pressure (HPP) units consist of a horizontal HP vessel to generate external pressure that will be transmitted uniformly and instantaneously throughout the selected food matrix. HPP and PEF technologies are being explored as a green innovative food processing alternative for food processing for aflatoxins and enniatins reduction or elimination in several foodstuffs without producing toxic residues or affecting the nutritive value, the palatability or the technological properties of the products. After the treatment, mycotoxins were extracted by dispersive liquid-liquid microextraction (DLLME) and determined by HPLC-MS/MS-IT. Both HPP and PEF techniques showed measurable impact on mycotoxins levels. Reduction percentages of 14–29% have been obtained with both HPP and PEF treatments for enniatins even higher reductions were obtained for AFB2 and AFG1 under PEF treatment, reaching reduction of 72 and 84% respectively. The effect of the explored techniques has also been compared with a thermal treatment performed at 90 ◦C during 21 s and resulted more effective than thermal treatment, being an effective tool to incorporate to food industry in order to reach mycotoxins reduction

Keywords: Mitigation; mycotoxins; PEF; HPP

Agradecimientos: Este trabajo ha sido financiado por el proyecto del Ministerio de Ciencia e Innovación de España (PID2020-115871RB-I00) y por el proyecto AICO/2021/037 de la Generalitat Valenciana.

Volumetric absorptive microsampling as an alternative tool for human biomonitoring of mycotoxins in blood

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Abstract

Human biomonitoring, which is the direct measurement of biomarkers of exposure and effect in biological fluids (e.g. blood is becoming an imperative tool in epidemiology an d public health sciences to assess internal exposure to food contaminants such as mycotoxins. Recently, there is a growing interest in the use of alternative non-invasive or minimally invasive sampling strategies to venous blood collection, such as volumetric absorptive microsampling (VAMS) VAMS). VAMS enables patients to sample themselves at home via a finger prick and features a wide range of advantages including a smaller sample volume requirement, improved analyte stability and ease of sample handling, storage and transportation for remote testing. A VAMS based multi mycotoxin method was developed for 24 different mycotoxins. The method was validated in terms of specificity, calibration range, apparent recovery, repeatability, reproducibility, limits of detection and quantification. The recovery of the different mycotoxins was in dependent of the haematocrit level and remained acceptable after storage of VAMS for 7 and 21 days in refrigeration (4 °C) and room temperature. Finally, a comparison was made between the novel methodology based on VAMS and a procedure for the analysis of liquid whole blood in 20 different blood samples. Four mycotoxins and metabolites (ochratoxin A, ochratoxin alpha, zearalenone and aflatoxin B1) were detected in these samples, resulting in similar detection capabilities for both methods. Given all the benefits associated with VAMS and the method developed, VAMS sampling may serve as an alternative to conventional venous blood collection for biomonitoring multiple mycotoxin exposure in blood, especially in resource limited areas where equipment facilities are scarce and there may be a considerable delay between sampling and analysis or in specific populations, such as neonates and anaemic patients.

Keywords: mycotoxins exposure bio monitoring; VAMS

Trends on human biomonitoring and risk assessment of mycotoxins

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Abstract

Mycotoxins are fungi secondary metabolites that have raised public health concern given their acute and long-term health effects. Human exposure to mycotoxins (and their mixtures) occurs mainly through ingestion of contaminated food and is expected to increase in Europe due to climate change. Under the HBM4EU Project, deoxynivalenol (DON), an immunotoxic, reprotoxic and a probable endocrine disruptor, and fumonisin B1 (FB1), a possible teratogen and carcinogen, were studied. The general aim was to estimate the European population exposure to these mycotoxins using human biomonitoring (HBM) and contribute to their risk assessment (RA). In studies aligned in several European regions, the internal exposure was evaluated through the determination of total DON (tDON, including free DON and its glucoronides) in urine (n = 1270) by quality assured laboratories. Concerning tDON risk characterization based on the generated HBM data, two approaches were followed: i) reverse dosimetry and comparison of the estimated external exposure with group TDI, and ii) direct comparison with the newly derived HBM guidance value. Data showed that, although exposure levels varied between the participating countries, adults are currently exposed to DON and a fraction of this population, particularly in Poland and France, is exposed to levels that might be harmful to their health. Due to the limited HBM data available, no risk assessment was performed for FB1. Noteworthy, the mechanistic data available was organized into an adverse outcome pathway for the first-time linking exposure during pregnancy to neural tube defects in the fetus. Under the new Partnership for the Assessment of Risks from Chemicals (PARC), mycotoxins will continue to be in focus. Although no new HBM data will be obtained during the first years, data gaps on the toxicity of Alternaria toxins and enniatins will be closed, to allow their regulation and prevent human exposure.

Keywords: Human biomonitoring; next generation risk assessment; mycotoxins; climate change

Acknowledgements: The HBM4EU Project (GA 733032) and the "mycotoxins' study group" are acknowledged.

POSTER COMMUNICATIONS

Mycotoxins beauvericin and enniatin B interfere in green algae growth

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Abstract

Aquaculture has been a growing sector of food production worldwide in the last decades. However, little is known about food safety regarding to potential toxigenic fungi in algal cultures and lesser about the environmental association between mycotoxins and aquatic organisms. Cereals and other plants are often used to complete or enrich fish meal in aquaculture diets a common practice used for intensive or semi-intensive aquaculture purposes, on which these products could be contaminated by mycotoxins and produce toxicity effects. Here, we studied phytotoxicity by the exposure mycotoxins, beauvericin (BEA) and enniatin B (ENN B) in four green algae phytoplankton strains, including *Acutodesmus sp., Chlamydomonas reinhardtii, Haematococcus pluvialis*, and *Monoraphidium griffithii*. Results revealed that *Acutodesmus sp.* and *C. reinhardtii* maintained or slightly tended to flow up and down growth rate without reaching values below 50% or 60%, respectively; thus, no IC_{50} values were reached. On the other hand, for *H. pluvialis* and *M. griffith,* IC_{50} values were reached for all times in individual treatment, while in mixtures it varied. It was observed that individual exposure of algae to BEA and ENN B produced high potential phytotoxicity in *Accutodesmus spp* and *Haematococcus pluvialis* reaching IC_{50} values; while in combination, only at very specific concentrations of BEA and ENN B phytotoxicity was reported.

Keywords: algae, enniatin B, beauvericin, aquaculture

Acknowledgment: This work has been funded by Spanish Ministry of Science and Innovation PID2020-115871RB-I00; FORTHEM Alliance Project (REF: 612489-EPP-1-2019-1-DEEPPKA2-EUR-UNIV).

Occurrence of AFM1 in raw cow milk from two Tunisian climatic regions

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Abstract

The contamination of food with toxic molecules has become one of the major problems nowadays. Among the food contaminants, mycotoxins cause a decrease in the food's quality and nutritional values, as well as serious health effects on animals and humans. Mycotoxins are a large group of secondary metabolites produced by filamentous fungi, especially *Aspergillus, Fusarium, Penicillium, Claviceps*, and *Alternaria*. Milk is one of the most complete nature food holding an important part of a good and healthy diet. Cow's milk is the most frequently consumed by humans and was previously considered to be the ideal follow-on milk after weaning for babies. Aflatoxin M1 (AFM1), which is the monohydroxylated metabolite of aflatoxin B1 (AFB1), was the most prevalent compound reported on cow milk. In fact, part of AFB1 ingested by cows through contaminated feed is liver biotransformed and excreted later as AFM1through milk. AFM1 has been classified as carcinogenic, cytotoxic, teratogenic, and a genotoxic toxin. In the present work the presence of AFM1 in Tunisian raw cow milk sampled in different geographical area corresponding to two Tunisian climatic regions was evaluated. For this purpose, the extraction method applied consisted in a first step of defatting with hexane, then the extraction was carried out with H2O and acetonitrile and a mixture of MgSO4, NaCl, DSCPH and TSCPH salts. The determination was carried out by Liquid Chromatography (HPLC) coupled to Fluorescence Detection (FD). The analytical parameters revealed good recoveries with intra day and inter day precision of 97 and 103%, respectively. The detection and quantification limits were 0.3 and 1 µg/L, respectively. Then, the methodology was applied to eight cow milk samples. The results revealed that at first sight that the evaluated samples resulted contaminated by mycotoxins levels ranging between 4,4 and 54,9 µg/L while the climatic zone correlation and the corresponding mycotoxin contamination is on current study.

Keywords: Aflatoxin M; Milk raw samples, HPLC-FD

Acknowledgments: This study was supported by the Spanish Ministry of Science and Innovation project (PID2020-115871RB-I00) and by the Generalitat Valenciana project (AICO/2021/037). Noelia Pallarés wishes to thank the post-PhD program of University of Valencia for the requalification of the Spanish University System from the Ministry of Universities of the Government of Spain, modality “Margarita Salas” (MS21-045)”, financed by the European Union, Next Generation EU.

Evaluation of pulsed electric field-assisted extraction on the recovery of antioxidant and phenolic compounds from *Ziziphus lotus*

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Abstract
Ziziphus lotus is a deciduous plant abundant in Tunisia that is commonly used for its medicinal properties as an infusion due to its high content of compounds with antioxidant capacity and phenolic compounds. However, not all parts of this plant have the same properties, being the leaves used for the infusion. For this reason, different parts of *Ziziphus lotus* (flowers, leaves, stem, root and fruits) were chosen to study the recovery of compounds with antioxidant and phenolic capacity using pulsed electric field-assisted extraction (PEF), a sustainable technology with considerable advantages over conventional methods using water as a solvent. 4g of each part were taken and treated by PEF (3 kV/cm, 100 kJ/kg, 1h in agitation), the results obtained were compared with conventional aqueous and hydroalcoholic extraction (80:20 v/v ethanol:water) with 1h in agitation using spectrophotometric methods. After analysis, statistically significant differences were observed for the different parts of the plant and extraction methodology, showing that flowers and leaves have the highest antioxidant capacity and phenolic compound content. Furthermore, the results obtained suggest that the PEF-assisted extraction is effective for the recovery of these compounds compared to the conventional methodology, being an interesting option for the achievement of the Sustainable Development Goals related to health and climate change, as well as for its application in matrices with high-added value compounds such as *Ziziphus lotus*.

Keywords: Pulsed electric field; ziziphus lotus; extraction; phenolic compounds; antioxidant

Acknowledgements: Este trabajo ha sido financiado por el proyecto del Ministerio de Ciencia e Innovación de España (PID2020-115871RB-I00) y por el proyecto AICO/2021/037 de la Generalitat Valenciana.

Citrinin and dihydrocitrinone in human urine samples: a systematic review

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Abstract
Citrinin (CIT) is a mycotoxin produced by several fungi species belonging to *Penicillium*, *Aspergillus* or *Monascus* genera. This toxin is widely distributed in stored grain and other plant-derived products and therefore breaking into the food chain, so human can be exposed to citrinin throughout the diet. For assessing the exposure to this nephrotoxic mycotoxin, the human biomonitoring (HBM) approach represent the most accurate alternative, quantifying the biomarker consisting in CIT plus its main metabolite dihydrocitrinone (DH-CIT) in urine which can be correlated to a reference value to characterize the risk. Therefore, this study aimed to review the available literature regarding the determination of CIT and/or DH-CIT in human urine for having a comprehensive overview of the analytical methodologies, sample treatment techniques and overall quantitative and qualitative results. A systematic review was performed until the end of October 2022 using the combination of keywords “citrinin” and “urine” either in title or abstract. Besides, the bibliography section of the retrieved articles was analyzed to find other useful articles. A total of 19 studies were considered, published from 2012 to 2022, that quantified CIT and/or DH-CIT in human urine samples from African, Asian, or European cohorts. Although many sample treatments were successfully applied, including immunoaffinity columns and “dilute and shoot” approaches among others, the gold standard analytical determination was liquid chromatography coupled to tandem mass spectrometry. Quantitative and qualitative data are comparable only for those studies with big sample sizes (n > 100), incidences above 50% of samples and at levels around or below 1 ng/mg creatinine for both toxins. Nevertheless, many studies provided results without normalization with creatinine, leading to non-reproducible results, and did not apply the biomarker excretion rate of 40% for further exposure assessment, which should be mandatory in future studies for a better understanding of the global impact of CIT.

Keywords: citrinin, human biomonitoring, biomarkers, exposure

Acknowledgements: This work was supported by Spanish Ministry of Science and Innovation Project (PID2020-115871RB-I00).

Protective capacity of digested coffee extracts against food contaminants such as acrylamide in SH-SY5Y

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Abstract

Acrylamide (AA), present in some heat-treated foods, is an important toxic compound for humans. The coffee beverage is one of the most consumed and important sources of AA, since the raw bean contains the reaction substrates and is processed at very high temperatures during roasting. On the other hand, coffee naturally contains a wide range of bioactive compounds with health benefits, including polyphenols, caffeic acid, lactones, and niacin, among others. Polyphenols are molecules that have one or several phenolic rings in their structure and are characterized by having antioxidant capacity. This work is focused on studying the protective capacity of digested coffee extracts against food contaminants such as AA in human neuroblastoma cells SH-SY5Y and, quantifying the total antioxidant capacity (CAT) through the ABTS-PERSULPHATE method and the content of total soluble phenols by the Folin-Ciocalteau method. Coffee-digested extracts were used to determine CAT as well as the content of total soluble phenols. SH-SY5Y cells were exposed to different AA concentration ranges, from 0 to 5 mM and digested coffee extract at 1:5 dilution and their mixture in 1:2 dilutions over 24, 48 and 72 h by the MTT (3-4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) assay was carried out. Results showed that AA cytotoxicity may be reduced with the coffee extract due to the fact that digested coffee extracts are high in antioxidants and phenols. The harmful effects of acrylamide present in coffee could be mitigated by the same antioxidants present in coffee.

Keywords: coffee, antioxidants, polyphenols, acrylamide, SH-SY5Y.

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In silico modelling for predicting toxicological endpoints of mycotoxins

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Abstract

Mycotoxins are toxic secondary metabolites produced by fungi. They are generated in crops and can seriously affect human health through the food chain.[1] Up to date, many hundreds of different mycotoxins have been described; however, a regulatory legislation only exists for a few of them, due to the lack of toxicity data. In this context, the aim of our work is the generation of toxicity data of a wide range of mycotoxins by *in silico* modeling, to provide useful data for their regulation.[2] For the generation of the models, extensive data from different databases and literature sources of mycotoxins and other natural products from the same chemical space have been collected. Different QSAR models have then been generated, testing several descriptor selections and modeling algorithms, and the best models have been selected after the evaluation of their accuracy, sensitivity and specificity, as well as their application to a set of compounds reserved for validation purposes. Robust models have been developed for the prediction of mutagenicity, cytotoxicity, genotoxicity and oral acute toxicity of mycotoxins, with values of accuracy, specificity and sensitivity of over 80% in most of cases. Our results provide a useful, simple and economic tool to predict toxicity data of mycotoxins. These models will be implemented in a technological platform intended to help industry and authorities in optimization and regulation purposes, respectively.

Keywords: QSAR; mutagenicity; mycotoxins; risk assessment

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Development of reproducible QSAR models for the prediction of mutagenicity of mycotoxins

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Abstract

Mycotoxins are common contaminants in a wide variety of food matrices, thus representing a threat to Public Health. About 600 mycotoxins have been described in literature, but only a few of them are regulated, because of the lack of data regarding their toxicity and mechanisms of action. Thus, it is necessary to evaluate the toxicity of some mycotoxins commonly found in foodstuffs but not yet regulated[1]. In this sense, *in silico* toxicology (IST) approaches can be used to rapidly assess chemical hazard. For instance, QSAR models have proven to be optimal computational methods for the prediction of toxicological endpoints[2]. The main objective of the present study was the development of scientifically valid QSAR models for predicting the mutagenicity of mycotoxins. Those models can be used later by the industry and the regulatory organisms to provide reliable safety levels for consumers, while reducing the use of *in vivo* assays. To generate input data, a survey of scientific literature on mutagenicity of mycotoxins has been done. A data base for bacterial mutagenicity has been generated with the Bacterial Reverse Mutation Test, that relies primarily on *Salmonella typhimurium* tester strains. Data have been extracted from different sources (Pubchem, EPA, ECVAM, EFSA OpenFoodTox), considering both the amount and quality of the data (homogeneity, reliability). Several chemical descriptors have been calculated for all compounds, and different QSAR strategies were followed to compare and select the best model. Two final models were built, and the best model was applied to an external validation set. The model was able to correctly classify more than 80% of the test compounds. Thus, we can conclude that the selected model is a valuable tool for screening mutagenicity of non-regulated mycotoxins, prioritizing mycotoxins for regulatory purposes.

Keywords: QSAR; mutagenicity; mycotoxins; risk assessment

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Genetic transformation in *Leishmania*: a tool for the study of *in vitro* and *in vivo* infections

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Abstract

Advances in genetic engineering of eukaryotic and prokaryotic organisms have led to great progress in the field of research and, more specifically, in the study of infections both in vitro and in vivo. The aim of this work is to genetically modify different species of the protozoan parasite Leishmania spp. responsible for fatal infections in humans in order to express proteins of interest, either fluorescent proteins (mCherry), proteins that serve as a model for immunological studies (ovalbumin), and/or proteins that allow monitoring infections in vivo (luciferase) by using the IVIS Optical Imaging System. Thus, we obtained a library of transformed Leishmania strains, including Leishmania infantum BCN150, L. donovani LV9 and L. major friedlin. To verify that the strains had correctly integrated the gene of our protein of interest in their genome, confocal microscopy images were acquired and Western blotting was performed. In the case of parasites with mCherry and luciferase, the protein had a cytoplasmic distribution. In parasites with ovalbumin, the presence of ovalbumin was observed on the cell surface. This is due to ovalbumin gene was cloned together with a leader peptide (HASPB), which allows it to anchor to the outer face of the plasma membrane. Thus, ovalbumin is much more exposed to the cells of the immune system, making it a good model for antigenic presentation studies.

Keywords: Leishmania; ovalbumin; luciferase; microscopy.

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Intestinal and hepatic 3D cell cultures for preclinical testing of drug efficacy and toxicity

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Abstract

The use of 3D cell cultures is spreading in all areas of biomedical research, since their characteristics allow to obtain results that resemble those that can be obtained in "in vivo" conditions, thereby reducing the use of experimental animals. Our group has started to develop a system for the study of chemical-biological interactions using three-dimensional models of established cell lines (CaCo2 and HepG2 cell spheroids from human intestine and liver), as well as organoids from BALB/c mouse intestinal explants (mouse miniguts), which preserve the cellularity of the organ from which they originate. We are currently establishing the ideal size and time for the development of spheroids, for their standardization in compound screening. In the case of mouse organoids, we have already established the optimal extraction and maintenance process and we are standardizing their use for compound screening and their possible use as an in vitro pharmacokinetic model.

Keywords: organoids; spheroids; 3D Cell culture; HTS; Drug discovery

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Tyrosol alleviates toxic effects induced by T-2 toxin in Caco-2 cells

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Abstract

Cereals stand out as a source of energy and fiber whose intake has been linked to multiple benefits at the intestinal, metabolic and diseases prevention level. Despite that, cereals are susceptible to natural contamination by fungi able to produce mycotoxins under certain conditions. On the other hand, extra virgin olive oil (EVOO) is the best fat reference due to its demonstrated cardioprotective properties and is the main fatty component of the Mediterranean diet. In this work, the toxicity alleviation produced by tyrosol (a polyphenol abundant in EVOO) in Caco-2 cells exposed to T-2 toxin was investigated. The cell viability was evaluated: (i) after pretreating the Caco-2 cells for 24 hours with tyrosol (25, 50 and 100 µM) and subsequent addition of T-2 toxin at 15 nM (lowest concentration that produced a significant decreased in cell viability) and (ii) after simultaneous exposure of tyrosol and T-2 toxin at same concentrations. The results showed that the pretreatment of Caco-2 cells with tyrosol for 24 h did not serve to attenuate the cytotoxic effects caused by exposure of T-2 toxin at any tested concentration. In the simultaneous treatment, 25 µM of tyrosol prevented the toxic effects produced in Caco-2 cells by the exposure of 15 nM of T-2 toxin. However, cytotoxic effects were observed in the rest of the combinations (50 µM tyrosol + 15 nM T-2 and 100 µM tyrosol + 15 nM T-2). These results suggest that tyrosol at low concentrations (25 µM) could exert a cytoprotective effect against 15 nM of T-2; but other studies are needed to corroborate this hypothesis.

Keywords: tyrosol; T-2 toxin; viability; Caco-2.

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Investigating the cytotoxic effects of patulin and citrinin on human neuroblastoma cells

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Abstract

Mycotoxins are toxic secondary metabolites produced by fungi. Research in this field is of crucial importance for the food industry as the toxins, which emerge in food and food storage, are capable of harming mammals even in little doses. Patulin (PAT) and Citrinin (CTN) are mycotoxins predominantly produced by fungi species *Penicillium* and *Aspergillus*. PAT mainly affects apples, while CTN is generally found in grains**.** This study aims to investigate the individual and combined cytotoxicity of PAT and CTN *in vitro* on the human neuroblastoma cell line SH-SY5Y. Cells were subjected to a variety of concentrations, ranging from 0 to 100 µM, in 1:2 dilutions. After exposure for 24 and 48 h, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed. This assay measures cell viability by reducing MTT, a soluble yellow tetrazolium salt, to an insoluble purple formazan through a mitochondrial-dependent reaction. For PAT, the highest concentration tested reduced cell viability to 38 % and 16 % after 24 h and 48 h. IC_{50} was detected to be 1.35 µM and 0.77 µM after 24 h and 48 h, respectively. With a maximum concentration of 3 µM, no value was obtained after 24 h; however, after 48h, an IC_{50} of 2.85 µM was observed. For CTN, the highest concentration tested reduced cell viability to 45 % for both times studied. IC_{50} was determined to be 62.5 µM and 51 µM at 24 h and 48 h. The combination also presented cytotoxicity. Both PAT and CTN show cytotoxic effects on SH-SY5Y cells. Although PAT appears to be more toxic than CTN, CTN still exhibits an adverse effect at low doses. Further research will be carried out to achieve a better understanding of these effects.

Keywords: Mycotoxin; Patulin; Citrinin; SH-SY5Y

Acknowledgments: This work has been supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-100 and the FORTHEM Alliance Project (REF: 612489-EPP-1-2019-1-DEEPPKA2-EUR-UNIV).

Cytotoxicity of chlorpyrifos in cell culture: a review

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Abstract

Chlorpyrifos (CPF) is a broad spectrum and efficient organophosphorus pesticide; it is one of the most widely used in the European Union. The CPF has multiple applications: insecticide, acaricide, thermiticide, nematocide, etc. The WHO has classified it as a moderately dangerous pesticide (class II). Its mechanism of action is based on the irreversible inhibition of the acetylcholinesterase (AchE). Chlorpyrifos causes respiratory, reproductive, digestive, cardiovascular, and immunological disorders. Because of its lipophilicity, CPF is able to cross the blood-brain barrier, causing neurological damage. Several studies find that it is a cause of neurobehavioral abnormalities, cognitive deterioration, and motor dysfunction. Traces of the CPF have been detected in several environmental (soil, groundwater) and food (water, fruit, vegetables, cereals) matrices, suggesting that the world population is chronically exposed to it. The aim of this study is to summarize the cytotoxic effects of CPF by *in vitro* methods. Results obtained demonstrated cytotoxic activity of CPF in different cell lines, with different exposure times and endpoints. The CPF concentration able to reach 50% inhibition of cellular proliferation (IC_{50}) was evaluated, and a wide range of values were obtained. The IC_{50} values ranged from 0.00142 µM in LMH human hepatocellular carcinoma cells to 2000 µM in HaCaT human keratinocyte cells at 24 h of exposure. At 48 h of CPF exposure, the IC_{50} values ranged from 1 to 1000 µM in JEG-3 human choriocarcinoma cell line. At 72 h of CPF exposure the IC_{50} range was from 17.5 µM to 30 µM in SHSY5Y neuroblastoma cells. Differences between IC_{50} values observed could be caused by the different cells used, supplements in the medium and assay conditions. The next objective is to develop further tryals to confirm the mechanisms of action of chlorpyrifos in producing these toxic effects at the cellular level.

Keywords: chlorpyrifos, cytotoxicity, MTT assay, IC_{50}

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Antioxidant activity of fish by-product hydrolysates

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Abstract

In recent years, significant growth in fish production worldwide has occurred, and as a consequence, the volume of organic waste produced by the fish processing industry has vastly increased. By-products are currently wasted, underutilized, or used to produce low-value-added products; however, they are an important source of proteins, which can be employed to produce biologically active peptides with nutritional and functional interest. The characteristics and quality of these fish by-product hydrolysates may be influenced by several factors. In this context, the aim of the present study was to evaluate the antioxidant properties of different fish by-product hydrolysates. For this purpose, Atlantic mackerel and sardine by-products were enzymatically hydrolysed using papain, pepsin, and Protamex® for 6 h at the optimal conditions of each enzyme. The degree of hydrolysis (DH) of the samples was determined by measuring the content of free amino groups with the TNBS method. The antioxidant properties of the hydrolysates were analysed using the DPPH radical scavenging activity assay, the ABTS radical scavenging capacity method, and the FRAP assay. The highest DH was observed for sardine by-products which varied between 47.15% and 93.64%. Moreover, Papain and Protamex resulted in higher DH values than pepsin, for both, mackerel and sardine by-products. The results showed a correlation between DH and the antioxidant activity of the hydrolysates. Sardine was the most valuable raw material for producing antioxidant peptides, and Papain and Protamex were more effective than pepsin for the production of antioxidant protein hydrolysates. These results highlight the importance of the raw material and the enzymes used during the hydrolysis process to obtain fish hydrolysates with high antioxidant activity. The use of fish by-products to obtain compounds of nutritional and functional interest is a promising strategy for promoting the sustainability and economic activity of the marine environment.

Keywords: peptides; bioactivity; enzymatic hydrolysis; fish waste

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Extracellular vescicles modulate chemotherapy response in a 3D Neuroblastoma spheroids model

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Abstract
Extracellular vesicles (EVs) are key mediators of cell-tumor microenvironment communication, promoting progression, metastasis, and stemness.[1,2] In Neuroblastoma (NB), a metastasis-prone embryonic malignancy of pre-scholar age, the prognosis for high risk (HR) patients is still grim.[3] This study investigates the role of EVs in driving NB resistance and dissemination. Considering the role of low oxygen tension (hypoxia) in cancer spread, we used a 3D spheroid model, naturally developing oxygen gradients and better reflecting *in vivo* tumor behavior. We formed spheroids using SK-N-AS (non-N-MYC-amplified) and IMR-32 (N-MYC-amplified) NB cell lines and cultured them up to a diameter of 400-500 µm. Immunofluorescence analysis confirmed the establishment of hypoxia in the inner portion of the spheroids, which were then treated with Doxorubicin (Doxo), a commonly used drug for first line treatment of NB. We assessed Doxo IC50 using MTT and ATP viability assays over the course of 48 hours. Hypothesizing that different oxygenation levels established in the spheroids might induce specific contributions to tumor behavior, we used migration on gelatin to isolate two cell populations: one derived from the inner mass (core) and the other from the external portion (periphery). We characterized the core and periphery using Western Blotting (WB) and real-time PCR evaluating expression of drug resistance pumps, stemness and hypoxia-related genes and proteins. We also performed cell cycle analyses and evaluated apoptosis following treatments. We then isolated EVs from the core and periphery, culturing cells in normoxic and in hypoxic conditions. EVs were characterized by WB, electron microscopy and Nanosight. We then proved that EVs increase Doxo resistance in NB cells. This study provides an optimized 3D NB model characterization, offering novel insights into EVs role as potential mediators of NB spread. Our findings could help developing alternative strategies for an early treatment of NB metastatic disease.

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Keywords: Drug resistance; Extracellular Vesicles; Neuroblastoma; 3D spheroids

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Parasympathetic Synapse Loss in Amyotrophic Lateral Sclerosis (ALS)

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Abstract
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive death of motor neurons (MNs) resulting in muscle atrophy, paralysis, respiratory failure, and death. Patients also show several physiopathological symptoms related to the autonomic nervous system (ANS): decrease intestinal motility and urination, changes in heart rate and blood pressure, reduction in lacrimal and salivary secretion, etc., all clinical features suggesting ANS involvement. On the other side, systemic treatment with GDNF (glial cell line derived neurotrophic factor) has proven to confer protection to spinal cord MNs against ALS. Thus, and giving the similarities between somatic and ANS neuronal circuitry, we decided to study: first, whether autonomic synaptic connections are affected by ALS; and second, if they are damaged, expose them to GDNF to try to rescue ANS synaptic connections. We simplify our study by only studying the parasympathetic nervous system (PNS); in particular, the one that controls salivary glands. In ALS mouse model (The human $SOD1^{G93A}$ transgenic mouse) at symptomatic stages (120 days), the expression of synaptic marker (Synapsin) was characterized by immunofluorescence. PNS synaptic boutons, synaptic coverage, cellular volume, number of cells, were quantified. We observed a significant reduction in the number and coverage of synapses per neuron in ALS PNS samples with respect to wild type mouse (WT), with no obvious cellular loss. Because one of our goals was to protect ALS afflicted ANS synapses with GDNF, we first characterized the expression of GDNF receptors (Gfra2) in our model. Indeed, RT-qPCR and WB assays confirmed the presence of receptor. Although we have not yet applied GDNF to ALS PNS samples, we do expect to protect these synapses from the disease. We will extend our findings to other ANS organs that posit severe complication in ALS such as the heart. In sum, ANS circuitry is severely affected by ALS.

Keywords: ALS; Synapse; Autonomic Nervous System (ANS); Parasympathetic Nervous System (PNS).

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Effect of beauvericin and enniatin B in gene expression of *Daphnia magna*

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Abstract

Daphnia magna is one of the most sensitive invertebrates to detect neuroactive compounds and represents an important trophic level in an aquatic food chain. *D. magna* provides an additional advantage in predicting cytotoxicity comparable with mammalian spp. and it also contributes in reporting the consequences in the environment of contaminants. Beauvericin (BEA) and enniatin B (ENN B) are mycotoxins often produced by *Fusarium spp*. and simultaneously detected in several feed. In this study, the *D. magna* invertebrate model was chosen to explore the potential toxicity of BEA and ENN B and evaluate the transcript levels of genes involved in xenobiotic metabolism (*mox, gst, abcc4, abcb1,* and *abcc5*), reproduction, and oxidative stress (*vtg-SOD*) by qPCR. A total of 120 daphnids were used to perform the assay by exposing them for 24 h individually to BEA at 8 µM and 2 µM, or ENN B at 1.6 µM and 0.8 µM. Two mixtures of BEA + ENN B, designed as "high mixture" for [8 + 1.6] µM and "low mixture" for [2 + 0.8] µM were also tested, for 24 h. Results demonstrated that BEA upregulated genes involved in phase I (*mox*), phase II (*gst*), and phase 0/III (*abcb1, abcc5*) of xenobiotic metabolism, whereas ENN B only either had no effect (*gst, abcc5*) or downregulated them (*abcb1, mox*). So that, exposure to environmental concentrations of mycotoxins ENN B and BEA can affect expression of genes involved in multiple systems as well as the additional value of the toxicological foot-print of mycotoxins in the environment.

Keywords: beauvericin; enniatin; Daphnia magna; gene expression

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Copresence of zearalenone and its metabolites in pig liver samples

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Abstract

Zearalenone (ZON) is produced by *Fusarium* fungi and occurs in cereal grains. Phase I metabolism of ZON results in the production of α-zearalenol (α-ZON), β-zearalenol (β-ZON), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL), zearalanone (ZAN). ZEN and its metabolites have oestrogenic activity. Different studies have shown large interspecies differences in ZON metabolism, which have been correlated with the species-sensitivity. Based on the exposure estimates, the risk of adverse health effect of feed containing ZON and its modified forms is very low. Pigs are the most sensitive species to ZON. Literature that addressed the ZON metabolism, conclude that α-ZON prevails in pigs. Compliance with Commission Recommendation (2006/576/EC) guidance values ensures that no adverse effects on animal health occur and no significant transfer of mycotoxins into animal foodstuffs occurs. Thus, the residue levels detected in studies analyzing animal foodstuff do not necessarily reflect levels in food on the market but give an indication of transfer and of the possible contribution of mycotoxin residues in animal products to the overall dietary exposure. Due to the high entero-hepatic recirculation of zearalenone and its metabolites, the liver is at special risk for possible contamination. In this study, 30 swine and pig livers from Valencian Community supermarkets were analyzed for ZON and its metabolites. Results showed that 54% of the samples were positive for ZON or one of its metabolites. ZAN showed the highest incidence (33%) and concentration of 1.17±0.26 ng/g for positive samples. The co-occurrence of ZON and its metabolites was observed (7%), as well as the prevalence of α-ZON (7%) over β-ZON. Accordingly, Hazard Analysis and Critical Control Point (HACCP) system should be implemented throughout the food chain to reduce the presence and production of mycotoxins in feed. Furthermore, control systems to analyze and monitoring mycotoxins need to be reinforced to protect animal and human health.

Keywords: zearalenone; feed; pig; liver

Evaluation of citrinin toxicity on SH-SY5Y cells and its prevention using spirulina ethanolic extract

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Abstract

Citrinin (CIT) is a mycotoxin produced by fungi from the genera *Penicillium*, *Aspergillus* and *Monascus*. It is mainly found in cereals, but it can also be detected in fruits, spices and even some herbs. Although its toxicity has been studied deeply at renal and hepatic level, there are no studies of its effect on neuronal cells. On the other hand, microalgae have become popular in recent years as an alternative source of nutrients. Spirulina, the most widely consumed microalgae worldwide, is rich in proteins and bioactive compounds (polyphenols and pigments such as phycocyanin). Therefore, the aim of the present work is to study, for the first time, the toxicity of CIT in SH-SY5Y cells, a cell line derived from human neuroblastoma, and its possible prevention using an ethanolic extract of spirulina. The CIT cytotoxicity was evaluated using the MTT assay at 24 and 48h of exposure, and its impact on the cell cycle was studied by flow cytometry. Finally, both assays were also performed with the combination of CIT and spirulina extract. The obtained results show an IC_{50} of 77.1±10.1 µM at 24h and 74.7±9.6 µM at 48h. Regarding the cell cycle, a cycle arrest was observed in the G_2/M phase, which is more evident when CIT dose increases. Finally, a significantly improvement of cell viability was observed using CIT in the range of 25.00-77.50 µM in combination with ethanolic extract of spirulina at concentrations of 31.25 and 62.5 µg/mL. These results demonstrate that CIT produces cell cycle disruption and that its cytotoxicity can be reduced when combined with microalgae extracts. However, further studies are needed to evaluate this combination and to shed light on the mechanism of CIT toxicity.

Keywords: citrinin, spirulina, cytotoxicity, cell cycle

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Evaluation of effects of hydrolysed fish products in Caco2 cells

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Abstract

Fish protein hydrolysates are desirable food ingredients due to their beneficial effect on human health. Moreover, dietary antioxidants have properties such as anti-inflammatory, anti-carcinogenic and anti-mutagenic. Numerous studies shown that the consumption of diets rich in fruits and vegetables, have a cytoprotective effect, since many antioxidants may act as free radical scavengers resulting in an augmentation of the endogenous defense system. This approach of ingesting fish products supplemented with antioxidants may provide a strategy to enhance the beneficial effect of fish waste products. So, the aims were: (i) to determine the cytotoxic effects of hydrolysed fish protein and collagen; (ii) to determine the ability of the antioxidants Vit C, Vit E, quercetin (QUE) and resveratrol (RSV) to reduce the damage induced by hydrolysed fish protein and collagen in human colon adenocarcinoma cells (Caco-2) and, (iii) to evaluate the content and, the bioaccessibility and bioavailability of the minerals K, Ca, Mg, Fe, Zn, as well as metals, provided by hydrolysed fish protein and collagen. Results determined a decrease in cell viability of bioaccesible fraction of hydrolysed fish protein and collagen in a concentration-dependent manner, showing cytotoxic effects at 0.2 mg/mL and 0.5 mg/mL concentrations of bioaccesible fractions. After the co-exposure with vit C, vit E and RSV, the cell viability of the bioaccesible fraction of one hydrolysed salmon fish protein and collagen increased compared to the bioaccesible fraction without the co-exposure. And, regarding to QUE, only the co-exposure with one hydrolysed salmon fish protein increased the cell viability compared to the bioaccesible fraction alone. The most abundant element was P for product all hydrolysed fish protein (366-565 mg/L) and Ca for collagen (633 mg/L). A decrease in all minerals occurred after the bioavailability process. For all products before and after the simulated gastrointestinal digestion, the most abundant element was As.

Keywords: fish hydrolysates; Caco-2 cells; antioxidants; bioaccesibility

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FLEXiGUT: Towards a comprehensive understanding of the life course impact of dietary and environmental exposure on chronic low grade gut inflammation

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Abstract

The Flemish exposome project, FLEX*i*GUT,is the first large-scale exposomics study focused on chronic low-gradegutinflammation.Gut inflammation is hypothesized to be related to biological processes that cause health deterioration and increase the risk of chronic diseases and/or accelerated biological ageing. Exposure to environmental and dietary contaminants has been associated with gut microbiota dysbiosis. Interestingly, the Bacteroides2 enterotype has recently been linked to gut inflammation, revealing the existence of a gut microbiome-inflammatory axis. FLEX*i*GUTaims to characterize human life course environmental exposure to assess and validate its impact on gut inflammation and related biological processes and diseases. Two Flemish prospective cohorts are used to cover the human life course: the “ENVIRONAGE birth cohort”, a mother-child cohort which provides follow-up from gestation to the age of 10, and the “Flemish Gut Flora Project longitudinal cohort”, a cohort of adults. Available biological samples include blood, urine, saliva, faeces and pregnancy-related samples (placenta and cord blood). Targeted and untargeted analysis of legacy and emerging contaminants, markers of air pollution, mycotoxins and the metabolome, including objective markers of e.g. food intake, ensure a comprehensive assessment of the exposures.The associated biological responses are investigated by applying -omics techniques, including metagenomics, DNA adductomics and metabolomics, as well as assessment of telomere length and measurement of inflammatory markers.The biomonitoring of contaminants and the gathering of -omics data for thebiological samples collected at different time points (n=400 mother-child pairs and n=400 adults), complemented with the information available from questionnaires, lifestyle and clinical data, will allow capturing the exposome from prenatal life onwards. An integrative multi-omics data processing approach will be applied to uncover associations between the exposuresand diseases, but also provide insights into the mechanisms by which the exposure might be exerting its effects.

Keywords: Exposome; Gut inflammation; Environmental and food contaminants; Microbiome

Immunological effects of gliotoxin and ochratoxin A on neuroblastome undifferentiated SH-SY5Y cells

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Abstract

Gliotoxin (GLI) and Ochratoxin A (OTA) have shown to exert an effect over neuronal activity. Different mechanisms of action by which they exercise their cytotoxicity have been attributed to GLI and OTA including the triggering of the production of inflammatory mediators such as interleukin-6 (IL-6) and necrotic tumoral factor (TNF- α). For this purpose, and taking our previous results of cytotoxicity, a measurement of the production of IL-6 and TNF- α and cell cycle assessment in neuroblastoma undifferentiated SH-SY5Y cells was performed when treated with GLI, OTA and its combination. A) production of IL-6 and TNF- α was determined by sandwich enzyme-Linked immunosorbent assay (ELISA) by using human specific monoclonal antibodies for both inmunomarkers; and B) Cell cycle distribution and cell proliferation study was performed by using Vidnelov´s PI solution for flow cytometry analysis. When cells were treated with GLI and OTA a marked increase in the production of IL-6 was observed coinciding proportionally with the results obtained in the cell viability assay; conversely, no TNF- α production was observed showing that TNF- α is not involved in GLI neither OTA cytotoxicity. The same pattern was observed when the combination of GLI and OTA was tested. Regarding cell cycle, a decrease in the G0/G1 was observed in both GLI and OTA treatment being more pronounced when GLI was tested; thus, showing a cell cycle disruption.

Keywords: Gliotoxin; Ochratoxin A; SH-SY5Y; neurotoxicity.

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Acrylamide extraction by accelerated solvent extraction and solid phase extraction in food

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Abstract

Acrylamide (AA) is a process contaminant formed in the Maillard Reaction in starchy foods (such as bread, potato, or coffee) heated at temperatures above 120 °C. The International Agency for Research on Cancer (IARC) classifies AA as probably carcinogenic and genotoxic. In addition, it is also associated with damage to reproductive functions and the nervous system. This study aimed to evaluate the advantages and limitations of two techniques, Accelerated Solvent Extraction (ASE) and Solid Phase Extraction (SPE), as extractions techniques of AA from food matrices. ASE is used for polar compounds through organic solvents and aqueous phases using high temperatures and pressures. The parameters studied were the type and volume of solvent and different formulations of the extractant cell content. SPE is based on extraction by affinity of the analyte by the solid phase. An extraction method was optimized for a carbon column considering the volume of elution and flow direction at different AA concentrations. Methanol with sea sand and 0.5 g of ZnSO4 and MgO inside the cell were the conditions that achieved the highest recovery percentage (90%). For SPE, the optimum elution conditions were 3 mL of methanol and reverse flow direction. Both techniques are useful in the AA extraction from food, but further studies are needed to increase their efficiency at low concentrations.

Keywords: acrylamide; extraction; ASE; SPE

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The epigenetic interplay of mycotoxins & EpsteinBarr virus towards childhood cancer

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Abstract

Vulnerable populations in low-and middle-income countries (LMICs)are daily exposed to dietary carcinogens such as mycotoxins. Infection by Epstein Barr virus (EBV) is linked to a childhood cancer called Burkitt lymphoma (BL) which is endemic in parts of sub-Saharan Africa where chronic mycotoxins' exposure co-exists. It was recently demonstrated *in vitro*that aflatoxin B1 (AFB1) modifiesthe DNA methylome andpromotesEBV infection in B-cells. In addition, both AFB1 and EBV may alter DNA methylation levels and deregulate the expression of cancer-related genes. The aim of this study is to assess theepigeneticinteraction between mycotoxins and EBV (and other infections) in affected populations and validate the underlying mechanisms using *in vitro*and *in vivo*models. Accurate exposure assessments of mycotoxins and oncogenic viruses will be conducted in an established cohort of African infants and children, embedded in the MISAME-III cohort. Furthermore, the epigenetic toxicity following mycotoxin exposure and EBV infection will be integrated with the gene expression profile, in the cohort, as well as in cell lines and humanized mice. The outcome of this research will elucidate the mechanistic pathway(s) of environmentally-induced cancer. Understanding the mechanisms by which mycotoxins and viruses interact to deregulate the epigenome and induce tumors will provide insights to both the scientific community and governmental officials on how to overcome this public health challengewith focus on LMICs.

Keywords: Mycotoxins; Epstein Barr virus; Epigenetics; Burkitt lymphoma

Analysis of genotoxicity and histone modifications resulting from multi-mycotoxin exposure in human intestinal Caco-2 cells: a protocol

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Abstract
Mycotoxins are toxic fungal secondary metabolites that can induce various health effects in humans, including cancer, affecting specific organs such as the kidneys, liver and intestines. Previous cohort-based research has shown chronic low-dose intake of multiple mycotoxins (*i.e.* deoxynivalenol (DON), patulin (PAT), sterigmatocystin (STERIG)) to be associated with an increased risk of colorectal cancer (CRC). Indeed, the link between (multiple) mycotoxin exposure and cancer has mainly been established in empirical studies but needs to be investigated for causal relationships. Besides sequence alternations in the genome, changes in the epigenetic status can also contribute to the development and progression of cancer. To this extent, mycotoxin-induced damage at the genome and histone level will be analyzed in human intestinal Caco-2 cells, priorly exposed to DON, PAT, STERIG, as well as emerging *Alternaria* mycotoxins. Genotoxicity testing will be performed byγ-H2AX immunofluorescence to detect DNA-double strand breaks. Post-translational histone modifications (PTMs), which are covalent enzymatic modifications of proteins and important determinants of the epigenetic status (*e.g.* acetylation and methylation of lysine's and arginine's residues), will be analyzed by liquid chromatography–tandem mass spectrometry, thoroughly validated for purpose. As a pioneer in the field, this research aims to map mycotoxin-induced genotoxicity and PTM induction, to investigate causal links between multiple mycotoxins and CRC risk, as well as prognosticate CRC outcome.

Keywords: Mycotoxins; colorectal cancer; genotoxicity; histone modifications

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Climate change impact on growth and mycotoxin production of *Aspergillus* and *Penicillium* fungi

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Abstract

According to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, Earth's climate is undergoing adverse global changes as an unequivocal result of anthropogenic activity. In a future in which greenhouse gasses emissions continue to grow, scientists forecast longer lasting frost-free and growing seasons, increased heavy precipitation events, more intense heat waves, and reduced soil moisture. Since climate represents the key driving force of fungal colonization and mycotoxin production, climate changes (CC) are expected to have an impact on fungal biodiversity patterns, which, in turn, can also have implications in food availability, food security and, thus, in public health. Hence, it is crucial to evaluate ecological responses of fungi to CC. The present study summarizes the available evidence regarding the impact of CC on growth and mycotoxin production by the key mycotoxigenic fungi belonging to the genera *Aspergillus* and *Penicillium*. Evidence suggests that future changes in the interacting environmental factors, such as temperature, precipitation and atmospheric CO_2 concentration, are expected to negatively affect crops worldwide in terms of loss of suitable cultivation areas and increase in mycotoxin contamination. The ability of mycotoxigenic fungi to respond to CC might induce a shift in their geographical distribution and in the pattern of mycotoxin occurrence. In particular, it seems likely that warmer climates will favour thermotolerant species, leading to the prevalence of *Aspergillus* over *Penicillium* species. The present available knowledge is discussed in the context of predictive models that predominantly consider two-way interactions (temperature X water activity). Very little information is available on the effect of three-way interactions (temperature, water activity, and CO_2). Integrated and interdisciplinary approaches are required to transfer the current approaches to a global level and support strategies to reduce risk areas and improve public and animal health under predicted future scenarios.

Keywords: Climate changes; Aspergillus; Penicillium; mycotoxins.

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