CONVEGNO MONOTEMATICO
SOCIETÀ ITALIANA DI FARMACOLOGIA

Gruppo di Lavoro Farmacognosia

FARMACOGNOSIA
nuove opportunità terapeutiche dal mondo vegetale

Hotel Royal Continental, Via Partenope, 38
NAPOLI 20-21 giugno 2014

Programma e Abstract

Comitato Scientifico
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Con il patrocinio di

Università degli Studi di Napoli Federico II

Ente Parco Nazionale del Vesuvio

Programma

Venerdi 20 Giugno

12.30-14.00 ***Registrazione dei Partecipanti***

14.00-14.20 Benvenuto (Presidente SIF, Comitato Organizzatore, Direttore Dipartimento di Farmacia Napoli Federico II)

14.20-15.00 Lettura magistrale: **Bioactive molecules from natural sources in the prevention of chronic degenerative diseases: my ten years research story with Sulforaphane**
Hrelia P. (Bologna)

15.00-16.00 Sessione Orale 1

Moderatori: Cantelli Forti G. (Bologna) – Cirino G. (Napoli)

15.00-15.15 **On the antitumor activity of *Citrus bergamia* juice**
Cirmi S, Ferlazzo N, Lombardo GE, Calapai G, Navarra M (Messina)

15.15-15.30 **Antileukemic activity of sulforaphane in hypoxic conditions and in primary blasts from patients affected by myelo- and lympho-proliferative disorders**
Turrini E, Sestili P, Carulli G, Cantelli-Forti G, Fimognari C, Hrelia P (Bologna, Urbino, Pisa)

15.30-15.45 **Cynaropicrin inhibits stat3 phosphorylation through gsh depletion and induces cell death in du145 cell line**
Chiavegato G, Butturini E, Carcereri de Prati A, Rigo A, Cavalieri E, Darra E, Mariotto S (Verona)

15.45-16.00 **Effect of the hydrogen sulfide (H_2S) donor diallyl trisulfide (DATS) on melanoma**
De Cicco P, Panza E, Armogida C, Bucci M, Cirino G, Ianaro A. (Napoli)

16.00-16.30 ***Coffee break***

16.30 -18.00 Sessione Orale 2

Moderatori: De Pasquale R. (Messina) – Calignano A. (Napoli)

16.30-16.45 **Neuroprotective effects of Co-ultraPEALut on inflammatory process induced by traumatic brain injury**
Impellizzeri D, Cordaro M, Paterniti I, Bruschetta G, Siracusa R, Cuzzocrea S, Esposito E (Messina)

16.45-17.00 **Phytocannabinoid modulation of neuropathic pain-associated neuroinflammation**
Guida F, Luongo L, Boccella S, de Novellis V, Maione S (Napoli)

17.00-17.15 ***In vitro*** **anti-inflammatory activity of *Fragaria* spp. in human gastric epithelial cells**

Sangiovanni E, Fumagalli M, Vrhovsek U, Colombo E, Brunelli C, Gasperotti M, Mattivi F, Bosisio E, De Fabiani E, Dell'Agli M (Milano, San Michele all'Adige)

17.15-17.30 ***Vitis vinifera* L.: anti-inflammatory activity on the gastrointestinal tract**
Colombo E, Sangiovanni E, Di Lorenzo C, Fumagalli M, Restani P, Dell'Agli M (Milano)

17.30-17.45 **Antinociceptive and anti-inflammatory effects of bergamot essential oil in *in vivo* models**
Lombardo GE, Mannucci C, Cirmi S, Ferlazzo N, Calapai G, Navarra M (Messina)

17.45-18.00 ***Astragalus membranaceus* extract is effective in a rat model of rheumatoid arthritis**
Di Cesare Mannelli L, Micheli L, Cinci L, Corti F, Bartolucci G, Vannacci A, Mugelli A, Ghelardini C (Firenze)

18.00-19.00 Riunione del Gruppo di Lavoro di Farmacognosia

20.30 Serata Pizza

Sabato 21 Giugno

8.30-10.30 Sessione Orale 3

Moderatori: Perfumi M. (Camerino) – Mascolo N. (Napoli)

8.30-8.45 **The Italian Phytovigilance System**
Menniti-Ippolito F, Raschetti R, Santuccio C, Bruno B, Scarpa B, Dalfrà S, Floridi F, Calapai G, Zuccotti G, Colombo ML, Firenzuoli F, Moro P, Valeri A, Mazzanti G (Roma, Messina, Milano, Torino, Firenze, Modena)

8.45-9.00 **Vascular L-type Ca^{2+} channel mediated activity of murrayafoline A from *Glycosmis stenocarpa*: electrophysiological and molecular docking studies**
Durante M, Sgaragli G, Huong TT, Cuong NM, Fusi F (Siena, Hanoi)

9.00-9.15 **Cardioprotective effects of naringenin in elderly rats submitted to ischemia-reperfusion.**
Testai L, Martelli A, Breschi MC, Calderone V (Pisa)

9.15-9.30 **Tanshinone IIA, a major component of *Salvia miltiorrhiza* Bunge, inhibits *in* vitro rat platelet activation via Erk-2 signaling pathway**
Maione F, De Feo V, Pilla A, Russo R, Caiazzo E, Pieretti S, Cicala C, Mascolo N (Napoli, Salerno, Roma)

9.30-9.45 **Cyanidin-3-O-glucoside prevents endothelial cells dysfunction via Nrf2 pathway**
Speciale A, Fratantonio D, Ferrari D, Anwar S, Cimino F, Saija A (Messina)

9.45-10.00 **Pharmacological activity of compounds isolated from the leaves of the tree *Artocarpus tonkinensis* used in Vietnamese traditional medicine**
Pozzesi N, Riccardi C, Delfino DV (Perugia)

10.00-11.00 Coffee break e sessione poster

11.00-12.45 Sessione Orale 4

Moderatori: Ghelardini C. (Firenze) **–** Russo A. (Catania)

11.00-11.15 **The non-psychotropic plant cannabinoids, cannabidivarin (cbdv) and cannabidiol (cbd), activate and desensitize transient receptor potential vanilloid 1 (trpv1) channels in vitro: potential for the treatment of neuronal hyperexcitability**
Iannotti FA, Hill CL, Leo A, Soubrane C, Mazzarella E, Russo E, Whalley BJ, Di Marzo V, Stephens GJ (Pozzuoli, Reading, Catanzaro)

11.15-11.30 **A new approach for the identification of natural drugs targeting Leukemic Stem Cell (LSC)**
Ferruzzi L, Mulaw MA, Ihme S, Fimognari C, Buske C (Bologna, Ulm)

11.30-11.45 **Role of salidroside, active compound of *Rhodiola rosea* L., in the prevention and treatment of morphine tolerance and dependence**
Mattioli L (Camerino)

11.45-12.00 **Bee propolis in the treatment of *Helicobacter pylori*: in the right way to clinical application**
Biagi M, Tilli V, Montini L (Siena)

12.00-12.15 **Evaluation of the interaction of plant polyphenolic derivatives with STAT1 as a likely mechanism of their inhibitory effect on cytokine signalling pathways**
Masullo M, Menegazzi M, Di Micco S, Bifulco G, Pizza C, Masiello P, Piacente S (Salerno, Verona, Pisa)

12.15-12.30 **Wild mediterranean dietary plants as anti-obesity agents**
Conforti F, Marrelli M, Menichini F (Cosenza)

12.30-12.45 **New hypotheses on *Serenoa repens* (Bartram) Small extract mechanism of action through *in silico* methods**
Governa P, De Vico L, Biagi M, Giachetti D, Manetti F (Siena, Copenaghen)

12.45 ***Premiazione e Chiusura del Convegno***

Sessione poster sabato 21 giugno ore 10.00-11.00

Sessione 1 – **Moderatore** Delfino G. (Perugia)

P1. **Neuroprotection by association of palmitoylethanolamide with luteolin in experimental Alzheimer's disease models: the control of neuroinflammation**
Siracusa R, Paterniti I, Cordaro M, Campolo M, Cornelius C, Navarra M, Cuzzocrea S, Esposito E (Messina, Catania)

P2. ***Astragalus membranaceus*** **extracts: a new natural resource for the management of chemoterapy-induced neurotoxicity**
Corti F, Zanardelli M, Di Cesare Mannelli L, Maresca M, Micheli L, Bilia AR, Mugelli A, Ghelardini C (Firenze)

P3. **Attenuation of morphine tolerance by an extract of *Astragalus membranaceus* root**
Micheli L, Corti F, Di Cesare Mannelli L, Zanardelli M, Tenci B, Bartolucci G, Mugelli A, Ghelardini C (Firenze)

P4. **Effect of cannabidivarin in an animal model of beta-amyloid induced toxicity**
Guarino A, Negro L, Maione F, Cristiano C, Aveta T, Vaia M, De Caro C, Di Marzo V, Calignano A, Iuvone T (Napoli, Pozzuoli)

P5. **Olea Europea-derived phenolic products attenuate antinociceptive morphine tolerance: an innovative strategic approach to treat cancer pain**
Muscoli C, Lauro F, Dagostino C, Ilari S, Giancotti La, Gliozzi M, Costa N, Carresi C, Musolino V, Casale F, Ventrice D, Oliverio M, Palma E, Nisticò S, Procopio A, Mollace V (Catanzaro, Roma)

Sessione 2 - **Moderatore**: Cicala C. (Napoli)

P6. **Natural isothiocyanates and inhibition of mast cells degranulation: is H_2S the real player?**
Martelli A, Citi V, Marino A, Testai L, Breschi MC, Calderone V (Pisa)

P7. **The anti-inflammatory and antioxidant effects of Bergamot juice (BJe) in an experimental model of inflammatory bowel disease**
Bruschetta G, Impellizzeri D, Di Paola R, Ahmad A, Campolo M, Cuzzocrea S, Esposito E, Navarra M (Messina, Manchester)

P8. **Antinociceptive effects of carnosol and carnosic acid, two *o*-diphenolic diterpenes from *Salvia officinalis* L.**
Maione F, Bisio A, Romussi G, Mascolo N, Pieretti S (Napoli, Genova, Roma)

P9. **Protective effects of Cyanidin-3-O-glucoside against LPS-induced damage in Caco-2 intestinal cells**
Ferrari D, Speciale A, Fratantonio D, Cristani M, Saija A, Cimino F (Messina)

P10. ***Armoracia rusticana*** **reduces inflammatory response and reactive oxygen species release in LPS-stimulated macrophages**

Adesso S, Larocca M, Del Regno M, Calabrone L, Padula MC, Autore G, Martelli G, Rossano R, Marzocco S (Salerno, Potenza)

P11. **Topical anti-inflammatory activity of the aerial parts of *Glechoma sardoa* Bég**
Sosa S, Pelin M, Masullo M, Sanna C, Ballero M, Tubaro A, Della Loggia R, Piacente S (Trieste, Salerno, Cagliari)

Sessione 3 – Moderatore Autore G. (Salerno)

P12. **Antiproliferative activity and radical scavenging properties of *Salvia ceratophylla* and *S. hydrangea* extracts**
Tundis R, Bonesi M, Salehi P, Menichini F, Loizzo MR (Cosenza, Tehran)

P13. ***Salvia verbenaca* L. as a potential source of anticancer agents for the melanoma treatment**
Russo A, Cardile V, Graziano ACE, Formisano C, Rigano D, Canzoneri M, Bruno M, Senatore F (Catania, Napoli, Palermo)

P14. ***Rubus ulmifolius* leaf extracts: testings on murine myeloma cells and chemical investigations**
Bernardini S, Triggiani D, Ceccarelli D, Salvini L, Taddei AR, Pollini D, Ovidi E, Tiezzi A (Viterbo, Siena)

P15. **Vinyl disulfides from asafoetida induce apoptosis of human melaloma cell lines**
Armogida C, Panza E, De Cicco P, Taglialatela-Scafati O, Cirino G, Ianaro A (Napoli)

P16. **Natural sesquiterpenes inhibit the genotoxicity induced by cigarette butts in the bacterial reverse mutation assay**
Di Giacomo S, Mazzanti G, Di Sotto A (Roma)

P17. **Effects of polyphenol hydroxytyrosol, an oleuropein metabolite, on hepatic inflammation and oxidative stress in a rat model of NAFLD**
Mattace Raso G, Pirozzi C, Simeoli R, Santoro A, Lama A, Di Guida F, Russo R, Berni Canani R[1], Calignano A, Meli R (Napoli)

Sessione 4 - **Moderatore**: Meli R. (Napoli)

P18. ***Betula aetnensis* Rafin extract in streptozotocin-induced diabetes**
Acquaviva R, Vanella L, Sorrenti V, Amodeo A, Mastrojeni S, Ragusa S, Di Giacomo C (Catania, Catanzaro)

P19. **Effects of *Tithonia diversifolia* (Hemsl.) A. Gray extract on adipocyte differentation of human mesenchymal stem cells**
Acquaviva R, Vanella L, Sorrenti V, Barbagallo I, Genovese C, Amodeo A, Mastrojeni S, Ragusa S, Di Giacomo C (Catania, Catanzaro)

P20. **The effect of bergamot-derived polyphenolic fraction on LDL small dense particles and non alcoholic fatty liver disease in patients with metabolic syndrome**
Gliozzi M, Carresi C, Musolino V, Palma E, Muscoli C, Vitale C, Gratteri S, Muscianisi G, Janda E,

Muscoli S, Romeo F, Ragusa S, Mollace R, Walker R, Ehrlich J, Mollace V (Catanzaro, Roma)

P21. **Bone regeneration in critical-size defects of rat calvaria treated with Human Amniotic Fluid Stem Cells seeded into a collagen scaffold: effect of the oral administration of Ferutinin**
Zavatti M, Bertoni L, Maraldi T, Resca E, Beretti F, Guida M, De Pol A (Modena, Bolzano)

P22. **Reinstatement of cocaine conditioned place preference induced by social defeat stress is blocked by *Hypericum perforatum* L.**
Fugazzotto F, García-Pardo MP, Miñarro J, Rodríguez-Arias M, Aguilar MA, Occhiuto F (Messina, Valenzia)

Sessione 5 - **Moderatore** Avato P. (Bari)

P23. **Luteolin derived flavonoids as bio-markers of *Passiflora loefgrenii* extracts**
Argentieri MP, Guzzo F, Levi M, Avato P (Bari, Verona)

P24. **Clinical investigations on products of vegetal origin when used as food additives**
Nunziata A (Pomezia)

P25. **Production of galanthamine from *Narcissus poeticus* L.**
Pace L, Fasciani P, Ferri D, Bacchetta L, Ubaldi C, Marcozzi G (L'Aquila, Roma)

P26. **Poliphenolic content, antioxidant properties and amylase inhibition by *Capsicum annuum* L. var. "Cornetto di Pontecorvo DOP"**
Di Sotto A, Toniolo C, Di Giacomo S, Vitalone A, Mazzanti G, Nicoletti M (Roma)

P27. **Testings of *Myrtus communis* leaf extracts on bacteria and mammalian cells**
Mastrogiovanni F, Bernardini S, Gallipoli L, Balestra GM, Pollini D, Gallorini V, Triggiani D, Ovidi E, Tiezzi A (Viterbo)

P28. **Stealth PLGA nanoparticles for intracellular Curcumin release**
Serri C, Argirò M, Biondi M, Crispi S, Diano N, Giarra S, Mayol L, Menale C, Mita DG, Mita L, Piccolo MT, Saija A (Messina, Napoli, Roma)

Sessione 6 – Moderatore Ialenti A. (Napoli)

P29. **Caffeic Acid attenuates high glucose-induced oxidative stress and endothelial dysfunction in human endothelial cells via modulating NF-kB pathway**
Fratantonio D, Speciale A, Ferrari D, Anwar S, Saija A, Cimino F (Messina)

P30. **Antioxidant and heavy metals absence in tomatoes grown in toxic muddy soils**
Marra N, Caporale A, Tommonaro G, Popolo A, De Prisco R, Nicolaus B, Essolito M, De Martino F, Sinicropi MS, Saturnino C, Autore G (Salerno, Pozzuoli, Cosenza)

P31. **Capability of Trichotecene mycotoxins to induce oxidative stress in a model of intestinal epithelial cells**
Del Regno M, Marzocco S, Adesso S, Popolo A, Chirollo C, Severino L, Autore G (Salerno, Napoli)

P32. **Antioxidant and antifungal activities of the Cameroonian medicinal plant *Annona muricata***
Donati M, Kamdem Simo M, Bertin R, Chen Z, Froldi G (Padova, Yaoundé)

P33. **Preliminary phytochemical analyses and *in vitro* evaluation of the antioxidant activity of a feed supplement containing Rose hip extract**
Brovedani V, Sosa S, Pelin M, D'Orlando E, Pacher T, Zitterl-Eglseer K, Tubaro A (Trieste, Vien)

P34. **Biological properties of Algerian *Thymelaea microphylla* Coss. & Dur. Extracts**
Dehimi K, Dahamna S, Speciale A, Cimino F, Cristani M (Messina, Algeria)

Sessione 7 – Moderatore Occhiuto F. (Messina**)**

P35. **Chemical composition, antioxidant activities and protective effects of *Sideritis italica* extract on C2C12 oxidative stress**
Leporini L, Pintore G, Tirillini B, Menghini L (Chieti-Pescara)

P36. **The leaves of the PGI "Nocciola di Giffoni" (*Corylus avellana* L.) as a rich source of antioxidant diarylheptanoids related to curcumin**
Masullo M, Cerulli A, Olas B, Pizza C, Piacente S (Salerno, Lodz)

P37. **Biovariability of caper species from Calabria: chemical and biological evaluation**
Conforti F, Marrelli M, Menichini F (Cosenza)

P38. **Red wine inhibits aggregation and increases ATP-diphosphohydrolase (CD39) activity of rat platelets**
Caiazzo E, Cinquegrana M, Tedesco I, Bilotto S, Spagnuolo C, Russo GL, Ialenti A, Cicala C (Napoli, Avellino)

P39. **BERGA*Met*®*:* a phytotherapic approach to cardiovascular disease**
Failla P, Salerno R, Casale F, Muscoli C, Gliozzi M, Palma E, Mollace V (Catanzaro, Roma)

P40. **Effects of a single dose of a green tea extract supplement on the Peroxidation of Leukocytes Index Ratio (PLIR) of healthy subjects**
Manafikhi H, Marocco I, Nardecchia F, Reggi R, Peluso I, Serafini M, Altieri F, Palmery M (Roma)

LETTURA MAGISTRALE

Bioactive molecules from natural sources in the prevention of chronic degenerative diseases: my ten years research story with Sulforaphane

Hrelia P

Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

Natural products play an important role in the prevention of chronic degenerative diseases due to the potential capacity of inhibiting and modulating several targets simultaneously. This pleiotropism might constitute an advantage against pathologies characterized by an enormous biological diversity, such as cancer. In the past 10 years my research interest was addressed to the study of Sulforaphane (SFN), an isothiocyanate found in cruciferous vegetables. First identified as an important inducer of phase II enzymes, SFN has proved to be an effective chemopreventive agent in cell cultures, in carcinogen-induced and genetic animal cancer models, as well as in xenografts models of cancer. It was proven to inhibit, reverse or delay the development of all the different stages of the carcinogenic process, it promoted potent cytostatic and cytotoxic effects orchestrated by the modulation of different molecular targets. Apoptotic events are regulated by cell-cycle-dependent mechanisms but are independent of a mutated p53status. Combination of SNF with cytotoxic therapy potentiate the chemotherapeutic effects, suggesting a therapeutic benefit in clinical setting. Moreover, SNF seems to possess a favorable toxicological profile, considering the absence of genotoxicity.

SNF was shown to increase intracellular signaling of the transcription factor Nrf2, a master regulator of the antioxidant response in humans. When activated, Nrf2 switches functions to increase the production of many detoxification, antioxidant-SOD, and catalase- and anti-inflammatory proteins. Because the Keap1-Nrf2 pathway regulates over 600 cytoprotective genes, it has been considered useful to protect against a broad range of diseases in which oxidative stress is a common feature, including renal, cardiovascular and neurological diseases. In this latter contest, SNF well fulfilled the requirement for a putative neuroprotective drug having diverse pharmacological activities. Neuroprotective effects of SUL are long-lasting, by inhibiting mitochondrial collapse and 6-OHDA-induced apoptosis and increasing Total Antioxidant Activity and GSH levels. The involvement of ERK and PI3-K/Akt pathways was demonstrated in the neuroprotective effects of SNF. Neuroprotection was confirmed in a rat model of PD, with behavioural, hystochemical and molecular effects.

Overall, SNF appears to be an effective and safe agent for the prevention of a broad range of inflammation and oxidative stress related chronic degenerative diseases.

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COMUNICAZIONI ORALI

On the antitumor activity of *Citrus bergamia* juice

Cirmi S[a], Ferlazzo N[a], Lombardo GE[a], Calapai G[b], Navarra M[a]

[a]*Department of Drug Sciences and Products for Health and* [b]*Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy*

Citrus bergamia Risso et Poiteau (Bergamot) is a small tree belonging to the family of Rutaceae, cultivated almost exclusively along the southern coast of Calabria region (Italy). Bergamot fruit is used mostly for the extraction of its essential oil widely used in perfume industry. Bergamot juice (BJ), instead, is considered just a secondary and discarded product. The increasing interest concerning the antitumor activity of several fruit juices and vegetable extracts has allowed to highlight that natural substances commonly assumed by the diet can have an important role in the prevention of cancer. Therefore, the object of our study was to evaluate the antitumor effects of BJ both in *in vitro* and in *in vivo* models.

First we assess the ability of BJ in reducing the growth rate of different cancer cell lines, observing the maximal antiproliferative effect in neuroblastoma cells. The SH-SY5Y growth inhibition elicited by BJ was not due to a cytotoxic action or apoptosis. Instead, BJ stimulated the arrest of cell cycle in the G1 phase and determined a modification in cellular morphology, causing a marked increase of detached cells. The inhibition of adhesive capacity on different physiologic substrates and on endothelial cells monolayer were correlated with impairment of actin filaments, reduction in the expression of the active form of focal adhesion kinase (FAK) that in turn caused inhibition of cell migration (Delle Monache et al., PLoS One, 2013, 8 (4): e61484).

The second task of this study was to evaluate the effect of BJ in a spontaneous metastatic neuroblastoma SCID mouse model. The results showed that BJ reduced the pulmonary metastases formation, although failed to reduce primary tumor weight (Navarra et al., Fitoterapia, 2014, 95: 83–92).

In order to assess which bioactive component of BJ is responsible for its antitumor activity, we focused on the flavonoid fraction of bergamot juice (BJe). Our results suggest that BJe inhibits HT-29 human colorectal carcinoma (CRC) cell growth and induces apoptosis by multiple mechanisms. Molecular assays revealed that higher concentration of BJe increases ROS production which causes loss in mitochondrial membrane potential and oxidative DNA damage. Instead, lower concentrations of BJe inhibits MAPK pathways and altering apoptosis-related proteins, that in turn induced cell cycle arrest and apoptosis.

In conclusion, we demonstrate that BJ exerts important antiproliferative effects *in vitro* by different mechanisms depending on the cell lines. Moreover, we show the ability of BJe in modulating specific molecular pathways involved in the regulation of apoptosis and cell growth, depending on its concentration. Finally, we suggest that the inhibitory effects on lung metastasis colonization in vivo may be due to the impairment of cell adhesiveness, migration and invasion observed *in vitro*. Thus, our study highlights the role of BJ as anti-cancer drug underlying its potential clinical interest and laying the basis for further investigation.

Antileukemic activity of sulforaphane in hypoxic conditions and in primary blasts from patients affected by myelo- and lympho-proliferative disorders

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[b]*Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy*
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Sulforaphane (SFN) is a dietary isothiocyanate found in cruciferous vegetables showing antileukemic activity. SFN is a highly reactive and hydrophobic compound that can alter many cellular processes. Inhibition of cell proliferation, increased apoptosis, anti-inflammatory and antioxidant activities, induction of phase-II detoxification enzymes, inhibition of cyclooxygenase 2, and various other mechanisms have been proposed to explain its anticancer effects (Fimognari and Hrelia, 2007). Leukemias are malignant neoplasms involving cells originally derived from hematopoietic precursor cells. All leukemias start in the bone marrow, that is diffusely replaced by abnormally proliferating neoplastic cells. The neoplastic cells may spill out of the bone marrow and reach the blood, where they may be present in large numbers, resulting in the clinical presentations of the disease. Despite the development of multiple new agents, chemoresistance frequently hampers the successful treatment of leukemias, and relapse continues to be the most common cause of death (Karp et al., 2003). Those therapeutic issues can also be imputable to tumor microenvironment, characterized by regions of hypoxia that can influence tumor cell sensitivity to drug treatment. Thus, leukemias remain a formidable therapeutic challenge that requires the identification and the development of novel agents for the treatment of this disease.
Based on these considerations and with the aim to extend the potential clinical impact of SFN in the oncological field, we investigated its antileukemic effect on blasts from patients affected by different types of leukemia and, taking into account the intrinsically hypoxic nature of bone marrow (Filippi et al., 2011), on a human lymphoblastic cell line maintained in hypoxic conditions. In particular, we tested SFN on patients with chronic lymphocytic leukemia, acute myeloid leukemia, T-cell acute lymphoblastic leukemia, B-cell acute lymphoblastic leukemia, and blastic NK cell leukemia. SFN dose-dependently induced apoptosis in blasts from patients diagnosed with acute lymphoblastic or myeloid leukemia. Moreover, it was able to cause apoptosis and to inhibit proliferation in hypoxic conditions on lymphoblastoid cell line. As to its cytotoxic mechanism, we found that SFN creates an oxidative cellular environment that induces DNA damage and Bax and p53 gene activation, which in turn helps trigger apoptosis.
On the whole, our *in vitro* and *ex vivo* results raise hopes that SFN might set the stage for a novel therapeutic principle complementing our growing armature against malignancies and advocate the exploration of SFN in a broader population of leukemic patients.

Filippi I, Naldini A, Carraro F. Role of the hypoxic microenvironment in the antitumor activity of tyrosine kinase inhibitors, Curr Med Chem 2011, 18:2885-92
Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer, Mutat Res 2007, 635:90-104
Karp JE, Ross DD, Yang W, Tidwell ML, Wei Y, et al. Timed sequential therapy of acute leukemia with flavopiridol: in vitro model for a phase I clinical trial. Clin Cancer Res 2003, 9:307-15

Cynaropicrin inhibits stat3 phosphorylation through gsh depletion and induces cell death in du145 cell line

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STAT3 is a transcription factor constitutively activated in highly malignant solid and hematological tumor that has a critical role in the inhibition of apoptosis and induction of chemoresistance. Inhibition of STAT3 signaling pathway suppresses cell survival signals and leads to apoptosis in cancer cells, suggesting that direct inhibition of STAT3 function is a viable therapeutic approach.
In this work, we identify the naturally occurring sesquiterpene lactone cynaropicrin as a potent inhibitor of both IL-6-inducible and constitutive STAT3 activation in the human macrophage-like cell line THP-1 and in human prostate cell line DU-145, respectively (IC50=12 μM). Cynaropicrin, that contains α-β unsatured carbonyl moiety and function as potent Michael reaction acceptor, induces dose- and time-dependently the drop in intracellular glutathione (GSH) concentration. This event is time compatible with the inhibition of STAT3 tyrosine phosphorylation. The concomitant ROS production concurs to the maintenance of low level of GSH and prevents the recovering of the GSH concentration. Finally, the glutathione ethylene ester (GEE), the cell permeable GSH form, prevents the inhibitory action of cynaropicrin on STAT3 tyrosine phosphorylation. The disturbance in the intracellular redox state induces S-glutathionylation of STAT3.
These findings suggest that cynaropicrin is able to induce redox-dependent post-translational modification of cysteine residues of STAT3 protein in order to regulate its function.
STAT3 inhibition leads to the suppression of two anti-apoptotic genes, Bcl-2 and Survivin, in DU145 cells that constitutively express active STAT3. This event may be responsible of the decline in cell viability after cynaropicrin treatment. As revealed by PI/Annexin-V staining, PARP cleavage and DNA ladder formation, cynaropicrin cytotoxicity is mediated by apoptosis.
Moreover, we found that cynaropicrin potentiated cytotoxic effect of two well-establish chemotherapeutic agent, cisplatin and docetaxel. Combination index study demonstrate that this sesquiterpene displayed a slight to strong synergism with these drugs. The combination of cynaropicrin and cisplatin allows a dose reduction up to 2.6 and 8-fold respectively and the combination of cynaropicrin and docetaxel allows a dose reduction up to 2 and 96-fold respectively. The favorable DRI (>1) allows dose-reduction that leads to toxicity reduction in the therapeutic applications.
Taken together our studies suggest that cynaropicrin suppresses the STAT3 pathway, leading to the down-regulation of STAT3-dependent gene expression and chemosensitization of tumor cells to chemotherapy.

Effect of the hydrogen sulfide (H_2S) donor diallyl trisulfide (DATS) on melanoma

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Research in recent decades has shown widespread pharmacological effects of *A. sativum* and its organosulfur compounds such as diallyl disulphide, S-allylcysteine, and diallyl trisulfide (DATS). Epidemiological studies have demonstrated a reciprocal relationship between garlic consumption and incidence of cancers[1]. DATS, one of the most potent polysulfides present in garlic, has been shown to release hydrogen sulfide (H_2S) under physiological conditions[2]. In the last few years, numerous physiological and pathophysiological roles have been proposed for the gasotransmitter H_2S along with a plethora of cellular and molecular targets. Malignant melanoma is an aggressive form of skin cancer with an increasing incidence and a death rate that frequently resists chemotherapy, so the search for new agents for its treatment is of great importance.
In this study, we evaluated the potential antitumor effect of the H_2S donor DATS both *in vitro* and *in vivo*.
We firstly demonstrated that DATS (10-30-100 μM) inhibited the growth of four human melanoma cell lines (A375, SK-Mel-5, SK-Mel-28 and PES43) in a time- and concentration-dependent manner.
Cytofluorimetric analysis with annexin V/PI staining demonstrated that the anti-proliferative effect of DATS was due to its ability to induce apoptosis of A375 cells. In fact, after incubation with DATS 100 μM for 48h almost all of the cells (93%) exhibited markers of late apoptosis. This effect was further confirmed by the time-dependent activation of caspase 3 and by the cleavage of its substrate poly (adenosine diphosphate-ribose) polymerase (PARP). Moreover, DATS (100 μM) induced time-dependent accumulation of G_0/G_1-phase population (31%) and a reciprocal reduction of cell ratio in S (-5%) and G_2/M-phases (-27%).
Several reports have shown that in melanoma the constitutive activation of NF-kB confers tumor survival capacity and avoidance of apoptosis[3]. Incubation of A375 cells with DATS 100 μM inhibited IkBα degradation and consequently NF-kB nuclear translocation and activation. Moreover, the expression of the anti-apoptotic proteins c-FLIP, XIAP and Bcl-2, that is transcriptionally regulated by NF-kB[4], was greatly reduced following treatment with DATS at 100 μM for 3h and 6h.
Two of the most frequently deregulated pathways in melanoma are Mitogen-Activated Protein Kinase (MAPK)/ERK and Phosphoinositide 3-Kinase (PI3K)/AKT[5]. These two pathways play an important role in melanoma development and progression and are involved in the mechanism of resistance to targeted therapy. Our study showed that DATS (100 μM) inhibited the phosphorylation and activation of both AKT and ERK. To further support our *in vitro* findings we also used DATS *in vivo* in a murine model of melanoma. Our results demonstrated that DATS (50 mg/Kg p.o) significantly reduced both tumor volume and weight as compared to control mice by 67% and 75% ($P<0.001$) respectively.
Therefore, DATS by donating H_2S induces apoptosis and cell cycle arrest of human melanoma cells and inhibits tumor growth *in vivo*. Our results open new and exciting therapeutic prospective in the treatment of metastatic melanoma.

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Neuroprotective effects of Co-ultraPEALut on inflammatory process induced by traumatic brain injury

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Traumatic brain injury (TBI) initiates a neuroinflammatory cascade that contributes to neuronal damage and behavioral impairment. N-palmitoylethanolamide (PEA) is an endogenous fatty acid amide belonging to the family of the N-acylethanolamines (NAEs). PEA is an important analgesic, anti-inflammatory and neuroprotective mediator, acting at several molecular targets in both central and sensory nervous systems as well as immune cells. However, PEA lacks a direct antioxidant capacity to prevent the formation of free radicals, and to counteract the damage of DNA, lipids and proteins. Luteolin (Lut), a common flavonoid present in many plants, has strong antioxidant and pharmacological activities, including a memory-improving effect. It displays excellent radical scavenging and cytoprotective properties, particularly when tested in complex biological systems where it can interact with other antioxidants, such as vitamins. Lut displays specific anti-inflammatory effects, which are only partly explained by its antioxidant capacities. The anti-inflammatory activity of Lut includes activation of antioxidative enzymes, suppression of the nuclear factor (NF)-κB pathway and inhibition of pro-inflammatory substances. In vivo, Lut reduces increased vascular permeability and is effective in animal models of CNS inflammation.
In the present study, we performed a widely-used model of TBI to determine the neuroprotective propriety of palmitoylethanolamide (PEA) and the antioxidant effect of a flavonoid luteolin (Lut), given as a co-ultramicronized compound Co-ultraPEALut. Specifically, TBI was induced in mice by controlled cortical impactor. Co-ultraPEALut (1 mg/kg, soluble 10% ethanol, i.p.) was administered 1 h after craniotomy. At 24 h after TBI, the brains were collected.
We demonstrated that the treatment with Co-ultraPEALut resulted in a significant improvement of motor and cognitive recovery after controlled cortical impact (CCI), as well as markedly reducing lesion volumes. Moreover, our results revealed the ability of Co-ultraPEALut, to reduce brain trauma through modulation of NF-kB activation. In addition, treatment with Co-ultraPEALut significantly enhanced the post-TBI expression of the neuroprotective neurotrophins GDNF compared to vehicle. Co-ultraPEALut at the dose of 1 mg/kg, also modulated apoptosis, the release of cytokine and ROS, the activation of chymase, tryptase and nitrotyrosine. Thus, our data demonstrated that Co-ultraPEALut at a lower dose compared to PEA alone, can exert neuroprotective effects and the combination of both could improve their ability to counteract the neurodegeneration and neuroinflammation induced by TBI.

Phytocannabinoid modulation of neuropathic pain-associated neuroinflammation

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Neuropathic pain is a severe chronic disease which affects the quality of the life. Recent reports have suggested that peripheral inflammation as well as central neuroinflammation contribute to the induction and maintenance of neuropathic pain. In particular, microglial cells seem to play a pivotal role in initiating the neuronal sensitization which is responsible of allodynia in several models of neuropathic pain (Clark et al., 2007, Luongo et al., 2008). Cannabinoids have been widely investigated in the central immune regulation specially as it regard the CB2 mediated effect on microglia (Racz et al., 2008, Luongo et al., 2010). Recent evidence also highlighted an antinociceptive effect of phytocannabinoids (Maione et al., 2011).
In the present study we have investigate the anti-hyperalgesic and anti-allodynic effect of the phytocannabinoids cannabidiol (CBD) and cannabichromene (CBC) in the spared nerve injury (SNI) animal model. Moreover, we have investigated the effect of those natural drugs on the mRNA and protein levels of the main caspases involved in maintaining allodynia (caspase-1, 12) and on microgliosis in the dorsal horn of spinal cord of injured mice.
We have observed that CBD and CBC reduced in a dose dependent manner the mechanical allodynia and thermal hyperalgesia induced by nerve injury. In addition, we have observed that CBD was more potent than CBC in preventing casp-1 increased levels, while was equipotent in reducing casp-12 in SNI animals. Moreover, phytocannabinoids reduced the activation of glia and microglia in the dorsal horn of spinal cord 7 days after peripheral injury. Finally, by using primary microglial cell cultures, we observed that Casp-1 translocation from nucleus to cytoplasm (active form) was completely prevented by CBD treatment in LPS-activated cells.
These findings demonstrate that the natural components of C*annabis sativa* are able to alleviate neuropathic pain symptoms and reduced the molecular target which are responsible of central sensitization associated with neuroinflammation.

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In vitro anti-inflammatory activity of *Fragaria* spp. in human gastric epithelial cells

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Strawberries (*Fragaria* spp.) are commonly consumed berries, and are the most popular choice among consumers, being eaten fresh, frozen and in different processed products. The biological activity of strawberries is correlated to the presence of several compounds, including proanthocyanidins, anthocyanins and ellagitannins.
The gastrointestinal tract represents an important barrier between the human hosts and microbial populations. During gastric inflammation, epithelial cells release higher levels of cytokines including IL-1β, TNFα, and IL-8, a potent neutrophil-activating chemokine that plays a central role in gastritis (Crabtree et al., 1995). This response strictly depends on the activation of NF-kB pathway (Yasumoto et al., 1992). Strawberry has been shown to inhibit ethanol-induced gastritis in rats, and the effect was related to the presence of anthocyanins (Alvarez-Suarez et al., 2011). However, the inhibitory effect of tannin-enriched fractions (TEFs) from strawberries against gastric inflammation was not previously described. The aim of the present work was to investigate if tannins from *Fragaria* spp. could contribute to inhibit gastric inflammation. For this purpose, berries were harvested at maturity and the extraction of polyphenols was carried out with a mixture acetone/water (70/30 v/v), as previously reported (Gasperotti et al., 2010). TEFs and agrimoniin, the most abundant ellagitannin occurring in *Fragaria* spp., were assayed to investigate a) the inhibition of NF-kB nuclear translocation and driven transcription; b) the effect on IL-8 release in gastric epithelial cell line (AGS) stimulated with TNFα and IL-1ß. Both TEFs from *Fragaria* x *ananassa* and *Fragaria vesca* inhibited TNFα-induced NF-kB driven transcription and nuclear translocation. IC_{50} on TNFα-induced NF-kB nuclear translocation were 0.25 and 1.01 μg/ml, respectively. Agrimoniin inhibited TNFα-induced NF-kB driven transcription and nuclear translocation in a concentration-dependent manner. When the stimulus was IL-1β, the effect of TEFs and agrimoniin were lower with respect to TNFα. Since IL-8, whose expression is strictly dependent on NF-kB activation, is widely involved in gastric inflammation, the following experiments evaluated the effect of the extracts/individual compound on IL-8 secretion in AGS cells. Both the extracts and agrimoniin were able to inhibit IL-8 release, induced by TNFα and IL-1β, in a concentration-dependent way. IC_{50} of *Fragaria* x *ananassa* and *Fragaria vesca* extracts on TNFα-induced IL-8 release were 0.09 and 0.29 μg/ml, respectively. The effect of the extracts on TNFα-induced IL-8 release was ten fold higher than that induced by IL-1β. Agrimoniin inhibited preferentially TNFα-induced IL-8 release as well (IC_{50} 0.042 μM). TEFs and agrimoniin also inhibited IL-8 promoter activity induced by TNFα in a concentration-dependent manner. Our results report that *Fragaria* spp., which are widely consumed as nutrients, show *in vitro* anti-inflammatory effect at the gastric level, being agrimoniin responsible, at least in part, for the biological activity exerted by the extracts. These results suggest that tannins, in addition to anthocyanins, could contribute to the beneficial effects of *Fragaria* spp. at the gastric level.

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Vitis vinifera L.: anti-inflammatory activity on the gastrointestinal tract

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The gastrointestinal tract represents an important barrier between the human host and microbial populations. One potential consequence of this interaction is the development of mucosal inflammation, which can lead to gastritis and ulcer in the stomach, and inflammatory bowel diseases (IBDs, i.e. ulcerative colitis and Crohn's disease) in the gut. *Helicobacter pylori* is the bacterium mainly involved in the pathogenesis of gastric diseases. In gastric inflammation, macrophages produce pro-inflammatory cytokines, including TNFα and IL-1β, which determine the activation of NF-kB. It has been extensively reported that NF-kB plays a crucial role in the pathogenesis of gastritis, ulcer and IBDs. This nuclear factor mediates the transcription and secretion of different pro-inflammatory mediators including IL-8, a potent chemokine leading to the activation of macrophages and neutrophils, and contributing to the maintenance of the inflammatory status. It has been previously demonstrated that different extracts from grape juice, skin and seeds show anti-inflammatory effects. The water extract of *Vitis vinifera* L. leaves is a component of various food supplements; however, there are no studies in the literature on the anti-inflammatory activity in the gastrointestinal tract. The aim of this study was to evaluate *in vitro* the effect of the water extract from *Vitis vinifera* L. against gastrointestinal inflammation. The extract was obtained from dried leaves of *Vitis vinifera* L., var. Teinturiers and characterized by pH differential method and HPLC-DAD analysis; the extract was assayed on human gastric epithelial cells (AGS) and colonocytes (Caco-2) stimulated with TNFα and IL-1β (both at 10 ng/mL). NF-kB driven transcription was evaluated by transient transfection assay with a plasmid containing the luciferase gene under the control of three NF-kB responsive elements. NF-kB nuclear translocation and IL-8 secretion were performed by an ELISA assay. Quantitative analysis showed that kaempferol-3-O-glucoside was the most abundant flavonoid, and cyanidin-3-O-glucoside the main anthocyanoside. In AGS cells, the extract inhibited NF-kB driven transcription in concentration-dependent way, after stimulation with both TNFα and IL-1β, with statistically significant inhibition starting from 5 μg/mL and 25 μg/mL, respectively. When TNF-α was used as stimulus, the extract exhibited 50% of inhibition at 25 μg/mL, while the effect on IL-1β-induced transcription was significant at 200 μg/mL (-40%). The extract inhibited also the NF-kB nuclear translocation in a concentration-dependent manner. Similarly to what was observed in AGS cells, inhibitory effect of the extract was dependent on the pro-inflammatory stimulus used in Caco-2 cells as well; water extract inhibited in a concentration-dependent manner both NF-kB driven transcription and nuclear translocation induced by TNFα, with a maximum effect at 50 μg/mL whereas the effect on IL-1β-induced NF-kB driven transcription was lower. Although TNFα was not able to induce IL-8 release in Caco-2 cells, the extract (50 μg/mL) significantly inhibited IL-1β-induced IL-8 release. Results obtained in this study provide some experimental evidence on the anti-inflammatory activity of *Vitis vinifera* L. water extract in the gastrointestinal tract. If these data will be confirmed by *in vivo* studies, the use of *Vitis vinifera* L. extracts might be useful against gastrointestinal inflammatory diseases.

Antinociceptive and anti-inflammatory effects of bergamot essential oil in *in vivo* models

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Renewed interest in natural products as potential source of drugs led us to investigate on the antinociceptive and anti-inflammatory activity of bergamot essential oil (BEO). *Citrus bergamia* Risso et Poiteau (bergamot), is a small tree belonging to the family Rutaceae. To date, ninety-five percent of worldwide bergamot production occurs in the Ionic costs of Reggio Calabria (Calabria, Italy), where an habitat particularly suitable for its cultivation exists. Bergamot fruit is used mostly for the extraction of its essential oil from the peel, widely used in perfume industries. BEO comprises a volatile fraction (93–96% of total) containing monoterpene and sesquiterpene hydrocarbons (such as limonene, α- and β-pinene) and oxygenated derivatives (such as linalool and linalyl acetate). The non-volatile fraction (4–7% of total) consisting essentially of coumarins and psoralens, such as bergapten and bergamottine (Costa et al., Flav Fragr J, 2010). Since the toxicity of bergapten is well known, our study has been performed using the BEO fraction deprived of bergapten (BEO-BF).
Carrageenan-induced edema in rats was used as a model of inflammation. As assessed by plethysmometer, treatment with BEO-BF led to a significant inhibition of paw oedema induced by a subplantar injection of carrageenan. Moreover, histological examination of paw biopsies showed a reduction of pathological changes typically of oedema in BEO-BF treated rats. In order to gain a better insight into BEO-BF's mechanism(s) of action, some mediators of early phase of inflammation were take in account. So, IL-6 and TNF-α levels in the paw homogenate were measured by ELISA assays. BEO-BF treatments decreased both the IL-6 and TNF-α levels with respect to the carrageenan control group. Furthermore, in BEO-BF-treated rats we observed a reduction of nitrite/nitrate levels, as measured by Griess reaction in exudates. The latter could be related to the free radicals scavenging properties of BEO-BF (DPPH test, chelating activity and reducing power).
The antinociceptive activity of BEO-BF was examined in two different pain models in mice: the acetic acid-induced writhing response, a model of inflammatory pain, and the hot plate test, a model of supraspinal analgesia. Results of the writhing test showed that BEO-BF elicited a pronounced analgesic response as demonstrated by a significant inhibition of constrictions in mice receiving acetic acid, with respect to control animals. On the other hands, administration of BEO-BF did not produced any significant effect in the hot-plate test of analgesia.
In summary, our study indicate that BEO-BF exerts protective effects in carrageenan-induced paw oedema, as well as inhibit the inflammatory pain, suggesting its potential role as anti-inflammatory drug.

Astragalus membranaceus extract is effective in a rat model of rheumatoid arthritis

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Rheumatoid arthritis is a chronic disease characterized by pain, swelling, and stiffness of multiple joints. This immune-mediated disease presents chronic progressive inflammation and destruction of joints and associated structures, as well as systemic symptoms. Its hallmarks are persistent inflammation of the joint synovial because of infiltration of macrophages and activated T cells, and the subsequent destruction of surrounding bone and cartilage tissue, both of which are responsible of functional disability. Rheumatoid arthritis is a highly disabling disease that limits mobility, hampers work, and reduces quality of life.
Astragalus membranaceus (Fisch.) Bge. is an adaptogenic herb from the traditional Chinese medicine. The root of this herb has extensive immunomodulatory actions and, recently, significant pain reliever properties have been described for a hydroalcoholic extract. Aimed at evaluating the efficacy of *Astragalus membranaceus* in articular pain, a hydroalcoholic extract from selected roots (Epo, Italy, 70% EtOH, D.E.R. 3:1) was tested in a rat model of rheumatoid arthritis induced by the intra-articular injection of Complete Freund Adjuvant (CFA). A single per os (p.o.) administration of 300 mg kg^{-1} Astragalus extract was able to significantly revert CFA-induced mechanical hyperalgesia. In a parallel experimental set the same dosage p.o. was daily administered for 14 days starting from the day of CFA injection. At day 14^{th}, treatment with the Astragalus extract significantly prevented the alterations in pain threshold evaluated by mechanical noxious (Paw pressure test) or non-noxious (Electronic Von Frey test) stimuli applied on the paw, as well as a decrease of articular pain when directly measured on the damaged joint (PAM test). Moreover, the Astragalus extract reduced postural unbalance (hind limb weight bearing alterations; Incapacitance test) considered a feature of spontaneous pain. Repeated treatment decreased the caliber of damaged joint and prevented the inflammatory infiltrate at periarticular level. Rats repeatedly treated with the Astragalus extract showed decreased levels of plasmatic inflammatory cytokines IL-1β and TNF-α.
In conclusion, the hydroalcoholic extract of *Astragalus membranaceus* relieves pain in a rat model of rheumatoid arthritis. The Astragalus extract reverts pain after a single administration and prevents the establishment of pathological alterations when repeatedly administered. The well known safety profile of Astragalus strongly highlights the relevance of this natural product for a possible application in painful diseases related to articular damages.

The Italian Phytovigilance System

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Introduction: Herbal preparations are used by a large and growing number of consumers and there is the need to monitor their use and safety in the population. Most of these products are not marketed in Italy as drugs, and thus the surveillance of their adverse events is not included in the Italian Pharmacovigilance System. An ad hoc surveillance system complementary to that used for drug monitoring has been set up.
Aim: The Phytovigilance System has been implemented in Italy since 2002. The project started as a pilot study and in 2012 the system was enhanced and officially adopted from the Ministry of Health as an alert system.
Methods: The surveillance is based on the collection and evaluation of spontaneous reports of suspected adverse reactions observed after the administration of: 1) food supplements; 2) herbal preparations and galenic formulations; 3) other preparations of natural origin (*e.g.* propolis); 4) homeopathic medicines. For serious adverse reactions, follow-up of the patient and the label of the suspected product are collected and, if available, the package of the product is also sent to the Istituto Superiore di Sanità for possible laboratory investigations.
A Scientific Committee of experts in phytotherapy, botanics, toxicology, pediatrics, pharmacology, pharmacognosy, pharmacoepidemiology and homeopathy evaluates causality relationship of serious events. A Steering Committee comprising experts of the National Institute of Health, Italian Medicines Agency and Ministry of Health supports the Scientific Committee.
Results: From April 2002 to April 2014, 883 reports were collected. In 50% of reports hospitalization was indicated, in 6% a life-threatening event was reported. The reactions were mainly related to the gastrointestinal tract, the skin, the nervous system. "Herbal" food supplements were the products more frequently associated with the reactions; 11% of all reports were related to homeopathic medicinal products. In 40% use of a concomitant drug was reported. Safety issues raised because of contamination/adulteration of products, home made preparations containing hepatotoxic plants, interactions between herbal containing products and drugs. The following signals were pointed out: hepatopathies associated with different products; allergic reactions to propolis containing products; myopathies associated with red yeast rice extracts.
Conclusions: The benefit/risk is unknown for most of the products on the market, furthermore these products are mostly marketed as food supplements, proposed to improve wellbeing and, thus, self administered. Encouraging spontaneous reporting and communication of possible risks can contribute to improve awareness among health personnel and patients about the risk profile of these remedies.

Vascular L-type Ca^{2+} channel mediated activity of murrayafoline A from *Glycosmis stenocarpa*: electrophysiological and molecular docking studies

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Murrayafoline A, isolated from the dried powdered roots of *Glycosmis stenocarpa*, was assessed for vasoactivity on rings and for its effects on L-type Ba^{2+} currents [$I_{Ba(L)}$] on single myocytes isolated from rat aorta. Murrayafoline A augmented concentration-dependently, in a bell-shaped fashion, phenylephrine-induced contraction of endothelium-intact rings. Removal of the endothelium markedly reduced this effect and disclosed a vasodilatory activity at concentrations ≥ 47.3 μM. The latter was essentially due to the inhibition of extracellular Ca^{2+} influx but not to reduction of Ca^{2+} release from the intracellular stores. The spasmolytic effect of murrayafoline A was more evident in rings stimulated with 60 mM as compared to those stimulated with 30 mM K^+; in the latter case low drug concentrations caused an increase of high K^+ induced tone. Murrayafoline A, at concentrations ≤ 14.2 μM, shifted to the left the concentration-response curve to K^+ whereas, at concentrations > 14.2 μM, antagonized (S)-(-)-Bay K 8644-induced contraction.
A 3D model of the rat L-type Ca^{2+} channel central pore was built to simulate docking of murrayafoline A and two dihydropyridine ligands, the antagonist nifedipine and the agonist (S)-(-)-Bay K8644. Binding free energy measurements gave comparable values for the three ligands, which interacted with thirteen aminoacid residues of the channel α_{1C} subunit binding pocket in a similar way.
In single myocytes, murrayafoline A stimulated, at low concentrations, or inhibited, at high concentrations, $I_{Ba(L)}$ with negligible effects on current kinetics. Findings demonstrate that murrayafoline A is a naturally-occurring vasoactive agent; its vasodilation or vasoconstriction, as a function of drug concentration, being ascribable to the block or stimulation, respectively, of L-type Ca^{2+} channel.

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Cardioprotective effects of naringenin in elderly rats submitted to ischemia-reperfusion

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The cardiac tolerance against the ischemic insult and the effectiveness of endogenous defences that ensure cardioprotection are reduced in the ageing. Damage and lethality degree of myocardial infarction are increased in elderly patients [1]. Ageing-related mitochondrial dysfunction, largely due to the oxidative stress, is likely to account for such an unsuccessfully cardioprotection: indeed a clear decline of mitochondrial functions in aged rats compared with the young ones have been observed [2].
A rational cardioprotective strategy against ischemia-reperfusion (I/R) injury in elderly patients should be focused to prevent the progress of mitochondrial dysfunction, however suitable therapies are not currently available.
A correlation between the intake of flavonoids, present in many vegetables usually assumed with the diet, and a reduced mortality for cardiovascular diseases has been widely demonstrated. The antioxidant and cardioprotective effects of many flavonoids have been clearly elucidated [3].
Noteworthy, recent data of ours suggest that naringenin (NAR), an abundant flavanone in the Citrus genus, besides already known vasoactive properties [4,5], is able to promote cardioprotective effects [6]. These are due, at least in part, to a mitochondria-targeted action: NAR is able to activate a mitochondrial calcium-dependent potassium channel (mitoBKCa), localized on the inner mitochondrial membrane, resulting in an influx of potassium ions, a mild depolarization, and then a decrease of calcium uptake into mitochondrial matrix [7].
On these basis, this work aimed to evaluate in elderly rats the potential NAR-induced effects on the improvement of mitochondrial function, on the recovery of effectiveness of the cardioprotective defences and on the achievement of a protection against I/R injury.
Aged rats (40-44 weeks) were daily treated with NAR (100 mg/Kg i.p.) for 6 days. At day 7, animals were sacrificed with an overdose of pentobarbital and their hearts were perfused on Langendorff apparatus. The functional recovery and ischemic injury were evaluated on hearts submitted to I/R (30'/120').
Aged hearts showed a more extensive myocardial damage than young ones, the heart rate-pressure of ventricular developed product (%RPP) value at the 120^{th} minute of reperfusion were 24% vs 39%, respectively, and the ischemic ventricular area were (%$A_{I/VS}$) 40% vs 32%. Moreover, a significant reduction in the mitochondrial function was observed, in agreement with literature data. Aged hearts treated daily with NAR presented a reduced damage: %RPP value was 54% and %$A_{I/VS}$ 27%, these values were superimposable with the young ones.
These preliminary results, represent a first interesting step to demonstrate cardioprotective effects of NAR also in elderly rats, suggesting a possible preventive effect of the flavanone on the decline mitochondrial function in ageing, with a clear translational and nutraceutical value.

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Tanshinone IIA, a major component of *Salvia milthorriza* Bunge, inhibits *in vitro* rat platelet activation via Erk-2 signaling pathway

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Introduction. The roots of *Salvia milthorriza* Bunge (Danshen) are used in Traditional Chinese Medicine as a remedy for activating blood and eliminating stasis. Tanshinone IIA (TIIA), a major component of *Salvia milthorriza*, is one of the active diterpenoids in Danshen that exhibites a significant improvement of the blood flow in the coronary circulatory system and a reduction of myocardial infarction. However, its effect on platelet and underlying mechanism of action remain unknown. *Materials and Methods*. In order to investigate the effect of TIIA on platelet functionality, rat PRP were incubated with TIIA for 1 min at 37°C prior the addition of the stimuli (ADP or collagen). Aggregation was monitored in a light transmission aggregometer measuring changes in turbidity with continuous observation up to 10 minutes after the addition of the stimuli. Mitogen-activated protein kinase (MAPK) signaling pathway and tubulin acetylation were analyzed by Western blot technique. Also, the effect of the TIIA was studied *in vivo* on bleeding time in mice. *Results*. Our results shown that TAII selectively inhibited rat platelet aggregation induced by reversible ADP stimuli (3μM) in a concentration-dependent manner (0.5-50μM). Nevertheless, TIIA was less active against the irreversible stimuli induced by ADP (10μM) and collagen (10μg/mL). Moreover, experiments performed on platelet lysates collected at different time-point after the addition of the stimuli shown that TIIA selectively modulated tubulin acetylation and inhibited mitogen-activated protein kinase (MAPK) signaling pathway such as Erk-2 phosphorylation. Concomitantly, TIIA administrated i.p. at 10mg/kg significantly amplified mice bleeding time with an increase of 58% compared to its control (2.06±0.29 minutes *vs* 1.30±0.07). Acetylsalicylic acid (ASA) was used as reference drug for *in vitro* and *in vivo* experiments. *Conclusions*. Together these results provide useful insights in understanding the mechanism of action of TIIA on platelet aggregation and support the pharmacological efficacy of "Danshen" in Chinese herbal medicine.

Cyanidin-3-O-glucoside prevents endothelial cells dysfunction via Nrf2 pathway

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Many plant antioxidants, taken through daily diet or plant-derived dietary supplements, have been shown able to prevent free radical-related diseases; however, it is now considered that the in vivo beneficial effects of these phytochemicals are unlikely to be explained just by their antioxidant capability. The discovery of specific genes (HO-1, NQO1, g-GCS) and pathways (redox sensitive Nrf2, NF-kB regulated signaling) affected by antioxidants, led to the hypothesis that some phytochemical may act instead modulating these cellular stress adaptive response pathways (Speciale et al., 2011). In particular, anthocyanins, one of the most interesting and widespread classes of flavonoids, seem to play a role in preventing human diseases related to oxidative stress (Cimino et al., 2006). The dietary consumption of these pigments has been proposed to be associated with a significant protection against several human pathological conditions.
Since activation of endogenous cellular defense mechanisms can represent an innovative approach to therapeutic intervention in pathological conditions characterized by chronic tissue damage, a better understanding of adaptive response mechanisms induced by dietary plant antioxidants at the cellular and molecular levels can lead to novel strategies for the prevention and treatment of many different diseases. At this aim, we investigated whether Cyanidin-3-O-glucoside (C3G), an anthocyanin commonly present in food and vegetables from Mediterranean Diet, may act as a modulator of gene regulation and signal transduction pathways in different inflammatory and oxidative stress conditions (induced by TNF-α and Palmitic acid [PA]) in Human Umbilical Vein Endothelial Cells (HUVECs). PA, the most abundant saturated free fatty acid in the bloodstream, is implicated in the activation of oxidative stress, not only by uncoupling oxidative phosphorylation and increasing the generation of oxygen species, but also by impairing endogenous antioxidant defenses.
In our experimental conditions, HUVECs challenging with PA and TNF-α induced inflammation and oxidative stress comporting a reduction of intracellular cell defense systems (antioxidant proteins and enzymes), the induction of an oxidative damage (decreased intracellular Total Antioxidant Activity, and increased intracellular H2O2, and lipid peroxidation byproducts), and activating NF-kB proinflammatory pathway (adhesion molecules, leukocyte adhesion, NF-kB nuclear accumulation). Endothelial cells C3G pretreatment was able to prevent PA and TNF-α induced alterations and increased intracellular antioxidant power. We further demonstrated that C3G was able to trigger Nrf2 pathway through the activation of specific kinases (ERK1/2), leading to the upregulation of Antioxidant Responsive Element (ARE)/regulated cytoprotective enzymes (HO-1 and NQO-1), effect abolished by the pharmacological inhibitor of these kinases, PD98059.
Our data confirm the hypothesis that natural Nrf2 inducers, such as C3G and other dietary phytochemicals, might represent a potential therapeutic strategy to protect vascular system against various stressors preventing several pathological conditions.

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Pharmacological activity of compounds isolated from the leaves of the tree Artocarpus tonkinensis used in Vietnamese traditional medicine

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The leaves of *Artocarpus tonkinensis* (*At*) are used in Vietnamese traditional medicine for treatment of arthritis, and the compound maesopsin 4-O-β-D-glucoside (TAT-2), isolated from them, inhibits the proliferation of activated T cells. Our goal was to test the anti-proliferative activity of TAT-2 on the T-cell leukemia, Jurkat, and on the acute myeloid leukemia, OCI-AML. TAT-2 inhibited the growth of OCI-AML (and additional acute myeloid leukemia cells, such as U937, KG-1, and HL-60) but not Jurkat cells. Growth inhibition was shown to be due to inhibition of proliferation and, at a lesser extent, to increase in cell death. Analysis of cytokine release showed that TAT-2 stimulated the release of TGF-β, yet TGF-β neutralization did not reverse the maesopsin-dependent effect.
The drugs currently used for the therapy of AML are anthracycline and cytarabine. In order to compare these drugs with TAT-2, we stimulated OCI-AML cells with sub-optimal concentrations of aracytidine (ARA-C), doxorubicine and TAT-2 alone or in combination. TAT-2 given together with either ARA-c or Doxorubicin significantly decreased the OCI-AML cell number to the same level of ARA-C plus doxorubicin given together. Thus, TAT-2 can significantly increase the effectiveness of the drugs used currently in the AML therapy.
Gene expression profiling determined that Maesopsin modulated 19 identifiable genes. Transcription factor CP2 was the gene most significantly modulated. Real-time PCR validated that up-regulation of sulphiredoxin 1 homolog (SRXN1), hemeoxygenase 1 (HMOX1), and breast carcinoma amplified sequence 3 (BCAS3) were consistently modulated. The role of HMOX1 has been analyzed in depth. It is an anti-oxidant protein that, generally, protects cells from cell death. A western blot analysis confirmed that HMOX1 mRNA was translated in its protein and both TAT-2 and the *At* leave decoction up-regulated its expression when compared to the untreated control. Moreover, ARA-C and doxorubicin, the drugs currently used in the therapy of AML, did not up-regulate the HMOX1 protein. Thus, also the western blot analysis indicate that TAT-2 but not aracytidine (ARA-C) or doxorubicin up-regulates HMOX1, confirming the data of microarray and RT-PCR for this particular gene. To see if HMOX1 was responsible for the TAT-2-dependent growth inhibition, OCI-AML cells were transfected with HMOX1 transgene. Results suggest that overexpression of HMOX1 did not decrease but rather significantly increased OCI-AML cell number, suggesting that HMOX1 overexpression was not responsible for TAT-2 dependent inhibition of OCI-AML cell growth.
Decoction of the leaves of *At* has also been tested for its activity in a model of collagen-induced arthritis in mice. In the thymus of these mice, a subclinical form of arthritis determined a block of differentiation of the step that bring to $CD4^+CD8^+$ double positive (DP) from $CD4^-CD8^-$ double negative (DN) thymocytes. The consequence was a dramatic increase in $CD4^-CD8^-$ DN and a parallel decrease of DP thymocytes. The administration of At decoction completely abrogated the differentiation block.

The non-psychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability

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Epilepsy is the most common neurological disorder in humans, with over 50 million people worldwide affected. Recent evidence suggests that the transient receptor potential cation channel subfamily V member 1 (TRPV1) may contribute to the onset and progression of some forms of epilepsy. Since the two non-psychotropic cannabinoids cannabidivarin (CBDV) and cannabidiol (CBD) exert anticonvulsant activity *in vivo* and produce TRPV1-mediated intracellular calcium elevation *in vitro*, we evaluated the effects of these two natural products on TRPV1 channel activation and desensitization and in an *in vitro* model of epileptiform activity.

Patch clamp analysis in transfected HEK293 cells demonstrated that CBD and CBDV dose-dependently (1-30 μM) activate and rapidly desensitize rat TRPV1, as well as rat TRP channels of subfamily V type 2 (TRPV2) and subfamily A type 1 (TRPA1). TRPV1 and TRPV2 transcripts were shown to be expressed in rat hippocampal slices by quantitative PCR, whereas TRPA1 was at the limit of detection.

When tested on epileptiform neuronal spike activity in such slices exposed to a Mg^{2+}-free solution using multi electrode arrays (MEAs), CBDV (10 μM) reduced both epileptiform burst amplitude and duration. The prototypical TRPV1 agonist, capsaicin (10 μM), produced similar, although not identical effects. CBDV effects were not always sensitive to IRTX (1 μM), a selective TRPV1 antagonist. These data suggest that CBDV anti-epileptiform effects in the Mg^{2+}-free model, are not uniquely mediated via activation of TRPV1.

As assessed by means of western blot analyses using a polyclonal antibody against its phosphorylated form, TRPV1 was strongly phosphorylated, and hence likely sensitized by capsaicin (10 μM) and CBDV (10 μM), but not IRTX (1 μM), in control hippocampal slices. An increase of TRPV1 phosphorylation was observed in hippocampal slices exposed to Mg^{2+} free solution. Interestingly, in this experimental condition, both capsaicin (10 μM) and CBDV (10 μM) caused instead dephosphorylation of TRPV1, consistent with its possible desensitization.

We propose that the fast desensitization of tonically activated TRPV1 determined by agonists and CBD or CBDV, could open a new therapeutic opportunity to treat neurological disorders caused by an excess of neuronal activity such as epilepsy, and that CBDV effects on TRP channels should be next assessed also in the context of *in vivo* models of epilepsy.

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A new approach for the identification of natural drugs targeting Leukemic Stem Cell (LSC)

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Cancer is the second leading cause of death worldwide. Chemotherapy is a major therapeutic approach for the treatment of localized and metastatic cancers. However, conventional cancer therapies cause clinically relevant side effects and favor selection of resistant cells. The interest in innovative strategies for cancer treatment is therefore escalating. Extensive research has evidenced that cancer is caused by dysregulation of as many as 500 different gene products. Most natural products target multiple gene products and thus are suited for treatment of cancer. Spices have been extensively used in the Eastern world for many aliments for millennia, and five centuries ago, they spread to the West world.
Leukemia stem cells (LSCs) play a pivotal role in the process of leukemia initiation, progression, and relapse. For these reasons, LSCs represent a potential and promising pharmacological target for the treatment of leukemia. Unfortunately, only few agents have been proven to have a selective effect in the eradication of LSCs. Eppert et al. (2011) defined LSC and hematopoietic stem cell (HSC) gene signatures based on the CD34+ subpopulation of 16 primary human acute myeloid leukemia (AML) samples.
The aim of the present study was to identify natural drugs able to act specifically on the human LSC compartment. For this purpose, we used the connectivity map (cmap), which is a collection of gene-expression profiles from five cultured human cells treated with more than 1300 small bioactive molecules. It represents a functional connection between drugs and gene expression (Lamb et al., 2006). We performed cmap analysis using the LSC signature and obtained a list of drugs ranked based on several parameters expressing their relative strength in up- or down-regulating the gene signature. We then selected and screened piperlongumine, derived from the fruit of *Piper longum*. Long pepper is a spice widely used in Ayurvedic medicine to treat many diseases, including tumors. Piperlongumine was picked as a compound of interest based on its ability to selectively block the Nrf2 program in cancer cells, sparing normal cells from toxicity (Raj et al., 2006). The cmap result was first validated by testing the pharmacological activities of piperlongumine on two leukemic cell lines (U937 and MOLM13). It induced apoptosis and inhibited cell proliferation in a dose-dependent manner. Afterwards, we investigated its activities on CD34+ sorted subpopulation from 6 primary human AML samples and healthy cord blood. Piperlongumine (0-14 μM) induced apoptosis in a dose-dependent manner and, at the highest tested dose, completely inhibited the colony formation in all patient samples. Of note, it did not show any significant effect on healthy CD34+ cells at all tested concentrations. Based on these results, we can conclude that the cmap is an efficient tool for the identification of natural drugs able to target LSCs and thereby leading to treatment and long-term remission of leukemia. Piperlongumine is thus a promising pharmacological candidate for the treatment of leukemia and deserves further investigations on *in vivo* models.

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Role of salidroside, active compound of R*hodiola rosea* l., in the prevention and treatment of morphine tolerance and dependence

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Drug addiction represents a major problem for both human health and for economic and social costs particularly relevant worldwide. Among the various addictions, opioid dependence is certainly among the most popular and it is also associated with high mortality. Detoxification is often the first step in the individual treatment in opioid dependence. However, the currently available pharmacological treatments are often not effective or associated with severe side effects. A novel approach for the treatment of adverse effects caused by the abuse of psychoactive drugs sees the use of natural products, especially with anxiolytic and antidepressant action, both for their effectiveness and low toxicity. Recently it was demonstrated the effectiveness of *Rhodiola rosea* L., a well-known traditional oriental medicine with adaptogenic, anxiolitic, antidepressive and antistress properties, in the prevention and treatment of morphine-induced withdrawal symptoms [1]. The therapeutic activities of *R. rosea* are due to the quality and quantity of the mix of components that characterize it. The biologically active substances include organic acids, phenolic compounds, including phenylpropane derivatives as rosavins (rosavin, rosine, rosarin) and phenylethane derivatives such as tyrosol and salidroside. The most recent studies indicate that salidroside is the most responsible for many *R. rosea* effects[2-5]. Therefore, the present work was aimed to evaluate in vivo the role of salidroside, psychoactive compound of *Rhodiola rosea* L., in the prevention and treatment of tolerance and withdrawal syndrome induced by morphine. For this purpose, male CD-1 mice were injected with repeated administration of morphine (10 mg/kg, s.c.) twice daily for 5 or 6 days, in order to make them tolerant or dependent. SDS (0, 0.1, 0.2 and 0.4 mg/kg) or 20 mg/kg of a hydroalcoholic extract of *Rhodiola rosea* L. (RHO), standardized to 3% rosavine and 1% salidroside and used as reference drug, were administered 60 min prior to each morphine injection (for acquisition) or prior the last injection of morphine or naloxone on test day (for tolerance or dependence expression respectively). Morphine tolerance was evaluated by testing its analgesic effect in the tail flick test at 1st and 5th days. Morphine dependence was evaluated by counting the number of withdrawal signs (jumping, rearing, forepaw tremor, teeth chatter) after naloxone injection (5 mg/kg; i.p.) on the test day (6th). Results demonstrate that acute administration of 0.2 mg/kg of SDS results in a significant reduction of the expression of morphine tolerance comparable to that obtained with the dose of 20 mg/kg of RHO. Furthermore, pretreatment with 0.2 mg/kg of SDS, 60 minutes prior each morphine injection, was more effective than the dose of 20 mg/kg of the extract in reducing the jumping but equally effective in reducing the totality of the withdrawal symptoms. These data show new vistas for the role of salidroside in the prevention and treatment of withdrawal symptoms induced by morphine, confirming its essential role in the effects of *Rhodiola rosea* L. on opioid dependence.

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Bee propolis in the treatment of *Helicobacter pylori*: in the right way to clinical application

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Helicobacter pylori (HP) is the infectious agent that cause the most common bacterial infection overall, over a billion people worldwide. Common gastric diseases, like chronic gastritis and duodenal ulcer and the most serious gastric lymphoma are strictly linked to HP infection with a correlation up to 90% [1]. The pathogenetic factors of HP, such as ureases, CagA and VacA proteins, determine a massive inflammatory response in the stomach and a progressive damage of mucosa [2]. Currently, HP infection treatment consists of two or three antibiotics and a proton pump inhibitor, even if eradication rate is statistically estimated not to be over 80% [3]. Clinical studies indicate that antioxidant supplementation increases HP eradication rate and drugs tolerability [4] and recently we experienced the possible role of natural products for gastro-protection, particularly polyphenols rich extracts [5], capable of antibacterial, anti-inflammatory and antioxidant activities. We focused our work on bee propolis and first comparing *in vitro* anti-HP activity of many different propolis dry extracts typologies and confirming that antimicrobial efficacy is strictly related to redox and antiradical properties of products [6], we deeply investigated a standardized propolis extract (50% total polyphenols) (SPE). SPE showed a very good antimicrobial activity both against metronidazole and clarithromycin resistant culture type HP strains and clinical isolates (with a strain from gastric cancer biopsy), against CagA+ and VacA+ strains with identical minimal inhibitory and bactericidal concentrations for all the tested HP strains (MIC=625 mg/l; MBC=1250 mg/l). Further *in vitro* tests highlighted that antimicrobial efficacy of SPE was not related to the bacterial concentration and that bactericidal effect was recorded after 16 hours of incubation and sustained up to three days of MBC monitoring. We furthermore tested SPE with all the antibiotics of I and II line therapy also considering levofloxacin with a checkerboard technique according to EUCAST documents [7] and we recorded synergistic effect in most cases or at least additive effect against metronidazole and clarithromycin resistant HP strains. These findings outlined a consistent profile of effectiveness of propolis against HP and as gastro-protective agent, ameliorated by the previously demonstrated anti-inflammatory and immunomodulant properties of this product [6] and permitted to obtain the authorization for a large clinical study in San Marino that will start in the autumn 2014 that will consider the supplementation of propolis to the conventional therapy to increase HP eradication rate and minimize side effects of antibiotical treatment.

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Evaluation of the interaction of plant polyphenolic derivatives with STAT1 as a likely mechanism of their inhibitory effect on cytokine signalling pathways

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STAT (Signal Transducer and Activator of Transcription) proteins are a family of latent cytoplasmic transcription factors which mediate broadly diverse biologic processes, including cell growth, differentiation, apoptosis, fetal development, transformation, inflammation, and immune response.[1] Recently, epigallocathechin-3-gallate (EGCG) has been demonstrated a strong and selective inhibitor of STAT-1 activation.[2]
In the frame of a project aimed at investigating the affinity of plant polyphenolics for STAT-1, flavonoids like myricetin and delphinidin,[3] proanthocyanidins or condensed tannins[4] were found to exhibit a good affinity for STAT-1.
Garcinol, a polyisoprenylated benzophenone isolated from *Garcinia* genus,[5] has been reported to exert anti-inflammatory activity by curtailing cytosolic phospholipase (cPL)A2 activation as well as COX-2 and iNOS expression and NO production in LPS-stimulated macrophages.[6]
In order to provide deeper insight into the effects of garcinol on cytokine signaling pathway and clarify the underlying molecular mechanisms, the extraction of garcinol from the fruits of *Garcinia cambogia*, has been carried out.
The fruits of *G. cambogia* afforded, as the main compounds, not only garcinol, but also guttiferones K, and guttiferone M,[5] two polyisoprenylated benzophenones which differ each other for the isoprenyl moieties and their position on the benzophenone core. The affinity of garcinol, guttiferones K and M for STAT-1 has been evaluated by Surface Plasmon Resonance (SPR) and molecular docking studies. The equilibrium dissociation constants K_D obtained indicated that garcinol and guttiferones have a good affinity for STAT-1, when compared to that reported for ECGC.[4] Molecular docking data of these compounds with the protein STAT-1 were in good agreement with the SPR results.
These compounds, by interaction with STAT-1, might interfere with cytokine signaling and consequent induction of pro-inflammatory genes. Thus, the isolated compounds have been tested for their ability to modulate cytokine signaling in MDA-MB-231 and INS-1E cell lines.
The obtained data showed that garcinol was able to inhibit IFN-γ -induced STAT-1 as well as TNF-α -elicited NF-kB activation in both cell lines in a dose dependent manner, as assessed by evaluation of DNA binding of the two transcription factors by EMSA. Guttiferones K and M also exerted an inhibitory effect on cytokine signaling pathways, but with differences in different cell lines.

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Wild Mediterranean Dietary Plants as Anti-Obesity Agents

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Obesity, which is characterized by excessive fat accumulation, occurs by fat absorption by lipase and sequential fat accumulation in adipocytes through adipocyte differentiation. Thus, inhibition of pancreatic lipase activity and adipocyte differentiation would be crucial for the prevention and treatment of obesity. Pancreatic lipase is considered as one of the safest target for diet-induced anti-obesity drug development. Natural products identified from traditional medicinal plants and microbial sources have always presented an exciting opportunity for the development of new types of therapeutics. About half of all compounds that have been successful in clinical trials during the past 20 years have been derived from natural origin. Despite this scenario, Orlistat, a semi-synthetic derivative of lipstatin (a natural product), is the only pancreatic lipase inhibitor approved for anti-obesity treatment till date and P57, an appetite suppressant, is in clinical trials for obesity.

Many plants are known to have not only nutritive and taste values but also physiological effects, as they are prescribed in various traditional preparations. Numerous trials have been conducted to find and develop new anti-obesity drugs through herbal sources, in order to minimize side effects associated with the present anti-obesity drugs. One of the most important strategy in the treatment of obesity includes the development of nutrient digestion and absorption inhibitors, in an attempt to reduce the energy intake through gastrointestinal mechanisms without altering any central mechanism. Pancreatic lipase is a key enzyme for triglycerides absorption in the small intestine.

This enzyme is secreted from the pancreas and hydrolyzes triglycerides. Therefore, the suppression of triglycerides absorption by lipase inhibition is a major approach for preventing obesity.

For this purpose different hydroalcoholic extracts of wild edible plants from Calabria Region (Italy) were evaluated for their in vitro pancreatic lipase inhibitory activity. Our studies identified some plants, such as *Capparis sicula* and *Clematis vitalba*, as new natural sources for pancreatic lipase inhibitors and they could be strong candidates as ingredients for botanical food supplements marketed for reducing body weight and fat mass.

In addition, hydroalcoholic extracts of edible plants from the Calabria region (Italy) were evaluated for their *in vitro* antioxidant and antiradical properties and *in vitro* and *in vivo* anti-inflammatory activity.

GC-MS and HPTLC analysis were used for the identification of plant constituents.

In conclusion natural products identified from traditional Mediterranean dietary plants represent an opportunity for the development of new types of therapeutics and/or preventive agents.

New hypotheses on *Serenoa repens* (Bartram) Small extract mechanism of action through *in silico* methods

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Benign prostatic hyperplasia (BPH) is a common disease in men aged over 50 years old, with an incidence increasing to more than 80% over the age of 70 [1]. As life expectancy is in continuous growth, BPH is increasingly going to attract pharmaceutical interest.
Within conventional therapies, such as α-adrenoreceptor antagonists and 5-α-reductase inhibitor, there is a large requirement for treatment with less adverse event on blood pressure and sexual function: phytotherapy may be the right way to fill this need, offering at least three different herbal medicines registered in many countries, such as *Serenoa repens* (Bartram) Small, *Prunus africana* (Hook. f.) Kalkman and *Urtica dioica* L. extracts [2].
Particularly, *Serenoa repens* standardized extract (SRE) has been widely studied and its ability to reduce lower urinary tract symptoms related to BPH is comprehensively described in literature. Many putative mechanisms of action have been proposed, including anti-androgenic actions, inhibition of 5-α-reductase (5AR), anti-inflammatory, anti-edematous and anti-oxidant effects, and anti-proliferative influence leading to apoptosis through the inhibition of growth factors. Nevertheless, many of these effects are supported only by *in vitro* enzymatic studies, being the actual mechanisms of action of SRE to be elucidated [3].
An innovative investigation on the mechanism of inhibition of 5AR by SRE active principles has been proposed in this work through computational (*in silico*) methods, using as a template the crystal structure of human liver 5-β-reductase: the results confirmed that both sterol and fatty acids can play a role in the inhibition of the enzyme. Surprisingly, docking simulations of fatty acids showed that, although completely different in structure and chemistry, most of the lipids of the extract can bind the active site in similar position to that assumed by the endogenous substrate (testosterone), the well-known inhibitor (finasteride) and the sterols contained in SRE, thus suggesting a competitive mechanism of inhibition.
Considering that more than the 75% of the extract is made of fatty acids, it can be said that sterols are useful, but fatty acids are essential for SRE activity.
Furthermore, being the prostatic bioavailability and the computated binding energies of finasteride and SRE compound comparable, even if more studies are needed, it can be assumed that one of the mechanisms by which SRE act in reducing LUTS related to BPH is the inhibition of prostatic 5AR.
If this is true, finally, this work propose a further confirmation for the rational use of other herbal products, sharing most of the chemical composition of the drug with SRE, in the management of BPH.

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POSTER

P1. **Neuroprotection by association of palmitoylethanolamide with luteolin in experimental Alzheimer's disease models: the control of neuroinflammation**

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. Its neuropathological hallmarks include deposition of beta amyloid (Aβ) fibrils in senile plaques. Numerous biochemical events leading to Aβ neurotoxicity in AD have been proposed. Neuroinflammation plays a prominent role among these, and includes production of oxygen free radicals, activation of the apoptotic cell cascade and neuronal cell degeneration. It has been proposed that the amyloid aggregates and microglia activation are able to favor the neurodegenerative process observed in AD patients. However, the role of inflammation in AD is controversial, because in early stages the inflammation could have a beneficial role in the pathology, since it has been thought that the microglia and astrocytes activated could be involved in Aβ clearance. Nevertheless the chronic activation of the microglia has been related with an increase of Aβ and possibly with tau phosphorylation. Studies in AD brains have shown an upregulation of complement molecules, pro-inflammatory cytokines, acute phase reactants and other inflammatory mediators that could contribute with the neurodegenerative process. However, clinical trials and animal models with non-steroidal anti-inflammatory drugs (NSAIDs) indicate that these drugs may decrease the risk of developing AD and apparently reduce Aβ deposition, further studies are needed to determine whether treatment with anti-inflammatory strategies, may decrease the neurodegenerative process that affects these patients. It is currently unknown whether brain inflammation in AD patients is the cause of the disease or a secondary phenomenon.
Thus, in the present study we used *in vitro* and *ex vivo* organotypic models of AD to assess the neuroprotective effects of a co-ultramicronized formulation of the fatty acid amide palmitoylethanolamide (PEA) and the antioxidant flavonoid luteolin (Lut), nominated co-ultraPEALut (PEA:Lut ratio of 10:1). In the *in vitro* model, retinoic acid (100 nM) differentiated human neuroblastoma SH-SY5Y cells were pre-treated with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27 μM PEA) for 2 h. Cell injury was induced by $A\beta_{1-42}$ stimulation (1 μM). Twenty-four hours later cell vitality was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, comet alkaline assay and Western blot analysis for IkBα and nuclear factor-kB. Co-ultraPEALut was also tested in an *ex vivo* organotypic model of AD. Hippocampal slice cultures prepared from postnatal day 6 mice were pre-treated on day 21 with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27 μM PEA) for 2 h and then incubated with $A\beta_{1-42}$ (1μg/ml) for 24 h. Slices were then processed for MTT assay, nitric oxide assay and Western blot analysis for brain-derived neurotrophic factor (BDNF) and apoptosis inducing factor levels (AIF). The aim of the present study is to better identified the cellular and molecular mediators involved in the inflammatory process associated with AD and several possible therapeutic approaches. Taken together our results clearly show that co-ultraPEALut is able to blunt Aβ-induced astrocyte activation and to exert a marked protective effect on glial cells. These findings suggest that the association of co-ultraPEALut may provide an effective strategy for AD.

P2. ***Astragalus membranaceus*** **extracts: a new natural resource for the management of chemoterapy-induced neurotoxicity**

Corti F, Zanardelli M, Di Cesare Mannelli L, Maresca M, Micheli L, Bilia AR, Mugelli A, Ghelardini C

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Oxaliplatin is a diamine cyclohexane platinum derivative used for the treatment of several solid tumor types, especially in some cisplatin-resistant cancers. Neurotoxicity is its major dose-limiting side effect, significantly affecting the quality of life of patients. The management of chemotherapy-induced neurotoxicity is based on symptomatic drugs that do not intervene on the neurodegenerative process. The unsatisfactory therapeutic strategies reflect the lack of knowledge about the biological events at the base of oxaliplatin neurotoxicity. In a rat model of painful oxaliplatin-induced neuropathy, we previously highlighted the oxidative stress as a relevant biochemical alteration induced by the antineoplastic drug and directly involved in pain development.
Astragalus membranaceus (Fisch.) Bge. is an adaptogenic plant from the traditional Chinese medicine and the root of this herb shows cytoprotective properties based on antioxidant activity. Aimed to evaluate the antineurotoxic properties of this plant, aqueous, alcoholic and hydroalcoholic extracts from selected roots of *A. membranaceus* were obtained and analyzed in an *in vitro* model, focused on the oxidative stress, of oxaliplatin neurotoxicity.
Focusing on different constituents of the central nervous system, we used the neuronal-derived cell line SH-SY5Y and primary cultures of rat cortical astrocytes. Oxaliplatin significantly increased superoxide anion production and induced lipid peroxidation (malonyl dialdehyde levels), protein (carbonylated proteins) and DNA oxidation (8-OH-2-dG levels). Astragalus extracts (50 μg/mL) were able to reduce the oxidative damage in both cell types. In particular, in astrocyte cell culture, the aqueous extract reduced by 55% the superoxide anion ($O_2^{\cdot-}$) production evoked by oxaliplatin; the hydroalcoholic extract reduced $O_2^{\cdot-}$ by 35% and the alcoholic one by 15%. The hydroalcoholic extract was the most active one in preventing the oxaliplatin-dependent apoptotic process evaluated as caspase 3 activity. Finally, the protective effect of *A. membranaceus* did not interfere with the oxaliplatin antineoplastic in vitro mechanism as evaluated on the human colon adenocarcinoma cell line (HT29).
The hydroalcoholic extract of *Astragalus membranaceus* promotes the rescue mechanisms that protect nervous tissue from the damages triggering chronic pain. Its well documented safety profile strongly suggests the usefulness of this natural product in oxaliplatin-induced neurotoxicity.

P3. **Attenuation of morphine tolerance by an extract of *Astragalus membranaceus* root**

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Morphine, the natural alkaloid from *Papaver somniferum*, is the analgesic drug most commonly used for moderate to severe pain management. In spite of the potency and efficacy of morphine, its clinical application for chronic persistent pain is limited by the development of tolerance to its antinociceptive effect. To overcome tolerance, increasingly large doses of morphine are required to elicit effective analgesia, thus enhancing the severity of side effects. The specific mechanisms underlying the development of morphine tolerance is still unclear and compounds able to prolong morphine antinociceptive efficacy are lacking. Recently, the involvement of glial cells has been suggested to play a role in the phenomenon since spinal microglia and astrocytes are activated in morphine-tolerant animals. Moreover, experimental glia inhibition is able to delay the development of tolerance.
Astragalus membranaceus (Fisch.) Bge. is able to prevent glial activation in models of chronic pain suggesting a glia regulatory effect. On this basis the hydroalcoholic extract from selected roots of *Astragalus membranaceus* (Epo, Italy, 70% EtOH, D.E.R. 3:1) was tested in morphine-treated rats.
Group 1 was daily treated with 600 mg kg^{-1} Astragalus extract per os (Astragalus + morphine treated rats), while group 2 received the vehicle (vehicle + morphine treated rats). On day 1 morphine treatment (10 mg kg^{-1} intraperitoneally) was started in both groups. Pain threshold measurement was daily performed by Paw pressure test 30 min after morphine injection. Morphine induced a significant analgesic effect till day 8 in group 2. On the contrary the alkaloid was effective up to day 16 in group 1, significantly delaying the development of tolerance. Astragalus extract did not show per se any analgesic effect. In order to evaluate the role of glial cells in Astragalus mechanism, the density of Iba1- (microglia) and GFAP- (astrocytes) positive cells were measured in the dorsal horn of the spinal cord. In group 2 (vehicle + morphine) the onset of tolerance was characterized by an increase of both microglia and astrocyte cell number. In the group 1 Astragalus treatment did not prevent the increase in glial cell density.
The hydroalcoholic extract of *Astragalus membranaceus* is able to double the duration of morphine antinociceptive efficacy by a mechanism that do not seem related to glia modulation. These data support a possible application of this natural product in opioid-based therapies.

P4. **Effect of cannabidivarin in a model of beta-amyloid induced toxicity**

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Alzheimer's Disease (AD) is the most common form of neurodegenerative disorders. The typical symptoms of AD include short and long term memory loss, irritability and confusion. The hallmarks of AD are the deposition of β amyloid peptide (Aβ) aggregates and neurofibrillary tangles of hyperphosphorylated *tau* protein. Several evidences show that Aβ deposition induces a severe reactive gliosis and neurotoxic effects in *in vivo* models of AD. Gliosis is a common feature in neurodegenerative diseases since activated glial cells release inflammatory and neurotoxic mediators partly accountable for AD signs and symptoms (*Guillot-Sestier MV et al. 2013. CNS Neurol Disord Drug Targets. 12(5):593-607*). Nowadays, cannabinoids (CBs) are considered promising candidates for the AD therapy because of their anti-inflammatory, anti- apoptotic and anti-oxidant effects. Evidence exists that CBs can reduce Aβ-dependent activation of microglia and astrocytes, thus providing neuroprotective effects. In particular, several experimental data pointed out the properties of cannabidiol (CBD), a non-psychotropic phytocannabinoid, to reduce the Aβ aggregation and toxicity *in vitro*, thus resulting neuroprotective in *in vivo* models of AD (*Iuvone T et al. 2009. CNS Neurosci Ther. 5(1):65-75*).
These findings encouraged us to carry on the study of the effect of CBD-related phytocannabinoids on Aβ toxicity. In particular, we focused our attention on cannabidivarin (CBDV), which resulted the most effective compound in reducing the proliferative and inflammatory response of glial cells stimulated with Aβ in our preliminary experiments.
Aim of the present study was to investigate CBDV effects in an *in vivo* model of AD (*Maurice T, et al. 1996. Brain Res. 706(2):181-93*). Male C57/BL6 mice received an icv injection of aggregated Aβ peptide (0,5μg/μL) and then were chronically treated with CBDV (20 mg/Kg). On the 21st day after the Aβ icv injection a behavioral test was performed and mice were euthanized. The ipsolateral hippocampus was isolated from the brains and the tissue homogenates were subjected, respectively, to western blot and ELISA assays. In our experiments, CBDV showed reduced glial cell proliferation induced by Aβ, evaluated as the amounts of GFAP and S100B protein. Moreover, the inflammatory response in Aβ-treated animals was lowered by CBDV treatment, as shown by reduced COX2 expression in CBDV-treated vs un-treated control mice. These results were paralleled by results obtained in the behavioral test. In fact, CBDV treatment resulted in an amelioration of cognitive performaces in comparison to un-treated mice in Y-maze test. Finally, CBDV treatment was able to restore the deregulation of endocannabinoid levels induced by Aβ.
Further experiments will be necessary to clarify CBDV mechanism of action in order to propose this phytocannabinoid as a new pharmacological tool in approaching AD.

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P5. ***Olea Europea*-derived phenolic products attenuate antinociceptive morphine tolerance: an innovative strategic approach to treat cancer pain.**

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Morphine and related opioid drugs are currently the major drugs for severe pain. Their clinical utility is limited in the management of severe cancer pain due to the rapid development of tolerance. Restoring opioid efficacy is therefore of great clinical importance. A great body of evidence suggests the key role of free radicals and posttranslational modulation in the development of tolerance to the analgesic activity of morphine. Epidemiological studies have shown a relationship between the Mediterranean diet and a reduced incidence of pathologies such as coronary heart disease and cancer. A central hallmark of this diet is the high consumption of virgin olive oil as the main source of fat which contains antioxidant components in the non-saponifiable fraction, including phenolic compounds absent in seed oils. Here, we show that in a rodent model of opiate tolerance, removal of the free radicals with phenolic compounds of olive oil such as hydroxytyrosol and oleuropein re-instates the analgesic action of morphine. Chronic injection of morphine in mice led to the development of tolerance and this was associated with increased nitrotyrosin and malondialdehyde (MDA) formation together with nitration and deactivation of MnSOD in the spinal cord. Removal of free radicals by hydroxytyrosol and oleuropein blocked morphine tolerance by inhibiting nitration and MDA formation and replacing the MnSOD activity. The phenolic fraction of virgin olive oil exerts antioxidant activities in vivo and free radicals generation occurring during chronic morphine administration play a crucial role in the development of opioid tolerance. Our data suggest novel therapeutic approach in the management of chronic cancer pain, in particular for those patients who require long-term opioid treatment for pain relief without development of tolerance.

P6. **Natural isothiocyanates and inhibition of mast cells degranulation: is H_2S the real player?**

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Hydrogen sulfide (H_2S) is an endogenous gasotransmitter involved in the regulation of many physiological mechanisms in the cardiovascular, respiratory, gastroenteric, endocrine and central nervous systems. Accordingly, an impaired production of endogenous H_2S contributes to the pathogenesis of important disorders[1]. Among its several functions, H_2S is deeply involved in tuning inflammation, although its role in inflammatory signalling is not yet clear[2]. To date, exogenous compounds, acting as H_2S-releasing agents, are viewed as promising and innovative pharmacotherapeutic agents. In a recent report, the H_2S-releasing properties of some synthetic aryl isothiocyanate (ITC) derivatives were reported, indicating that the ITC function can be viewed as a suitable slow H_2S-releasing moiety, endowed with those pharmacological effects typical of this gasotransmitter[3]. Noteworthy, many ITC derivatives (deriving from a myrosinase-mediated transformation of glucosinolates) are well-known secondary metabolites of plants belonging to *Brassicaceae*, a widest botanical family comprising many edible species. The phytotherapic and nutraceutic usefulness of *Brassicaceae* in the prevention of important human diseases, such as cancer and neurodegenerative pathologies, cardiovascular and inflammatory diseases, has been widely discussed in the scientific literature[4,5]. Although the above effects are largely attributed to ITCs, the exact mechanism of action is still unknown. In this experimental work, we aim first to investigate the possible H_2S-releasing properties of some natural ITCs, and then to evaluate their effect on mast cells (MC)-like RBL-2H3 cell line (Rat Basophilic Leukemia) degranulation. H_2S release was detected by amperometric recordings. The release of β-hexosaminidase has been measured as a reliable marker of MC degranulation by means of spectrophotometric assays. NaHS, used as a fast H_2S-donor reference drug, inhibited in a concentration-dependent manner the DNP-induced MC activation. IS-176, a synthetic benzothioamide, employed as a slow H_2S-donor reference drug, turned out to be able to inhibit MC degranulation with a concentration-dependent manner comparable to NaHS. As concerns natural compounds, different glucosinolates and ITCs belonging to the *Brassicaceae* family, such as sinigrin, allyl-ITC (AITC), phenyl-ITC (PhITC) and 4-hydroxy-benzyl-ITC (4-OH-BnzITC), have been tested. They all induced inhibition of MC degranulation, even if with different degrees of potency. In conclusion, the tested natural isothiocyanates exhibit significant H_2S-release and inhibition of mast cell degranulation leading us to hypothesize that H_2S may be, at least in part, the real player accounting for several biological effects of *Brassicaceae* as the anti-inflammatory one.

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P7. **The anti-inflammatory and antioxidant effects of Bergamot juice (BJe) in an experimental model of inflammatory bowel disease**

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The beneficial properties of the flavonoidic fraction of bergamot juice (BJe) have been raising interest and have been the subject of recent studies, considering the potentiality of its health promoting substances. Flavonoids have demonstrated radical-scavenging and anti-inflammatory activities. The aim of the present study was to examine the effects of BJe in mice subjected to experimental colitis. Colitis was induced in mice by intracolonic instillation of dinitrobenzene sulfonic acid (DNBS). BJe was administered daily orally (20 mg/kg). Four days after DNBS administration, colon nuclear factor NF-kB and MAP kinase phospho-JNK activation was increased as well as cytokine production such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Neutrophil infiltration, by myeloperoxidase (MPO) activity, in the mucosa was associated with up-regulation of adhesion molecules (ICAM-1 and P-selectin). Immunohistochemistry for nitrotyrosine and poly ADP-ribose (PAR) also showed an intense staining in the inflamed colon. Treatment with BJe significantly reduced the appearance of diarrhea and body weight loss. This was associated with a significant reduction in colonic MPO activity. BJe reduced NF-kB and p-JNK activation, the pro-inflammatory cytokines release, the appearance of nitrotyrosine and PAR in the colon and reduced the up-regulation of ICAM-1 and P-selectin. In addition, colon inflammation was also associated with apoptotic damage. Treatment with BJe caused a decrease of pro-apoptotic Bax expression and an increase of anti-apoptotic Bcl-2 expression. The results of this study suggested that administration of BJe may be beneficial for treatment of inflammatory bowel disease.

P8. **Antinociceptive effects of carnosol and carnosic acid, two *o*-diphenolic diterpenes from *Salvia officinalis* L.**

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Introduction: Diterpenes are secondary metabolites that can be found in higher plants, fungi, insects and marine organisms and display a great deal of biological activities. The anti-inflammatory characteristics of some members of diterpenoid family, especially those isolated from plants, have been described and mostly involve the multiple signaling pathways that are deregulated during inflammation and inflammatory pain syndrome, including nuclear factor kB, p38 mitogen-activated protein kinase and phosphatidylinositol-3-kinase. Among the multiple polyphenols identified in leaves of *Salvia officinalis* L., the o-diphenolic diterpenes carnosol and carnosic acid are most abundant and exert potent anti-inflammatory effects. Since few data are reported on the antinociceptive effects of carnosol and carnosic acid, in the present study the effects of diterpens in pain models of inflammatory pain were investigated. *Materials and methods*: Briefly, the two diterpenes isolated from leaves of *Salvia officinalis* were administrated subcutaneously (s.c.) in a dose dependent manner (1-100 μg/20μl) 30 minutes before the injections of 1% formalin (20μl; s.c.) or 1% carrageenan (50μl; s.c.) into the dorsal hind paw of the mice. Successively, antinociceptive properties of carnosol and carnosic acid were investigated using the formalin and carrageenan-induced hyperalgesia assays. *Results*: The s.c. administration of carnosol and carnosic acid increased nociceptive threshold. Carnosol and carnosic acid inhibited only the late phase of formalin test (266.6±36.40 vs 97.00±12.66; $P<0.001$ and 257.70±40.72 vs 76.94±16.15; $P<0.001$ for carnosol and carnosic acid respectively), while both compounds displayed at 3 and 4h a significant anti-inflammatory and anti-nociceptive effects (55.13±12.23 vs 89.20±15.16; $P<0.01$ and 45.12±15.16 vs 95.12±21.10; $P<0.05$ for carnosol and carnosic acid respectively) in carrageenan-induced hyperalgesia in mice. *Conclusions*: These results demonstrate that carnosol and carnosic acid present significant anti-inflammatory and also anti-nociceptive effects on chemical models of nociception that might contribute for the antinociceptive property of the *Salvia officinalis* L.

P9. **Protective effects of Cyanidin-3-O-glucoside against LPS-induced damage in Caco-2 intestinal cells**

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The intestinal mucosal barrier plays an important role in the body's protection against luminal pathogens and antigenic molecules; a dysregulation of one of its components, especially changes in paracellular permeability, can lead to severe intestinal disorders. Inflammatory bowel diseases (IBDs), the collective name for Crohn's disease and ulcerative colitis, are characterized by persistent and unpredictable attacks of inflammation of the intestine, causing weight loss, diarrhoea, rectal bleeding, abdominal pain, fever and anemia (Cao *et al*, 2013). Recent studies support beneficial effects of anthocyanins, one class of flavonoid compounds that are widely distributed in mediterranean diet, in various chronic inflammatory diseases, for example, the inflammatory bowel diseases. Inhibition of NF-kB activation by these compounds could explain part of their anti-inflammatory properties, but few data are available on the intestine.
At this aim we employed an *in vitro* model of the acute phase of intestinal inflammation using Caco-2 cells, a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells, exposed to lipopolysaccharide (LPS). LPS was found to promote gut-barrier dysfunction through an oxidative mechanism and increased epithelial permeability (Hirotani *et al*, 2008). We considered the main signalling pathways involved in intestinal inflammation, especially those mediated by the transcription factor NF-kB. We then investigated the potential beneficial effects induced by cyanidine-3-O-glucoside (C3G). Caco-2 cells exposure to LPS 100 μg/mL for 6 h induced an alteration of cellular redox state (increased ROS production), activated NF-kB proinflammatory pathway (p65 nuclear localization) and altered Caco-2 cells barrier permeability. Cells pre-treatment for 24h with C3G 20 μM was effective in preventing LPS-induced oxidative stress, and attenuated permeability changes; moreover, C3G 20 μM pre-treatment prevented LPS-induced nuclear translocation, thus inhibiting the activation of the inflammatory pathway.
Finally, our data suggest that C3G may have protective effects against LPS-mediated intestinal mucosal damage and impairment barrier function in intestinal epithelial cells.

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P10. ***Armoracia rusticana*** **reduces inflammatory response and reactive oxygen species release in LPS-stimulated macrophages**

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Horseradish (*Armoracia rusticana* Gaertn, Mey, Scherb) is a perennial crop belonging to the *Cruciferae* family. Due to its extremely pungent root, horseradish is used fresh grated as condiment for meat and fish products or into sauces. Moreover the ethno-medical uses of *Armoracia rusticana* leaves and roots have a long history. Horseradish is rich in glucosinolates (GLSs) that provide the characteristic flavour and aroma as a result of their breakdown into isothiocyanates (ITCs) and others sulfur compounds. Horseradish, as well as the other members of *Brassicaceae* family, represents a rich source of health-promoting phytochemicals, their beneficial effects have been principally attributed to the anticancer properties of GLSs and their ITCs derivatives and to complex mixture of phenolic compounds possessing antioxidant activity. Among the horseradish enzymes, myrosinase (EC 3.2.3.147), a thioglucoside glucohydrolase responsible of the hydrolysis of glucosinolates in the correspondents isotiocyanates, and peroxidase (EC 1.11.1.7), a glycoprotein commonly used as a reagent for clinical diagnosis and analytical immunoassays, are the most investigated. In this study we report the antinflammatory effect of four different genotypes of horseradish (*Armoracia rusticana* Gaertn, Mey, and Scherb) samples (GUA, FAR, MIN, TRI) harvested from a fields grown in Southern Italy (Accettura - latitude 40° 29' N, longitude 16° 9' E - Basilicata, Italy) and the contribute of mirosinase to the *Armoracia rusticana* activity against inflammatory response induced by *Escherichia coli* lipopolysaccharide (LPS) in J774A.1 murine macrophages. Our results show that, except for FAR sample, all the horseradish samples significantly reduced nitric oxide (NO) release and inducible NO synthase (iNOS) and cycloxygenase-2 (COX-2) expression in LPS-treated macrophage. In particular, among tested samples, the stronger anti-inflammatory effect was highlighted for TRI, able to induce also cellular defence mechanisms, as heme-oxygenase enzyme expression. The presence of myrosinase further enhanced TRI anti-inflammatory effect, respect to TRI sample alone. In this study we also determine, in the four horseradish samples, their antioxidant compounds content and antioxidant activity. Among tested samples, TRI shows the highest value for total phenols and flavonoids content and for antioxidant activity measured on the basis of free radical scavenging properties of $DPPH^{\cdot}$. Moreover also reactive oxygen species (ROS) release by LPS-treated macrophage resulted significantly inhibited by TRI. Considering that the four *Armoracia rusticana* samples don't show differences in sinigrin concentration, the dominat glucosinolate in this plant, their different anti-inflammatory activity may be the result of the combined effect between glusosinolates-isothiocyanates and antioxidant compounds content, these latter differently present in the four samples. In fact, the highest phenol and flavonoid contents and the highest antioxidant activity of sample TRI could explain its higher anti-inflammatory activity respect to the other horseradish samples. Thus this study reports the antinflammatory and antioxidant effect of *Armoracia rusticana,* also highlighting differences among various subculture, and suggesting its potential health-promoting benefits.

P11. **Topical anti-inflammatory activity of the aerial parts of *Glechoma sardoa* Bég.**

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Glechoma sardoa Bég. (Lamiaceae) is a perennial herb endemic to Sardinia, characterized by a prostrate stem and blue-violet bilabiate flowers arising from the leaf axils. Traditionally, its aerial parts have been used for their wound healing, antineuralgic and antirheumatic properties [1], which suggest also possible anti-inflammatory effects.
To verify the topical anti-inflammatory properties of *G. sardoa*, an ethanol extract of its aerial parts was evaluated for the ability to inhibit the phorbol myristate acetate (PMA)-induced ear dermatitis in mice [2]. The extract (100-1000 $\mu g/cm^2$) induced a dose-dependent oedema reduction, with an ID_{50} (dose inducing 50 % oedema inhibition) of 741 $\mu g/cm^2$, and was about only seven times less active than the reference non steroidal anti-inflammatory drug indomethacin (ID_{50} = 95 $\mu g/cm^2$). By column chromatography, the extract was separated in six fractions (A-F), among which fractions A, C, D and E gave a significant contribution to its anti-inflammatory activity. Phytochemical studies of these fractions by RP-HPLC, NMR and mass spectrometry techniques showed the presence of glycolipids along with a new diterpene derivative in fraction A, glycolipids in fraction B, free sugars in fraction C, while fraction D was characterized by the presence of sesquiterpenes (litseacassifolide and glechomafurane). Fraction E contained a series of phenol derivatives, such as quercetin-3-O-rutinoside, kampferol-3-O-rutinoside, chlorogenic acid, cinnamic acid and the flavonoid glucoside camaroside, never reported in the genus *Glechoma*. Fraction F was also purified yielding rosmarinic acid and its methoxy derivative shinobashiric acid B, which showed slight but significant topical anti-inflammatory activity. Further studies are in progress to investigate the anti-inflammatory activity of the most abundant compounds and the relevant mechanism of action.

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P12. **Antiproliferative activity and radical scavenging properties of *Salvia ceratophylla* and *S. hydrangea* extracts**

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Cancer is one of the main causes of death all over the world. The world health organization (WHO) estimates that 84 million people would die of cancer between 2005 and 2015.[1]
Cancers, numerous inflammatory processes that lead to cancer, and some autoimmune diseases, have been attributed to the direct or indirect effect of free radical-induced oxidative stress.[2]
Supported by a growing increase of scientific research attesting the health properties of spices, today they are used not only for their culinary use but also for their potential impact on human health.
Salvia is one of the most consumed spices, known also for their biological properties.[3-5] In this context two *Salvia* species, *S. ceratophylla* and *S. hydrangea*, were investigated for their antiproliferative activity against a panel of human cancer cell lines namely ACHN, C32, COR-L23, A549, LNCaP, and Huh-7D12.
The cytotoxicity was evaluated using the sulphorhodamine B (SRB) assay.[5] The antioxidant activity was determined as antiradical efficiency with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The total phenol and total flavonoid content was also determined. *S. ceratophylla* exhibited the strongest activity against C32 cells with an IC_{50} value of 20.8 μg/ml. Interesting results on human breast cancer and hormone dependent prostate carcinoma were found when *S. hydrangea* was applied to cell cultures with IC_{50} values of 37.3 and 31.6 μg/ml, respectively. A selective activity against tumor cells was demonstrated since both *Salvia* species not affected the proliferation of skin fibroblasts 142BR, used as control cell line. Moreover, both species showed a good radical scavenging activity with IC_{50} values of 5.3 and 5.5 mg/ml, for *S. hydrangea* and *S. ceratophylla*, respectively.
In conclusion, extracts of both *Salvia* species could be considered as potential sources of antiproliferative compounds. Additional studies, which should include the isolation of active substances and the *in vivo* experimentation, must be carried out in order to define mechanisms of action.

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P13. ***Salvia verbenaca* L. as a potential source of anticancer agents for the melanoma treatment**

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The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal genus of the Lamiaceae family, comprising about 900 species widespread throughout the world. Several species are used in folk medicine all around the world to treat microbial infections, cancer, malaria, inflammation and to disinfect homes after sickness. *Salvia verbenaca* L., known in Italy as *Salvia minore,* is a species distributed in the Mediterranean Area but it is also common in the Northern Europe. In Spain it is called "*gallocresta*" while in England "*wild english clary*" or "*vervain sage*". In Italy is found frequently throughout the territory with the exclusion of the Alps. In the Sicilian traditional medicine, *Salvia verbenaca* is known as "*spaccapetri*" and its leaves and flowering aerial parts are used to resolve cases of kidney stones. The plant is also known as bactericide against respiratory ailments, as healing in wounds and ulcers. In the continuation of our investigations on the essential oils of *Salvia* species, in this study we investigated the potential anticancer activity of the essential oil of *S. verbenaca* growing in natural sites (Piano Battaglia, Sicily). The chemical composition and the biological activity of the oil were compared with those of cultivated plants in the Botanical Gardens of Palermo. The essential oils obtained by dried aerial parts of natural and cultivated plants were analyzed by GC and GC/MS. The growth-inhibitory and proapoptotic effects of the essential oils from wild and cultivated populations of *S. verbenaca* were evaluated in the human melanoma cell line, M14. The oil from wild populations of *S. verbenaca* was strongly characterized by fatty acids (39.5%) and carbonylic compounds (21.2%), with hexadecanoic acid (23.1%) and (*Z*)-9-octadecenoic acid (11.1%) as the main constituents. The oil from cultivated plants was also rich of fatty acids (22.6%) and carbonylic compounds (17.8%), but contained a higher quantity of hexahydrofarnesyl acetone (9.7%), sesquiterpenes (15.4%) and phenolic compounds (5.5%). Both the two essential oils were able to inhibit the growth of the cancer cells examined after 72 h of treatment, but the essential oil from cultivated populations exhibited the major effects with IC_{50} value of 8.1 μg/ml. Our data also demonstrate that the essential oils induced apoptotic cell death, that could be related to an overall action of the compounds present, but in particular to phenolic and sesquiterpene compounds. In summary, these results demonstrate that the cultivation conditions influence the chemical composition of the essential oils of the *Salvia verbenaca* plants and suggest that this aromatic plant may be considered a source of molecules for the prevention and treatment of neoplastic diseases, including melanoma.

P14. ***Rubus ulmifolius*** **leaf extracts: testings on murine myeloma cells and chemical investigations**

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Rubus ulmifolius Schott is a plant widely diffused in Italy and well known as "rovo". It has been already studied both at molecular and pharmacological level and some properties have been found, including antioxidant and anti-inflammatory activities (Dall'Acqua et al., 2008) whereas fresh leaves and decoctions are used in Italian folk medicine for skin and intestinal problems (Uncini Manganelli and Tomei, 1999). Rubus leaves extract contains molecules having pharmaceutical properties such as triterpenes, fatty acids, tannins, flavonoids (Panizzi, et al., 2001).
In our lab we carried out investigations to evaluate if Rubus ulmifolius leaves could contain molecules having additional and unknown biological properties. In this view, in the present study, we investigated cell death and cytotoxicity potential properties tested on P3X myeloma murine cell model. Morphological features of treated cells were studied both by light microscopy, scanning (SEM) and transmission (TEM) electron microscopy; chemical procedures such as TLC, HPLC and Mass Spectrometry allowed to isolate and investigate the extract fraction responsible of cell death and cytotoxicity affections.

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P15. **Vinyl disulfides from asafoetida induce apoptosis of human melaloma cell lines**

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Malignant melanoma is an aggressive form of skin cancer which frequently resists chemotherapy, therefore the search for new agents for its treatment is of great importance.
Ferula assa-foetida L. is a major source of asafoetida, a foul-smelling gum-resin of dietary and medicinal relevance. Asafoetida is characterized by an unpleasant sulfurous odor, reminiscent of garlic, rotten meat and sweat (Mahendra, and Bisht, 2012).
In this study, several vinyl disulfide compounds, isolated and purified from asafoetida, were assessed for their cytotoxic effect on human melanoma cell lines.
The potential anti-proliferative effect of the vinyl disulfides was tested in vitro on four different human melanoma cell lines: A375, PES43, SK-Mel-5 and SK-Mel-28. Among all the compounds tested the most effective in suppressing proliferation of melanoma cells resulted the RTFA16C compound. Moreover, among the cell lines used the most sensitive resulted to be the PES43 whose growth was inhibited by RTFA 16C (10-30-100 μM) by 24%, 57% and 70% respectively at 72 h. Thus, this cell line was selected for the subsequent molecular studies.
In order to understand whether the anti-proliferative effect of the vinyl disulfide was due to the induction of apoptosis a western blot analysis was carried out on the whole cell extracts of PES43 treated with RTFA 16C 30 μM for 3-6 and 24 h. Our results demonstrated an activation of caspase-3 and cleavage of its substrate poly (ADP ribose) polymerase (PARP-1) suggesting the involvement of the apoptotic process.
It is well known that in melanoma constitutive activation of NF-kB confers tumour survival capacity and escape from apoptosis (Ueda and Richmond, 2006). Thus we hypothesized that the RTFA 16C induction of apoptosis was associated with suppression of NF-kB activation.
As hypothesized, western blot analysis carried out on the nuclear extracts of PES43 cells incubated with RTFA 16C for 3-6-24h resulted in a time-dependent reduction of p65 nuclear translocation and activation. Moreover, the expression of the anti-apoptotic proteins c-FLIP, XIAP and Bcl-2, that is transcriptionally regulated by NF-kB (Ben-Neriah et al., 2011), was greatly reduced following treatment of cells with RTFA 16C (30 μM) for 3-6 and 24h.
Two of the most frequently deregulated pathways in melanoma are Mitogen-Activated Protein Kinase (MAPK)/ERK and Phosphoinositide 3-Kinase (PI3K)/AKT (Hodis et al., 2012). These two pathways play an important role in melanoma development and progression and are involved in the mechanism of resistance to targeted therapy. Western blot analysis revealed that treatment of PES43 cells with RTFA 16C (30 μM) inhibited the phosphorylation and activation of both AKT and ERK at all time considered (3-6-24h).
Induction of apoptosis by RTFA 16C in human metastatic melanoma cell line has high pharmacological value and NF-kB inhibition is considered a very promising strategy to improve the fight against cancer.

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P16. **Natural sesquiterpenes inhibit the genotoxicity induced by cigarette butts in the bacterial reverse mutation assay**

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Tobacco smoke is one of the greatest threats to human health and the leading cause of preventable death in the industrialized society [1]. Among tobacco-containing products, cigarette represents the most commonly used. After cigarette combustion, a waste portion, defined as cigarette butt, is discarded. It consists of a filter, which retains in the matrix toxic smoke components, among which carcinogens and irritant substances [1]. In spite of their benefits to smokers, cigarette filter pose a serious litter and toxic waste disposal problem, due to their not biodegradability and the persistence of many toxic chemicals. Therefore, cigarette butts need to be manipulated as special waste, with potential risks to human and environmental health [2]. In present study, the genotoxicity risk of the cigarette butts was evaluated in the bacterial reverse mutation assay. Then, the ability of the natural sesquiterpenes β-caryophyllene (CRY) and β-caryophyllene oxide (CRYO) to inhibit their mutagenicity was studied, as a possible prevention strategy. A methanolic extract from the smoked Marlboro Silver cigarette residues (CBE, cigarette butt extract) was used as butt sample. The mutagenic effect of CBE was evaluated on *Salmonella tiphymurium* TA98 and TA100 and *Escherichia coli* WP2*uvr*A strains, both in the absence and presence of an exogenous CYP450-enriched metabolic activator (S9). Thereafter, the antimutagenicity of CRY and CRYO was studied against CBE. In order to study the potential mechanism involved in the antimutagenicity of the extracts, three different protocols (pre-, co- and post-treatment) were applied. Results obtained showed that CBE produced mutagenic effects (increasing about twice the the number of revertant colonies) in all the strains tested but only in the presence of the exogenous metabolic activator S9. The concentration of 0.3 mg/ml produced a sub-maximal mutagenic effect in all strains and was used in the antimutagenicity assay. Both sesquiterpenes (CRY 0.43-1.7 mg/m and CRYO 0.09-0.34 mg/ml) significantly reduced the CBE-induced revertant colonies, although with different potency and specificity. Antimutagenicity of CRY and CRYO was similar in *S. tiphymurium* strains, in all treatments. In contrast, CRYO was the most active compound in *E. coli* WP2uvrA, being the antimutagenic effect strong at all concentrations and protocols, while the CBE-mjutagenicity inhibition produced by CRY was mostly moderate. In cell survival experiments, test compounds never produced cytotoxic effect s in the presence of CBE. Present results allow hypothesize the involvement of multiple mechanisms (both desmutagenic and bioantimutagenic) in the antimutagenicity of the sesquiterpenes tested [3]. Taking into account the potential toxicity due to cigarette butt exposure, also considering the mutagenic power here highlighted, CRY and CRYO appear to be possible further candidates as environmental decontaminants against this hazardous waste.

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P17. **Effects of polyphenol hydroxytyrosol, an oleuropein metabolite, on hepatic inflammation and oxidative stress in a rat model of NAFLD**

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Introduction: Clinical and experimental data suggested the importance of olive oil rich diet as potential nutritional strategy able to prevent obesity and related disorders including non-alcoholic fatty liver disease (NAFLD). The beneficial and protective effects of the olive oil, one of the most important component of the mediterranean diet, have been attributed largely to the hydroxytyrosol, the major metabolite of the polyphenol oleuropein [1]. **Aim:** The potential mechanisms of action of polyphenols in NAFLD are still largely unexplored. To this purpose, we used an experimental model of steatosis, feeding young rats with a high fat diet (HFD) rich in usaturated and saturated fats and treating them with hydroxytyrosol. This type of diet is used as nutritional model to induce insulin-resistance (IR) and NAFLD in non-genetically modified animals [2]. **Materials and methods**: In our experiment, we administered HFD for 6 weeks to induce the early events of NAFLD due to fat overnutrition in young animals to exclude age and gender influences. After weaning, male Sprague-Dawley rats were divided into three groups as following: 1) a control group receiving the standard diet (STD; 10.5% fat, 16.4% proteins, and 73.1% carbohydrates; 4.06Kcal/g); 2) a HFD fed group receiving vehicle (HFD; 58.0% fat, 16.4% protein, and 25.5 carbohydrates; 5.6 kcal/g); and 3) HFD-fed rats treated by gavage with hydroxytyrosol (HFD+HT, 10 mg/kg/die) [3]. After 5 weeks all rats were subjected to the oral glucose tolerance test (OGTT). After 6 weeks blood sample was collected by cardiac puncture and serum obtained. Liver tissue was excised and immediately frozen for following determinations. **Results:** HFD rats showed a marked increase in serum AST, ALT and cholesterol. Fasting glucose levels were strongly increased, and this parameter was associated to an impairment of glucose tolerance. Hepatic damage, inflammation and oxidative stress were assessed by evaluation of pro-inflammatory cytokines (TNF-α and IL-6), reactive oxygen species (ROS) production and malondialdehyde (MDA) levels. In HFD fed rats hydroxytyrosol treatment reduced serum parameters of hepatic damage and showed an euglycaemic effect in fasting rats and in OGTT. Moreover, the treatment with this polyphenolic compound reduced liver inflammation and oxidative stress decreasing the transcriptional levels of TNF- α and IL-6, and the production of ROS and MDA. Finally, hydroxytyrosol treatment was able to ameliorate hepatic functionality preventing nitrosylation of liver proteins and restoring PPAR- α mRNA amount. **Conclusions:** Our data demonstrate a protective effect of hydroxytyrosol in preventing and limiting the early inflammatory events responsible of insulin-resistance and steatosis onset. In particular, this oleuropein derivative was able to reduce hepatic inflammation and oxidative damage and to restore glucose homeostasis. So, we can conclude that beneficial effects of mediterranean diet assumption might be, at least in part, related to hydroxytyrosol presence in olive oil. Indeed, consumption of olive oil enriched in hydroxytyrosol could be considered an available strategy to prevent liver steatosis and its related complications.

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P18. ***Betula aetnensis*** **Rafin extract in streptozotocin-induced diabetes**

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Several studies suggested that the increased oxidative stress plays an important pathogenic role in development and progression of diabetes; in fact oxidative stress may cause damage of cellular membranes and changes in structural and functional integrity of subcellular organelles which may contribute to development of diabetic complications [1-3].
Much attention has been focused on phytoconstituents present in fruits, vegetables, and medicinal herbs that may be helpful in preventing complications related to metabolic syndrome.
In view of results of our recent study indicating that *B. aetnensis* Rafin. (Birch Etna) extract possesses significant antioxidant activity, in this study we investigated the effects of *Betula aetnensis* bark alcoholic extract in streptozotocin (STZ)-induced diabetic rats [4].
Betula aetnensis Rafin. (Birch Etna) is a medium-sized deciduous tree, typically reaching 5–20 m tall, which belongs to the family *Betulaceae*. It grows on the eastern slope of Etna, at an altitude between 1200 and 2000 m [5,6]. Many *Betula* species are used in folk medicine to treat skin diseases, infections, inflammations, rheumatism and urinary disorders [7]. Nearly, all species contain flavonoids, tannins, saponins, sterols and pentacyclic triterpenoids, such as betulin, betulinic acid and ursolic acid which have shown multiple biological activities with apparent effects on glucose absorption/uptake, insulin secretion, diabetic vascular dysfunction, retinopathy and nephropathy [8].
In the present study anti-hyperglicemic activity of *Betula aetnensis* Rafin. bark alcoholic extract was evaluated. In addition several markers of oxidative stress were determinated in liver, pancreas and brain.
Results obtained in the present study confirm that *Betula aetnensis* Rafin. exhibits interesting health promoting properties suggesting that protective effects of *Betula aetnensis* Rafin. may be not verely due to its antioxidant capacity.

In Memoriam
This work is dedicated to the memory of Prof. Liliana Iauk

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P19. **Effects of *Tithonia diversifolia* (Hemsl.) A. Gray extract on adipocyte differentation of human mesenchymal stem cells**

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The genus *Tithonia* (Asteraceae) comprises 13 taxa, which are distributed in eleven species. *Tithonia diversifolia* (Hemsl.) A. Gray is native to Mexico and also grows in parts of Africa, Australia, Asia, and other countries of North America. *Tithonia diversifolia* and its extracts are traditionally used for the treatment of diabetes, diarrhea, menstrual pain, malaria, hematomas, hepatitis, hepatomas, and wound healing [1]. Recently it has been suggested that these effects might be ascribed to terpenoids and flavonoids contained in the aerial parts of *Tithonia diversifolia* [2]. Several studies investigated anti-inflammatory, analgesic, antimalarial, antimicrobial and antidiabetic activities [3], however studies are needed in order to understand the molecular modes of action of *Tithonia* and its extracts.
There is increasing interest on the in vivo protective effects of natural compounds contained in plants against oxidative damage caused from reactive oxygen species (ROS).
When free-radical formation exceeds protective antioxidant mechanisms, or the later are compromised, oxidative stress occurs; increasing evidence from research show that oxidative stress is associated with the pathogenesis of obesity and it has been demonstrated that *in vitro* pre-adipocyte proliferation and differentiation can be controlled by redox metabolism [4] suggesting that ROS may be involved in adipocyte differentiation.
The 5'-adenosine monophoshate-activated protein kinase (AMPK) has been proposed to act as a main metabolic switch in response to changes in cellular meabolism [5]. AMPK also acts as a fuel sensor in regulating glucose and lipid homeostasis in adipocytes by many additional effects both on genes and specific enzymes [6].
In the present study the total phenolic and flavonoid contents of aqueous, methanol and dichloromethane extracts of leaves of *Tithonia diversifolia* (Hemsl.) A. Gray were determined; furthermore, free radical scavenging capacity of each extract and the ability of these extracts to inhibit *in vitro* plasma lipid peroxidation were also evaluated. In order to test the hypothesis that *Tithonia* extract may also affect adipocyte differentiation, human mesenchymal stem cell coltures were treated with *Tithonia diversifolia* aqueous extract and cell viability, free radical levels, Oil-Red O staining and western bolt analysis for HO-1 and AMPK were carried out. Results obtained in the present study provide evidence that *Tithonia diversifolia* (Hemsl.) A. Gray exhibits interesting health promoting properties, resulting both from its free radical scavenger capacity and also by induction of protective cellular systems involved in cellular stress defenses and in adipogenesis of mesenchymal cells.

In Memoriam
This work is dedicated to the memory of Prof. Liliana Iauk

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P20. **The effect of bergamot-derived polyphenolic fraction on LDL small dense particles and non alcoholic fatty liver disease in patients with metabolic syndrome**

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The occurrence of Metabolic Syndrome (MS) represents an independent risk factor for developing cardiovascular disease states in patients suffering from type 2 diabetes mellitus. Moreover, both the size of LDL particles and liver dysfunction identified as non alcoholic fatty liver disease (NAFLD) represent important biomarkers for the development of cardiometabolic risk in patients with MS. We studied the effect of bergamot polyphenolic fraction (BPF) in patients with MS and NAFLD. 107 patients were enrolled at the San Raffaele IRCCS (Rome). All of them showed ultrasonografic evidences of NAFLD and at least three out of five previous identified criteria for the diagnosis of MS. Patients were divided into two groups: one receiving placebo and the second receiving BPF 650 mg twice a day for 120 consecutive days. In the group receiving BPF 650 mg twice a day, a significant reduction of fasting plasma glucose, serum LDL cholesterol and triglycerides alongside with an increase of HDL cholesterol was found. This effect was accompanied by a significant reduction of both ultrasonographic and metabolic biomarkers of NAFLD as well as by a decrease of small dense LDL particles.
Overall, our data show that bergamot-deriving polyphenolic fraction given in patients with MS and NAFLD, leads to concomitant amelioration of the lipemic and glycemic serum profile and to substantial reduction of liver steatosis. This effect, alongside with a reduction of pro-atherogenic small dense LDL and enhancement of anti-atherogenic high dense HDL, shed new light on the potential use of bergamot-extract for reducing cardiometabolic risk in patients with MS.

P21. **Bone regeneration in critical-size defects of rat calvaria treated with Human Amniotic Fluid Stem Cells seeded into a collagen scaffold: effect of the oral administration of Ferutinin**

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A great challenge for regenerative medicine is the repairing of bone loss in a wide range of disease including osteoarthritis, osteoporosis, osteogenesis imperfecta along with traumatic injury and orthopedic surgery. Critical-size bone defects are not capable of repairing itself. In this perspective many scaffolds have been investigated as potential alternatives to bone graft for bone defect repair. Among them collagen type I, the major component of extracellular matrix (ECM), is the most popular biologic material used to produce tissue-engineered grafts because of its high availability, easy purification from living organisms, non-antigenicity, non-toxicity and biological plasticity. Moreover bone tissue engineering merges scaffolds with cells and growth factors to create a tissue engineered construct to enhance bone regeneration. We have already demonstrated that a construct, consisting of collagen type I and human amniotic fluid stem cells (AFSCs) predifferentiated towards osteogenic lineage, is a good system for bone lesion repair (Maraldi et al., 2013).
This study aims to evaluate the bone regeneration in a critical size bone defect of rat calvaria treated with this construct after oral administration of ferutinin at the dose of 2 mg/kg/5 ml, solubilized in 0.5% Tween 80 and water. Ferutinin is a daucane sesquiterpene from the roots of *Ferula hermonis* Boiss with estrogenic properties and able to stimulate the proliferation and osteogenic differentiation of AFSCs (Zavatti et al., 2013).
In male rats (n=10) 12 weeks aged we performed two symmetric full-thickness cranial defects on each parietal region and a scaffold was implanted into each cranial defect. Rats were divided into four groups: 1) collagen scaffold, 2) collagen scaffold + ferutinin, 3) collagen scaffold + AFSCs, 4) collagen scaffold + AFSCs + ferutinin. Rats were sacrificed after 4 weeks and calvarias were removed, fixed in 4% paraformaldehyde, embedded in paraffin and cut in sections of 7 μm thickness. Histomorphometric analysis on H & E stained sections showed a significant increase (about 30%, $p<0.0001$) in the regenerated area of the 4th group compared to the others. Immunohistochemistry performed with human anti-mitochondrial antibody showed the presence of AFSCs after four weeks from the transplant. Immunofluorescence analysis revealed the presence of osteocalcin (a marker of osteogenic differentiation), estrogen receptors (ERα and GPR30) in all groups with a greater expression of all markers in samples where the scaffold was treated with AFSCs and rats were orally administered with ferutinin.
Our results demonstrated that the construct collagen type 1-AFSC, in association with an oral administration of ferutinin, is able to successfully regenerate bone in a critical-size bone defect in an animal model.

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P22. **Reinstatement of cocaine conditioned place preference induced by social defeat stress is blocked by *Hypericum perforatum* L.**

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Cocaine addiction has become one of the most serious problems worldwide however, there are no approved medications for this disorder. A novel approach to the treatment of adverse effects of drugs of abuse is one which makes use of natural products. *Hypericum perforatum* L. (St John's wort) possesses antidepressant effects and inhibits the re-uptake of norepinephrine, serotonin and dopamine, relevant neurotransmitters for the behavioural effects and addictive properties of cocaine. The objective of the present work was to test the effectiveness of *Hypericum perforatum* standardized extract (hypericum), containing 0.3% hypericin, as a treatment for cocaine addiction in an animal model. The conditioned place preference (CPP) paradigm is used to assess the rewarding properties of drugs and reinstatement of CPP after extinction is a model of the relapse that characterises drug addiction. Besides cocaine priming, stress can induce the reinstatement of cocaine CPP. Since emotional stressors are primary activators of a stress response in humans, social defeat in an agonistic encounter is considered a stressor of ecological and ethological validity in rodents, that closely mimics real-life situations in a human context. We evaluated whether treatment with hypericum altered the reinstatement of cocaine CPP induced by social defeat stress in mice. Four groups of adult male mice of the OF1 strain (n = 8-9) were conditioned with 25 mg/kg of cocaine following an unbiased procedure of place conditioning with 3 phases: Pre-conditioning (Pre-C, days 1-3), Conditioning (days 4-7) and Post-conditioning (Post-C, day 8). After the Post-C test, animals underwent an extinction session every week which consisted of placing the animals in the apparatus for 15 min. After confirmation to extinction, the reinstating effects of social stress (alone or with hypericum) were evaluated. Reinstatement tests were the same as those for Post-C (free ambulation for 15 min), except that the animals were tested after inflicting social defeat. The four groups of mice received vehicle, 75, 150, or 300 mg/kg of hypericum extract i.p. 30 min before the reinstatement test, performed immediately after social defeat. The social stress consisted of a 10-min agonistic encounter in a neutral cage with a defeat result for the experimental mouse. Each experimental mouse was confronted with an aggressive opponent and presented avoidance/flee and defensive/submissive behaviours after suffering aggression (threat and attack) from an opponent. Data were analysed with an ANOVA with a variable "Hypericum" with four levels (0, 75, 150 and 300) and "Days" with four levels (Pre-C, Post-C, Extinction and Reinstatement). All groups showed CPP in Post-C (a significant increase in the time spent in drug-paired compartment in Post-C respect to that spent in Pre-C) and similar values of extinction. Mice exposed to social defeat alone showed a reinstatement of CPP after social defeat exposure. Hypericum dose-dependently reduces the reinstating effects of social defeat. We hypothesised that the blockade of stress-induced reinstatement of cocaine CPP by hypericum can be related with their antidepressant effects and inhibition of catecholamine reuptake. Although more studies are needed our results suggested the usefulness of hypericum as a natural treatment for cocaine dependence.

P23. **Luteolin derived flavonoids as bio-markers of *Passiflora loefgrenii* extracts**

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The genus *Passiflora* comprises about 500 species which are especially known for their peculiar flowers. Among them, *P. incarnata*, passionflower, is the most studied species. It is approved both in Europe and USA as a natural source of food flavouring and can be added to foodstuff. Moreover, the aerial parts of this species*, Passiflorae herba,* are listed in the European Pharmacopeia for the relief of mild symptoms of mental stress and as aid to sleep. In view of the important applications of *P. incarnata* for human health, several other species have been investigated for their chemical and pharmacological features. The present communication describes the chemical composition of the aerial parts of *P. loefgrenii*, garlic onion passiflora, a rare species native to Brazil, where its fruits are traditionally used as food. To the best of our knowledge, no phytochemical study on this species has been reported previously. Our research aimed to describe the flavonoid compositional profile to disclose a potential use of *P. loefgrenii* for the prevention and treatment of pathological conditions. Inspection of the metabolic profile combining HPLC-DAD and ESI-MS/MS data showed that the extract from the aerial parts contained glycosylated flavonoids with luteolin as the main aglycone. Main constituents were orientin (luteolin-8-*C*-glucoside), luteolin-6-*C*-rhamnosyl-7-*O*-glucoside and luteolin-6-*C*-rhamnoside. Some isoorientin (luteolin-6-*C*-glucoside) was also present. Comparison of the flavonoid profile of *P. loefgrenii* and *P. incarnata* showed some differences, in particular the absence in *P. loefgrenii* of vitexin (apigenin-8-*C*-glucoside) and isovitexin (apigenin-6-*C*-glucoside), used as bio-markers for *P. incarnata* and possibly implied in the sedative effect of this drug. Luteolin and/or luteolin glycosides are among the most common flavonoids present in edible plants and in plants used in traditional medicine to treat a variety of pathologies. Numerous preclinical studies have shown that they have antioxidant and anti-inflammatory properties and there are several *in vivo* studies suggesting their cancer chemopreventive potential. Thus *P. loefgrenii*, rich in luteolin glycosides, represents a good candidate to be explored for other pharmacological and therapeutic properties than the sedative one.

P24. **Clinical investigations on products of vegetal origin when used as food additives**

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The design and realization of clinical studies on products of vegetal origin doesn't present any problems in terms of rules and regulations to be applied, when such products are developed as drugs. In this case, the studies must be conducted (when in EU) according to the EMA regulations for drugs.

On the contrary, when products of vegetal origin are used as food additives, the design of the clinical investigation and the regulatory framework pose a number of practical constraints and doubts.

Products of vegetal origin can be used as food additives and can be marketed using the claims described by the Health Ministry and approved by EFSA. The use of the products of vegetal origin is derived from the traditional medicine or from the modern Evidence Based Medicine.

Clinical investigations on products of vegetal origin used as food additives should collect data of adequate quality on a proper sample size. At the same time, the cost of such investigations should be sustainable, considering that the economic margin of food additives is lower and that their lifecycle is shorter than the ones of drugs.

The author describes the use of web based systems to collect data on products of vegetal origin when used as food additives. More specifically, the author describes how to design surveys where the data are pooled at the origin so that the survey complies with the recommendations of Italian Data Protection Authority without the need of obtaining the informed consent of the individual subject. The surveys should be designed in order to guarantee the data integrity, the subject privacy, the quality of data and reliability of results.

The described systems can be used to obtain data from end-users or from healthcare professionals. These tools allow to carry on clinical investigations able to increase the knowledge on the studied products and able to generate data acceptable by the Ministry of Health and EFSA.

P25. **Production of galanthamine from *Narcissus poeticus* L.**

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Galanthamine is a benzazepine alkaloid being the most interesting for its use in the treatment of Alzheimer's disease as a cholinesterase inhibitor. The cholinergic hypothesis postulates that memory impairment in patients with Alzheimer's disease results from a deficit of cholinergic function in the brain [Lopez et al. 2002]. In the commercial drugs is present as active ingredient the galanthamine bromohydrate by chemical synthesis through an expensive and complex process.
Potential sources for large-scale extraction of the alkaloid are species of genus *Narcissus*, a monocotyledon belonging to the *Amaryllidaceae* family; the bulb usually accumulates the highest amount of alkaloids and levels of galanthamine vary between species and cultivars from trace amounts to 2.5% of dry weight [Lubbe et al, 2009].
Our group extracted essential oil from *Narcissus poeticus* L. of Rocca di Mezzo (AQ- Italy) in a locality called Terranera, at an altitude of 1300-1400 m, in grass pasture, subject in winter to snowfalls and in spring to temporary flooding because of melting snow. The climate is humid, the zone is in full sunlight and the soil is Karst [Ferri et al, 2009].
In the last two seasons we investigated the presence of galanthamine in all parts of the plant (flower, stem, bulb and rootlet) and we found discrete levels of alkaloid not only in the bulbs [Ferri et al., 2013].
In this paper we illustrate *in vitro* micropropagation of *Narcissus poeticus* L. that can represent a resource for the production of the biomolecules of interest. Some tests have been carried out for obtaining part of plants *in vitro*, with the use of phytohormones such as BAP and NAA (data in press). The organs including bulbs, microtubers, corms, somatic embryos and shoots, are suitable for mass propagation by using bioreactors. Bioreactor techniques for large-scale propagation of geophytes were described for *Gladiolus*, *Narcissus* and *Crocus* [Benschop et al., 2010].
Next step is to assess anticholinesterase activity (AChE) of galanthamine in the extracts, which may be masked or enhanced by the presence of other secondary metabolites.

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P26. **Poliphenolic content, antioxidant properties and amylase inhibition by *Capsicum annuum* L. var. "Cornetto di Pontecorvo DOP"**

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The present study investigated the potential beneficial properties of *Capsicum annuum* L. var. "Cornetto di Pontecorvo DOP" (CPP), in order to highlight its potential role as a functional food. Particularly, the antioxidant activity and the *in vitro* inhibitory potential against α-amylase of CPP were studied in relation to its polyphenolic composition. α-Amylase inhibitors have been shown to play an important role in managing hyperglycemia and diabete, by delaying carbohydrate digestion and reducing glucose absorption [1]. Hyperglycemia triggers free radical generation and development of oxidative stress. Therefore, improving the antioxidant defences is essential as a support therapeutic strategy for diabetes treatment [2]. Polyphenols, which are widespread constituents in plants and edible vegetables, among which pepper and tomato, have been shown to possess both antioxidant and α-amylase inhibitory properties [3,4]. In this context, CPP supplementations could represent a new dietary strategy for managing hyperglicemia complications. CPP vegetables, carrying DOP certification, were obtained by the producer's association at the end of August 2013. Extracts of peel (CPe), pulp (CPu), seeds (CPs) and whole fruit without seeds (CPf) were prepared by 70% ethanol maceration, then the solvent was evaporated under vacuum. The polyphenolic profiles were evaluated by high-performance thin-layer chromatography (HPTLC) and densitometric analysis; in addition, the total polyphenol amount was determined colorimetrically. Different antioxidant mechanisms, among which radical scavenging power against the synthetic DPPH and ABTS radicals, and against the ROS (oxygen reactive species) compounds, superoxide anion and hydroxyl radical, the inhibition of ROS-mediated lipid peroxidation and the ROS generation block (by reducing and/or chelating mechanisms) were studied [5]. Finally, the α-amylase inhibition was evaluated by 3,5-dinitrosalicylic acid method [1]. The HPTLC analysis showed the presence of different polyphenols in all the extracts tested, highlighted as fluorescent spots before NPR and anisaldehyde derivatization. Total polyphenols were particularly high in CPf and CPs, although a significant content was also present in CPe and CPu. As regard the antioxidant activity, the extracts CPs, CPf and CPe were in general more active than CPu, being able to strongly scavenge both the synthetic radicals and the ROS species, to inhibit the ROS-mediated lipid peroxidation and to inhibit ROS generation by chelating mechanisms. All the extracts were also active as α-amylase inhibitors. On the basis of these results, the biological activity of the tested extracts seems to be related to the polyphenol content. For instance, CPs evidenced strong radical-scavenging activity, lipoperoxidation and α-amylase inhibition, associated with a high polyphenol levels. Although further evaluations are needed for identifying the polyphenols involved in the bioactivities found, present results suggest a possible role of CPP supplementation (as both whole product or fractions) for supporting conventional diabetes therapies.

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P27. **Testings of *Myrtus communis* leaf extracts on bacteria and mammalian cells**

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Myrtus communis, known at popular level as "mirto", is a plant widely used in traditional medicine for its anti-inflammatory properties. In our Lab investigations have been carried out to establish possible further biological properties of such plant. *Myrtus communis* leaves were collected from plants and air dried; dried leaves were powdered and extracted with EtOH for 50 min in the dark. The ethanol extract was then centrifuged, the pellet discarded and the supernatant dried by a nitrogen flow. The residue was dissolved in a solution of DMSO 10% in distilled water to a concentration of 10mg/ml and filtered through a 0,22 μm cellulose syringe filter. The extract was then tested on *Bacillus cereus* and *Pseudomonas syringae pv actinidiae* in order to establish possible antibacterial properties and on murine myeloma cells to evaluate possible antiproliferative activity of the extract. Extracts of *Mirtus communis* leaves showed antibacterial properties on both tested bacteria and the proliferation on murine myeloma cells consistently decreased after *Mirtus communis* extract treatment. Presently chemical investigations for the characterization of the bioactive molecules are in progress.

P28. **Stealth PLGA nanoparticles for intracellular Curcumin release**

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Curcumin (CURC), a polyphenol extracted from the rhizome of *Curcuma Longa Linn*, possesses numerous pharmacological activities, including anti-inflammatory, antioxidant and antimicrobial. CURC can be potentially used in cancer treatment taking advantage of its ability to block the proliferation of many tumor cells. Even more interestingly, CURC seems to induce a selective cytotoxic effect mainly towards cancer cells, even in the presence of healthy cells.
Unfortunately, the pharmacological potential of CURC is severely restricted by its low water solubility/absorption, short half-life and extremely poor bioavailability. CURC encapsulation in biodegradable nanoparticles (NPs) made up of poly(lactic-co-glycolic acid) (PLGA) allowed to increase its circulation times/half-life, resistance to metabolic degradation and also promotes controlled release and targeting. It must be underlined that size, surface charge and hydrophilicity of NPs in which the drug is loaded strongly affect their pharmacokinetics. For example, if hydrophilic polymer such as poly(ethylene oxide) (PEO) is superficially exposed on NPs, it hampers the adsorption of opsonin proteins, thus conferring *stealth* properties to NPs and increasing their probability to passively accumulate in peripheral diseased sites with an enhanced vascular permeability and retention (EPR effect).
In this context, we formulated stealth PEO coated PLGA NPs by a modified double emulsion-solvent evaporation technique without any chemical reaction between the two polymers, thus avoiding the presence of chemical reaction solvents and wastes. The obtained NPs were loaded with CURC which, for the above mentioned solubility problems, was previously complexed with hydroxylpropyl β-cyclodextrin HPβCD after dissolution in ethyl alcohol (EtOH) followed by solvent evaporation. The obtained NPs were characterized in terms of size and zeta potential (ZP) by Photon Correlation Spectroscopy (PCS). Morphology was investigated by trasmission electron microscopy (TEM). Moreover, *in vitro* drug release studies were carried out to evaluate the ability of the formulated NPs to control and prolong CURC release. NP stability was evaluated by measuring their size over time both in aqueous media and serum. To evaluate CURC loaded-NPs biological efficacy, the malignant mesothelioma cell line MSTO-211H was used as a cancer model. Cell growth and cell cycle progression were analysed by Crystal Violet assay and FACS analysis.
PLGA and PEO coated PLGA NPs were spherical and with a mean size of approximately 200 nm and, for all formulations, ZP ranged between -12 and -15 mV. Morphological, ZP and size analyses confirmed an effective, hydrophilic PEO coating on NPs. This also promoted NP stability over time; indeed, NP size was roughly stable for at least 30 days in water, while immediate aggregation was observed in the case of uncoated PLGA NPs. CURC was efficiently loaded into the produced NPs and preliminary *in vitro* release studies demonstrated their ability to sustain drug delivery. *In vitro* studies on MSTO-211H cells showed that CURC loaded NPs were able to inhibit cell growth and promoted a prolonged cell cycle arrest in the G0/G1 phase, thus improving CURC bioavailability and half-life.

P29. **Caffeic Acid attenuates high glucose-induced oxidative stress and endothelial dysfunction in human endothelial cells via modulating NF-kB pathway**

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Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. The metabolic abnormalities of diabetes, mainly hyperglycemia, cause reactive oxygen species (ROS) overproduction in endothelial cells leading to endothelial dysfunction. It has been demonstrated that the transcription factor NF-kB, functionally dependent on cellular redox status, plays a key role in endothelial activation triggering adhesion molecules gene expression and then leukocyte adhesion to the endothelium.
Several studies have shown that natural antioxidants ameliorate a number of altered physiological and metabolic parameters that occur as a result of type 2 diabetes. Recent review reported that these compounds are able to induce Nuclear factor erythroid 2-related factor 2 (Nrf2) that in turn upregulates the expression of antioxidant genes involved in the protection of the cells from oxidative damage (Speciale et al, 2011). Among various phenolic compounds, caffeic acid, found in many types of fruit and coffee in high concentrations, has exhibited pharmacological antioxidant activity and is known to have an antidiabetic effect in streptozotocin-induced diabetic rats (Jung et al., 2006).
In this study we investigated if physiological concentrations of CA (10 nM) were able to protect human endothelial cells against alterations induced by high glucose levels (HG) in Human Umbilical Vein Endothelial Cells (HUVECs). Exposure of HUVECs to HG 25 mM for 24h up-regulated NF-kB nuclear translocation and reduced GSH, SOD and total antioxidant power (TAS) levels. Interestingly, CA cotreatment restored antioxidant levels and prevented NF-kB activation. Furthermore, physiological concentration of CA was able to induce Nrf2 nuclear translocation and HO-1 gene expression in HUVECs exposed or not to HG. These data support the involvement of the cellular adaptive response, activated by CA, able to counteract the damage induced by HG.
The findings might be of clinical significance as endothelial dysfunction, that could lead to cardiovascular disease, is reversed by caffeic acid consumption, as well as other dietary plant polyphenols, suggesting that this approach could be applied to the prevention of diseases associated with inflammation and oxidative stress, including atherosclerosis.

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P30. **Antioxidant and heavy metals absence in tomatoes grown in toxic muddy soils**

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Fruits of *Solanum lycopersicum*, the common tomatoes, have a high content of essential nutritive elements for human health [1]. They are rich in vitamins (C, A, B group, D, K) and minerals (P, Fe, Ca, Mg, Mn, Cu, K, Na) besides to be very rich in flavonoids and lycopene [2]. These characteristics make tomatoes the best allies against cancer disease and an essential food for a good diet [3]. Our investigation is focused on tomatoes grown in polluted soils to check their phytochemical and nutritive features. With this aim, we have extracted skin, juice and seeds from tomatoes grown in muddy soils and have verified the antioxidant properties and the anticancer activity of their bioactive metabolites, further the presence of possible heavy metals. We have performed antioxidant assay on ethanol extracts using DDPH; while the antioxidant activity of ethereal extracts has been evaluated according to ABTS assay. The results of antioxidant assay show tomatoes keep an high antioxidant activity, mostly in their lipophilic fraction, rich of the most representative compounds. Citotoxicity assay have been performed on HeLa, PDAC, A375, cells line and the results, both for chloroformic and ethanol extracts, for the seeds and for tomato juice are negative, because of they don't show any antiproliferative activity. As regards heavy metal presence, it has been evaluated using spectroscopy of atomic absorption with graphite oven, and the performed tests showed the absence of heavy metals. These results have a great scientific role, because they open a promising pathway to follow for the primary prevention of several widespread diseases.
Keywords: Antioxidant activity, tomatoes

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P31. **Capability of Trichothecene mycotoxins to induce oxidative stress in a model of intestinal epithelial cells**

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Mycotoxins are food contaminants produced by secondary metabolism of fungi found primarily in cereal grains and derived products. They are not essential to mold growth but sporadically contaminate crops, causing major economic losses every year. More than 400 different mycotoxins have been isolated and chemically characterized and data from the FAO showed that about 25% of food world production is contaminated by at least one mycotoxin. The gastrointestinal tract plays a major role in the processes of food decomposition and enzyme involved at the level of the digestive tract and its integrity is one of the most important systems in defense against ingested toxins. Following ingestion of contaminated food, intestinal epithelial cells are exposed to high concentrations of mycotoxins which could induce severe mycotoxicosis in the host. Nivalenol (Niv) is a trichothecene mycotoxin, found in cereals and processed grain, frequently associated with another widespread contaminant, Deoxynivalenol (Don). A data collection on the occurrence of Fusarium toxins in food in the European Union showed a 57% incidence of positive samples for Don and 16% for Niv, out of several thousands of samples analyzed. In this study we investigated the effects of Niv and Don, alone and in combination, on intestinal epithelial cell line (IEC-6). Oxidative stress plays often an important role in maintaining intestinal mucosal integrity: in particular in IEC-6 cells, mitochondria are the major source of reactive oxygen species (ROS) and also a sensitive target for ROS-mediated damage. Increased ROS generation causes oxidative stress and is also responsible for intestinal mucosal injury. In this study we report that both Niv and Don induced ROS production, reflecting an induction of oxidative stress state in IEC-6. Mycotoxins-induced ROS in IEC-6 have been decreased in the presence of the NAD(P)H inhibitor, DPI. In addition NAC treatment, an antioxidant which is a precursor of reduced glutathione, was able to reduce ROS production induced by Niv and Don in IEC-6, although to a lesser extent respect to DPI inhibition. Moreover in mycotoxins treated IEC-6 variations in calcium homeostasis have been also observed. Therefore, our results highlighted the risks of Niv and Don associated in food intake for the intestinal homeostasis and remark the importance of mycotoxin contamination assessment in products of vegetal source.

P32. **Antioxidant and antifungal activities of the Cameroonian medicinal plant *Annona muricata***

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Annona muricata L. (Annonaceae), commonly known as Soursop, Guanàbana or Graviola, is a small, upright evergreen tree of 5-6 m, with large, glossy, dark green leaves, which produces an edible fruit well known for its health properties. Although it is native of America, *Annona muricata* become established in many tropical countries of the world. The plant has been used in traditional medicine for many diseases, especially against infections and cancer. Effectively, it has been used in African herbal medicine systems for its sedative and antispasmodic properties, and also as hypotensive, insecticide, vermifuge, and for coughs, fevers, pain and skin diseases. Several chemical compounds have been isolated from the plant *Annona muricata*, and phytochemical investigations revealed the presence of alkaloids, tannins, coumarins, flavonoids, terpenoids. Among these compounds, researchers evidenced isoquinoline alkaloid annonaines, and acetogenins as the main active principles from Annona muricata.
The aim of this research was to investigate the antioxidant capacity and antifungal activity *in vitro* of ethanolic extracts obtained from leaf, stem and root of *Annona muricata*. The antioxidant capacity has been evaluated using assays based on different mechanisms: the Oxygen Radical Absorbance Capacity (ORAC) assay, based on HAT reaction, and DPPH radical scavenging capacity assay, based on SET reaction. We also determined the Total Flavonoid Content (TFC) and the Total Phenolic Content (TPC) for each extract. Furthermore, the antifungal activity was studied by evaluated the MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) in different strains of *Candida*.
In DPPH and ORAC assays all the *Annona muricata* extracts showed an appreciable antioxidant activity; among these, the root extract showed the highest activity both in ORAC and DPPH assays, with an EC_{50} of 18.8 μg/ml, and a TEAC value of 11282 μmol TE/g, respectively. All the extracts contain phenolic and flavonoid compounds even if in different entity, particularly, the root extract has the highest level of TPC. The antifungal assays showed for all *Annona muricata* extracts an inhibitory activity on *Candida glabrata*, and the leaf extract showed also a high activity against *Candida albicans*.
In conclusion, the present data showed that *Annona muricata* has antioxidant activity, and also could be useful to inhibit candidiasis *in vivo*. Phytochemical studies are in development to determine the compounds responsible of these activities.

P33. **Preliminary phytochemical analyses and *in vitro* evaluation of the antioxidant activity of a feed supplement containing Rose hip extract**

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Rosa canina L. (Rosaceae) is a shrub characterized by red-orange oval hips, widespread in all Europe. The hips are traditionally used in the European folk medicine especially against osteoarthritis due to their antioxidant and anti-inflammatory activity. Furthermore, *R. canina* hip water extracts are combined to hydrolysed collagen in feed supplements for horses affected by ostheoarthritis. This study was aimed to characterize the chemistry of a preparation containing a *R. canina* aqueous extract and hydrolysed collagen, with particular attention to the content of polyphenols and triterpenes, as well as to evaluate its antioxidant activity.
The preparation was submitted to ultrasonic extraction using polar solvents and the relevant extracts were screened for their antioxidant and free radical scavenging activity by Ferric Reducing Antioxidant Power (FRAP) and 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays. Each extract (20 mg/ml) showed a marked activity with respect to the reference substance, the antioxidant one (FRAP assay) being related to the total phenolic content. The antioxidant effects of these extracts were evaluated also in human monocytic THP-1 cells stimulated by phorbol myristate acetate (PMA). Almost all the extracts, devoid of significant cytotoxic (MTT assay) and ROS induction (NBT assay) effects reduced the oxidative stress induced by PMA. Phytochemical analyses of the extracts by High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) revealed the presence of low amounts of quercetin and ellagic acid.
The preparation was submitted also to sequential extraction by increasing polarity solvents using Soxhlet apparatus. HPLC-DAD analysis showed the highest concentration of flavonoids in the methanol extract after Solid Phase Extraction (SPE), quantifying quercetin at 13.4 μg/g. Furthermore, the triterpenes oleanolic acid (25.5 μg/g), betulinic acid (7.3 μg/g) and ursolic acid (93.6 μg/g) were identified and quantified in the dichloromethane extract by Gas Chromatography-Mass Spectrometry (GC-MS). Further studies are in progress to investigate the anti-oxidant activity of these extracts as well as to characterize the role of their constituents in the activity of the whole *R. canina* preparation.

P34. **Biological properties of Algerian *Thymelaea microphylla* Coss. & Dur. extracts**

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Thymelaea microphylla Coss. & Dur. is an endemic annual plant very common in arid and desert pastures. In traditional medicine, it is used against abscesses and for anticancer, anti-inflammatory and antidiabetic properties. However few is reported in the international literature on its chemical composition and bioproperties. This work aims to better study the phenolic profile and the antioxidant/free radical scavenger properties of different aqueous and organic (obtained by ethanol, acetone, ethyl acetate and hexane) extracts of leaves and flowers of *Thymelaea microphylla* collected from the region of M'sila in East Algeria.
The phenolic profile of the extracts under study was determined by means of HPLC-DAD analysis; moreover the total content of flavonoids, flavonols and condensed tannins were estimated in all extracts by means of spectrophotometric methods. Polyphenols are naturally occurring secondary metabolites in all plant materials, and play a wide range of biological effects which have been attributed to their antioxidant activity by several mechanisms. HPLC-DAD analyses revealed the presence of phenolic acids in the water extract, while the organic extracts were characterized mainly by the presence of flavonoids. According to these findings, the aqueous extract was found to be the richest in phenols while the acetone and ethyl acetate extracts had the highest content of flavonoids and flavonols, and the acetone and ethyl acetate extracts contained the highest amounts of condensed tannins.
The biochemical experiments confirmed our previous observations on the good antioxidant capacity of these extracts tested in a battery of redox-based assays differing in the mechanisms involved and the chemical environment used (Folin-Ciocalteau assay; bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical; trolox equivalent antioxidant capacity assay; ferric reducing/antioxidant power test; beta-carotene bleaching test). In these tests, the following potency order was observed: aqueous > acetone > ethyl acetate > ethanol >> hexane. Interestingly, in the beta-carotene bleanching assay (that is carried out in a linoleic acid system) the potency order was: acetone ≥ hexane >> ethyl acetate ≥ ethanol >> water. Furthermore, other assays were employed to evaluate the protective properties of the extracts under study against oxidative agents/conditions which can cause damage to cell macromolecules and have role in development of several affections such as diabetes complications and inflammation. Thus we evaluated the capacity of extracts to scavenge the superoxide anion, to inhibit the formation of advanced glycation end products (AGEs), and to prevent albumin denaturation. The aqueous extract showed the best capacity to inhibit the formation of AGEs among all extracts, while the acetone and ethyl acetate extracts were the most efficient against albumin denaturation in the superoxide dismutase - mimetic assay.
In conclusion the biological activities of *Thymelaea microphylla* extracts are most likely related to their chemical composition and to the different phenolic acids and flavonoids present in its extracts. Our data confirm that aqueous and organic extracts *Thymelaea microphylla* extractsfloweres and leaves may be a good source of bioactive compounds potentially effective against pathological conditions in which oxidative stress play a significant role.

P35. **Chemical composition, antioxidant activities and protective effects of *Sideritis italica* extract on C2C12 oxidative stress**

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The genus *Sideritis* is present in Italy with the species *S. montana* L., *S. romana* L., and the endemic *S. italica* (Mill.) (1). *Sideritis italica* is a medicinal plant used in the form of decotion of aerial parts as diuretic and digestive; the species *S. italica* was only marginally investigated. This work describes the results of chemical investigation on primary and secondary plant metabolites focused on pigments, amino acids, total proteins and main class of phenols distribution in S. *italica*. We also compared the composition of water, ethanol and hydroalcoholic extracts The antioxidant activity of the extracts was investigated in chemical assays as well as in *in vitro* assays (antiproliferative activity, ROS and DNA damage induced by hydrogen peroxide) on myoblast cell line (C2C12) as modulators of oxidative stress.
The ultrastructure of aerial parts and quantitative distribution of pigments, including chlorophylls and amino acids, as well as the main class of secondary metabolites were investigated by SEM.
SEM confirms the presence of pharmacognostic characteristics, such as glandular and non-glandular trichomes on aerial green parts. The chemical analysis indicates that the leaves are the most important part of the plant, and ethanol/water 70/30 is the preferable extraction solvent because the highest concentration of all metabolites was found in 70% ethanol extract of leaves. The presence of glandular trichomes justifies the pleasant smell of *S.italica* and the small numbers can be related to low yield in essential oil (2). The antiradical assays and the *in vitro* tests on mouse myoblast cells C2C12 confirm the biological activities of the extract. C2C12 culture medium supplemented with the extract, at doses (5-200μg/ml) not interfering with cell viability, was seen to modulate the ROS production and balance the increased oxidative stress induced by hydrogen peroxide. The treatment of C2C12 cells with 200 μg/ml of extract results in a high percentage reduction of ROS, compared to untreated and H2O2 treated groups. The quantitative reduction of 8-hydroxy-2'-deoxyguanosine, which is a biomarker of free radical DNA damage, confirms the protective effect of *S. italica* extract on oxidative stress at basal condition as well as in presence of exogenous stimuli). The results obtained in the present study support the rational base for the medicinal use of plant and extracts in modulating the free radical metabolism and balancing the oxidative stress.

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P36. **The leaves of the PGI "Nocciola di Giffoni" (*Corylus avellana* L.) as a rich source of antioxidant diarylheptanoids related to curcumin**

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In Campania region there are currently 16 registered agriculture products, among which the PGI (Protected Geographical Indication) product "Nocciola di Giffoni" (*Corylus avellana*).[1] Even if the nutritive features of this product are well documented,[2] to our knowledge little is known about its metabolome and its by-products.
Hazelnut (*Corylus avellana* L.), which belongs to the family Betulaceae, is one of the most popular tree nuts. Campania is the first Italian region in the hazelnut production, and in Salerno the 90% of the production is given by "Nocciola di Giffoni". The hazelnut hard shell, containing a kernel, is the nut of commerce and hazelnut skin, hazelnut hard shell, and hazelnut green leafy cover as well as hazelnut tree leaf are byproducts of roasting, cracking, shelling/hulling, and harvesting processes, respectively. Among these, none has any commercial value except the hazelnut hard shell, which is currently used as a heating source upon burning. Hazelnut is known as a source of nutritious food with a high content of healthful lipids while little is known about the biological activity of its by-products.[2]
In order to achieve deeper insights into the chemical composition of the waste products of the PGI Campania product "Nocciola di Giffoni" and to highlight the occurrence of biologically active phytochemicals, the investigation of the leaves has been carried out. The MeOH extract of the leaves has been purified by different chromatographic steps affording new polyphenolic compounds. Their structures have been elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, TOCSY, ROESY) experiments as well as ESIMS analysis. Isolated compounds have been determined as diarylheptanoid-type molecules, oxidized at different positions of the heptanoid chain. Within the diarylheptanoid class, curcumin, the well known polyphenolic molecule isolated from the rhizome of *Curcuma longa* (Zingiberaceae), appears as a promising chemopreventive compound able to reverse, inhibit or prevent the development of cancer by inhibiting specific molecular signaling pathways involved in carcinogenesis, and to affect molecular events implicated in inflammation.[3] Curcumin is reported to prevent lipid peroxidation, histological alterations and oxidative tissue damage.[3] In the frame of a project aimed at evaluating for diarylheptanoids isolated from the leaves of "Nocciola di Giffoni" some of the activities reported for curcumin, the effects of the MeOH extract and isolated compounds on human plasma lipid peroxidation induced by H_2O_2 and H_2O_2/Fe^{2+} have been tested and compared with those exerted by curcumin. Lipid peroxidation has been quantified by measuring the concentration of TBARS. All compounds and curcumin have been tested at concentrations ranging from 0.1 to 100 μM. Most of the compounds were more active than curcumin at the same concentration.

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P37. **Biovariability of caper species from Calabria: chemical and biological evaluation**

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The potential health benefits of active principles from fruits and vegetables against various diseases and conditions, such as diabetes, cancer and obesity, has recently gained renewed interest [1].
Many *Capparis* species are used in diet or medicine [2]. These shrubs mainly grow on rocky and sandy substrata in southern America, Europe (Mediterranean area), Africa, Madagascar, Asia, Australia, and the Pacific Islands. *Capparis* sect. *capparis* has its maximum diversity in the Mediterranean Region [3].
The phytochemical composition of *Capparis* species depends on ecological conditions at the site of collection (soil, exposition, water regime etc.). In order to cover all taxa and morphotypes present in Calabria, twenty samples of two different species of caper, *Capparis orientalis* Veill. and *C. Sicula* Veill. ssp. *sicula*. were studied.
As regards the first species, both possible habitats (sea cliffs and the walls of old buildings) were examined
(samples C4 and C10). *C. sicula* is a variable species with five subspecies. In Calabria ssp. *sicula* occurs with two morphotypes: one on calcareous rocks (C9 and C18), and the other one on buildings and clayish soil.
Aerial parts (leaves and fruits) of *Capparis* sp. were extracted through maceration. The flavonoid glycoside rutin was clearly recognized by means of HPTLC. Quantitative analyses were performed in order to underline differences in the rutin content of different samples. Moreover, extracts were assayed for their *in vitro* activity against porcine pancreatic lipase using 4-nitrophenyl caprylate as synthetic substrate. Samples showed good inhibitory activity with IC_{50} values ranging from 0.73 to 2.32 mg/ml.

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P38. **Red wine inhibits aggregation and increases ATP-diphosphohydrolase (CD39) activity of rat platelets**

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Wine is an alcoholic beverage deriving by bacterial fermentation of grapes harvested from *Vitis vinifera*. Wine, in particular red wine, has been shown to exert a peculiar cardioprotective effect compared to other alcoholic beverages; inhibition of platelet aggregation seems to be one of the mechanisms underlying this beneficial effect. The antiplatelet effect of red wine has been supposed to be due to a reduced prostanoid synthesis, an increased nitric oxide production and/or an enhanced platelet c-AMP level (1,2); however, the mechanism is still unclear. CD39 (ATP-diphosphohydrolase), an integral membrane glycoprotein metabolizing ATP and ADP to AMP that in concert with CD73 contributes to extracellular adenosine accumulation, is considered a key modulator of thrombus formation; the loss of CD39 activity from endothelium sustains platelet aggregation and thrombogenesis (3). There is evidence that moderated red wine consumption increases CD39 activity in platelets from streptozotocin-induced diabetic rats (4), thus suggesting an interaction between red wine consumption and adenosine signaling. Here we evaluated the effect of red wine on rat platelet aggregation and CD39 activity, in vitro.
We used two different red wines, "R" (GAE, gallic acid equivalent, 1.09 mg/ml) and "F" (GAE, 2.19 mg/ml), produced from Aglianico grapes in 2008 and 2011 respectively. Rat platelets were incubated with unfractionated red wine or with an equal volume (10 μl) of ethanol (12 % v/v) for 2 min at 37 °C and platelet response to ADP (1 – 30 μM) was evaluated. In another set of experiments, following incubation with red wines, as described above, on platelet lysates CD39 expression was evaluated by Western blot analysis; furthermore, CD39 ATPase and ADPase activity was evaluated as inorganic phosphate (Pi) released following incubation with ATP or ADP, quantified by a colorimetric assay and expressed as nmol Pi released *per* μg of protein.
Both wines, "R" and "F", significantly inhibited ADP (1 and 3 μM) - induced platelet aggregation. Interestingly, "F" was effective in inhibiting also the effect of high ADP concentrations (10 and 30 μM). CD39 expressed on platelets was not modified by wines. Both wines increased platelet ATP-diphosphohydrolase (CD39) activity.
Our results confirm that red wine inhibits platelet aggregation and suggest that this effect may be dependent on its ability to increase platelet CD39 activity.

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P39. **BERGA*Met*®: a phytotherapic approach to cardiovascular disease**

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The increased consumption of fruits and vegetables was considered crucial in prevention and cure against a variety of diseases, such as cancer, diabetes, neurodegenerative diseases, heart and brain vascular diseases. Many studies demonstrated that their protective properties result from the presence of low-molecular antioxidants (phenols, polyphenols and tannins) that protect the cells and their structures against oxidative damage. The identification of bioactive compounds extracted from medicinal vegetable is a promising strategy for the development of pharmaceutical product. BERGA*Met*®, formed by Citrus Bergamia Risso (endemic plant in Calabria), Olea Europea (or more commonly olive) and ascorbic acid (hydrosolubile vitamin with antioxidant properties) is a product with important phytotherapeutic properties on

- cholesterol levels
- glycemia control
- weight management

Bergamot determine cholesterol, triglycerides and glycemia control with an antioxidant action. The efficacy of the Bergamot Polyphenol Fraction (BPF) has now been established in animal models and human studies, against cardiovascular disease [1]. A significant reduction of low dense lipoprotein (LDL) was found after BPF treatment [2] [3]. The Olea Europea has a beneficial effect on lipids and carbohydrates metabolism, also with an antioxidant activity. The ascorbic acid protected against free radicals, it is essential for the correct functioning of the immune system and for the synthesis of collagen.
Cardiovascular diseases are still the main cause of death in the world. The World Health Organization estimates there will be about 20 million cardiovascular deaths in 2015, accounting for 30 percent of all deaths worldwide. Physical exercise associated with a proper diet with the aid of phytotherapic products proved an important role in the prevention and treatment of cardiovascular diseases. Therefore, the BergaMet® represents a valid strategy against cardiovascular disease.

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P40. **Effects of a single dose of a green tea extract supplement on the Peroxidation of Leukocytes Index Ratio (PLIR) of healthy subjects.**

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The Peroxidation of Leukocytes Index Ratio (PLIR) measures both the resistance of leukocytes to an exogenous oxidative stress and their functional capacity of the oxidative burst in response to activation. PLIR discriminates between reducing and scavenger activities of antioxidants and is able to appreciate the potentially dangerous effect of uric acid (UA) on innate immune response.
Some effects of Epigallocatechin-3-gallate (EGCG), the main flavonoid of Green Tea (GT), are not imputable to the antioxidant activity, but involve modulation of antioxidant enzymes and UA levels.
We aimed to evaluate the effect of a single dose of a Green Tea Extract (GTE) supplement on the PLIR of 4 healthy subjects (2 men and 2 women).
The PLIR has been calculated on an Accuri C6 BD cytometer, before (T 0), 30 minutes (T 30 min.) and 3 hours (T 3 h) after a single dose of a commercially available Green Tea Extract (GTE, 400mg EGCG). Statistical analysis was carried out with repeated measures analysis of variance (RM ANOVA), with time as within-subjects factor. Student-Newman-Keuls post hoc analysis, was used to isolate differences between groups.
Differential effects has been observed on PLIR of lymphocytes, monocytes and neutrophils After GTE consumption PLIR of neutrophils increased at T 3 h ($p<0.05$), while the increase observed in lymphocytes was near to significance ($p=0.067$) and no significant effects were found in monocytes. On the other hand, at T 30 min opposite effects were observed in men (decrease) and women (increase) in all cells.
These time, gender and cell type differential effect could be due to the regulation of gene expression, as well as of total antioxidant capacity and UA levels. We have planned to investigate these possibility and the molecular mechanisms, in a larger study, however our preliminary results suggest that the GTE modulate the redox status of leukocytes.

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