HERBAL PROTECTION Report Summary

Project ID: 316067
Funded under: FP7-PEOPLE
Country: Bulgaria

Final Report Summary - HERBAL PROTECTION (Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation)

This research has pointed towards the use of natural compounds in herbs as universal protectors or mitigators against toxicity of some deleterious agents such as mycotoxins and/or radiation. Such protective effects were seen for naturally occurring dietary ingredients, mainly containing flavonoids. Some herbs from South Africa and India were studied for their biological activity and protective effects using various cell lines or animal models. The studied herbs were from families: Fabaceae, Menispermaceae, Zingiberaceae, Leguminosae. Some of the studied herbs such as Centella asiatica, Withania somnifera, Silybum marianum, Tinospora cordifolia, Glycyrrhiza glabra, Stem bark of the trees Piptadeniastrium africanum, Millettia Laurentii, Haberlea rhodopensis, Curcuma Longa (Turmeric) and Ginger (the rhizome of the Zingiber officinale) were found to have some target anti-bacterial, anti-fungal, anti-inflammatory, immuno-stimulating and/or antioxidative activities as well as protective effects on kidneys and liver or to be useful for wound healing. Some of them were found to have a high content of flavonoids and to ameliorate gamma-radiation-induced lesions or to protect against deleterious effects of some mycotoxins. 16 herbal extracts were analyzed for the presence of bio-active constituents and antioxidant potential. The bioactivity analysis of selected herbs revealed following fingerprints: Anti-lipid peroxidation (60-70%); Nitric Oxide Scavenging (50-70%); Site Specific Hydroxyl Radical Scavenging (70-80%); Non-Site Specific Hydroxyl Radical Scavenging (30-40%). Qualitative analysis of classes of phyto-chemicals revealed following ranges: Alkaloids (Moderate to Extremely High); Tannins (Very Low to Low); Terpenoids (Moderate to Extremely High); Saponins (Low to Moderate); Glycosides (Moderate to High); Anthraquinones (Very Low to Moderate); Proteins (Moderate or otherwise absent). The phytochemical fingerprint analysis of these herbs revealed: Phenolic content of herbs ranges from 60 to 85% with respect to gallic acid used as standard equivalence; Flavonoid content of herbs ranges from 30 to 70% with quercetin used as standard equivalence; On the basis of the above results 7 herbs were further screened in order to test their efficacy against targeted mycotoxins: Glycyrrhiza glabra, Tinospora cordifolia, Zingiber officinale, Curcuma longa, Centella asiatica, Silybum marianum, Withania somnifera. Various in vivo or ex vivo enzymatic and non-enzymatic experiments e.g., Catalase, Glutathione Reductase, Glutathione Peroxidase and Superoxide dismutase, etc at liver and intestine tissues revealed the protective effects of some target herbs against mycotoxin-induced toxicity.

The algorithm for assessing radio protective potential of plant extracts and natural products was assessed via some target in vitro and in vivo tests and clinical trials. The mechanisms of radio protective action of the tested extracts and natural products was analysed.

A number of formulation approaches have been employed to increase the solubility and oral absorption of some herbal extracts and products and subsequently to enhance their bioavailability and therapeutic activity.

The antioxidant capability of the resulting plant extracts against a stable radical DPPH was evaluated and the most of plant extracts showed high efficiency in the DPPH test.

The following Indian herbs Tinospora cordifolia (in dose 300 mg/kg bw or 4000 ppm via the feed) and Glycyrrhiza glabra (in dose 400-600 mg/kg bw or 6600 ppm via the feed) and following South African herbs: Centella asiatica (in dose 300-400 mg/kg bw or 4600 ppm via the feed), Withania somnifera (in dose 200-400 mg/kg bw or 4000 ppm via the feed) and Silybum marianum (in dose 80 mg/kg bw or 1100 ppm via the feed) appeared to have a good protective effect in broiler chick (breed
Silymarin (a purified extract of seeds of milk thistle Silybum marianum L., Asteraceae,) was found to have a good capacity of minimizing the deleterious effects of radiation. The radioprotective efficacy of silymarin was evaluated by antioxidant enzymatic and non-enzymatic assays using liver and intestine, hematological parameters and immunological studies. We found that silymarin increase cell viability and chologenic cell survivability. Nanoformulation of Silymarin with higher efficacy has been developed. SNEDDS (Self Nanoemulsifying Drug Delivery Systems) was prepared to increase the solubility and oral absorption for achieving better bioavailability and therapeutic activity of Silymarin. The radioprotective efficacy and preliminary studies against mycotoxin toxicity revealed that Silymarin nanoemulsion has promising results better than the parent silymarin compound. The radioprotective efficacy of Silymarin as a dietary supplement comprises of a mixture of flavonolignans containing silybin (main constituent), isosilybin, silychristin, silydianin and taxifoline commonly found in the dried fruit of milk thistle plant Silybum marianum. It can reduce gamma-radiation-induced micro nuclei formation and reactive oxygen species levels, apoptosis and DNA damage (as measured by Comet assay and flow-cytometry) and mitochondrial membrane disruption. The silymarin nanoemulsion-pretreated (10µg/ml) irradiated group (Balb/c mice) showed lower frequency of apoptotic bodies of human embryonic kidney (HEK) cells as compared to radiation alone group. Survival studies using Balb/c mice confirmed that silymarin exhibits maximum protection at 50 mg/kg b/w against 9 Gy gamma-irradiation. Pre-irradiated treatment with silymarin could restore total lymphocyte counts (TLC) by the 15th day to normal. Based on the series in vivo and in vitro (MTT assay and Annexin V-PI studies, Comet assay and Flow-cytometry) studies, the analysis of data revealed that there is a shift in antioxidant balance upon administration of silymarin that leads to radioprotection. Protection against radiation-induced cell-death and DNA damage by silymarin could be attributed to a reduction in ROS induced by gamma-radiation. In vitro and in vivo experiments showed that silymarin is a promising, effective and safe radiation countermeasure agent and has potential for use during nuclear/radiological emergencies. Our results have clearly shown that the radioprotective efficacy of silymarin nanoformulation is better than silymarin parent compound and preliminary studies indicate its potential ability to reduce mycotoxin-induced toxicity. Therefore, nanosilymarin could be considered as useful source for mitigating both radiation and mycotoxin-induced toxicity warranting further studies to validate its efficacy in in vivo models.

EPR in vitro spectroscopy studies demonstrated that the naturally isolated Piptadenastrium africanum and Haberlea rhodopensis extracts exhibited well expressed DPPH scavenging capacity either before or after UV irradiation. In conclusion, we suggest that further detailed EPR in vitro and in vivo studies for possible application of those extracts as potential radical scavengers and UV protectors in experimental animal models have to be carried out.

The binding ability of ochratoxin A using nano-enabled materials to mitigate exposure was also evaluated. All tested sample materials exhibited strong binding affinity toward OTA in solution. The use of these nanoparticles as feed additives in ameliorating the toxicity of OTA in animals and humans seemed promising. Further studies using some animal models are still required to ascertain the potentials of these materials for use as OTA binders.

Chitosan nanoparticles functionalized with plant extracts for the inhibition of the toxic effects of aflatoxin B1 and ochratoxin A were evaluated (green nanotechnology) with possible applications in preventing damages caused by these mycotoxins with the aim to improve food safety and boost human and animal health. The chitosan nanoparticles with extracts from medicinal plants (Menta Longifolia and Leonotis leonurus) were synthesised and characterised. The antioxidant ability of extracts was evaluated before being incorporated into chitosan using DPPH radical scavenging assay.
Protective effects of samples from leaves and stem bark of Erythrina caffra were found via MTT assay (cell viability method) on the lymphocyte cells in the presence of T-2 toxin.

Millettia macrophylla was found to have estrogenic effects and to prevent postmenopausal osteoporosis in Wistar rats. The identification of its secondary metabolites (13 metabolites) and the evaluation of their estrogenicity and cytotoxicity toward tumoural cells was also done.

The extracts or whole powder from South African herbs Centella asiatica, Withania somnifera, Silybum marianum and Indian herbs Glycyrrhiza glabra, Tinospora cordifolia, Ginger (the rhizome of the Zingiber officinale) and Curcuma Longa (Turmeric) were found to have wound-healing activity and/or anti-inflammatory activity and/or antibacterial or antifungal activities in the form unguents or sprays.

The antibacterial activity of the medium polar extracts of T. potatoria leaves and stem bark was found against Mycobacterium smegmatis. The compounds possibly contributing to this activity, and which may therefore be promising precursors to be used for the development of novel anti-TB drugs were established. Seven compounds were isolated from the medium polar extract [MeOH/DCM (1:1, v/v)] of T. potatoria stem bark. Two novel secondary metabolites named tetraceranoate and N-hydroxy imidate-tetracerane were isolated and identified. Tetraceranoate exhibited the best activity against M. smegmatis with a minimum inhibitory concentration (MIC) of 7.8 μg/mL, while β-stigmasterol, betulinic acid and betulin showed appreciable antimycobacterial activity (MIC 15 μg/mL). The isolated compound tetraceranoate showed antibacterial activity against M. smegmatis as high as rifampicin (one of a three drug regimen recommended in the initial phase short-course anti-tuberculosis therapy). Thus, tetraceranoate might be an interesting target for systematic testing of anti-TB treatment and management.

This finding supports the use of T. potatoria in African traditional medicine for the treatment of tuberculosis related symptoms. The leaves and stems of A. cordifolia exhibited varied antibacterial activity against four Gram-positive bacteria, i.e. Bacillus cereus ATCC11778, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923 and S. saprophyticus ATCC 15305, as well as four Gram-negative bacterial strains, i.e. Escherichia coll ATCC 25922, Klebsiella pneumoniae ATCC 13883, Moraxella catarrhalis ATCC 23246 and Proteus mirabilis ATCC 43071. Seven constituents [stigmasterol (1), stigmasta-4,22-dien-3-one (2), friedelin (3), friedelane-3-one-28-al (4), 3-O-acetyl-aleuritolicacid (5), 3-O-acetyl-erythrodiol (6) and methyl-3,4,5-trihydroxybenzoate(methylgallate) (7)] were isolated from the stem MeOH extract of A. cordifolia. All these compounds displayed some antibacterial activity against the eight pathogens with highest activity against S. saprophyticus (2 mg/ml). The study demonstrated that the antibacterial activities of A. cordifolia extracts may be due to the presence of the seven isolated compounds, where compounds 3–6 showed the best activity. The observed activity against gastrointestinal, skin, respiratory and urinary tract pathogens supports the traditional use for the treatment of such ailments.

The investigation of the protective effect of the extracts derived from the plants Gunnera perpensa and Hydnora abyssinica against the mycotoxin T-2 revealed no significant protection.

Studies on in-vitro efficacy of herbal extracts against Ochratoxin A using Normal Kidney Epithelial cells (NKE) and tumorigenic Kidney cell line (ACHN) revealed antioxidant potential of the most target extracts. NF-κB activation ability of 6 herbal extracts with radiation was determined on Lac-Z reporter cells and two extracts were found to have highest activity.

Studies on ex-vivo or in vivo efficacy of herbal extracts [RDP03, RDP06, RDP10, RDP09 and RDP011, e.g. Zingiber officinale, Tinospora cordifolia, Curcuma longa, Glycyrrhiza glabra extract] against Ochratoxin A with the help of EPR revealed a high efficiency. It was found that the levels of oxidative stress markers reduced significantly on addition of extract RDP03. In conclusion, we consider further more detailed in vitro and in vivo studies for possible application of those extracts as potential radical scavengers and protectors against different environmental stress causing oxidative damage.

Studies on influence of ochratoxin-A and an extract of Tinospora cordifolia against biochemical and oxidative changes in mice spleen tissue homogenates using EPR spectroscopy revealed that combination of OTA with oral administration of Tinospora cordifolia extract led to significant improvement in the levels of oxidative stress biomarkers in mice spleen. It seems that Tinospora cordifolia extract behaves as a good scavenger of ROS and RNS and might find application in the pharmaceutical and food industry as a protector against various diseases, e.g. mycotoxices.

Studies on protective effect of two essential oils isolated from Rosa damascene Mill. and Lavandula angustifolia Mill, and two classic antioxidants against L-dopa oxidative toxicity induced in healthy mice revealed that combining the L-dopa therapy (in the Parkinson’s disease treatment) with antioxidants can reduce related side effects and provide symptomatic relief. The natural antioxidants can be isolated from any plant parts such as seeds, leaves, roots, bark, etc., and their extracts riched in...
phenols can retard the oxidative degradation of the lipids, proteins and DNA. Thus, study suggests that combination of essential oils (Rose oil and Lavender oil), Vitamin C and Trolox with L-dopa can reduce oxidative toxicity, and may play a key role in ROS/RNS disarm.

Studies on ex vivo effect of Glycyrrhiza glabra root extract on some “real time” biomarkers of oxidative stress via EPR spectroscopy revealed that Glycyrrhiza glabra (Licoric) exhibited good anti-inflammatory, antiviral, antimicrobial, antioxidative, anticancer, immunomodulatory, hepatoprotective and cardio-protective properties and excellent ex vivo radical scavenging capacity, which are relevant to radioprotection. It was established that in almost all organs of the treated mice the levels of biomarkers tested were close to those of the untreated controls. Significantly lower levels of nitrite and ascorbate radicals were measured only in the spleens and the hearts of the treated mice compared to controls. This EPR ex vivo study characterizes Glycyrrhiza glabra water extract as a good antioxidant.

The anticancer potential of the dichloromethane / methanol extract of Crateva adansonii stem barks was investigated using human breast cancer cell and 7,12 dimethylibenz(a)anthracene (DMBA)-induced mammary tumorigenesis model in rats The results suggest that the C. adansonii extract may possess antitumor constituents, which could combat breast cancer and prevent chemically-induced breast cancer in rats. C. adansonii extract significantly (p<0.001) revealed in vivo the reduction of the cumulative tumour yield (87.23%), total tumour burden (88.64%), average tumour weight (71.11%) and tumour volume (78.07%) at the dose of 75 mg/kg as compared to DMBA control group. This extract showed a moderate hyperplasia at the dose of 75 mg/kg while at 300 mg/kg no significant change was noted as compared to DMBA group. It protected rats from the DNA alteration induced by DMBA and increased antioxidant enzymes activities in mammary gland tissue homogenates. In addition, Ultra-High Performance Liquid Chromatography / ESI-QTOF-Mass Spectrometry analysis of C. adansonii extract detected structure-related of many well-known anticancer agents such as flavane gallate, flavonol, phenylpropanoids, sesquiterpene derivatives, gallotannins and lignans. The LD50 of C. adansonii was estimated to be greater than 5000 mg/kg. The transfer of knowledge and training activities (workshops) were done in various work packages via the following activities:

- Training courses or specializations in different areas of research organized by various participants in different countries for receiving target skills.
- Screening of Herbal extracts for their anti-toxin efficacy performed on both normal and transformed cells.
- Standardization of bioassay protocols to evaluate nutraceutical standardization; antioxidant activity in both lipid and aqueous phase, free radical induced flux and; ex vivo systems for anti-lipid per-oxidation potential. These assays are used to standardize the nutraceuticals for its efficacy, which reduces with time (due to varied storage conditions). Such assays were carried out jointly and necessary training done.
- In silico biprospection model: A standardized mathematical model developed in house at the laboratory has been shared and necessary training imparted to use this model for selection of nutraceuticals based of multi-parametric based matrix analysis.
- Process standardized for herbal preparation preventing loss of thermolabiles compounds was shared and jointly performed for development of multiple solvent-system based nutraceuticals.
- The extraction of plant materials and compound isolation in Rhodes University (South Africa) was carried out with participation of visiting Marie Curie fellows by using various chromatographic techniques including low pressure column chromatography, preparative thin layer chromatography, high pressure liquid chromatography, high speed counter current chromatography.
- Characterization of plant metabolites in Rhodes University (South Africa) was carried out with participation of visiting Marie Curie fellows by using nuclear magnetic resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR), ultraviolet (UV), elemental analyses (EA), Mass Spectroscopy (MS), Raman spectroscopy (RS), Mossbauer analyses (MA), etc.
- Training courses and acquired skills of visiting Marie Curie fellows in University of Johannesburg (South Africa) was realized in the extraction of active medicinal plant components and characterizing them using various chromatographic techniques including TLC and GC-MS/MS, MTT assay and Comet assay.
- The extraction and characterization of plant materials and compound isolation in TU (Bulgaria) was carried out with participation of visiting Marie Curie fellows by using EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy) on “Fiber Optic Spectrometer”, etc.
- Participation in various in vitro or in vivo experiments and exchange of knowledge or receiving some experience in various technics such as magnetic resonance imaging and spectroscopy, positron emission tomography, MTT assay, EPR (Electron
Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), DPPH radical scavenging assay, (ABTS diamonium salt radical cation decolorization test is also used as a radical scavenging test), Comet assay, Annexin V-PI (propidium iodide) studies, flow-cytometry, etc were realized.

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Last updated on 2017-05-11
Retrieved on 2018-11-13

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