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14th Scientific and Training Conference of the Polish Society of Toxicology

Poznań, Poland
September 4–6, 2024

ABSTRACT BOOK

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14th Scientific and Training Conference of the Polish Society of Toxicology

Poznań, Poland

September 4–6, 2024

ABSTRACT BOOK

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Ladies and Gentlemen,

We are pleased to present you a special issue of our journal – *Advances in Clinical and Experimental Medicine*, in which we present scientific materials in the form of abstracts submitted to the 14th Scientific and Training Conference of the Polish Toxicological Society titled "Prospects and challenges of modern toxicology", which took place on September 4–6, 2024, in Poznań, Poland. The conference was organized by two Polish Toxicological Society branches – the Poznań branch and the Wrocław branch, which joined forces to provide conference participants with experiences at the highest scientific level. The conference was held under the honorary patronage of the rectors of both universities – Professor Andrzej Tykarski, rector of the Poznan University of Medical Sciences, and Professor Piotr Ponikowski, rector of the Wroclaw Medical University.

The 2024 conference received numerous applications on various topics, covering theoretical and practical aspects of toxicology, which is an interdisciplinary field of knowledge that uses the experience of basic and clinical science. The current conference gathered leading scientists, researchers and practitioners in the field of toxicology who shared the results of their latest research, innovative research methods used and practical applications in various aspects of toxicology.

In this issue, you will find summaries of works presented in the form of posters during this year's Polish Toxicological Society Scientific and Training Conference. They reflect the wide range of topics discussed at the conference, in line with the idea of the interdisciplinary nature of modern toxicology. The sessions during the event included topics such as: experimental toxicology, molecular/genetic toxicology, in vitro toxicology, nanotoxicology, toxicological analysis, clinical toxicology, forensic toxicology, addiction toxicology, veterinary toxicology, industrial and environmental toxicology, food toxicology, cosmetics toxicology, zebrafish – animal model in toxicology, among many others.

We hope that the summaries included in this issue will be an interesting encounter with contemporary toxicology and a valuable source of knowledge and inspiration for further research and cooperation in the field of toxicology, in accordance with its interdisciplinary nature.

We would like to thank all authors and conference participants for their contribution and commitment to the development of this important field of science and for sharing their research, passions and achievements.

Prof. Ewa Florek
Prof. Agnieszka Piwowar

CONFERENCE PROGRAM

Prospects and challenges of modern toxicology

Poznań, September 4–6, 2024

September 4, 2024, Wednesday

Hours	Room
08:00–16:00	**REGISTRATION** **Hall**
10:00–12:00	**WORKSHOPS** **Congress Hall A**
10:00–12:00	**Application of in silico toxicology methods for toxicity testing on the example of poisonous warfare agents and N-nitrosamines in food** Maciej Noga, Adrian Frydrych, Kamil Jurowski
	Application of portable X-ray fluorescence spectroscopy (pXRF) in toxicological analysis in the context of forensic, regulatory and environmental toxicology Damian Kobylarz, Łukasz Niżnik, Kamil Jurowski, Alicja Krośniak
12:00–12:30	**COFFEE BREAK** **Hall**
12:30–12:45	**OPENING CEREMONY** **Congress Hall A** prof. dr hab. Ewa Florek, prof. dr hab. Agnieszka Piwowar
12:45–13:30	**PLENARY LECTURE** **Congress Hall A** **Role of inflammatory macrophages in lung injury and disease pathogenesis induced by chemical toxicants** Debra L. Laskin Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, USA
13:30–14:15	**LUNCH BREAK** **Hall**
14:15–16:00	**SESSION I** **Congress Hall A** prof. dr hab. Marcin Kruszewski, dr hab. Bartosz Wielgomas, prof. GUMed
14:15–14:30	**Graphene quantum dots: Nanotoxicity aspect** Tuba Oz, Sheetal Kaushik Bhardwaj, Ajeet Kaushik, Małgorzata Kujawska
14:30–14:45	**Biosafety and toxicity of polydopamine nanoparticles with the potential for cancer treatment** Marta Szukalska, Tomasz Zalewski, Marta Witkowska, Bartosz F. Grześkowiak, Magdalena Bigaj-Józefowska, Emilia Cicha, Patrycja Sujka-Kordowska, Izabela Miechowicz, Michał Nowicki, Radosław Mrówczyński, Ewa Florek
14:45–15:00	**In vitro toxicity evaluation of graphene oxide functionalized with selenium** Tuba Oz, Palaniappan Nagarajan, Ivan Cole, Małgorzata Kujawska
15:00–15:15	**Factors influencing the toxicity of micro- and nanoplastics** Bożena Bukowska, Kamil Płuciennik
15:15–15:30	**The toxicological analysis of heavy metals in low-cost jewelry from Chinese e-commerce platforms using portable XRF spectroscopy** Kamil Jurowski
15:30–15:45	**The influence of plastic nanoparticles on blood hematology parameters and colon lymphocyte profiles: In vivo model studies** Katarzyna Dziendzikowska, Wojciech Grodzicki, Malwina Czerwińska, Małgorzata Gajewska, Kinga Majchrzak-Kuligowska, Michał Oczkowski, Joanna Gromadzka-Ostrowska, Marcin Kruszewski
15:45–16:00	**Nanosilver modulates expression of inflammation related lncRNA in neural cells in culture** Kamil Brzóska, Marcin Kruszewski

September 4, 2024, Wednesday

Hours	Room
16:00–16.30	**COFFEE BREAK** **Hall**
10:00–16:30	**POSTER SESSION I** **Hall** dr hab. Kamil Jurowski prof. UR, prof. IEM, dr hab. Ewa Sawicka, dr hab. Mariola Śliwińska-Mossoń
10:00–16:30	**Tris(2,3-dibromopropyl) isocyanurate (TBC) – new brominated flame retardant initiates the autophagy process in mouse spermatogenic cells (GC-1 (spg)) in vitro** Dominika Szlachcikowska, Bartosz Skóra, Konrad Szychowski, Anna Tabęcka-Łonczyńska
	Evaluation of the synergistic anticancer effect of sorafenib and adipose tissue stem cell culture medium Aleksandra Gładyś, Aleksandra Skubis-Sikora,Patrycja Wieczorek, Bartosz Sikora, Adam Mazurski, Piotr Czekaj
	Cytotoxicity of extracellular vesicles loaded with glucose oxidase in lung cancer cells Magdalena Bamburowicz-Klimkowska, Monika Ruzżcka-Ayoush, Małgorzata Sikorska, Agnieszka Stawarska, Ireneusz P. Grudziński
	The effect of liposomal DMU-212 on differentiation of human ovarian granulosa cells in a primary 3D culture model Małgorzata Józkowiak, Paulina Skupin-Mrugalska, Mikołaj Czajkowski, Robert Z. Spaczyński, Hanna Piotrowska-Kempisty
	The role of baicalin in chemoprevention of ovarian cancer cells exposed to cadmium ions Agnieszka Piwowar, Ewa Sawicka
	Drug-induced QT prolongation: from molecular mechanisms to clinical management Anna Szoszkiewicz, Aleksander Jamsheer
	Endothelin-1 as potential target in the predictive medicine of pancreatitis Milena Ściskalska, Marta Kepinska
	Interactions of ethanol with levetiracetam or brivaracetam on GABA and glutamate levels in selected rat brain structures Joanna Stragierowicz, Michał Klimczak, Anna Kilanowicz, Bogusława Pietrzak, Ewa Zwierzyńska
	Effects of cannabidiol on toxicity veterinary drug using HepG2 cells Eryka Pankowska, Oliwia Kończak, Tatiana Wojciechowska, Maciej Gogulski, Lidia Radko
	Studies on the effect of different folic acid dosing alone and in the situation of exposure to ethanol in the zebrafish embryonic model Agnieszka Stawarska, Karolina Masiukiewicz, Anna Zamielska, Aleksandra Kulesza, Anna Małkowska
	Study of the interactions of paracetamol with xenobiotics in the zebrafish embryonic model Anna Małkowska, Julia Bonder, Karolina Wieczorek, Sylwia Giziewicz, Kacper Pawlak
	Pro-metastatic activity of silver nanoparticles related to epithelial-mesenchymal transition mechanism Magdalena Matysiak-Kucharek, Krzysztof Sawicki, Lucyna Kapka-Skrzypczak
	Susceptibility of CACO-2 and HT29MTX cells to silver nanoparticles in combination with polystyrene nanoparticles Sylwia Męczyńska-Wielgosz, Katarzyna Sikorska, Marcin Kruszewski
	The impact of silver and polystyrene nanoparticles on gut cellular model Katarzyna Sikorska, Sylwia Męczyńska-Wielgosz, Kamil Brzóska, Marcin Kruszewski
	The influence of fullerenes as doxorubicin nanocarriers on the triple-negative breast cancer cells proliferation Natalia Zaręba, Marta Kepinska
	Long-term exposure to multi-walled carbon nanotubes for potential cartilage tissue engineering applications Marta Szukalska, Marta Witkowska, Magdalena Richter, Tomasz Trzeciak, Izabela Miechowicz, Andrzej Marszałek, Wojciech Piekoszewski, Michał Giersig, Ewa Florek

September 4, 2024, Wednesday

Hours	Room
10:00–16:30	**Synthesis and cytotoxicity effects of some carboxychalcones** Dorota Olender, Milena Kasprzak, Bartosz Skóra, Katarzyna Sowa-Kasprzak, Anna Pawełczyk, Konrad A. Szychowski
	Synthesis of novel fluorescent probes based on azadipyrromethene and aza-BODIPY derivatives Aleksandra Pawska, Barbara Wicher, Michał Kryjewski
	Purines with alkyl disulfide substituents: Synthesis, physicochemical characterization and preliminary toxicity study Piotr Szyk, Maciej Kulawik, Jakub Kubiak, Dariusz T. Młynarczyk, Agnieszka Zgoła-Grześkowiak, Tomasz Gośliński
	Microneedle system with porphyrin-based photosensitizer of potential use in medicine and pharmacy Beata Czarczyńska-Goślińska, Piotr Szyk, Jakub Kubiak, Maciej Kulawik, Anna Froelich, Agata Roszak, Irena Budnik, Dariusz T. Młynarczyk, Tomasz Gośliński, Tomasz Osmałek
	Circulating microbiota-derived metabolites in female patients with relapsing-remitting multiple sclerosis Ewa Żurawska-Płaksej, Hanna Czapor-Irzabek, Vincent Wu, Justyna Chojdak-Łukasiewicz, Anna Pokryszko-Dragan, Sławomir Budrewicz, Agnieszka Piwowar, Nicola Zamboni

September 5, 2024, Thursday

08:00–15:00	**REGISTRATION** **Hall**
09:00–10:30	**SESSION II** dr hab. Małgorzata Kujawska prof. UMP, prof. dr hab. Ireneusz Grudziński
09:00–09:30	**Acute carbon dioxide inhalation leads to acute cognitive impairment and systemic vascular inflammation in humans** Howard Kipen, Frederic T. Lu, Disha Gupta, Nancy Fiedler, Usha Satish, Kathleen G. Black, Adriana De Resende, Leonardo D. Calderón, Changjiang Guo, Andrew Gow Rutgers University, Environmental and Occupational Health Sciences Institute (EOHSI), Piscataway, NJ, USA
09:30–09:45	**Circadian rhythm and xenobiotics** Edyta Reszka
09:45–10:00	**Electroporation as a method to improve the assessment of the toxic effects of xenobiotics in a zebrafish model** Anna Małkowska, Anna M. Nowicka, Łukasz Szymański, Weronika Skarpetowska, Agnieszka Szarek
10:00–10:15	**The effect of indoxyl sulfate-uremic toxin on the coagulation and fibrinolysis process in an animal model of thrombosis** Dariusz Pawlak, Małgorzata Karbowska, Tomasz Kamiński, Beata Znorko, Tomasz Domaniewski, Tomasz Misztal, Tomasz Rusak, Natalia Marcińczyk, Krystyna Pawlak
10:15–10:30	**Evaluation of chemicals for estrogen and (anti)androgen disorders** Mateusz Paleczny, Natalia Ślezińska, Daniel Krakowian, Justyna Faron, Dominika Gądarowska, Robert Sornat, Katarzyna Gruszka, Przemysław Żemła, Inga Mrzyk
10:30–11:00	**COFFEE BREAK** **Hall**
11:00–12:30	**SESSION III** **Congress Hall A** prof. dr hab. Edyta Reszka, prof. dr hab. Piotr Czekaj
11:00–11:15	**Toxicovigilance 2.0: Modern approaches for the hazard identification and risk assessment of poisons** Marta Sowińska, Łukasz Niżnik, Kamil Jurowski
11:15–11:30	**The use of liquid chromatography coupled with triple quadrupole mass spectrometry for identifying the causes of acute poisonings** Wiktoria Jiers, Karina Sommerfeld-Klatta, Magdalena Łukasik-Głębocka, Barbara Zielińska-Psuja, Artur Teżyk, Czesław Żaba

September 5, 2024, Wednesday

11:30–11:45	**Unveiling the power of antidotes: Exploring their versatile and overlooked applications** Joanna Toporowska-Kaźmierak, Kamil Jurowski
11:45–12:00	**A driver with 'auto-brewery syndrome'?** Paweł Papierz
12:00–12:15	**Retrospective clinical analysis of acute poisonings with antipsychotics and antidepressants among patients hospitalized at the Toxicology Department in Poznań** Maria Hareńska, Izabela Gałązka, Michał Bardan, Monika Lewandowska, Katarzyna Tylkowska, Michał Kozicki, Artur Teżyk, Magdalena Łukasik-Głębocka, Karina Sommerfeld-Klatta
12:15–12:30	**In search of optimal solutions in comparative analyzes of the toxicity of xenobiotics using publicly available toxicogenomic databases on the example of the Comparative Toxicogenomics Database** Michalina Złomańczuk, Maciej Gawlik
12:30–13:15	**LUNCH BREAK** **Hall**
13:15–14:45	**SESSION IV** **Congress Hall A** prof. dr hab. Anna Kilanowicz-Sapota, dr hab. Anna Bizoń
13:15–13:30	**The problem of using psychoactive substances by hard coal miners during work: A case report of fatal combined α-PiHP and tramadol poisoning** Rafał Skowronek, Paulina Wachholz, Beata Bujak-Giżycka, Rafał Celiński, Natalia Pawlas
13:30–13:45	**Development of direct, non-invasive and rapid methods for determining chosen toxic elements using pXRF (portable X-ray fluorescence spectrometer) in bones for forensic laboratory toxicology purposes** Damian Kobylarz, Kamil Jurowski
13:45–14:00	**Challenges in liquid mercury spill incidents: Current status and strategies using specialized kit for mercury absorption** Agnieszka Świdniak, Łukasz Niżnik, Kamil Jurowski
14:00–14:15	**Non-harmonized temperament as a factor in worse cognitive but not executive functioning in alcohol-related cerebral atrophy** Natalia Nowaczyk, Michał Mikołajczak, Lidia Cierpiałkowska
14:15–14:30	**I would rather die than withdraw: Addressing the opioid epidemic** Rochanne L. Honarvar, Sara M. Roberts, Jarosław R. Romaniuk
14:30–15:00	**COFFEE BREAK** **Hall**
9:00–15:00	**POSTER SESSION II** **Hall** dr hab. Małgorzata Kujawska prof. UMP, dr hab. Marta Kepinska prof. UMW, dr Marta Szukalska
9:00–15:00	**Preliminary evaluation of selected parameters reflecting carbohydrate disorders in people living with HIV in the aspect of antiretroviral therapy** Beata Szymańska, Brygida Knysz, Hubert Ciepłucha, Agnieszka Piwowar
	Liquid chromatography–tandem mass spectrometry method for the determination of antibaterial drugs in plasma samples Izabela Malczak, Anna Gajda
	The development of LC-MS/MS method for determination of six ionophores in liver tissue Agnieszka Chłodowska, Małgorzata Olejnik
	The devil is not as black as he is painted: Prediction of acute toxicity and environmental fate (hydrolysis and biodegradation) of the Novichoks – organophosphorus nerve agents – using in silico toxicology methods Maciej Noga, Kamil Jurowski
	Determination of ciprofloxacin in *Lucilia sericata* larvae using capillary electrophoresis combined with mass spectrometry Magdalena Czuma, Maria Walczak

September 5, 2024, Wednesday

9:00–15:00	**Immunohistochemical assessment of the expression of CYP1A1, CYP2A13 and CYP2E1 enzymes in lung cancer tissues of smoking patients** Mariola Śliwinska-Mossoń, Julianna Piłkowska, Piotr Błasiak, Adam Rzechonek
	The impact of exposure to tobacco smoke on selected metabolic and hormonal parameters in the serum of women with polycystic ovary syndrome Anna Bizoń, Justyna Niepsuj, Grzegorz Franik, Agnieszka Piwowar
	Virtual odyssey of AP-238 fate in the body (ADME): Application of selected in silico toxicology methods for rapid and reliable prediction of new psychoactive substance fate in the body on the example of AP-238 Alicja Krośniak, Kamil Jurowski
	Research strategy in the assessment and identification of endocrine-disrupting chemicals Przemysław Żemła, Dominika Gądarowska, Daniel Krakowian, Inga Mrzyk
	Analysis of the presence of pesticide substances in the Pilica catchment area and their potential toxicity Weronika Misztal, Aleksandra Chamerska, Katarzyna Zagibajło, Paweł Jarosiewicz
	Wastewater-based epidemiology study to assess illicit drug use in Greater Poland Voivodeship Agnieszka Klupczyńska-Gabryszak, Szymon Plewa, Eliza Matuszewska, Dagmara Pietkiewicz, Natalia Rzetecka, Jan Matysiak
	Are environmental phenols capable of migrating into the follicular fluid? Anna Klimowska, Joanna Jurewicz, Michał Radwan, Paweł Radwan, Bartosz Wielgomas
	Assessment of the relationship between bone mineral density, bone turnover markers and Cd concentration in blood and urine of women from the Łódź macroregion Rafał Kusak, Marzenna Nasiadek, Joanna Stragierowicz, Anna Kilanowicz
	Unlocking effective first aid strategies for human envenomation caused by marine animal species Aleksandra Adamczyk, Łukasz Niżnik, Kamil Jurowski
	The beneficial effect of *Aronia melanocarpa* L. berries extract against cadmium impact on the concentrations of bone morphogenetic proteins: A study in an in vivo model of current environmental human exposure to this heavy metal Małgorzata Michalina Brzóska, Joanna Rogalska, Alicja Roszczenko
	Activity of caspases and calpains in breast cancer cel lines after exposure to estrogens and metalloestrogens Ewa Sawicka, Marta Matyja, Patrycja Rzepka, Agnieszka Piwowar
	ADHD and Pycnogenol®: Common ground for new treatment possibilities Anna Sośnicka, Adriana Kubis-Kubiak
	Prediction of selected toxicity parameters for clephedrone Łukasz Niżnik, Kamil Jurowski
	The bromoderivatives of curcumin: Synthesis, physicochemical characteristics and toxicity studies Eduard Potapskyi., Julian Myszkiewicz, Dariusz Młynarczyk, Dawid Łażewski, Gabriela Korzańska, Roman Lesyk, Marcin Wierzchowski
	Quantitative analysis of chosen OH-polycyclic aromatic hydrocarbons (OH-PAHs) level in urine samples after consumption of grilled marshmallows Magdalena Szumska, Beta Janoszka, Maciej Maciejczyk, Aleksandra Damasiewicz-Bodzek, Krystyna Tyrpień-Golder
	Levels of selected toxic and essential elements and 5-hydroxymethylfurfural (HMF) in honeys available for retail sale Michał Klimczak, Mateusz Głowacki, Jakub Stasiński, Anna Kilanowicz, Adam Daragó
	Might modifying diet manage exposure to perfluoroalkyl substances? Dominika Sikora, Piotr Rzymski
	Use of bioindication method to assess toxicity of extracts of selected fruiting bodies of macrofungi Maja Paterska, Marcin Szymański, Judyta Cielecka-Piontek

September 6, 2024, Friday

08:00–11:00	**REGISTRATION** **Hall**
09:00–10:30	**SESSION V** **Congress Hall A** prof. dr hab. Hanna Piotrowska-Kempisty, dr hab. Marta Mendel, prof. SGGW
09:00–09:15	**Impact of beauvericin on porcine intestinal barrier integrity** Joanna Polak, Urszula Latek, Wojciech Karlik, Magdalena Chłopecka, Marta Mendel
09:15–09:30	**Histopathological and molecular background of salinomycin toxicity in turkeys** Ilksen Berfin Ekinci, Agnieszka Chłodowska, Anna Sławińska, Kacper Żukowski, Monika Olszewska-Tomczyk, Małgorzata Olejnik
09:30–09:45	**New methods to control of antibacterials use in pigs and poultry farms** Anna Gajda, Ewelina Nowacka-Kozak
09:45–10:00	**A preliminary investigation of the poultry body weight effect of essential oils in litter and residue in organ tissue of exposed chickens** Tomasz Śniegocki, Bartosz Sell
10:00–10:15	**Assessment of fipronil exposure in humans after ectoparasiticide application on household pets** Wojciech Rodzaj, Małgorzata Wacławik, Joanna Jurewicz, Bartosz Wielgomas
10:15–10:30	**Assessment of patterns of exposure to synthetic pyrethroids among pet owners: A longitudinal study using silicone wristbands** Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas
10:30–11:00	**COFFEE BREAK** **Hall**
11:00–12:15	**SESSION VI** **Congress Hall A** prof. dr hab. Małgorzata Brzóska, prof. dr hab. Agnieszka Piwowar
11:00–11.15	**The effect of black chokeberry (*Aronia melanocarpa* L.) extract on the concentration of interleukin 6 in the liver: A study in an in vivo model of human environmental exposure to cadmium** Magdalena Kozłowska, Małgorzata Michalina Brzóska, Joanna Rogalska
11:15–11:30	**Carcinogenicity of selected pharmacologically active dyes and health risk for consumers of contaminated fish** Kamila Mitrowska
11:30–11:45	**Identification of polyester oligomers and epoxy coatings as potential migrants from food contact materials** Monika Beszterda-Buszczak, Rafał Frański
11:45–12:00	**Toxicological analysis and safety assessment of selected elements in foods for special medical purposes for oncology patients** Adrian Frydrych, Kamil Jurowski
12:15–13:00	**AWARDS CEREMONY** **CONFERENCE CLOSING** prof. dr hab. Ewa Florek, prof. dr hab. Agnieszka Piwowar

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Contents

Day 1. Keynote lecture

Day 1. Session I

Day 1. Poster session 1

Day 2. Session 2

52 Mateusz Paleczny, Natalia Ślezińska, Daniel Krakowian, Justyna Faron, Dominika Gądarowska, Robert Sornat, Katarzyna Gruszka, Przemysław Żemła, Inga Mrzyk
Evaluation of chemicals for estrogen and (anti)androgen disorders

Day 2. Session 3

53 Marta Sowińska, Łukasz Niżnik, Kamil Jurowski
Toxicovigilance 2.0: Modern approaches for the hazard identification and risk assessment of poisons

54 Wiktoria Jiers, Karina Sommerfeld-Klatta, Magdalena Łukasik-Głębocka, Barbara Zielińska-Psuja, Artur Teżyk, Czesław Żaba
The use of liquid chromatography coupled with triple quadrupole mass spectrometry for identifying the causes of acute poisonings

55 Joanna Toporowska-Kaźmierak, Kamil Jurowski
Unveiling the power of antidotes: Exploring their versatile and overlooked applications

56 Paweł Papierz
A driver with 'auto-brewery syndrome'

57 Maria Hareńska, Izabela Gałązka, Michał Bardan, Monika Lewandowska, Katarzyna Tylkowska, Michał Kozicki, Artur Teżyk, Magdalena Łukasik-Głębocka, Karina Sommerfeld-Klatta
Retrospective clinical analysis of acute poisonings with antipsychotics and antidepressants among patients hospitalized at the Toxicology Department in Poznań

58 Michalina Złomańczuk, Maciej Gawlik
In search of optimal solutions in comparative analyses of the toxicity of xenobiotics using publicly available toxicogenomic databases on the example of the Comparative Toxicogenomics Database

Day 2. Session 4

59 Rafał Skowronek, Paulina Wachholz, Beata Bujak-Giżycka, Rafał Celiński, Natalia Pawlas
The problem of using psychoactive substances by hard coal miners during work: A case report of fatal combined α-PiHP and tramadol poisoning

60 Damian Kobylarz, Kamil Jurowski
Development of direct, non-invasive and rapid methods for determining chosen toxic elements using pXRF (portable X-ray fluorescence spectrometer) in bones for forensic laboratory toxicology purposes

61 Agnieszka Świdniak, Łukasz Niżnik, Kamil Jurowski
Challenges in liquid mercury spill incidents: Current status and strategies using specialized kit for mercury absorption

62 Natalia Nowaczyk, Michał Mikołajczak, Lidia Cierpiałkowska
Non-harmonized temperament as a factor in worse cognitive but not executive functioning in alcohol-related cerebral atrophy

63 Rochanne L. Honarvar, Sara M. Roberts, Jarosław R. Romaniuk
I would rather die than withdraw: Addressing the opioid epidemic

Day 2. Poster session 2

64 Beata Szymańska, Brygida Knysz, Hubert Ciepłucha, Agnieszka Piwowar
Preliminary evaluation of selected parameters reflecting carbohydrate disorders in people living with HIV in the aspect of antiretroviral therapy

65 Izabela Malczak, Anna Gajda
Liquid chromatography–tandem mass spectrometry method for the determination of antibaterial drugs in plasma samples

66 Agnieszka Chłodowska, Małgorzata Olejnik
The development of LC-MS/MS method for determination of 6 ionophores in liver tissue

67 Maciej Noga, Kamil Jurowski
The devil is not as black as he is painted: Prediction of acute toxicity and environmental fate (hydrolysis and biodegradation) of the Novichoks organophosphorus nerve agents using in silico toxicology methods

Day 3. Session 5

Day 3. Session 6

Role of inflammatory macrophages in lung injury and disease pathogenesis induced by chemical toxicants

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Abstract

Background and objectives. Mustard vesicants including sulfur mustard and nitrogen mustard (NM) are a bifunctional alkylating agents known to cause pulmonary injury that can progress to fibrosis. They are both considered high priority chemical threat agents. Our research is focused on elucidating inflammatory mechanisms of lung injury induced by mustard vesicants, with the overall goal of identifying targets for the development of drugs to mitigate toxicity.

Materials and methods. For our studies, we use as rat model of NM mustard lung toxicity. Treatment of rats with NM (0.125 mg/kg, i.t.) results in a persistent increase in macrophages in the lung beginning 3 days post exposure.

Results. These cells consist of pro-inflammatory/cytotoxic M1 macrophages and anti-inflammatory/wound repair M2 macrophages, as determined by flow cytometric analysis and expression of phenotype specific genes and proteins. Whereas M1 macrophages were prominent 1–3 days after NM, M2 macrophages were most notable at 28 days. At this time, the macrophages were enlarged and vacuolated, and contained high levels of oxidized lipids, consistent with a profibrotic phenotype. The appearance of M1 in the lung correlated with early NM-induced acute injury and M2 macrophages with the development of fibrosis, suggesting a potential role of these macrophage subpopulations in the pathogenic response. This is supported by findings that blocking M1 macrophages prevented acute lung injury, while suppressing M2 macrophages, inhibited the development of fibrosis.

Farnesoid X receptor (FXR) is a nuclear receptor involved in bile acid and lipid homeostasis; it also has anti-inflammatory activity. Following NM exposure, FXR activity is reduced in the lung. To analyze the role of FXR in macrophage activation and NM toxicity, we used obeticholic acid (OCA), a synthetic FXR agonist. Administration of OCA (15 mg/kg) attenuated NM-induced histopathology, oxidative stress, inflammation, dyslipidemia, and altered lung function. OCA also mitigated the effects of NM on pro-inflammatory and profibrotic macrophages activation and mediators released by these cells. Aberrant regulation of lipid homeostasis is known to be central to the development of inflammatory diseases including fibrosis and emphysema, which are long term consequences of mustard vesicant exposure.

Conclusions. Our findings are important as they provide new mechanistic information about the pathogenesis of mustard lung toxicity that may lead to the development of more specific therapeutics to treat diseases associated with aberrant lipid handling and inflammation.

Key words: nitrogen mustard, inflammatory macrophages, farnesoid X receptor, obeticholic acid, rat model

Graphene quantum dots: Nanotoxicity aspect

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Abstract

Background. Graphene-based nanomaterials are revolutionizing various aspects of medicine by promoting novel sensing, diagnostic, and therapeutic approaches. In our project, we study GBNs as an active agent targeting α-synuclein inclusions. At the same time, concerns have been raised about their nanotoxicity.

Objectives. The object of our study is blue luminescent graphene quantum dots (GQDs) synthesized from carbonized citric acid using a microwave-assisted synthesis approach.

Materials and methods. The cytotoxicity was assessed in normal human dermal fibroblasts (HDFs). Using the MTT test, we assessed the relative viability of HDFs exposed to GQDs in a concentration range from 5 µg/mL to 200 µg/mL for 24 h, 48 h and 72 h. After interpolating unknowns from the standard curve, the IC_{50} doses were calculated. Moreover, we evaluated DNA damage and inflammatory responses in cell lysates and culture medium, respectively. The expression of 27 DNA damage response-associated proteins using a membrane antibody array system and 40 human inflammatory factors using a multiplex ELISA array were semiquantitatively determined.

Results. Compared to the control (untreated cells), the cytotoxic activity of GQDs was remarkably above the concentration of 125 µg/mL. IC_{50} was time-dependent, with the highest value of 70.29 µg/mL at 24 h. Several proteins involved in DNA damage and pro-inflammatory responses have been identified.

Conclusions. GQDs exhibited time- and concentration-dependent cytotoxicity, which should be considered in further stages of the research.

Key words: graphene, cytotoxicity, antibody microarrays

Biosafety and toxicity of polydopamine nanoparticles with the potential for cancer treatment

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None declared

Abstract

Polydopamine nanoparticles (PDA NPs) are multifunctional nanoparticles that have attracted growing interest in cancer treatment and other biomedical applications such as biosensing, drug delivery, tissue engineering, and clinical diagnosis. Although the possibilities of using PDA-NPs in medicine are still growing, a full assessment of their toxicological profile has not been carried out, which makes the introduction of nanoparticles into clinical trials very difficult.

The main goal of our research was to investigate the in vivo biodistribution and toxicity of polydopamine nanoparticles. Since the chemical structure, modifications, size, route of administration and exposure dose of PDA-NPs may affect their toxicity, these parameters were taken into account in our studies. Then, the synthesized nanoparticles were functionalized with metal ions (Fe^{3+} and Mn^{2+}) with contrasting properties in nuclear magnetic resonance imaging (MRI) to assess their biodistribution. In addition, we also functionalized PDA nanoparticles for potential applications in the treatment of liver cancer. The obtained materials were characterized using modern techniques such as: DLS, zeta potential, SEM, TEM, FTIR, ICPOES, MRI imaging, in order to determine their physicochemical properties. The key task of the project was to assess the biodistribution and toxicity of synthesized nanomaterials based on PDA-NPs in vivo using rat and mouse models. We performed histological evaluation and determination of well-known oxidative stress biomarkers: total protein (TP), reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), trolox equivalent antioxidant capacity (TEAC), nitric oxide (NO), S-nitrosothiols (RSNO), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) in tissues.

The research topics we have undertaken are pioneering studies focusing on the assessment of the biodistribution, short-term and long-term toxicity of PDA-NPs in vivo, which has not been described so far. The obtained results allowed the establishment of certain toxicity criteria, thanks to which it will be possible to plan further stages of research towards potential biomedical applications.

Key words: polydopamine nanoparticles, biosafety, toxicity, cancer treatment, in vivo study

In vitro toxicity evaluation of graphene oxide functionalized with selenium

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Abstract

Background. Nanomaterials are utilized in biomedical sciences for treating disorders like infections, neurodegenerative diseases, and cancer due to their unique physicochemical properties and increased surface contact with the environment. The eco-friendliness of graphene oxide functionalized with selenium (GO-Se) has been demonstrated to hold great promise for various biological applications.

Objectives. The aim of our study is the cytotoxicity and DNA damage response-associated proteins of graphene oxide selenium decorated one-dimensional rods on magnesium alloy surfaces in 3.5% NaCl medium.

Materials and methods. The mammal cell viability assay was carried out with a normal human dermal fibroblast cell line for 24 h, 48 h and 72 h of exposure. Using a membrane antibody array, the expression of 27 DNA damage response-associated proteins in cell lysates was applied for 24 h of exposure in cytotoxic concentration (IC_{50}).

Results. Compared to the untreated cells, the cytotoxic activity of GQDs was remarkable. Nevertheless, at concentration values as high as 200 µg/mL, the cell viability is still nearly 57% compared with untreated cells. The IC_{50} doses were calculated as 275 µg mL^{-1}, 163 µg mL^{-1} and 110 µg mL^{-1} at 24 h, 48 h and 72 h, respectively. DNA damage response-associated proteins including cell cycle checkpoints and regulatory key proteins, have been identified.

Conclusions. As per the findings of this research, GO-Se exhibited relatively low cytotoxicity. Nevertheless, we plan further studies to understand the particular toxicity mechanism of GOSe, apart from studies on its biomedical application.

Key words: graphene oxide, cell death, DNA damage response

Factors influencing the toxicity of micro and nanoplastics

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Abstract

Micro and nanoplastic particles (MNPs) are widespread in the environment posing a potential threat to living organisms. It has been found that they enter living organisms and accumulate in the trophic chain. Numerous studies have reported the presence of MPs in humans, but unfortunately little data have been collected regarding NPs. MPs have been detected in human lungs, sputum, stool and urine. They have also been determined in the male reproductive system and in human blood. Other reports have mentioned a higher amount of MPs in tumor tissue in patients with colorectal adenocarcinoma in comparison to controls.

The widespread presence of these particles impels the assessment of their potential toxicity on the human body. Numerous scientific studies conducted so far on the toxicity of micro and nanoplastics (NMPs) have shown that their cytotoxicity, oxidative properties and genotoxicity depend on their concentration, time of action and particles diameter.

Different extrinsic and intrinsic factors contribute to the toxicity of micro/nanoplastics. Sensitivity to NMPs depends on the cell type, with normal cells being more sensitive than cancer cells. The harmful effects of NPs and MPs also depend on their functionalization with anionic or carboxyl groups due to greater interaction with cell membrane components. Moreover, MNPs with positive zeta potential are more toxic than those with negative zeta potential because the cells are negatively charged, which provokes stronger interactions. Cationic MNPs are particularly toxic due to their greater cellular uptake and/or their effects on cells. The toxicity of MNPs may be increased by environmental factors, including UV radiation, because it causes shrinking of the particles and change their shape, which is particularly important issue when working with environmentally altered MNPs. Also eco-corona changes properties of micro/nanoplastics and increases environmental risks. The associations described above play an important role in assessing the risk of environmental contamination with plastics and the threat to the health of living organisms.

Key words: microplastics, nanoplastics, genotoxicity, cytotoxicity, UV radiation

The toxicological analysis of heavy metals in low-cost jewelry from Chinese e-commerce platforms using portable XRF spectroscopy

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Abstract

Background. Presence of heavy metals (Pb, Cd and Hg) in low-cost jewelry poses significant health and environmental risks. This alarming discovery marks the first scientific report revealing the hidden dangers associated with popular jewelry products among the Polish population. Prior to this study, there has been a significant gap in understanding the quality and safety of these widely-used accessories. The findings underscore the urgent need for heightened awareness and regulatory measures to protect public health and the environment from these toxic substances.

Objectives. This study aims to assess the levels of heavy metals in low-cost jewelry for adults from Chinese e-commerce platforms using portable X-ray fluorescence (pXRF) analysis.

Materials and methods. A total of 106 jewelry samples, including charms and bracelets, were purchased from various Chinese e-commerce platforms. Utilizing the pXRF (portable X-ray fluorescence) technique, an direct, non-destructive, rapid and 'green' analysis was conducted to determine the concentrations (µg/g) of Pb, Cd and Hg in the investigated samples.

Results. The analysis revealed significant contamination of the jewelry with heavy metals. Mercury was the most frequently detected element, present in 90% of the products with an average concentration of 2,946.25 µg/g. Cadmium and lead were detected in 51% and 21% of the samples, respectively, with average concentrations of 1,192.77 µg/g and 1,525.5 µg/g. Notably, 71% of the samples exceeded the EU limit for lead, and 51% for cadmium.

Conclusions. The study highlights the severe contamination of low-cost jewelry with hazardous heavy metals and underscores the need for stricter regulations and monitoring of the jewelry industry. The use of pXRF demonstrated its effectiveness as a rapid, non-destructive method for rapid identifying heavy metals in consumer products.

Key words: portable X-ray fluorescence, hazardous elements, jewelry, in situ analysis, toxicological risk assessment

The influence of plastic nanoparticles on blood hematology parameters and colon lymphocyte profiles: In vivo model studies

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Conflict of interest
None declared

Abstract

Background. In recent years, plastic pollution has become a global problem, impacting both the environment and human health. Nanoplastics, ranging from 1 nm to 1,000 nm, pose significant risks due to their potential toxicity, prevalence, persistence, and interactions with organisms.

Objectives. The aim of the study was to assess the systemic toxicity of plastic nanoparticles through hematological analysis, as well as their effect on immune cells in gut-associated lymphoid tissue.

Materials and methods. The study was conducted on male and female Wistar rats, which were orally administered different substances based on group assignment: silver nanoparticles (AgNPs), commercial plastic nanoparticles (NPs), a mixture of silver and plastic nanoparticles, and plastic nanoparticles from a rice bag for 28 days. After 4 weeks, the rats were sacrificed, and blood and colon samples were collected. Blood cell counts were analyzed using the electrical impedance method, and the profiles of intraepithelial and colon lamina propria lymphocytes were analyzed by flow cytometry.

Results. The results showed that nanoparticle administration significantly influenced the population of immune cells both in the colon and systemic circulation. Specifically, the analysis revealed a reduction in leukocyte levels in the group treated with AgNPs and a decrease in NK cells in the intestinal wall among those receiving commercial nanoplastic particles. Additionally, there was an increased number of CD161 lymphocytes in the intestinal wall of the group that received the mixture of NPs and AgNPs.

Conclusions. The results indicate that exposure to plastic particles modulated systemic blood parameters and significantly affected the inflammatory cell profile in the colon. Given the relevance and potential health implications of chronic exposure to nanoplastic particles, further research is crucial to fully understand the associated risks and threats.

Key words: plastic nanoparticles, colon, immunotoxicity, gut-associated lymphoid tissue

Nanosilver modulates expression of inflammation related lncRNA in neural cells in culture

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Conflict of interest
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Abstract

Background. Long non-coding RNA (lncRNA), is defined as RNA longer than 200 nucleotides that is not translated into functional proteins. Depending on their localization and their specific interactions with DNA, RNA and proteins, lncRNAs can modulate chromatin function, alter the stability and translation of cytoplasmic mRNAs and interfere with signaling pathways.

Objectives. Since expression of many lncRNAs is regulated, we studied changes in inflammation related lncRNAs expression upon treatment with 20 nm silver nanoparticles (AgNPs) in non-differentiated human neuronal cell line – LUHMES.

Materials and methods. Spherical AgNPs of nominal size 20 ±5 nm were purchased from NanoComposix (Prague, Czech Republic). LUHMES cells (CRL-2927) were grown in monolayer in NunclonDTM cell culture flasks coated with poly-L-ornithine and human plasma fibronectin in Advanced DMEM/F-12 medium with supplements. Silver nanoparticles were added to the cell culture for 6 h to the final concentration 0.28 µg/mL (0.062 µg/cm^2), then cells were harvested, total RNA was isolated and reverse-transcribed to cDNA. Inflammation-related lncRNA expression was profiled using the Inflammation lncRNA H96 PrimePCR PCR Array (BioRad) according to the manufacturer's instructions. Twofold increase or decrease in expression among statistically significant differences were assumed as important.

Results. Silver nanoparticles treatment resulted in increase of 10 and decrease of 2 lncRNAs expression, as compared with untreated control. According to LncRNAWiki 2.0 database the modified lncRNAs were associated with Pi3k/akt signaling, Wnt/β-catenin signaling. Nf-κb signaling, Mapk signaling, and P53 signaling.

Conclusions. Nanosilver modify expression of lncRNA that might be a prerequisite to its ability to modify gene expression. Further, as alteration of lncRNAs expression is observed in numerous diseases, a chronic exposure to AgNPs might affect human health.

Key words: non-coding RNA, LUHMES cells, silver nanoparticles, inflammation

Tris(2,3-dibromopropyl) isocyanurate (TBC): New brominated flame retardant initiates the autophagy process in mouse spermatogenic cells (GC-1 (spg)) in vitro

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Conflict of interest
None declared

Abstract

Background. Tris(2,3-dibromopropyl)isocyanurate (TBC) has been widely used in plastics, textiles, and electronic devices. Due to its high hydrophobicity, it has a high bioaccumulation potential. Moreover, TBC is an endocrine disruptor, which can negatively affect male fertility. Whereas, the mechanism of action of TBC on GC-1 (spg) cells is poorly understood.

Objectives. The study aimed to evaluate the effect of TBC alone and in cotreatment with PP242 (mTOR inhibitor) and GW9662 (peroxisome proliferator-activated receptor gamma (PPARγ) antagonist) on the basic metabolic parameters as well as to determine whether the autophagy process is involved in the cellular response to TBC exposition in GC-1 (spg) cells.

Materials and methods. The resazurin reduction test allowed for the assessment of cell activity and metabolism, and the impact of TBC on the cell cycle was assessed using flow cytometry. The expression of PPARγ gene and proteins involved in autophagy was assessed using RT-PCR and western blot methods, respectively.

Results. Tris(2,3-dibromopropyl)isocyanurate (10 μM) decreased metabolic activity and increased PPARγ mRNA level in GC-1 (spg) cells. Moreover, a decrease in p-mTOR and increased ATG5 protein expression were observed. It was also shown that TBC caused an increase in the number of cells in the G0/G1 phase.

Conclusions. Tris(2,3-dibromopropyl)isocyanurate negatively affects GC-1 (SPG) cells by inducing autophagy and inhibiting cell cycle progression at the G0/G1 phase.

Key words: autophagy, GC-1 spg, TBC, spermatogonia, flame retardant

Evaluation of the synergistic anticancer effect of sorafenib and adipose tissue stem cell culture medium

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Conflict of interest
None declared

Abstract

Background. Taking into account that a crucial role in the development of hepatocellular carcinoma (HCC) play alterations in the Ras/Raf/MEK/ERK signaling pathway, the standard treatment includes oral tyrosine kinases inhibitors, as sorafenib (first-line treatment). Due to poor prognosis in advanced HCC, new treatment opportunities are being investigated. It is thought that the regenerative potential of stem cells, including adipose-derived stem cells (ADSCs) may be useful in treating this disease as an alternative or complementary treatment to pharmacological therapy. Some doubts arise from observations that ADSC secretome may both inhibit and support cancer cell growth. Thus, the effect of ADSCs, a possible anti-cancer agent, on the growth of malignant tumors is not entirely clear, and studies have yielded conflicting results.

Objectives. The aim of the study was to compare the effect of sorafenib and adipose-derived stem cell medium (CM-ADSC) on Ras/Raf/MEK/ERK cell signaling pathway and the characteristics of HepG2 cells.

Materials and methods. HepG2 cells were cultured with 7.5 μM sorafenib and/or with CM- ADSC diluted 1:1 with standard medium for 48 h. Ras/Raf/MEK/ERK gene expression (RT-qPCR), cell viability (MTT assay), and apoptosis (flow cytometry and RT-qPCR) were assessed.

Results. Sorafenib upregulated *CCNE1*, but decreased *MAP2K1* gene expression as well as cell viability. Apoptosis assay showed a reduction in the number of viable cells, an increase in HepG2 in early and late apoptosis, and increased expression of *BAX*, *CASP3* and *TP53*. CM- ADSC significantly upregulated *KRAS*, *CCNE1*, *CCND1* and *MAP2K1* expression, as well as *BAX*, *CASP7* and *TP53* expression, and increased HepG2 viability (1.59 times).

Conclusions. Against the background of sorafenib which showed a clear anti-cancerous effect, CM-ADSC displayed pro-apoptotic activity at mRNA level of some genes, but not at the cellular level. Furthermore, it increased the viability of HepG2 cells, which may be considered as pro-cancerous effect. Thus, the observed effects of sorafenib and CM-ADSC did not appear to be synergistic.

Key words: sorafenib, adipose-derived stem cells, conditioned medium, hepatocellular carcinoma

Cytotoxicity of extracellular vesicles loaded with glucose oxidase in lung cancer cells

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Conflict of interest
None declared

Abstract

Background. Extracellular vesicles (EVs) are small membrane-bound vesicles secreted by various cells including cancer cells. Due to their low immunogenicity, stability in biological fluids, and ability to cross biological barriers, the EVs are being studied as novel biological carriers for different chemical and biological cargos including enzymatic proteins. Glucose oxidase (GOX) catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide that induces severe toxicities to different cells and tissues. In recent studies, this enzyme served as a tumor starving molecule due to glucose consumption based on the catalyzed enzymatic reaction.

Objectives. To optimize the electroporation technique for uploading of GOX into EVs collected from lung cancer cells and to use the as-developed GOX-EVs construct in cytotoxic studies using the lung cancer cells from which the EVs was obtained.

Materials and methods. Exosomes were collected from A549 cells using a multi-step centrifugation, filtration and ultracentrifugation protocol. Electroporation of EVs was conducted based on the square wave pulse using 4 mm gap cuvettes with different voltage levels, times, and pulse numbers. The as-produced GOX-EVs were isolated using ultracentrifugation. The enzymatic cargo was analyzed based on protein levels (BCA), FAD (HPLC) and nanoparticle tracking analysis (NTA). Alamar Blue assay was applied to study the cytotoxic effect of the as-produced construct on lung cancer cells.

Results. Uploading of GOX into EVs was optimized for 250 mV, 10 ms and 1 pulse protocol resulting in obtaining the stable GOX-EVs nanoconstructs ranging in the size of 164–188 nm. These enzyme-loaded EVs produced severe cytotoxicity on lung cancer A549 cells.

Conclusions. The enzymatic cargo (GOX) was successfully uploaded into EVs based on the electroporation method. The as-obtained GOX-EVs construct induced severe cytotoxic effects on lung cancer cells.

Key words: extracellular vesicles, glucose oxidase, electroporation, cytotoxicity, lung cancer cells

The effect of liposomal DMU-212 on differentiation of human ovarian granulosa cells in a primary 3D culture model

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Conflict of interest
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Abstract

Background. Granulosa cells (GCs) are crucial for ovarian follicle development and function, exhibiting multipotency and the ability to differentiate into neuronal cells, chondrocytes, and osteoblasts in vitro. 3,4,5,4′-tetramethoxystilbene (DMU-212) is a methylated derivative of well-described resveratrol, a natural polyphenol found in grapes and berries, with a wide spectrum of biological activities, including notable anti-cancer properties. Interestingly, DMU-212 exhibits cytotoxic effects predominantly on cancer cells while sparing non-cancerous ones.

Objectives. This study aimed to investigate the effects of liposomal formulation of methylated resveratrol analog – lipDMU-212, on the osteogenic differentiation ability of human ovarian GCs in a primary 3D cell culture model.

Materials and methods. GCs were isolated from follicular fluid obtained via transvaginal ultrasound-guided aspiration from women undergoing the IVF-ICSI procedure. A cell viability assay was conducted to evaluate the non-cytotoxic effects of lipDMU-212 on GCs. Based on these results, a concentration of 5 µM was selected to investigate the impact of lipDMU-212 on the differentiation capacity of human GCs in a primary 3D cell culture model. Differentiation was induced over a 15-day period, with assessments conducted at 3 time points: day 1, day 7 and day 15. The differentiation process was characterized qualitatively through alizarin red staining and quantitatively by measuring alkaline phosphatase (ALP) activity.

Results and conclusions. Our studies revealed that lipDMU-212 promotes osteogenic differentiation of GCs in a primary 3D cell culture model, as evidenced by increased mineralization. The ability of GCs to differentiate into various tissue-specific cells highlights their potential utility in regenerative medicine. Given the aging population and increasing life expectancy, exploring novel therapeutic agents and strategies to mitigate bone loss and improve outcomes for orthopedic degenerative diseases is of high significance. This study contributes to the understanding of the role of lipDMU-212 in osteogenic differentiation, offering promising insights for future regenerative medicine applications.

Key words: 3,4,5,4′-tetramethoxystilbene (DMU-212), granulosa cells, differentiation, osteoblasts

The role of baicalin in chemoprevention of ovarian cancer cells exposed to cadmium ions

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Abstract

Background. Ovarian cancer is still common hormone-dependent cancer among women and has a high mortality rate. Its development is influenced by metalloestrogens, such as cadmium ions, which are present in polluted environments and interact with the estrogen receptor. The effectiveness of chemotherapy can be weakened by the development of multidrug resistance (MDR). Searching of natural substances which can act as chemopreventive agents, increasing the sensitivity of cancer cells to cytostatics is interesting. Such properties are attributed to baicalin, a flavonoid present in the root of *Scutellaria baicalensis*.

Objectives. The aim of the study was to evaluate the chemopreventive properties of baicalin of the human ovarian cancer cell line MDAH-2774 exposed to the toxic action of cadmium ions and additionally in relation to the detoxification capacity of glutathione-S-transferase (GST), especially its π form associated with MDR.

Materials and methods. MDAH-2774 cells were exposed for 24 h individually to cadmium ions (as $CdCl_2$) and baicalin at concentrations ranging from 0.1 μM to 50 μM and 10 μM to 100 μM, respectively, as well as to a combination of these compounds. The mitochondrial activity of MDAH-2774 cells was assessed by the MTT test and an immunocytochemical examination of GST-π expression by ABCAM test.

Results. In relation to the independent effect of baicalin on MDAH-2774 cells, a decrease in mitochondrial activity was observed with increasing concentrations of the flavonoid. In combined exposure, baicalin demonstrated a stronger protective effect on MDAH-2774 cells compared to single exposure to the toxic effects of $CdCl_2$. The flavonoid also reduced the expression of GST-π, which was initially stimulated by cadmium ions, at the highest concentrations.

Conclusions. The obtained results suggest that baicalin may inhibit the toxic effects of cadmium ions and be an effective chemopreventive agent, as well as enhance the efficacy of chemotherapy, possibly through a beneficial impact on MDR.

Key words: ovarian cancer, cadmium, baicalein, chemoprevention, MDR

Drug-induced QT prolongation: From molecular mechanisms to clinical management

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Abstract

Drug-induced QT prolongation is a critical concern in clinical pharmacology and toxicology due to its potential to trigger life-threatening arrhythmias such as torsade de pointes. The QT interval, measured on the electrocardiogram (ECG), commences with the initiation of ventricular depolarization, signified by the Q wave, and culminates with the end of repolarization, as indicated by the T wave. Prolongation of the QT interval, especially when it co-occurs with risk factors such as heart diseases, female gender, low potassium levels, or old age, is associated with an elevated risk of lethal cardiac arrhythmias. Many drugs can induce QT interval prolongation, such as antibiotics, anticholinergics, antidepressants, opioids, and anticancer drugs. Drug-induced QT prolongation results from multiple risk factors, including genetic variations, particularly in genes related to drug metabolism (e.g., cytochrome P450 genes) and cardiac electrophysiology (e.g., genes encoding ion channels such as *KCNH2*). A salient ramification of drug-induced QT prolongation is the market withdrawal of numerous medications. Nonetheless, these drugs may retain safety and therapeutic efficacy for distinct subsets of patients. Identification of common genetic variants associated with drug-induced QT prolongation and mapping their functional consequences are imperative for tailoring pharmacotherapy. Such personalization aims to avoid the unnecessary discontinuation of potentially valuable drugs. This review poster summarizes the molecular background of drug- induced QT prolongation and management options to prevent the toxicological implications of this condition.

Key words: cardiotoxicity, torsades de pointes, toxicology, pharmacogenetics

Endothelin-1 as potential target in the predictive medicine of pancreatitis

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Abstract

Background. Endothelin-1 (ET-1), a strong vasoconstriction factor, contributes to damage of pancreatic tissue and its hypoxia. This process may be aggravated by exposure to smoke xenobiotics. ET-1 intensifies the pro-inflammatory response. It is believed that ET-1 concentration should be taken into the account as a predictor of severe acute pancreatitis.

Objectives. Assessment of the influence of single nucleotide polymorphism (SNP) rs5370 and rs5333 in gene encoding ET-1 and the ETA receptor (respectively) on changes in ET-1 concentration in healthy people and patients with acute pancreatitis (AP), taking into account the exposure to tobacco smoke.

Materials and methods. Material was venous blood. The SNPs: rs5370 (*EDN1* gene) and rs5333 (*EDNRA* gene) were determined using the PCR-RFLP method, plasma ET-1 concentration – using ELISA method.

Results. The ET-1 concentration in smokers was higher than non-smokers. The dynamics of changes in ET-1 concentration during hospitalization of patients with acute pancreatitis showed that in the group of smokers, the concentration of ET-1 in the blood is significantly higher on the 3rd day compared to the 1st day of hospitalization. Similarly, in the AP patients group with the T/G genotype (SNP rs5370), a statistically significant higher ET-1 concentration on the 3rd day of hospitalization compared to the 1st day was observed, in contrast to the group of AP patients with the G/G genotype where this change was not statistically significant. The analysis of odds ratio showed that the risk of AP occurrence is almost twofold increased for individuals with the T/C genotypes for SNP rs5333 compared to people with the T/T genotype.

Conclusions. Smoking increases the ET-1 concentration in the blood. The presence of the T/G genotype (SNP rs5370) can contribute to an increased sensitivity to endothelial damage in the course of AP. This genotype is associated with the elevated (twofold) risk of AP occurrence.

Key words: acute pancreatitis, endothelin-1, single nucleotide polymorphism, smoking

Interactions of ethanol with levetiracetam or brivaracetam on GABA and glutamate levels in selected rat brain structures

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Abstract

Background. Disruption of GABA and glutamate is one of the mechanism behind inducing seizures. Levetiracetam and brivaracetam, novel anticonvulsants with few adverse effects, act via the synaptic vesicle protein 2A influencing the release of GABA and/or glutamate in the CNS. However, the interactions of 2 above with ethanol on these neurotransmitters are hardly described and ethanol is well-known of disturbing GABA and glutamate transmission.

Objectives. The aim of the study was to preliminary assess the interaction of levetiracetam and brivaracetam with ethanol by determining GABA and glutamate concentrations in 3 structures rat brain structures.

Materials and methods. The experiment was approved by the Local Ethical Committee for Experimentation on Animals (resolution No. 9/ŁB231/2022). Adult male Wistar rats (n = 46) were divided into 6 groups: 1) control group (1% methylcellulose); 2) ethanol group (20% ethanol); 3) levetiracetam group (300 mg/kg bw.); 4) brivaracetam group (6 mg/kg bw.); 5) levetiracetam with ethanol; 6) brivaracetam with ethanol. The above substances were administrated daily by oral gavage for 28 days. After the sacrifice of animals, their brains were collected. GABA and glutamate concentrations in 3 structures, i.e., cerebellum, hippocampus and cerebral cortex were determined using the UPLC-PDA method.

Results and conclusions. There were no changes in GABA and glutamate concentrations in the hippocampus in all study groups. In the cerebellum and cerebral cortex, administration of brivaracetam decreased GABA concentration in these structures. In rats exposed to both, brivaracetam and ethanol, GABA concentration in the cerebellum were similar to those in the control group. However, no such effect was observed in the cerebral cortex. The administration of levetiracetam with or without ethanol did not significantly affect the concentration of both neurotransmitters in all structures. The results indicate the need for further research to understand the mechanism underlying the interactions of these drugs with ethanol.

Key words: GABA, glutamate, brivaracetam, levetiracetam, ethanol

Effects of cannabidiol on toxicity veterinary drug using HepG2 cells

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Conflict of interest
None declared

Abstract

Background. Growing awareness and the need to protect public health, including food safety, call for a thorough investigation of the mechanism of action of veterinary drugs in consumers and the reduction of their adverse effects on humans. Improper use of veterinary drugs such as thiamulin leads to the appearance of residues in animal tissues and occurrence of adverse effects in consumers. The use of natural substances of plant origin, extracted from the seeds of the hemp plant (*Cannabis sativa L.*) such as cannabidiol (CBD) is one solution to minimize the negative effects of thiamulin.

Objectives. The study aimed to determine the effect of CBD on the cytotoxic effect of thiamulin in consumers using in vitro tests.

Materials and methods. The cytotoxic activity of thiamulin and the effect of CBD were tested after 72-h exposure to HepG2 cells. Two concentrations of cannabidiol showing no toxic activity were selected for the study. The cytotoxic concentrations (IC50) of the test drug and in combination with CBD were evaluated using 4 biochemical endpoints: mitochondrial activity (MTT assay), lysosomal activity (NRU assay), proliferation (TPC assay) and cell membrane integrity (LDH assay). Effects on DNA synthesis (BrdU assay), oxidative stress, and cell death were also evaluated. The nature of the interaction between the veterinary drug and CBD was assessed by isobolographic analysis. Statistical analysis was performed using GraphPad software.

Results. The long-term action of thiamulin disrupted mitochondrial activity and cell proliferation. Decreased cells viability was observed from a concentration of 12.5 µg/mL of the drug. IC50 values were ranged from 26.7 µg/mL to 39.5 µg/mL. IC50 values for the veterinary drug in combination with CBD were higher from 28.7 µg/mL to 79.1 µg/mL. The decreased cell DNA synthesis and reduced levels of oxidative stress after the mixture was demonstrated. An increased percentage of apoptotic cells was shown in the mixture with a low concentration of CBD. Interactions between the veterinary drug and CBD showed a concentration-dependent nature, ranging from antagonistic to synergistic effects at high concentrations of the drug.

Conclusions. The increased human health risks associated with the presence of the veterinary drug in food products and the protective nature of CBD use underscore the importance of these studies in food toxicology and require further research.

Key words: veterinary drug, cannabidiol, HepG2 cells, interaction

Studies on the effect of different folic acid dosing alone and in the situation of exposure to ethanol in the zebrafish embryonic model

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Abstract

Background. Folic acid is necessary for the proper development of pregnancy. Its deficiency may result in congenital defects in the fetus. Therefore, folic acid is one of the vitamins recommended for supplementation by all pregnant women, as well as women planning to get pregnant. However, in recent years there have been reports that the use of folic acid in higher doses is associated with potentially harmful effects on the fetus including cardiotoxicity. Additionally, folic acid could inhibit the teratogenic effect of ethanol. Its concentration and time of administration are essential to achieve this saving effect.

Objectives. The aim of the study was to estimate the effect of high doses of folic acid on the development of *Danio rerio*, with particular emphasis on the cardiovascular system. Additionally, small doses were tested for the protective effects of folic acid with simultaneous exposure to ethanol.

Materials and methods. An analysis of lethal and sublethal changes occurring in zebrafish within 96 h of exposure to the tested substances was conducted.

Results. Studies have confirmed that the use of high doses of folic acid affects the development of embryos. The most frequently observed change was coagulation. However, no other developmental changes were found, such as larval pigmentation, edema, tail curvature, and delayed chorion detachment. As the dose of folic acid was increased, the heart rate decreased. The administration of folic acid in zebrafish embryos and exposure to high ethanol concentration did not increase morphological abnormalities.

Conclusions. High doses of folic acid affect the cardiovascular system in the zebrafish model. This effect is dose dependent. In turn, the use of lower doses of folic acid, despite previous reports, is not always able to eliminate all the adverse effects of substances that possibly cause damage, an example of which is alcohol in high doses.

Key words: folic acid, cardiotoxicity, hypervitaminosis, zebrafish, ethanol

Study of the interactions of paracetamol with xenobiotics in the zebrafish embryonic model

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Abstract

Background. Paracetamol is one of the most widely used over-the-counter painkillers and antipyretic drugs. It is considered well-tolerated and safe, even during pregnancy. However, adverse reactions, including effects on fetus, associated with paracetamol intake are observed and lead to considerations related to dose reduction. Ethanol and caffeine are the most popular psychoactive substances and their possible influence on the toxicity of the paracetamol was featured. Their popularity and prevalence mean that they are often used simultaneously with paracetamol. While caffeine and ethanol are widely known to have adverse effects on zebrafish embryos alone, the extent of their simultaneous effects, particularly interactions with paracetamol, were not previously studied.

Objectives. Our study aimed to investigate the possible interaction between paracetamol and ethanol or caffeine and determine the type of interaction in zebrafish model.

Materials and methods. An analysis of lethal and sublethal changes occurring in zebrafish within 96 h of the tested substances exposure was conducted.

Results. The study's findings are of significant importance, confirming that combining ethanol or caffeine with paracetamol increases the occurrence of developmental defects compared to using these xenobiotics separately. The results show that the paracetamol-ethanol and paracetamol-caffeine mixture increase larval mortality. Significant reductions in eye size and shorter larval length were also noted in combination with paracetamol and ethanol. Both substances significantly intensified the decreased pigmentation caused by paracetamol used alone. The effect was more significant with the increased concentration of caffeine or ethanol at a constant paracetamol concentration. Changes in body shape and curvatures, number of edemas and survival rate were observed when paracetamol and caffeine were administered simultaneously. The Chou–Talalay method confirmed the synergistic nature of this type of interaction in both tested combinations.

Conclusions. The zebrafish model, with its unique ability to not only assess the toxicity of individual substances but also help assess interactions between various xenobiotics, has revealed potential significant implications for the field of toxicology and pharmacology. The findings of this study could potentially influence the way we understand and manage the use of paracetamol, ethanol, and caffeine in various contexts.

Key words: zebrafish, paracetamol, ethanol, caffeine, interaction, combined exposure

Pro-metastatic activity of silver nanoparticles related to epithelial-mesenchymal transition mechanism

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Abstract

A wide spectrum of nanoparticles toxicity has been described, including cytotoxic effects, oxidative stress induction, pro-inflammatory proteins secretion, apoptosis, and genotoxicity. Recent years have brought reports of new toxicity mechanisms, including profibrogenic and pro-metastatic effects, e.g., through epithelial-mesenchymal transition (EMT). The aim of the study was to determine the pro-metastatic activity of silver nanoparticles (AgNPs) on lung adenocarcinoma A549 and MDA-MB-436 breast cancer cells. The cytotoxicity was determined using MTS test, examining cell's metabolic activity. A neutral red test was used to determine the effect of AgNPs on the level of cytoplasmic membranes integrity. A real-time RT-PCR was used to measure the mRNA expression of EMT markers – cadherin N and cadherin E. The impact of AgNPs on cell migration was assessed by QCM Chemotaxis Cell Migration Assay and wound healing assay. Finally, pro-inflammatory proteins secretion profile was presented using the Human Cytokine Array Kit technique. Studies have shown that AgNPs are toxic to cells of both tested cell lines. At the same time, however, they seem to increase prometastatic cell potential by increasing mesenchymal N-cadherin and decreasing epithelial E-cadherin mRNA expression. A relatively minor increase in the level of migration of cells as a result of AgNPs treatment was demonstrated. Moreover, it was shown that as a result of AgNPs treatment, cells of both lines secrete a number of pro-inflammatory proteins, including interleukin 6 and interleukin 8, key cytokines in the process of EMT induction and modulation. To summarize, studies have shown that AgNPs increase the prometastatic potential of cancer cells. The obtained results encourage further analyses, taking into account the dynamic development of nanotechnology and the disturbing cancer statistics.

Key words: metastasis, cancer cells, silver nanoparticles, epithelial–mesenchymal transition

Susceptibility of CACO-2 and HT29MTX cells to silver nanoparticles in combination with polystyrene nanoparticles

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Conflict of interest
None declared

Abstract

Background and objectives. As per the definition, MNPlastic particles are less than 5 mm in size and are sourced from various everyday products, textiles, agriculture, and waste. A subset of these particles, those less than 1 nm in length, are called nanoplastics. Despite their emergence as contaminants in living organisms and ecosystems, the extraction and analysis of MNPlastics pose significant challenges. This study, therefore, presents a novel approach to evaluate the toxicological effects of in vitro exposure of CACO-2 and HT29MTX cells to AgNPs in combination with polystyrene nanobeads (PS).

Materials and methods. Cells were incubated with different doses of AgNPs in combination with polystyrene beads for 24–72 h, and cytotoxicity, apoptosis, and oxidation stress were assessed by flow cytometry. In addition, the impact of these mixtures on the uptake in CACO-2 and HT29MTX cells was evaluated.

Results. Our findings reveal that exposure to a mixture of nanoparticles led to a significant decrease in the viability of CACO-2 cells, accompanied by an increase in cytotoxic effects. Interestingly, this effect was not observed with HT29MTX cells. Furthermore, higher doses of the AgNPs and PS mixture resulted in a substantial increase in the level of ROS in cells and an elevation in late apoptotic and necrotic cells. Notably, our study also demonstrated a time and dose-response decrease in the uptake of PS due to the presence of AgNPs.

Conclusions. Our study fills a significant gap in the current understanding of nanoparticle toxicity. While numerous studies have focused on the toxicity of individual nanoparticles, there is a need for more information on the effects of nanoparticle mixtures on the environment and human health. Our results underscore the potential for the toxicity of nanoparticles in mixtures to differ from that of single nanoparticles. Moreover, we demonstrate that the toxicity induced by a single nanoparticle can be altered, either increased or decreased, by co-exposure to nanoparticles with a different composition, thereby highlighting the complex nature of nanoparticle toxicity.

Key words: silver nanoparticles, polystyrene nanobeads, toxicity, oxidation stress

The impact of silver and polystyrene nanoparticles on gut cellular model

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Conflict of interest
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Abstract

Background and objectives. Nanoparticles (NPs) have many bioapplications as therapeutics, transfection vectors, antibacterial, fluorescent labels. Silver nanoparticles (AgNPs) with strong antimicrobial properties are widely used in various medical applications. On the other hand, the negative impact of nanoplastics to aquatic organisms are also widely described. There is still a lack of information about the toxicity of nanoplastics to humans and it seems that should be investigated. The aim of this study was to analyze the cytotoxicity of nanoplastics and AgNPs on 3D gut cellular model. Three kinds of cell line were used: Caco-2, HT29-MTX and Raji.

Materials and methods. Cytotoxicity of AgNPs and polystyrene NPs was estimated using flow cytometry. Gut cellular model was exposed to AgNPs (0.1 µg/mL and 1.5 µg/mL) and polystyrene NPs (1 µg/mL and 10 µg/mL). The cytotoxicity of tested NPs was investigated by analysis of number of cells, detection of reactive oxigen species and apoptosis process.

Results. There was lack of toxicity of tested NPs in gut cellular model. Reactive oxygen species level did not show statistically significant differences. The late apoptosis process was only observed after treatment of cells with AgNPs at higher concentration.

Conclusions. Our results showed that tested NPs can have some negative effect on cells by their impact on apoptosis process in cellular model. It seems that NPs should be tested in further experiments in order to estimate their impact on human and another organisms.

Key words: silver nanoparticles, nanoplastics, cytotoxicity, cellular model

The influence of fullerenes as doxorubicin nanocarriers on the triple-negative breast cancer cells proliferation

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Abstract

Background. Chemotherapy is a primary method for treating breast cancer. However, its impact on both cancerous and healthy cells remains a significant challenge in clinical practice. Nanomedicine aims to address this by enabling the targeted delivery of anti-cancer drugs.

Objectives. The aim of this study was to investigate the influence of fullerenes – C_{60} as a carrier of doxorubicin (DOX) on breast cancer cell line MDA-MB-231, that is considered the most aggressive subtype, with a high likelihood of metastasis and the development of chemoresistance.

Materials and methods. The triple-negative breast cancer cells, which do not express any of hormone receptors neither human epidermal growth factor receptor-2 (MDA-MB-231) and non-tumorigenic mammary epithelial cells MCF-10a were treated with C_{60}, DOX and C_{60}-DOX. C_{60} and C_{60}-DOX were subjected to size and zeta potential measurements. Proliferation of cells and toxicity of treating agents was observed on a real-time cell analyzer for 72 h.

Results. After 72 h of C_{60}-DOX treatment to MDA-MB-231 results in significantly lower cell proliferation than cells treated with DOX alone. The different behavior was observed for MCF-10a cells, whose C_{60}-DOX treatment resulted in significantly higher proliferation than cells treated with DOX alone. Interestingly, fullerenes C_{60} alone do not significantly affect the reduction of proliferation in any cell line compared to untreated cells.

Conclusions. These studies indicate that fullerenes C_{60} combined with DOX play a dual role – they enhance the drug's toxicity toward cancer cells, increasing the effectiveness of chemotherapy, while simultaneously reducing the drug's toxicity toward non-cancerous cells, protecting them from undesirable side effects.

Key words: fullerenes, doxorubicin, nanocarriers, toxicity, breast cancer

Long-term exposure to multi-walled carbon nanotubes for potential cartilage tissue engineering applications

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Conflict of interest
None declared

Abstract

Background. Over the last decade interest in the technology of tissue regeneration has grown enormously, especially with respect to reparative techniques. Novel treatment methods of articular cartilage injuries based on nanomaterials such as multi-walled carbon nanotubes (MWCNTs) are challenging for scientists. The elucidation of toxicity determinants of MWCNT is still incomplete.

Objectives. The aim of this study was to evaluate the long-term toxicity and oxidative stress of multi-walled carbon nanotubes in male rats. Our animal model studies include histological examination and measurement of oxidative stress parameters in the body fluid and tissues of animals after long-term exposure to of MWCNTs.

Materials and methods. Wistar male rats were administrated a single injection of MWCNTs (diameter ~15–30 nm, length ~15–20 μm) to the knee joint at 3 concentrations: 0.03 mg/mL, 0.25 mg/mL and 0.5 mg/mL. Twelve and 18 months after injection, the rats were euthanized and tissue samples collected. Histopathological examination was conducted. Relevant markers of oxidative stress and biochemical parameters were determined in serum, liver, and kidney: total protein (TP), reduced glutathione (GSH), glutathione S– transferase (GST) activity, thiobarbituric acid reactive substances (TBARS), nitric oxide (NO), Trolox equivalent antioxidant capacity (TEAC) and catalase (CAT) activity.

Results. Our study, after 12 months, showed a statistically significant reduction of 62.19% in serum GSH levels in rats exposed to nanotubes at higher concentrations compared to lower concentrations. The 18-month exposure resulted in a statistically significant increase in GST activity by 53.93% in the group of rats exposed to nanotubes at higher concentrations compared to animals receiving MWCNTs at lower concentrations and by 58.99% compared to the control group. Moreover, a 35.79% TEAC decrease was observed in rats receiving nanotubes at higher concentrations compared to the group receiving lower concentrations of MWCNTs. Longer exposure time to the carbon nanotube solution results in higher NO levels in the rats' kidneys.

Conclusions. An analysis of oxidative stress parameters can be a key indicator of the toxic potential of multi-walled carbon nanotubes, among others.

Key words: long-term toxicity, in vivo model, multi-walled carbon nanotubes, oxidative stress parameters

Synthesis and cytotoxicity effects of some carboxychalcones

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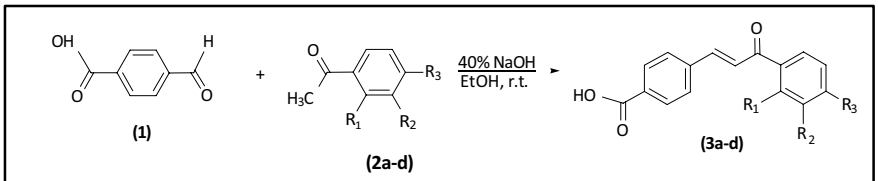
Abstract

Background. Chalcone is an aromatic ketone that forms the central core of many important biological compounds. Structurally, it consists of 2 aromatic rings connected by a 3-carbon, unsaturated aliphatic chain with a carbonyl group. Chalcones are biogenetic precursors of flavonoids and isoflavonoids. Their analogues have been reported to exert neuroprotective effects and have been extensively studied to prevent neurodegenerative disorders and suggested to be used as a multi-functional candidate against multi-resistant Alzheimer's disease in the future.

Objectives. The synthesis of some chalcones and confirming their structures using spectral methods (FT-IR, MS, NMR). Evaluation of the cytotoxic activity potential of the compounds obtained using various assays.

Materials and methods. The proposed derivatives were obtained in the classical Claisen- Schmidt condensation in an alkaline water-alcohol medium, at room temperature using terephthalaldehydic acid (1) and appropriate aromatic ketones (2a-d) as substrates. The influence of some chalcones synthesized on the mouse hippocampal neuronal HT-22 cell line was studied by resazurin reduction assay, the LDH release of the compounds tested and using flow cytometry-based methods.



2a, **3a**: R_1=H, R_2=H, R_3=H; **2b**, **3b**: R_1=H, R_2=OCH_3, R_3=OH; **2c**, **3c**: R_1=OH, R_2=H, R_3=OCH_3; **2d**, **3d**: R_1=H, R_2=H, R_3=OH

Results. The carboxychalcones (3a–3d) were synthesized in one-pot reactions with good yields (75–80%). The resazurin reduction and LDH release assay showed the time- and dose-dependent effect of compounds tested in HT-22 cells. However, the higher toxicity effect was observed only in 100 µM concentration. Moreover, the flow cytometry analyzes evidenced the ability of compounds 3b and 3c to significantly decrease the intracellular ROS level, affecting the cell cycle.

Conclusions. The carboxychalcones were obtained in one-pot reactions with good yields. The compounds tested are characterized by a low cytotoxicity effect on HT-22. The antioxidant character of 3b and 3c derivatives suggests the potentially beneficial application of these compounds in further studies.

Key words: chalcones, Claisen–Schmidt reaction, anti-neurodegenerative agents, cytotoxicity, cell cycle

Synthesis of novel fluorescent probes based on azadipyrromethene and aza-BODIPY derivatives

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Abstract

Background. Fluorescent probes are essential in toxicology for cellular imaging, allowing researchers to visualize and understand cellular responses to toxicants at a molecular level. They help elucidate mechanisms of toxicity and monitor various cellular processes such as ROS detection. Aza-BODIPYs, derivatives of azadipyrromethene (ADPM) containing $-BF_2$ groups, exhibit strong visible light and near-infrared absorption. Their high fluorescence efficiency makes aza-BODIPY and ADPMs ideal as fluorescence molecular probes.

Objectives. The aim of the study was to synthesize new ADPMs and aza-BODIPYs and to analyze their spectral properties.

Materials and methods. All reactions were carried out in oven-heated glassware under inert gas atmosphere. Flash column chromatography on silica gel was used for purification of the synthesized products. Multi-step reaction pathway, utilizing, e.g., aldol condensation or Michael addition was used.

Results. ADPMs and aza-BODIPYs were obtained. NMR experiments, including 2D, were used to ascribe signals to the atoms. Fluorescence properties were studied to assess their potential application as fluorescent probes.

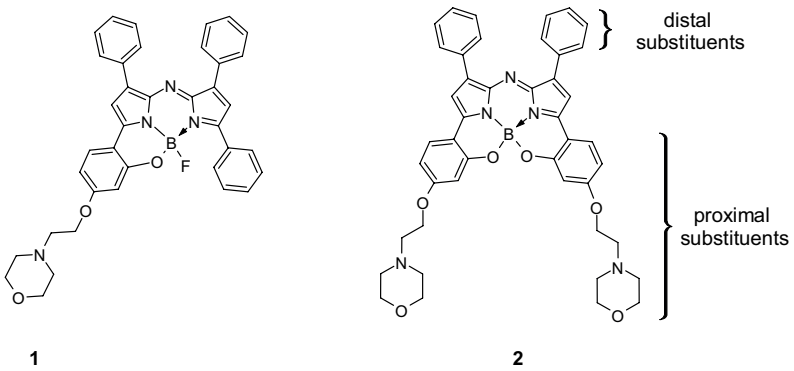

Fig. 1. Obtained aza-BODIPY

Conclusions. Novel aza-BODIPYs were synthesized and their spectral properties, including light absorption and emission were established.

Key words: aza-BODIPY, fluorescent probes, nuclear magnetic resonance, UV-VIS spectroscopy

Purines with alkyl disulfide substituents: Synthesis, physicochemical characterization and preliminary toxicity study

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Abstract

Background. Purine analogues constitute a group of heterocycles with fused pyrimidine and imidazole rings. These agents present a broad spectrum of activities, from antiviral to cytotoxic in eukaryotic cells. Many derivatives of adenosine and guanosine have established medical activity. A very interesting group is represented by 6-mercaptopurine and its analogues of antiproliferative and immunosuppressive activity. 6-mercaptopurine belongs to antimetabolites, and its pro-drug is azathioprine. Nevertheless, the introduction of methyl nitroimidazole moiety is associated with an increased risk of skin cancer due to its degradation in skin tissue after sunlight exposure. Due to the unique action of these compounds, there is great interest in further analogues.

Objectives. The research focused on the synthesis of purine derivatives. The reactions of disulfanediylbis(ethane-2,1-diyl) diacetate with 6-mercaptopurine and adenosine were studied towards novel purine derivatives with alkyl disulfide substituents.

Materials and methods. The reaction was carried out using classical and microwave-assisted methods. The reaction conditions were optimized using different initiators, reaction times, and solvents. The products were purified by flash column chromatography. Physiological properties and biological activity – acute toxicity using the Microtox system were collected and analyzed.

Results. Various synthetic routes and separation procedures for cross-dehydrogenative coupling between disulfanediylbis(ethane-2,1-diyl) diacetate and 6-mercaptopurine or adenosine were assessed. New purines with alkyl disulfide substituents were characterized using NMR and MS as well as subjected to Microtox acute toxicity assessment.

Conclusions. Optimization of reaction conditions allowed the obtaining of new purines with alkyl disulfide substituents that have potential applications in medicine and pharmacy.

Key words: acute toxicity, heterocycles, immunosuppression, organosulfur

Microneedle system with porphyrin-based photosensitizer of potential use in medicine and pharmacy

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Abstract

Background. Skin cancer is a leading cause of death worldwide. Traditional treatments such as chemotherapy, radiation, surgery, cryotherapy and radiation reveal several drawbacks, including weak specificity and short effects. Photosensitizers, such as protoporphyrin derivative – protoporphyrin IX, are mostly used in traditional topical dosage forms of lower bioavailability. Microneedle systems constitute a promising and modern drug delivery method for skin applications.

Objectives. Four goals were established: 1) printing of microneedles and their coating with the selected hydrogel containing 0.1% PPIX disodium (PPIX); 2) microscopic characterization of the selected hydrogel and the coated solid microneedle system; 3) the release study to monitor the topical delivery of PPIX; and 4) acute toxicity assessment.

Materials and methods. Microneedles were designed and manufactured by a 3D printer using Phrozen Aqua-Blue photocurable resin. The 10% poloxamer-based hydrogel containing 0.1% PPIX was analyzed under the optical microscope. Microneedles were immersed in 10% poloxamer-based hydrogel containing 0.1% PPIX and then taken out for drying for 24 h. The coating procedure was repeated. The coated microneedles were observed under SEM microscope. The release of PPIX in time from coated microneedles was assessed by the use of Franz cells. Acute toxicity studies were performed using the Microtox system.

Results. In poloxamer-based hydrogel, fine PPIX particles of dark color, irregular shapes and distribution were observed under the microscope. The diameters of 4 randomly selected particles were 1.5–1.8 μm. Images of the system were also captured with SEM microscope. The amount of PPIX released after a 4-h test in 3 Franz cells was 0.2569 mg/cm^2. The acute toxicity study will be presented.

Conclusions. The 3D printing technology is an effective way of producing microneedles. However, the printing and coating procedures of these systems require further optimization.

Key words: 3D printing, microneedle system, porphyrin-based photosensitizer

Circulating microbiota-derived metabolites in female patients with relapsing-remitting multiple sclerosis

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Conflict of interest
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Abstract

Background. Multiple sclerosis (MS) is a chronic condition affecting the central nervous system (CNS), associated with autoimmune inflammation and neurodegeneration processes. Recent research has highlighted the role of gut microbiota and their metabolites in modulating immune responses within the CNS and regulating metabolic pathways involved in MS pathology. The metabolites produced by the microbiota can be classified into diet-derived, host-derived and de novo synthesized.

Objectives. This study aimed to identify metabolites associated with microbiota activity that differentiate female patients with relapsing-remitting multiple sclerosis (RRMS) and healthy individuals.

Materials and methods. Blood serum samples from 140 women with RRMS and 25 healthy ones were subjected to untargeted metabolomics. Metabolites were extracted with methanol:water (6.5:1) solution and analysis was performed with quadrupole-time-of-flight mass spectrometer (Agilent iFunnel 6650, Santa Clara, USA) by direct injection technique (FIA-MS). Data was processed with SLAW software and analyzed by MetaboAnalyst 6.0 platform.

Results. Final data set contained 314 metabolites, of which 91 differed significantly between patients and controls (FDR < 0.05). Among them, at least 40 metabolites can be linked to microbiota, e.g., 4hydroxyphenylpyruvic acid, homovanillic acid, catechol, coumarate, sphinganine 1-phosphate, hexadecanal, indole-3-acetate, 1-methyluric acid, deoxycholic acid 3-glucuronide, 4-methyl-2oxopentanoate or hippurate. Metabolite set enrichment analysis based on the selected compounds identified pathways significantly impacted in RRMS: amino acid (phenylalanine, tyrosine, tryptophan, arginine and histidine), catecholamine neurotransmitters, sphingolipid metabolism and retinoic acid signaling pathway. Shifts in steroid metabolism (including bile acid metabolism and mevalonate pathway) and purine degradation pathway were also observed.

Conclusions. Understanding the gut-brain axis and the role of microbiota-derived metabolites in MS may provide novel therapeutic approaches. Targeting gut microbiota to regulate diverse metabolic pathways could potentially influence disease course and symptom management. Further investigations are needed to elucidate the specific mechanisms underlying microbiota-mediated effects in MS and to develop personalized interventions based on microbiota modulation.

Key words: microbiota, relapsing-remitting multiple sclerosis, metabolites

Acute carbon dioxide inhalation leads to acute cognitive impairment and systemic vascular inflammation in humans

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Abstract

Background and objectives. Acute human exposures to common indoor concentrations of 1,000–2,500 ppm of CO_2 are well documented to cause cognitive deficits in challenging measures of executive function. Rodent studies have shown that 2 h at these concentrations leads to PMN activation, oxidative burst, and systemic vascular inflammation as well as vascular leak in brain and other tissues, potentially providing a mechanism for the observed declines in executive function. We sought to test if mechanistic findings in rodents are observed in human subjects using a non-invasive measure of cerebrovascular fluid leak.

Materials and methods. Twelve healthy young students completed standardized tests of executive function (Strategic Management System) for 2 h while breathing, 1 week apart, either filtered ambient air (CO_2 = 600 ppm) or air with CO_2 at 2,500 ppm, in a blinded, randomized within subject cross-over design. Venous blood was sampled before, immediately following, and 6 h after exposure. We examined isolated PMNs by differential centrifugation for evidence of activation, as measured by oxidative burst. Oxygen consumption rate was measured using a Seahorse analyzer. Oxidative burst, following stimulation with PMA, and metabolic function were assessed by paired pre post analysis. Evidence of cerebral vascular leak is being obtained via fMRI using a 3D diffusion-prepared arterial spin labeling (ASL) perfusion sequence to allow mapping of water exchange across the blood-brain barrier without contrast.

Results. Carbon dioxide exposure increased baseline oxygen consumption from 13 ±2.9 pM/min to 21 ±3.3 pM/min. For oxidative burst, neutrophils were treated with PMA, and basal oxygen consumption was measured. In response to PMA there was a significant increase in oxygen consumption; however, relative to air, CO_2 exposure delayed this time to peak from 56 ±3.5 min to 98 ±9.2 min. Additionally, CO_2 reduced the total oxidative burst as determined by the area under the curve (11 ±0.9 nM vs 8 ±1.3 nM). All of these changes were statistically significant. We will also report human fMRI results for evidence of cerebrovascular water leak as was seen in the prior rodent study.

Conclusions. We showed that CO_2 exposure increased non-mitochondrial oxygen consumption without external stimulation. This was observed in all subjects and is indicative of increased NADPH oxidase function. Most importantly, CO_2 abrogated the PMA-mediated oxidative burst presumably as the cells were already stimulated. These data support an inflammatory mechanism for the loss of executive function that is observed in military and civilian subjects upon acute CO_2 exposure and will be further elucidated by pending fMRI results.

Key words: carbon dioxide toxicity, acute human exposure, vascular inflammation, oxygen consumption

Circadian rhythm and xenobiotics

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None declared

Abstract

Circadian rhythm is an important protective mechanism in living organisms that controls many aspects of animal physiology and behavior. These include, adjustment to daily environmental changes such as the light-dark cycle, temperature fluctuations and food availability. Animal experiments conducted since the end of the last century indicate that the toxicity of many environmental xenobiotics and drugs depends on the time of day of exposure. Recent studies have nailed down the molecular basis of circadian changes in sensitivity to xenobiotic exposure. It is well known that part of the transcriptome and proteome in animals undergoes cyclic changes in expression throughout the day, including many key genes involved in xenobiotic metabolism and transport. In addition, many xenobiotics can directly alter the expression of genes controlling circadian rhythms. Many studies have been undertaken toward pharmacological modulation of circadian components, which may represent a new and promising anticancer strategy. Similarly, knowledge of the relationship between circadian rhythms and occupational exposures may be important for the occupational hygiene and safety of various groups of workers, especially those working in continuous or shift work.

Key words: circadian rhythm, xenobiotics, bladder cancer, clock genes

Electroporation as a method to improve the assessment of the toxic effects of xenobiotics in a zebrafish model

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Abstract

Background. Assessing acute toxicity using zebrafish embryos (FET) has been a valuable tool in toxicological research. This model is unique due to various advantages, including its high compatibility with the human genome (70% similarity of genetic material), rapid development and uncomplicated and accessible research investigation. However, research conducted with large mass compounds, approximately higher than 3kDa, is much more difficult due to the chorion, which surrounds and protects the embryos in the early developmental stages. Several methods have been proposed to increase chorion permeation, including dechorionation or microinjection. However, these techniques are associated with significant limitations, especially a high percentage of embryo mortality in the first hours after fertilization.

Objectives. This study introduces an electroporation, which remarkably enhances the permeability of compounds through the chorion. This novel approach identifies potentially harmful effects of drugs/chemicals in the first hours of exposure, representing a significant advancement in our field.

Materials and methods. The electroporation method was developed to enhance the permeability of sparingly soluble compounds through the chorion. Extensive optimization was conducted to ensure that substances could pass through the chorion without harming the embryos. Our research revealed that electroporation using 20/10/3 (voltage/pulse time/number of pulses) parameters is the safest for embryos. S-thalidomide and curcumin, 2 substances with limited chorion permeability, were assessed to determine whether the proposed electroporation conditions are sufficient for chorion penetration.

Results. Pronounced and visible morphological variations were observed after thalidomide exposure with electroporation. In addition, the content of thalidomide in embryos was determined using voltammetric sensors. The amount of S-thalidomide after electroporation and incubation with S-thalidomide was much higher than in embryos without electroporation. In the case of curcumin, which is intensely yellow, the color of the embryos suggests its better penetration through the chorion. However, only a slightly more significant number of morphological changes were observed in embryos simultaneously subjected to electroporation.

Conclusions. This innovative approach, electroporation, holds significant promise in toxicology, particularly in assessing the toxic effect of compounds at early developmental stages.

Key words: zebrafish, electroporation, chorion permeability, toxicity

The effect of indoxyl sulfate-uremic toxin on the coagulation and fibrinolysis process in an animal model of thrombosis

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Conflict of interest
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Abstract

Background. Reduction of excretory kidney function is not only connected with increased risk of cardiovascular diseases, but also with retention of uremic toxins, especially indoxyl sulfate (IS). The IS is one of the most potent protein-bound uremic toxin that accumulates during CKD, and exerts aggressive and multidirectional effect on the body. Its concentration can be increased even by 80 times during renal insufficiency. Moreover, there are no currently available treatment options that allow to remove effectively IS.

Objectives. The purpose of the study was to assess influence of IS on hemostatic system as well as thrombus formation and clot firmness in animal models.

Materials and methods. The research was conducted using male Wistar Crl:WI (cmdb) rats and male C57BL6/cmdb mice in 2 stages. Firstly, we assessed IS impact on thrombotic process after acute exposure (intravenous administration). Further we examined IS influence after chronic exposure (IS was administered in 2 doses 100 mg/kg or 200 mg/kg of b.w./day in drinking water for 4 weeks). In addition, we performed thromboelastometric analysis, and evaluated IS impact on blood morphology and coagulation parameters. We also investigated IS impact on platelet activity, aortic contents of sirtuin 1 and 3, and parameters of hemostatic system.

Results. Obtained data indicate that IS creates prothrombotic state and accelerates thrombotic response. Indoxyl sulfate can enhance primary hemostasis leading to augmented formation of platelet plug through increased number and activity of platelets, and can affect secondary hemostasis through the influence on plasma coagulation and fibrinolysis, mainly by increased levels of complex tissue factor/factor VII, and PAI-1. Moreover, IS increased kinetics and strength of clot formation. Chronic exposure to IS also reduced aortic contents of sirtuin 1 and 3.

Conclusions. The findings described suggest that IS can be the one of crucial uremic factors promoting thrombotic events in patients suffering from CKD.

Key words: indoxyl sulfate, coagulation, fibrinolysis

Evaluation of chemicals for estrogen and (anti)androgen disorders

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Abstract

Background. In 2023, new hazard classes were introduced into the CLP regulation, i.e., endocrine disruptors in relation to human health or the environment. An endocrine disruptor is an exogenous substance/mixture that affects the function of the endocrine system producing adverse effects in a healthy organism and/or its progeny. The strategy and methods for evaluation of the substance are based on 5 tiers, where tier 3 includes the in vivo uterotrophic bioassay and the Hershberger bioassay.

Objectives. The aim of the study was to characterize changes resulting from exposure to reference substances.

Materials and methods. Studies were conducted according to OECD 440/441 guidelines. In the uterotrophic bioassay, 19-day-old female rats were divided into 5 groups of 6 animals each and were orally administered 17α-ethinylestradiol (0.3 µg/kg/day; 1 µg/kg/day; 3 µg/kg/day; 10 µg/kg/day) or corn oil (control group) once daily for 3 days. In the Hershberger test, castrated male rats aged 54–56 days were divided into 3 groups of 6 animals each. Once a day for 10 days, the animals were administered androgen (testosterone propionate, 0.4 mg/kg/day, s.c.) or both androgen and antiandrogen (flutamide, 3 mg/kg/day, per os) or corn oil (control group, per os). After the end of exposure, the animals were euthanized and their hormone-dependent organs/structures were collected and analyzed.

Results. In the uterotrophic bioassay wet and dry weights of uteruses were analyzed obtaining statistically significant and dose-dependent increase in animals exposed to doses of 1 µg/kg/day, 3 µg/kg/day and 10 µg/kg/day compared to the control group. The Hershberger test analyzed the weights of ventral prostate, seminal vesicle, levator ani-bulbocavernosus muscle, Cowper's glands and glans penis obtaining statistically significant differences in the weights of all androgen-dependent organs/structures compared to the control group. In both tests, the acceptance criteria were met.

Conclusions. Based on the obtained statistically significant differences in the weights of hormone-dependent organs, the estrogenic and (anti)androgenic effects of the used reference materials were confirmed meeting the tests acceptance criteria.

Key words: endocrine disruptors, uterotrophic/Hershberger bioassay

Toxicovigilance 2.0: Modern approaches for the hazard identification and risk assessment of poisons

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Conflict of interest
None declared

Abstract

Background. Toxicovigilance can be defined as toxicological prevention. Nowadays toxicological exposure is increasing which is why new tools are needed to help traditional methods of collecting and analyzing data used in poison centers to improve toxicological surveillance.

Objectives. Introducing the topic of toxicovigilance and presenting the modern approaches in toxicological monitoring.

Materials and methods. Material for preparing the work are generally available articles, regulatory and legal documents describing toxicovigilance from recent year, found using scientific search engines for keywords such as prevention, social network, toxicovigilance. The data from selected articles, analyzed and elaborated, enabled the formulation of conclusions.

Results. The development of technological thought creates an opportunity to use social networks to improve toxicovigilance. There is a correlation between the appearance of substance abuse among people using social networks and the content they post. Therefore, these data can be used to effective toxicological surveillance. The most helpful methods to extract key information are: natural language processing (NLP), analysis of the frequency of occurrence of specific words in the text, clustering, classification and analysis of the so-called n-grams.

Conclusions. At the time of increasing toxicological threats, methods of effective toxicovigilance are needed. Traditional methods supported by modern technologies will allow more accurate and faster assessment of toxicological exposure, as well as its prediction.

Key words: prevention, social network, toxicovigilance

The use of liquid chromatography coupled with triple quadrupole mass spectrometry for identifying the causes of acute poisonings

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Abstract

Background. Antipsychotic drugs (AP) represent a large group of drugs that exhibit significant differences in their mechanisms of action and impact on neurotransmitter systems. The clinical picture of acute AP poisoning includes many nonspecific, diverse symptoms, resulting in considerable diagnostic difficulties. Therefore, toxicological studies, enabling qualitative and/or quantitative determination of the drug, play a crucial role in AP poisoning cases.

Objectives. The aim of the study was to identify the cause of poisoning in patients suspected of AP overdose using the liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS). Qualitative analysis followed the isolation of analytes from patient biological samples. For this purpose, the Biotage Extrahera device was used for protein precipitation. Medical documentation allowed for the comparison of information obtained during the patient interview with the results of the toxicological analysis.

Materials and methods. The study included 61 patients admitted to the Toxicology Department of the Franciszek Raszeja Municipal Hospital in Poznań between March 25, 2023, and January 31, 2024. As the study material, serum/plasma samples collected from patients at the time of admission were used.

Results. AP poisoning was confirmed in 60 patients. Only in 1 case were no drugs detected in the patient's blood. Quetiapine was the most frequently identified substance, found in 67% of patients. In 85% of cases, not only was the drug intake declared in the medical documentation confirmed, but the analysis also detected the intake of many other drugs not declared by the patients.

Conclusions. The conducted analysis enabled the identification of drugs ingested by patients suspected of AP overdose, thereby confirmed the poisoning. However, due to the numerous additional medications detected in patient's blood, the analysis results did not correlate with interview data. This indicates the superiority of toxicological analyses over interviews and highlights the need for their conduction in cases of suspected acute drug poisoning.

Key words: antipsychotic drugs, acute poisoning, LC-MS/MS

Unveiling the power of antidotes: Exploring their versatile and overlooked applications

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None declared

Abstract

Background. Antidotes are often an underrated aspect of medicine and toxicology. Few specialists dedicate their attention to researching antidotes, even though they play a crucial role in saving lives and ensuring patient health. In emergency situations, where every second counts, the proper use of an antidote can mean the difference between life and death. Therefore, research and development in the field of antidotes require appropriate attention and greater commitment from the medical community. An antidote, commonly known as a countermeasure, has the ability to neutralize a poison or reduce its toxic effects, which are dangerous to health and life. Exposure to toxic substances increases year by year, making education and public awareness about first aid in cases of acute poisoning critically important.

Objectives. An overview of the most commonly used antidotes in the emergency treatment of acute poisonings.

Materials and methods. The materials were gathered from publicly available scientific articles, publications, and studies on websites such as PubMed, Google Scholar, Science Direct, and others, as well as from textbooks and scholarly works.

Results. In the case of acute poisonings, treatment involves the use of antidotes, along with decontamination and accelerating the elimination of the poison. Antidotes can be a key component of pre-hospital care, such as the use of atropine, oxygen therapy, glucagon, naloxone, or flumazenil. This is crucial for further therapeutic management and patient prognosis.

Conclusions. The diversity of xenobiotics poses a serious challenge in cases of acute poisonings. Only prompt medical intervention with the use of appropriate antidotes can mitigate or reduce the side effects of commonly encountered toxins and improve patient prognosis. The results were published in article: Kobylarz, D., Noga, M., Frydrych,. . .. Jurowski, K. (2023). Antidotes in Clinical – Toxicology Critical Review. *Toxics*, 11(9), 723.

Key words: antidote, antidotes, xenobiotics, poisoning

A driver with 'auto-brewery syndrome'

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Abstract

Auto-brewery syndrome (ABS), also known as gut fermentation syndrome (GFS), is an extremely rare and under-researched disease. The pathophysiology of ABS is probably caused by gut dysbiosis, which leads to the fermentation of certain carbohydrates into ethanol. Although it is rare, in a person with auto-brewery syndrome the concentration of endogenous ethanol may exceed the legal threshold for the post-use state and even for the intoxicated state. It may also show symptoms of alcohol poisoning with a noticeable, specific odor in the exhaled air. It should be borne in mind that drivers who are aware of the phenomenon of endogenous alcohol formation may use it as a method to avoid punishment. On the other hand, people who actually suffer from the disease may be unfairly punished for an act they did not commit. The report presents the case of a driver whose exhaled breath contained ethanol at a level of 2–3%, and such a high content was supposed to result from the consumption of carbohydrates, not from drinking alcohol.

Key words: autobrewery complex, endogenous ethanol

Retrospective clinical analysis of acute poisonings with antipsychotics and antidepressants among patients hospitalized at the Toxicology Department in Poznań

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Abstract

Background. The prescription of antipsychotic drugs and antidepressants has increased in recent times. Due to the multidirectional mechanisms of action, several drugs are often advised at the same time. This treatment system plays a significant role in both accidental and intentional poisonings.

Objectives. The aim of the presented study was to assess the clinical picture of the poisonings with antipsychotics and antidepressants.

Materials and methods. The retrospective study included 52 patients poisoned with drugs, hospitalized at the Toxicology Department between March 2023 and February 2024. The analysis was performed based on available medical records. Initial diagnoses were made in accordance with the ICD-10 classification.

Results. The study group included 39 women and 13 men. With the average age of the group being 36 years old. The initial diagnosis of T43 (poisoning with psychotropic drugs, not classified elsewhere) concerned 10 patients, which constitutes 19.2% of the entire group. The diagnosis of T43.5 (other and unspecified antipsychotics and neuroleptics) was made 15 times. In 26 cases, drug poisoning was accompanied by ethanol consumption. The most frequently used form of treatment was: administration of anticoagulants, infusions with electrolytes and glucose, and proton pump inhibitors. Gastric lavage was performed on 3 patients. The average hospitalization time was 4 days.

Conclusions. From 52 cases of poisoning with antipsychotic and antidepressants drugs, 24 patients took several drugs at the same time (46.2%). Administration of drugs in 37 cases was related to a suicide attempt (71.2%). Symptoms that frequently occurred were: shortness of breath, confusion, and dizziness. Twenty people were unconscious as a result of the poisoning. The most common score according to the PSS scale was 2 points (26 patients). In conclusion, there is a lack of epidemiological data on acute poisonings in Poland compared to global data.

Key words: acute poisoning, drugs, clinical toxicology

In search of optimal solutions in comparative analyses of the toxicity of xenobiotics using publicly available toxicogenomic databases on the example of the Comparative Toxicogenomics Database

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Abstract

Over the years and experience gained, toxicogenomics is becoming more and more useful in toxicological research, especially in the assessment of the risk of exposure to xenobiotics in the human living and working environment. Standardization of toxicogenomic tests, such as the DNA microarray test, allows for the creation of publicly available electronic databases for in silico research without the need to organize a toxicogenomic laboratory. One of the most extensive databases offered on the Internet is the Comparative Toxicogenomics Database (CTD) offered by the North Carolina State University (USA).

The authors of the presented work attempted to compare the risk of specific cancer and immunological diseases of environmental origin based on data obtained from CTD. An original algorithm was adopted to assess the similarity of the impact on the expression of selected genes to reference substances with proven toxic effects. The influence on expression was concluded based on the database resources in 4 directions: increasing gene expression, decreasing gene expression, ambivalent effects (increasing or decreasing gene expression under certain conditions) and no effect on expression. The percentage similarity of selected environmental factors to the reference substance was determined. The assessed diseases included: breast cancer, lung cancer, ulcerative colitis and psoriasis, and among the factors assessed were tobacco smoke components, synthetic food additives and cosmetic ingredients.

The authors additionally presented a simulation of data differentiation in the CTD database in terms of reliability and its impact on the final result of the analyses. Three parameters were taken into account: the organism or model (e.g., in vitro) used for the source experiments, the impact factor if of the journal publishing the results and the date of publication. During the simulation, 3 levels of data credibility were adopted: balanced (weighting coefficients in the range of 0.8–1.0), intermediate (coefficients 0.7–1.0) and restrictive (coefficients 0.6–1.0).

Key words: toxicogenomic databases, toxicity, xenobiotics

The problem of using psychoactive substances by hard coal miners during work: A case report of fatal combined α-PiHP and tramadol poisoning

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Abstract

Background. Working in an underground coal mine is undoubtedly associated with a lot of stress. This promotes the abuse of psychoactive substances – ethyl alcohol, but also medicines and narcotics, which is sometimes the subject of media reports. The scale of the problem in Poland is unknown. Drug tests are not routinely performed in mines.

Case report. A 40-year-old hard coal miner suddenly fell while working and lost consciousness, followed by a seizure. Sudden cardiac arrest occurred, which could not be restored by cardiopulmonary resuscitation. The forensic autopsy did not determine the cause of death. However, it allowed to exclude traumatic death and show few pathological changes. Toxicological analyses of blood and urine performed using liquid chromatography coupled with mass spectrometry showed, among others: the presence of α-PiHP (one of the most popular cathinone derivatives) at a concentration of 0.062 µg/mL and 0.163 µg/mL, respectively, the metabolite of α-PiHP (OH-α-PiHP) at a concentration of 0.080 µg/mL and 1.50 µg/mL, and tramadol at concentrations of 1.60 µg/mL and 15.9 µg/mL (toxic concentration in blood). Finally, the forensic pathologist determined that the man's sudden death was the result of acute cardiorespiratory failure, which developed in the course of acute poisoning with the combination of tramadol and α-PiHP.

Conclusions. In the case of hard coal miner's death, it is always justified to collect biological material for toxicological tests and to order analyses for the presence not only of ethyl alcohol, but also of medicines and narcotics. This may be crucial to determining the cause of death and for post-accident investigation in the workplace.

Key words: hard coal mining, drugs of abuse, medicines, poisoning

Development of direct, non-invasive and rapid methods for determining chosen toxic elements using pXRF (portable X-ray fluorescence spectrometer) in bones for forensic laboratory toxicology purposes

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Abstract

Background. Bones are rarely the subject of toxicological tests, although they can provide valuable forensic information about exposure to toxic elements, which is important especially in the absence of other evidence. Current methods of analyzing elements in bones, such as ICP-MS or AAS, are invasive and destroy evidence, and also require complicated sample preparation. Portable Xray fluorescence (pXRF) offers a non-destructive, fast and low-cost alternative.

Objectives. The aim of our research was to develop a non-destructive method for the analysis of toxic elements in bones using a portable X-ray fluorescence spectrometer (pXRF). This method allows direct in situ analysis, without the need to prepare the sample, which allows the evidence to be kept intact.

Materials and methods. A portable X-ray fluorescence spectrometer (pXRF) Thermo Fisher Scientific Niton XL3t 950GOLDD+ purchased by the Institute of Medical Expertise in Łódź was used for the research. An in situ analysis method using pXRF was developed for the analysis of heavy metals and selected toxic elements in bones. In order to improve the quality and accuracy of the analyses, correction of spectrum fluctuations and fundamental parameters (FP) was used based on hydroxyapatite and calcium phosphate standards created using the pressing technique with a Hercules40t hydraulic press. Additionally, hydroxyapatite was synthesized with the addition of a standard of appropriate concentration to better mimic the actual bone structure. A specific analytical calibration procedure was also developed that enabled precise analysis of the elemental composition of bones using pXRF.

Results. We created patterns from hydroxyapatite and calcium phosphate with a diameter of 32 mm using the pressing technique. The calibration standards showed increasing concentrations of the tested elements within the range most commonly found in actual human bone samples described in the scientific literature. Moreover, we synthesized hydroxyapatite with the addition of a standard of appropriate concentration, which allowed us to take into account signal fluctuations caused by the matrix structure.

Conclusions. The research allowed the development of effective methods for in situ toxicological analysis of elements in bones using pXRF. It has been shown that the pXRF technique, supported by appropriate calibration standards and advanced spectral analysis methods, can provide precise and accurate results in a short time, without destroying samples.

Key words: X-ray fluorescence (pXRF), laboratory forensic toxicology, toxic elements, white analytical chemistry (WAC), bones

Challenges in liquid mercury spill incidents: Current status and strategies using specialized kit for mercury absorption

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Abstract

Background. Mercury remains a significant concern in various environments despite increased efforts to restrict its use. While mercury thermometers have been largely phased out and replaced with safer alternatives, they are still available in pharmaceutical archives and storage facilities. Additionally, a wide array of measurement instruments containing mercury are still in operation, particularly in hospitals and laboratories. These devices, including sphygmomanometers, barometers, and certain types of pressure gauges, continue to pose a risk due to the hazardous nature of mercury.

Objectives. The primary objectives of this study are to develop and validate an effective procedure for the disposal of liquid mercury using a commercially available specialized disposal kit.

Materials and methods. This procedure for mercury spill cleanup was performed using the Chemizorb® Hg kit, following the manufacturer's instructions with some modifications based on our observations.

Results. We have developed a modified procedure for securing mercury, which is a cheaper but equally effective method of neutralizing and utilizing this element. The proper utilization of the Chemizorb® Hg kit with our modifications included is represented in the form of video material.

Conclusions. These findings allow for the development of effective methods to properly utilize commercially available mercury disposal kits. By understanding the precise mechanisms and best practices for mercury containment and disposal, we can ensure that these kits are used to their fullest potential. This not only enhances environmental safety by reducing mercury contamination but also protect public health by minimizing exposure to this toxic element.

Key words: mercury poisoning, mercury exposure, mercury toxicity, neutralization

Non-harmonized temperament as a factor in worse cognitive but not executive functioning in alcohol-related cerebral atrophy

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Abstract

Long-term alcohol abuse duration is related to structural changes in the brain, leading to cortical and/or subcortical atrophy. However, cerebral atrophy is also observed in patients with Korsakoff syndrome and Wernikce's encephalopathy; specific structural changes are found in their brains (especially in the frontal lobes, corpus callosum, thalamus, and mammillary bodies). General cortical and subcortical atrophy can be observed in alcohol-related dementia, but the role of non-harmonized temperament (with dysfunctions of emotional reactivity and activity) in cognitive dysfunction in alcohol-dependent men is not well-known. The structure of the emotional response mode in alcohol-dependent men was considered in the light of Jan Strelau's regulatory theory of temperament. One of the most important goals of our research was to evaluate temperament structure as a moderator of the relationship between structural changes in the brain and the efficiency of cognitive and executive functions. One hundred and 3 men with alcohol use disorder were examined by means of screening, neuroimaging, and neuropsychological methods. Our research showed the moderating role of temperament structure in the relationship between gradual cerebral atrophy and cognitive decline, but not in the relationship between gradual cerebral atrophy and executive functioning impairment. Among alcohol-dependent men with a non-harmonized temperament structure (and a high degree of nicotinism), there is a stronger correlation between subcortical or general cerebral atrophy (increased volume of cerebrospinal fluid in the whole brain and/or in the ventricular system) and worsening cognitive functioning (impaired memory, learning efficiency, and overall level of cognitive functions). On the other hand, in patients with harmonized temperament, the associations between cerebral atrophy and cognitive or executive functioning are not significant. These results should contribute to the development of dependence research and may be useful in the diagnosis and treatment of dependencies and other mental and behavioral disorders that co-occur with abnormal alcohol use.

Key words: alcohol-related cerebral atrophy, temperament, alcohol

I would rather die than withdraw: Addressing the opioid epidemic

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Abstract

Over the past decade, the use of opioids worldwide has climbed. According to the United Nations Office on Drugs and Crime in 2020, 1.2% (61 million) of the global population engaged in opioid use with opioids accounting for 2/3 of drug-related deaths. In the USA alone, the Centers for Disease Control and Prevention (CDC) attribute 645,000 deaths to opioid overdose between 1999 and 2021. While the USA has seen a decrease in use over 2023, the use of opioids continues to be alarming. The state of Ohio opioid related deaths is nearly double the average of the USA with fentanyl overdoses accounting for 98% of the drug-related deaths in 2023. In response to the ongoing opioid crisis the National Institutes of Health (NIH) and National Institute on Drug Abuse have invested millions of US dollars to research intervention approaches. These efforts have focused on multistate initiatives utilizing a community-based participatory research approach for intervention and prevention in opioid use.

Following suit, Poland has experienced increased opioid use. This presentation will discuss the trend in the opioid epidemic in the USA and the current intervention and prevention efforts to combat opioid-related deaths by describing the findings along with implications of the collaborative efforts to remedy this problem. At Case Western Reserve University (CWRU) in Cleveland, Ohio, located in the USA, 2 research initiatives focus on managing the opioid epidemic. First the NIH Helping to End Addiction Long-term (HEAL) initiative is a multi-state collaborative community participatory research initiative with the goal of reducing opioid overdose deaths particularly within marginalized populations. With a research team based out of CWRU, HEAL has resulted in ongoing community-based initiatives. The Begun Center, also a part of CWRU, has been instrumental in the scientific response to the opioid epidemic in conducting several intervention studies. Results from the Begun Center include public health efforts involving law enforcement and other social welfare stakeholders. Common actions of both sites at CWRU include harm reduction, education, and collaboration with healthcare facilities and first responders. Implications from these initiatives include collaborative efforts to address the opioid epidemic from federal, state, and local stakeholders along with education dissemination for harm reduction leading to preliminary outcomes of decreased opioid-related deaths.

Key words: opioids, fentanyl, prevention, intervention, harm reduction

Preliminary evaluation of selected parameters reflecting carbohydrate disorders in people living with HIV in the aspect of antiretroviral therapy

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Abstract

Background. Thanks to significant progress in the therapy of HIV infection, it has become a chronic disease. However, the need for constant use of drugs in combined antiretroviral therapy (cART) is unfortunately associated with an increased risk of developing of accompanying metabolic disorders, especially carbohydrates.

Objectives. The aim of the study was to assess 4 non-routine parameters related to carbohydrate metabolism: irisin (IRS), myostatin (MSTN), glucagon-like peptide 1 (GLP-1) and fetuin-A (FETU-A) in men living with HIV (MLWH) undergoing 5 years of cART compared to people not infected with HIV. The obtained parameter results after 5 years of cART were compared with the results from before therapy and after 1 year of cART.

Materials and methods. The research included 30 MLWH (average age 40 years), under the care of the Center for the Prevention and Treatment of Infectious Diseases and Addiction Therapy in Wrocław and the Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiencies of the Wroclaw Medical University, and 25 HIV-uninfected men of similar age, constituting the control group. Two treatment regimens were used in MLWH. The parameters were determined in plasma using the enzyme immunoassay method.

Results. Statistically significantly higher levels of IRS and MSTN and lower FETU-A levels were found in the MLWH group compared to the control group. Higher, although statistically insignificant, levels of parameters were demonstrated in the MLWH subgroup treated with NRTI (nucleoside reverse transcriptase inhibitors) +INSTI (integrase inhibitors). There were significant increases in IRS, GLP-A and FETU-A levels over 5 years of cART in MLWH.

Conclusions. Preliminary studies may indicate the usefulness of the determined parameters in the assessment of carbohydrate metabolism in the aspect of cART used. However, the topic requires further research to understand the role of selected parameters in the carbohydrate disorders in MLWH.

Key words: HIV, combined antiretroviral therapy, parameters of carbohydrate metabolism

Liquid chromatography–tandem mass spectrometry method for the determination of antibaterial drugs in plasma samples

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Abstract

In toxicological and pharmacological research, one of the most frequently used materials is plasma. Therefore, the method of determining antimicrobials in plasma using liquid chromatography–tandem mass spectrometry (LC-MS/MS) was developed. This method allows for the determination of antibacterial drugs from the groups of beta-lactams, tetracyclines, fluoroquinolones, pleuromutilins, macrolides and sulfonamides. Optimized extraction method uses ammonium acetate with pH = 4.5, methanol and acetonitrile. To obtain better recovery, the plasma sample was concentrated by evaporation in a stream of nitrogen and then dissolved in the mixture of mobile phases. The mobile phases were 0.2% formic acid in water and 0.1% formic acid in acetonitrile in gradient mode, and the analytes were separated using a C18 analytical column. This method has been validated and satisfactory results for the linearity, precision and recovery were obtained. Optimized method will be used to study changes in the pharmacokinetics of the most commonly used antibacterials in pigs exposed to deoxynivalenol. Pharmacokinetic studies generate a large number of samples to be examined, so a short sample preparation and chromatography analysis time will help in efficient material testing.

Key words: LC-MS/MS, antimicrobials, plasma, method validation

The development of LC-MS/MS method for determination of 6 ionophores in liver tissue

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Abstract

Background. Ionophores are commonly used to prevent coccidiosis in various animal species, including chickens and turkeys. Liquid chromatography-tandem mass spectrometry is the most commonly used technique for monitoring of residues in animal tissues. Due to ionophores' high potential toxicity, and to ensure the safety of food of animal origin, it is crucial to develop new methods for ionophore identification and quantitation.

Objectives. We aimed to develop an LC-MS/MS method for the simultaneous determination of 6 ionophore coccidiostats (lasalocid, maduramicin, monensin, narasin, salinomycin, semduramicin) in liver tissue.

Materials and methods. The sample preparation was based on extraction with acetonitrile and clean-up on silica columns. The separation of analytes was conducted using Synergi™ 4 µm Fusion-RP 80 Å, 50 × 2 mm column (Phenomonex) with a gradient of acetonitrile and ammonium formate. Two precursor ions were selected for each analyte: sodium adduct $[M+Na]^+$ and ammonium adduct $[M+NH_4]^+$, each with at least 2 product ions. Blank liver and muscle tissues were spiked with ionophores at levels corresponding to 1, 5, 20, and 100 µg per kg of tissue pre- and post-extraction. Calibration curves were prepared by the injection of mixed standard solutions at the same concentration levels.

Results. The method was found sensitive (LOD below 0.5 µg/kg) and selective. For the ammonium adduct $[M+NH_4]^+$, the values ranged from 20.3% (lasalocid) to 66.1% (semduramicin); from 20.3% (lasalocid) to 66.1% (semduramicin); from 28.2% (salinomycin) to 151% (monensin); from 13.0% (lasalocid) to 35.2% (monensin), for recovery, matrix effects and accuracy, respectively. Regarding the sodium adduct $[M+Na]^+$, the values varied from 23.6% (narasin) to 154% (semduramicin); from 30.7% (salinomycin) to 143% (monensin); from 13.2% (lasalocid) to 66.0% (semduramicin), for recovery, matrix effects and accuracy, respectively.

Conclusions. Due to high matrix effects, the method requires calibration curve preparation using standard solutions. The differences between specific ions need to be further studied.

Key words: ionophores, LC-MS/MS, ammonium adduct, sodium adduct

The devil is not as black as he is painted: Prediction of acute toxicity and environmental fate (hydrolysis and biodegradation) of the Novichoks organophosphorus nerve agents using in silico toxicology methods

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Conflict of interest
None declared

Abstract

Background. Novichok is a class of organophosphorus chemical warfare agents characterized by extreme toxicity. Following their use in Salisbury, a broad public debate on their safety ensued, leading to a greater understanding of their nature and potential threat. It is crucial to study their toxic properties and environmental fate from a public safety perspective. The list of hypothetical structural formulas for Novichoks is extensive, and their high potential toxicity and reactivity make experimental studies for each of them extremely labor-intensive and risky.

Objectives. The aim of the study was to apply selected in silico toxicology methods to predict acute toxicity (rat, oral administration) and extrapolate to humans, as well as predict the environmental fate (hydrolysis and biodegradation) of selected Novichoks (n = 17).

Materials and methods. The QSAR Toolbox and T.E.S.T software, recommended by OECD and US EPA, were used to fill data gaps on the toxicity of chemical compounds. HYDROWIN and BIOWIN models, integrated into the EPI Suite package developed by US EPA and Syracuse Research Corporation, were also used to support in silico analyses. These models are based on the group contributions method and have been validated using independent sets of reference substances (structural analogs meeting in silico validation requirements).

Results. The results for acute toxicity showed that the most toxic Novichoks were A-232 (0.21 mg/kg), A-230 (0.35 mg/kg) and A-234 (0.58 mg/kg). The least toxic were the "Iranian" Novichok (178.96 mg/kg) and C01-A038 (310.04 mg/kg). Regarding environmental fate, the study results indicate that Novichoks released into the environment hydrolyze at varying rates – from very fast (less than 1 day) to very slow (over a year). Most of the studied compounds underwent complete biodegradation within a few weeks to several months. The biodegradation predictions classify these compounds as relatively difficult to biodegrade.

Conclusions. The use of in silico toxicology methods to predict acute toxicity and environmental parameters provided results for supertoxic substances without using laboratory animals. These studies provide significant information on the prediction of acute toxicity, hydrolysis rate, and biodegradation, which would be impossible in this case due to experimental challenges.

Key words: Novichok, organophosphorus compounds, nerve agents, chemical warfare agents, in silico

Determination of ciprofloxacin in *Lucilla sericata* larvae using capillary electrophoresis combined with mass spectrometry

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Abstract

Substances derived from insects can be used for therapeutic purposes due to their diverse biological properties. In this paper, special attention is paid to the species *Lucilia sericata* and the benefits of larval therapy in patients who, as a result of hospitalization, have developed pressure ulcers and other difficult-to-heal wounds.

Entomotoxicology relies on toxicological analyses to identify xenobiotics that might be present in the body of a deceased person and have been consumed by necrophagous insects along with tissues. Nevertheless, this is just one application of insects in the field of entomology. In addition to therapeutic use, larvae may be a useful alternative material not only for entomotoxicological studies to detect substances ingested at toxic doses, but also at therapeutic doses. The present work describes a procedure for assaying of ciprofloxacin in *Lucilia sericata* larvae obtained from a patient receiving this antibiotic during larval therapy using capillary electrophoresis coupled with mass spectrometry.

The method has been optimized and validated, taking into account the voltage selection, the method of conditioning the capillary, the volume of the dosed sample and the quadrupole operation mode. Validation of the method included determination of precision, repeatability, accuracy, recovery, LOD and LOQ. The analytical procedure was linear for concentrations ranging from 100 ng/mL to 1,000 ng/mL with determination coefficients higher than 0.9976 for all the analytes. An optimized procedure for assaying of ciprofloxacin using capillary electrophoresis combined with mass detection allowed the determination of the antibiotic in the larval material. Referring to the conclusions obtained, ciprofloxacin was identified in the test sample.

The compound migrated within 10 min and its concentration calculated from the regression curve formula was 750 ng/g. The presence of ciprofloxacin in the larvae sample is due to the fact that the patient received an antibiotic 500 mg twice daily during larvae therapy, corresponding to a ciprofloxacin plasma concentration of 1.5–2.9 µg/mL. Thus, ciprofloxacin has been shown to penetrate tissues, which was associated with the detection of antibiotic in larval material.

Key words: entomotoxicology, *Lucilia sericata*, ciprofloxacin, capillary electrophoresis, mass spectrometry

Immunohistochemical assessment of the expression of CYP1A1, CYP2A13 and CYP2E1 enzymes in lung cancer tissues of smoking patients

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Conflict of interest
None declared

Abstract

Background. Smoking is the most important risk factor for lung cancer. About 90% of patients with this cancer smoke or have smoked cigarettes in the past. Additionally, over 60 carcinogenic compounds have been identified in tobacco smoke, and most of these compounds require metabolic activation, which occurs with the participation of cytochrome P450 enzymes, and in the case of the metabolic activation of tobacco smoke components, enzymes such as CYP1A1, CYP2A13 and CYP2E1. These enzymes have been suggested to be important in carcinogenicity and respiratory toxicity, but their expression in lung cancer tissues remains to be determined.

Objectives. The aim of the study was to assess the precise localization and intensity of expression of CYP1A1, CYP2A13 and CYP2E1 enzymes in tumor and peri-tumor tissues of the lungs of smoking patients.

Materials and methods. The study group consisted of 50 patients with lung cancer qualified for surgical treatment at the Lower Silesian Center for Oncology, Pulmonology and Hematology (KB No. 456/2023). Immunohistochemical localization of CYP1A1, CYP2A13 and CYP2E1 enzymes was performed using the Agilent Dako EnVision FLEX Kit and monoclonal antibodies. Enzymes expression was assessed using H-score values.

Results. In cancer tissues from smoking patients, enzyme expression was strong and moderate, while in peri-tumor tissues and tissues from non-smokers, enzyme expression was moderate, weak or absent. For all 3 enzymes, the average H-score values in smoking patients were statistically significantly higher than the average values in non-smoking patients and peri-tumor tissue.

Conclusions. The increased risk of cancer development in tobacco smokers is associated with the expression of CYP1A1, CYP2A13 and CYP2E1 enzymes responsible for the metabolism of xenobiotics in cigarette smoke. High enzyme expression in smoking patients may be associated with lung cancer progression.

Key words: lung cancer, smoking, enzymes, CYP1A1, CYP2A13, CYP2E1

The impact of exposure to tobacco smoke on selected metabolic and hormonal parameters in the serum of women with polycystic ovary syndrome

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Conflict of interest
None declared

Abstract

Background. Exposure to tobacco smoke has significant implications for the endocrine system. It was well documented that smoking exacerbates metabolic syndrome and hyperandrogenism in women with polycystic ovary syndrome (PCOS). One of the potential etiological factor of PCOS is abdominal obesity, which through the action of secreted adipokines, can influence hormonal and metabolic status.

Objectives. This study aims to investigate selected metabolic and hormonal parameters, including apelin, chemerin, and lipocalin-2 concentrations, in the serum of non-smoking and smoking women suffering from PCOS.

Materials and methods. The study was conducted on the serum of 60 women with PCOS, of whom 37 were non-smoking and 23 were smoking. Selected metabolic and hormonal parameters were conducted during hospitalization at the Department of Endocrinological Gynecology in 2022, while the concentrations of cotinine, apelin, chemerin, and lipocalin-2 were assayed using commercial tests.

Results. The mean values of age and BMI of the women were comparable in both non-smoking and smoking subgroups. We observed significant changes in the concentrations of high-density lipoprotein (HDL-C), sex hormone binding globulin (SHBG), vitamin D3, and the free androgen index (FAI) between non-smoking and smoking women. Among the investigated peptide hormones, significant changes between the studied groups were found only in the case of lipokalin-2. The correlation coefficients between the concentration of cotinine and SHBG, HDL-C or vitamin D3 levels were negative, while those with the concentration of fasting glucose or the value of FAI were positive. In case of peptide hormones, only the concentration of lipokalin-2 was significantly correlated with cotinine concentration, while apelin concentration was not significantly associated with any assayed parameters.

Conclusions. Smoking exacerbates adverse metabolic and hormonal profiles in women with PCOS. The correlation of lipocalin-2 with cotinine concentration further underscores the specific endocrine disruptions associated with tobacco exposure, while chemerine or apelin did not correlate with cotinine concentration, indicating a selective impact of smoking on certain hormonal pathways.

Key words: polycystic ovary syndrome, tobacco smoke, metabolism, hormones

Virtual odyssey of AP-238 fate in the body (ADME): Application of selected in silico toxicology methods for rapid and reliable prediction of new psychoactive substance fate in the body on the example of AP-238

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Abstract

Background. AP-238 (1-(4-cinnamyl-2,6-dimethylpiperazin-1-yl)propan-1-one) is a synthetic opioid classified as a new psychoactive substance (NPS). It emerged due to restrictions on fentanyl analogs, leading to the creation of "legal" opioids like AP-238. This substance poses a significant risk due to its high potential for causing respiratory depression. Its unique chemical structure impacts its toxic properties. Given the lack of knowledge about its behavior in the body and the increasing risks, predicting absorption, distribution, metabolism, and excretion (ADME) using in silico methods is essential and justified.

Objectives. The study aimed to estimate the ADME profile of AP-238 using selected in silico toxicology methods relevant to clinical and forensic toxicology. Specific parameters were determined for:

- A (Absorption): logP, logD, water solubility, gastrointestinal absorption, bioavailability, passive diffusion;
- D (Distribution): plasma protein binding (PPB), log Ka, unbound fractions, volume of distribution, blood-brain barrier (BBB) permeability;
- M (Metabolism): inhibition and substrate for CYP450 3A4, 2D6, 2C9, 2C19, 1A2; potential phase I (N-dealkylation, epoxidation, oxidation, reduction, hydrolysis) and phase II (conjugation with UGT, glutathione, DNA) reactions;
- E (Excretion): renal clearance, substrate for OCT2 transporter.

Materials and methods. To predict the ADME profile of AP-238, 4 specialized software tools were used: ACD/Labs Percepta (licensed by the Institute of Forensic Research in Łódź), pkCSM, XenoSite, and SwissADME.

Results. Absorption parameters suggest high oral bioavailability. Reduced water solubility at higher pH levels may affect its bioavailability across different body compartments. AP-238 shows moderate plasma protein binding (78.25%), influencing its distribution. Distribution analysis revealed that AP-238 can cross the BBB. Metabolism predictions indicate that AP-238 is a substrate for CYP450 enzymes, suggesting potential interactions with other drugs metabolized by enzymes. Phase I and II metabolic pathways were also hypothesized. Excretion parameters suggest that AP-238 is primarily eliminated via the kidneys.

Conclusions. ADME predictions for AP-238 indicate high bioavailability, effective BBB penetration, and complex metabolism. The results suggest significant interactions with substances metabolized by CYP450 enzymes, which should be considered in clinical and legal contexts. In silico methods provided quick and reliable preliminary ADME parameters, crucial for further toxicity studies of AP-238.

Key words: NPS, AP-238, in silico, ADME

Research strategy in the assessment and identification of endocrine-disrupting chemicals

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Abstract

The continuous economic development of the world leads to the production of new chemical substances used in various areas of life and everyday products. According to Endocrine Society, 1,000 of 85,000 human-made chemicals may act as endocrine disruptors. Endocrine disruptor (ED) is the exogenous substance/mixture that affects the function of the endocrine system producing adverse effects in a healthy organism and/or its progeny.

For hazard identification and assessment a multi-level toxicological testing strategy is used to assess chemicals for endocrine disrupting functions. Procedures related to the evaluation and identification of chemicals (potential ED's) are divided into 5 levels in accordance with OECD No. 150.

Firstly, in silico (Level 1) methods are used to search for existing data that can help characterize chemicals and predict their potential physical and/or structural capacity for endocrine disruptions. Selection of the most appropriate tests has to be done on a case-by-case basis, considering the need to minimize animal testing. In order to properly assess endocrine disrupting effects in vitro (Level 2) tests should be used. OECD 456 method is performed firstly to measure ED effect on estradiol or/and testosterone production. Obtaining positive results at OECD 456 obliges to extend in vitro testing and involve 3 other methods OECD 493, OECD 455 and/or OECD 458 connected with estrogen and/or androgen receptor binding disruption. The further step in ED in vitro assessment should be thyroid-hormone disruption pathway. Thyroid is an important organ and thyroid's ED may have the negative impact on neuronal and physical health and development. Despite the procedures for identification, thyroid disrupting chemicals are developed and validated but the OECD test guidelines are not introduced yet. New methods for different ED's targets should be still introduced and improved. Further Levels 3–5 related to ED's identification are connected with in vivo methods.

Key words: endocrine disruptor, alternative methods, cell culture

Analysis of the presence of pesticide substances in the Pilica catchment area and their potential toxicity

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Abstract

Pesticides, widely used to control pests, weeds, and plant diseases, enter rivers and lakes mainly through surface runoff from agricultural land. This causes pollution and negatively impacts aquatic organisms, disrupting food webs and reducing biodiversity. However, most pesticides used in agriculture are not monitored in aquatic ecosystems by EU Member States. This study aimed to monitor pesticides in the Pilica River catchment seasonally and assess their potential toxicity to gauge ecological risk.

Samples were taken in 2022 (June, September, December) at 35 points and in 2023 (August, November) at 43 points in the Pilica catchment. Monitoring included in-situ physico-chemical analyses and pesticide detection using liquid chromatograph coupled to a mass spectrometer (LC-MS/MS), which can identify 95 pesticide substances. Results showed significant seasonal variations, with the highest concentrations in spring and summer due to intensive agriculture. High levels were found in the river's upper section, decreasing downstream, suggesting the river's potential for self-purification.

Table 1. Data showing the potential toxicity of pesticides on selected model organisms (data taken from Pesticide Properties DataBase)

	EC50 (Daphnia magna [mg/dcm^3], 48 h	Birds (Anas platyrhynchos) [mg/kg]	Fish (Oncorhynchus mykiss) [mg/L], 21 days	LD50 (rat) [mg/kg]	EC50 (Aquatic plants) [mg/dcm^3], 7 days, biomass	EC50 (Algae Pseudokirchneriella subcapitata) [mg/L], 72 h, growth
The most toxic	0.0116 (chlorantraniliprole)	98 (acetamiprid)	0.0032 (carbendazim)	146 (acetamiprid)	0.0247 (pethoxamid)	0.0024 (terbutryn)
The last toxic	>200 (mecoprop)	>4640 (terbutryn)	109 (mecoprop)	>10 000 (chlorotoluron and carbendazim)	40.2 (mecoprop)	>149 (pethoxamid)

The research was carried out as part of the LIFE PILICA project (Implementation of River Basin Management Plan in the Vistula basin on the example of Pilica river catchment; Contract No. LIFE19 IPE/PL/000005 – acronym IP LIFE PL Pilica Basin CTRL) and as part of a Student Research Grant funded by the Faculty of Biology and Environmental Protection of the University of Lodz.

In 2023, a report was published showing that the Rykolanka River, a left-bank tributary of the Pilica River in the Masovian Voivodeship, has the highest concentrations of the metabolite AMPA in Europe. Databases (PubChem and Pesticide Properties Database) were reviewed to determine the potential toxicity of pesticide compounds detected in the Pilica catchment to various model organisms: zooplankton; mammalian model; bird; fish; aquatic plants and algae. The compounds posing the greatest risk were shown to vary according to the model organism (Table 1). For some models, adequate toxicological studies with the analyzed substances have not yet been conducted. The results indicate the need for more detailed studies of surface water pollution by pesticide substances.

Key words: pesticide substances, surface water quality, agriculture, pollution

Wastewater-based epidemiology study to assess illicit drug use in Greater Poland Voivodeship

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Abstract

Background. Wastewater-based epidemiology (WBE) is based on the assumption that the amounts of illicit drugs and their metabolites present in raw wastewater reflect the use of drugs by the population of a given area. In contrast to indirect methods, like population surveys and police statistics, the WBE provides objective data that covers the entire communities and enables monitoring near-real time trends in drug consumption. Due to encouraging results and advances in analytical techniques, allowing for determination of excreted drugs at very low concentration levels, the use of WBE approach for mapping illicit drug use in urban populations has become popular in many countries in the world.

Objectives. The aim of the research was to apply the WBE approach for the determination of illicit drugs in wastewater samples collected in cities located Greater Poland Voivodeship, Poland.

Materials and methods. Raw wastewater samples were subjected to a multi-step preparation procedure consisting of addiction of internal standard mixture, filtration, and solid-phase extraction. Determination of illicit drugs and their metabolites were conducted using high-performance liquid chromatography coupled to tandem mass spectrometry with triple quadrupole mass analyzer. The method covered ten analytes, including amphetamine and their derivatives, metabolites of cocaine and tetrahydrocannabinol, and new psychoactive substances (ketamine and mephedrone).

Results and conclusions. The research showed that the profiles of illicit drug consumption determined for cities located in the Greater Poland Voivodeship were generally similar; however, some differences could be noticed. The most abundant illicit drug residues found in wastewater samples were amphetamine and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol. WBE provided valuable data and can serve as an additional data source for monitoring the trends in drug abuse. Future investigations should include the cities from other regions of Poland to study spatial differences in illicit drug consumption in the country.

Key words: wastewater, drugs of abuse, liquid chromatography–tandem mass spectrometry

Are environmental phenols capable of migrating into the follicular fluid?

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Conflict of interest
None declared

Abstract

Background. The success of the fertilization process is strongly influenced by the presence, or lack thereof, of essential biomolecules in the microenvironment of oocyte development, known as follicular fluid (FF). Hence, the presence of emerging pollutants in FF can alter the concentration of those biomolecules or mimic their actions.

Objectives. The study aimed to compare concentrations of 14 common phenols in urine and FF samples of women attending a fertility clinic. In addition, it was verified whether urine, as a more easily accessible specimen, can be used to predict the FF concentrations of the targeted compounds.

Materials and methods. Two groups of women were enrolled in the study – subjects with unknown cause of pair infertility and controls with pair infertility diagnosed on the male side. Urine and FF samples collected from each participant were analyzed with optimized and validated SPE-GC-MS/MS methods. Free and total concentrations in FF samples and total urinary concentrations were subjected to statistical analysis.

Results. Detection frequency greater than 50% was observed for total concentration of 8 and 3 phenols in urine and FF samples, respectively. However, only methylparaben in its free form was detected in more than 50% of FF samples. Free and total methylparaben concentrations in FF were significantly correlated ($r = 0.533$, $p < 0.0001$). Differences in concentrations between subjects and controls were statistically significant only in case of benzophenone 3, with lower median estimated for subjects. Also, weak correlations between total paraben concentrations in urine and FF samples were observed.

Conclusions. This study proved that some environmental phenols are capable to migrate into the FF. However, the similar results obtained for both groups, may suggest that there is no correlation between environmental exposure to studied compounds and women fertility. Further research is needed to confirm above observations.

Key words: endocrine disrupting compounds, female reproductive health, follicular fluid

Assessment of the relationship between bone mineral density, bone turnover markers and Cd concentration in blood and urine of women from the Łódź macroregion

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Abstract

Background. Chronic cadmium (Cd) toxicity is a serious health problem, and the mechanism of its long-term exposure to bone mineral density (BMD) has not been fully elucidated as yet.

Objectives. The study aimed to assess the association between BMD, bone markers remodeling and formation, and renal function and long-term environmental exposure to Cd in women from the Łódź macroregion.

Materials and methods. The study population consisted of 74 women residing for more than 15 years in the Łódź macroregion with known bone disorders treated at an osteoporosis clinic and healthy women without bone lesions. Patients were assessed for BMD using *T* score, markers of bone formation (bone-specific alkaline phosphatase (BALP) and bone resorption (tartrate-resistant acid phosphatase (TRAP)). Urine Cd concentration (UCd) and blood Cd concentration (BCd) were determined. Renal function was assessed by serum creatinine concentration and eGFR.

Results. In women with *T* score values indicating osteopenia and/or osteoporosis (<–1), UCd levels, but not BCd, were significantly higher (twofold) compared to the group without bone disorders. Also in the group with osteopenia, the mean eGFR was statistically significantly lower compared to the group without osteopenia. There was a statistically significant correlation between UCd levels and serum bone resorption marker (TRAP) levels which may suggest a direct effect of Cd on bone loss. No such correlation was shown for the bone formation marker (BALP).

Conclusions. Our study suggests that environmental exposure to low-dose Cd may be an important risk factor for the development of osteopenia, whilst measuring UCd concentration, rather than BCd, appears to be a reliable marker. Further studies are needed to assess whether the risk of reduced BMD by Cd is related to renal function or its direct effect on bone resorption.

Key words: cadmium, women, bone, bone mineral density

Unlocking effective first aid strategies for human envenomation caused by marine animal species

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Conflict of interest
None declared

Abstract

Background. Marine envenomation is the serious global concern that affects both local residents and tourists in coastal regions. Symptoms of marine envenomation are various, depending on the type of marine organism involved and the severity of the envenomation – from mild local reactions to life-threatening systemic manifestations. Moreover, the toxin present in the victim's body can cause delayed complications, which may lead to severe multi-organ failure.

Objectives. The aim of this study is to raise awareness about the problem of the marine envenomation and to present first aid methods in such cases described in the literature.

Materials and methods. When conducting literature search, we utilized PubMed and Google Scholar, employing various combinations of keywords such as 'marine envenomation', 'hot', 'hot water', 'ice', 'ice packs', 'cold', 'cold water', 'treatment', and 'first aid'. Initially, we screened titles and abstracts for relevance, followed by a thorough evaluation of complete texts to ensure inclusion of reliable information.

Results. Treatment of the marine envenomation mainly focuses on alleviation of symptoms and prevention of later complications. The choice of the proper strategy depends on the species of marine organism, as well as on the severity of the sting and the location of the injury. Hot water immersion (HWI) is the most common first aid method – it involves immersing affected body part in hot water for a period of time. Another often used technique is applying the cold compress to the injured area – the ice pack treatment (IPT). Both of these methods are believed to provide pain relief, reduce inflammation and promote tissue healing and their effectiveness is comparable.

Conclusions. Due to the diversity of species of venomous marine animals and significant differences in the response of organisms to their toxins, it is difficult to determine a universal scheme of first aid in poisoning. Treatment should be selected individually, although in most of the described cases the use of the HWI or the IPT has had a positive effect.

Key words: marine envenomation, first aid, treatment

The beneficial effect of *Aronia melanocarpa* L. berries extract against cadmium impact on the concentrations of bone morphogenetic proteins: A study in an in vivo model of current environmental human exposure to this heavy metal

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Abstract

Background. Epidemiological studies prove that current environmental exposure to cadmium in industrialized countries poses a health risk to the general population, including damage to the skeleton. Thus, the attention of scientists has been focused on looking for effective protective strategies. Our recent studies in a female rat model of low-level and moderate lifetime human exposure to cadmium show that supplementation with products based on the berries of *Aronia melanocarpa* L. seems to be that strategy.

Objectives. The study aimed to investigate if the damaging action of low-to-moderate chronic exposure to cadmium and the protective effect of co-administration of an extract from *A. melanocarpa* berries on the skeleton may be related to their impact on bone morphogenetic proteins (BMPs).

Materials and methods. Female rats (192 young animals) were treated with cadmium in diet ("Morawski" Kcynia) at the concentration of 0 mg/kg, 1 mg/kg or 5 mg/kg and/or 0.1% aqueous extract from *A. melanocarpa* berries (prepared from commercial powdered extract by Adamed) for 3–24 months. The concentrations of BMPs such as BMP-2, BMP-4, BMP-6, and BMP-9 were determined in the distal femoral epiphysis using rat-specific ELISA kits by FineTest.

Results. The exposure to the 1 mg Cd/kg diet decreased the concentration of BMP-2 after 17 months. In the animals treated with the 5 mg Cd/kg diet, the concentration of BMP-2 was reduced throughout the whole experiment and BMP-9 concentration was also lower than in the control group but only after 24 months. The concentrations of BMP-2 and BMP-9 in the animals co-administered with cadmium and *A. melanocarpa* berries extract were within the ranges of values determined in the control group. The treatment with cadmium had no impact on the concentrations of BMP-4 and BMP-6.

Conclusions. The supplementation with *A. melanocarpa* berries extract during low-to-moderate exposure to cadmium may prevent this heavy metal-induced decrease in the concentrations of BMPs and thus protect from its damaging impact on the bone tissue.

Key words: *Aronia melanocarpa* L., cadmium, bone morphogenetic proteins, bone tissue, rat

Activity of caspases and calpains in breast cancer cel lines after exposure to estrogens and metalloestrogens

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Abstract

Background. Pollution with heavy metals such as cadmium and chromium(VI) is increasing in the environment. Both metals belongs to carcinogens and endocrine disruptors because can bind to the estrogen receptor, inducing the development of breast cancer. Elimination of cancer cells takes place by apoptosis. One of the most important components determining the programmed death are caspases. Activation of executive caspases, e.g., caspase-3, depends on caspase-8 and -9, i.e., initiator caspases. Calpains, belonging to proteolytic enzymes, are also involved in the apoptosis process. Reduced apoptosis results in pathological changes leading to the formation of cancer cells in which the expression of caspases and calpains is changed. Objectives. The aim of the study was to assess whether metalloestrogens present in occupational exposure – chromium(VI), or in cigarette smoke – cadmium, as well as estrogens, -17β-estradiol, affect the apoptosis of breast cancer cells. Due to the fact that the biotransformation of estrogens causes greater toxicity, the metabolite: 4-hydroxyestradiol was studied. The individual effects of the compounds, as well as the interactions of estrogen with metal on the activity of caspases and calpains, were assessed.

Materials and methods. The study used 2 breast cancer cell lines MDA-MB-175-VII and MCF-7, exposed to Cr(VI), Cd(II), 17β-estradiol, 4-OHE2 and the combined action of estrogens with metals. After exposure of cell lines to compounds, cell lysates were prepared. Using Abcam tests, the activity of caspase-3, caspase-9 and calpains in the lysates was examined fluorimetrically.

Results and conclusions. The combined effect of metals and estrogens significantly inhibits apoptosis by reducing the activity of caspase-3 and -9, but the activity of calpains has a differential effect. The combined effect of metalloestrogens with estrogens seems to be more harmful than the effect of individual compounds, i.e., environmental exposure to mixtures may adversely affect the apoptosis of estrogen-dependent breast cancer cells.

Key words: breast cancer, metalloestrogens, caspases, calpains, apoptosis

ADHD and Pycnogenol®: Common ground for new treatment possibilities

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Abstract

Background. Neuronal organoids, specifically, mimic the structural and functional complexity of the human brain, allowing for the investigation of intricate neural processes and interactions. We investigated whether Pycnogenol® – patented extract derived from the bark of the *Pinus pinaster*, could mimic the action of atomoxetine, a second-line ADHD medication.

Objectives. We hypothesise that it could act as a norepinephrine reuptake inhibitor, but without causing adverse effects. Research on Pycnogenol®, specifically for ADHD treatment, is limited. Preliminary studies suggest its antioxidant and anti-inflammatory properties might also offer benefits for managing ADHD symptoms.

Materials and methods. We cultured human dopaminergic neurons derived from induced pluripotent stem cells aged 3 weeks, as well as forebrain organoids aged 3 months. These cultures were treated with either 10 µM or 25 µM Atomoxetine or 10 µg/mL or 100 µg/mL Pycnogenol®. We then measured cytotoxicity levels, concentrations of norepinephrine and its transporter, and assessed their effects on neuritogenesis and organoid growth.

Results. Our research suggests that treatment with Pycnogenol® may lead to an increase in norepinephrine levels. Interestingly, alongside we observed a significant reduction in norepinephrine transporters. This dual impact suggests that Pycnogenol® exerts a complex modulation of the neurotransmitters system, enhancing norepinephrine availability while simultaneously affecting its reuptake mechanism by downregulating transporters. Our comprehensive analysis indicates that, similar to Atomoxetine, Pycnogenol® activates neuritogenesis, fostering the formation of new neurites, which could have significant implications for neural development, particularly in ADHD, where disruptions in early synaptic formation may occur.

Conclusions. These findings offer valuable insights into the intricate pharmacological actions of Pycnogenol® on neurotransmitter regulation. However, further investigations are necessary to fully understand the underlying mechanisms and potential implications of this dual modulation, facilitating a more comprehensive comprehension of Pycnogenol's® impact on neurotransmitters dynamics.

Key words: ADHD, Pycnogenol®, organoids, therapy

Prediction of selected toxicity parameters for clephedrone

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Abstract

Background. From an applied toxicology perspective, clephedrone can be classified as a new psychoactive substance (NPS). This stimulant NPS is a synthetic analogue of cathinone – a substance found naturally in the leaves of the khat plant (*Catha edulis*).

Objectives. The aim of the research was to utilize in silico tools to determine selected toxicity endpoints, including acute toxicity, health effects, genotoxicity, eye and skin irritation, cardiotoxicity, and disruption of the endocrine system.

Materials and methods. For the determination of selected toxicity parameters, selected in silico tools were utilized, including: ACD/Labs Percepta 2023.1.2, ADMETLab 2.0, AdmetSAR 3.0, OCHEM, ProTox 3.0, StopTox, TEST 5.1.2, VEGA QSAR 1.2.3. To ensure consistency, an indicator in the form of SMILES was introduced when using each method: ClC1=CC=C(C(C(C)NC)=O)C=C1.

Results. The obtained results allow for a preliminary risk assessment associated with contact with klefedrone, a source of intoxication among drug users. The probability of acute toxicity varies depending on the route of administration, with oral toxicity ranging between 65% and 85%. The estimated LD50 value also varies significantly depending on the route of administration and species, with the most reliable value being 260 mg/kg body weight, administered intraperitoneally to mice. In terms of adverse health effects, the highest risk is associated with the gastrointestinal system (occurrence of nausea). Regarding genotoxicity, the results indicate a low risk of occurrence in the Ames test. The probability of eye and skin irritation occurring is 57% and 74%, respectively. The possible cardiotoxic and endocrine system effects appear to be low.

Conclusions. Conclusions regarding the use of in silico methods for predicting selected toxicity parameters indicate a novel approach both in the context of judiciary and medicine. Although these models do not fully replace experimental research, they offer rapid, costeffective, and ethical preliminary prediction capabilities, supporting regulatory bodies in making informed decisions and assisting medical professionals in managing health issues associated with NPS. Further development of in silico methods along with their proper validation based on empirical data is essential to fully confirm their reliability and comprehensiveness in toxicity prediction.

Key words: 4-CMC, 4-chloromethcathinone, clephedrone, new psychoactive substance (NPS), in silico toxicology

The bromoderivatives of curcumin: Synthesis, physicochemical characteristics and toxicity studies

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Abstract

Background. Curcumin is a biologically active substance of natural origin. It is a compound with a broad spectrum of therapeutic activities (anti-cancer, anti-inflammatory, antioxidant, hepatoprotective, anti-bacterial) while having low toxicity. Due to its poor water solubility and bioavailability, curcumin is not used in therapy. Modification of the molecule with short alcohol chains, on the other hand, may improve the pharmacokinetics and pharmacodynamics of the molecule. Although curcumin shows activity in PDT, the efficiency of photodynamic processes is insignificant. This can be improved by the so-called "heavy atom effect" - the introduction of bromine into the structure of the molecule can significantly enhance the photodynamic effect. Moreover, halogenation of a drug molecule is usually associated with an increase in therapeutic activity.

Objectives. The synthesis of curcuminoids with short-chain alcohol and bromine moieties, confirming their structures using spectral methods (UV-Vis, NMR) as well as evaluation of the toxicological studies using Microtox® test.

Materials and methods. The proposed derivatives were obtained using the base aldol condensation reaction using appropriate aldehydes and acetylacetone complex with BF_2. Then decomplexation reaction was performed to obtain curcuminoids with free keto-enol moiety. Their purity and structure were confirmed by using TLC and NMR techniques in Agilent DD2 800 spectrometer. The toxicity of obtained derivatives was evaluated by exposing bacteria *Vibrio fischeri* to these compounds in Microtox® test.

Results. Four new curcuminoids (1-4) were synthesized in 2- or 3-step reactions. NMR spectra show high purity of those compounds. The Microtox® test showed different levels of toxicity of obtained derivatives.

Conclusions. Aldol condensation reaction is a good way to obtain curcuminoids with good yields (41.1–74.86%). Unfortunately, the introduction of short-chain alcohol moieties did not improve the solubility in water. As the test showed varying levels of toxicity of the compounds obtained, further cytotoxicity studies (for example, on fibroblasts) are needed.

Key words: curcumin, bromine, anticancer activity, antimicrobial activity, photodynamic therapy

Quantitative analysis of chosen OH- polycyclic aromatic hydrocarbons (OH-PAHs) level in urine samples after consumption of grilled marshmallows

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Conflict of interest
None declared

Abstract

Background. Marshmallows are confectionery products popular among children, teenagers and adults all over the world. One of the popular way of marshmallows consumption is grilling over the bonfire. The grilling process may introduce certain changes in product chemical composition, leading to the formation of potentially harmful compounds. Therefore grilled marshmallows can be a source of exposure to harmful compounds, including carcinogenic polycyclic aromatic hydrocarbons (PAHs).

Objectives. The aim of the study was evaluation of exposure, especially among children, to carcinogenic PAHs from grilled marshmallows by developing a chromatographic method of OH-PAHs analysis (9-OH-phenathrene and 1-OH-pyrene) in urine samples.

Materials and methods. The study group included children and adults (n = 22). Urine samples were collected at various intervals after eating grilled marshmallows. Samples pretreatment included enzymatic hydrolysis and SPE clean-up. Quantitative analysis of OH-PAHs was performed using the HPLC analytical system with fluorescence detector. For the separation of PAHs Hypersil Green PAH column was used. The separations were performed under gradient conditions using methanol and water.

Results. Applied method enabled to determine 9-OH-phenathrene and 1-OH-pyrene in urine samples. The levels of detected compounds were higher in the samples of individuals who ate colored grilled marshmallows (0.228 ng/mL and 2.948 ng/mL for 1-OH-pyrene and 9-OH-phenathrene, respectively) comparing to the levels of OH-PAHs in samples of individuals who ate only white grilled marshmallows (0.164 ng/mL and 1.834 ng/mL for 1-OH-pyrene and 9-OH-phenathrene, respectively). The highest level of analyzed compounds were observed in urine samples collected at 2nd and 3rd interval.

Conclusions. Grilled marshmallows are an additional source of exposure to PAHs, especially in the group of children. The lack of knowledge regarding carcinogenic substance content in popular, especially among young people, confectionery grilled products represents a serious social and health problem.

Key words: OH-PAHs, grilled marshmallows, HPLC, children, urine

Levels of selected toxic and essential elements and 5-hydroxymethylfurfural (HMF) in honeys available for retail sale

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Abstract

Background. Honey is a natural food product produced by bees, which collect pollen from an area of approx. 7 km^2. Therefore they are constantly exposed for example to metals from contaminated plants and air. Moreover, sugars in honey may be formed into toxic 5- hydroxymethylfurfural (HMF) and its content increases with thermal treatment or with storage time of honeys.

Objectives. The aim of the study was to determine the concentrations of 1) selected toxic metals (cadmium, lead); 2) essential elements (copper, zinc, chromium, manganese, iron, magnesium and calcium); and 3) HMF in 21 honeys available for retail sale in the Łódź macroregion.

Materials and methods. The analysis of the elements' concentrations was performed using atomic absorption spectrometry using the flameless technique (GF-AAS) for Cd, Cu, Pb and Cr and the flame technique (FAAS) for Mg, Ca, Zn, Mn, and Fe after mineralization of honey samples in a microwave oven. HMF content in honey was determined using the UPLC-PDA method after dissolving samples in deionized water and filtered with through a PTFE syringe filter (0.22 µm pore size).

Results and conclusions. Among the essential elements, calcium and magnesium were found in the highest concentrations (6.7 mg/kg ±0.15 and 4.0 ±0.04 mg/kg, respectively), so they can be used to properly balance the diet, while concentration of Cd and Pb in studied honeys did not exceeded maximum levels according to the Commission Regulation (EU) 2023/915, i.e., 0.005 mg/kg and 0.1 mg/kg. On the other hand, in 25% of the tested honeys HMF concentrations exceeded the limit set by Council Directive 2001/110/EC, that is 40 mg/kg (up to 3 times), which may indicate improper storage of honey or its adulteration with sugar syrup.

Key words: honey, toxic metals, essential elements, 5-hydroxymethylfurfural (HMF)

Might modifying diet manage exposure to perfluoroalkyl substances?

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Abstract

Perfluoroalkyl substances (PFASs) are synthetic organofluorine chemicals with numerous industrial applications, e.g., water and stain-resistant coatings for fabrics, oil-resistant coatings for paper and cardboard food contact materials, insecticides, fire-fighting foams, and floor polishes. Due to their increased use, emission, persistence, mobility, toxicity, and bioaccumulation ability, PFASs are considered emerging environmental contaminants. Exposure to these chemicals, mostly occurring through diet, was associated with a broad range of effects, including immunosuppression, alterations in lipid profiles, liver enzymes, hormone production, pregnancy-induced hypertension and preeclampsia, decreased birth weight, and testicular and kidney cancer. In 2022, the European Commission recommended monitoring PFASs levels in food, especially animal-derived, yet elevated levels of PFASs do not affect food introduction on the market. We review the main nutrition-related exposures to PFASs and options for minimizing them before introducing the appropriate control system for PFASs content in the European Union. The data shows that consumption of tea, coffee, pork, beef, processed meat, fried fish, and shellfish correlates with PFASs plasma concentration. On the contrary higher consumption of vegetables, fruits, fruit juice, low-fat dairy, or soy products revealed an inverse relation with PFASs levels in plasma. Also, the same fast-food meals prepared at home were inversely associated with PFASs concentration in plasma. Interestingly, for some products, the dependence on PFASs concentration changes with the degree of processing e.g., nuts and seeds revealed a negative association between plasma PFASs concentration in opposite to butter based on them. In addition, PFASs have been detected in tap water supplies across many regions, calling for improved detection, monitoring, removal, and regulation of PFASs in drinking water. Due to the omnipresence and bioaccumulation of PFASs in aquatic and terrestrial food chains, current legal action may not be sufficient. Therefore, specific dietary habits remain key to mitigating non-occupational PFASs exposure.

Key words: perfluoroalkyl substances, food contamination, water contamination, dietary exposure, human exposure

Use of bioindication method to assess toxicity of extracts of selected fruiting bodies of macrofungi

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Abstract

Background. Currently, no apparatus is designed to test the toxicity of chemicals or extracts from mushrooms' fruiting bodies. Evaluation relies solely on living organisms across various levels of organization. This study investigates the potentially toxic effects of ethanolic and aqueous extracts from *Hericium erinaceus*, *Ganoderma lucidum*, and *Coprinus comatus* fruiting bodies.

Objectives. The study aims to develop a straightforward method for preliminary testing of the chemical effects of mushroom fruiting body extracts on living organisms.

Materials and methods. Invertebrates – *Daphnia pulex*, *Chaoborus* sp. (larva) and *Chironomus aprilinus* (larvae) – were selected as test organisms. Their reactions to mushroom fruiting body extracts were recorded using a multimedia projector, capturing images every 10 s. Toxicological parameters such as IC50 (concentration causing 50% mortality) and IT50 (time causing 50% mortality) were determined for each extract.

Results. Dry mushroom fruiting body extracts were dissolved in the test organisms' living water. All aqueous extracts were found to be non-toxic. The ethanol extract from *Coprinus comatus* was most toxic to *Daphnia* and *Chironomidae*, causing 50% mortality in about 8 h and 40 min, respectively, at a 2 mL dose. *Ganoderma lucidum* showed lower toxicity, with over 24 h needed for 50% mortality. The ethanol extract of *Hericium erinaceus* was the least toxic to the invertebrates.

Conclusions. The developed biotest provides a cost-effective alternative to commercial tests for assessing the toxicity of fungal and plant extracts. It offers a rapid screening method for potential chemical effects on organisms, enhancing environmental and health safety assessments.

Key words: biotest, bioindication, biotoxicity, macrofungi

Impact of beauvericin on porcine intestinal barrier integrity

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Abstract

Mycotoxins, secondary metabolites produced by fungi, are the most prevalent natural contaminants found in food and feed. Consequently, animal exposure to these toxins is unavoidable and continuous. Beauvericin (BEA), an "emerging mycotoxin", belongs to a relatively novel category of mycotoxins whose effects are not yet fully understood and remain largely unregulated. Different mycotoxins, including BEA, among their other harmful effects, have been shown to negatively affect the intestinal barrier, leading to increased permeability and compromised intestinal integrity. Therefore, the presented study aims to investigate the influence of BEA on the intestinal tissue of pigs, focusing on the integrity and permeability of the intestinal barrier. The study was performed ex vivo using Ussing-type Chambers. Intestinal tissue was obtained from routinely slaughtered adult pigs. Measurements to assess the effects on intestinal tissue were conducted at defined intervals, evaluating factors such as Transepithelial Electrical Resistance (TEER), transport of Lucifer Yellow (LY) and Horseradish Peroxidase (HRP) across the explant wall, as well as Lactate Dehydrogenase (LDH) release. At the concentration used in the experiment, BEA caused significant changes in the parameters TEER and transport of LY compared to the control group. The study demonstrated that BEA affects the intestinal tissue and intestinal barrier function of pigs. However, some results were unexpected, highlighting the complexity of the effects of BEA and underscoring the need for further investigation to fully understand its impact and mechanisms.

Key words: mycotoxins, beauvericin, intestinal barrier, porcine

Histopathological and molecular background of salinomycin toxicity in turkeys

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Abstract

Background. Salinomycin, a commonly used ionophore coccidiostat, is toxic to susceptible species, including turkeys, with field cases of intoxication reported at exposures as low as 26 mg/kg feed.

Objectives. This research aimed to characterize salinomycin toxicity in turkey heart and liver through histopathology and transcriptomics.

Materials and methods. Turkeys were exposed to salinomycin in feed for 2 weeks (21 mg/kg feed). Livers and hearts were sampled from control and treated groups, processed, and stained with hematoxylin and eosin for light microscopy analysis. mRNA isolated from tissues was sequenced, and differentially expressed genes were detected. Gene ontology (EnrichR) and pathway analysis (Webgestalt, Reactome) were applied.

Results. No clinical or gross changes were observed. Liver histopathology revealed necrotic lesions, degeneration of hepatocytes, lymphocyte infiltration, and hepatic steatosis, distorted hepatic architecture due to numerous round cytoplasmic vacuoles and peripheral displacement of hepatocyte nuclei. In the heart, pronounced changes included cardiomyocyte vacuolation, connective tissue hyperplasia, proliferation of cardiomyocyte nuclei, loss of cross-striations, fragmentation, and necrosis in some specimens. Gene onology identified spindle assembly checkpoint signaling and translation as the most significant biological processes in heart and liver, respectively. Enriched cellular components included intracellular non-membrane-bounded organelle and mitochondrial membrane. RNA binding was found the most significantly affected molecular function for both tissues. In the heart, 4 pathways were upregulated and 7 downregulated in the salinomycin-exposed group, with significant pathways including the transport of small molecules, M phase, and cell cycle. In the liver, 14 pathways were significantly regulated, with translation being the most significant.

Conclusions. Salinomycin toxicity in turkeys was confirmed at both histopathological and molecular levels, strongly affecting the heart and liver. The identified molecular features align with salinomycin's mode of action.

Key words: salinomycin, turkey, histopathology, RNAseq

New methods to control of antibacterials use in pigs and poultry farms

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Abstract

The control of veterinary medicines use is an important element in animal husbandry. In practice, antibiotics and other antimicrobial drugs, in addition to their therapeutic indications, can be also used without any justification. Consequently, the misuse of antibiotics in animals, can lead to the presence of their residues in food, which may be a potential source of health risks for consumers. Considering the risks involved, new non-invasive methods are being searched for ante-mortem monitoring of the presence of antibiotics in pigs and poultry to determine whether antibiotics have been used correctly during breeding, in accordance with veterinary recommendations.

So far, antibiotics in food-producing animals are mainly analyzed in tissues, as a part of the National Chemical Residue Control Programme. However, these surveys do not enable ante-mortem monitoring of antibiotics use on livestock farms.

An alternative to tissue analysis in pigs for the control of veterinary drug application seems to be the use of oral fluid. In our study, the transfer and detectability in oral fluid of several antibiotics (oxytetracycline, sulphadoxine, trimethoprim, lincomycin, thiamulin, tylosin, amoxicillin) after their therapeutic administration to pigs was demonstrated. In the case of oxytetracycline, a high correlation was shown between oral fluid and tissue concentrations on the withdrawal day of the applied drug. In poultry, the least invasive tool to combat the illegal use of antibiotics appears to be the testing of chicken feathers, as an alternative to other biological matrices. After experimental administration of doxycycline to broiler chickens, high concentrations of the antibiotic were detected that persisted for long periods of time. In the control of the use of veterinary medicines, animal drinking water is also tested. However, the testing of water allows the identification of the source of possibly drug contamination, not their residues in the animals' bodies.

Key words: antibacterials, control, new methods, poultry, pig

A preliminary investigation of the poultry body weight effect of essential oils in litter and residue in organ tissue of exposed chickens

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Abstract

The use of aromatic and flavor supplements, including herbs, is increasing in animal nutrition and treatment. The rising interest in these supplements stems from a trend towards healthy nutrition and natural raw materials in animal feed. The reduction or ban of antibiotics in animal nutrition has also boosted interest in supplements that replicate the growth-enhancing effects previously achieved with antibiotics. Alternative feed additives, such as essential oils, can be incorporated into litter. For example, eucalyptus leaves can be added as a natural herbicide. In this study, a litter additive containing carvacrol was prepared using plasticizers and potato starch. Carvacrol, an essential oil, possesses strong antifungal, antiviral, and antimicrobial properties, reducing parasites responsible for gastrointestinal diseases.

The aim of this study was to evaluate the effect of the addition of carvacrol to litter on weight gain and evaluate the occurrence of residues in chicken tissues. A method for determining carvacrol in plasma, lung, muscles, and liver tissues was used to detect possible residues.

The analysis of residues after 42 days of exposure to carvacrol in litter clearly indicated that the animals were in contact with the test substance. When analyzing the concentrations in individual tissues, it could be stated that the contact occurred through the lungs, because the highest concentrations of carvacrol residue was in this tissue. To the best of our knowledge, this is the first attempt to determine carvacrol residues in poultry tissue after exposure to the substance contained in litter, and these are the first data to confirm its residues after such exposure.

Key words: carvacrol, essential oils, litter, liquid chromatography–mass spectrometry, poultry

Assessment of fipronil exposure in humans after ectoparasiticide application on household pets

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Conflict of interest
None declared

Abstract

Background. Ectoparasiticides are commonly used on household pets such as cats and dogs to treat and prevent tick, flea and lice infestations. Human interaction with treated pet may lead to (prolonged) exposure of the former to active ingredients present in the drug, which might cause negative health effects. Fipronil is one of the most commonly used active substances in such products.

Objectives. The main aim of the study was to assess fipronil exposure among pet owners using human biomonitoring; moreover, routes of fipronil exposure were investigated using silicone wristbands. To achieve these goals, sensitive and accurate analytical methods were developed and validated.

Materials and methods. Study participants collected urine samples and wore silicone wristbands before and after application of a fipronil-containing ectoparasiticide on their pets. Sample preparation for urine samples was based on solid phase extraction, whereas for silicone wristbands sequential solid-liquid and liquid-liquid extraction was performed. Both methods used liquid chromatography and tandem mass spectrometry as separation and detection technique, respectively, and quantitated fipronil as well as its relevant degradants/metabolites (fiproles), such as fipronil-sulfone and fipronil-desulfinyl.

Results. Carefully developed analytical methods allowed to quantify fipronil at levels as low as 1 pg/mL (urine) and 0.1 ng/g (silicone wristbands). Analysis of collected samples showed a notable increase in fiproles' levels after ectoparasiticide application. For instance, fipronil detection rate in urine before and after application were 4% and 60%, respectively; at the same time, median fipronil level in silicone wristbands skyrocketed from 1.9 ng/g to 650.4 ng/g, suggesting significant dermal exposure.

Conclusions. The study has shown that even single application of fipronil-based product on companion animal may lead to significant increase of fiproles' levels in urine and silicone wristbands collected from humans. However, further research is necessary to determine whether measured exposure may pose a threat to human health.

Key words: fipronil, pet owners, exposure assessment, human biomonitoring, silicone wristbands

Assessment of patterns of exposure to synthetic pyrethroids among pet owners: A longitudinal study using silicone wristbands

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Abstract

Background. Synthetic pyrethroids are commonly employed as active components of veterinary anti-ectoparasitic products. Usage of such products has increased considerably worldwide, especially among pet owners. A longitudinal study involving 15 pet-owning volunteers has been conducted, that included scheduled veterinary drug application on pet, collection of both biological samples and silicone wristbands, that served the purpose of personal passive samplers of exposure.

Objectives. The aim of the study was to assess both the magnitude of exposure to pyrethroids resulting in their use on pets, as well as the time trends of said exposure.

Materials and methods. Repeated collection of spot urine samples and silicone wristbands worn on the wrist of dominant hand had been employed both prior to and after the veterinary drug application among all study volunteers ($n = 15$). Pyrethroid metabolites had been determined in urine samples with the use of GC-MS, while wristbands extracts containing native pyrethroids had been subjected to instrumental analysis by GC-ECD. Additionally, 2 urine samples had been collected at 2 and 4 weeks post-application.

Results. A significant ($p < 0.05$) increase in concentrations of urinary metabolites, and permethrin in wristbands samples collected during a week following the drug application had been noted. Metabolite concentrations had been very strongly correlated ($r_s = 0.9161$, $p < 0.05$) with wristband pyrethroid concentrations in samples collected following the drug application. Urinary metabolite concentrations noted in samples collected 4 weeks after the drug employment had been notably higher than levels assessed prior to product use.

Conclusions. Silicone wristband had proven to be a useful tool for exposure assessment to synthetic pyrethroids, which complements biomonitoring greatly. Study observations point to a possibility of pyrethroids being accumulated indoors after repetitive applications. Patterns of exposure produced in this study tentatively point to behavioral factors being drivers of the magnitude of exposure.

Key words: synthetic pyrethroids, silicone wristband, pet

The effect of black chokeberry (*Aronia melanocarpa* L.) extract on the concentration of interleukin 6 in the liver: A study in an in vivo model of human environmental exposure to cadmium

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Abstract

Background. Cadmium (Cd) occurrence in human's natural and occupational environment poses a significant threat to health, and the forecasts indicate that the general population exposure to this toxic metal in industrialized countries will increase. Our recent findings show that black chokeberry (*Aronia melanocarpa* L.), a rich source of biologically active compounds, especially polyphenols, may effectively prevent Cd toxicity, including damage to the liver.

Objectives. The study aimed to investigate the effect of administering a polyphenol-rich extract from *A. melanocarpa* L. berries on the concentration of pro-inflammatory cytokine – interleukin 6 (IL-6) in the liver of rats chronically exposed to Cd.

Materials and methods. The experiment was carried out on 192 female Wistar rats, which were administered Cd ("Morawski" Kcynia) at a concentration of 1 or 5 mg/kg of diet and/or 0.1% aqueous extract of black chokeberry (Adamed) for 3, 10, 17, and 24 months. The concentration of IL-6 was determined in the aliquots of 10% liver homogenates using a Quantikine® ELISA diagnostic kit by R&D Systems.

Results. The exposure to the 1 mg Cd/kg diet led to an increase in the concentration of IL-6 after 10, 17 and 24 months of the study, while the simultaneous administration of the *A. melanocarpa* L. berry extract provided complete protection against this action of Cd. In the rats intoxicated with the 5 mg Cd/kg diet, the concentration of IL-6 was increased only after 24 months of the experiment, and the co-administration of the extract allowed maintaining the values of this parameter at the level noted in the control group.

Conclusions. The study results allow us to conclude that *A. melanocarpa* berry extract consumption may protect against an increase in the concentration of IL-6 in the liver under repeated exposure to Cd, and therefore inhibit the development of inflammatory processes in this organ.

Key words: *Aronia melanocarpa*, cadmium, interleukin 6, liver, rat

Carcinogenicity of selected pharmacologically active dyes and health risk for consumers of contaminated fish

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Abstract

Recently, interest in the fate of pharmacologically active substances (PASs) in aquatic environments has increased. So far, there have been a lot of reports published on the occurrence of PASs, such as antibiotics, lipid-regulators or nonsteroidal anti-inflammatory drugs, but there has been limited data on the presence of synthetic organic dyes used as PASs in water bodies. Due to coloring properties, many synthetic organic dyes are used as industrial colorants in many areas, including textile, leather and paper industries, as well as in the food processing and cosmetic sectors. However, some synthetic organic dyes are also PASs with a broad spectrum of fungicidal, ectoparasiticidal, and bactericidal activities. These pharmacologically active dyes (PADs) are used in human medicine as aseptic agents and in veterinary medicine to control pathogenic fungi, protozoan ectoparasites and bacteria in fish. Nevertheless, the dyes have never been registered for food-producing animals in the European Union due to their potential carcinogenicity. The most often found dye in farmed fish and free-living fish of Polish lakes and rivers is malachite green, and its main metabolite – leucomalachite green.

To date, the carcinogenicity of only a few PADs have been evaluated by the International Agency for Research on Cancer (IARC). Gentian violet, leucomalachite green were classified to Group 2B as "possibly carcinogenic to humans", while malachite green, leucogentian violet, methylene blue were classified to Group 3 as "not classifiable as to its carcinogenicity in humans".

This paper discusses the existing scientific evidence for the PADs' carcinogenicity as well as the cancer risk for humans associated with consumption of fish contaminated with the PADs.

Key words: PAS, malachite green, gentian violet, leuco, methylene blue, risk assessment

Identification of polyester oligomers and epoxy coatings as potential migrants from food contact materials

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Abstract

Background. Food packaging has an irreplaceable role worldwide protecting the food against chemical and microbiological contamination. Coatings are applied internally on cans to prevent from the corrosion of metal plates and the migration of metal ions into the food. Polyester coatings were developed to replace epoxy-phenolic coatings as the latest was demonstrated to release bisphenol A and its derivatives, questioning their safety.

Objectives. In this work we applied HPLC-MS analysis with the cone voltage-induced fragmentation insource (CID in source) and HPLC-QTOF-MS analysis in order to identify the most common classes of potential migrants present in the can coating materials marketed in Poland.

Materials and methods. A total number of 30 canned food and beverages samples were collected from supermarkets in Western Poland in early 2024. The extraction process was performed by stirring the empty cans filled with appropriate volume of acetonitrile. Prior to the HPLC-MS analysis, the sample was further filtered through syringe filters.

Results. The polyester derived migrants were detected in the extracts of inner coatings of fish and meat products-containing cans. Most of above migrants can be described as cooligoesters, cyclic and linear, which consisted of 1 diol monomer, namely neopentyl glycol (NPG), and 2 diacid comonomers, namely isophthalic acid/hexahydrophthalic acid (iPA/HHA). In all tested beverages bisphenol A diglycidyl ether (BADGE) conjugates were detected, namely butoxyethanol adducts with BADGE.

Conclusions. Determination of the structure of non-intentionally added substances (NIAS) is the area of research which has been recently widely developed in the field of food packaging materials, however, the reported data devoted to the polyester derived migrants are definitely less numerous than those devoted to the epoxy resin derived migrants and requires further analysis.

Key words: can-coating material, polyesters, epoxy resins

Toxicological analysis and safety assessment of selected elements in foods for special medical purposes for oncology patients

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Abstract

Background and objectives. Foods for special medical purposes (FSMP), dedicated to oncological patients and available in pharmacies, play an unquestionable role in their nutrition (nutritional pharmacotherapy, clinical nutrition). However, the scientific literature lacks comprehensive studies in the field of regulatory toxicology for elements such as heavy metals (Hg, Pb, Cd, and As) and physiologically essential metals (Cu, Mn, Fe, and Zn) regarding their content and comprehensive toxicological health risk assessment in accordance with ESPEN and EFSA requirements.

Materials and methods. First, the FSMP samples were mineralized using microwave-assisted mineralization. Then, the elements were determined using the ICP-MS technique (inductively coupled plasma mass spectrometry). Next, appropriate strategies were used to estimate the exposure of oncology patients to dietary elements in relation to the manufacturers' recommendations. This involved estimating the daily (ADI, µg/kg bw/day) or weekly (PTWI, µg/kg bw/week) dietary exposure, depending on the element under consideration, in the regulatory context of EFSA and ESPEN guidelines.

Results. With regard to heavy metals, spectacular results were obtained for cadmium, with alarming exposure levels found in several samples (approx. 90% of the PTWI for Cd). For physiologically essential elements, numerous discrepancies were found between the actual and declared product compositions, depending on the method of administration and the recommended intake.

Conclusions. All products were deemed safe for cancer patients based on current guidelines and available evidence. However, due to the significant findings, it is recommended to develop clear and unambiguous guidelines for FSMPs, as these products play a crucial role in oncological care and should be treated similarly to drugs. This group of dietary products receives very little attention (both in academic teaching and scientific research), highlighting the need for further investigation.

Key words: heavy metals, FSMP, cancer patients, toxicological risk assessment, food safety, clinical nutrition

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