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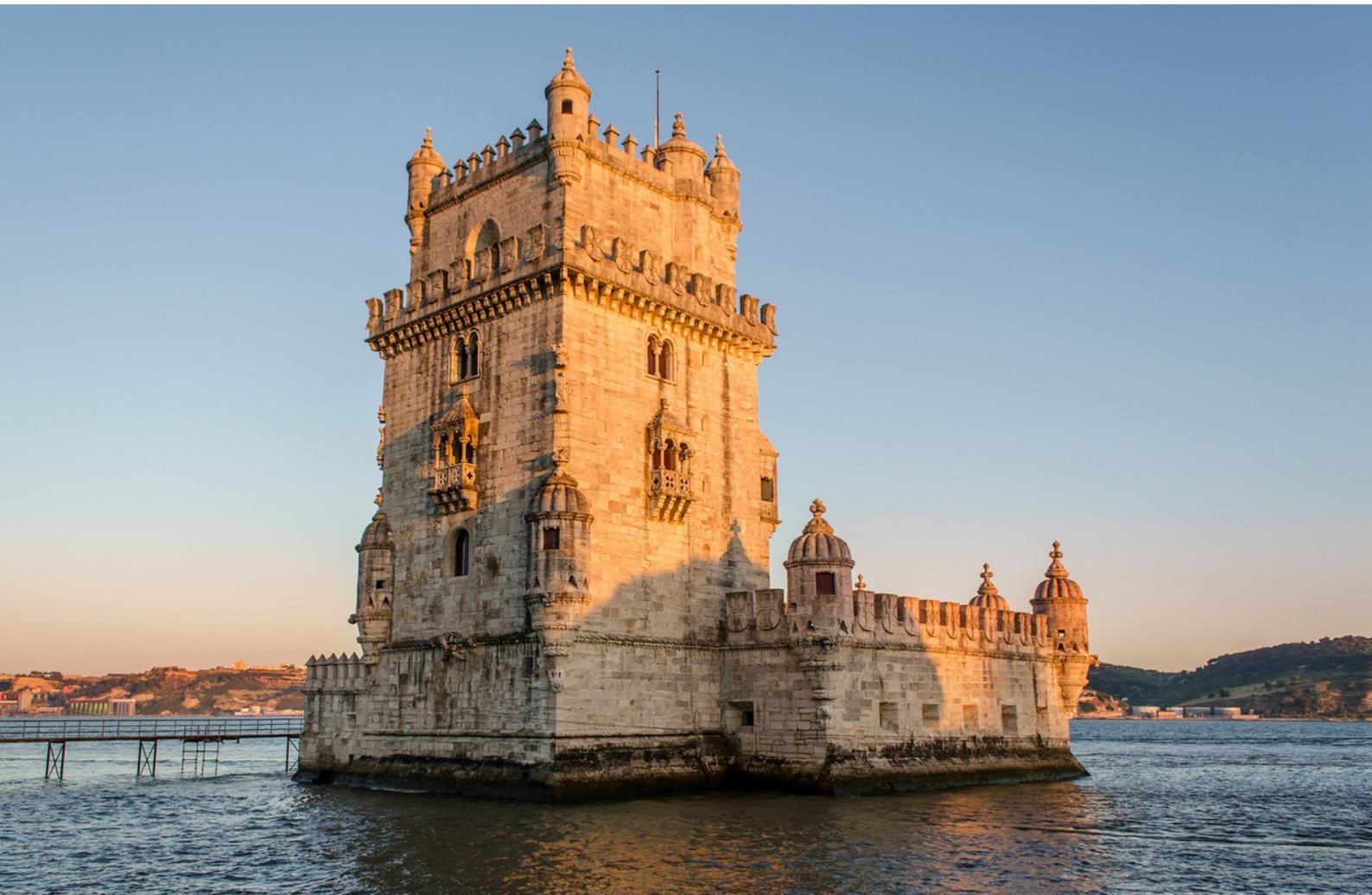
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

NutRedOX
COST Action
CA 16112

Lisbon, 2-4th October

Universidade Lusófona, Campo Grande



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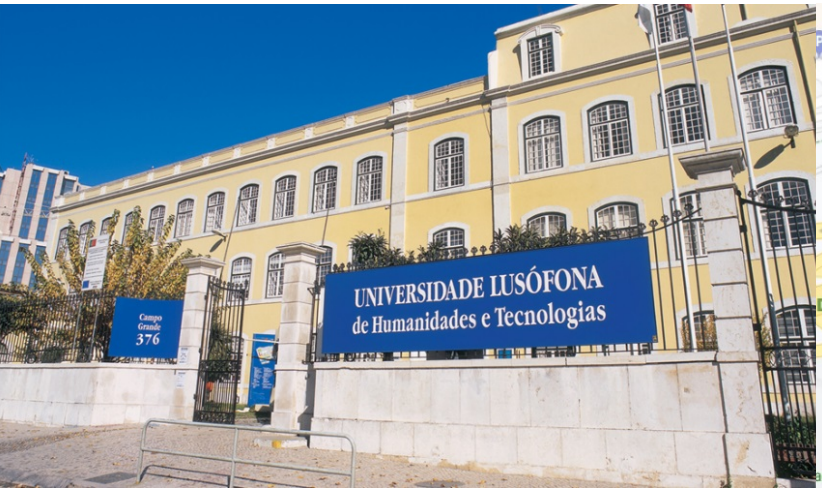
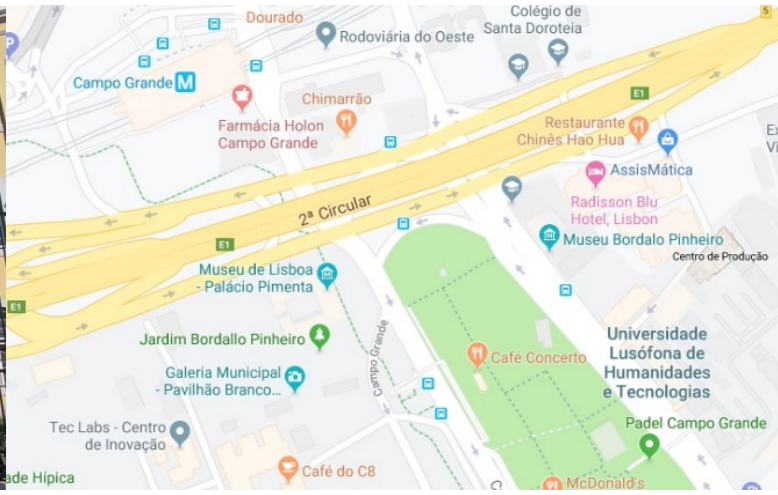
LOCATION





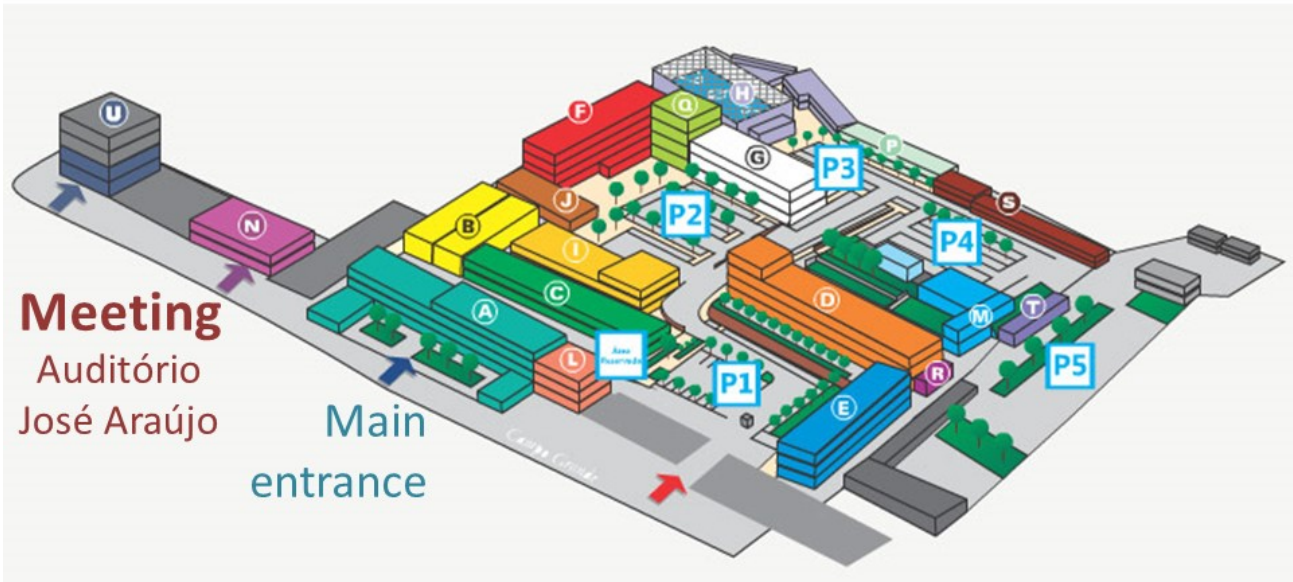
- Address:
  Auditório José Araújo
  Universidade Lusófona de Humanidades e Tecnologias
  Campo Grande, nº 376
  1749-024 Lisboa – Portugal

How to get there:

- Universidade Lusófona is near the Metro Station of Campo Grande with access to the **Green** and **Yellow** lines.
- You can also reach it by BUS:

  701 – Campo Ourique (Prazeres) → Campo Grande (Metro)
  717 – Praça do Chile → Fetais
  736 – Cais do Sodré → Odivelas
  767 – Campo Mártires da Pátria → Reboleira (Metro)

Lusófona University campus



IMPORTANT INFORMATION

WIFI CONNECTION AT THE UNIVERSITY

freeulht (no password needed)

COMMITTEE CONTACTS:

Ana Fernandes – p3378@ulusofona.pt

Cláudia Nunes dos Santos – claudia.nunes.santos@nms.unl.pt

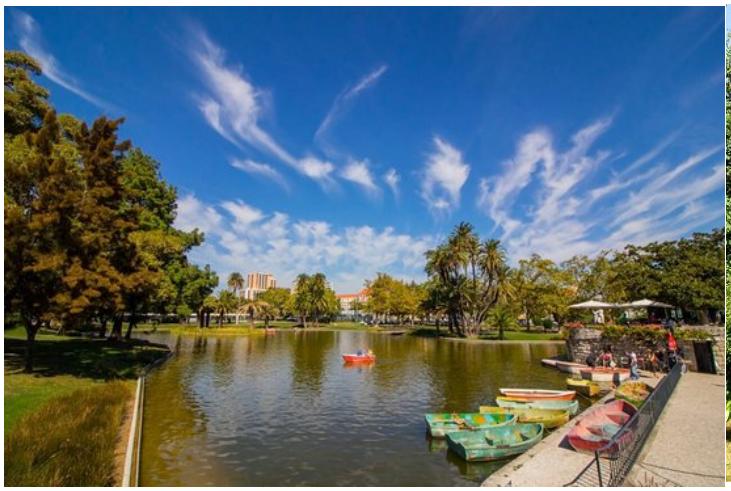
Catarina Pinto – 00351 927563407

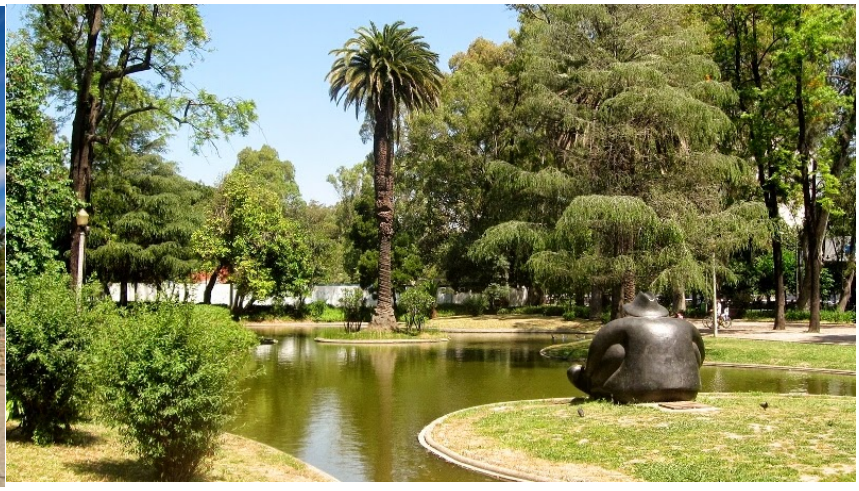
MEETING DINNER

The networking dinner will be on October 3rd at the restaurant D. Afonso, o Gordo, in Rua Santo António da Sé nº18, 1100-500 Lisboa

Also,

In front of the University you can visit the Gardens of Campo Grande!





Ana Sofia Fernandes
CBIOS, Universidade Lusófona

Cláudia Nunes dos Santos
CEDOC, iBET, ITQB

Nuno Saraiva
CBIOS, Universidade Lusófona

Catarina Pinto
CEDOC

João Costa
CBIOS, Universidade Lusófona

Cíntia Pego
CBIOS, Universidade Lusófona

Marisa Nicolai
CBIOS, Universidade Lusófona

2nd October

14:30	**Welcome** Mustapha Malki, Ana Fernandes, Cláudia Nunes dos Santos
15:00 – 16:30	**Local Organizers session** *Chairs: Ana Fernandes and Mustapha Malki*
15:00	Nutrition to avoid cancer: inflammation and angiogenesis as a target *Douglas M. Noonan, Univ. degli Studi dell'Insubria, Italy*
15:30	Implications of thiol dynamics in health and disease *Sofia Pereira, Nova Medical School, Portugal*
16:00	Effects of physical exercise on proteostasis in ageing and disease models *Mustafa Atalay, Univ. Eastern Finland*
16:30 – 17:00	**Coffee break**
17:00 – 17:40	**WG1 session** *Chair: Agnieszka Bartoszek*
17:00	Nutrition and Ageing: funding opportunities in Horizon 2020 and Horizon Europe *Patrícia Calado, ANI - National Innovation Agency, Portugal*
17:20	COST action CA16205 - European Network on Understanding Gastrointestinal Absorption-Related Processes (UNGAPp) *Kateřina Valentová, Institute of Microbiology, Czech Academy of Sciences, Czech Republic*
17:40	**Posters Session WG2** *Welcome reception*

3rd October

9:00 – 10:30	**WG2 session** *Chair: Josep Tur*
9:00	Translational methods for tracking anthocyanins in the rat organs *Sabina Passamonti, Univ. Trieste, Italy*
9:20	Phytochemicals in cardiac diseases *Antigone Lazou, Aristotle Univ. of Thessaloniki, Greece*
9:40	Effects of *Lippia citriodora* leaves extract on lipid and oxidative blood profile of volunteers with hypercholesterolemia: a preliminary study *Alfonso Di Costanzo, Univ. Molise, Italy*
10:00	In vivo and In vitro Beneficial effects of *Salvia officinalis* (sage) leaf extract on glucose tolerance, insulin sensitivity, inflammation and oxidative stress *Mohamed S. Zaibi, Univ. Buckingham, UK*
10:30 – 11:00	**Coffee break**
11:00 – 12:30	**Young Investigators Session** *Chairs: Caroline Gaucher and Mourad Elhabiri*
11:00	The use of an in vitro biotransformation model and data analysis workflow to characterize anti-inflammatory lead compounds derived from *Filipendula ulmaria* *Anastasia Van der Auwera, Univ. Antwerp, Belgium*
11:20	Beneficial effects of a berry-enriched diet in hypertensive rats: metabolic fate of (poly)phenols and the role of gut microbiota *Andreia Filipa Gomes, IBET, CEDOC, Portugal*
11:40	Therapeutic potential of s-nitrosothiols in the prevention of atherosclerosis: modulation of monocytes and smooth muscle cells metaplasia into foam cells *Justine Bonetti, Univ. Lorraine, France*
12:00	Anticancer properties of thymoquinone In 786-o renal cancer cells *João G. Costa, CBIOS – Univ. Lusófona, Portugal*
12:00 – 14:00	**Lunch**
12:30 – 14:00	Core Group Meeting

14:00 – 15:00	WG separate meetings
15:00 - 15:30	Follow up of WG meetings *Chair: Claudia Nunes dos Santos*
15:30 – 16:30	**Science Communication Session** *Chair: Claudia Nunes dos Santos* 15:30 Communicate science to scientists: beyond writing articles *Maria Serrano Correia, CEDOC, Portugal* 16:00 Science communication to society *David Marçal, Ciência Viva-Agência Nacional Para A Cultura Científica E Tecnológica, Lisboa, Portugal*
16:30 – 17:00	**Coffee break**
17:00 – 18:30	**WG3 session** *Chair: Nina Hermans* 17:00 Polyphenol intake and intestinal permeability in the older subjects: the contribution of the MaPLE project *Patrizia Riso, DeFENS – Univ. degli Studi di Milano, Italy* 17:20 The role of Bile acids in Nutrition and Health – cross talk at the host-microbe interface *Susan Joyce, Univ. College Cork, Ireland* 17:40 Platelet mitochondrial DNA methylation in L-carnitine supplemented aged women *Rosita Gabbianelli, Univ. Camerino, Italy, PLATELET* 18:00 Low molecular weight gut polyphenols metabolites: brain permeability and effects at neuroinflammation *Claudia Nunes dos Santos, CEDOC, Nova Medical School, Portugal*
19:00	**Dinner**

4th October

9:00 – 10:30	**WG4 session** *Chair: Elke Richling*
9:00	The micronutrient selenium in health and disease *John Mackrill, Univ. College Cork, Ireland*
9:20	Luteolin and S-Allyl cysteine alleviate β-amyloid, glycative, and oxidative injury in a primary rat hippocampal neuron model for hyperglycemia-induced Alzheimer's disease *Çimen Karasu, Gazi Univ., Turkey*
9:40	Resveratrol prevention of aged-linked alteration. Inflammation in osteo-arthritis *Norbert Latruffe, Laboratoire BioPeroxIL, Univ. Bourgogne-Franche Comté, France*
10:00	Beyond antioxidative effects: combinatory estrogenic effects of beer polyphenols *Doris Marko, Univ. Vienna, Austria,*
10:30h – 11:00h	Coffee break
11:00h – 13:00h	MC Meeting
13:00	Closing session

LOCAL ORGANIZERS SESSION

EFFECTS OF PHYSICAL EXERCISE ON PROTEOSTASIS IN AGEING AND DISEASE MODELS

Mustafa Atalay[1], Juha Hulmi[2], Irina Belaya[1], Jaakko Hentilä[2], Ayhan Korkmaz[1], Shuzo Kumagai[4], Niku Oksala[4]

[1] Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland
[2] University of Jyväskylä, Department of Biology of Physical Activity, Finland
[3] Center for Health Science and Couceling, Kyushu University, Kasuga, Fukuoka, Japan
[4] Division of Vascular Surgery, Tampere University Hospital, Tampere, Finland.
mustafa.atalay@uef.fi

Protein homeostasis, proteostasis, is maintained through several integrated pathways and its regulation is disrupted in ageing and pathological conditions due to increased oxidative stress and compromised antioxidant defence and overall tissue protection. Our studies revealed that stress protein: heat shock protein (HSP) defence was impaired in experimental diabetes and endoplasmic reticulum (ER) stress was induced in ageing and Duchenne type dystrophy in skeletal muscle. Although an acute bout of exhaustive exercise may cause oxidative and metabolic stress, our studies demonstrated that chronic regular physical activity significantly, and antioxidant supplementations partly enhanced antioxidant and HSP protections. Regular physical activity restored proteostasis through enhanced redox regulation, attenuated ER stress-related apoptosis signalling in ageing in skeletal muscle, and improved HSP defence in experimental diabetes in various tissues. Nevertheless, these improvements were less evident in dystrophic muscle. These results provide further evidence that long-term physical activity has a protective effect against compromised proteostasis in ageing and in diabetes.

References:

1. Atalay M, Oksala N K J, Laaksonen D E, Khanna S, Nakao C, Lappalainen J, Roy S, Hänninen O, Sen C K: Exercise training modulates heat shock protein response in diabetic rats, J Appl Physiol, 97:(2):605-11, 2004.
2. Oksala NKJ, Laaksonen D, Lappalainen J, Khanna S, Sen CK, Journal of Sports Science M.: Alpha-lipoic Acid modulates heat shock factor-1 expression in streptozotocin-induced diabetic rat kidney. Antioxidant and Redox Signalling, Volume 9 Number 4, 497-506, 2007.
3. Hulmi J.J., Hentilä J., DeRuisseau K.C., Oliveira B.M., Papaioannou K.G., Autio R., Kujala U.M., Ritvos O., Kainulainen H., Korkmaz A., **Atalay M.** Effects of muscular dystrophy, exercise and blocking activin receptor IIB ligands on the unfolded protein response and oxidative stress, Free Radical Biology & Medicine, 2016 Oct;99:308-322.
4. Belaya I, Suwa M, Chen T, Giniatullin R, Kanninen KM, Kumagai S, **Atalay M** Long-term exercise protects against cellular stresses in aged mice, Oxidative Medicine and Cellular Longevity, 2018 Mar 25;2018:2894247.

COST ACTION CA16205 - EUROPEAN NETWORK ON UNDERSTANDING GASTROINTESTINAL ABSORPTION-RELATED PROCESSES (UNGAP)

[1]Valentová, K., & [2]Augustijns, P.

[1] Institute of Microbiology of the Czech Academy of Sciences, Prague, Czechia
[2] KU Leuven, Leuven, Belgium

kata.valentova@email.cz

Oral administration is the most common drug delivery route. Absorption of a drug from the gut into the bloodstream involves disintegration of the dosage form, dissolution of the API, and transport across the gut wall. The efficiency of these processes is determined by highly complex and dynamic interactions between the gastrointestinal tract, the dosage form and the API. The fraction absorbed of the drug is affected by various factors including physiological variables, pathological conditions, local differences in gut permeability, the intraluminal behaviour of the formulation, and food effects. This complex interplay determines drug delivery performance and may cause large interindividual variability, but is poorly understood. Furthermore, comparison between drug absorption studies is hampered due to knowledge fragmentation and lack of standardisation across pharmaceutical subdisciplines. As a result, the available knowledge is underutilized in drug development and clinical treatment.
The European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP) is a multidisciplinary Network of scientists aiming to advance the field of intestinal drug absorption by focussing on 4 major challenges: (i) differences between specific patient populations, (ii) regional differences along the gastrointestinal tract, (iii) the intraluminal behaviour of advanced formulations, and (iv) the food-drug interface. The integration of knowledge, combined with the exchange of best practices across sectors and disciplines, will help improve our understanding of intestinal drug absorption and spur future developments in the field. The Action also aims to advance the career of young, talented researchers from across Europe, thereby strengthening Europe's leading position in pharmaceutical sciences.

This presentation is supported by the COST Action CA16205 UNGAP and Czech Ministry of Education, Youth and Sport (LTC19039).

TRANSLATIONAL METHODS FOR TRACKING ANTHOCYANINS IN THE RAT ORGANS

Passamonti, S.

University of Trieste, Department of Life Sciences, Italy

spassamonti@units.it

Introduction

Anthocyanins (AC) are regarded as agents that enhance cellular vitality. To advance nutrition science, it is essential to assess their levels and kinetics in plasma and tissues of living organisms, such as the rat. The results should indicate if it reasonable to consider these flavonoids as the direct effectors of given cellular or molecular responses, or, rather, the latter are mediated by other interposed chemical entities.

Objectives

We have applied in vivo methods to track AC from the stomach to the blood and other target organs (brain, liver, kidneys). In support of the idea that AC effects may be mediated by other chemical entities, we have both tracked polyphenols microbial metabolites (PPM) in the rat tissues and tested the ability of AC to interfere with bilirubin homeostasis.

Materials and methods

We have developed and applied in the rat the following methods: i) in situ isolated stomach; ii) short-term distribution kinetics; iii) in situ isolated perfused liver.

Results

We have found that: i) in few minutes AC are absorbed form the stomach, metabolized and excreted by both the liver and the kidneys; ii) in few seconds both AC and PPM reach the brain and other organs, where they are metabolized; iii) AC inhibit the hepatic uptake of bilirubin.

Conclusions

The organism expresses the molecular machinery for the ultra-rapid absorption and distribution of AC and PPM. These interactions may cause the organ homeostasis to oscillate, and this may be beneficial for organ physiology.

Supported by Agrotur II (Interreg Programme Italy-Slovenia 2014-2020, ERDF and national cofounding), NutriRedOx (Cost Action CA 16112), several other public grants. Shared merit with internal and external research colleagues.

EFFECTS OF LIPPIA CITRIODORA LEAVES EXTRACT ON LIPID AND OXIDATIVE BLOOD PROFILE OF VOLUNTEERS WITH HYPERCHOLESTEROLEMIA: A PRELIMINARY STUDY

[1]A. Angiolillo, [2]M. Palazzo, [3]F. Vizzarri, [2]D. Casamassima, [4]C. Corino & [1]A. Di Costanzo

[1] Centre for Research and Training in Medicine for Aging, Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy
[2] Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy
[3] Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Bari, Italy
[4] Department of Health, Animal Science and Food Safety, University of Milano, Milano, Italy

angiolillo@unimol.it

Lippia citriodora is a plant traditionally used for its anti-inflammatory, antioxidant and antispasmodic effects. Biological activities such as antioxidant, anxiolytic, neuroprotective, anticancer, anesthetic, antimicrobial and sedative effects were proved in cell cultures, in animal studies, as well as in a few human clinical trials. The plant has also showed a marked improvement in blood lipid profile in some animal species, such as rabbit, brown hare, pig, horse, sheep and donkey. In the present study, we investigated the effect of *Lippia citriodora* extract on lipid and oxidative blood profile of hypercholesterolemic volunteers. This was a preliminary, open-label, single-arm, phase I clinical study. Twelve adult volunteers with hypercholesterolemia received capsules containing *L. citriodora* leaves extract, containing 23% of phenylpropanoids, (100 mg, once a day) for 16 weeks. Selected blood parameters and plasma oxidative markers were measured at baseline and after 4, 8 and 16 weeks of treatment. Compared to baseline, total cholesterol levels resulted significantly decreased and HDL cholesterol significantly increased with none or mild side effects. Triglycerides and LDL cholesterol showed a downward trend after 8 and 16 weeks. Oxidative status was improved due to significant decrease in concentration of Total Oxidant Status (TOS) and Reactive Oxigen metabolites (ROMs) and increase of Ferric Reducing Ability of Plasma (FRAP), vitamin A and vitamin E. The obtained results suggest that dietary supplementation with *L. citriodora* extract can improve the lipid profile and enhance the antioxidant power of blood. However, further studies are necessary to confirm the therapeutic benefits of this plant.

IN VIVO AND *IN VITRO* BENEFICIAL EFFECTS OF *SALVIA OFFICINALIS* (SAGE) LEAF EXTRACT ON GLUCOSE TOLERANCE, INSULIN SENSITIVITY, INFLAMMATION AND OXIDATIVE STRESS

Mohamed S. Zaibi[1], Mohamed R. Ben Khdher[2], Gemma Margetts[1], Kieron Edwards[3], John C. Clapham[1], Claire J. Stocker[1], David C. Hislop[1] and Dominic Eze[4].

[1] *Buckingham Institute for Translational Medicine, University of Buckingham, Buckingham, UK.*
[2] *Nutrition Functional Food & Vascular Health Laboratory, University of Monastir, Tunisia.*
[3] *Sibelius Natural Products, Oxford UK*
[4] *Medical School, University of Buckingham, Buckingham, United Kingdom*

Mohamed.zaibi@buckingham.ac.uk

Background and aim: Sage has been used for centuries in traditional medicine. We investigated its effects in a mouse model of obesity, inflammation and insulin resistance, along with its effects on oxidative stress, lipolysis and lipogenesis in 3T3-L1 cells, and anti-inflammatory properties in human cells.
Methods: DIO mice were treated for 5 weeks with sage extract (100 and 400 mg kg^{-1}/day). EE, bodyweight, fat mass, liver glycogen and lipids, blood glucose, plasma insulin, lipids, leptin, and pro- and anti-inflammatory cytokines were evaluated. ROS, lipolysis and lipogenesis were tested in 3T3-L1 cells. Inflammation response was measured in human adipocytes, intestinal cells, and neuroblastoma cell line.
Results: Sage and rosiglitazone showed very similar effects. Sage decreased blood glucose and plasma insulin during OGTT, the insulin tolerance test (ITT) confirmed the insulin sensitivity improvement. Sage reduced TG, NEFA and pro-inflammatory cytokines and increased the levels of anti-inflammatory cytokines. In 3T3-L1 cells, sage exhibited a significant anti-oxidant effect and reduced in a dose-related manner the accumulation of lipid droplets without affecting lipolysis. In human adipocytes, sage reduced MCP-1 levels under normal and inflammatory conditions, decreased CRP, SAA, ICAM-1 and VCAM-1 levels in human inflamed intestinal cells and protected nerve cells against ACM-induced inflammation.
Conclusions: *In vivo*, sage reduces inflammation, improves insulin sensitivity and glucose tolerance. *In vitro*, it protects against oxidative stress, inhibits lipogenesis in mice adipocytes and reduces inflammation in human adipocytes, intestinal and nerve cells. Sage presents an alternative for diabetes treatment and other diseases associated with oxidative stress and inflammation.

THE USE OF AN *IN VITRO* BIOTRANSFORMATION MODEL AND DATA ANALYSIS WORKFLOW TO CHARACTERIZE ANTI-INFLAMMATORY LEAD COMPOUNDS DERIVED FROM *FILIPENDULA ULMARIA*

[1]Van der Auwera, A., [1]Peeters, L., [2] Beirnaert, C., [1]Bijttebier, S., [1]Foubert, K., [1]Pieters, L., & [1]Hermans, N.

[1] Natural products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Belgium

[2] Adrem Data Lab, Department of Mathematics and Computer Science, University of Antwerp, Belgium

Anastasia.VanderAuwera@uantwerpen.be

Since many NSAIDs show severe gastrointestinal side effects, there is a high need for new drugs.[1] An integrated strategy is developed to characterize new anti-inflammatory lead compounds derived from *Filipendula ulmaria*. Firstly, the phytochemical composition was explored in a comprehensive manner using UPLC-DAD-HRMS. A rich diversity of phenolic constituents was (tentatively) identified.[2,3] Natural products are often pro-drugs, e.g. glycosides, which undergo extensive biotransformation after oral intake. This urges the need for identification and activity profiling of the intestinal metabolites. Therefore, a *Filipendula ulmaria* extract was subjected to *in vitro* gastrointestinal biotransformation, which mimics the gastric, intestinal and colonic phase, including fecal fermentation.[4] Samples before, during and after biotransformation were analyzed with UPLC-DAD-HRMS. An in-house automated data-analysis workflow for multiclass longitudinal data was used to screen interesting biotransformation profiles.[5,6] In the colon phase, a decrease in relative abundance was observed for different glycosylated flavonoids like rutin, spiraeoside and avicularin. Consequently, the relative abundance of aglycons such as quercetin increased, indicating microbial deglycosylation. As a last step, the activity will be evaluated with *in vitro* anti-inflammatory assays, focusing on cyclooxygenase (COX).[7] The non-biotransformed *Filipendula ulmaria* extract (50 µg/mL) already showed a 76.6 ± 2.4% inhibition on the COX-1 and 43.7 ± 9.9% on the COX-2 enzyme. However, the same extract (20 µg/mL) had no inhibitory effect on the cell-based COX-2 gene expression assay. Nevertheless, metabolites often display different biological activities compared to their precursors. The activity of the biotransformed samples will therefore be evaluated by subjecting them to these *in vitro* assays.

[1]K. Brune, et al. J Pain Res 2015, 8, 105; [2]S. Bijttebier, et al. Planta Med 2016, 82, 559; [3]S. Bijttebier, et al. Anal Chim Acta 2016, 935, 136; [4]A. Breynaert, et al. Planta Med 2015, 81, 1075; [5]L. Peeters, et al. J Chromatogr A **2019**, 1595, 240; [6]C. Beirnaert, et al. Metabolites 2019, 9, 54; [7]J. Katanić, et al. J Ethnopharmacol 2016, 193, 627.

BENEFICIAL EFFECTS OF A BERRY-ENRICHED DIET IN HYPERTENSIVE RATS: METABOLIC FATE OF (POLY)PHENOLS AND THE ROLE OF GUT MICROBIOTA

Gomes A.[1,2], Oudot C.[3], Macià A.[4], Foito A.[5], Carregosa D.[1,2,6], Stewart D.[5,7], Van de Wiele T.[8], Berry D.[9], Matzapetakis M.[2], Motilva M.J. [5,10], Brenner C.[4], Santos CN[1,2,6]

[1]Instituto de Biologia Experimental e Tecnológica, Portugal;
andreia.gomes@nms.unl.pt;
[2]Instituto de Tecnologia Química e Biológica, UNL, Portugal;
[3]INSERM UMR-S 1180- University Paris-Sud, France;
[4]Food Technology Department, University of Lleida, Spain;
[5]Environmental and Biochemical Sciences, James Hutton Institute, Scotland, UK;
[6]Centro de Estudos de Doenças Crónicas (CEDOC), Portugal;
[7]Institute of Mechanical Process and Energy Engineering, Heriot Watt University, Scotland, UK;
[8]Center for Microbial Ecology and Technology (CMET), Ghent University, Belgium;
[9]Division of Microbial Ecology, University of Vienna, Austria;
[10]Instituto de Ciencias de la Vid y del Vino-ICVV, CSIC-Universidad de La Rioja-Gobierno de La Rioja, Spain
andreia.gomes@nms.unl.pt

Epidemiological studies have shown a consistent beneficial relationship between the consumption of fruits and vegetables, rich sources of (poly)phenols, and a reduced risk of cardiovascular diseases. While the absorption and metabolism of (poly)phenols has been described in healthy conditions, it is not clear how their metabolic fate is affected under pathological conditions. This study evaluated how the metabolic fate of berry (poly)phenols is modulated in an *in vivo* model of hypertension as well the associated microbiota alterations.

Dahl salt-sensitive rats were fed with a low salt diet (LS, 0.26% NaCl) or a high salt diet (HS, 8% NaCl) with or without a berry mixture (LSB or HSB; blueberries, blackberries, raspberries, Portuguese crowberry and strawberry tree fruit) for 9 weeks.

An increase in kidney weight index in HS rats was detected that was attenuated in HSB rats. Histological analysis of HS rat's kidney tissues showed sclerotic glomeruli but in less extent in the HSB rat's kidneys. Salt enriched diet promoted an increase in urinary excretion of berry's (poly)phenol metabolites, opposing to a reduction in faeces. Moreover, salt and berries modulate gut microbiota composition as demonstrated by 16S rRNA analysis. Some changes in the microbiota caused by the high salt diet such as expansion of the families *Proteobacteria* and *Erysipelotrichaceae* were suppressed by berries.

Our study clearly demonstrated the beneficial effects of a (poly)phenol-enriched diet ingestion in a chronic model of hypertension. Thus, beneficial effects of (poly)phenols could be related with these interlinked modifications, between metabolites and microbiota environments.

Funding

ANR (ANR-13-ISV1-0001-01), FCT (FCTANR/BEX-BCM/0001/2013; SFRH/BD/103155/2014; IF/01097/2013) and iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344).

THERAPEUTIC POTENTIAL OF *S*-NITROSOTHIOLS IN THE PREVENTION OF ATHEROSCLEROSIS: MODULATION OF MONYCTES AND SMOOTH MUSCLE CELLS METAPLASIA INTO FOAM CELLS

[1]Bonetti, J., [2]Corti, A., [1]Fries, I., [2]Pompella, A., & [1]Gaucher C.

[1] Université de Lorraine, CITHEFOR, F-54000 Nancy, France
[2]Laboratorio di Redox Signalling-Dip. Ricerca Translazionale NTMC, Scuola Medica, Università di Pisa, Italy

justine.bonetti@univ-lorraine.fr

During atherosclerosis development, oxidative stress and inflammation potentiate the decrease of nitric oxide (NO) bioavailability either in its free or storage forms (*S*-nitrosothiols). As *in vivo*, *S*-nitrosothiols, like *S*-nitrosoglutathione (GSNO), are the physiological form of NO storage and transport, they can be used as therapeutics to restore NO bioavailability and to counteract the different steps of atherosclerosis development. Indeed, GSNO has been shown to modulate proteins activity and/or expression by *S*-nitrosation process, which also counteract oxidative stress (Belcastro *et al*., 2017). So, GSNO may have potential to modulate the differentiation/dedifferentiation (metaplasia) of human monocytes and human smooth muscle cells (hSMC) into foam cells. To that purpose, we developed a model of hSMC dedifferentiation, mimicking atherosclerosis development, induced by oxidative stress (2,2'-Azobis(2-methylpropionamidine) dihydrochloride, AAPH). The induction of oxidative stress was validated by a significant decrease of glutathione (GSH) amount as well as a slight increase in lipid peroxidation. Oxidative stress-induced dedifferentiation was validated by the decrease of the expression of contractile proteins like α-actin, vimentin and transgelin. In parallel, we also developed an inflammation-induced model of monocytes differentiation into M1- like (LPS/IFN-γ) or M2-like (IL-4 or IL-10) macrophages. The activity of GSH/GSNO-related enzymes like glutathione peroxidase and gamma-glutamyl transferase were modulated by inflammation with a decrease of glutathione peroxidase activity in M2-like macrophages, compared to M0-like macrophages. The production of foam cells as well as GSNO potential to limit or reverse the systems described above are still under investigation.

Financial support:
The CITHEFOR EA3452 lab was supported by the "Impact Biomolecules" project of the "*Lorraine Université d'Excellence*" (*Investissements d'avenir – ANR*).

Reference:
E. Belcastro, W. Wu, I. Fries-Raeth, A. Corti, A. Pompella, P. Leroy, I. Lartaud, C. Gaucher, Oxidative stress enhances and modulates protein *S*-nitrosation in smooth muscle cells exposed to *S*-nitrosoglutathione, Nitric Oxide Biol. Chem. 69 (2017) 10–21.

ANTICANCER PROPERTIES OF THYMOQUINONE IN 786-O RENAL CANCER CELLS

[1,2]Costa, J.G., [2]Keser, V., [2]Jackson, C., [1]Saraiva, N., [1]Almeida, N., [2]Camões, S.P., [2]Manguinhas, R., [2]Castro, M., [2]Miranda, J.P., [1]Fernandes, A.S., [2]Oliveira, N.G.

[1] CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal
[2] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

jgcosta@ulusofona.pt

Thymoquinone (TQ) is a monoterpene isolated from the oil of *Nigella sativa* seeds, commonly known as black seed or black cumin, used as spice and in folk medicine. The aim of this work was to evaluate the cytotoxic effects induced by TQ and its impact on the migration and invasion potential of 786-O human renal cancer cells. Cell viability was assessed using the Crystal Violet and MTS assays. TQ treatment clearly decreased cell viability in a concentration- and time-dependent manner. Co-incubation with reduced glutathione (GSH) altered the cytotoxic pattern displayed by TQ, markedly increasing cell viability. In addition, the effects of TQ in the cell cycle distribution and apoptosis were evaluated using flow cytometry, and an increase in the sub-G1 population and in the % of apoptosis was observed. Regarding the effect of TQ on renal cancer progression, it was observed that this compound, at a non-cytotoxic concentration, significantly decreased the collective migration of 786-O cells, whereas it had no effect in terms of chemotactic migration. TQ also decreased the invasiveness potential of 786-O cells, as evaluated by a transwell cell invasion assay. Overall, these results suggest that TQ presents an important anticancer potential in renal cancer cell carcinoma that warrants further investigation.

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PLATELET MITOCHONDRIAL DNA METHYLATION IN L-CARNITINE SUPPLEMENTED AGED WOMEN

Bordoni L. [1], Sawicka A. K.[2], Szarmach A. [3], Winklewski P. J. [4], Olek R.A.[2*],

Gabbianelli R.[1*]

[1] School of Pharmacy, Unit of Molecular Biology, University of Camerino, Italy
[2] Department of Bioenergetics and Nutrition, Gdansk University Physical Education and Sport, Poland
[3] 2nd Department of Radiology, Faculty of Health Sciences, Medical University of Gdansk, Poland
[4] Department of Human Physiology, Faculty of Health Sciences, Medical University of Gdansk, Poland
*These authors share senior authorship

rosita.gabbianelli@unicam.it

Introduction: Positive effect of L-Carnitine supplementation in cardiovascular diseases has been recently questioned due to the evidence on trimethylamine-N-oxide (TMAO) production by gut microbiota.
Objectives: The aim of this study was to evaluate the impact of L-carnitine supplementation on TMAO level, lipid profile and platelets mitochondrial DNA methylation (mtDNA) at two selected regions (*MTCO1*, D-loop) in a group of aged women that conducted a resistance training protocol (24-weeks, twice a week, with or without supplementation).
Material and Methods: The study protocol has been approved by the Independent Bioethics Commission for Research at Medical University of Gdansk (NKBBN/354-201/2017). Thirty women, from 62 to 72 years old were analysed in the study. Plasma TMAO (ng/ml) was determined by the UPLC/MS/MS method. Platelet mtDNA extraction was performed using the Genomic DNA extraction kit (Norgen Biotek Corp., Canada). MtDNA of *MTCO1* and D-loop was measured, after the conversion of mtDNA with bisulfite, by the PyroMark PCR Kit (Qiagen, Germany). Lipid profile was measured by automatic analyzer Cobas6000.
Results: Data analyses revealed that L-Carnitine supplementation significantly modify platelet mtDNA methylation at D-loop region and this change was associated with an improved lipid profile.
Conclusions: A complex correlation among TMAO, mtDNA and lipid profile suggests that L-carnitine supplementation together with resistance training activity can exert a positive effect on cardiovascular health.

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S-ALLYL-L-CYSTEINE AND LUTEOLIN PREVENTS HYPERGLYCEMIA INDUCED $A\beta_{1-42}$ INJURY IN AN ALZHEIMER'S DISEASE CELL MODEL

[1] Zubeyir Elmazoglu, [1]Dilara Oguz, [1]Zehra Aydin Bek, [2]Edgar Rangel-López, [2]Abel Santamaria & [1]Cimen Karasu

[1] Gazi University Faculty of Medicine, Turkey
[2] National Institute of Neurology and Neurosurgery, Mexico

cimenkrs@gmail.com; karasu@gazi.edu.tr

Chronic hyperglycemia (HG) is a characteristic feature of Diabetes mellitus (DM) and a potential risk factor for the progression of Alzheimer's Disease (AD). Current therapeutic strategies are not sufficient enough to overcome hyperglycemia induced oxidative stress, inflammation and mitochondrial dysfunction leading cognitive impairment in AD. Thus we aimed to investigate the potential redox modulatory effects of S-Allyl-L-cysteine (SAC) and Luteolin (LUT) to overcome $A\beta_{1-42}$ -induced neurodegeneration using a HG-AD *in vitro* model.

AD model was designed by using embryonic primary hippocampal neurons. Long incubation periods (48 h) and high glucose (150 mM) were chosen to mimic chronic hyperglycemia. For the protection experiments, cells were pre-treated with SAC or LUT, then co-treated with glucose (24 h) and followed by incubation with 500 nM oligomeric Aβ1-42 (24 h) in high glucose conditions. Cell cytotoxicity analysis, ROS generation (DCFDA), inflammatory markers (iNOS, IL-1β, Tnf-α) were measured. In addition, advanced oxidation end products (AGE, HNE, 3-NT) and intrinsic antioxidant enzymes (SOD, CAT, GPx, GRx) were examined to support the potential redox modulatory effects of SAC and LUT.

SAC and LUT diminished the $A\beta_{1-42}$ induced neuronal loss under hyperglycemia conditions with decreased ROS levels concomitant with the increment of SOD, CAT, GPx, GRx. While, advanced oxidation end products and inflammatory cytokines were increased in HG-AD model, SAC treatment not only ameliorated the AGE, HNE, 3-NT levels but also reduce inflammatory parameters, more than LUT.

Our results suggested that both SAC and LUT showed neuroprotective effects in HG-AD via lowering oxidative stress-related end products and neuroinflammation along with higher antioxidant response.

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RESVERATROL PREVENTION OF AGED-LINKED ALTERATION. INFLAMMATION IN OSTEO-ARTHRITIS

Norbert Latruffe*[1], Emeric Limagne[1,2], Allan Lançon[1], Dominique Delmas[1,2], Mustapha Cherkaoui Malki[1].

[1]Laboratoire BioPeroxIL EA 7270, Université de Bourgogne-Franche Comté. Faculté des Sciences Gabriel, Dijon, F21000, France
[2] INSERM U866, Dijon, F21000, France

Norbert.Latruffe@u-bourgogne.fr

Osteo-arthritis is a painful articular disease characterized by cartilage degradation, osteophyte formation and local inflammation and frequent in senior poeple. So far, there is no efficient medicine to delay cartilage breakdown. We investigate pro-inflammatory paracrine interactions between human primary chondrocytes and macrophages following interleukin-1-β (IL-1β) treatment; and evaluated the molecular mechanism responsible for the inhibitory effect of resveratrol (1). The activation of NF-κB in chondrocytes by IL-1β induced IL-6 secretion which triggers STAT3 protein activation in macrophages. Interestingly, STAT3 positively regulates IL-6 secretion. These experiments illustrate the usefulness of the co-culture model in the inflammatory arthritis linked process which mimic situation in the synovial joint than separated chondrocytes and macrophages. We showed the presence of an inflammatory amplification loop induced by IL-1β. Resveratrol provided a strong inhibitory effect on the pro-inflammatory markers' secretion. The decreased of IL-6 secretion is dependent on the NFκB inhibition in the chondrocytes. Leads of IL-6 level decrease and limit STAT3 activation in the macrophages leading to the interruption of the inflammatory amplification loop.

These results open new potential approaches to prevent and treat osteoarthritis by dietary resveratrol.

(1) Limagne E, Lançon A, Delmas D, Cherkaoui Malki M, Latruffe N (2016). *Nutrients,* May 11;8(5). pii: E280.

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BEYOND ANTIOXIDATIVE EFFECTS: COMBINATORY ESTROGENIC EFFECTS OF BEER POLYPHENOLS

[1]Aichinger, G., [1]Marko, D.

[1]Department of Food Chemistry and Toxicology, University of Vienna, Austria

doris.marko@univie.ac.at

Hop containing products including beer represent a considerable source for a spectrum of polyphenols with antioxidative properties. However, several of these polyphenols also possess estrogenic properties. By interfering with estrogen receptors (ERs), they might act as endocrine disruptors, disturbing the natural hormonal balance. Respective effects have been described of the prenylated chalcone 8-prenylnaringenin (8PN). Its parent compound, xanthohumol (XAN), is not estrogenic, but has been associated with beneficial effects on human health. But these phytoestrogens are not the only source of hormonally active compounds in food. Especially cereal-based food is well-known to be prone to contamination with mycotoxins. Among this very heterogeneous class of potential food contaminants, there are also estrogenic compounds like the *Fusarium* metabolites zearalenone (ZEN) and α-zearalenol (α-ZEL), found mainly in cereal-based foods such as bread, breakfast cereals, but also in beer.

We addressed the question whether hop polyphenols are able to modulate the endocrine activity of *Fusarium* toxins. Experiments were conducted in estrogen-sensitive Ishikawa cells, incubating with ZEN, α-ZEL, XAN, 8PN and combinations thereof. The estrogenic stimulus was determined as ER-dependent alkaline phosphatase activity.
By using mathematical models to assess interactions, we found strong antagonistic effects, especially of XAN, on the estrogenicity of *Fusarium* toxins. Our results suggest that hop polyphenols - in addition to the anti-oxidative properties - may also have a protective effect against endocrine effects of mycotoxins. This would be of particular interest in the light of recent efforts to produce XAN-enriched beers or food supplements containing hop preparations.

1

WINE, MEDITERRANEAN DIET AND COGNITIVE FUNCTION IN NORMAL AGING

[1]Silva, P., Latruffe, N.[2]

[1]Laboratory of Histology and Embryology, Institute of Biomedical Sciences Abel Salazar (ICBAS), Rua de Jorge Viterbo Ferreira nº228, 4050-313 Porto, Portugal
[2] BioPeroxIL laboratory, Université de Bourgogne, 6, Boulevard Gabriel, 21000 Dijon, France

psilva@icbas.up.pt

Numerous studies suggest that Mediterranean diet (MD) is one of the healthiest dietary patterns in the world associated with low morbidity and mortality among old age subjects. MD may be beneficial with respect to reducing the incidence of many age-related diseases, including the neurodegenerative ones. This diet is based on abundant and variable plant foods, high consumption of cereals, olive oil as the main fat, low intake of red meat and moderate consumption of wine. Regarding the latter there is some controversy due to the favorable and unfavorable effects of alcohol on health. Excess of alcohol increases the risk of liver cirrhosis and cancer, mainly those of the upper digestive and respiratory tract and is the risk of accident death. On other hand, wine is composed by various phenolic compounds that have antioxidant characteristics which are assumed to be responsible for their beneficial health properties. Several *in vitro* and *in vivo* studies suggested that wine phenolic compounds protect brain damage by other mechanisms beyond the antioxidant ones, including: reducing neuroinflammation, interfering in antiapoptotic processes, activating endothelial NO production and release, affecting the synthesis and degradation of amyloid β peptide, reducing tau aggregation, acting as signaling molecules that interact with intracellular signaling pathways, and modulating the expression of genes, microRNAs and proteins. In conclusion, scientific research indicates that moderate consumption of wine, and especially red wine, could play a key role in the prevention of cognitive decline when consumed together with a mixture of healthy foods and with healthy lifestyle patterns.

2

CENTAURIUM ERYTHRAEA EXTRACT MEDIATES PRO-SURVIVAL PATHWAYS AND INSULIN EXPRESSION IN STZ-TREATED BETA-CELLS

[1]Đorđević, M., [1]Grdović, N., [1]Mihailović, M., [1]Arambašić Jovanović, J., [1]Uskoković, A., [1]Rajić, J., [1]Sinadinović, M., [1]Tolić, A., [2]Mišić, D., [2]Šiler, B., [1]Poznanović, G., [1]Vidaković, M. & [1]Dinić, S.

[1] Department of Molecular Biology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060, Belgrade, Serbia
[2] Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060, Belgrade, Serbia

sdinic@ibiss.bg.ac.rs

Diabetes is characterized by hyperglycaemia resulting from a deficiency in insulin secretion and/or action leading to severe diabetic complications. Despite numerous efforts, recovery and maintenance of functional beta-cells is still an unresolved task in diabetes therapy. Considering anti-diabetic properties of medicinal herb *Centaurium erythraea* Rafn (CE), this study aimed to analyze protective effects of the CE extract on Rin-5F beta-cell line exposed to diabetogenic agent streptozotocin (STZ). Cytoprotective concentration of CE extract (0.25 mg/mL) and IC_{50} dose of STZ (12mM) were determined using cell viability assay (MTT). The level of insulin mRNA and the concentration of insulin released from beta-cells in a culture medium were analyzed by RT-qPCR and ELISA, respectively. Activity of Akt, ERK and p38 kinases, as well as nuclear levels of islet-enriched Pdx1 and MafA proteins were assessed by Western blot analysis. In comparison to STZ-treated cells, CE extract/STZ co-treatment increased viability of Rin-5F cells for 12%. STZ-treated beta-cells displayed reduced mRNA level of insulin to 63% and reduced insulin secretion to 76% in comparison to controls, while application of CE extract improved insulin mRNA level to 77% and insulin secretion to 90% of the control level. Improved viability and functionality of beta-cells could be ascribed to a CE extract-mediated modulation of the activities of pro-survival Akt, ERK and p38 kinases and Pdx-1 and MafA factors involved in regulation of beta-cell proliferation and insulin expression/secretion. The results of this study suggest that CE extract promotes proliferative and pro-survival pathways in beta-cells and improves their functional properties.

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3

CHOKEBERRY EXTRACT AMELIORATES PLASMA LIPIDS ALTERATIONS IN OBESE DIABETIC RATS

[1]Vucic, V., [1]Arsic, A., [2]Milic, P., [3]Mitrovic, M., [4]Jeremic, J. & [4]Jakovljevic, V.

[1] Institute for Medical Research, University of Belgrade, Serbia

[2] High Medical School of Professional Studies, Cuprija, Serbia

[3]Pharmanova, Belgrade, Serbia

[4]Faculty of Medical Sciences, University of Kragujevac, Serbia

@vesna.vucic.imr@gmail.com

This study investigated the effects of polyphenol rich standardized chokeberry (*Aronia melanocarpa*) extract (SAE), on plasma lipids and fatty acid (FA) profiles in obese diabetic rats. Forty male *Wistar albino* rats, 6 weeks old, were randomly divided into two groups (n=20), fed with standard diet (Sd), or high fat diet (HFd) for one month. Afterwards, HFd fed rats received streptozotocin intraperitoneally (25 mg/kg), to induce diabetes, and were returned to Sd. Ten rats from each group received SAE 0.45 mL/kg/day by oral gavage for 4 weeks. Thus, we had 4 groups: control rats (Ctrl), healthy rats supplemented with SAE (SAE), obese rats with diabetes (OD) and obese diabetic rats treated with SAE (OD+SAE).

We found that OD rats had higher levels of total and LDL-cholesterol and tryglicerydes (TG) than Ctrl group. OD+SAE group had significantly lower levels of LDL-cholesterol and TG than OD group, suggesting cardioprotective effects of SAE. Palmitic acid, saturated FA, palmitoleic acid and monounsaturated FA were lower, while n-6 and total polyunsaturated FA (PUFA), were significantly higher in OD and OD+SAE than in the Ctrl and SAE groups. In OD+SAE, α-linolenic acid (n-3) was higher as compared to SAE group, and linoleic acid (n-6) was higher than in the Ctrl group.

Differences in FA profiles were mostly found between OD groups and healthy rats independently of SAE consumption. Nevertheless, these alterations are alleviated in OD+SAE group indicating that SAE might have beneficial effects on FA profiles. Further studies of longer duration and/or higher SAE doses are needed.

4

VASORELAXANT EFFECT OF COCOA POWDER EXTRACT

[1]Todorovic, V., [2]Jankovic, G., [2]Marinko, M., [1]Sobajic, S. & [2]Novakovic, A.

[1] Department of Bromatology, University of Belgrade – Faculty of Pharmacy, Serbia
[2] Department of Pharmacology, University of Belgrade – Faculty of Pharmacy, Serbia

vanja.todorovic@hotmail.com

Research has suggested a number of cardiovascular benefits achieved due to consumption of cocoa flavonoids. Their cardioprotective abilities, at least in part, could be attributed to vasodilator properties. The main cocoa flavonoid is (-)-epicatechin (60-85 % of total flavonoids) and it has raising research attention as a single active compound but as well as a component in a complex matrix like cocoa powder. Therefore, the aim of the present study was to investigate vasorelaxant effect of cocoa powder extract and compare this effect to (-)-epicatechin vasodilator properties on human saphenous vein (HSV) and human internal mammary artery (HIMA).

HSV and HIMA grafts were supplied from patients undergoing coronary artery revascularization and giving their consent for the excision of remaining tissue. The rings were pre-contracted with phenylephrine (10 μM) to reach stable and sustained contraction, followed by exposing the rings to increasing concentration of (-)-epicatechin (0.1–10 μM) or ethanol cocoa powder extract ((-)-epicatechin in the same concentration range) to obtain concentration-response curves.

Cocoa powder extract (CPE) induced a concentration-dependent relaxation of HIMA rings with maximal response 97.6±4.8% (n=5) or HSV rings with maximal response 90.3±5.7%; (n=5). On the other hand, when (-)-epicatechin was applied, almost half relaxant CPE response was estimated on both of blood vessels (45.7±7% (n=5) - HIMA and 37.3±5.8% (n=5) - HSV). In conclusion, cocoa powder extract caused more relaxation of HIMA and HSV compared to epicatechin and this result contributes to concept that complex natural mixtures often possess higher biological potential than their individual components.

5

BIOSYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACTS: AN UPDATE

[1]Smilkov, K., [1]Gjorgieva Ackova, D.

[1] Department of Applied Pharmacy, Division of Pharmacy, Faculty of Medical Sciences, University Goce Delčev, Štip, North Macedonia

katarina.smilkov@ugd.edu.mk, darinka.gorgieva@ugd.edu.mk

In the recent period, different types of nanoparticles (NPs) have been proposed to improve antioxidant, but also antimicrobial properties of various natural compounds. Much attention has been dedicated to synthesis of NPs using biogenic enzymatic processes. The biosynthesis of NPs has been claimed to be superior to chemical synthesis, especially because of the opportunity of producing more environment-friendly and less toxic products. Among the numerous types of NPs, bioreduction-produced silver NPs from ionic silver-containing solutions are receiving much attention.

In this work, we present an update on our investigation on biosynthesis of silver NPs (AgNPs), thus presenting a method of reduction of silver nitrate solution, using a plant decoct from black pepper fruit (*Piper nigrum*, L). Namely, we present biosynthesis of AgNPs from 1 mmol/L $AgNO_3$ solution, by bioreduction that was provided from the complex composition of pepper fruit extract, obtained by decoction. The formation of the silver NPs was monitored by UV/VIS spectrophotometry and this technique was used for determination of the optimal incubation time for the bioreduction. Here we present the method introduced and the techniques for characterization of the AgNPs that have been performed so far. In addition, we present several ideas of further examination and application of the obtained product, especially in the direction of assessment of their antioxidant properties.

6

2-YEAR MEDITERRANEAN DIET NUTRITIONAL INTERVENTION REDUCED INFLAMMATION AND OXIDATIVE STRESS IN METABOLIC SYNDROME PATIENTS

[1]Sureda, A., [1]Monserrat-Mesquida M., [1]Quetglas-Llabrés, M., [1]Mascaró, C.M., [1]Mateos, D., [1,2]Ugarriza, L., [1]Pons, A. & [1]Tur J.A.

[1] Research Group in Community Nutrition and Oxidative Stress, University of Balearic Islands & CIBEROBN (Physiopathology of Obesity and Nutrition) & IDISBA, E-07122, Palma, Spain.
[2] CS Camp Redó, IBSalut, E-07120 Palma, Spain.

antoni.sureda@uib.es

Metabolic syndrome is a disorder characterised by the existence of multiple risk factors, including central obesity, hyperglycaemia, hypertriglyceridemia, low plasma HDL-cholesterol and hypertension. This syndrome is also associated with low-grade chronic inflammation and oxidative stress. The aim of the present study was to evaluate the effects of a nutritional intervention based on Mediterranean Diet on plasma pro-inflammatory and oxidative stress biomarkers and peripheral blood mononuclear cells (PBMCs) and neutrophils capabilities to produce reactive oxygen species (ROS) after immune stimulation. Subjects aged 55 to 75 years, with overweight/obesity and metabolic syndrome participated in the study. Subjects were divided in two groups: 1) Intensive lifestyle intervention with a hypocaloric Mediterranean diet, physical activity and behavioural therapy and 2) a control group assigned to non-intensive advice on healthy diet (also Mediterranean type) following habitual clinical practice. Plasma levels of the cytokines – interleukin 1β and monocyte chemoattractant protein-1 – and malondialdehyde were significantly reduced in both groups after 2-year intervention. Plasma activity of myeloperoxidase was significantly reduced only in the intervention group after 2 years. ROS production decreased in both PBMCs and neutrophils after the intervention period when stimulated with zymosan and lipopolysaccharide, without significant differences between groups. In conclusion, both dietary interventions reduced pro-inflammatory parameters and the production of ROS by immune cells. The analysis of a greater number of individuals and a longer follow-up period are necessary to determine if the intensive intervention induces additional improvements.

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7

NRF2 PATHWAY MODULATION BY RESVERATROL IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

[1]Spaleniak, W.K., [1]Laoubi, S., [1]Cuendet, M.

[1] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, 1211 Geneva, Switzerland

weronika.spaleniak@unige.ch

Pancreatic cancer (PC) is the most lethal gastrointestinal cancer with a 5-year survival rate of only 9%. Thus, there is an urgent need for improving chemoprevention strategies and therapies. It is assumed that a diet rich in vegetables and fruits might be correlated with lower risk of PC. A natural compound, sulforaphane, has been shown to counteract the aggressiveness of PC through Nrf2 pathway activation [1]. On the other hand, an elevated level of Nrf2 has been demonstrated to support cancer cell survival and progression [2]. Therefore, it is necessary to evaluate the impact of natural compounds present in the diet on PC in regards to Nrf2 pathway modulation. The aim of the study was to investigate mechanisms used by resveratrol in relation to this pathway in pancreatic ductal adenocarcinoma (PDAC) cells. Two cell lines – CFPAC1 and MIA PaCa-2 – were treated with resveratrol. Cytotoxicity was assessed by the MTT assay. Western blots and immunocytochemistry were applied to study protein levels. The antioxidant properties were assessed by measuring ROS level. Treatment with 100 µM resveratrol decreased cell viability and simultaneously increased expression of Nrf2 downstream proteins. However, the investigation of Nrf2 translocation to the nucleus did not lead to a clear outcome. Decreased ROS production was observed in cells pretreated with 10 and 100 µM resveratrol. Given these results, resveratrol is involved in oxidative stress response in PDAC cell lines. However, its effect on the Nrf2 pathway should be further investigated.

References:
[1] Chen X, Jiang Z, Zhou C, Chen K, Li X, Wang Z, et al. Cell Physiol Biochem 2018; 50: 1201-15.
[2] Jaramillo MC, Zhang DD. Genes Dev 2013; 27: 2179-91.

8

CHARACTERIZATION OF PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF FLOWERS AND CLADODES EXTRACTS FROM DIFFERENT *OPUNTIA* CACTUS SPECIES

Youssef El Kharrassi [1], Ezzouhra El Maaiden[1,2], Khadija Moustaid [2],Abdelkhalid Essamadi[1], Pierre Andreoletti[3], Mustapha Cherkaoui-Malki[3]and Boubker[1] Nasser

[1] Université Hassan 1er, FST, Laboratoire Biochemie et Neuroscience, BP 577, Settat 26000, Morocco,
[2]Université Hassan 1er, Laboratoire de Chimie Appliquée et Environment, BP 577, 26000, Settat – Morocco,
[3]Université Bourgogne Franche-Comté, Laboratoire Bio-PeroxIL EA7270, UFR SVTE, Dijon 21000, France.

boubker.nasser@uhp.ac.ma

In the last decade, growing scientific datarelated to O*puntia spp*have been published, revealing their diverse biological activities, anti-inflammatory, anti-microbial and neuroprotective properties among others. The aim of this work was to study the chemical composition and the antioxidant propertiesof the flowers and cladodes of several *Opuntia* species.We prepared two flower-extracts: a chloroform extract for phytosterols content analysis by gas chromatography and a methanol extract for phenolic contentevaluation.Comparatively, two cladode-extracts were prepared: ahexane extract for gas chromatography analysisof phytosterols content and amethanol extract for testing the antioxidant activity, which has been assessed using 1,1-diphenyl-2-picrylhydrazyl, ferric reducing/antioxidant power, total antioxidant capacity and Metal chelating activity assays.

The results of the phytochemical studies showed that the methanol extract of flower bellowing to *O. ficusindica,* who gives a red/pink fruit,revealedthe highest value of polyphenol 1.826 mg/100g of dry matter. For flavonoids, only two ecotypes belonging to *O. ficusindica*(red/pink fruit) and (orange fruit)presented high content 0.998 and 1.064 mg/100g of dry matter respectively. On the other hand, we found large quantities of β-Sitosterol in the cladodes of three species *O. megacantha, O. leucotricha* and *O. dillenii:* 61.33, 51.59 and 42.54% of total phytosterol contentrespectively.Our study revealed also that *O.dillenii* has a powerful anti-radical effect.These data highlight the potential use of *Opuntia* flowers as source of polyphenols and flavonoids and for their antioxidative capacities that would counteract oxidative stress and inflammation in different age-related diseases.

Keywords: Antioxidant activity, flavonoid, flower, cladode, *Opuntia,* phytosterols, polyphenol.

9

RUMEX OBTUSIFOLIUS AND *HIPERICUM ALPESTRE* HERBS AS SOURCES FOR NEW ANTIOXIDANT COMPOUNDS

[1,2,3]Sahakyan, N., [1,2]Ginovyan, M.,[3,4]Andreoletti, P., [c,d]Cherkaoui-Malki, M., [1]Petrosyan, M., [1,2]Trchounian, A.

[1]Department of Biochemistry, Microbiology & Biotechnology, Biology Faculty, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia; [2]Research Institute of Biology, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia; [3]Laboratoire BioPeroxIL, Biochimie du Peroxysome, Inflammation et Métabolisme Lipidique, EA 7270,Unité de Formation et de Recherche des Sciences Vie, Terre et Environnement, 21000 Dijon, France; [4]Laboratoire BioPeroxIL, Université Bourgogne-FrancheComté, 6 Bd Gabriel, 21000 Dijon, France

Trchounian@ysu.am

The goal was to reveal antioxidant properties of herbs *Rumex obtusifolius* Willd and *Hypericum alpestre* subsp. *polygonifolium*, used in Armenian traditional medicine.
R. obtusifolius methanol extract (ME) had the lowest IC_{50} value (25.29 μg mL^{-1}). At the presence of 100 μg mL^{-1}, the highest H_2O_2 reducing activity was observed with *H. alpestre* ME and acetone extracts (99.9% and 98.30%, respectively). Both herbs ME exhibited the metal chelating activity at the concentration of 125 μg mL^{-1} by reducing the number of Fe^{2+}-ferrozine complexes by 31.75% and 73.02%, respectively. Lipid peroxidation inhibitory activity was non-marked. The antioxidant compounds completely maintained DPPH scavenging activity even after treatment at 121^0C for 30 min. The chemical composition was determined by GC-MS analysis. Palmitic acid, hexadecanoic acid and ethyl ester could have a contribution in antioxidant activity of *R. obtusifolius*. Catechol, guaiacol, vanillic acid and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one possessing antioxidant properties were identified in *H. alpestre*. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test showed that the sub-cytotoxic concentration for both BV-2 microglial wild type cells and acyl-CoA oxidase type 1 (ACOX1) deficient cell lines was 1 mg mL^{-1}. The specific catalase activity was increased in both cell lines after 48-hour treatment with both extracts. 24-hour treatment with *H. alpestre* extract was needed to activate palmytoil oxidase in both cell lines, but in *R. obtusifolius* this enzyme increased its activity after 48-hour treatment. Both extracts possessed the increasing of SOD activity in both cell lines.
The results revealed good potential of *H. alpestre* and *R. obtusifolius* herbs as sources for new antioxidant compounds.

1

EFFECTS OF SOMATOSTATIN, CURCUMIN AND QUERCETIN ON THE SIGNALLING PATHWAYS IN BREAST CANCER CELLS

[1]A. Hanikoglu, [2]E. Kucuksayan, [3]F. Hanikoglu, [1,#,*]T. Ozben, [4]G. Menounou, [4]A. Sansone, [4]C. Chatgilialoglu, [5]G. Di Bella & [4,#]C. Ferreri

[1]Department of Biochemistry, Faculty of Medicine, Akdeniz University, 07070 Antalya, Turkey.
[2]Department of Biochemistry, Faculty of Medicine, Alanya Alaaddin Keykubat University, Antalya, Turkey.
[3]Department of Biochemistry, Faculty of Medicine, Biruni University, Istanbul, Turkey.
[4]Consiglio Nazionale delle Ricerche, ISOF, Via Piero Gobetti 101, 40129 Bologna, Italy
[5]Di Bella Foundation, Via G. Marconi 51, 40122 Bologna, Italy
[#]Senior authors contributed equally,
[*]Presenting author: ozben@akdeniz.edu.tr

Objective: Breast cancer is the second main cause of cancer death in women. Curcumin, an active component of spice turmeric, has attracted interest because of its anti-inflammatory and anticancer activities. Quercetin has antioxidative and anti-inflammatory effects. Somatostatin, also known as growth hormone–inhibiting hormone regulates the endocrine system and affects neurotransmission and cell proliferation. There is no study in the literature investigating the effects of somatostatin, curcumin and quercetin and their combinations on signalling pathways in human breast cancer cells.
Material and Methods: The doses of somatostatin, curcumin and quercetin for incubations were determined by MTT test. We used MCF7 and MDA-MB231 breast cancer cells incubated with Cur and Que for 24h, in the absence and presence of SST, at their EC_{50} concentrations to evaluate their effects on EGFR and MAPK signalling pathways.
Results: Distinct signalling pathway changes were found in both cell lines. In MCF7 cells, separate or combined incubations with SST and Que, significantly decreased EGFR and incubation with Cur decreased MAPK signalling. In MDA-MB231 cells, incubation with Cur decreased AKT1 and p-AKT1(Thr308) levels. Incubation with Cur and Que decreased the EGFR levels.
Conclusion: Our results showed that somatostatin, curcumin and quercetin treatments can be combined to induce changes in signalling pathways. Phosphorylation of AKT is an important link in cell apoptosis process. Phosphorylated AKT (p-AKT) proteins cause cell death and regulate various signalling pathways. This study contributes to the literature showing the effects and mechanisms of these antioxidants in combination with somatostatin in breast cancer cell signalling.

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2

METABOLIC PROFILE ELUCIDATION AFTER *IN VITRO* BIOTRANSFORMATION OF *HERNIARIA HIRSUTA* BY AN INNOVATIVE DATA ANALYSIS STRATEGY FOR DYNAMIC MULTICLASS EXPERIMENTS

[1]Peeters, L., [2]Beirnaert, C., [1]Van der Auwera, A., [1]De Bruyne T., [2]Laukens, K., [1]Pieters L., [1]Foubert, K., [1]Hermans, N.

[1] Natural products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Belgium
[2] Adrem Data Lab, Department of Mathematics – Computer Sciences, University of Antwerp, Middelheimlaan 1, 2020 Antwerp, Belgium

Laura.Peeters@uantwerpen.be

Urinary stone disease is considered as an important healthcare problem. An aqueous extract of the aerial parts of *H. hirsuta* (Caryophyllaceae) is a widely used herbal medicine. However little is known about the active compounds and the mechanism of action.[1,2] Previous phytochemical research on *Herniaria* species revealed the presence of saponins, flavonoids and coumarins.[2] Metabolites of phytochemicals present in *H. hirsuta* (most likely saponins) may be responsible for the beneficial effects.
In vitro gastrointestinal biotransformation followed by automated data analysis was optimized using hederacoside C as a model compound for saponins.[3,4] Samples were analyzed with UHPLC-UV-HRMS before, during and after biotransformation. To analyze the longitudinal multiclass data, XCMS and EDGE were used to extract significant differential profiles from the raw data.[5,6] An interactive Shiny app was developed in R and used to train a random forest model for predicting experts response.[4,7] The maximal score of 1 corresponds to the model labeling this feature as interesting, the minimal score of 0 defines an uninteresting feature.
As an example, herniariasaponin H, the most abundant saponin in the extract, showed stepwise elimination of sugar moieties resulting in medicagenic acid. This aglycon showed a score of 1.
Subsequent *in vitro* hepatic biotransformation (phase I and II) was performed with medicagenic acid. Suspect screening, using Meteor Nexus software, and non-target screening were combined leading to the tentative identification of ten metabolites.[8] These hydrophilic metabolites can be excreted via the kidneys. However, it is possible that one of these metabolites is responsible for the urolithiatic activity.

[1] Atmani F, et al. J Ethnopharmacol. 2004;95(1):87-93.
[2] van Dooren I, et al. Planta Med. 2016;82(18):1576-83.
[3] Breynaert A, et al. Planta Med. 2015;81(12-13):1075-83.
[4] Peeters L, et al. Journal of Chromatography A. 2019;1597:54.
[5] Storey JD, et al. PNAS 2005;102(36):12837-12842
[6] Leek JT, et al. Bioinformatics 2005;22(4):507-508
[7] Beirnaert C, et al. Metabolites. 2019;9(3):54.
[8] Vervliet et al. Drug Test Anal 2019;11:479.

3

XANTHOHUMOL AND PHENETHYL ISOTHIOCYANATE COMBINATION IS THE POTENT INDUCER OF ACTIVATION OF NRF2 AND EXPRESSION OF ANTIOXIDANT ENZYMES IN PANCREATIC CANCER CELLS

Violetta Krajka-Kuźniak, Marta Cykowiak, Wanda Baer-Dubowska

Department of Pharmaceutical Biochemistry Poznan University of Medical Sciences, Poznań, Poland

Email: baerw@ump.edu.pl

Pancreatic cancers possess the worst prognosis and have one of the highest mortality rates. Advancing age is a highrisk factor for this type of cancer. Thus, the management of pancreatic cancer in the aging population is becoming increasingly important. In this study we evaluated the effect of selected phytochemicals and their combinations on the activation of Nrf2 and the expression of genes controlled by this transcription factor in the human pancreatic cancer cell line MIA-Pa-Ca-2. Treatment for 24 h with xanthohumol (XAN), resveratrol (RES), indole-3-carbinol (I3C) or phenethyl isothiocyanate (PEITC) alone at the concentration of 5 and 10 μM enhanced Nrf2 activation and expression, but combinations of these phytochemicals were more efficient suggesting their synergistic interaction. This observation confirmed the results of our preliminary investigation in which the concentration of 1 μM of these phytochemicals was applied. The combination of XAN and PEITC and to lesser extent the mixture of RES and PEITC increased mostly the expression of *SOD, catalase* and *GPx*, the Nrf2 target genes which products deactivate ROS.
No differences were found considering the effect of single phytochemicals and their combination on the expression of *GSTP* and *NQO1* genes, which products are involved in reactive electrophiles detoxification. These results indicate that combinations of phytochemicals resembling that occurring in natural diets may efficiently modulate the signaling pathways, which proper function is important for pancreatic cancer prophylaxis or improving the results of conventional therapy.

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4

GUT MICROBIOTA COMPOSITION INFLUENCES THE EXTENT OF WEIGHT LOSS AFTER HYPOCALORIC DIET IN OBESE MALE ADOLESCENTS

[1]Djordjevic, A.D., [2]Vujkovic Cvijin, I.I., [3]Lešović, S.J., [1]Gligorovska, Lj.N., [1]Bursac B.N., [1]Vojnović-Milutinović D.D. & [1]Matić, G.M.

[1] Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia
[2] National Institute of Allergy and Infectious Disease, NIH, Bethesda, USA
[3] Special Hospital for Thyroid Gland Diseases and Metabolic Diseases „Cigota", Zlatibor, Serbia

djordjevica@ibiss.bg.ac.rs

Prevalence of obesity among adolescents has been constantly increasing in the last decades. The treatment of obesity requires multidisciplinary approach, which includes dietary management. However, not all people respond to dietary intervention in the same way. Since gut microbiota has been tightly linked to obesity, the aim of this pilot study was to assess whether microbiota composition affects the outcome of the hypocaloric diet on weight loss in obese male adolescents.

Forty-four obese male adolescents (average BMI>95th percentile), 12-15 years old, were selected from the large cohort of 500 patients. Their body composition was assessed before and after 3-week balanced hypocaloric diet (1200-1700 kcal) with preserved nutritional value. Microbial DNA was extracted from cryopreserved fecal samples collected before the dietary intervention. Alterations of the gut microbiota were analyzed using MiSeq 16S rRNA gene sequencing.

The primary outcome of the diet was the change in body weight and BMI. Subjects were divided in 2 groups according to significant differences in delta BMI after the dietary intervention ($P < 0.001$). The values for delta BMIs were 1.93 and 2.66 for groups 1 and 2, respectively. The observed differences were associated with fecal microbiome composition. Group 2 subjects, which have lost more weight, originally had less *Firmicutes spp.* bacteria, more specifically from families *Lachnospiraceae* and *Desulfovibrionaceae.*

These preliminary results show that the ability for diet-induced weight loss could be associated with microbiota composition. Whether certain bacterial taxa represent facilitating or resilience factor for weight loss is yet to be determined in future experiments.

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5

ACTIVITY OF USNIC ACIDS DERIVATIVES TOWARDS CANCER CELLS

Anna Herman-Antosiewicz[1], Agnieszka Pyrczak-Felczykowska[2], Anna Pawlik[1], Kamil Ryś[1], Aleksandra Hać[1], Beata Guzow-Krzemińska[3], Rajeshwar Narlawar[4] & Michael Kassiou[4]

[1] University of Gdańsk, Department of Medical Biology and Genetics, Poland;
[2] Medical University of Gdańsk, Chair and Department of Physiology, Poland;
[3] University of Gdańsk, Department of Plant Taxonomy and Nature Conservation, Poland;
[4] School of Chemistry, The University of Sydney, Australia;

anna.herman-antosiewicz@biol.ug.edu.pl

The emerging increase in morbidity and mortality caused by cancer inclines to constant development of drugs with high potency and selectivity towards cancer cells. Natural products and their structures continue to play a significant role in drug discovery. Usnic acid (UA) is an abundant secondary metabolite found in lichens. It possesses broad spectrum of biological activities, such as antimicrobial, antiviral, antiprotozoal, anti-inflammatory, wound-healing, analgesic as well as antiproliferative, anti-metastatic and anti-angiogenic. However, the high hepatotoxicity and low water solubility of UA restricts its practical use in anticancer therapy. Thus, efforts are made to obtain UA derivatives with more favourable biological properties.

The aim of this work was synthesis and selection of UA derivatives with strong and selective activity against cancer cells and elucidation of the mechanisms of their action. Biological activities have been tested using MTT viability test, microscopy techniques, clonogenicity assay, flow cytometric evaluation of cell cycle and cell death.

Among synthesized UA derivatives isoxazole derivatives 2a and 2b revealed potent, as compared with parent compound, antiproliferative activity towards cancer cells of different origin, such as breast, prostate, cervical, lung and liver cancers. Moreover, healthy human cells appeared less sensitive than cancer cells to tested compounds. Mechanism of action of 2a and 2b derivatives in MCF-7 breast cancer cells relied on G0/G1 cell cycle arrest, induction of massive cytoplasmic vacuolization and apoptosis. These results indicate that 2a and 2b derivatives might be promising anticancer drugs although their antitumor potency has to be validated in *in vivo* models.

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6

EFFECTS OF THE FOOD BIOACTIVE ERUCIN ON HUMAN RENAL CANCER CELLS

Vidovic, B.[1], Guerreiro, I[2], Costa, J.G.[2], Oliveira, N.G.[3], Saraiva, N.[2], Fernandes, A.S.[2]

[1]Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[2]CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal
[3]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal

bojana.vidovic@pharmacy.bg.ac.rs

Epidemiological evidence associates the consumption of cruciferous vegetables with reduced risk of several types of cancer, including renal cell carcinoma. Erucin, 4-(methylthio) butyl isothiocyanate is a structurally-related analog of the well-known sulforaphane. Erucin can be generated both by *in vivo* reduction of sulforaphane, and by enzymatic hydrolysis of glucoerucin, a glucosinolate found at high levels in rockets species. Contrarily to sulforaphane, only limited studies have addressed the anticancer properties of erucin. The goal of this study is therefore to evaluate the cytotoxic effects of erucin and its effects on renal carcinoma cell motility and intracellular ROS levels.
Exposure to erucin (10-100 μM) induced a concentration-dependent decrease in cell viability (MTT assay). Cytotoxic effects were more pronounced in 786-O human renal cancer cells than in Vero cells (non-cancer origin), used as a control cell line. Non-cytotoxic concentrations (up to 50 μM; MTT and PI staining) were chosen for the subsequent studies. Erucin decreased intracellular ROS levels in Vero cells, but not in 786-O cells (DCFH-DA). Erucin decreased collective cell migration (Wound-healing assay) and chemotaxis/chemoinvasion (transwell-based assay) of renal cancer cells. Our results show for the first time that erucin has favorable effects *in vitro* against human renal carcinoma, that require further investigation.

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7

STUDY OF THE EFFECTS OF HONEY ON THE MECHANISMS OF AUTOPHAGY IN MACROPHAGES

Jérémy VERBEKE[1], Mathieu LIMAUGE[1], Robin VARSEBROUCQ[1], Virginie TEVEL[2], Patricia RENARD[1], Thierry ARNOULD[1], Michel JADOT[2]
[1]Research Unit in Cell Biology (URBC)
[2]Research Unit in Molecular Physiology (URPhyM), Physiological Chemistry Lab
Namur Research Institute for Life Sciences (NARILIS), University of Namur (UNamur), Belgium

jeremy.verbeke@unamur.be

For thousands of years, honey has been a staple of traditional medicine. It was used as an ointment to treat various ailments such as skin and gastro-intestinal injuries. In the last few decades, honey has regained interest in the eyes of the scientific and medical communities. Its biological properties have been extensively studied and honey is now recognized as having wound-healing properties. Indeed, honey is a potent antimicrobial, antioxidant and immunomodulatory agent (Almasaudi *et al.*, 2017; Alvarez-Suarez *et al.*, 2014; Kwakman *et al.*, 2011). While these properties have been clearly demonstrated, little is known about the cellular and molecular processes underlying the effects of honey on eukaryotic cells and organelles.

As macrophages are actively involved in both inflammation and wound healing, it is interesting to analyze their biological responses to honey exposure. This project thus aims to study the response of RAW264.7 macrophages to the presence of several types of honey, from medical grade honeys (Revamil® and Manuka) to artisanal honeys harvested in spring and summer time, with a special focus on the biology of mitochondria and lysosomes as well as on autophagic flux.

In this study, we show that macrophages incubated in the presence of Revamil®, Manuka and summer honeys display an increased abundance of the autophagy markers LC3-II and p62. In addition, neither trehalose nor methylglyoxal, two components of honey, seem to be responsible for these effects. The exact nature of the modulation of autophagy by honey remains currently unknown but seems to be independent of mTOR activation. However, the accumulation of these autophagic markers could be caused by either a stimulation of the initiation of the autophagic flux, or by the inhibition of the terminal degradation stages. Future work will thus be aimed at testing these hypotheses by analyzing the signaling pathways controlling autophagic flux in macrophages, as well as attempting to identify the potential compound(s) that is (are) responsible for these effects.

Almasaudi, S. B., Abbas, A. T., Al-Hindi, R. R., El-Shitany, N. A., Abdel-Dayem, U. A., Ali, S. S., … Harakeh, S. M. (2017). Manuka Honey Exerts Antioxidant and Anti-Inflammatory Activities That Promote Healing of Acetic Acid-Induced Gastric Ulcer in Rats. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–13.

Alvarez-Suarez, J., Gasparrini, M., Forbes-Hernández, T., Mazzoni, L., & Giampieri, F. (2014). The Composition and Biological Activity of Honey: A Focus on Manuka Honey. *Foods*, *3*(3), 420–432.

Kwakman, P. H. S., te Velde, A. A., de Boer, L., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2011). Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE*, *6*(3), 3–9.

ANTI-PROSTATE CANCER ACTIVITY OF A NEW ORGANOSELENIUM COMPOUNDS-ROLE OF IRON METABOLISM

Kaczor, K. B.[1], Juhas, U.[2], Borkowska, A.[1], Pawlik, A.[3], Scianowski, J.[4], Paculа, A.[4], Obieziurska, M.[4], Wojtowicz, A.[1], Antosiewicz, J.[1]

[1]Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk, Poland
[2]Department of Immunology, Medical University of Gdansk, Poland
[3]Department of Biochemistry, Gdansk University of Physical Education and Sport, Poland
[4]Department of Organic Chemistry, Faculty of Chemistry, Nicolaus Copernicus University, Poland

andzelika.borkowska@gumed.edu.pl

The main goal of this study was to evaluate anticancer activity of newly synthetized organoselenium compounds (OSCs) and get insight on mechanism of their activity.
OSCs have been shown to possess a variety of biological activities including cytostatic and cytotoxic action against tumour cells. In this study, the cytotoxic effect and anticancer mechanism of action of OSCs was investigated on two phenotypically different prostate cancer cell lines DU 145 and PC-3. The influence of studied compounds on viability parameter was also assessed on normal prostate cell line PNT1A. The results showed that some of the OSCs efficiently inhibited cancer cell proliferation, whereas normal PNT1A cells were less sensitive. Conversely cytotoxic activity of some OSCs was observed only in very high concentrations. Inhibition of cancer cell growth was associated with induction G2/M cell cycle arrest and prompted cell death thorough apoptosis. Interestingly some compounds increase cell death which was partially blocked by iron chelator what indicates for ferroptotic cell death. These assumptions is confirmed by observed changes in iron metabolism in cells treated with OSCs.
In conclusion, our data demonstrate that newly synthesized OSCs despite of being strong antioxidants *in vitro*, induced oxidative stress dependent cell death in prostate cancer cells. In addition, action of some of these compounds is related to changes in iron metabolism.

9

ANTIMICROBIAL ACTIVITY AND IMMUNOMODULATING POTENTIAL OF *LACTOBACILLUS PLANTARUM* M2 ISOLATED FROM DONKEY MILK

[1]Kostelac, D., [2]Gerić, M., [2]Gajski, G., [1]Markov, K., [1]Čanak, I., [1]Jakopović, Ž., [1]Frece, J.

[1]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000, Zagreb, Croatia
[2]Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia

dkostelac@pbf.hr

Traditionally donkey milk has been used as an alternative to human milk for infants or children mainly because of its similarities in nutrient composition, hypoallergenicity, immune modulation and antimicrobial activity. As a highly nutritious substrate it has a great potential for probiotic bacteria isolation. Beneficial health effects of probiotic bacteria used in functional foods and pharmaceutical products are based on the ability and capacity of these microorganisms in stimulation of host microbiota and immune system.
The aim of the study was to assess *Lactobacillus plantarum* M2's probiotic potential through antimicrobial activity and immunomodulation potential.
L. plantarum M2 was isolated from donkey milk and identified using biochemical and genetic tests. Antimicrobial activity against several pathogens was determined using turbidimetric method. TNF-α production of LPS-stimulated human blood mononuclear cells (PBMC) was determined using commercial kit.
L. plantarum M2 demonstrated great antimicrobial activity against all tested pathogens during 72h, namely *S. aureus* (96% inhibition), *S. typhimurium* (99% inhibition), *L. monocytogenes* (93% inhibition), *C. utilis* (48% inhibition) and *E. coli* (70% inhibition during 48 h). The LPS-stimulated PBMCs treated with *L. plantarum* M2 extracellular metabolites showed significant 66% suppression of TNF-α production (87.7 pg/mL compared to 262.5 pg/mL).
Based on the results it can be assumed that donkey milk has a great potential as a source of probiotic bacteria contributing to the antimicrobial and anti-inflammatory human health benefits.
Study supported by Institutional financing.

10

SYNTHESIS OF SILVER NANOPARTICLES BY *ARTEMISIA ANNUA* PLANT EXTRACT AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

[1,2]Aghajanyan A., [1,2]Trchounian A.

[1]Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 0025 Yerevan, Armenia; Research Institute of Biology, Yerevan State University, 0025 Yerevan, Armenia

Trchounian@ysu.am

Among the noble metal nanoparticles (NPs) Ag has several applications, such as antibacterial agent. *Artemisia annua* L. plant has various biological activities due to antibacterial, anti-inflammatory and antitumor effects [1]. *A. annua* is the source for the production of artemisin that is used in Armenian folk medicine. The aim was to adopt a low cost and simple green method of the synthesis AgNPs using extract of *A. annua* and to evaluate their antibacterial activity.

A. annua grown by hydroponics method was used for extraction, as a reducing agent for the development of AgNPs. 10 mL of extract was added to 90 mL of 1 mM $AgNO_3$, and it was shaken at room temperature for 1 h [2]. The color of the reaction mixture turned from yellowish brown to dark brown, indicating the formation of AgNPs. The reduction in $AgNO_3$ to AgNPs was observed by recording UV-Vis absorption spectrum. The antibacterial activity of synthesized AgNPs was evaluated against Gram-negative (*Escherichia coli* BW 25113) and Gram-positive (*Enterococcus hirae* ATCC 9790) bacteria. The minimum inhibitory concentrations of AgNPs against these bacteria were of 100 and 150 μg mL^{-1}, respectively. The synthesized AgNPs not only inhibited the growth of *E. coli* but also killed them after 24 hours.

The results demonstrated a significant effect of of *A. annua* extract AgNPs against both Gram-negative and Gram-positive bacteria.

References

1. Isani G. et al. Oxidative Medicine and Cellular Longevity, 2019, ID 1615758, 9 pages.
2. Singh T. et al. Journal of Genetic Engineering and Biotechnology, 2017,15:31-39.

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