Dipartimento di Agraria

Dipartimento di Eccellenza 2018-2022

Dipartimento di Fisiologia e Farmacologia Vittorio Erspamer
Sapienza Università di Roma

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SOCIETÀ ITALIANA DI FARMACOLOGIA

Gruppo di Lavoro
Farmacognosia, Fitoterapia e Nutraceutica

CONVEGNO MONOTEMATICO

Le Basi Farmacologiche dei Nutraceutici

Hotel Royal Continental, Via Partenope, 38
NAPOLI 29-30 marzo 2019

Programma e Abstract

Comitato Organizzatore
Raffaele Capasso, Angelo A. Izzo, Gabriela Mazzanti

Presidente Onorario
Francesco Capasso

Comitato Scientifico
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Comitato Organizzatore Locale:
Barbara Romano, Ester Pagano, Dipartimento di Farmacia, Università degli Studi di Napoli Federico II

Con il patrocinio di

Università degli Studi di Napoli Federico II

Programma

Venerdi 29 Marzo

12.30-14.00 Registrazione dei Partecipanti

Sala Vesuvio
14.00-14.30 Saluti di benvenuto (Magnifico Rettore dell'Università degli Studi di Napoli Federico II, Presidente SIF, Comitato Organizzatore, Direttore Dipartimento di Farmacia Napoli Federico II, Direttore Dipartimento di Agraria Napoli Federico II)

14.30-15.00 Lettura magistrale: **Impact of Mediterranean diet on gut microbiota**
Ercolini D. (Napoli)

15.00-16.45 Sessione: Farmacologia clinica **Sala Vesuvio**

Moderatori: Mugelli A (Firenze) – Racagni G (Milano)

15.00-15.15 **Clinical evaluation of a new Hydroxytyrosol and Vitamin E-based nutraceutical formulation on pediatric NAFLD**
Smeriglio A, Nobili N, Alisi A, Mosca A, Crudele A, Zaffina S, Denaro M, Trombetta D (Messina, Roma)

15.15-15.30 **Caffeine intake during pregnancy and neonatal low birth weight: data from a multicenter Italian retrospective study**
Mannucci C, Calapai F, Facchinetti F, D'Anna R, Santamaria A, Lenti MC, Vannacci A, Perone M, Cardia L,. Sorbara EE, Oteri A, Calapai G (Messina)

15.30-15.45 **Vitamin D replacement ameliorates serum lipoprotein functions, adipokine profile and subclinical atherosclerosis in pre-menopausal women**
Zimetti F, Greco D, Kocyigit D, Adorni MP, Marchi C, Ronda N, Gurses KM, Canpinar H, Guc D, Oguz SH, Gurlek A, Strazzella A, Simonelli S, Tokgozoglu L, Bernini F (Parma, Ankara, Konya, Milano)

15.45-16.00 **Effect of a novel nutraceutical combination on serum lipoprotein functional profile and circulating PCSK9**
Adorni MP, Ferri N, Marchianò S, Trimarco V, Rozza F, Izzo R, Zimetti F, Bernini F (Parma, Padova, Milano, Napoli)

16.00-16.15 **Whey protein isolate supplementation improves body composition, muscle strength and treatment tolerance in malnourished advanced cancer patients undergoing chemotherapy**
Cereda E, Ferrari A, Turri A, Klersy C, Cappello S, Imarisio I, Caraccia M, Zuccarini A, Cicognini D, Borioli V, Monaco T, Stella GM, Arcaini L, Pedrazzoli P, Caccialanza R (Pavia)

16.15-16.30 **A novel nutraceutial formulation from winemaking by-products for the control of circulating oxidative stress markers: a randomized clinical trial**
Annunziata G, Caruso D, Buonomo G, D'Avino M, Tenore GC, Novellino E (Napoli, Benevento)

16.30-16.45 **Polyphenol health effects on cardiovascular disorders: myth or truth?**
Potì F, Zimetti F, Spaggiari G, Santi D, Zanotti I (Parma, Modena-Reggio Emilia)

16.45-17.15 **Coffee break**

17.15-19.00 Sessione: Infiammazione **Sala Vesuvio**

Moderatori: Ghelardini C (Firenze) – Cirino G (Napoli)

17.15-17.30 **Protective effects of cyanidin-3-O-glucoside on human epithelial cells exposed to palmitic acid**
Muscarà C, Bashllari R, Molonia MS, Occhiuto C, Cristani M, Saija A, Cimino F, Speciale A (Messina)

17.30-17.45 **Hydroxytyrosol-3O sulfate prevents Endothelial-to-Mesenchymal Transition induced by inflammatory environment**
Nannelli G, Terzuoli E, Ziche M, Donnini S, Morbidelli L (Siena)

17.45-18.00 **Erucin, a H_2S-donor from *Eruca sativa* Mill. exhibits protective effects at vascular level against oxidative and pro-inflammatory stimuli**
Martelli A, Piragine E, Citi V, Testai L, Matteo R, Ugolini L, Pagnotta E, Lazzeri L, Di Cesare Mannelli L, Vellecco V, Brancaleone V, Bucci M, Ghelardini C, Calderone V (Pisa, Bologna, Firenze, Napoli, Potenza)

18.00-18.15 **Anti-ageing activity of *Salvia haenkei* in an *in vitro* epidermal model**
Cocetta V, Cadau J, Catanzaro D, Saponaro M, Miolo G, Ragazzi E, Alimonti A, Montopoli M (Padova, Bellinzona)

18.15-18.30 **Tannins from Chestnut (*Castanea sativa* Mill.) leaves and fruits show promising in vitro anti-inflammatory properties in gastric epithelial cells**
Piazza S, Sangiovanni E, Vrhovsek U, Fumagalli M, Khalilpour S, Masuero D, Colombo L, Mattivi F, De Fabiani E, Dell'Agli M (Milano, San Michele all'Adige, Varese)

18.30-18.45 **Plumericin reduces intestinal inflammation and oxidative stress: *in vitro* and *in vivo* studies**
Rapa SF, Waltenberger B, Di Paola R, Autore G, Cuzzocrea S, Stuppner H, Marzocco S. (Salerno, Innsbruck, Messina)

18.45-19.00 **Graminex pollen: protective effects in immortalized prostate cells (PC3) and rat prostate challenged with LPS**
Recinella L, Locatelli M, Macchione N, Ferrante C, Chiavaroli A, Carradori S, Zengin G, Cesa S, Leporini L, Leone S, Brunetti L, Menghini L, Orlando G **(**Chieti**,** Milano, Konya, Roma)

17.15-19.00 Sessione: Neuroscienze **Sala Posillipo**

Moderatori: Calapai G (Messina) - Calignano A (Napoli)

17.15-17.30 **Resveratrol treatment reduces the vulnerability of SH-SY5Y cells and cortical neurons overexpressing SOD1-G93A to Thimerosal toxicity**
Sirabella R, Laudati G, Mascolo L, Guida N, Pizzorusso V, Serani A, Di Renzo G, Canzoniero L MT, Formisano L (Napoli)

17.30-17.45 **Effects of *Ginkgo biloba* L. (GK501) and *Panax ginseng* C.A. Meyer (G115) and their combination in organotypic hippocampal slice cultures and mixed cortical cells exposed to excitotoxic insults**
Landucci E**,** Bilia AR, Pellegrini-Giampietro D, Bergonzi MC (Firenze)

17.45-18.00 **Purple corn extract as adjuvant therapy for the prevention and treatment of trigeminal pain: role of microglia and of the gut microbiota**
Magni G, Marinelli A, Riccio D, Lecca D, Milani C, Ventura M, Abbracchio MP, Tonelli C, Petroni K, Ceruti S (Milano)

18.00-18.15 **Implication of gluten peptides on neurological disorders: study of molecular mechanisms in experimental models of epilepsy**
Gerace E, Resta F, Landucci E, Renzi D, Masi A, Pellegrini-Giampietro D E, Calabrò A, Mannaioni G (Firenze)

18.15-18.30 **Pomegranate-derived nutraceuticals: from gut health to abdominal pain relief**
Lucarini E, Parisio C, Micheli L, Mulinacci N, Ghelardini C, Di Cesare Mannelli L (Firenze)

18.30-18.45 **Altered gut microbiota and endocannabinoid system tone in vitamin D deficiency-mediated chronic pain**
Boccella S, Guida F, Belardo C, Iannotta M, Piscitelli F, De Filippis F, Paino S, Ricciardi F, Siniscalco D, Marabese I, Luongo L, Ercolini D, Di Marzo V, Maione S (Napoli, Pozzuoli)

18.45-19.00 **Dietary depletion of chicken egg exosomes and their cargo affects the gene expression profile in right and left hippocampus and impairs cognitive performance in C57BL/6 mice**
Fratantonio D, Shu J, Cui J, Zempleni J (Roma)

20.30 **Serata Pizza**

Sabato 30 Marzo — Sala Partenope

8.45-10.45 Sessione: Metabolismo ed obesità

Moderatori: Mazzanti G (Roma) – Bernini F (Parma)

8.45-9.00 **Extra virgin olive oil polyphenols modulate the expression of key inflammatory genes and miRNAs in human adipocytes**
Carpi S, Massaro M, Polini B, Digiacomo M, Manera M, Scoditti E, Nieri P (Pisa, Lecce)

9.00-9.15 **Oleacein, a polyphenol of olive oil, modulates expression of adipogenesis markers in adipose tissue of high-fat diet fed mice**
Maggisano V, Lepore SM, Bulotta S, Mignogna C, Arcidiacono B, Iannone M, Brunetti A, Russo D, Celano M (Catanzaro)

9.15-9.30 **A study about the effect of fruit phytochemicals on the glucose and phenols intestinal absorption in a Caco-2TC7 monolayer model, by means of glucose and polyphenols amperometric biosensors**
Rocchitta G, Bacciu A, Arrigo P, Barberis A, Spissu Y, Bazzu G, Serra PA (Sassari)

9.30-9.45 **Lycopene activates brown adipose tissue through PPARγ stimulation and might be effective for the treatment of obesity**
Irrera N, Mannino F, Pallio G, Altavilla D, Squadrito F, Bitto A (Messina)

9.45-10.00 **Water-soluble extract of *Morus alba* is able to lower the expression of proprotein convertase subtilisin/kexin type 9 in hepatoma cell lines with benefic effects on LDL cholesterol-uptake**
Lupo MG, Macchi C, Marchianò S, Corsini A, Ruscica M, Ferri N (Padova, Milano)

10.00-10.15 **The marine microalgae *Tisochrysis lutea* attenuate high-fat-induced Metabolic Syndrome in Rat.**
Cinci L, D'ambrosio M, Bigagli E, Zambelli F, Cesario G, Niccolai A, Biondi N, Rodolfi L, Tredici MR, Luceri C (Firenze)

10.15-10.30 ***Citrus bergamia* juice extract and its major flavanones: a treasure trove for the interaction with the AMPK/SIRT1 axis**
Maugeri A, Ferlazzo N, Musumeci L, Russo C, Gitto R, De Luca L, Navarra M (Messina)

10.30-10.45 ***In vitro* protective effects of a *Glycyrrhiza glabra* L. leaf extract on palmitate-induced insulin resistance in endothelial cells**
Molonia MS, Occhiuto C, Cristani M, Siracusa L, Rocco C, Ruberto G, Saija A, Cimino F, Speciale A (Messina, Catania)

10.45-11.45 Coffee break e sessione poster

11.45-13.00 Sessione: Antitumorali ed antinfettivi

Moderatori: Mascolo N (Napoli) – Calderone V (Pisa)

11.45-12.00 **Natural products from various sources prevent colon carcinogenesis and increase apoptosis in the Pirc rat (*F344/NTAC-Apc ^{am1137}*), a genetic model of colorectal cancer**
Tortora K, Femia AP, Luceri C, Giovannelli L, Caderni G (Firenze)

12.00-12.15 **Potential chemopreventive and therapeutic effects of cynaropicrin in metastatic melanoma**
De Cicco P, Busà R, Ercolano G, Formisano C, Taglialatela-Scafati O, Ianaro A (Napoli, Palermo)

12.15-12.30 **The anti-proliferative and anti-metastatic effects of oleacein in SH-SY5Y human neuroblastoma cells**
Cirmi S, Lombardo GE, Musumeci L, Russo C, Maggisano V, Celano M, Russo D, Navarra M (Messina, Catanzaro)

12.30-12.45 **Saffron byproducts as sources of bioactive extracts: pharmacological and toxicological focus on anthers**
Ferrante C, Menghini L, Chichiriccò G, Recinella L, Leone S, Chiavaroli A, Brunetti L, Di Simone S, Ronci M, Piccone P, Lanza B, Cesa S, Poma A, Vecchiotti G, Orlando G (Chieti)

12.45-13.00 **New potential nutraceutical application and chemical characterization of Humic extract from green compost.**
Verrillo M, Salzano M, Cozzolino V, Spaccini R, Piccolo A (Napoli)

13.00 **Premiazione e Chiusura del Convegno**

13.30-14.00 **Riunione del Gruppo di Lavoro di Farmacognosia, Fitoterapia e Nutraceutica**

Sessione poster sabato 30 Marzo ore 10.45-11.45 Sala Giardino

Moderatori: Testai L (Pisa) - Russo A (Catania) - Morbidelli L (Siena) - Caderni G (Firenze) - Dell'Agli M (Milano) – Orlando G (Chieti) – Avato P (Bari)

Sessione 1

P1. **Effect of 8-week n-3 fatty-acids supplementation on oxidative stress and inflammation in middle and long distance running athletes**
Buonocore D, Verri M, Doria E, Ghitti M, Cattaneo L, Dossena M (Pavia)

P2. **May natural supplements alter the urinary LH and steroid's profiles in antidoping analyses? A pilot study**
Alberti F, Braganò MC, de la Torre X, Iannone M, Botrè F (Roma)

P3. **Pros and cons of pure molecules and plant extracts in nutraceutical field: the ideain *vs* pistachio hull extract case**
Denaro M, Smeriglio A, Trombetta D (Messina)

P4. **Role of nutrition in neurodegenerative disease**
Rizzi L, Bianchi V (Milano)

P5. **Pharmacological effects of the standardized Lipidosterolic Extract from *Kigelia africana* fruits in experimental Benign Prostatic Hyperplasia induced by testosterone in Sprague Dawley Rats**
De Pasquale D, Occhiuto C, Aloisi I, Santoro G, Tranchida PQ, Mondello L, Puglisi G, Occhiuto F (Messina)

P6. **The paradox of toxicological studies on nutraceuticals**
Nunziata A, Bianco S, Le Donne M (Pomezia)

Sessione 2

P7. **Antioxidant and Antiinflammatory Activities of Polyphenolic Rich Ethyl Acetate Fraction from *C. incanus* Leaves**
D'Ambrosio M, Bigagli E, Cinci L, Gori A, Brunetti C, Luceri C (Firenze)

P8. **Efficacy of xyloglucan against *Escherichia coli* urinary tract infection: *in vivo* study**
Campolo M, Casili G, Lanza M., Franco D, Fazio E, Filippone A, Paterniti I, Peritore A, Cuzzocrea S and Esposito E (Messina)

P9. **Strawberry tannins as potential ingredients of nutraceuticals to counteract *H. pylori*-induced gastric inflammation**
Martinelli G, Fumagalli M, Sperandeo P, Sangiovanni E, Piazza S, Polissi A, De Fabiani E, Dell'Agli M (Milano)

P10. **Sodium propionate protects from inflammation and ROS stress**
Filippone A, Paterniti I, Campolo M, Lanza M, Casili G, Cuzzocrea S, Esposito E (Messina)

P11. **Vitamin E supplementation prevents allergen sensitization in the mouse**
Cerqua I, Koeberle A, Riemma MA, Rossi A, Cirino G, Roviezzo F (Napoli, Jena)

P12. **α-T-13'-carboxychromanol, an endogenous metabolite of vitamin E, limits inflammation by targeting 5-lipoxygenase**
Bilancia R, Pein H, Pace S, Ialenti A, Sautebin L, Rossi A, Werz O, Koeberle A (Napoli, Jena)

P13. **Evidence for a beneficial effect of caffeine in psoriasis-like inflammation**
Caiazzo E, Morello S, Ialenti A, Cicala C (Napoli, Salerno)

Sessione 3

P14. **Peptides released after simulated gastrointestinal digestion of dehydrated chips exert antinflammatory activity in intestinal epithelial cells**
Rapa SF, Cianciarulo D, Basilicata MG, Pepe G, Sommella E, Manfra M, Rago R, Rago G, Autore G, Campiglia P, Marzocco S (Salerno, Potenza, Battipaglia)

P15. **Polyphenolic extract from Tarocco (*Citrus sinesis* L.Osbeck) clone "Lempso" exerts anti-inflammatory and antioxidant effects via NF-kB and Nrf-2 activation in murine macrophages**
Cianciarulo D, Basilicata MG, Pepe G, Sommella E, Russo M, Manfra M, Autore G, Campiglia P, Marzocco S (Salerno, Potenza, Reggio Calabria)

P16. ***Aloe vera* gel and *Punica granatum* onconutraceutical potential in intestinal epitelial cells during oxidative stress and inflammatory conditions**
Cianciarulo D, Rapa SF, Merciai F, Salviati E, Pepe G, Sommella E, Profili R, Manfra M, Autore G, Campiglia P, Marzocco S (Salerno, Potenza)

P17. **Antinflammatory effects of T-isochrysis lutea and Arthrospira platensis M2 extracts and of their main bioactive compounds**
Luceri C, Bigagli E, Cinci L, D'Ambrosio M, Niccolai A, Biondi N, Rodolfi L, Tredici MR (Firenze)

P18. **Polyphenolic composition, enzyme inhibitory effects ex-vivo and in-vivo studies on two Brassicaceae of north-central Italy**
Leone S, Orlando G, Ferrante C, Menghini L, Recinella L, Chiavaroli A, Leporini L, Di Nisio C, Mollica A, Stefanucci A, Zengin G, Locatelli M, Macedonio G, Tayrab E, Ali I, Musa TH, Musa HH, Ahmed AA, Brunetti L (Chieti, Konya, Khartoum, Nanjing)

P19. ***Crocus sativus*, *Serenoa repens* and *Pinus massoniana* extracts modulate inflammatory response in isolated rat prostate challenged with LPS**
Chiavaroli A, Recinella L, Ferrante C, Locatelli M, Carradori S, Macchione N, Zengin G, Leporini L, Leone S, Martinotti S,Brunetti L, Vacca M, Menghini L, Orlando G (Chieti, Milano, Konya)

P20. **Anti-inflammatory and antioxidant effects of anchovy (*Engraulis encrasicolus*) by-products protein hydrolysates**
Lanza M, Mangano V, Salvo A, Filippone A, Casili G, Lanteri G, Briguglio G, Capparucci F, Esposito E, Macrì F (Messina)

Sessione 4

P21. **Ultra-micronized palmitoylethanolamide rescues the cognitive decline-associated loss of neural plasticity in the neuropathic mouse entorhinal cortex-dentate gyrus pathway**
Iannotta M, Boccella S, Cristiano C, Belardo C, Romano R, , Farina A, Guida F, Piscitelli F, Palazzo E, Mazzitelli M, Imperatore R, Tunisi L, de Novellis V, Cristino L, Di Marzo V, Calignano A, Maione S, Luongo L (Napoli, Benevento, Pozzuoli, Lubbock)

P22. ***In vitro*** **and** ***in vivo*** **evidence for the use of** ***Vitis vinifera*** **hydroalcoholic extract against oxaliplatin neurotoxicity**
Micheli L, Ghelardini C, Mattoli L, Maidecchi A, Parisio C, Lucarini E, Pacini A, Di Cesare Mannelli L (Firenze, Sansepolcro)

P23. **D-Aspartate drinking solution alleviates pain and cognitive impairment in neuropathic mice**
Belardo C, Palazzo E, Luongo L, Guida F, Marabese I, Romano R, Iannotta M, Rossi F, D'Aniello A, Stella L, Marmo F, Usiello A, de Bartolomeis A, Maione S, de Novellis V (Napoli)

P24. **Effect of Ribodiet®, a ribonucleotide-based formulation, in a mouse model of Alzheimer's disease**
Raucci F, Sgherbini A, Pedretti N, Saviano A, Russo M, Casillo GM, Daglia M, Maione F (Napoli, Bergamo, Pavia)

P25. **Neuroprotective effect of sodium propionate in** ***in vitro*** **and** ***in vivo*** **models**
Paterniti I, Filippone A, Campolo M, Lanza M, Casili G, Cuzzocrea S, Esposito E (Messina)

P26. **Gender and age- dependency of the effects of Bud extracts from** ***Tilia tomentosa*** **Moench in central nervous system**
Olivero G, Vallarino G, Cervia I, Turrini F, Boggia R, Zunin P, Donno D, Beccaro GL, Pittaluga A, Grilli M (Genova, Torino)

P27. **Effect of** ***Eruca sativa*** **meal and glucoerucin in diabetic neuropathic pain in mice**
Parisio C, Pagnotta E, Lucarini E, Micheli L, Testai L, Martelli A, Di Cesare Mannelli L, Calderone V, Lazzeri L, Matteo R, Ugolini R, Ghelardini C (Firenze, Bologna, Pisa)

Sessione 5

P28. **Nutraceutical effects of an** ***Eruca sativa*** **seed extract in an experimental model of metabolic syndrome**
Flori L, Piragine E, Citi V, Martelli A, Pagnotta E, Ugolini L, Matteo R, Di Cesare Mannelli L, Lazzeri L, Ghelardini C, Calderone V, Testai L (Pisa, Firenze, Bologna)

P29. **Discovery of potential pancreatic lipase inhibitors from** ***Salvia miltiorrhiza*** **Bunge**
Conforti F, Marrelli M, Grande F, Occhiuzzi MA, Maione F, Mascolo N (Rende, Napoli)

P30. **Silybin as a new tool to counteract doxorubicin resistance by targeting glucose uptake**
Giacomini I, Catanzaro D, Gabbia D , Cocetta V, Biagi M, Ragazzi E, Montopoli M, Carrara M (Padova, Siena)

P31. ***Allium cepa* L. var. Tropea: a source of nutraceuticals with anti-obesity potential**
Marrelli M, Meleleo D, Mallamaci R, Argentieri MP, Conforti F, Avato P (Rende, Bari)
P32. **Antihyperglycemic effects of the ethyl acetate extract from the peel of *Punica granatum* L. var. Dente di cavallo: a possible nutraceutical application of a food waste**
Di Giacomo S, Locatelli M, Toniolo C, Cacciagrano F, Vitalone A, Mazzanti G, Carradori S, Cesa S, Di Sotto A (Roma, Chieti)

P33. **Preventing Adolescent Stress-induced Cognitive and Microbiome Changes by Omega 3 – PUFA/Vitamin A Diet Enrichment**
Provensi G, Schmidt SD, Boehme M, Rani B, Costa A, Blandina P, Izquierdo I, Cryan JF, Passani MB (Firenze Porto Alegre, Cork)

Sessione 6

P34. **Effect of polygodial on human melanoma cells: Role of Hsp70 protein**
Russo A, Cardile V, Graziano ACE, Avola R, Madrid A (Catania, Valparaíso)

P35. **Quercetin and Cisplatin combined treatment alter cell cycle and sensitize resistant cancer cell lines**
Catanzaro D, Giacomini I, Vianello C, Ragazzi E, Montopoli M (Padova)

P6. **Preclinical evaluation of tanshinones from *Salvia miltiorrhiza Bunge* on human glioblastoma models *in vitro***
Piccolo M, Ferraro MG, Maione F, Tammaro C, Raucci F, Fattorusso A, Santamaria R, Irace C (Napoli)

P37. **Biological activity of *Capsicum annum* cv Senise on IHH cell line**
Sinisgalli C, Ostuni A, Castiglione Morelli MA, Tirrico S, De Benedettis MG, Milella L (Potenza)

P38. **Curcuma and garlic as backbone for new nature-inspired hybrids as multi-pharmacological agents: focus on BDNF modulation**
Catanzaro M, Fagiani F, Basagni F, Govoni S, Racchi M, Rosini M, Lanni C (Pavia, Bologna)

P39. **Evaluation of onconutraceutical potential of vegetable smoothies in cardiomyocites and breast cancer cell lines**
Rapa SF, Salviati E, Cianciarulo D, Pepe G, Sommella E, Coppola D, Coppola L, Manfra M, Autore G, Marzocco S, Campiglia P (Salerno, Scafati, Potenza)

Sessione 7

P40. **Chemical Compounds Extracted From Agricultural Waste: Characterization And Evaluation Of Antioxidant Activity**
Salzano M , Verrillo M , Cozzolino V , Vinci G , Spaccini R , Piccolo A (Napoli)

P41. **Design and characterization of an integrate dialytic system coupled with amperometric microsensors for the dynamic quantification of ascorbic acid and total polyphenols in the Pompia fruits.**
Bacciu A, Arrigo P, Pala S , Bazzu G, Migheli R, Serra PA, Rocchitta G (Sassari)

P42. **Development and characterization of a new microdialitic-electrochemical device for the extraction and quantification of total polyphenols present in Extra Virgin Olive Oil (EVOO)**
Arrigo P, Molinu MG, Dore A, Bacciu A, Mastinu M, Bazzu G, Rocchitta G, Serra PA (Sassari)

P43. **Phytochemical and biological characterization of methanolic extracts from Tunisian *Rumex algeriensis* and *Rumex tunetanus***
Occhiuto C, Abidi J, Cimino F, Speciale A, Saija A , Ruberto G, Siracusa L, Bouaziz M, Cristani M (Messina, Sfax, Catania)

P44. **Evaluation of the phytochemical composition and biological activities of the ethanolic extracts from "Bianco di Sperlonga" PGI celery ecotype: a multimethodological study**
Di Sotto A, Carradori S, Locatelli M, Ingallina C, Mannina L, Toniolo C, Vitalone A, Giusti AM, Di Giacomo S (Roma, Chieti)

P45. **Antimicrobial and Phytotoxic activity of essential oil of *Origanum vulgare* growing in Cilento**
Della Pepa T, Elshafie HS, Capasso R, De Feo V, De Martino L, Camele I, Gutierrez Pacheco MM, Vazquez Armenta FJ, Ayala Zavala JF, Nazzaro F, Caputo L (Napoli, Salerno, Potenza, Sonora, Avellino)

P46. **Assessment of consumer perception of olive oil's characteristics and health claims related to nutraceutical attributes**
Lombardi A, de Gennaro B, Cavallo C, Roselli L, Del Giudice T, Vecchio R, Cicia G (Napoli)

P47. **Microdispersions of ellagic acid and pomegranate extracts as new potential nutraceutical ingredients**
Turrini F, Boggia R, Pittaluga AM, Grilli M, Zunin P (Genova)

LETTURA MAGISTRALE

Impact of Mediterranean diet on gut microbiota

Ercolini D

Department of Agricultural Sciences, University of Naples Federico II, Portici
Task Force on Microbiome Studies, University of Naples Federico II

Food sources have played a major role in guiding the evolution of *Homo sapiens*. The role of diet in influencing the composition of the gut microbiota is widely recognized. However, until recently, not many studies have broadly and systematically considered the association between long-term dietary habits and gut microbiota. Access to foods is the first factor affecting the gut microbiota as it can vary according to how differently some populations eat. Specific patterns of the gut microbiota have been also linked to diet, clearly correlating a specific composition of the microbiota with animal fat and protein-based versus vegetable-based diets. In addition, several studies have shown an association between consumption of fiber and increased microbial richness, and others have shown how a sudden dietary change induces changes in microbiota composition. Intestinal microbiome can be considered a useful biomarker of long-term consumption of healthy and unhealthy diets. Therefore, it is important to pose the question on whether and to what extent long-term dietary choices can impact on the composition of the microbiota and how this can influence the production of beneficial microbial metabolites.
To investigate the potential effects of the Mediterranean diet (MD), we report the results of a cross-sectional survey assessing the gut microbiota and metabolome in a cohort of healthy Italian subjects in relation to their habitual diets. In addition, an intervention study is on-going aiming to evaluate the effect of the consumption of a Mediterranean diet in subjects at cardiovascular risk and with unhealthy dietary and lifestyle.
Subjects can be stratified according to diet and microbiota. In addition, the consumption of fruit vegetables and legumes in individuals with the highest adherence to MD is clearly linked to higher levels of fecal short chain fatty acids, this happening even considering the omnivore population alone. The fiber consumption was also significantly associated to higher levels of fibre-degrading Firmicutes and *Prevotella*. On the other hand, higher urinary trimethylamine oxide levels were detected in individuals with lower adherence to the MD. Dissecting shotgun metagenomes from the same subjects we also investigated the possible presence of different *P. copri* strains and their functional potential, in order to further explore the presence of a diet-induced selection. High diversity within *P. copri* was highlighted: strains present in omnivore subjects were clearly different compared to those in vegetarians/vegans. In addition, significantly different genes occurred in *P. copri* strains from Western and non Western populations.
Using a Mediterranean diet based isocaloric nutritional intervention in overweight to obese subjects, we observed a significant increase of fibre-degrading bacteria, associated to a reduction of total cholesterol, bile acids, carnitine and to an increase in metabolic markers of fruit, vegetables and legumes consumption associated to a Mediterranean diet. In addition, baseline microbiota was linked to urolithin production from nuts based ellagic acid.
High-level consumption of plant foodstuffs consistent with a MD is associated with beneficial microbiome-related metabolomic profiles in healthy subjects. The study of sub-genus and strain level diversity highlight a current oversimplification of diet-dependent microbe-host associations and suggest that strain-level differences may occur following diet-dependent selection of the microbiota and this can have fundamental functional consequences correlated with possible healt benefits.

COMUNICAZIONI ORALI

Clinical evaluation of a new Hydroxytyrosol and Vitamin E-based nutraceutical formulation on pediatric NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in children and adults. Several evidences suggest that the improvement of oxidative stress is a possible therapeutic strategy for paediatric NASH[1].
In light of this, we performed a randomized double-blind placebo-controlled trial to test the potential efficacy, assessed by improvement of oxidative stress parameters and liver ultrasound, and tolerability of a new hydroxytyrosol (HXT) and vitamin E-based nutraceutical formulation in 80 children and adolescents with biopsy-proven NAFLD[2].
Seventy patients completed the 4-months study period. The combined therapy was well tolerated, and there were no adverse events. No significant changes of oxidative stress and metabolic parameters, and no improvement of steatosis were observed in the placebo arm. On the other and, patients in the treatment arm, showed a decrease of insulin resistance, triglyceride levels, oxidative stress parameters and steatosis grade. Noteworthy, the steatosis improvement correlates with the levels of advanced glycation end products and carbonylated proteins.
In conclusion, 4 months-treatment with this new nutraceutical formulation is enough to improve the main oxidative stress parameters, insulin resistance and steatosis in children with NAFLD revealing a promising strategy that could be easily integrated to the diet to improve NAFLD-related liver damage in children. The promising results are certainly attributable to the pharmaceutical formulation used, which differs from all those commercially available for the delivery of the active ingredients in extra virgin olive oil. Indeed, the lipophilic vehicle substantially increases the absorption and bioavailability of the active ingredients and synergizes them, for its richness in polyphenols by itself, increasing the biological activity observed[2,3].

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Caffeine intake during pregnancy and neonatal low birth weight: data from a multicenter Italian retrospective study

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Caffeine (1,3,7-trimethylxanthine) is one of the most consumed substances worldwide. It can be found in coffee, tea, soft drinks, chocolate, and many other food products [1]. Orally-ingested caffeine is rapidly absorbed and reaches peak plasma concentration within 30–45 min and its plasmatic half-life is 5 to 6 h [2]. Over 95% of caffeine is transformed in the liver through demethylation by the microsomal CYP1A2 enzyme into the primary metabolites paraxanthine, theophylline and theobromine [3, 4]. Caffeine intake originating from different food products such as chocolate, tea and coffees is common during pregnancy. The influence of caffeine on pregnancy outcomes has not been still fully determined. Some studies show that high caffeine intake could cause miscarriage, preterm birth or reduction of fetal growth, but other findings do not support this view. The aim of the study was to point on caffeine intake during pregnancy through the analysis of data collected during a multicenter study named PHYTOVIGGEST that we carried out between 2014 and 2017 in the maternity units of three Italian general hospitals. Principal outcomes of pregnancy and caffeine exposure during pregnancy were taken into consideration. Data of 5405 pregnancies were collected by direct questionnaire supplemented with data from patients' clinical records. Results showed that principal source of caffeine intake in our sample was coffee and that 42.3% of pregnant women have assumed at least one coffee a day during the pregnancy. Analysis of dose response effects show that in pregnancy women consuming to 3 or more coffees a day (about ≥195 mg caffeine/day) there was a statistically significant association between caffeine exposure and babies with low birth weight (< 2500 g), while the other outcomes taken in account for the study were not modified.

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Vitamin D replacement ameliorates serum lipoprotein functions, adipokine profile and subclinical atherosclerosis in pre-menopausal women

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Background and aims: Low vitamin D (vitD) has been linked to increased cardiovascular (CV) risk, but the effects of vitD supplementation are not clarified. We evaluated the impact of vitD normalization on HDL cholesterol efflux capacity (CEC), which inversely correlates with CV risk, the proatherogenic serum cholesterol loading capacity (CLC), adipokine profile and subclinical atherosclerosis.
Methods and results: Healthy premenopausal women with vitD deficiency (n = 31) underwent supplementation. Subclinical atherosclerosis was evaluated by flow-mediated dilation (FMD), pulse wave velocity (PWV) and augmentation index (AIx), measured with standard techniques. HDL CEC and serum CLC were measured by a radioisotopic and fluorimetric assay, respectively. Malondialdehyde (MDA) in HDL was quantified by the TBARS assay. Pre-β HDL was assessed by 2D-electrophoresis. Serum adipokines were measured by ELISA.
VitD replacement restored normal levels of serum 25-hydroxyvitamin D (25OHD) and significantly improved FMD (+4%; $p < 0.001$), PWV (-4.1%: $p < 0.001$) and AIx (-16.1%; $p < 0.001$). Total CEC was significantly improved (+19.5%; $p = 0.003$), with a specific increase in the ABCA1-mediated CEC (+70.8%; $p < 0.001$). HDL-MDA slightly but significantly decreased (-9.6%; $p = 0.027$), while no difference was detected in pre-β HDL. No change was observed in aqueous diffusion nor in the ABCG1-mediated CEC. Serum CLC was significantly reduced (-13.3%; $p = 0.026$). Levels of adiponectin were increased (+ 50.6%; $p < 0.0001$) and resistin levels were decreased (- 24.3%; $p < 0.0001$). In women after vitD replacement, the ABCA1-mediated CEC directly correlated with pre-β HDL ($r^2 = 0.346$; $p < 0.001$) and inversely with resistin ($r^2 = 0.220$; $p < 0.009$).
Conclusion: Our data support vitD supplementation for CV risk prevention.

Effect of a novel nutraceutical combination on serum lipoprotein functional profile and circulating PCSK9

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Background: Beside the reduction of cholesterolemia, a beneficial effect on cardiovascular risk may be obtained by improving lipid-related serum lipoprotein functions such as high-density lipoproteins (HDLs) cholesterol efflux capacity (CEC) and serum cholesterol loading capacity (CLC), by reducing proprotein convertase subtilisin kexin type 9 (PCSK9), independently of lipoprotein concentrations. Another possible strategy to counteract atherosclerosis may be inflammation attenuation.
Aim: We aimed to evaluate the effect of an innovative nutraceutical (NUT) combination containing red yeast rice (monacolin K 3.3 mg), berberine 531.25 mg and leaf extract of Morus Alba 200 mg (LopiGLIK®), on serum HDL-CEC, serum CLC and on circulating PCSK9 levels. We also evaluated in an in vitro model of human macrophages, some anti-atherosclerotic effects of Morus Alba extract, the innovative compound present in the above combination, focusing on pro-inflammatory cytokines release and ATP-Citrate Lyase activity, a key enzyme in the pathway of cytosolic acetyl-CoA synthesis. **Materials and methods**: Twenty three dyslipidemic subjects were treated for 4 weeks with the above NUT combination. HDL-CEC was measured using specific cell-based radioisotopic assays; serum CLC and PCSK9 concentrations were measured fluorimetrically and by enzyme-linked immunosorbent (ELISA) assay, respectively. Quantification of cytokines in the medium of macrophages treated with Morus Alba extract was performed by ELISA kit. ATP-Citrate Lyase activity was determined by fluorimetric assay. **Results**: The NUT combination significantly reduced plasma level of the total cholesterol and low-density lipoprotein cholesterol (-9.8% and -12.6%, respectively). Despite no changes in HDL-cholesterol, the NUT combination improved total HDL-CEC in 83% of the patients, by an average of 16%, as a consequence of the increase mainly of the ATP-binding cassette A1-mediated CEC (+28.5%). The NUT combination significantly reduced serum CLC (-11.4%) while it did not change PCSK9 plasma levels (312.9±69.4 ng/mL vs 334.8±103.5 mg/L, before and after treatment, respectively). Given the presence of Morus alba in the above innovative NUT combination, we studied the effect of this single component on cytokine release from human macrophages and we observed a marked inhibition of the amount of pro-inflammatory cytokines IL-6 and TNFα (-71.7% and -97.2%, respectively) in the medium. Morus Alba extract was also able to significantly inhibit the activity of the enzyme ATP-Citrate Lyase (-35%). **Conclusions**: The present NUT combination improves the serum lipoprotein functional profile providing complementary beneficial effects, without any detrimental increase of PCSK9 plasma levels. In addition, the innovative component of the combination, Morus Alba extract, inhibits cytokine productions and the activity of ATP-Citrate Lyase further contributing to the antiatherosclerotic effect of this NUT combination.

Whey protein isolate supplementation improves body composition, muscle strength and treatment tolerance in malnourished advanced cancer patients undergoing chemotherapy

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Background. To evaluate the benefit of whey protein isolate (WPI) supplementation in addition to nutritional counseling in malnourished advanced cancer patients undergoing chemotherapy (CT).
Methods. In a single-center, randomized, pragmatic, parallel-group controlled trial (ClinicalTrials.gov: NCT02065726; February 2014 - June 2018), 166 malnourished advanced cancer patients with mixed tumor entities candidate to or undergoing CT were randomly assigned to receive nutritional counseling with (N=82) or without (N=84) WPI supplementation (20 grams/daily) for 3 months. Primary endpoint was the change in phase angle (PhA). Secondary endpoints included changes in standardized PhA (SPA), fat-free mass index (FFMI), body weight, muscle strength, quality of life and CT toxicity (CTCAE 4.0 events).
Results. In patients with the primary endpoint assessed (modified intention-to-treat population), counseling plus WPI (N=66) resulted in improved PhA compared to nutritional counseling alone (N=69): mean difference, 0.48° [95%CI, 0.05 to 0.90] (P=0.027). Imputation of missing outcomes yielded consistent findings. WPI supplementation resulted also in improved SPA (P=0.021), FFMI (P=0.041), body weight (P=0.023), muscle strength (P<0.001) and in reduced risk of CT toxicity (risk difference, -9.8% [95%CI, -16.9 to -2.6]; P=0.009), particularly of severe (grade ≥3) events (risk difference, -30.4% [95%CI, -44.4 to -16.5]; P=0.001).
Conclusion. In malnourished advanced cancer patients undergoing CT and receiving nutritional counseling, 3-month supplementation with WPI resulted in improved body composition, muscle strength, body weight and reduced CT toxicity. Further trials, aimed at verifying the efficacy of this nutritional intervention on mid and long-term primary clinical endpoints in newly diagnosed specific cancer types are warranted.

A novel nutraceutical formulation from winemaking by-products for the control of circulating oxidative stress markers: a randomized clinical trial

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Growing evidences indicated trimethylamine N-oxide (TMAO), a microbiota-derived metabolite, as a novel risk factor for cardiovascular diseases. Polyphenols have been demonstrated to inhibit the growth of TMA-producing bacterial strains, and resveratrol (RSV) reduced TMAO levels in mice. In this study, we evaluated the TMAO-reducing effect of a novel nutraceutical formulation based on a polyphenolic extract from grape pomace in humans (Taurisolo®). The polyphenolic profile of Taurisolo® was assessed by a High Performance Liquid Chromatography-diode-array detector (HPLC-DAD) method, and RSV was monitored as an indicative marker. After in vitro GI digestion, intestinal bioaccessibility of RSV was 92.3%. A randomized, placebo-controlled, cross-over trial was carried out to evaluate the TMAO-reducing effect of Taurisolo® in a cohort of healthy subjects. In acute, the maximum levels of RSV were detected both in serum and whole blood 60 min after the administration of Taurisolo®; in chronic, a significant increase of RSV was detected in serum after the 4-week treatment. After 4-week treatment, the TMAO serum levels were significantly decreased in the treatment group compared to placebo (63.6% vs. 0.54%, respectively, $P < 0.0001$). In conclusion, our data show that Taurisolo® may represent a novel and useful natural remedy to reduce prognostic markers for incident cardiovascular events. Undoubtedly, further in vitro and in vivo studies need to be performed in order to elucidate possible mechanisms of action and corroborate our preliminary results.

Polyphenol health effects on cardiovascular disorders: myth or truth?

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Background: Polyphenols are proposed as protective factors against the occurrence of degenerative diseases affecting several systems, including cardiovascular ones. The beneficial effect of polyphenol-enriched diets is assumed to be multifactorial, including direct antioxidant and anti-inflammatory properties, as well as the polyphenols-related capability to modulate lipid metabolism and gut microbiota function. However, the prediction of polyphenols impact on human health remains uncertain, since available long-term studies provide controversial results.
Aim of the study: we performed a meta-analysis to assess the effect of polyphenols as food supplement or isolated compounds on cardiovascular parameters, to clarify their beneficial properties on human health.
Methods: The literature search was conducted to identify double-blind, randomized, controlled clinical trials published in English language until November 2018 and evaluating chronic polyphenols administration with a treatment period over 2 weeks on cardiovascular health. All available polyphenol-enriched compounds were considered eligible.
Results: High heterogeneity has been found in the literature search, in terms of treatment formulation, dose, source and compounds evaluated. Thirty-four studies were included, evaluating cardiovascular health parameters. Polyphenols administration reduced both systolic (-1.01 mmHg, 95%CI:-2.04;0.02, p=0.005) and diastolic (-1.32 mmHg, 95%CI:-2.37;-0.27, p=0.001) pressure, as well as low-density-lipoprotein cholesterol levels (-4.39 mg/dL, 95%CI:-7.66;-1.11, p=0.009). On the contrary, the polyphenols assumption significantly increased high-density lipoprotein cholesterol serum levels (2.68 mg/dL, 95%CI:2.43;2.92, p<0.001) and the brachial artery flow mediated dilation (0.89%, 95%CI:0.40;1.38, p<0.001).
Conclusion: The meta-analysis revealed a significant effect of polyphenols in positively modulating the cardiovascular health parameters. Although definitive recommendations for the use of these compounds in the prevention of cardiovascular disease are currently not applicable, a potential role of polyphenol compounds for cardiovascular diseases prevention is clearly evident.

Protective effects of cyanidin-3-O-glucoside on human epithelial cells exposed to palmitic acid

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The intestinal mucosal barrier represents a defensive barrier between the human body and the surrounding environment providing protection against luminal pathogens and antigenic molecules. An important factor of intestinal homeostasis and inflammation is the barrier integrity so that the dysfunction of intestinal mucosal barrier is key to the occurrence of severe intestinal disorders, including inflammatory bowel diseases (IBDs). Recently, epidemiological studies reported obesity and metabolic syndrome as risk factors in IBDs development. In fact, increased serum free fatty acids (FFA), or adipokines and cytokines, taken up into the enterocyte via the basolateral membrane, are supposed to be potential modulators of intestinal inflammation. In addition, increased FFA uptake is associated to notice able morphological alterations and cytokines and chemokines release from the intestinal cells so activating and recruiting immune cells. However, even if the mechanisms involved in the observed effects remain unclear, it has been suggested the involvement of arachidonic acid cascade with resultant increased derivatives (PGE2, TBXs, etc), altered cell membrane fluidity, intracellular oxidative stress, and the activation of NF-kB proinflammatory pathway. Many studies reported the effects of anthocyanins, a class of flavonoid compounds widely distributed in the Mediterranean diet, in various chronic inflammatory diseases, such as IBDs. In particular, anthocyanins are able to inhibit the release of proinflammatory cytokines, acting via reduced NF-κB expression and translocation, apoptosis, and oxidative stress probably via activation of cellular adaptive responses triggered by the Nrf2 transcription factor.
The aim of this work was to evaluate some of the intracellular mechanisms involved in fatty acid modulation of intestinal epithelial inflammation by using an *in vitro* intestinal epithelial system consisting of filter grown Caco-2 monolayers, and the effects exerted by cyanidin-3-O-glucoside (C3G) pretreatment. Caco-2 cells basolateral exposure to palmitic acid (PA) for 6 h induced intracellular oxidative stress. Interestingly, C3G pretreatment was able to reduce reactive oxygen species and intracellular total antioxidant power. Furthermore, PA activated NF-kB proinflammatory pathway and induced IL8 gene and COX-2 protein expression. On the contrary, cells pretreatment for 24h with C3G was effective in preventing PA-induced changes.
In conclusion, C3G improved intracellular redox status altered by PA in Caco-2 cells and showed anti-inflammatory properties through the modulation of NF-kB pathway. These data suggest that anthocyanins could contribute, as complementary approaches to the conventional already existing therapeutic approaches (i.e. non-steroidal anti-inflammatory drugs) to the management of IBDs.

Hydroxytyrosol-3O sulfate prevents Endothelial-to-Mesenchymal Transition induced by inflammatory environment

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Purpose: HydroxyTyrosol (HT) is one of the major phytocompound found in olive oil. HT and, particularly, its major plasma metabolite HT-3O sulfate (HT-3Os) are endowed with antioxidant and anti-inflammatory properties. Many beneficial effects have been attributed to these compounds against vascular diseases by improving endothelial function. Endothelial-to-mesenchymal transition (EndMT) has been implicated in the pathogenesis of a number of inflammatory-related diseases as atherosclerosis or eye disorders. However, to date the role of HT-3Os in EndMT is not well known. Thus, the aim of this study was to investigate the protective role of HT-3Os against EndMT process.
Methods: To this end, endothelial cells (EC) [human umbilical vein EC (HUVEC) and human retinal EC (HREC)] were exposed to interleukin-1β (IL-1β). This challenge represents an *in vitro* condition mimicking inflammatory environment able to induce the EndMT process. HREC were used as a specific model to investigate HT-3Os effects on vascular retinal diseases. In this study, we addressed whether HT-3Os recovers EndMT by evaluating ECs morphology and EndMt markers.
Results: The results indicate that both EC models exposed to IL-1β up to 7 days underwent EndMT, through cell morphology change. HT3-Os attenuated the multiple steps of EndMT process induced by IL-1β, recovering cell morphology and phenotype. Mechanicistically, HT-3Os in presence of IL-1β, targeting FGFR1 and *let-7 miRNA* levels, regulated transforming growth factor beta (TGF-β) signalling by attenuating the downregulation of the transcription factor *SNAI1* and *ZEB2* expression and gene expression of EndMT markers (*FN1, VIM, NOTCH3, CNN1, MMP2* and *MMP9*).
Conclusion: These results demonstrate that HT-3Os is able to counteract the pathological EndMT in inflamed EC, maintaining high *let-7* miRNA expression and preventing the activation of TGF-β signalling. Thus these data reinforce the beneficial effects of active compounds present in extravergin olive oil, namely HT and its circulating metabolite, on the vascular system and in particular the endothelium.

Erucin, a H_2S-donor from *Eruca sativa* Mill. exhibits protective effects at vascular level against oxidative and pro-inflammatory stimuli

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Vascular inflammation is a pathological state that underlies several cardiovascular (CV) and non-CV diseases such as hypertension, diabetes, atherosclerosis, neurodegeneration and so on. The research of novel compounds, and in particular nutraceutical products based on botanicals, able to preserve the vascular wall from oxidative and pro-inflammatory stimuli, represents an important challenge for pharmacology. Among the several classes of natural compounds, we selected erucin, an isothiocyanate deriving from the hydrolysis of the glucosinolate gluoerucin present in *Eruca sativa* Mill. (rocket salad or arugula), as potential nutraceutical compound able to exert vascular protection. In previous works we demonstrated that erucin was a "smart" H_2S-donor, able to release hydrogen sulfide (H_2S) in a slow and gradual way[1,2]. Accordingly, we demonstrated also that it was a vasorelaxant and anti-hypertensive agent and hence useful to be administered in case of endogenous H_2S deficiency[3]. Indeed, H_2S plays a fundamental role in the maintenance of homeostasis in several districts and in particular at CV level where it exhibits anti-oxidant, anti-inflammatory and vasorelaxant properties. In this study, in particular, we investigated the potential protective effects of erucin at vascular level. The experiments were performed on both Human Umbilical Vein Endothelial Cells (HUVECs) and Human Aortic Smooth Muscle Cells (HASMCs), and evaluated the ability of erucin to release H_2S-inside cells and to exert anti-oxidant and anti-inflammatory activities. For the evaluation of intracellular H_2S-release a fluorescent dye (WSP-1), able to detect selectively H_2S, was incubated in HUVECs and HASMCs. Instead, to investigate the protective effects, both HUVECs and HASMCs were treated with oxidative stimuli (i.e. H_2O_2 200μM) or pro-inflammatory stimuli (serum-free medium medicated with high-glucose concentration, 25mM) and then the ability of erucin (1 or 3 μM) to protect cells from death, reactive oxygen species (ROS) production and apoptotic events, was recorded. The results showed that erucin released H_2S inside HUVECs and HASMCs in a concentration dependent manner, protected both cell lines from oxidative damage and, in particular, prevented ROS production after H_2O_2 exposure in HASMCs. Moreover, in the cellular model of vascular inflammation, erucin preserved cell viability and prevented caspase 3/7 release as a marker of apoptosis.
In conclusion, this preliminary study paves the way for the use of erucin as nutraceutic agent deriving from *Brassicaceae* edible plants, able to act as a protector of the vascular tree integrity. Further experiments will be addressed to evaluate erucin influence on inflammation markers (e.g. TNF-α, IL-6, IL-1β) and to set up other models of vascular inflammation (i.e. lipopolysaccharide, LPS).

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Anti-ageing activity of *Salvia haenkei* in an *in vitro* epidermal model

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Skin is a very complex system formed by numerous inter-related components that act as a barrier for the body, playing multiple roles including homeostatic regulation, immune surveillance, temperature maintenance, etc. Endogenous factors, in concert with external assaults, continuously affect the skin, leading to structural changes that influence its appearance and its various physiological functions. Modification of these components results in altered barrier function, which increase the risk of developing pathologies and related reactions. It is thus clear that the maintenance of the integrity of the epidermal barrier and the slowdown of the skin aging processes is important in prevention of skin diseases. Previous studies as demonstrated that an extract of the Bolivian plant *Salvia haenkei* is a potential antisenescence agent, acting reducing senescent cells affecting the IL1a release and reducing the ROS formation.
This study was designed to investigate the effect of an extract of *Salvia haenkei* on HaCat human keratinocytes cell line as model of epidermis. HaCat cells were exposed to stress factors related to premature aging of cells such as ultraviolet radiation and radicals. The ROS scavenger activity was firstly confirmed in keratinocytes; interestingly it was found that SH is able to restore the barrier integrity compromised by UV induced stress acting by reinforcing the cytoskeleton structure and sealing the tight junctions. Moreover, SH was found to be able to increase the migration velocity of the cells, suggesting an interesting role in improving barrier functions of the skin.
The results of this study identify *Salvia haenkei* as a compound useful for anti-aging skin treatment and open new perspectives for clinical use of this extract.

Tannins from Chestnut (*Castanea sativa* Mill.) leaves and fruits show promising in vitro anti-inflammatory properties in gastric epithelial cells

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Extracts from leaves and fruits of chestnut (*Castanea sativa* Mill.) are included in the BELFRIT list as ingredients for food supplements. Despite their traditional use in gastrointestinal disorders, mostly due to the presence of high amount of tannins, their polyphenolic composition and biological activities are poorly investigated.
In search of nutraceuticals with potential anti-gastritis properties, two different varieties of chestnut, namely *venegon* and *verdesa*, were selected, and the hydroalcoholic extracts from leaves and fruits were characterized for their tannin content. Ellagitannins castalagin and vescalagin occurred in leaves whereas fruits showed high amount of condensed tannins.
Since the traditional use of *Castanea sativa* is scarcely supported by scientific evidence and tannins are receiving growing attention for their potential anti-inflammatory activity, the effect of extracts from fruits and leaves to counteract TNFα-induced IL-8 release and NF-κB pathway in gastric epithelial cells (AGS) was investigated.
Both leaves and fruits inhibited the release of IL-8, a chemokine involved in *H. pylori*-induced gastritis. In fruit, the amount of tannins paralleled the effect on IL-8 inhibition. In particular, extracts from pericarp and episperm, which were rich in condensed tannins, showed the highest inhibitory effect (IC_{50}s < 0.4 μg/mL) whereas kernel, which was poor in tannins, was inactive. The inhibitory effect of the extracts on the TNFα-induced NF-κB driven transcription partially explained the IL-8 inhibition.
Similarly, ellagitannins castalagin and vescalagin occurring in leaves extracts exerted high inhibitory effect on IL-8 release (IC_{50s} 0.04 μM); the effect was partially due to the impairment of the NF-κB pathway (2.6-3.0 μM); this effect seems to contribute to the anti-inflammatory activity elicited by leaves extracts. Chestnuts are widely used in food industry for sweets and flour production, through procedures that require heating. Therefore, we evaluated the anti-inflammatory property of hydroalcoholic extracts from *verdesa* and *venegon* chestnut varieties upon exposure to mild (50 °C) and high (100 °C) temperature. Our data demonstrated that, when heated at 50°C, up to 6 h, both extracts maintained the inhibitory activity on IL-8 secretion. However, both extracts exhibited reduced activity upon exposure at 100 °C in a time dependent manner; of note, the inhibitory effect was almost completely lost after 2 h of incubation at 100 °C.
Moreover, comparing three different harvesting years of the cultivar *venegon*, we observed only slight changes in IL-8 inhibition and negligible differences in the phenolic content.
The interest in tannin-enriched extracts and food against gastric inflammation is supported by the stability of tannins in the gastric environment, as previously established in the literature. After *in vitro* simulated gastric digestion of chestnut fruit extract, we did not observe a relevant impairment of IL-8 inhibition.
Our data support for the first time the use of tannin-enriched extract from leaves and fruits of *Castanea sativa* Mill. as anti-inflammatory agents. Furthermore, this study supports the use of leaves and fruit skin, which actually represent by-products of the chestnut production, for nutraceutical purposes.

Plumericin reduces intestinal inflammation and oxidative stress: *in vitro* and *in vivo* studies

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Inflammation is an innate and non-specific defence response operated by our body in response to an exogenous or endogenous insults whose goal is the elimination of the cause of damage, as well as the start of the breakdown process. When inflammation is not properly regulated it might become chronic and might lead to a host of diseases. In particular, the prevalence of chronic intestinal inflammatory diseases (IBD) are rapidly and continuously increasing in all age groups, both in the industrialized and in the developing world. Oxidative stress, dysbiosis and the intestinal barrier dysfunctions, as well as genetic factors seem to be involved in the etiopathogenesis of IBD [1]. Currently, no satisfying treatment of IBD is available. Thus, there is an urgent need for new drugs with anti-inflammatory and anti-oxidant activities. In the course of this study we evaluated the effect of Plumericin, one of the main bioactive components extracted from the bark of Himatanthus sucuuba (Woodson), a typical tree of the Amazon rainforest which belongs to the Apocynaceae family, on intestinal inflammation. A rat intestinal epithelial cell (IEC-6) assay and a 2,4,6-dinitrobenzene sulfonic acid-induced colitis (DNBS) *in vivo* assay were selected as models to evaluate the anti-inflammatory and anti-oxidant activity of plumericin. Our results indicated that Plumericin significantly reduces the levels of factors primarily involved in intestinal inflammation, such as tumor necrosis factor-α (TNF-α), as well as the expression of cyclooxygenase type 2 (COX-2), inducible nitric oxide synthase (iNOS) and the formation of nitrotyrosine. Plumericin is also able to inhibit the nuclear translocation of the p65 subunit of the nuclear-kB transcription factor (NF-kB), responsible for the gene expression of the above mentioned enzymes involved in the inflammatory process. Moreover, Plumericin reduced significantly the intracellular levels of reactive oxygen species (ROS), activated the nuclear factor erythroid-derived 2 (Nrf2) and also enzymes related to oxidative stress condition, such as heme oxygenase-1 (HO-1), NAD(P)H dehydrogenase (NQO1) and superoxide dismutase (SOD). Furthermore, Plumericin also inhibits inflammasome activation. Results obtained in these *in vitro* experiments were also confirmed *in vivo*. In conclusion, Plumericin shows promising anti-inflammatory and antioxidant activities and therefore could be useful in the treatment of IBD.

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Graminex pollen: protective effects in immortalized prostate cells (PC3) and rat prostate challenged with LPS

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Prostatitis, a general term describing prostate inflammation, is a common disease that could be sustained by bacterial or non-bacterial infectious agents. Considering the inflammatory pathways involved in prostatitis, the protective effects of herbal extracts for blunting the burden of inflammation and oxidative stress are under investigation. Pollen extracts have been previously reported as promising agents in managing clinical symptoms related to prostatitis.
To this regard, the aim of the present work was to evaluate the antiproliferative activity of Graminex pollen (Graminex™, Deshler, OH, USA) on immortalized prostate cells (PC3), and the protective effects in an experimental model of tissue inflammation constituted by *ex vivo* prostate specimens challenged with *Escherichia coli* lipopolysaccharide (LPS). In this context, we studied the putative mechanism of action of pollen on multiple inflammatory pathways, including the impact on prostaglandin E_2 (PGE_2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), whose activities were significantly increased by inflammatory stimuli, including LPS. Furthermore, we investigated the protective ability of pollen regarding radical oxygen species (ROS) production and the levels of malondialdehyde (MDA), a well-established marker of lipid peroxidation. Finally, we characterized the Graminex pollen from a phytochemical point of view, with regards to phenolic fingerprint, antiradical and enzyme inhibition profile.
We found that Graminex pollen displayed scavenging effects in phosphomolybdenum, 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid, cupric ion reducing activity, and ferric reducing antioxidant power tests. In addition, Graminex pollen was able to reduce ROS production by PC3 cells challenged with hydrogen peroxide, NFkB gene expression and MDA and PGE_2 levels, in rat prostate specimens. Finally, Graminex pollen was also revealed to be a potential cholinesterase, tyrosinase, α-amylase and α-glucosidase enzyme inhibitory agent.
Taken together, Graminex pollen appears to be a promising natural product for the management of the inflammatory components in the prostate.

Resveratrol treatment reduces the vulnerability of SH-SY5Y cells and cortical neurons overexpressing SOD1-G93A to Thimerosal toxicity

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Amyotrophic Lateral Sclerosis (ALS) is an idiopathic neuronal disease of the motor system characterized by degeneration of cortical, brainstem and spinal motor neurons. In humans, mutation of glycine 93 to alanine of Cu^{++}/Zn^{++} superoxide dismutase type-1 (SOD1-G93A) has been associated to some familial forms of ALS (fALS). Several evidence proposed the involvement of environmental pollutants that like mercury could accelerate ALS symptoms. In this study, SH-SY5Y cells stably transfected with SOD1 and G93A mutant of SOD1 constructs were exposed to non-toxic concentrations (0.01 μM) of ethylmercury thiosalicylate (Thimerosal) for 24 h. Intringuingly, we found that Thimerosal, in SOD1-G93A cells, but not in SOD1 cells, reduced cell survival. Moreover, Thimerosal-induced cell death occurred in a concentration dependent-manner and was prevented by the Sirtuin 1 (SIRT1) activator Resveratrol (RSV). Interestingly, several studies reported that RSV *in vitro* is protective against enviromental and non-enviromental neurotoxicants such as PCB, MeHg, and methamphetamine and improves motor function and survival in ALS mice. Furthermore, Thimerosal decreased the protein expression of transcription factor Downstream Regulatory Element Antagonist Modulator (DREAM), but not DREAM gene. Interestingly, DREAM reduction was blocked by cotreatment with RSV, suggesting the participation of SIRT1 in determining this effect. Immunoprecipitation experiments in SOD1-G93A cells exposed to Thimerosal demonstrated that RSV increased DREAM deacetylation and reduced its polyubiquitination. In addition, RSV counteracted Thimerosal-enhanced Prodynorphin (PDYN) mRNA, a DREAM target gene. Furthermore, cortical neurons transiently transfected with SOD1-G93A construct and exposed to Thimerosal (0.5 μM/24 h) showed a reduction of DREAM and an up-regulation of the Prodynorphin gene. Interestingly, both the treatment with RSV or the transfection of siRNA against Prodynorphin significantly reduced Thimerosal-induced neurotoxicity, while DREAM knocking-down potentiated Thimerosal reduced cell survival. These results demonstrate the particular vulnerability of SOD1-G93A neuronal cells to Thimerosal and that RSV via SIRT1 counteracts the neurodetrimental effect of this toxicant by preventing DREAM reduction and Prodynorphin up-regulation.

Effects of *Ginkgo biloba* (GK501) and *Panax ginseng* C.A. Meyer (G115) and their combination in organotypic hippocampal slice cultures and mixed cortical cells exposed to excitotoxic insults

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Introduction: Many neurological diseases are associated with an increase of glutamate levels in CNS and excitotoxicity. Several studies have shown that *Ginkgo biloba* and *Panax ginseng* have neuroprotective and antioxidants properties that can contribute to counteract CNS diseases, without producing significant side effects [1].
Aim of the Study: The aim of our study was to investigate the protective effect of two specific standardized extracts of *Ginkgo biloba* (GK501) and *Panax ginseng* (G115) alone or in combination in two different models: rat organotypic hippocampal slice cultures and mixed cortical cells exposed to excitotoxic insults and to investigate their molecular mechanisms.
Materials and Methods: *Ginkgo biloba* (G115) and *Panax ginseng* (GK501) extracts alone or in combination were used in two *in vitro* experimental models of primary cultures exposed to excitotoxicity: rat organotypic hippocampal slices exposed to either 5 μM kainic acid or 10 μM N-Methyl-D-aspartate for 24 hours, and mixed cortical cells exposed to 300 μM for 10 min. Cell death in the CA3 or CA1 subregions of slices was quantified by measuring propidium iodide fluorescence [2], whereas murine mixed cortical cells were exposed to 300 μM NMDA for 10 minutes and 24 h later cell damage was quantitatively evaluated by measuring the amount of lactate dehydrogenase (LDH) released from injured cells into the extracellular fluid [3].
Results and Discussion: Hippocampal slices exposed up to 24 h - 48 h to various concentrations of *Ginkgo biloba* (GK501) and *Panax ginseng* (G115) extracts alone or in combination displayed no apparent signs of injury. However, when present in the incubation medium during NMDA or kainate insult both extracts elicited a dose-dependent neuroprotective effect that reached its maximal significance at 0.01 mg/ml for ginseng, 0.017 mg/ml for gingko and 0.027 for the mix. Murine mixed cortical cells exposed up to 24-48 h to different concentrations of extract displayed no apparent signs of injury. When present in the incubation medium, during the 10 min period of NMDA and the subsequent 24 h recovery period *Ginkgo biloba* (GK501) and *Panax ginseng* (G115) extracts alone or in combination elicited a dose-dependent neuroprotective effect that reached its maximal significance at at 0.01 mg/ml for ginseng, 0.017 mg/ml for gingko and 0.027 for the mix. Our results show a greater neuroprotective effect of the combination as compared to the single extracts.
Conclusions: Our results suggest that *Ginkgo biloba* (GK501) and *Panax ginseng* (G115) extracts alone or in combination have neuroprotective effect and that it was increased when in combination, presuming a additive or synergistic effect of the two extracts. These results suggest a role of this combination as a new potential approach for the treatment and prevention of neurodegenerative diseases.
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Purple corn extract as adjuvant therapy for the prevention and treatment of trigeminal pain: role of microglia and of the gut microbiota

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Trigeminal pain is a highly debilitating condition whose pharmacological treatment still represents an unmet medical need. In the search of new approaches, the pivotal role of a correct diet in promoting health is clearly emerging beyond drugs. Studies performed with different sources of anthocyanins (ACNs) showed that they can protect against several inflammation-related diseases (*Tsuda, Mol Nutr Food Res 2012*), but very few data are available on pain syndromes, with no hints on TG pain. Additionally, accumulating evidence introduces the concept of "gut-brain axis" as a bidirectional signaling between the gut microbiota and the central nervous system (CNS), in both physiology and pathology. With the present project we tested, for the first time, the role of an ACN-rich dietary supplement in an *in vivo* model of trigeminal sensitization, evaluating its effect in combination with an already known analgesic, i.e. acetylsalicylic acid (ASA). Moreover, we elucidated the role of microglial cells in the CNS and infiltrating macrophages in the PNS in the development of orofacial pain and their modulation by ACN-enriched dietary supplement. Finally, we investigated the effect of ACNs on the modulation of specific members of the gut microbiota with known effect on brain inflammation.

We utilized isogenic maize model foods: purple corn with increased ACN content, and yellow corn without ACNs as control, both provided as water-soluble granules (*Petroni et al., Planta 2014*). Dietary supplements were tested on a model of trigeminal sensitization *in vivo* based on the unilateral injection of Complete Freund's Adjuvant (CFA) in the temporomandibular joint of male rats. Mechanical allodynia was measured by probing the orofacial skin regions with Von Frey filaments (*Magni et al., Glia 2015*). The expression of the microglia/macrophages marker Iba1 was evaluated by immunohistochemistry. For the analysis of microbiota composition, the bacterial taxonomic profile was reconstructed from fecal samples by means of 16S rRNA profiling protocol (*Milani et al., PLoS One 2013*).

Animals receiving water and yellow corn developed ipsilateral orofacial allodynia, maintained up to 72 hours which was instead significantly reduced in rats that received purple corn. The effect was fully comparable to the anti-allodynic action exerted by ASA in animals drinking water and yellow corn. Moreover, purple corn extract was as effective as ASA in inhibiting the TG infiltration of $Iba1^+$ macrophages in CFA-injected rats. Conversely, purple corn alone significantly reduced microglial activation in the brainstem, with no effect exerted by ASA. This latter result was confirmed *in vitro*, since the treatment of LPS-activated microglia with purple corn extract reduced the production of pro-inflammatory mediators and promoted a shift towards an anti-inflammatory phenotype. Finally, purple corn administration significantly modified the gut microbiota toward an anti-inflammatory taxonomic profile.

Based on results, we speculate that purple corn extract acts to prevent inflammatory pain through different cellular/molecular mechanisms that could also involve the gut-brain axis. Therefore, we foresee a possible application of ACN-rich dietary supplements as co-adjuvant to pharmacological treatments or as new preventive strategy against TG pain, aimed at reducing drugs dosage and side effects and improving patients' compliance to therapy.

Implication of gluten peptides on neurological disorders: study of molecular mechanisms in experimental models of epilepsy.

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Background: Some nutrients are able to influence neuronal functions and synaptic plasticity by acting on molecular systems that are vital for maintaining cognitive function and essential for brain development. Diet is linked to physiological but also pathological conditions and what we consume seems to have significant implications for the brain. For example, gluten related disorders (GRD) are frequently associated with neurological and psychiatric manifestations (Julian et al., 2018). In particular, people with epilepsy diagnosed with celiac disease (CD) seems to be characterized by intractable seizure. In these patients, gluten restriction diet has ensued in a reduction of both seizure frequency and antiepileptic medication (Bashiri, H., *et al.*, 2016). However, the molecular mechanisms that associates GRD and epileptogenesis are yet unknown. The gliadin peptides 31-43 (p31-43) (involved in innate immunity) and 57-68 (p57-68) (the immunodominant peptide that induce the adaptive immunoresponse in CD), are two of the main gliadin peptides that remain undigested by the intestine (Shan et al., 2002). In particular, p31-43 has been shown to induce toxicity both in *in vitro* and *in vivo* tissues obtained from patients with CD (Maiuri, L. *et al.*, 1996, Ciclitira, P. J. & Ellis, H. J., 1998).
Aim: the aim of our study was to examine the neurotoxic effects of the gliadin peptides p31-43 and p57-68 in *in vivo* and *in vitro* models of kainate-induced-epilepsy, in order to characterize the molecular mechanisms that correlates GRD and epileptogenesis.
Methods: For *in vivo* experiments, we used C57/B mice intraperitoneally injected either with kainate or with p31-43 or their combination and then observed for 90 min in order to assess latency, type and duration of epileptic seizures.
For *in vitro* experiments, we used organotypic hippocampal slices exposed to kainate (5 μM) for 24 h, a classical model of temporal epilepsy (Morin-Brureau *et al.*, 2013), alone or in combination with p31-43 or p57-68. Neuronal cell death was evaluated with propidium iodide fluorescence. We then analyzed the electrophysiological responses of p31-43 alone on spontaneous excitatory synaptic currents and the role of transglutaminases.
Results: The administration of p31-43 exacerbates the number and the duration of seizures induced by kainate in *in vivo* experiments and worsens the CA3 injury induced by kainate in *in vitro* experiments, showing a correlation between p31-43 and epilepsy. Moreover, the electrophysiological responses of p31-43 on CA3 pyramidal neurons showed an increase of spontaneous excitatory synaptic currents and in the total number of evoked APs in neurons, indicating an enhanced neuronal excitability. Furthermore, p31-43 significantly increased the expression of TG2 and TG6, indicating that its neurotoxic effect involve the transglutaminase family.
Conclusions: Our study associates the toxic effects of gluten to epilepsy and evidence mechanisms of induced toxicity by the gliadin peptide p31-43 that could consider gluten free diet as a possible therapeutic strategy for intractable seizure in patients affected by GRD.

Pomegranate-derived nutraceuticals: from gut health to abdominal pain relief

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The management of chronic or recurrent visceral pain resulting from an intestinal damage, as in the case of Inflammatory Bowel Diseases (IBDs), is still a clinical problem. In many patients affected by IBDs the pharmacological therapies offer little benefit for abdominal symptoms such as pain and bloating and their prolonged use is limited by the side effects. The beneficial antioxidant and anti-inflammatory properties of pomegranate in several gastrointestinal diseases was recently highlighted, though its effect on visceral pain has not yet investigated. The purpose of this study was to evaluate the efficacy of different pomegranate preparations in preventing the development of abdominal pain induced by colitis in rats. The effect of the nutraceutical components contained in the fruit was investigated. Colitis was induced in the animals by the intrarectal injection of DNBS (30 mg in 0.25 mL EtOH 50%). The pomegranate decoction (300 mg kg^{-1}, containing 11.5% polysaccharides and 15% ellagitannins) as well as the derived polysaccharides (300 mg kg^{-1}) and ellagitannins (45 mg kg^{-1}, equivalent to the decoction content) fractions were orally administered once daily for 14 days, starting from DNBS injection. Visceral sensitivity was assessed in the animals by measuring the viscero-motor and the abdominal withdrawal response to colo-rectal distension 7 and 15 days after DNBS injection, 24h after the last treatment. The effect of the treatments on the intestinal inflammation, fibrosis and increase in mast cells induced by DNBS was histologically evaluated on day 15. The pomegranate decoction as well as the polysaccharides and the ellagitannins fractions significantly reduced the development of visceral pain in the animals (as observed on day 7 and 14), without showing significant difference of efficacy among them. The effect of ellagitannins (45 mg kg^{-1}) was comparable to that of the total decoction (300 mg kg^{-1}). By contrast, polysaccharides need a dose 9 times higher than that present in the decoction to reach the same efficacy. Moreover, although all the preparations significantly reduced the intestinal damage induced by DNBS, the decoction and the ellagitannins resulted more effective than the polysaccharides. In conclusion ellagitannins seem to be responsible for most of the beneficial effects of pomegranate on DNBS-induced colitis. On the other hand, the polysaccharides contained in this fruit support and enhance the effect of ellagitannins. Pomegranate-derived nutraceuticals could represent a complementary approach to the conventional therapies for promoting abdominal pain relief in IBD patients.

Altered gut microbiota and endocannabinoid system tone in vitamin D deficiency-mediated chronic pain

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Recent evidence points to the gut microbiota as a regulator of brain and behavior, although it remains to be determined if gut bacteria play a role in chronic pain. The endocannabinoid system is implicated in inflammation and chronic pain processing at both the gut and central nervous system (CNS) levels. In the present study, we used low Vitamin D dietary intake in mice and evaluated possible changes in gut microbiota, pain processing and endocannabinoid system signaling.
Vitamin D deficient mice showed gut microbiota perturbation, chronic pain development, and specific alterations of endocannabinoidome members at the gut and spinal cord level. An anti-inflammatory *N*-acylethanolamine, palmitoylethanolamide counteracted most of these behavioral and biochemical changes whilst affecting gut microbiota composition.
Our data suggest that Vitamin D deficiency, and associated changes in gut bacterial composition, cause altered responses in pain behaviors via molecular mechanisms involving the endocannabinoid and *N*-acylethanolamine signaling systems.

Dietary depletion of chicken egg exosomes and their cargo affects the gene expression profile in right and left hippocampus and impairs cognitive performance in C57BL/6 mice

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Backgroung: Exosomes play essential roles in cell-to-cell communication and deliver cargos such as RNAs, lipids and proteins to recipient cells, in which can regulate genes and metabolism. Exosomes are also present in foods such as milk and chicken eggs and the encapsulation renders labile cargo resistant to the degradation during food processing and in the intestinal tract.
Hypothesis: RNAs in chicken eggs are bioavailable and elicit phenotypes across species boundaries. Assess the bioavailability and distribution of chicken egg exosomes. 2) Assess the effects of exosome-RNAs sufficient (ERS) and exosome-RNAs depleted (ERD) diets on spatial and learning memory (SLM) in mice.
Methods: Exosomes were isolated from chicken egg yolk using ultracentrifugation, and labeled using the fluorophore, 1,1-dioctadecyl-3,3,3,3 tetramethylindotricarbocyanine iodide (DiR) 2) Exosome were administrated to mice orally by gavage (1.0 x 1012exosomes/g). Absorption and distribution of exosomes was monitored using Odyssey® CLx Imaging System. The intestine was flushed with saline prior to analysis. 3) Studies of spatial learning and memory were assessed in mice fed a modified AIN-93G-based ERD ERS diet. SLM were assessed using the Barnes maze (BM) and the Morris water maze (MWM), and controlled for hippocampal synaptic plasticity using a startle response protocol 4) Hippocampus sites (right and left) were collected and the gene expression was assessed by RNAseq.
Results: The diets had no effect on food and water intake, respiratory exchange rate, physical activity. Spatial learning and memory was impaired in mice fed the ERD diet compared with ERS controls with a noticeable effect in the offspring compared to breeder mice.
Conclusions: Chicken egg exosomes labeled with fluorophores accumulated in liver, spleen and brain following oral administration in mice. When synthetic, fluorophore-labeled microRNAs were transfected into exosomes and administered to mice, distinct species of microRNAs showed unique distribution profiles and accumulated in intestinal mucosa, spleen, liver, kidneys or brain. SLM was impaired in mice fed the ERD diet compared with ERS controls in Barnes Maze or to in the Morris Water Maze. Changes in SLM were associated with differential expression of 83 genes in the left and 163 genes in the right hippocampus, including 18 pathway implicated in learning and memory in the right hippocampus.

Extra virgin olive oil polyphenols modulate the expression of key inflammatory genes and miRNAs in human adipocytes

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Inflammation of adipose tissue plays an important role in the development of many chronic diseases associated with obesity. Underlying regulatory networks involve inflammatory gene products and miRNAs (small non-coding RNAs). Polyphenols are naturally occurring antioxidants and integral components of the healthful Mediterranean diet. They have been reported to promote health through many mechanisms including anti-inflammatory activities. In particular, some polyphenols of extra virgin olive oil (EVOO) including secoiridoids, oleocanthal (OC) and oleacein (OA), and simple phenols, such as hydroxytyrosol (HT), are responsible for many nutraceutical characteristics of EVOO. However, their role in obesity-associated adipocyte inflammation and related miRNAs deregulation has not been fully elucidated.
In this study, we investigated the impact of OC, OA and HT on the expression of genes and miRNAs associated with inflammatory and dysmetabolic responses in human adipocytes. To this aim, fully differentiated Simpson-Golabi-Behmel syndrome (SGBS) adipocytes were pre-treated with EVOO polyphenols (1-25 μmol/L) before stimulation with the cytokine tumor necrosis factor (TNF)-α. Levels of mRNA gene expression as well as cell and exosomal miRNAs were measured by real-time PCR.
We identified a panel of three miRNAs (miR-155-5p, miR-34a-5p and let-7c-5p) deregulated by TNF-α in both human adipocytes and in related exosomes. Interestingly, the miRNAs modulation by TNF-α was significantly counteracted by EVOO polyphenols. In agreements with miRNAs modulation, EVOO polyphenols significantly reduced the mRNA expression of genes implicated in adipocyte inflammation (IL-1β, COX-2), angiogenesis (VEGF, KDR, MMPs), oxidative stress (NADPH oxidase subunits), leukocytes chemotaxis and infiltration (MCP-1, CXCL-10, MCS-F).
This study demonstrates, for the first time, that polyphenols isolated from EVOO (OC, OA and HT) counteract the expression of inflammatory miRNAs in adipocytes and adipocyte-derived exosomes and concomitantly modulate the expression of pro-inflammatory genes, showing a protective profile. Therefore, these compounds could be novel dietary tools for the prevention of inflammatory diseases associated with obesity.

Oleacein, a polyphenol of olive oil, modulates expression of adipogenesis markers in adipose tissue of high-fat diet fed mice.

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Olives and extra virgin olive oil (EVOO) are the major sources of fat components of the Mediterranean diet and several studies have suggested that their phenolic constituents are mainly involved in the healthy action of this diet. Among these molecules, phenolic alcohols and their secoiridoids derivatives have clearly demonstrated to possess antioxidant, anti-inflammatory and anti-proliferative activities responsible for their beneficial properties. Oleuropein is the major phenolic compound in the olive leaves and drupes. However, during EVOO extraction, oleuropein is hydrolyzed by endogenous glycosidases which drastically reduce its concentration, so that only some of its bioactive compounds, and in particular oleacein (dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; 3,4-DHPEA-EDA) remains as major secoiridoid derivative in EVOO. Oleacein has shown protective effects on some metabolic alterations in mice fed with high-fat diet (HFD). Herein, we evaluated the molecular mechanisms involved in oleacein action on adipose tissue. Histological and molecular analysis was performed in abdominal adipose tissue of C57BL/6JOlaHsd mice fed with normocaloric diet (NCD), HFD, and HFD supplemented with 20 mg/kg of oleacein (HFD-OLEAC) for 5 and additional 8 weeks (in obese mice). Reduced areas of adipocytes were always present in HFD-OLEAC *vs* HFD groups. After five weeks of treatment, oleacein prevented the HFD-induced increase of peroxisome proliferator-activated receptor γ (PPARγ), fatty acid synthase (FAS) and sterol regulatory element-binding transcription factor-1 (SREBP-1), and the reduction of adiponectin expression. Similar effects were observed in obese mice treated with oleacein for additional 8 weeks, except for the levels of FAS which remained unchanged. These finding demonstrate that the oleacein protection against HFD-induced adiposity in mice is mediated by the modulation of the main regulators of adipogenesis process. Further studies on humans will clarify the potential role of this nutraceutical as additive for prevention and treatment of obesity.

A study about the effect of fruit phytochemicals on the glucose and phenols intestinal absorption in a Caco-2TC7 monolayer model, by means of glucose and polyphenols amperometric biosensors.

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Type 2 diabetes mellitus (T2DM) is currently affecting hundreds of million people worldwide. Fluctuations in blood glucose and hyperglycemia are one of the causes of reactive oxygen species production, thus determining several disfunctions to pancreatic β-cells and also insulin resistance and impaired glucose tolerance. So, it became extremely important to know how to manage post prandial hyperglycemia. (Ceriello and Motz, 2004, Wright Jr. *et al.*, 2006). In light of this, nowadays diets based on bioactive molecules, such as polyphenols, are paying special attention in order to modulate glucose intestinal absorption.
In this work, we present an original electrochemical device for real-time dynamic monitoring of the effects of natural compounds on the absorption of glucose and polyphenols through Caco-2TC7 monolayer cells.
The device combines a glucose oxidase-based biosensor coupled with a laccase/tyrosinase-based biosensor, placed in the basal portion of a cell culture plate. Both types of biosensors are coupled with their respective sentinel sensors, in order to eliminate the impact of eventual interfering currents deriving from the matrix.
The Caco-2TC7 monolayer, grown in a special insert, separates the apical compartment, simulating the intestinal lumen, from the basolateral portion, which virtually represents the bloodstream.
In a first series of experiments, the cell culture was treated, in the apical compartment, with glucose 1 mM while the glucose biosensor recorded the currents derived from the glucose passed in the basolateral compartment, obtaining a bioavailability of about 5.1%. The further treatment with Phlorizin and Phloretin, inhibitors of SGLT1 and GLUT2 glucose transporters, produced a 10-fold reduction in glucose transport. By means of laccase/tyrosinase biosensor it was also possible to detect, in the basolateral compartment, low levels of absorbed Phlorizin and Phloretin, that showed bioavailability of 0.13% and 0.49% respectively.
Then, in a successive series of experiments, blueberry and pomegranate juices, that contain relatively quite high amount of polyphenols (about 33% anthocyanins and about 53% anthocyanins), were also studied to evaluate their hypoglycemic potential. Actually, a treatment with those juices on Caco-2TC7 monolayer determined a sustained reduction in the glucose absorption: in particular, pomegranate juice determined a 0.8% of glucose bioavailability, while blueberry determined only the 0.17%, strongly demonstrating that blueberry and pomegranate juices exert a hypoglycemic effect on the monolayer. In this experiment, the polyphenols absorption was also evaluated, by means of laccase/tyrosinase biosensor, in the basolateral compartment. For both juices the same trend was highlighted. In fact, polyphenols' concentrations showed an increase in the first 50 minutes of monitoring, then a slow decrease was monitored, demonstrating an active polyphenols' transport through Caco-2-TC7 cell monolayer.

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Lycopene activates brown adipose tissue through PPARγ stimulation and might be effective for the treatment of obesity

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Brown adipose tissue (BAT) is specialized in energy expenditure and may be considered as a potential target for anti-obesity therapies. The adipose organ differentiates to whitening or browning depending on the increase/decrease of UCP1-expression (uncoupling protein 1). BAT is activated following exposure to cold, however, therapies based on cold exposure are clinically not feasible so that alternative strategies must be explored. Lycopene is a carotenoid found in tomatoes with a potent anti-inflammatory activity and acts on adipocytes through PPAR*γ* activation. Therefore, Lycopene was tested as a potential browning agent to induce white-to-brown adipocyte transdifferentiation.
3T3-L1 cells were differentiated into adipocytes under appropriate culturing conditions. Cells were treated with different concentrations of Lycopene (0.5, 1 or 2 μM) for 24 hours. Lipid accumulation was evaluated by oil-red-O staining and total mRNA was extracted to study browning specific genes (UCP-1, PPARγ, CIDEA, and Dio2) expression by qPCR.
Oil-red-O staining demonstrated that Lycopene reduced lipid accumulation compared to control cells at all tested doses. Moreover, Lycopene caused a significant increase ($p<.0001$ vs CTRL, each gene) of all browning specific genes expression and also of PPARγ gene expression following 24hrs of treatment. These results suggest that Lycopene caused the white-to-brown adipose differentiation through PPARγ activation and might be considered as a browning agent and as a future anti-obesity therapeutic approach.

Water-soluble extract of *Morus alba* is able to lower the expression of proprotein convertase subtilisin/kexin type 9 in hepatoma cell lines with benefic effects on LDL cholesterol-uptake

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BACKGROUND AND AIM: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a pivotal regulator of low-density lipoprotein cholesterol (LDL-C) plasma levels. An innovative hypolipidemic and hypoglycemic nutraceutical combination [red yeast rice (RYR - Monacolin K 3.3 mg), *Berberis aristata* cortex extract (BCE - Berberine 531.25 mg) and *Morus alba* leaves extract (MLE - 1-Deoxynojirimycin 4mg)] has been shown to do not alter PCSK9 plasma levels. Thus, the aim of the present study was to define the effect of this nutraceutical combination on genes involved in cholesterol homeostasis, including PCSK9, and thus the molecular mechanism underlying the hypocholesterolemic effect of *Morus alba* leaves extract.
MATERIAL AND METHODS: HepG2 and HuH7 cell lines were incubated with RYR (50μg/ml), BCE (40μg/ml) and MLE (1mg/ml), alone or in combination, for 24h. Their effects on PCSK9 expression (western blot), secretion (ELISA), transcription (Luciferase Promoter assay and RT-qPCR) and on LDL-uptake (flow cytometry) were then determined.
RESULTS: We confirmed the already-known effect of RYR and BCE on PCSK9 (the former enhancing its expression, the latter reducing it). Interestingly, MLE determined a concentration-dependent inhibition of mRNA PCSK9, with a maximal reduction at 1mg/ml (-54.8%±0.7%). The same concentration of MLE reduced PCSK9 expression levels (pro-PCSK9: -17.1%; active form: -59.3%), and its release into the cultured media (-43.8%±23.6%). MLE did not alter the PCSK9 promoter activity, suggesting a different mechanism of action compared to BCE. The combination of the three elements reduced the PCSK9 mRNA (-77.3%±0.8%), the PCSK9 expression (pro-PCSK9: -96.6%; active form: -93.3%), the extracellular protein levels (-74.4%±14.9%), and the PCSK9 promoter activity (-76.0%±9.6%). The same analyses performed on LDLR (the hepatocyte receptor for low-density lipoprotein) showed an increase in its expression level by both RYR, BCE and MLE. MLE was able to positively modulate the LDL-uptake (+3.0±0.01-fold), strengthening the RYR and BCE positive action when in combination.
CONCLUSIONS: BCE and MLE actively counteract the induction of PCSK9 by RYR, an effect that could explain the unchanged plasma levels of PCSK9 in patients treated with the nutraceutical combination. In addition, the use of MLE together with RYR and BCE, positively increased LDL-uptake by HepG2, supporting the rational of using this nutraceutical combination to control both hyperlipidemic and hyperglycemic conditions.

The marine microalgae *T-isochrysis Lutea* attenuate high-fat-induced metabolic syndrome in rat.

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The incidence of high fat diet induced obesity is rapidly increasing worldwide representing a major heath concern since it is associated with several chronic and life threatening diseases such as cardiovascular diseases, type 2 diabetes, fatty liver and other metabolic disorders collectively defined as metabolic syndrome. The western diet, rich in calories, fat and sugar, associated with physical inactivity, is one of the causes leading to this significant increase. The need for preventive and therapeutic intervention is evident and begins with teaching patients about healthy lifestyle behaviors and improved dietary habits.
Microalgae are a natural source of bioactive compounds and received much attention from researchers and companies in the last period for their potential applications in different scientific fields. By testing the safety and tolerability of a diet rich in the marine microalgae, *Tisochrysis Lutea (T. Lutea),* we previously noted that it exerted positive effects on lipid profiles by modulating lipid metabolism related genes, suggesting its potential ability to control dyslipidemias.
We exposed the human hepatocarcinoma cell line HepG2 to methanol extracts from a series of microalgal strains observing fibrate-like effects only in those treated with the *T. Lutea* extract. Later on, the effects of a diet enriched with 5% of *T. Lutea* biomass was tested in a model of high-fat diet induced metabolic syndrome in rats, over three months, and compared to those exerted by the pharmacological treatment with fenofibrate, 100 mg/kg.
T. Lutea was able to control a number of metabolic syndrome features: the microalga-rich diet in fact, significantly counteracted the increased levels of blood glucose and diastolic pressure induced by the high fat diet and reduced the amount of renal fat. Fenofibrate treatment had similar effects on blood glucose, controlled weight gain but had no effect on blood pressure. Both interventions increased the fecal excretion of lipids and positively modified the blood lipid profiles. However, the dietary intervention did not show hepato damaging effects associated to fenofibrate treatment.
In conclusion, these preliminary results suggest that *T. lutea* biomass might be useful in controlling some features of the metabolic syndrome; further studies will elucidate the mechanisms related to these effects.

Citrus bergamia juice extract and its major flavanones: a treasure trove for the interaction with the AMPK/SIRT1 axis

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A previous report indicated that a flavonoid-rich extract of bergamot juice (BJe) exerts anti-inflammatory effects through the activation of SIRT1 in leukemic monocytes THP-1 stressed with lipopolysaccharide (LPS) (Risitano et al., 2014).
The aim of this study was to deeply investigate the mode of action of BJe, along with its major flavonoids, on SIRT1 enzyme. In order to investigate the direct interaction between BJe or its major flavonoids (naringenin, hesperetin, naringin, neoeriocitrin, neohesperidin) and SIRT1, an enzymatic assay was employed. In the cell-free assay, all the tested compounds as well as the whole BJe inhibited the deacetylase activity of SIRT1, as reinforced by the results of *in silico* study, indicating that the major flavonoids of BJe could bind to the inhibitory site of this sirtuin. Surprisingly, these data disagreed with what was previously stated (Risitano et al., 2014). Consequently, we decided to employ an *in vitro* model in which THP-1 cells were first treated with the whole extract or each single flavonoid, and then stressed with LPS. In the whole cells insulted with LPS, a reduction of SIRT1 activity was observed, effect that was reverted by the pre-incubation with either BJe or its major flavonoids. This effect was also observed at gene level. Employing an activator and an inhibitor of AMPK (AICAR and dorsomorphin, respectively), we found that this kinase is involved in the activation of SIRT1 elicited by BJe or its major flavonoids in this experimental model.
Our study indicates the dual role of BJe and its components, depending on the employed experimental model, as well as reveals their mode of action on the AMPK/SIRT1 axis, suggesting them as promising candidates in pathologies in which this axis is implied.

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In vitro protective effects of a *Glycyrrhiza glabra* L. leaf extract on palmitate-induced insulin resistance in endothelial cells

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Liquorice (*Glycyrrhiza glabra* L., family Leguminosae) is one of the most popular medicinal plants known worldwide. The major value of this plant lies in the valuable compounds found in its roots, some of which are associated with positive health effects. However, little attention have been given to liquorice aerial parts, always been considered merely a waste, known to contain inositols and dihydrostilbenes, among the others, able to exert anti-inflammatory and antioxidant effects. Recently, inositols are receiving particular and growing interest owing to their several biological properties such as insulin sensitizing agent. In this study we chemically characterized a methanolic extract of *Glycyrrhiza glabra* leaf (GGLME) and then evaluated the *in vitro* effects on insulin resistance in human endothelial cells (HUVECs) exposed to palmitic acid (PA).
Liquid chromatography analysis of GGLME reported identification and characterization of over thirty components, including new dihydrostilbenes, and a consistent amount of the inositol D-pinitol. To investigate the protective effect of GGLME and D-pinitol on PA-induced insulin resistance, HUVECs were pretreated for 24 h with GGLME (40-80 μg/mL) or D-pinitol (40 μM), then exposed for 3 h to 100 μM PA, and finally treated with 100 nM insulin for 15 min. D-pinitol or GGLME pretreatment significantly protected HUVECs from PA-induced toxicity at all the tested concentrations. Our results indicate that PA exposure effectively inhibited insulin-mediated IRS-1 tyrosine phosphorylation, thus blocking the PI3K/Akt/eNOS pathway with subsequent insulin resistance. Both GGLME (in a dose-dependent way) and D-pinitol pretreatments were associated to an increased tyrosine phosphorylation of IRS-1. Interestingly, this effect was observed also without PA exposure, thus demonstrating an insulin sensitizing activity of the tested compounds. Furthermore, we demonstrated an impaired PI3K/Akt/eNOS insulin signaling following PA exposure. GGLME (in a dose-dependent way) or D-pinitol pretreatments effectively increased PI3K, Akt, and eNOS activation, and these effects were observed also without PA exposure.
Finally, our data suggest that the methanolic extract of *Glycyrrhiza glabra* leaf and its main compound D-pinitol are potentially able to ameliorate insulin resistance-related endothelial dysfunction caused by lipotoxicity. These observations contribute to provide a molecular background to the beneficial effects of *Glycyrrhiza glabra* leaf, from vegetable waste to new agent in the prevention and treatment of cardiovascular diseases related to insulin resistance, diabetes, and metabolic syndrome.

Natural products from various sources prevent colon carcinogenesis and increase apoptosis in the Pirc rat (*F344/NTAC-Apc ^{am1137}*), a genetic model of colorectal cancer

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New cancer cases are estimated to pass from 14.1 million in 2012 to 21.6 million by 2030, with colon cancer (CRC) representing the 2nd cancer related cause of death: consequently, the implementation of efficient preventive strategies has a key role in this multifactorial challenge http://www.who.int/cancer/media/news/cancer-prevention-resolution/en/). Three are the prevention levels: primary prevention aims at completely avoiding cancer onset in the population, promoting healthy lifestyles. Secondary and tertiary preventions are directed to population with high cancer risk such as those with genetic alterations or a personal history of neoplasia (Labianca et al., 2010). Colon carcinogenesis is a multistep process meaning that final event of carcinoma development could be prevented by interrupting the process. Natural compounds endowed with anti-inflammatory and/or anti-oxidant properties have been studied to assess their capability to prevent CRC carcinogenesis and develop chemopreventive strategies (Aggarwal et al., 2013). In line with this effort, we recently studied the potential chemopreventive activity of three natural products: bergamot juice extract (BJe, in collaboration with Michele Navarra, University of Messina), pomegranate mesocarp decoction (PMD) and morin, a polyphenol present in some fruits like strawberries and figs. These products were studied *in vivo* in the Pirc rat, a genetic model of CRC bearing a mutation in *Apc,* the key gene in CRC, which prompts the early development of both pre-neoplastic lesions (Mucin Depleted Foci, MDFs), and tumors along the intestine, especially in the colon (Femia et al., 2015). BJe treatment was able to reduce colon tumors in Pirc rats in a dose-dependent manner: indeed, 35 mg/kg dose reduced the number of colon tumors with a significant reduction achieved with 70 mg/kg, along with the down and up regulation of anti and pro apoptotic genes respectively, and an increase in apoptotic cells in the tumors. Both doses significantly reduced the number of MDFs. A significant reduction in the number of MDFs was also observed treating Pirc rats with PMD or with morin: the former, was able to induce also a decrease in the size of these lesions and a significant increase in apoptosis compared with MDFs in the untreated animals (Tortora et al., 2018). Morin reduced the number of MDFs and, interestingly, it also reduced the expression of the oncoprotein low-molecular weight-protein tyrosin phosphatase (LMW-PTP), restoring the response to 5-Fluorouracil in the colon mucosa of Pirc rats resistant to apoptosis (Lori et al., 2018). Our data suggest that these compounds act, at least in part, increasing apoptosis. They also suggest the possibility to implement efficient and safe chemopreventive strategies with the use of combined natural compounds from different sources, also by-products. Moreover, the example given by morin suggests the capability of natural molecules to work synergistically with therapeutics allowing to improve their efficacy.

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Potential chemopreventive and therapeutic effects of cynaropicrin in metastatic melanoma

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There is increasing evidence that dietary phytochemicals possess chemopreventive properties and hold promising potential for the treatment of cancer[1]. Cynaropicrin is a sesquiterpene lactone with many biological activities. It was first isolated from artichoke (*Cynara scolymus* L.) in 1960 and, more recently, from the aerial parts of *Centaurea drabifolia* subsp. a plant endemic of Turkey[2-3].
In the present study, we explored the potential anti-cancer effect of cynaropicrin against melanoma cells. Melanoma is an aggressive form of skin cancer with high metastatic properties frequently resisting to chemotherapy and immunotherapy. Malignant melanocyte transformation has been recognized to be associated with oxidative stress. Notable, reactive oxygen species (ROS) promote many aspects of tumor development and progression including: (a) cellular proliferation; (b) evasion of apoptosis; (c) tissue invasion and metastasis[4].
Cynaropicrin is a well-known potent antioxidant[2]. Indeed, we found that cynaropicrin reduced ROS generation and induced the transcription of antioxidant enzymes such as heme oxigenase-1 and glutamate-cysteine ligase trough the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) in A375 melanoma cells. Moreover, we demonstrated that cynaropicrin significantly inhibited the proliferation of several melanoma cell lines (A375, SK-Mel 28, WM983B, WM3060) in time- and dose-dependent manner. The anti-proliferative effect of cynaropicrin was related to induction of apoptosis according to cytofluorimetric analysis with annexin V/PI staining. The pro-apoptotic activity was also confirmed by the time-dependent activation of caspase-3 and by the cleavage of its substrate poly (adenosine diphosphate-ribose) polymerase (PARP). Furthermore, cynaropicrin reduced the expression of the antiapoptotic proteins c-FLIP, XIAP and Bcl-2 whose expression is transcriptionally regulated by NF-κB. Cell cycle analysis revealed that cynaropicrin induced cell cycle arrest in the G0/G1 phase by downregulating the expression levels of cyclin D1 and cyclin□dependent kinase (CDK) 4. Finally, we investigated the effect of cynaropicrin on several malignant features of human melanoma cells such migration, invasion and colonies formation, which are essential steps in the metastasis course[5]. We demonstrated that the migration rate of A375 cells, their invasiveness and the ability to form colonies were markedly inhibited by exposure to cynaropicrin. In addition, cynaropicrin inhibited the activation of Mitogen-Activated Protein Kinase (MAPK)/ERK, one of the main deregulated pathway involved in melanoma development and progression[6]. In conclusion, in this study we demonstrated that cynaropicrin represent a new effective natural chemopreventive agent able to inhibit melanoma growth and progression by regulating the cellular redox balance.

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The anti-proliferative and anti-metastatic effects of oleacein in SH-SY5Y human neuroblastoma cells

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Neuroblastoma is one of the most frequent cancers in childhood, often characterized by both rapid progression and fatal outcome. The therapy is frequently unsuccessful, thus underlining the importance of new pharmacological agents able to fight this neoplasm. Recently, some nutraceuticals from foods and vegetables have been proposed for the treatment of malignancies. In this line, the anti-cancer properties have been demonstrated for certain phenolic compounds present in small quantities in extra-virgin olive oil, among which oleuropein and hydroxytyrosol have been extensively studied, while minor attention has been addressed to oleacein.
The aim of our research was to study the molecular mechanisms at the basis of the anti-proliferative and the anti-metastatic capacity of oleacein on the SH-SY5Y human neuroblastoma cell line.
Our results demonstrate that oleacein is able to reduce the proliferation of SH-SY5Y cells in a concentration-dependent manner. This effect is correlated to a cytotoxic effect, a cell cycle block in S phase and the induction of apoptotic cell death, due to an increase of both Bax and p53, as well as a reduction of Bcl2 expression. Moreover, the treatment of SH-SY5Y cells with oleacein determined a reduction of cell adhesion and migration, through the inhibition of STAT3 phosphorylation.
Overall, our results demonstrated the anti-tumour properties of oleacein, suggesting the involvement of both pro- and anti- apoptotic proteins at gene and protein level, as well as that of MAP kinases.

Saffron byproducts as sources of bioactive extracts: pharmacological and toxicological focus on anthers

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Saffron (*Crocus sativus* L.), the most expensive spice in the world, has been long described as a protective agent in experimental models of oxidative stress, inflammation and cancer. Intriguingly, multiple studies also revealed the potential application of high quality saffron byproducts as cheap sources of antioxidants. In this context and in collaboration with the Consortium of L'Aquila Saffron (Consorzio dello Zafferano dell'Aquila), the aim of the present work was to characterize the phytochemical profile of the whole byproduct fraction, tepal and anther (CTA) water extracts. Additionally, we evaluated CTA effects on reactive oxygen species (ROS) levels and lactate dehydrogenase (LDH) activity on mouse myoblast (C2C12) and human colon cancer (HCT116) cell lines. The results were compared with the activity of saffron stigmas (CST) water extract, used as elective comparison agent. CST and CTA extracts were equally effective in blunting hydrogen peroxide-induced oxidative stress, in both cell lines. Furthermore, CST and CTA extracts reduced LDH activity in HCT116 cells, in the same experimental conditions. Subsequently, in order to further characterize saffron byproduct quality, we analyze their content of heavy metals, finding a more significant tendency to accumulate lead and cadmium in tepals compared to anthers. Considering this, we explored the pharmacological and toxicological potential of isolated anthers, by evaluating genotoxic and protective effects in multiple cell lines and rat tissues challenged with E. Coli lipopolysaccharide (LPS). The results of the toxicological evaluation indicated that anther extracts were well tolerated by the employed biological models. Particularly, water anther extract did not exert cytostatic, cytotoxic and genotoxic effects in Hs27 cells, in the range (10-500 μg/mL). Anther extract also revealed to be well tolerated by MCF7 and C2C12 cell lines, in the same concentration range, as showed by the results of viability (MTT) test. Additionally, we tested anther effects on basal and hydrogen peroxide-induced burden of oxidative stress, in both C2C12 and MCF7 cell lines, finding a significant blunting effect induced by the extract on ROS level. Based on this evidence, we further tested anther extract on isolated rat peripheral and central tissues, such as bladder, kidney, stomach, esophagus, lung, prostate, cortex and hypothalamus challenged with LPS, as pro-inflammatory stimulus. Anther extract revealed effective in blunting LPS-induced levels of pro-oxidant biomarkers such as nitrites and malonildialdehyde (MDA), suggesting protective effects in inflamed tissues. Moreover, in HCT116 cell line we explored anther effects in an experimental model of wound healing. The null effect on cell migration ruled out any significant alteration induced by the anther extract on migration and invasion capacities of human cancer cells. Taken together, the decreased tissue levels of nitrites and MDA induced by anther extracts suggest the valorization of saffron anthers, which are usually discarded, as potential protective agents against the increased burden of oxidative stress in inflammatory conditions. Finally, in agreement with the accepted principle of "Circular Economy", our findings further support an intriguing approach to innovatively improve the high quality byproduct fraction, with the final goal to optimize and develop the productive chain of Abruzzo saffron.

New potenzial nutraceutical application and chemical characterization of Humic extract from green compost

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A sustainable practice in agriculture is the use of compost as a source of organic matter [1]. In particular, humic substances (HS) and compost tea (CT) extracted from composted vegetable waste are increasingly regarded as efficient biostimulants to improve crop productivity and induce positive responses to biotic and abiotic stress in plants [2]. Moreover, the increasing frequency of antibiotic-resistant infections clearly demonstrates that research of new natural antibiotics are critical for modern medicine [3]. In this context, HS may act as effective carrier of specific pharmacological proprieties such as anti-inflammatory, antioxidant and anti-viral activity and, particularly, in animals may support immune-modulating and anticoagulant effects [4]. A limited literature is currently available on research activities on the antimicrobial activity of humic exctracts; only oxifulvic acids were previously indicated to exert an antimicrobial activity against bacterial strain involved in common human diseases [5]. The impact of HS on human health and the use of these natural products as therapeutic drugs can be an important topic for future research works. The aim of this work was to characterize the molecular composition of HS and CT extracted from different composted biomasses (artichoke, coffee, pepper and citrus) and evaluate their antioxidant proprieties and antimicrobial activities on human common bacterial strains such *as Staphylococcus aureus (ATCC 6538P) Pseudomonas Aeruginosa (KK27), Salmonella typhimurium (ATCC14028), Klebsiella pneumoniae (ATCC700603), Enterococcus faecalis (ATCC29212).* Artichoke, coffee, pepper and citrus composts were processed in the composting plant at the Castel Volturno experimental farm of the University of Napoli Federico II. Chemical characterization oare carried out by elemental analysis, solid state Nuclear Magnetic Resonance spectroscopy (^{13}C CPMAS NMR), Solution-state ^{1}H NMR spectroscopy, thermochemolysis-Gas Chromatography-Mass Spectrometry (THM-GC-MS) and FTIR–DRIFT Spectroscopy. The antioxidant activity was performed using ABTS method and DPPH assay [6]. Antimicrobial activity proprieties were evaluated by the agar dilution plate viable-count and Minimal Inhibitory Concentration (MIC) method [7]. The molecular characterization performed by ^{13}C-CPMAS-NMR and THM-GC-MS, showed a prevalence of alkyl, aromatic and carboxyl carbons in coffee-HS, while larger content of methoxyl and phenolic from lignin moieties was found in artichoke-HS. The coffe-CT showed a larger amount of phenolic compounds compared to the other CT. These data have been also confirmed by Folin-Ciocalteau assay. The results of ABTS and DPPH assay showed that all extracts scavenged the radical to different extents and showed antioxidant activity, though the highest value was observed for artichoke-HS and coffe-CT. We suggest that the large antioxidant activity of these material may be mainly related to the content of aromatic and phenolic compounds. Furthermore, HS and CT were tested for antimicrobial activity against human common bacterial strains. The HS from artichoke revealed an inhibition of bacterial growth (98%) on all tested strains, while the HS from coffee showed a lower inhibition activity (45%) against *Pseudomonas Auuruginosa.* Coffee-CT revealed a larger inhibition of bacterial growth (58%) thus exhibiting a considerable MIC values (55.5) towards *Staphylococcus aureus* bacterial strains tested. These results support the use of compost extracts as promising potential applications in the biomedical field as natural nutraceuticals.

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POSTER

P1. **Effect of 8-week n-3 fatty-acids supplementation on oxidative stress and inflammation in middle and long distance running Athletes**

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Background. Long-chain n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may alter oxidative status and immune function after exercise. The aim of this preliminary study was to determine the effects of n-3 supplementation on middle and long-distance running Athletes on oxidative status and immune function. **Methods.** Twenty-nine subjects of 17-30 years old, males and females, divided in two groups: 1) (n=21) trained Athletes (middle distance runners: 800m, 1500m, 3000m steeplechase; long distance runners: 5000m, 10000m, marathon); 2) (n= 8) Sedentary subjects. All subjects were randomly assigned to 4 g/day of n-3 supplementation, rich in EPA and DHA, for 8 weeks. Blood, saliva and urine samples were collected pre- (T0) and post- (T1) supplementation. The hematologic parameters (trygliceride, total cholesterol, HDL, CPK, LDH, HGH, IGF-1), oxidative markers (MDA, 8-OHdG, PCc), antioxidant parameters (GPx, SOD, CAT, DPPH scavenger), exercise-imposed stress markers (testosterone and cortisol) and inflammatory marker (TNF-α) were measured. **Results.** The results evidenced that MDA, cortisol and TNF-αlevels significantly decreased after supplementation in both Athletes and Sedentary subjects: variation was greater in Athletes than in Sedentary control subjects. C**onclusion**. These preliminary data allowed us to understand that the supplementation of 4 g/day of n-3 PUFAs rich in EPA and DHA for 8 weeks may be useful as a nutritional countermeasure to strenuous exercise-induced oxidative stress and inflammation in Athletes, but further studies, increasing the sample size for example, will be necessary.

P2. **May natural supplements alter the urinary lh and steroid's profiles in antidoping analyses? A pilot study**

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Background: Luteinizing Hormone (LH) induces testosterone secretion in male. Based on its biological activity, LH could be abused by male athletes for doping purposes, mainly to restore the functionality of gonads, suppressed by the administration of anabolic steroids. The urinary LH levels and the steroid profile are routinely examined in antidoping laboratories to reveal the intake of pseudo-endogenous steroids (testosterone and its precursors).
Natural supplements are commonly taken both by athletes and amateurs. Some natural products are reported to produce alterations of the LH levels and/or of the steroid urinary profile.
Fenugreek seems to increase testosterone levels in male serum, probably due to the presence of saponins or furostanol glycosides (1). It is reported that Ginger increases LH in infertile men (2), probably because of 6-gingerol. Finally, Cordyceps sinensis has shown in man an increase of salivary testosterone and of Testosterone/Cortisol ratio (3), that could be correlated to the presence of natural isoflavones (Orobol) (4) and/or to the sterol H-1A (5), an analogue of testosterone. **Aim:** The aim of this work was to investigate the possible alterations of both the LH levels and the steroidal basal urinary profile, following the intake of three natural supplement containing Fenugreek, Ginger and Cordyceps sinensis. Specifically, our study aimed to assess whether one or more of the above products could determine atypical results (false positives) in antidoping controls. **Experimental design:** Three different natural supplements containing Fenugreek, Ginger or Cordyceps sinensis were selected for this pilot study. Two healthy male volunteers were asked to take around 1000 mg/day of one of the selected supplements, for 5 consecutive days. The administration was repeated at the same conditions for each supplement and each volunteer. The urine samples, collected before and after the intake, were analyzed to measure the urinary concentration of LH (the total hormone and specifically the "intact" fraction) and testosterone. The following ratios were also calculated: testosterone/total LH (T/LH) and intact LH/total LH (LH int/tot). **Results and discussion:** Fenugreek: testosterone decreases in both the volunteers. Intact LH and T/LH increase in vol.1, but decrease in vol.2. Ginger: suppression of LH (intact and total) in both the volunteers. T/LH increases with testosterone (initially) in vol.2. The data reported above do not confirm the (scarce) data reported in the literature, where testosterone always increased.
Cordyceps: LH int/tot ratio decreases in both the volunteers. Total LH, testosterone and T/LH increase, in vol.1, while decrease in vol.2. In the case of Cordyceps, the increase of testosterone (observed in vol.1) partially agrees with literature data. An additional factor of variability is represented by the considered fraction of the fungus. **Final conclusions:** As mentioned previously, this work was designed as a pilot study, to shed light on a topic that could be relevant in the antidoping context. Our preliminary data show that the intake of natural supplements containing Fenugreek, or Ginger, or Cordyceps sinensis, could induce an alteration of the LH and/or steroid's levels, causing a false positive result at the antidoping control. Further studies are needed, extending the number of volunteers, evaluating the measured effects at higher doses and for a longer duration of the administration, to minimize effects correlated to the *intra-* and *inter-*individual variability.

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P3. **Pros and cons of pure molecules and plant extracts in nutraceutical field: the ideain *vs* pistachio hull extract case**

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Pistachio hull (*P. vera* L., Bronte variety) is an attractive source of health-promoting compounds potentially helpful in preventing the onset of various oxidative stress-related disorders[1,2]. Among polyphenols, Ideain (Cyanidin-3-*O*-galactoside) represents the most abundant compound with remarkable antioxidant, free radical scavenging and cytoprotective properties[2]. Recently we investigated, for the first time, the absorption efficiency and anti-inflammatory properties of Ideain across Caco-2 transwell model.

Transport studies showed an Ideain absorption efficiency of about 79% according to previous investigations on other glycosylated anthocyanins in the same experimental model. Absorption efficiency observed was not related to the Ideain concentration, confirming an active transport involvement by sodium-glucose carriers (SGLT 1 and 2) across the Caco-2 cell monolayer[3]. No absorption efficiency alteration was observed after Verapamil apical treatment; on the contrary, an increase of Ideain absorption efficiency (>50%, $P<0.001$) was observed after Verapamil basolateral treatment. This result confirms that Ideain, under physiological conditions, is expelled from the basolateral compartment by the P-gp, showing an asymmetric transport[4]. EDTA-treatment at lowest concentration (1 mM) no alters significantly the Ideain behavior while at higher concentrations (2.5-5 mM) a significant absorption efficiency decrease (~50%, $P<0.001$) was observed. This effect could be due to hydrophobic interactions between EDTA and Ideain, which is no longer recognized by SGLTs[5]. Moreover, Ideain pre-treatment of Caco-2 cells decreases the LPS-induced interleukins release with respect to the control ($P<0.001$) without showing any cytotoxicity or alteration of the barrier system.

In light of this, we decided to focus our attention on the best natural source of Ideain, the pistachio hull extract (PHE), in order to investigate how the plant matrix could improve or decrease the absorbtion, modify the transport mechanisms and/or the anti-inflammatory activity of the pure bioactive compound.

Results showed, independently from the treatment compartment chosen, that the Ideain absorption efficiency within the PHE was comparable with that observed in the basolateral treatment with the pure compound (~48 %). Moreover, PHE pre-treatment of Caco-2 cells decreases, more than Ideain ($P<0.001$), the LPS-induced interleukins release with respect to the control without showing any cytotoxicity or alteration of the barrier system.

In conclusion, our results show that Ideain absorbtion, likewise other glycosilated anthocyanins, is mediated by SGLTs but that, when administered as plant extract, the other glycosilated polyphenols probably compete with it decreasing its absorbtion efficiency by sodium-glucose carriers. Nevertheless, although the Ideain concentration within the Caco-2 cells was decreased, the PHE showed a greater anti-inflammatory activity than the pure molecule suggesting a synergistic mechanism among the polyphenols, which advises a more promising use of PHE with respect to the pure bioactive compound in the nutraceutical field.

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P4. **Role of nutrition in neurodegenerative disease**

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Introduction. Neurodegenerative diseases are characterized by the progressive loss of neuronal function in the brain causing cognitive impairment. The most common form is Alzheimer disease but is also included amyotrophic lateral sclerosis (ALS) and Parkinson disease (PD). Despite inflammation and hormonal deficiencies play an essential role in the pathogenesis of neurodegeneration, nutrition is one of the modifiable factors that has been included in the physiopathology of these diseases. **Methods**. A systematic literature search was performed using PubMed Medline and Cochrane Central Register of Controlled Trials. A combination of the following keywords was used: "nutrition" with "Alzheimer's' disease," "nutrition" with Amyotrophic lateral sclerosis," and "nutrition" with "neurodegeneration." The search included the filter "clinical trials" and "humans." Uncompleted studies that did not evaluate the mental impairment have been excluded. **Results**. We found 124 articles, of these 27 have been selected including 9058 patients with a mean age of 71,5+7,7. A significant difference between the duration of the studies, (varying from 3 weeks until 51 months) and the number of patients (from 24 until 2911) has been observed. The most common date emerged is that malnutrition and low body mass index correlated with the higher development of dementia and mortality, showing that nutrition is involved in the neurodegenerative process and SLA [1]. The administration of polyunsaturated fatty acids, either alone or in combination, had no significant effects on cognitive decline. High-dose B vitamin supplementation seemed beneficial in patients with cognitive dysfunction. Also, the administration of ginkgo biloba did not support any improving cognitive function A high-glycemic diet was associated with the higher cerebral amyloid burden and AD development. Dietary supplementation with protein showed a positive effect on cognitive function.
Discussion. The pathogenesis of motoneuron degeneration is not yet entirely understood, but nutrition represents a critical co-factor regulating the development of the disease. High protein diet and ketogenic diet seem to be the most effective increasing the IGF-1 plasma level and stimulating the IGF-1 receptor expression in the brain [2]. Circulating IGF-1 level has a protective effect on the brain and mediates the formation of new neurons in the adult hippocampus [3]. Insulin signaling is a specific independent inhibitor of neurons regeneration in aging [4], while the insulin/IGF-1 signaling has a neuroprotective effect [5]. So that favoring lower insulin and higher IGF-1 plasma level has a protective effect on the neuron, and a low carbohydrates-high protein diet seems to support this hormonal attitude while a high-fat diet predisposes to neuroinflammation in central and peripheral nervous systems. **Conclusions**. Nutrition plays a fundamental role in maintaining brain health and reduce neurodegenerative diseases development. However, an insufficient number of the clinical trial has investigated the interaction of macronutrients, hormonal impact and the decline of brain function.

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P5. **Pharmacological effects of the standardized Lipidosterolic Extract from *Kigelia africana* fruits in experimental Benign Prostatic Hyperplasia induced by testosterone in Sprague Dawley Rats**

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The use of nutraceuticals is very common in patients with prostatic diseases, sexual disorders and male infertility, and many combinations are commercially available. Various vegetable products used as nutraceuticals or drugs are attributed the capacity to exert benefic effects on reproductive system, and most of these drugs have a rich and varied lipidosterolic fraction, primarily responsible for the effects related to the male genital sphere. *Kigelia africana* (Lam.) Benth. (Bignoniaceae) is a plant used in African folk medicine as a vegetal remedy for various diseases, including some disorders of the male reproductive system, but its potential activities have not yet been fully explored. The main phytochemical constituents of *K. africana* fruits are iridoids, naphtoquinones, lignan, flavonoids, as well as phytosterols and fatty acids [1,2]. Benign prostatic hyperplasia (BPH) is a common disease seen in aged men, characterized by a non-malignant prostatic swelling associated with histomorphological changes, mainly due to the proliferation of luminal epithelium and fibromuscular tissue. The aim of the present study was to investigate whether the lipidosterolic hexane extract (LHE) from *K. africana* fruits, identified and quantified by using comprehensive two-dimensional gas chromatography-mass spectrometry/flame ionization detection (GC×GC-MS/FID), can prevent or reverse benign prostatic hyperplasia in rats. BPH was induced in experimental groups by daily subcutaneous injections of testosterone propionate (TP) for four weeks. β-sitosterolo (β-s) was used as positive control. A first series of experiment was conducted to investigate the ability of LHE to prevent BPH, using five groups of 5 rats for group: testosterone group (5 mg/Kg b.w., for 4 weeks); LHE groups (administered daily orally at a dose of 10 or 100 mg/kg b.w. along with the TP injections, for 4 weeks); β-s (administered daily orally at a dose of 1 mg/kg b.w. together with the TP injections, for 4 weeks); control group (vehicle by oral gavage and soy oil by subcutaneous injection). The second series of experiments was carried out, in two groups of 5 animals, to investigate the ability of LHE to reverse BPH. The rats were treated daily with testosterone. After 2 weeks of testosterone-treatment, the rats were orally treated with LHE (100 mg/kg b.w) or β-s (1 mg/kg b.w) together with the TP injections for other 2 weeks. On day 28, the animals were sacrificed by cervical dislocation after anesthesia. Prostates were excised, weighed, and used for macroscopic and histological studies. Testosterone and dihydrotestosterone (DHT) levels in prostate were measured. The results showed that LHE significantly reduced the prostatic weight, prostatic index, prostatic levels of testosterone and DHT, and the histopathological alterations (including the epithelial thickness, stromal proliferation and lumen area) induced by testosterone. These effects were superior to those demonstrated by β-s and appears to be due to a partial antiandrogenic activity of LHE and support the traditional use in some disorders of the prostate.

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P6. **The paradox of toxicological studies on nutraceuticals**

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There are very lively scientific debates between different international authorities on natural extracts. New European legislation entrusting EFSA with the safety assessment of natural extracts and the scientific and technical attitude of the US authorities sometimes generate controversy in the scientific, industrial and consumer worlds. The authors discuss two examples of such cases: the assessment of Aloe extract and Ginkgo Biloba extract.
In the USA, the FDA entrusted the National Toxicology program (NTP) with the assessment of the long-term risk and carcinogenicity of above-mentioned extracts. The results were negative, reinforced by penalizing toxicological evaluations.
We think, like many independent bodies, that there are many questionable aspects in the execution and evaluation of the tests. In both cases the test material was not the one reported by the pharmacopoeias and the composition limits imposed by the manufacturers. The doses used in long-term studies were much greater than 100 times those in human use (100 times is the limit that toxicologists consider representative). In addition to that the highest dose tested was not, as required by the relevant guidelines, the first dose that produces a toxic effect; on the contrary, in the tests were used massive doses that put a strain on the system of homeostasis of the animal in addition to altering the normal dietary profile of the animal.
The negative results therefore affect the propensity to use the plant extracts as a nutraceutical, and influence other global health authorities such as IARC, EFSA and other countries as they are obliged to take into account the results of such studies. This with major consequences on the freedom of choice of consumers and on the industrial sector of countries producing such extracts.

P7. **Antioxidant and Antiinflammatory Activities of Polyphenolic Rich Ethyl Acetate Fraction from *C. incanus* Leaves**

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Plants in the Mediterranean ecosystems are exposed to multiple stress factors, particularly during the summer, when water shortages are associated with high solar radiation and high temperatures: these conditions increase Reactive Oxygen Species (ROS) that may result in oxidative stress induced damage in several cellular components if antioxidant defense systems do not properly work. Polyphenols have been widely reported to protect plants from oxidative stress by directly scavenging ROS or by modulating glutathione biosynthesis activation of NF-kB, and the activation of the nuclear redox factor (Nrf2). *C. incanus* (Cistaceae) is a Mediterranean semi-deciduous shrub used as general remedy in traditional medicine since ancient times. We previously demonstrated that *C. incanus* leaf extracts, thanks to its high content of polyphenols, exert antioxidant effects in a cell free model. In particular, the ethyl acetate fraction (EAF) showed the highest activity in terms of radical scavenging capacity.

In this study, we evaluated the anti-inflammatory and the antioxidant effects of *C. incanus* leaf extracts in lipopolysaccharide (LPS)-stimulated murine macrophage RAW 264.7 cells, an *in vitro* model of inflammation. We firstly evaluated the ability of four different polyphenolic enriched extracts, namely CEE (crude ethanolic leaf extract), EAF (Ethyl Acetate Fraction), AF1 and AF2 (Aqueous Fractions), to counteract NO production. The EAF extract showed the highest activity, reducing NO production by more than 60%. The effects of EAF, 1 μM, were further analyzed by measuring the expression of COX2, iNOs, IL-6, IL-10, SOD2 and heme oxygenase-1 (HO-1) genes in the cellular lysate, by RT-PCR, and the production of PGE_2 in the culture medium, by ELISA immunoassay. Moreover, the involvement of Nrf2 antioxidant signaling pathway was evaluated by immunocytochemistry.

EAF treatment significantly reduce COX2 expression ($p<0.05$) and this effect was associated to a significant reduction of PGE2 ($p<0.05$). EAF significantly increased the nuclear translocation of Nrf2 and expression of its main target gene: HO-1. The expression of IL10 was significantly up-regulated whereas that of IL6 was significantly reduced further supporting the antinflammatory effects of EAF Finally, the antioxidant effects of EAF was confirmed by the significantly reduction of SOD2 expression.

In conclusion, these data indicate that the EAF extract from *C. incanus* leaf inhibits inflammatory responses mainly through the up-regulation of Nrf2/HO-1 pathway, providing scientific support for its traditional use in the treatment of various inflammation-related disorders.

P8. **Efficacy of xyloglucan against Escherichia coli urinary tract infection: *in vivo* study**

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Urinary tract infection (UTI) is the third most common infection after gastro-intestinal and respiratory infections, especially in women, occurring both in the community and hospital sceneries, named as HAI (hospital acquired infections), where 80% of them, are estimated to be catheter-associated. Natural approaches to conventional pharmaceutical treatments for UTIs attracted more attention in the recent years, due to high recurrence rates resulting in repeated drug exposure. In this study was evaluating the protective effect of xyloglucan, obtained from the seeds of the tamarind tree, and gelose, a biocompatible polysaccharide, in an *in vivo* model of UTI.
The infection was induced by a transurethral injection of *Escherichia coli (E. coli)* ($2x10^7$ CFU/ml), the most relevant uropathogen, for 7 days. Two days before the *E. coli* transurethral administration, preventive xyloglucan oral treatments were performed by gavage every day until the seventh day. Our results clearly demonstrated the protective barrier effect of xyloglucan, evaluated by electro-microscopy analysis of *E.coli* fimbriae; moreover, we support the potential beneficial effect in preventing urinary tract infections and gastroenteritis of xyloglucan and xyloglucan plus gelose, by means of the reduction of the *E. coli* positive CFU in urinary tract and by a reduction in histological changes and neutrophil infiltration in both bladder and intestine following *E. coli* infection. Therefore, xyloglucan can be considered an optimal oral medical device suitable for early clinical translation for UTIs especially acquired in hospital, which are becoming increasingly problematic in many clinical settings.

P9. **Strawberry tannins as potential ingredients of nutraceuticals to counteract *H. pylori*-induced gastric inflammation**

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Gastritis is an inflammatory-based disease, affecting millions of people in the world. *Helicobacter pylori* is the bacterium mostly responsible for its development. The infection of gastric epithelium by *H. pylori* induces an inflammatory response through the activation of several transcription factors including NF-κB and AP-1, ultimately leading to the release of pro-inflammatory cytokines such as IL-8, IL-6 and TNF-α.

Beside the classical pharmacological treatment based on bacterial eradication, food components could be used to reduce the consequences of *H. pylori* infection due to the anti-inflammatory and antibacterial properties displayed by several botanicals and the traditional use of some of them in gastritis.

Strawberry is one of the most frequently consumed fruits in the world; it contains sugars, vitamins, fibers, micronutrients and various polyphenols, including anthocyanosides and tannins, especially ellagitannins, such as agrimoniin and casuarictin. Gastric effects of strawberry have been documented by some *in vitro* and *in vivo* studies; the tannin-enriched extract inhibits IL-8 secretion in TNFα-treated human gastric epithelial cells by dampening NF-κB signaling.

The aim of this work was to evaluate the anti-inflammatory effect of a tannin-enriched extract from *Fragaria* X *ananassa* Duch. in two *in vitro* models of gastric inflammation. In addition, individual strawberry ellagitannins were investigated to assess their contribution to the anti-inflammatory action of the extract.

The strawberry extract was assayed on two gastric cell lines, AGS, the most used *in vitro* model to study gastric inflammation, and GES-1 (non tumoral gastric epithelial cells), less studied in the literature. Cells were co-cultured with TNF-α or with *H. pylori* to mimic *in vivo* infection. Both cell lines were infected with two different strains of *H. pylori*, the well-known and characterized ATCC® 700392 strain and one *H. pylori* clinical isolate.

The results obtained in this study showed that both pro-inflammatory stimuli TNF-α and *H. pylori* induced the expression and release of different inflammatory mediators in the two cell lines; IL-8 release was induced in both cell models, while IL-6 release in GES-1 cells only. The anti-inflammatory activity of *Fragaria* X *ananassa* extract was maintained in gastric epithelial cells GES-1: the release of IL-8 induced by TNF-α was inhibited at concentrations around 1-2 μg/mL. Approximately at the same concentrations, the extract inhibited the release of IL-6, in GES-1 cells only. The activity of the extract was also maintained in both cell lines after infection with the two different strains of *H. pylori*, however the IC_{50s} were higher compared to those effective in TNF-α-treated cells, but still reachable after a dietary consumption of strawberries. Furthermore, pure ellagitannins significantly attenuated the release of IL-8 and IL-6 by TNF-α-treated cells, at concentrations lower than 1 μM.

In conclusion, this study demonstrates that strawberry tannins, either as enriched extract or as pure compounds, act on gastric epithelial cells by inhibiting the inflammatory response induced by either TNF-α or *H. pylori*, at nutritionally relevant concentrations. These properties make these compounds exploitable as preventive or co-adjuvant agents in gastric diseases.

P10. **Sodium propionate protects from inflammation and ROS stress**

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The major end□products of dietary fibres fermentation by the gut microbiota are the short□chain fatty acids (SCFAs) acetate, propionate and butyrate, which have been shown to modulate host metabolism via effects on metabolic pathways and receptor□mediated mechanisms at different tissue sites. The beneficial effects of SCFAs on gut physiology, barrier function, and metabolism are well documented. Moreover, SCFAs can promote intestinal homeostasis and suppress intestinal inflammation. Recently, several studies described inhibitory effects of SCFA on NF-kB, one of the key transcription factors regulating genes implicated in innate immunity, cell cycle control, and apoptosis. Few reports devoted their efforts to investigate the properties of propionate, although it is abundant as such as butyrate in the gut and blood. Thus, the purpose of this study is to explore the protective mechanisms of sodium propionate (SP) in inflammatory and oxidative stress responses. The first step was to evaluate the anti-inflammatory and anti-oxidant mechanism of SP, by a classic in vitro model of inflammation and oxidative stress on murine macrophage cell line. For this reason J774-A1 was stimulated with lipopolysaccharide (LPS) from E. coli or with H_2O_2, and then treated with SP at different concentrations (0.1-10 μM). Moreover, to evaluate the effect on acute in vivo model of inflammation, carrageenan (CAR)-induced rat paw injection was performed, and rats were orally treated with 3 different doses of SP (10 mg/kg, 30 mg/kg and 100 mg/kg). Our in vitro results showed that SP (0.1-1-10 μM) significantly decreased in concentration-dependent-manner the expression of pro-inflammatory mediators, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) following LPS stimulation. Moreover, SP (0.1-1-10 μM) was able to enhance antioxidant enzyme production such as manganese-dependent superoxide dismutase (MnSOD) and heme oxygenase-1 (HO-1) following H2O2 stimulation. In addition, in in vivo model, SP (30 mg/kg and 100 mg/kg) markedly reduced paw inflammation, thermal hyperalgesia and tissue damage induced by CAR injection. Our results clearly demonstrated the anti-inflammatory and anti-oxidant properties of SP; therefore, we propose that SP may be an effective strategy for the treatment of inflammatory diseases.

P11**. Vitamin E supplementation prevents allergen sensitization in the mouse**

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The incidence of allergic disorders has dramatically increased in the developed world and a variety of factors (environment, genetics, hygiene, diet, etc.) are known to impact on their development. Recent advances in the understanding of the pathophysiology of allergy have revealed a series of complex immune cell interactions that can be manipulated to influence either sensitization to the allergen (prevention) or help alleviate allergic manifestations (treatment). To date, observational studies have reported that low Vitamin E intakes are associated with a higher prevalence of asthma. Here, we have investigated on the role of Vitamin E metabolites in an experimental model of asthma. BALB/c mice received subcutaneous administration of ovalbumin (OVA,100μ/g) at days 0 and 8. Part of the mice were intraperitoneally treated with the endogenous vitamin E metabolite 13'-carboxy-α-tocopherol (α-T-13'-COOH; 10 mg/kg i.p. 30 min before OVA) or the semi-synthetic lead compound α-amplexichromanol (α-T-13'-diOH; 10 mg/kg i.p. 30 min before OVA). IgE plasma dosage evidenced a significant reduction in OVA-induced IgE release following treatments with both compounds. The protective effects of both compounds were confirmed by functional study. Indeed, both α-T-13'-COOH and α-T-13'-dioH caused a significant reduction of bronchial hyper-responsiveness ($p<0.001$). However the semisynthetic compound α-T-13'-diOH showed a more marked effects ($P<0.05$). The major efficacy of α-T-13'-dioH in modulating OVA-airway hyper-responsiveness was confirmed by pulmonary LT (LTB4) dosage as well as by cytokine measurements (IL-13 and IL-4). In conclusion our data suggest that vitamin E supplementation prevents the development of cardinal features of asthma like disease such as airway hyper-responsiveness by modulating the molecular mechanisms underlying sensitization.

P12. **α-T-13'-carboxychromanol, an endogenous metabolite of vitamin E, limits inflammation by targeting 5-lipoxygenase**

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α-tocopherol (α-T), a naturally-occurring vitamin E form contained in peanuts, almonds and sunflower seeds, has potent antiinflammatory effects, some of which are independent of its antioxidant properties[1,2]. The effect of vitamin E in the production of leukotrienes (LTs), active lipid mediators with key roles in inflammation, has never been studied in detail so far.
The aim of this study was to evaluate the effect of vitamin E (α-T) and in particular its physiologically formed metabolite, α13'-carboxychromanol (α-T-13'-COOH), in mouse zymosan-induced peritonitis, an *in vivo* model of inflammation related to LTs.
Analysis of plasma and peritoneal exudate of mice treated *per os* with α-T revealed α-T-13'-COOH as major systemic vitamin E metabolite. α-T-13'-COOH (10 mg/kg i.p. 30 min before zymosan injection, 1 mg/mouse) reduced LTC_4 and LTB_4 peritoneal levels 30 min and 4 h after peritonitis induction, respectively. This effect was associated to inhibition of acute inflammatory reaction evaluated as vascular permeability (30 min after peritonitis induction) and peritoneal cell recruitment (4h after peritonitis induction). α-T (10 mg/kg, i.p) was less effective in reducing exudate levels of LTC_4. Thus, α-T-13'-COOH, which accumulated at sites of inflammation, possesses anti-inflammatory activity by suppressing LT formation.
The effects of α-T-13'-COOH on resolution phase of murine peritonitis was also studied (18 h after zymosan injection). At this time in the peritoneal exudate lipid profiles showed that pro-inflammatory mediators decreased (cyclooxygenase- and 5-lipoxygenase products), whereas pro-resolving mediators slightly increased (protectins, maresins). α-T-13'-COOH did not inhibit cell infiltration at the resolution phase. On the contrary it strongly increased the circulating resolvin E3 levels in plasma.
In conclution our data suggest that α-T -13'-COOH is the main endogenous bioactive metabolite of α-T, which contributes to the antiinflammatory properties of α-T by targeting 5-lipoxygenase.
For reference see Pein H, *et al* "Endogenous metabolites of vitamin E limit inflammation by targeting 5-lipoxygenase" Nat Commun. 2018; 9: 3834

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P13. **Evidence for a beneficial effect of caffeine in psoriasis-like inflammation**

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Psoriasis is a chronic immune-mediated inflammatory skin disease characterized by hyperproliferation and abnormal differentiation of keratinocytes [1]. Caffeine (1,2,7 trimethylxanthine) is found in many beverages and some of our foods, such as coffees, teas, colas, and even chocolate. Caffeine can act as antagonist of A_1 and A_2 adenosine receptors and at very high concentration as inhibitor of phosphodiesterase enzyme and by promoting intracellular Ca^{2+} release [2]. It has been suggested that caffeine can reduce inflammation in patients with eczema and psoriasis [3].
Here, we investigated the effect of caffeine in psoriasis-like inflammation by experiments in vitro, on human keratinocytes (HaCaT) cell line, and in vivo, in the mouse model of imiquimod-(IMQ)-induced psoriasis-like skin lesions.
Following incubation with caffeine (6, 24 or 48 hours at 0.1-10 mM), HaCaT cells were stimulated with a mixture of five pro-inflammatory cytokines (TNF-α, IL-17A, IL-6, IL-1α, 10 ng/ml). Cell proliferation was then evaluated by performing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.
C57BL6/J mice (8–11 weeks of age) received a topical application of IMQ cream (5%) to the external surface of the right ear, for 5 consecutive days, corresponding to a daily dose of 62.5 mg/ear. Groups of mice received topical application of caffeine (1, 10 and 30 % in acetone), of vehicle (acetone) or of the reference drug clobetasol (0.05 % ointment), 30 min before IMQ application. The severity of inflammation of the ear skin was measured on a daily basis using an adapted version of the clinical Psoriasis Area and Severity Index. Erythema and acantosis were scored independently on a scale ranging from 0 to 4 according to the degree of the inflammation. The thickness of right ears was measured using a caliper and the percentage change from baseline value was calculated.
We found that caffeine (3 and 10 mM) significantly reduced cell proliferation of human keratinocytes both unstimulated and following stimulation for 6, 24 or 48 hours with the mixture of pro-inflammatory cytokines (***$p<0.001$, $n=6$).
In addition, in the mouse model of psoriasis-like skin lesion, we observed that epidermal thickness, erythema and acanthosis were greatly reduced following topical application of caffeine at all concentration tested (**$p<0.01$ versus IMQ-acetone, $n=6$).
Our preliminary findings demonstrate a beneficial effect for caffeine in psoriasis-like inflammation.

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P14. **Peptides released after simulated gastrointestinal digestion of dehydrated chips exert antinflammatory activity in intestinal epithelial cells**

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Inflammatory bowel disease (IBD) is one of the most prevalent gastrointestinal disorders, and it includes ulcerative colitis and Crohn's disease [1]. In particular, it has been reported that intestinal epithelial cells (IECs) have emerged as key players in the generation and persistence of intestinal inflammation during IBD [2]. Currently research is demonstrating a growing interest in the discovery of diet-derived products, which have an anti-inflammatory and anti-oxidant potential useful for the treatment of IBD. In this context, this project aim to the characterization and biological evaluation of the bioactive peptides contained in the dehydrated potatoes. Peptides deriving from potatoes possess multiple healthy properties, that cannot be exerted as long as they are encrypted in parent proteins. To evaluate the biological activities of encrypted peptide sequences from dehydrated chips, a simulated gastrointestinal (GI) digestion was performed. The GI digest was fractionated by ultra-filtration with different cut-off membranes obtaining three fractions. The peptide fraction with a molecular weight of less than 1000 Daltons was further concentrated and purified by solid phase extraction (SPE). The peptide fractions were monitored and characterized by LC-HRMS experiments. Pharmacological evaluation was performed on rat intestinal epithelial cells (IEC-6), treated with *E. coli* lipopolysaccharide (LPS) plus interferon-γ (IFN) as pro-inflammatory agents. Our results indicate that the three different tested peptides, inhibit the expression of enzymes involved in the mechanisms of inflammation, such as cyclooxygenase-2 and inducible nitric oxide synthase. Moreover a reduction in the nitrotyrosine formation, an important marker of oxidative stress, was also observed in IEC-6 tested with the peptides in inflammatory conditions. The three peptides have also been shown to reduce the intracellular release of reactive oxygen species during the inflammatory response in IEC-6 cells. The obtained results indicate a possible use of these peptides to reduce the inflammatory state and oxidative stress at the intestinal level.

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P15**. Polyphenolic extract from Tarocco (*Citrus sinensis* L.Osbeck) clone "Lempso" exerts anti-inflammatory and antioxidant effects via NF-kB and Nrf-2 activation in murine macrophages**

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Citrus belongs to the family of Rutaceae, and it is one of the most important fruit tree crop in the world [1]. Several investigations reported a large number of healthy properties of *Citrus* flavonoids, such as hypolipidemic, hypoglycaemic, anti-inflammatory, and antioxidant properties [2]. Among *Citrus*, the variety, "Lempso", a typical hybrid of the Calabria region (Southern Italy), has been reported to possess superior antioxidant activity when compared to other common *Citrus* varieties. The aim of this study was to investigate *in vitro* the nutraceutical value of the Tarocco clone, "Lempso", highlighting its anti-inflammatory and antioxidant potential. A post-column 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging assay for the screening of antioxidant compounds in these complex matrices was developed. Subsequently, polyphenolic extract was tested on a murine macrophage cell line (J774A.1) under inflammatory conditions induced by lipopolysaccharide from *E.coli* (LPS). Macrophages are involved in host defence during inflammatory and immune response. It is known that, in response to LPS, a component of Gram-negative bacteria cell walls, macrophages produce and release inflammatory mediators, including cytokines, pro-inflammatory enzymes, as inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2), and highly reactive species, as nitric oxide (NO) and reactive oxygen species (ROS). In our experimental conditions the Lempso extract resulted to significantly inhibit NO and tumor necrosis factor-α and interleukin-6 release as well as iNOS and COX-2 expression. The inhibition of these pro-inflammatory factors was associated to Nuclear factor-kB (NF-kB) nuclear translocation inhibition. Our results also indicate an anti-oxidant potential of the extract from Lempso as indicated by the inhibition of ROS release and by the activation of the nuclear factor E2-related factor-2 (Nrf-2) pathway in macrophages. The obtained results highlight the anti-inflammatory and antioxidant potential of Lempso extract and its high nutraceutical value.

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P16. ***Aloe vera*** **gel and** ***Punica granatum*** **onconutraceutical potential in intestinal epitelial cells during oxidative stress and inflammatory conditions**

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Intestinal epithelial cells (IECs) play a pivotal role in maintaining intestinal homeostasis. Different noxious agents, such as chemical, physical, infectious, and inflammatory injuries can damage the intestinal epithelial integrity. This damage results also associated to anticancer therapies. Chemotherapy not only targets cancer cells, but also normal rapidly dividing cells, especially those lining the gastrointestinal tract. These activities result in an overproduction of reactive oxygen species (ROS) and pro-inflammatory factors. Gastrointestinal mucositis is a frequent and severe side effect of chemotherapy and radiotherapy in cancer patients [1,2]. Approximately 50 to 80% of patients suffered from mucositis, with the occurrence being dependent on the type of chemotherapy; vomiting, abdominal pain, and severe diarrhoea [3] were the most common symptoms experienced. Currently, no effective treatment exists for chemotherapy-induced mucositis, prompting the need to develop an anti-mucositis agent for use in clinics. Among the various *Aloe vera* is a cactus-like plant that grows readily in hot, dry climates. It belongs to the Liliacea family, of which there are about 360 species. Only two species are grown commercially: *Aloe barbadensis* Miller and *Aloe aborescens*. The parenchymatous cells in the fresh leaves of *Aloe vera* secrete colorless mucilaginous gel (i.e., *Aloe vera* gel) that contains 98-99% water and 1-2% active compounds can damage the intestinal epithelial integrity. In this study, we investigated the effects of polysaccharides isolated from *Aloe barbadensis* gel (200-6.25 μg/mL) in a model of oxidative stress and inflammation in intestinal epithelial cell line (IEC-6).

The *Aloe vera* gel was washed with 4 volumes of 95% (v/v) ethanol to achieve polysaccharide's precipitation, then the residual proteins were removed by TCA method. The IR spectroscopy analysis of the obtained extract has indicated the presence of typical functional groups of polysaccharides. Our results indicated that polysaccharides isolated from *Aloe vera* gel (200-6.25 μg/mL) was able to significantly inhibit ROS release from hydrogen peroxide-treated IEC-6 and NF-kB dependent pro-inflammatory factors (e.g. iNOS and COX-2) in lipopolysaccharide from *E. coli* (LPS) plus interferon-γ (IFN)-treated IEC-6 cells. The polysaccharide extract effect on pro-inflammatory factors in IEC-6 was further enhanced by pomegranate polyphenol extract (PPE) addition. PPE was analyzed by RP-UHPLC-UV-ESI-IT-TOF and roughly 30 polyphenolic compounds have been identified, belonging to: phenolic acid, flavonoids, ellagitannin and gallotannin. While the anthocyanin profile, analyzed by UPLC-PDA-MS, has revealed the presence of 7 different compounds, such as: pelargonidin 3,5-diglucoside and cyanidin 3,5-diglucoside.

Our results indicate that *Aloe vera* and *Punica granatum* could be useful to reduce the oxidative stress- and inflammatory-mediated complications associated to chemotherapy, as mucositis, at intestinal level.

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P17. **Antinflammatory effects of *Tisochrysis lutea* and *Arthrospira platensis* M2 extracts and of their main bioactive compounds**

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There is an increasing demand for natural bioactive compounds able to provide health benefits when included and consumed in a functional food or in a nutraceutical. In this regard, microalgae in addition to their nutritional value, are promising natural sources with great potential. In the present investigation, we prepared extracts from *Tisochrysis lutea* and *Arthrospira platensis* M2 biomasses, to evaluate their potential anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated RAW264.7 cells and the mechanisms involved .
The effects of the methanolic extract of *T. lutea* and the aqueous extract of *A. platensis* were compared to those of their main bioactive compounds, fucoxathin (FX) and C-phycocyanin (C-PC). FX is a xanthophyll with a unique structure featuring an unusual allenic bond and a monoepoxide within its molecule, exhibiting several biological functions including antioxidant, UV-protective and anti-inflammatory activities. C-PC is one of the major biliproteins of the blue-green microalgae *Spirulina platensis*. This water soluble protein pigment has significant antioxidant, radical scavenging and anti-inflammatory properties.
Cell cytotoxicity assay suggested that these extracts were not cytotoxic to macrophages at concentrations up to 1 mg/ml for the aqueous extract of *A. platensis* and up to 0.1mg/ml for the methanolic extract of *T. lutea*.
The expression of COX2, iNOs and heme oxygenase-1 (HO-1) genes were analysed by RT-PCR in the cellular lysate and PGE2 concentration was quantified in the culture medium, by using a competitive enzyme immunoassay.
The methanolic extract from *T. lutea* was able to reduce the LPS-stimulated production of PGE2, dose-dependently, exhibiting a higher effect compared to that of FX at concentration comparable to that calculated to be present in the microalgal biomass. Only the water soluble phycobiliprotein C-PC, modulated significantly the expression of the pro-inflammatory genes COX2 and iNOs. However, both extracts and single compounds up-regulated the expression of HO-1 in the presence of LPS, suggesting the involvement of the Nrf2/ARE signaling pathway that play an important role in inhibiting the production of pro-inflammatory cytokines.
In conclusion, these preliminary data indicated that *T. lutea* and *A. platensis* M2 biomasses inhibit inflammatory responses via the up-regulation of Nrf2/HO-1 pathway, suggesting that these two microalgae could potentially be novel functional foods candidate in the control of inflammatory chronic diseases.

P18. **Polyphenolic composition, enzyme inhibitory effects ex-vivo and *in-vivo* studies on two Brassicaceae of north-central Italy**

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Cruciferous vegetables are a precious source of bioactive compounds such as polyphenols and antioxidants that provide several health benefits. Three different extracts (soxhlet, microwave and decoction) from two species of broccoli: Brassica oleracea L. convar. Italica botrytis (L.) Alef. var. cymosa Duch. (Broccolo Fiolaro) and Brassica oleracea acephala L. convar. acephala (DC.) Alef. var. sabellica L. (Cavolo Nero), which are commonly spread in north-central Italy, were tested for their enzyme inhibitory effects. Enzyme inhibitory effects were investigated against cholinesterases, tyrosinase, α-amylase and α-glucosidase. The soxhlet extracts had the highest inhibitory AChE effects with 1.08 mgGALAE/g (in Cavolo Nero) and 0.90 mgGALAE/g (in Broccolo Fiolaro). The significant tyrosinase inhibitory effect was observed in the soxhlet extract of Cavolo Nero with 11.93 mgKAE/g. In addition, we evaluated the antioxidant activity of Broccolo Fiolaro and Cavolo Nero on lipopolysaccharide (LPS)-stimulated bladder, kidney and liver specimens, ex vivo. We observed a significant reduction of both nitrite and malondialdehyde (MDA) following treatment that indicates a significant inhibitory effect on oxidative/nitrosative stress and lipoperoxidation, respectively. Additionally, the blunting effect induced by extracts on LPS-induced lactate dehydrogenase (LDH) activity further support a protective effect by both Broccolo Fiolaro and Cavolo Nero in bladder, kidney and liver. HPLC analysis revealed that catechin, epicatechin, vanillic and 3-hydroxy benzoic acids were the major components. The phenolic components may contribute to the observed enzyme inhibitory effects. *in vivo* tests also demonstrated that the extracts decreased the biochemical parameters in diabetic rats. Particularly, we observed the reduction of plasma glucose levels, urea and total cholesterol following oral administration, with the higher inhibitory effects exerted by Broccolo Fiolaro compared to Cavolo Nero. Overall, our results could provide new insights on the use of these Broccoli species not only as foods but also as functional and nutraceutical supplements.

P19. ***Crocus sativus*, *Serenoa repens* and *Pinus massoniana* extracts modulate inflammatory response in isolated rat prostate challenged with LPS**

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Prostatitis is a common prostate disease that could be promoted by bacterial or non-bacterial infectious agents. In addition, inflammatory pathways involved in prostatitis have been increasingly studied, and herbal extracts endowed with anti-inflammatory effects are under investigation, individually or in combination, for their efficacy in alleviating the burden of inflammation, with possible improvements in symptoms. *Serenoa repens* (Serenoa), in combination with *Crocus sativus* (Crocus) and *Pinus massoniana* (Pinus), has previously shown to improve sexual function and limit urinary symptoms in patients suffering from concomitant erectile dysfunction and lower urinary tract symptoms.
In this context, the aim of the present study is to evaluate the efficacy of Serenoa, Crocus and Pinus extracts, either alone or in combination, on immortalized prostate cells (PC3) and in an experimental model of bacterial prostatitis constituted by *ex vivo* prostate specimens changelled with *Escherichia coli* lipopolysaccharide (LPS).
For the evaluation of their effiacy, we considered the effects of the single extracts and their pharmacological association on several markers of oxidative stress and tissue inflammation, such as prostaglandin E_2 (PGE_2) and nuclear factor kappa-light-chian-enhancer of activated B cells (NFkB), the activities of which were significantly increased by inflammatory stimuli related to prostatitis, including LPS.
We found that the tested extracts were able to reduce ROS production by PC3 cells and NFkB and PGE_2 activity in prostate specimens changelled with LPS.
The results about pharmacological association of the extracts, displayed synergistic effects, support a rational use of the mixture of the tested extracts as a novel anti-oxidant and anti-inflammatory formulation in bacterial prostatitis.

P20. **Anti-inflammatory and antioxidant effects of anchovy (*Engraulis encrasicolus*) by-products protein hydrolysates**

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Fish viscera constitute approximately 20% of the fresh fish biomass and are a rich source of protein and polyunsaturated lipids. Viscera are considered low quality raw materials or waste and might cause environmental, health and economic problems. However, converting proteinaceous fish waste into hydrolysates might increase biological, nutritional and economic value. Hydrolysates are produced by solubilization of the protein source, forming different size of peptides and amino acids. The aim of this study was to investigate the anti-inflammatory and anti-oxidant properties of anchovy (ENGRAULIS ENCRASICOLUS) by-products protein hydrolysates in an *in vitro* model of inflammation and oxidative stress, using a murine macrophage cell line. Raw 264.7 cells were stimulated with lipopolysaccharide (LPS) from Escherichia coli or with H_2O_2 and then treated with protein hydrolysates at different concentrations (0.01-0.05-0.1mg/mL). The protein expression of pro-inflammatory molecules, such as NF-kB , IkB-α, COX-2 and iNOS expressions was assayed by western blot analysis and IL-1β, TNF-α and IL-6 gene expression was evaluated by polymerase chain reaction. The treatment with hydrolisates (0.05-0.1mg/mL) exerted a significant protection against LPS-induced inflammation, decreasing the expression of pro-inflammatory mediators, such as COX-2, preventing the degradation of IκB-α and inhibiting the nuclear translocation of NF-κB. Moreover, hydrolisates significantly decreased iNOS expression following LPS stimulation. Protein hydrolysates were able to induce an activation of MnSOD and HO-1, decreasing the severity of oxidative stress.
In a preliminary *in vivo* studies on ApoE KO mice, we investigated the effect of protein hydrolysates on the development of atherosclerosis and changes in lipid profile. Our results revealed that protein hydrolysates treatment reduced the extent of atherosclerotic lesions.
Moreover, *in vitro* results suggest that the protein hydrolysates from Engraulis Encrasicolus may exert their protective effects through oxidative stress reduction by modulation of Mn-SOD and HO-1 and reducing pro-inflammatory mediators through NF-kB pathway, thus supporting the use of protein hydrolysates as anti-inflammatory and antioxidant agents.

P21. **Ultra-micronized palmitoylethanolamide rescues the cognitive decline-associated loss of neural plasticity in the neuropathic mouse entorhinal cortex-dentate gyrus pathway.**

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Chronic pain is associated with cognitive deficits. Palmitoylethanolamide (PEA) has been shown to ameliorate pain and pain-related cognitive impairments by restoring glutamatergic synapses functioning in the spared nerve injury (SNI) of the sciatic nerve in mice. SNI reduced mechanical and thermal threshold, spatial memory and LTP at the lateral entorhinal cortex (LEC)-dentate gyrus (DG) pathway.It decreased also postsynaptic density, volume and dendrite arborization of DGand increased the expression of metabotropic glutamate receptor 1 and 7 (mGluR1and mGluR7), of the GluR1, GluR1s845 and GluR1s831 subunits of AMPA receptor and the levels of glutamate in the DG. The level of the endocannabinoid2-arachidonoylglycerol (2-AG) was instead increased in the LEC. Chronic treatment with PEA, starting from when neuropathic pain was fully developed, was able to reverse mechanical allodynia and thermal hyperalgesia, memory deficit and LTP in SNI wild type, but not in PPARα null, mice. PEA also restored the level of glutamate and the expression of phosphorylated GluR1 subunits, postsynaptic density and neurogenesis. Altogether, these results suggest that neuropathic pain negatively affects cognitive behavior and related LTP, glutamatergic synapse and synaptogenesis in the DG. In these conditions PEA treatment alleviates pain and cognitive impairment by restoring LTP and synaptic maladaptative changes in the LEC-DG pathway. These outcomes open new perspectives for the use of the N-acylethanolamines, such as PEA, for the treatment of neuropathic pain and its central behavioural *sequelae*.

P22. ***In vitro* and *in vivo* evidence for the use of *Vitis vinifera* hydroalcoholic extract against oxaliplatin neurotoxicity**

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Oxaliplatin treatment is associated with the development of a dose-limiting painful neuropathy that leads to reduction or discontinuation of the anticancer therapy impairing patient's quality of life. Since oxidative unbalance is a relevant mechanism of oxaliplatin neurotoxicity, we assessed the potential antioxidant properties of *Vitis vinifera* hydroalcoholic extract in reducing oxaliplatin-induced neuropathy as a valuable therapeutic opportunity.
The hydroalcoholic extract of *Vitis vinifera* red leaf was characterized and tested in primary rat astrocyte cells treated with oxaliplatin (100 μM). Oxaliplatin lethality in the human colon cancer cell line HT-29 was evaluated in the absence and presence of the extract. *In vivo*, pain hypersensitivity was measured by behavioural tests in a rat model of oxaliplatin-induced neuropathy. Molecular targets of redox balance and morphometric alterations were studied in the nervous system by western blot, PCR and immunohistochemical analysis. *Vitis vinifera* extract (50 μg mL^{-1}, 4 h incubation) significantly reduced the oxaliplatin-dependent superoxide anion increase and lipid peroxidation in rat astrocytes. The extract did not interfere with the mortality elicited by oxaliplatin in HT-29 cancer cells. In oxaliplatin-treated rats, a repeated daily administration of the *Vitis vinifera* extract (300 mg kg^{-1}, p.o.) counteracted the loss of body weight induced by oxaliplatin and significantly prevented mechanical and thermal hypersensitivity to noxious and non-noxious stimuli both on day 14 and 21 after treatment as shown by Paw pressure test, von Frey test and Cold plate test. mRNA and protein levels of Nrf2 were normalized in spinal cord and DRGs. Moreover, oxaliplatin-induced neuropathy is linked with the numeric increased of astrocytes but not microglia cells in the spinal cord. Repeated treatment with *Vitis vinifera* extract significantly decreased the activation of astrocytes labelled as number of GFAP-positive cells in the dorsal horn of the spinal cord.
In conclusion, the hydroalcoholic extract of *Vitis vinifera* exerts beneficial effects against oxaliplatin neurotoxicity reducing oxidative damages and relieving pain without influencing oxaliplatin effect against cancer cells.

P23. **D-Aspartate drinking solution alleviates pain and cognitive impairment in neuropathic mice**

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D-Aspartate (D-Asp) is a free D-amino acid detected in multiple brain regions and putative precursor of endogenous N-methyl-D-aspartate (NMDA) acting as agonist at NMDA receptors. In this study, we investigated whether D-Asp (20 mM) in drinking solution for 1 month affects pain responses and pain-related emotional, and cognitive behaviour in a model of neuropathic pain induced by the spared nerve injury (SNI) of the sciatic nerve in mice. SNI mice developed mechanical allodynia and motor coordination impairment 30 days after SNI surgery. SNI mice showed cognitive impairment, anxiety and depression-like behaviour, reduced sociability in the three chamber sociability paradigm, increased expression of NR2B subunit of NMDA receptor and Homer 1a in the medial prefrontal cortex (mPFC). The expression of (post synaptic density) PSD-95 and Shank 1was instead unaffected in the mPFC of the SNI mice. Treatment with D-Asp drinking solution, started right after the SNI (day 0), alleviated mechanical allodynia, improved cognition and motor coordination and increased social interaction. D-Asp also restored the levels of extracellular D-Asp, Homer 1a and NR2B subunit of the NMDA receptor to physiological levels and reduced Shank1 and PSD-95 protein levels in the mPFC. Amitriptyline, a tricyclic antidepressant used also to alleviate neuropathic pain in humans, reverted mechanical allodynia and cognitive impairment, and unlike D-Asp, was effective in reducing depression and anxiety-like behaviour in the SNI mice and increased PSD protein level.
Altogether these findings demonstrate that D-Asp improves sensorial, motor and cognitive-like symptoms related to chronic pain possibly through glutamate neurotransmission normalization in neuropathic mice.

P24. **Effect of Ribodiet®, a ribonucleotide-based formulation, in a mouse model of Alzheimer's disease**

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Alzheimer's disease (AD) is one of the most common form of dementia characterized by the deposition of extracellular amyloid-β (Aβ) peptides in the brain. The neuronal death, that characterizes this neurodegenerative disorder, mainly involves the hippocampal and the neocortical area causing irreversible cognitive impairment and behavioral alteration. Beside synthetic drugs, the use of natural-derived products and/or nutraceuticals could represent a future medical option in industrialized countries.
Nutraceuticals represent a challenge for the future of drug-based pharmacotherapy, and, at the same time, are a powerful tool for the prevention of chronic disease. They are not proposed as an alternative to drugs, but instead, can be helpful to complement a pharmacological therapy and prevent the onset of chronic diseases. In this context, nucleotides in combination with other specific nutrient substances have demonstrated to improve the clinical outcomes in immunosuppressive and inflammatory-based diseases.
Given the well-known contribution of inflammatory pathways on AD (and other neurodegenerative diseases) onset and development, here we aimed to evaluate the potential therapeutic efficacy of Ribodiet® (a Prosol S.p.A. natural product extracted from yeast cells of Kluyveromyces fragilis and Saccharomyces cerevisiae, source of nucleotides, nucleosides, oligo nucleotides and ribonucleic acid fragments) in a non-genetic mouse model of AD. To assess the neuro-protective potential of Ribodiet®, mice were injected intracerebroventricularly (i.c.v.) with $A\beta_{1-42}$ peptide (3μg/3μl) and with Ribodiet® (0.1-10.0 mg/mouse) orally (o.s.) 3 times weekly for 21 days (experimental end-point). The mnemonic and cognitive decline, typical of $A\beta_{1-42}$ administration, was then evaluated by object recognition, olfactory discrimination and Y-maze test. Successively, by the aim of proteome profiler ElisaSpot, we have assessed the modulation of different cyto-chemokines on mice brain homogenates.
Our results indicate that the Ribodiet®, in a dose dependent manner, is able to revert the state of mnemonic decline and cognitive deficit typical of AD. Moreover, the *ex vivo* results seem to indicate a modulation, at the CNS level, of the main cytokines and chemokines (such as BCL, C5a, I-309, ICAM, IL-1α, IL-16, IP-10, KC, MIPs, SDF-1, TIMP-1, TREM-1 and HSP60) involved in the neuro-inflammation and neuro-degeneration process. The results of this study suggest the potential role of Ribodiet® as nutraceutical product useful for neurodegenerative disease such as AD, thanks to its anti-inflammatory and neuroprotective effect.

P25. **Neuroprotective effect of sodium propionate in *in vitro* and *in vivo* models**

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Sodium propionate (SP) is one of the main Short Chain Fatty Acids (SCFA) that can be produced naturally through host metabolic pathways. The physiological effects of SP have been documented and include the reduction of pro-inflammatory mediators. The aim of this study is to demonstrate the protective effect of SP both in *in vitro* model of Alzheimer's disease induced by oligomeric $A\beta_{1-42}$ stimulation and in a mouse model of spinal cord injury (SCI). For *in vitro* neuroinflammatory model, the human neuroblastoma SHSY-5Y cell line was stimulated by oligomeric $A\beta_{1-42}$ and treated with SP at three different concentration (0.1-10 μM). Therefore, in *in vivo* model SCI was induced by extradural compression of the spinal cord for 1 min at the T6-7 level using an aneurysm clip, and SP (10-30-100 mg/kg) was administered by oral gavage 1 and 6 h after SCI. Our results in *in vitro* neuroinflammatory model by $A\beta_{1-42}$ damage showed that pre-treatment of SP, significantly reduced nuclear factor-kB translocation, restored IkBα degradation, attenuates cicloxigenase-2 and nitric oxide synthase expression evaluated by Western blot analysis. In addition, we showed that SP treatment significantly ameliorated histopathology changes and improved recovery of motor function changes in spinal cord damage in a dose-dependent manner. Moreover, we demonstrated that SP modulated the NF-κB pathway showing a significant reduction in cytokine expression. In conclusion, our results demonstrated that SP possesses neuroprotective effects suggesting it could be considered an effective strategy for treatment of neuroinflammatory diseases.

P26. **Gender and age- dependency of the effects of Bud extracts from *Tilia tomentosa* Moench in central nervous system**

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Bud-derivatives, which are obtained by macerating meristematic fresh tissues of trees and herbaceous plants, represent a relatively new category of botanicals. In particular, in the most countries of the EU, bud-derivatives, named also gemmoderivatives or embrioextracts, are classified as plant food supplements. In a recent paper, Allio et al., (2015, J. Ethnopharmacol, 172:288) investigated the impact of *Tilia tomentosa* bud extracts (TTBEs) at GABAergic synapses by performing post-synaptic voltage-clamp recordings in hippocampal neurons. Direct application of TTBEs on post-synaptic terminals activated a chloride current in a way consistent with the activation of $GABA_A$ receptors. The involvement of these receptors was confirmed by the observations that either bicuculline and picrotoxin prevented the TTBEs-induced effects.
Based on these observations, we investigated whether TTBE can modulate the presynaptic release regulating $GABA_A$ receptors located on noradrenergic nerve terminals (Schmid et al., 1996, neuroscience , 73:3). Noradrenergic nerve terminals were isolated from the cortex of adult male mice and preloaded with [^{3}H]noradrenaline ([^{3}H]NA) to monitor the release of the endogenous amine. Exposure of synaptosomes to muscimol (10 μM) in superfusion elicited a significant release of the radioactive tracer. TTBEs (1÷1000), inactive on its own, significantly potentiated the muscimol-evoked releasing activity, consistent with a positive allosteric activity of the bud derivative on the presynaptic release-regulating $GABA_A$ receptors.
It is known that *Tilia tomentosa* Moench bud derivatives exert sedative and anxiolytic effects in the central nervous system. The finding that the TTBEs modulate the $GABA_A$-mediated functions is well consistent with these biological properties, but a direct demonstration of the impact of "*in vivo*" chronic administration of TTBEs on animal behaviour is still lacking. In an attempt to fill the gap, we investigated the effect of "*in vivo*" oral administration of TTBEs on the behavioural performances of both adult (3-6 months old) and aged (20-22 months old) male and female mice in the hole-board maze and in the light-dark box. In particular, we analysed the behavioural skills related to the spontaneous motor activity, the curiosity and the anxiety of young and old animals administered with the Tilia bud derivative. TTBE (1÷2000 dilution) was dissolved in the drinking water and animals were monitored for the water daily intake and for the gain of weight. Behavioural tests were performed before and at the end of the TTBEs supplementation. Our results suggest that the TTBE administration impacts in a gender and an age-dependent manner the curiosity, measured as number of dipping in the hole-board maze and as "time in the light" in the light-dark maze, as well as the spontaneous motor activity. These findings add new insights on the impact of these bud derivatives in CNS.
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P27. **Effect of *Eruca sativa* meal and glucoerucin in diabetic neuropathic pain in mice**

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Diabetes is a leading cause of neuropathy: around 50% of diabetic patients develop peripheral neuropathy, that severely limits patient's daily functions. The few approved therapies for pain management have limited efficacy and side effects. Therefore, the discovery and development of new drug candidates for treating diabetes-related neuropathic pain remains a major challenge in pharmaceutical field. Nutraceutical compounds received a lot of attention due to their potential therapeutic effects, but also thanks to their safety. Glucosinolates (GLSs) are among the most investigated bioactive compounds: many data demonstrate the antinociceptive effects mediated by their capacity of H_2S release. *Eruca sativa* (*E. sativa* Mill sel. Nemat), belonging to the *Brassicaceae* family and rich in GLSs, is known for its antioxidant, antiplatelet, antithrombotic, anticancer and antimicrobial properties.
The purpose of this study was to evaluate the antihyperalgesic properties of *E. sativa* defatted seed meal, and of its glucosinolate glucoerucin (GRE) in streptozotocin (STZ)-induced diabetic mice. *E. sativa* defatted seed meal was characterized: the GSL content accounted for total 138 μmol g^{-1}, with 98.6 % GER on the total GSL. Mice were treated with intraperitoneal administration of STZ at 100 mg kg^{-1}, and after three days with a second dose of 50 mg kg^{-1}. Acute administration of *E. sativa* (1 g kg^{-1} p.o.) and GRE (100 μmol kg^{-1} p.o., equimolar to meal content) did not reduce neuropathic pain in mice, while their co-administration in mixture with myrosinase enzyme (Myr 32 U mL^{-1}), which hydrolyses glucosinolates in isothiocyanates, showed an antihyperalgesic effect in STZ-diabetic mice in a dose-dependent manner. In particular, the antihyperalgic effect obtained with *E. sativa*+Myr was better than that shown by GRE+Myr. The co-administration of *E. sativa*+Myr and GRE+Myr with both the H_2S binding molecule, haemoglobin (300 mg kg^{-1} p.o.), and glutathione (20 mg kg^{-1} p.o. and s.c.), important for the catabolism of H_2S, abolished their pain-relieving effect. Antihyperalgic effect of *E. sativa*+Myr and GRE+Myr was fully prevented by XE991 (1 mg kg^{-1} i.p.), a selective blocker of K_v7 potassium channels. After a repeated administration of *E. sativa*+Myr for 8 days, the antihyperalgesic effect was maintained similar to that induced by a single without the onset of tolerance.
E. sativa+Myr and GRE+Myr reduce neuropathic pain by a mechanism involving a slow H_2S release and K_v7 channels modulation. *E. sativa* meal can be suggested as a new valid candidate for the nutraceutical treatment of diabetes-induced neuropathic pain.

P28. **Nutraceutical effects of an *Eruca sativa* seed extract in an experimental model of metabolic syndrome.**

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Several studies demonstrated the positive effects of the bioactive compounds of Brassicaceae on chronic diseases. Noteworthy, Brassicacae biosynthetize and store high levels of glucosinolates which, in turn, are converted into the corresponding isothiocyanates by myrosinase enzyme [1]. These compounds can induce the expression of lipolysis-related genes in white adipocytes and reduce the total cholesterol/HDL-cholesterol ratio, free fatty acid and adipsin levels [2]. Recently, the isothiocyanate functional group has been described as an effective hydrogen sulfide (H_2S) releasing moiety [3]. Therefore, isothiocyanate derivatives are expected to be endowed with many protective effects typical of this gastransmitter on the cardiovascular system; indeed, these effects have been already demonstrated for some synthetic isothiocyanate compounds [4-6]. *Eruca sativa* Mill. (ES, Brassicaceae) is a potential candidate for the treatment of metabolic syndrome and cardiovascular diseases (CVDs), and glucosinolates, flavonoids and isothiocyanates are the major constituents. Therefore, this work aims to evaluate the activity of ES seeds extract against metabolic syndrome. Male BALB-C mice were fed for 10 weeks with standard diet (Std), with High Fat (HF) diet, with Std diet enriched with an ES seed extract (Std + ES) or with HF diet enriched with the same extract (HF + ES). ES seed extract, titled in glucoerucin and glucoraphanin (400 μmol/g), was added at 0.75% p/p. At the end of the treatment, we measured waist circumference, weight, BMI (g/cm^2), glycemia from caudal vein of each fasted mouse (24h) and we evaluated the lipid panel (cholesterol, HDL, LDL), glycated hemoglobin and insulin levels. Moreover, the abdominal white adipose tissue was collected, weighed and used for the analysis of citrate synthase, an index of the metabolic activity of the adipocytes. Heart, liver, femur and brain were also collected from each mice.
The HF diet significantly increased the BMI, the adipose tissue and the body weight. The presence of ES extract in HF diet reduced significantly the BMI value and the weight increase. Moreover, ES extract in HF diet seemed to induce an interesting reduction of the amount of adipose tissue and an enhancement of citrate synthase activity. Then, we observed that the presence of ES extract contributed to a marked reduction of glycemic values and glycated hemoglobin (HbA1C) levels in animals treated with HF diet. In conclusion, these preliminary results showed a significant reduction of BMI, glycated hemoglobin levels and glycemia suggesting a positive effect of ES seed extract against metabolic syndrome, with an interesting translational and nutraceutical value.
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P29. **Discovery of potential pancreatic lipase inhibitors from *Salvia miltiorrhiza* Bunge**

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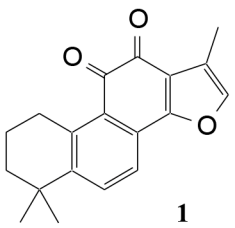
Salvia miltiorrhiza Burge (Danshen) belongs to the *Salvia* genus (Lamiaceae). It has been widely used in Asian countries for treating different diseases and early pharmacological studies demonstrated that *S. miltiorrhiza* possesses multiple bioactivities: cardiovascular and cerebrovascular effects as well as antioxidant, anti-inflammatory, anti-tumor and antidiabetic properties [1, 2]. These activities are due to the presence, in the dried root of rhizome, of lipophilic constituents (i.e. tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, dihydrotanshinone) as well as hydrophilic constituents (i.e. danshensu, salvianolic acid A and B, protocatechuic aldehyde) suggesting a potential synergism among these compounds. In the present study, in order to identify further mechanisms of action involved in lipid metabolism, *S. miltiorrhiza* extract was investigated for its lipase inhibitory activity. Pancreatic lipase, a triacylglycerol acyl hydrolase, is an important enzyme involved in the digestion of dietary fats in the gastrointestinal tract. The lyophilized extract of *S. miltiorrhiza* (E.S. 1% tanshinones) showed a good lipase inhibitory activity as revealed by enzyme-based *in vitro* assay using *p*-nitrophenyl caprilate as the probe substrate (IC_{50} value of 3.54 ± 0.22 mg/ml). Two compounds, cryptotanshinone and tanshinone IIA (the major lipophilic compounds of *S. miltiorrhiza*), have been selected as potential ligands of pancreatic lipase and their interaction mode with the enzymatic active site had been investigated. Molecular docking studies on the crystallographic structures of pancreatic lipase have been carried out to investigate the binding mode of the two major lipophilic *S. miltiorrhiza* components with the enzyme binding site. The potential contribution of tanshinone IIA (**1**) and cryptotanshinone (**2**) was observed.



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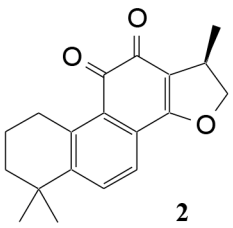

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P30. **Silybin as a new tool to counteract doxorubicin resistance by targeting glucose uptake**

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Nowadays, drug resistance still remains one of the major causes that hampers the effectiveness of cancer therapy. Recent studies support the idea that a dysregulation of cancer metabolism can be related to drug resistance onset. The aim of this study is to identify natural compounds able to target specific alterations in the metabolic pathway of resistant cells in order to prevent or overcome drug resistance. Firstly, the metabolic profile of human colorectal adenocarcinoma cells sensitive (LoVo WT) and resistant to doxorubicin (LoVo DOX) was delineated. Results showed that resistant cancer cells remodel their metabolism toward a glycolytic phenotype. In particular, it was observed that doxorubicin-resistant cancer cells exhibit an increased dependency from glucose for their survival, associated with overexpression of the glycolytic pathway. Moreover, both GLUT1s mRNA and protein expression significantly increase in LoVo DOX cells.
Among other compounds, silybin has been selected as a potential candidate to overcome doxorubicin resistance thanks to its ability to modulate GLUTs. Results showed that silybin is more active in resistant cells than in wild type cells and, moreover, that there is a synergic effect in LoVo DOX cells with the combined treatment with the chemotherapeutic drug. These data support the idea that targeting GLUTs can be a useful strategy to restore doxorubicin sensitivity; even if more investigations about the molecular mechanism are necessary, our work encourages to deeply explore the efficacy of silybin as natural compound useful for an association therapy to overcome doxorubicin resistance.

P31. ***Allium cepa* L. var. Tropea: a source of nutraceuticals with anti-obesity potential**

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Obesity represents the most prevalent nutritional disease and a major public health problem that, according to WHO, has reached epidemic proportions worldwide. Obesity is a factor of risk for several chronic diseases including diabetes, cardiovascular diseases and cancer. There are several strategies to prevent or treat it, one of them is the inhibition of gastrointestinal lipases. In the recent years, the side effects of some synthetic lipase inhibitors indicated the need for alternative drugs with this activity, including natural products. Plants provide a major dietary source for nutraceuticals with anti-obesity potential and, in some cases, their plausible mechanism of action has been highilighted. Following previous studies on plant investigation as a source of inhibitors of pancreatic lipase, we present here the phytochemical and biological profile of a dry skin hydroalcoholic extract of *Allium cepa* L. var. Tropea.

Flavonoid glycosides were the main specialized metabolites present in the extract, with quercetin-4'-*O*-glucoside (43±0.002 μg/mg) and quercetin (84±0.004 μg/mg) as the two main components and cyanidin-*4*-glucoside (1±0.001 μg/mg) as a minor component.

The ability to inhibit pancreatic lipase was evaluated *in vitro* by monitoring the hydrolysis of *p*-nitrophenyl caprilate, which releases the yellow chromogen *p*-nitrophenol. The sample was tested at different concentrations and showed a very good inhibitory activity with IC_{50} value of 0.77 ± 0.03 mg/ml.

It has been shown that obesity can impair intestinal barrier function inducing alteration in permeability and eventually causing inflammation. The effect of the extract on model Planar Lipid Membranes (PLMs) made up of dioleoyl-phosphatidylserine: dioleoyl-phosphatidylethanolamine: palmitoyl-oleoyl-phosphatidylcholine (27:27:18,w:w:w), a surrogate of intestinal membranes, has also been investigated. The extract of *A. cepa* L. var. Tropea was effective in forming channel-like pathways in the lipid bilayer. Electrophysiological data demonstrated that the extract interacts and forms stable pores in PLMs when added on the *cis* side of the medium facing the membranes. The effect of different concentrations (0.01 and 0.02mg/mL) in the ranges of applied voltages from 20 to 120mV and from -40 to -120mV was studied. The conductance values seem to be dependent on applied voltages decreasing as the voltage increases thus suggesting that lower applied voltages promote the ionic flux.

Overall, results from this study suggest that it is worth to further investigate the pharmacological potential of this extract from *A. cepa* L. var. Tropea and its main nutraceutical constituents as safer therapeutic agents in anti-obesity therapy.

P32. **Antihyperglycemic effects of the ethyl acetate extract from the peel of *Punica granatum* L. var. Dente di cavallo: a possible nutraceutical application of a food waste**

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Hyperglycemia represents the main pathogenic factor for the development of diabetes complications and has been found associated to mitochondrial dysfunction and oxidative stress, which in turn increase cell disfunction [1]. Emerging evidence suggested that natural products exerted antidiabetic effects by affecting hyperglycemia and related oxidative stress, mainly through the presence of polyphenolic constituents [2]. In line with this evidence and taking into account the beneficial properties associated with consumption of pomegranate juice [3], in the present study we assayed the potential anti-hyperglycemic activity of an ethyl acetate extract from the peel of *Punica granatum* L. var. Dente di cavallo (PGE). The extract was studied for its ability to affect the glucidic metabolism, particularly to inhibit α-amylase and α-glucosidase, two digestive enzymes responsible for the hydrolysis of dietary carbohydrates: their inhibition can delay carbohydrate digestion and reduce glucose absorption, with beneficial effects in the management of hyperglycemia [4]. Also, the PGE ability to block the release of advanced glycated end-products (AGEs), whose accumulation is known to be responsible for diabetic vascular complications, was studied [5]. The iron reducing and chelating activities, which are the primary mechanisms by which AGE inhibitors stop their metal-catalyzed formation, were evaluated as possible antioxidant mechanisms. At last, the phenolic content of PGE was characterized by chromatographic and spectrophotometric methods [2].
PGE was found able to strongly inhibit α-amylase enzyme in a similar way than the positive control: the IC_{50} values were 52.2 (CL 27.7 - 101.2) μg/ml and 35.6 (CL 22.8 - 55.5) μg/ml for acarbose and PGE, respectively. PGE also inhibited the α-glucosidase enzyme with about a 25 higher potency than the positive controls acarbose and quercetin. Furthermore, the extract exhibited ferrous and ferric ion chelating ability, with a maximum effect of 82.1% and 80.6% at concentration of 250 μg/ml respectively, and reducing properties, reaching the maximum effect of 80.5% at concentration of 10 μg/ml. At last, PGE was found able to inhibit the AGE production (maximum inhibition of 82.2% at the concentration of 1000 μg/ml), although with lower potency respect to the positive control rutin. The phytochemical analysis of PGE displayed the presence of high levels of total polyphenols, tannins and flavonoids, among which ellagic acid, gallic acid and catechin were identified.
Altogether these data highlight the ability of PGE to control the carbohydrate metabolism at different levels, both by inhibiting the metabolic enzymes and by affecting the AGE formation likely by chelating mechanisms. It is also noteworthy that peel from pomegranate, although being a waste of juice production, can be reviewed as a nutraceutical source. In conclusion, present results suggest the possible role of PGE as a remedy for preventing hyperglycemia complications and encourage further *in vivo* studies.

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P33. **Preventing Adolescent Stress-induced Cognitive and Microbiome Changes by Omega 3 – PUFA/Vitamin A Diet Enrichment**

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Early-life stress in humans and rodents has been shown to represent a neurodevelopmental risk with implications to subsequent cognitive abilities during adulthood. Likewise, poor nutritional habits are closely intertwined with mood regulation, stress perception and stress responses. Evidence suggests that a healthy diet, rich in polyphenols, vitamins, omega-3 polyunsaturated fatty acids (ω-3 PUFAs) and vitamins, exerts positive effects on cognitive performance, stress reactivity and neuroinflammation. However, little is known about the influence of these micronutrients on the cognitive and neurochemical consequences of chronic stress during adolescence. Therefore in this study we evaluated the impact of diet enrichement with ω-3 PUFA (0.79g/100g), and vitamin A (45 IU/g) in the acute and long-term behavioural, neurochemical and gut microbiota alterations induced by the social instability stress. Male Wistar rats fed with normal or enriched diet were submitted to a stress protocol that included a combination of repeated 1h daily isolation in a small container (akin to restraint) followed by pairing with a new partner in new cage. As rodents use social bonds to moderate stress, the social instability blunts habituation to repeated isolation. The stressful procedure was repeated for 15 days starting on post-natal day (PND) 30. The behavioural repertoire of the animals was acessed at the end of stress protocol, i.e. during adolescence (PND 45-50) as well as in adulthood (PND 70-75) using a battery of tests comprehensive of several domains affected by stress: mood (sucrose preference), anxiety (open field, elevated plus maze) and cognition (novel object recognition, fear conditioning). One day after the end of behavioural tests, animals were sacrificed and brains and cecal content were collected for neurochemical and gut microbiota composition analysis, respectively. Results from stressed animals were compared with a control group fed with normal chow that did not received stressful manipulations. Our study highlighted the beneficial effect of the enriched diet on cognitive memory impairment induced by social instability stress, as rats fed the enriched diet exhibited performance in both emotional and reference memory test undistinguishable from non-stressed rats. Furthermore, the decline of brain derived neurotrophic factor (BDNF) expression in the hippocampus and microbiota shifts in composition observed in stressed rats were normalized by the enriched diet. The detrimental behavioral and neurochemical effects of adolescent stress as well as the protective effect of the enriched diet were maintained through adulthood, long after the exposure to the stressful environment was terminated. No signs of anhedonia nor anxiety were found in stressed animals. Taken together, our results strongly suggest a beneficial role of nutritional components to ameliorate stress-related behaviors and associated neurochemical and microbiota changes, opening new venues in the field of nutraceutics.

P34. **Effect of polygodial on human melanoma cells: Role of Hsp70 protein**

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Numerous studies have reported that certain compounds present in spices, such as black pepper, ginger, chilli, turmeric, possess anti-cancer properties (Brown et al., 2010). One such natural compound is polygodial, a pungent component of water pepper, dorrigo pepper and mountain pepper. It was originally isolated from the plant water pepper (*Polygonum hydropiper,* Polygonaceae). It is the most widely occurring sesquiterpene dialdehyde, having been found in flowering plants, ferns, liverworts, fungi, and marine molluscs from all around the world (Huq et al., 2014). Polygodial is known to possess several pharmacological benefits such as antibacterial antifungal, anti-allergic and anti-inflammatory properties (Montenegro et al., 2014). In an ongoing to identify new natural anticancer compounds for the treatment and/or prevention of melanoma, we study the effects of polygodial on two melanoma cells A2058 and A375.
The cell viability was measured by 3(4,5-dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) test and lactate dehydrogenase (LDH) release was used to quantify necrosis cell death. Genomic DNA, caspase-3 activity, expression of cleaved caspase-9, B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X (Bax), cleaved caspase-9 and Hsp70 proteins were evaluated in order to study the apoptotic process. Generation of reactive oxygen species (ROS) was measured by using a fluorescent probe (Russo et al., 2018).
The treatment of A2058 and A375 cells with polygodial resulted in a significant reduction in cell viability, confirming that the specific structural features of the dialdehyde functional group are important for cell growth inhibition (Dasari et al., 2014). In addition, we demonstrated an apoptotic response after treatment of cancer cells with polygodial at 6.25-25 μM concentrations. But the central and novel finding in the present pre-clinical study is that this secondary metabolite down-regulates Hsp70 expression with a potential involvement in the apoptotic process. Alternatively, the inhibition of the caspase cascade at higher concentrations, correlated with additional reactive oxygen species increase, probably switched the mode of product-induced cell death from apoptosis to necrosis. Therefore, the combination of polygodial with other anti-melanoma cancer therapies could be considered a promising strategy that warrants further *in vivo* evaluation.

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P35. **Quercetin and Cisplatin combined treatment alter cell cycle and sensitize resistant cancer cell lines**

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One of the major problems in cancer treatment is the onset of resistance phenomena to chemotherapeutic drugs. In order to overcome the resistance, identification of natural compounds with a safety profile for a therapy of association with the drug is a challenging but relevant research field. In this study, it was investigated the cytotoxic effect and the modulation of the cell cycle by a well- known flavonoid: quercetin. A ovarian carcinoma cell line (SKOV3), an osteosarcoma human cell line (U2OS) and the corresponding clones resistant to cisplatin (SKOV3/CDDP and U2OS pt) were used. The obtained results showed the ability of quercetin (10-50 μM) to change the distribution of cell cycle phases in ovarian resistant clones (SKOV3/CDDP). Quercetin (50 μM) was incubated for 48 hours in all cell lines and, by Western blot technique, the levels of cyclin D1 and cyclin B1 were determined. The treatment with quercetin decreased significantly the cyclin D1 expression in SKOV3 and U2OSPt cells, but not in SKOV3/CDDP and U2OS cells. The reduction of cyclin D1 level could be linked to the G1/S phase alteration found in quercetin-treated cells. Despite our observations that this flavonoid influenced the G2/M phase of the cell cycle, and although cyclin B1 is required for the G2/M phase, quercetin did not affect cyclin B1 levels in none of the cell lines. These data indicate that is likely that other mechanisms are involved. Quercetin is compound that exceeds the resistance to CDDP and it could be interesting to evaluate cytotoxic activity in combination with chemotherapy drugs.

P36. **Preclinical evaluation of Tanshinones from *Salvia miltiorrhiza Bunge* on human glioblastoma models *in vitro***

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Medicinal plants and herbal extracts from traditional Chinese medicine are used increasingly commonly worldwide for their benefits to health and quality of life as dietary supplements or as nutraceuticals in functional foods. Among them, Salvia miltiorrhiza Bunge - a natural strong remedy for the treatment of a variety of conditions - is traditionally used for centuries in Asian countries as antioxidant, anticancer, and anti-inflammatory agent. Its pharmacological beneficial effects are mainly ascribed to the presence of lipophilic diterpenoid like-compounds such as dihydrotanshinone (DTA), tanshinone IA (TIA), tanshinone IIA (TIIA)and cryptotanshinone (CRY) that per se are able to penetrate the blood-brain barrier and to inhibit Aβ peptide aggregation, disaggregate amyloid fibrils, and protect neurons from an inflammatory condition. Moreover, several evidences support the hypothesis that tanshinones extracted from the roots (also known as Danshen) of Salvia miltiorrhiza exert significant cytotoxic and/or antiproliferative activity in vitro. Thus, the aim of the present study was to investigate the bioactivity profile of Danshen and its active constituents tanshinone IIA (TIIA) and cryptotanshinone (CRY) in human healthy and cancer models, including glioblastoma and colorectal cancer cells. In this way the anticancer activity of the selected compounds was determined in different cells lines, proving that Danshen and its active constituents were endowed with remarkable and selective inhibitory activities on glioblastoma cell lines LN-229 and U-87 MG (IC_{50} in the low micromolar range). Although many advances in antineoplastic therapy ha×ve taken place, gliomas have so far shown a poor response to antiproliferative drugs, because of their resistance to most chemotherapeutic agents. However, further experiments are ongoing to give an insight on the molecular mechanisms of action involved in the activation of cell death pathways by tanshinones. Therefore, besides being useful as food complements, preliminary outcome from preclinical evaluations suggest that Salvia miltiorrhiza roots could be considered as a new potential source of bioactive compounds valuable for the development of future anticancer treatments.

P37. **Chemical Compounds Extracted From Agricultural Waste: Characterization And Evaluation Of Antioxidant Activity**

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The growing world food demand forces the adoption of intensive agricultural practices to enhance the productivity of agrofood crops. These practices request the massive and continuous use of mineral fertilizers and of energetic inputs (e.g. irrigation, pesticides, etc.). Despite these approaches may provide an increase in crop yields they produce negative effect in the equilibrium of agroecosystem in the medium to long term, such as the loss of soil organic matter and the depletion of soil fertility Therefore, it is necessary to identify eco-sustainable techniques that can combine the preservation of soil quality with the suitable improvement of crop yield and quality. The use of "green compost" represent an effective and valuable alternative to the traditional practices, able to match the current indication and provisions provided by Governments and Institutions to implement the virtuous OM recycle and the circular economy in agro-ecosystems [1-2]. Besides the positive effect on soil fertility the "green" compost may represent valuable source of natural bioactive compounds to improve crop yield and quality, acting as bioeffectors [3-4]. The aim of this work was to develop an innovative extraction protocol of bioactive compounds from green compost, and to determine molecular features and antioxidant properties. Mature compost samples from artichoke and citrus residues were sieved at 2 mm and used to extract antioxidant molecules using organic solvent (EtOH:H_2O, 70/30, v/v). The different fractions were obtained during the first extraction cycle performed at 25°C for 24-48-72 hours. Subsequently each residue underwent a second extraction cycle, at 70°C for 24 hours. All ethanol extracts were separated from the residues by centrifugation, concentrated by rotavapor, recovered in water and finally freeze-dried. the molecular characteristic of isolated The fractions were hence characterized by ^{1}H-NMR spectroscopy in liquid phase, analyzing the potential antioxidant activity by the ABTS assay [4] and the effect on the metabolomic pathway. The results showed higher yields after the second extraction cycle conducted on the residues attained from the previous extraction at 25°C after 72 hours for artichoke and 48 hours for citrus composts. The ABTS test showed a greater antioxidant activity in all the extracts deriving from the second extraction cycle. The results of ^{1}H-NMR spectroscopy displayed significant differences in the molecular composition of the different fractions. In particular, the isolates from the first extraction cycle had a higher content of alkyl and hydroxyl-alkyl compounds while the extracts resulting from the second cycle exhibited a prevalence of aromatic compounds and amide derivatives. The data support the hypothesis that the larger antioxidant activity in green compost is related to the presence of aromatic and phenolic compounds [5]. In conclusion, our results indicate that green compost can be used not only as soil amendment and biostimulants, but also as a source of bioactive molecules characterized by antioxidant properties. These compounds could be used for formulations of new products to be applied in the agronomic sector.

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P38. **Curcuma and Garlic as backbone for new nature-inspired hybrids as multi-pharmacological agents: focus on BDNF modulation**

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Brain-derived neurotrophic factor (BDNF) is the most abundant and widely distributed neurotrophin in the central nervous system (CNS). Initially isolated as a secretory protein capable of promoting the survival of peripheral neurons, BDNF is now recognized as a plethoric factor able to regulate a wide repertoire of neural functions.
Nature has always been a rich source of products showing therapeutic properties. In our previous papers, we combined molecular fragments deriving from garlic and curcumin into new chemical entities, producing nature-inspired hybrids, previously tested for their ability to counteract Aβ-aggregation and oxidative stress (Simoni et al., 2015; Simoni et al., 2017). Since data from literature reported that natural compounds increase BDNF levels targeting TrKB/CREB pathway, the aim of our project is to evaluate if our nature-inspired molecules, beside their well-established antioxidant activity, are capable to exert neuroprotective effects acting on BDNF in a view of multi-pharmacological compounds.
We investigated the capability of our compounds to modulate BDNF protein levels by western blot in a cellular model of human neuroblastoma (SH-SY5Y). To better disclosure the molecular mechanism through which the compounds might affect BDNF levels, the expression of TrKB, CREB and BDNF mRNA has been evaluated by RT-PCR. Moreover, we tested whether our compounds may rescue the reduction of BDNF levels induced by cortisol.
Our results suggest that the new nature-inspired molecules are capable to significantly increase both BDNF protein amount and mRNA expression, either in basal conditions and following cortisol-induced stress conditions, thus suggesting that our compounds may exert neuroprotective effect.

P39. **Biological activity of *Capsicum annum* cv Senise on IHH cell line**

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Overproduction of oxidants in the human body is responsible of oxidative stress associated to several diseases. It has been reported that intake of vegetables and fruits is inversely associated with the risk of many chronic diseases. In fact, they are source of compounds that possess free radical scavenging abilities as well as anti-cancer, anti-inflammatory and protective activity for obesity, diabetes, neurodegenerative and cardiovascular disease [1,2].
Capsicum annuum L. cv Senise is a sweet pepper growing in Basilicata region. It is an important source of polyphenols, carotenoids, capsinoids which can play a key role in human health [3]. In this study biological activity of pepper extract was evaluated on immortalized human hepatocytes (IHH) cell line. Pepper extracts showed no cytotoxic activity on cells and reported protective effect to oxidative stress keeping the levels of ROS equal to the control in stressed condition. Moreover *C. annuum* promoted the expression of endogenous antioxidant like catalase, superoxide dismutase and glutathione peroxidase evaluated by RT-PCR. Additionally, pepper extracts reduced lipid accumulation in IHH cells exposed to oleic acid detected by Oil-red-O staining probably due to increase of AMPK expression. These first results suggest new potential application of *Capsicum annuum* cv Senise estracts in nutraceutical and pharmaceuticl field.

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P40. **Evaluation of onconutraceutical potential of vegetable smoothies in cardiomyocytes and breast cancer cell lines**

Rapa SF[a], Salviati E[a,b], Cianciarulo D[a], Pepe G[a], Sommella E[a], Coppola D[c], Coppola L[c], Manfra M[d], Autore G[a], Marzocco S[a], Campiglia P[a,e]

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The interest towards onconutraceutical products that could be useful not only for the prevention of cancer but also a valid support to the pharmacological therapies is a topic of growing interest. Breast cancer is the third most frequent cancer type in the world and the most common in female sex. Its prognosis may be so poor that it is second only to lung cancer as a cause of death by neoplasia. Furthermore, the incidence of breast cancer has been steadily increasing over the last few decades [1]. The main treatment for breast cancer is by chemotherapeutic drugs, targeted to the destruction of malignant cells. One of the most used drugs is the doxorubicin, but like all other anticancer drugs, it possesses important side effects, including cardiac toxicity. In fact, at high doses doxorubicin causes congestive heart failure [2]. Our project aims to identify possible nutraceutical matrices able to reduce the cardiotoxicity due to doxorubicin, without modifying its antineoplastic activity. In this study, we firstly carried out a chemical characterization of the main nutraceutical compounds contained in the orange (*Citrus sinensis*) and red grape (*Vitis vinifera* L. cv. Aglianico) smoothies by ultra-high performance liquid chromatography-tandem mass spectrometry experiments. In order to evaluate in a cellular system their pharmacological potential, we performed the antiproliferative activity of orange and grape extracts and three different mixes, composed by different ratios of the two food matrices. The experiments were conducted in parallel on two cellular lines: embryonic rat heart-derived cells (H9c2) and human breast adenocarcinoma cell line (MCF-7), in order to evaluate the antiproliferative activity of the tested extracts, also in presence of doxorubicin. Our results indicate that the orange and grape, as well as the relative mixes, don't exhibit a significant antiproliferative activity on H9C2 cardiomyocytes. Moreover, in presence of doxorubicin, particularly the orange extracts, but also the grapes and the 3 mixes in a comparable way, reduce the antiproliferative activity induced by doxorubicin on H9C2, thus indicating a reduced toxic activity of the chemioterapic agents on cardiomyocites.
On MCF-7 breast cancer cells, orange and grape, as well as their respective mixes, don't exhibit a significant antiproliferative activity. Interestingly, the doxorubicin antiproliferative activity on MCF-7 cells was unaltered by the contemporary presence of the tested extracts. These results indicate that the tested extracts could be useful to reduce the cardiotoxic effects of doxorubicin in cardiomyocytes, without affecting its pharmacological on breast cancer cells, thus highlighting their potential application in the onconutraceutic field.
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P41. **Design and characterization of an integrate dialytic system coupled with amperometric microsensors for the dynamic quantification of ascorbic acid and total polyphenols in the Pompia fruits.**

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Several studies have been carried out to highlight the importance of the biological and non-biological role of antioxidant compounds present in foods, in counteracting oxidative stress and the different diseases associated with it, such as atherosclerosis, diabetes mellitus, chronic inflammation and neurodegenerative disorders: these studies aimed to quantify the antioxidant capacity of various compounds and substances in a standardized way (Carlsen et al 2010, Del Rio et al 2004). The aim of the present work was to study the total antioxidant capacity in the whole fruit of Pompia *Citrus limon* var. pompia Camarda), a variety of endemic lemon from Sardinia, by quantifying ascorbic acid and polyphenols content in the endocarp of the mature fruit by means of a microdialytic system coupled to an amperometric microsensor. The use of small microdialysis probes, determining a minimum invasiveness on the fruit, and the high temporal resolution of the electrochemical analysis allow an in-situ analysis of the sample (user-free sample) and an almost real-time monitoring of it. The device consisted in a microdialysis probe inserted in the endocarp of the fruit, that was connected through polyethylene tubes to a miniaturized electrochemical cell of a volume of about 100 μl. By means of a microinfusion pump, connected to the probe, an acetate buffer solution was perfused. The evaluation of the total antioxidant capacity of the fruit, due to the content of ascorbic acid and of total polyphenols, was electrochemically obtained using graphite microsensors by applying different potentials. In particular, a fixed potential of +150 mV was applied for the detection of ascorbic acid alone and +500 mV for monitoring the total antioxidant capacity (polyphenols plus ascorbic acid). Data were calculated in equivalents of ascorbic acid. The amount of ascorbic acid was calculated being around 1.36 ± 0.16 mM, while the total antioxidant capacity was 1.65 ± 0.18 mM. From those values the concentration of polyphenols was derived by difference (0.29 ± 0.05 mM). The integrated microdialysis/microsensor system has proved to be an effective, reliable and repeatable tool, able to provide an extraction of the ascorbic acid and the polyphenols from the matrix in a short time and, in the experimental configuration used in this work, with rapid monitoring and, in the experimental configuration used in this work, with rapid monitoring and in "real-time".

P42. **Development and characterization of a new microdialitic-electrochemical device for the extraction and quantification of total polyphenols present in Extra Virgin Olive Oil (EVOO)**

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Polyphenols are the largest group of phytochemicals and they have been found in plant-based foods like fruits, vegetables, wine, tea, chocolate and cocoa-based products but also in extra-virgin olive oil (EVOO). In plants those compounds are linked to the survival of the plants, in fact they are synthetized as defense against parasites and microbes (Del Rio et al., 2003).
Recently, researchers strongly support the role of polyphenols in the prevention of degenerative diseases, such as tumors, cardiovascular diseases, but also neurodegenerative diseases as Alzheimer's and Parkinson's, shifting their attention from treatment to prevention and conceiving the intake of nutrients in the diet as a support for drug therapy.
Several epidemiological studies support the belief that diet regimens such as the Asian diet and the Mediterranean diet, which provides, in particular, extra virgin olive oil as the main lipid source, are associated with a reduced incidence of age-related diseases (Visioli et al 2018)
In recent years, several studies have confirmed the importance of plant polyphenols as a source of beneficial properties, proposing these molecules as nutraceuticals and important functional foods, so their determination in different food matrices has become of crucial importance
The extraction of polyphenols from olive oil is usually carried out through a liquid-liquid extraction (LLE) and a solid-phase extraction (SPE). Liquid-liquid extraction (LLE) has some disadvantages, determined by the need to use a rather large amount of solvents and much longer extraction times, if compared to the microdialysis technique. Typically, in the traditional liquid-liquid method (LLE) 2 to 60 g of olive oil and from 7 to 80 mL solvents are required.
This study aimed to develop a microdialysis-based extraction as an alternative to the liquid-liquid extraction method (LLE). This dialytic method allowed to extract the phenolic components of EVOO in a faster and reproducible way, reducing the amount of products and solvents (Bazzu G et al 2017). In order to improve the applicability of this type of extraction, the composition ox extraction fluid was modified and the ratio that provided the maximum extraction capacity of the polyphenols from the matrix was 80% of methanol and 20% of water, the same ratio that is used in the LLE.
Moreover, this dialytic method was coupled with an electrochemical monitoring system able to evaluate, in real time, the concentration of polyphenols extracted by extra virgin olive oil, by means of the constant potential amperometry technique, on a platinum-based microsensor, polarized to +500 mV. The potential has been chosen because, at higher potentials, polymerization phenomena on the electrode surface occur.
This dialytic-amperometric method allowed to monitor that fraction of polyphenols present in olive oil having a redox potential lower than the potential applied to the electrode.

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P43. **Phytochemical and biological characterization of methanolic extracts from Tunisian *Rumex algeriensis* and *Rumex tunetanus***

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Local environmental resources derived from plants play an important role not only for the provision of foods but also for their content of bioactive compounds useful for human wellbeing. The *Rumex* species, belonging to the Polygonaceae family, comprise about 200 species widely distributed around the World, including North Africa regions. Some species have been used traditionally, besides that as vegetable foods, also for their medicinal properties. The extracts of these plants, and compounds isolated from them, have been demonstrated to possess various pharmacological activities, including anti-inflammatory, antioxidant, antitumour, antibacterial, antiviral and antifungal properties, both in *in vitro* and *in vivo* studies. In the context of a research project aimed to investigate plants from North Africa as potential sources of bioactive product useful for healthy purposes, we have studied the phytochemical profile and the biochemical properties of methanolic extracts from different parts (flowers, leaves and stems) of Tunisian *Rumex algeriensis* and *Rumex tunetanus*. In fact Tunisia flora is known for its diversity of medicinal plants, but phytochemical and biological of these two *Rumex* species, endemic in Tunisia, have not been deeply investigated up today.
The phytochemical analyses were performed using standard colorimetric procedures (total flavonoids, total flavonols, total tannins, hydrolysable tannins, condensed tannins) and HPLC-DAD and HPLC-DAD-ESI-MS. Then, several in vitro cell-free assays have been used to estimate the antioxidant/free radical scavenging capability of the extracts (total polyphenols, DPPH, TEAC, ORAC, FRAP, β-carotene bleaching, NO scavenging-activity, SOD-like activity, HOCl-induced albumin degradation). Moreover, cell culture tests have been performed to evaluate the biocompatibility (on NIH/3T3 fibroblast cells) and the anti-inflammatory potential (on THP-1 cells) of the extracts under investigation. All extracts appeared particularly rich in phenolic compounds, being flowers and leaves richer than stems for both *Rumex* species. The main compounds were respectively: catechin, quercetin glycoside methyl derivatives, epicatechingallate for *Rumex algeriensis* flowers; dicaffeoylquinic and caffeoylquinic acids, epicatechingallate, quercetin glucoside methyl derivatives, quercitrin for *Rumex algeriensis* stems; quercetin glucoside methyl derivatives, catechin, quercetin hexoside deoxyhexoside, epicatechingallate for *Rumex tunetanus* flowers; quercetin glucoside methyl derivatives, quercetin hexoside deoxyhexoside, epicatechingallate, catechin for *Rumex tunetanus* stems; quercetin glucoside methyl derivatives, quercetin hexoside deoxyhexoside, kaempferol 3-O-glucoside, kaempferol hexoside deoxyhexoside, chlorogenic acid for *Rumex tunetanus* leaves. All extracts appeared endowed with excellent antioxidant/free radical scavenging properties. In particular the extracts from both *Rumex algeriensis* and *tunetanus* flowers and the extract from *Rumex algeriensis* stems are characterized by a remarkable SOD-like and NO-scavenging activity, as well as by the capability to protect albumin against degradation. Taking into account also the results concerning the cytotoxicity on NIH/3T3 cells, the anti-inflammatory properties of these extracts have been confirmed on THP-1 cells. In conclusion, *Rumex algeriensis* and *tunetanus* flowers and stems showed to be a potential source of bioactive products, to be used, due to their significant antioxidant properties, as nutraceuticals as well as in the food industry.

P44. **Evaluation of the phytochemical composition and biological activities of the ethanolic extracts from "Bianco di Sperlonga" PGI celery ecotype: a multimethodological study**

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In line with emerging evidence on the health benefits associated with celery consumption [1] and the role of local biodiversity as possible nutraceutical source, a multimethodological study was performed to characterize the phytochemical composition and biological properties of Italian PGI (*Protected Geographical Indication*) "Bianco di Sperlonga" (*Apium graveolens* L. variety *dulce*) celery, an ecotype cultivated in the Lazio Region of Central Italy. Celery samples, carefully divided in petioles, blade leaves and heart, were subjected to acidified ethanolic maceration, then analysed. Spectrophotometric and chromatographic analyses, based on both HPTLC and HPLC techniques, were performed to characterize the phytochemical composition of the extracts [2]; moreover, the antimutagenicity towards the oxidative DNA-damage induced by tert-butylhydroperoxide (tBOOH), the antioxidant activity (i.e. radical scavenger power and inhibition of lipoperoxidation) and the inhibition of digestive enzymes (i.e. α-amylase and lipase) were assessed, according to previous published methods [3-4].
Phytochemical characterization highlighted that blade leaves, followed by heart samples, contained the highest amount of polyphenols, tannins, flavonoids, chlorophylls and total carotenoids, whereas the concentration of these compounds was poorly significant in petioles. Chromatographic analyses identified rutin, catechin, benzoic, 3-hydroxybenzoic, syringic and p-coumaric acids as peculiar constituents of heart and blade leaves.
In spite of a weak antimutagenicity of heart celery extracts, the samples from both blade leaves and petioles significantly inhibited tBOOH-mutagenicity both in the absence and presence of the exogenous metabolic activator (S9). The involvement of desmutagenic mechanisms, through the prevention of tBOOH-mutagenicity by directly interfering at extra- or intracellular level, is expected. The strong antimutagenicity of petioles in the presence of S9 allowed us to hypothesize the presence in the extracts of compounds, requiring bioactivation to antimutagenic agents. The extracts were also able to significantly scavenge DPPH and ABTS radicals, while produced a slight inhibition of lipoperoxidation and did not affect the function of α-amylase and lipase. These biological activities appear likely due to phenolic compounds, although the contribution of other unidentified phytoconstituents (among which chlorophylls, pheophytins, and phthalides) cannot be excluded.
Present results demonstrate that combining different methodologies represents a suitable approach to analyze a complex food matrix overcoming limitations of any single technique and suggest the potential role of celery as a nutraceutical food.

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P45. **Antimicrobial and Phytotoxic activity of essential oil of *Origanum vulgare* growing in Cilento**

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Recent studies demonstrated that aromatic plants are a good source of nutraceuticals with a positive impact in human and vegetal health. Moreover, there is a growing interest in use of essential oils as possible alternatives for traditional antibiotics, pesticides and herbicides. *Origanum vulgare* L. is an aromatic perennial herb, belonging to Lamiaceae family, with many health benefits.This study was carried out to characterize the chemical composition of *O. vulgare* essential oil (EO) and to evaluate *in vitro* its antimicrobial and phytotoxic activities. The antimicrobial activity was performed against some post-harvest phytopathogenic fungi (*Botrytis cinerea*, *Penicilliumexpansum*, *Aspergillus niger* and *Moniliniafructicola*) and some phytopathogenic bacteria (*Bacillus megateriumand Clavibactermichiganensis* (G+ve) and *Xanthomonas campestris, Pseudomonas fluorescens*, and *P. syringae*pv.*phaseolicola* (G-ve). In *O.vulgare* EO, 35 compounds were identified, accounting 97.8% of the total oil. The main constituent is carvacrol (77.8%), followed by *p*-cymene (5.3%) and γ-terpinene (4.9%). The phytotoxicity of the studied EO was evaluated against the germination and initial radicle elongation of *Raphanussativus* L. (radish), *Lactuca sativa* L. (lettuce), *Lepidiumsativum* L. (garden cress), *Solanum lycopersicum* L. (tomato). Results demonstrated that the studied EO has a promising *in vitro* antimicrobial activity against all tested phytopathogens at all tested concentrations. It showed an interesting effect - in terms of Minimal Inhibitory Concentration and Minimal Bactericidal Concentration- against *Listeria innocua* and *Listeria monocytogenes* indeed. Moreover, both germination and radical elongation of selected plants were sensitive to the studied EO.

P46. **Assessment of consumer perception of olive oil's characteristics and health claims related to nutraceutical attibutes**

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The objective of this study is to investigate which are attributes (intrinsic and extrinsic) that mainly influence consumers' purchase decision of olive oil, including the four official health claims. To that end, three of the main attributes in the purchase process of olive oil, such as "organic olive oil" "market leader brand", "Italian olive oil", together with the attribute "presence of a specific health claim on the bottle" are used through Conjoint Analysis. Interviewed are invited to express their preference between eight profiles with different combination of attributes. Results show that "Italian olive oil" attribute is the most important for consumers (72%) in the olive oil purchase process, followed by "organic olive oil" (about 19%), "presence of health claim" on the bottle (8,3%) and lastly "market leader brand" attribute (0,82%). Going deeply in the relative consumers' importance health claim, results show that the health claim "*Replacing saturated fats with unsaturated fats in the diet has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease*" is the favourite compared to the other three. The second step of the research is dedicated to a specific section of questions on the consumers' level of comprehension, reliability, interest, importance, newness, intention to purchase and qualitative perception of olive oil with each health claim. Results confirm consumers' preference for the health claim "*Replacing saturated fats with unsaturated fats in the diet has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease*", that is considered the most understandable, credible and important compared to the others. Taking into account the level of newness and qualitative perception of the olive oil, the health claim "*Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The beneficial effect is obtained with a daily intake of 20 g of olive oil*" wins the comparison with the others, while the health claim "*Replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels. Oleic acid is an unsaturated fat*" is considered the most interesting and with the higher probability to be bought. As most studies on consumers' understanding of health claims are based on subjective understanding, this remains an area for more investigation. Next step of research will analyse the main drivers and relations that influence consumers' comprehension and acceptability of health claim in olive oil using a representative Italian sample.

P47. **Microdispersions of ellagic acid and pomegranate extracts as new potential nutraceutical ingredients**

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The health properties attributed to several fruits (i.e. pomegranates, raspberries, strawberries, blackberry, chestnuts, walnuts, pecan), herbs (tea) and seeds (berries seeds) are attributed to an important group of natural polyphenols classified as hydrolysable tannins (HT) named Ellagitannins (ETs), that have shown in vitro multi-target biological properties relevant to the treatment of several human diseases. In vivo, ETs are rather not absorbed, and they are hydrolysed providing mainly Ellagic acid (EA). EA is endowed with the same biological properties of ETs and it could be considered as the responsible of their health benefits. Unfortunately, EA cannot be exploited for in vivo applications because of its poor water solubility (9.7 μg/mL) and accordingly low bioavailability.
At first, aiming to increase EA solubility, an EA solid microdispersion (EA-md) was realized by employing only water and low methoxylated pectin, as a food compatible excipient, by applying spray drying technology. EA-md showed a 22% (w/w) Drug Loading (DL), a 30 times improved water solubility maintaining a remarkable radical scavenging activity [1]. It has been analytically characterised and used for in vivo pharmacological treatments in order to evaluate it as potential nutraceutical ingredient.
Adult (3-6 months old) and old (20-22 old months) male mice were chronically administered EA-md dissolved in the drinking water (about 150 mg/Kg) for 14 days. During this period, animals were monitored for the spontaneous motor activity and for curiosity before, during and at the end of the EA-md treatment. Adult and old mice were then sacrificed for "ex vivo, in vitro" analysis to test the efficiency of noradrenaline release from cortical nerve endings. It is known that noradrenaline exocytosis from cortical nerve endings is significantly impaired during ageing. We found that the chronic administration of EA-md did not alter the noradrenaline exocytosis from cortical nerve endings of adult mice, but significantly recovered the reduced noradrenaline overflow in aged mice. Further investigations are needed to explore the cellular cascade of events accounting for the beneficial effect.
In a second step, pomegranate, as a natural source of EA, has been considered to similarly prepare and investigate an analogous formulation. Since pomegranate fruit is recognized as one of the most important sources of ETs, mainly localized in the by-products obtained after industrial juice squeezing, a method to convert the squeezing marcs into a potential nutraceutical ingredient has been explored. In particular, Pulsed Ultrasound-Assisted Extraction (PUAE), using just water as solvent, resulted to be suitable for extracting the water-soluble bioactive molecules (PEx), whose content in hydrolysable tannins, standardized in EA, has been determined. Furthermore, the already mentioned spray drying microdispersion has been employed to formulate and to stabilize it over time. This last formulation (PEx-md) will be subjected to the already mentioned pharmacological experiments in order to study its nutraceutical properties too.

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