EUROPEAN COMMISSION
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Marie Curie Actions – International Research Staff Exchange Scheme

**Project No:** 316067

**Project Acronym:** HERBAL PROTECTION

**Project Full Name:** Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation

# Marie Curie Actions

# Final Report

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TRAKIA UNIVERSITY

Final Report

# PROJECT FINAL REPORT

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| **Grant Agreement number:** | 316067 |
| **Project acronym:** | HERBAL PROTECTION |
| **Project title:** | Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation |
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# 1. FINAL PUBLISHABLE SUMMARY REPORT

This section should normally not exceed 2 pages.

This is a comprehensive summary overview of results, conclusions and the socio-economic impacts of the project. The publishable report must be formatted to be printed as a stand alone paper document. This report should address a wide audience, including the general public. Moreover, do not include in the summary report any confidential information, whose publication might undermine the protection of commercial interests, including intellectual property, or privacy and the integrity of the individuals, in particular in accordance with Community legislation regarding the protection of personal data.

Please ensure that it:

- Is of suitable quality to enable direct publication by the REA or the Commission.

- Is comprehensive, and describes the work carried out to achieve the project's objectives; the main results, conclusions and their potential impact and use and any socio-economic impact of the project. Please mention any target groups such as policy makers or civil society for whom the research could be relevant.

- Includes where appropriate, diagrams or photographs and the project logo, illustrating and promoting the work of the project.

- Provides the address of the project Website (if applicable) as well as relevant contact details.

Publishable Summary:

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 modells. The studied herbs were from families: Fabaceae, Menispermaceae, Zingiberaceae, Leguminiaceae. Some of the studied herbs such as Centella asiatica, Withania somnifera, Silybum marianum, Tinospora cordifolia, Glycyrrhiza glabra, Stem bark of the trees Piptadenastrium 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 ROSS) against various toxic effects of mycotoxin ochratoxin A on the body weight, relative organ weight, biochemical indices and humoral immune response. A hepatoprotective effect was seen for Tinospora cordifolia and Glycyrrhiza glabra being stronger for chicks additionally supplemented with Glycyrrhiza glabra as can be seen from the pathomorphological findings and the lower levels of ASAT and ALAT. Protective effects of herbal feed additives Silybum marianum or Withania somnifera and slightly of Centella asiatica against the growth inhibitory effect of ochratoxin A and associated immunosuppression and biochemical or pathomorphological changes were seen, e.g. protective effects on the kidneys (strongest for Silybum marianum) and/or liver. The strong immunesuppressive effect of OTA on humoral immune response against Newcastle disease was completely prevented in chicks given the herbal additives Withania somnifera or Silybum marianum, which was additionally supported by the higher relative weight of immunocompetent (lymphoid) organs in the same chicks. The same herbs or appropriate mixture between them could be used as a practical approach for safely utilizing of OTA-contaminated feed.

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, hemathological 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 anti-mycobacterial 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 coli 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) andmethyl-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, RDP010, 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. mycotoxicoses.

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 dimethylbenz(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, phenylpropanoïds, 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 chromatograhopy.
- 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.

List of Keywords:

V201 Natural Resources Exploration and Exploitation, V307 Environmental toxicology, mycotoxins, toxicity, protective effects, herbs

Websites where additional information may be found:

http://www.uni-sz.bg/node/1601

# REPORT ON THE WORK PERFORMED AND RESULTS

Please report on the work performed and on the results of the research, addressing the following points clearly and concisely:

a) Accomplishment of the research objectives as presented in the original proposal

b) New objectives established during the course of work and new lines of research

The following structure should be used in the description of points a) and b) for each objective separately

- Objective of the research;

- Work performed (mentioning also unsuccessful approaches and unforeseen developments);

- Results and degree to which the objectives were met;

- List specific training received on scientific and technical aspects;

- Relevance for basic and applied science and for applications including industrial links.

Changes to original proposal: Note that the REA has to be informed in advance of any changes to the original proposal. For point a) it is important that any deviations form the original proposal are clearly indicated.

In order to help illustrate the work carried out during the fellowship, please enclose copies of the most relevant publications and reports as well as abstracts of the other publications and manuscripts.

Note that this is in addition to the free-text report requested above.

Additional information such as Word documents, graphs, tables, etc. can be uploaded as attachments using the upload functionality (attachments button)

Work Progress:

A short Description of the modifications:

All of the objectives and the tasks are achieved. There are only some amendments in regards to the time and destination of some secondments between the partners and beneficiaries, because of some organization changes and a subsequent transfer of some secondments and tasks from the partner DRDO to the beneficiary TU as follow:

-Work package 1 – without changes and all the tasks and scientific objectives were achieved

-Work package 2 – without changes and all the tasks and scientific objectives were achieved. A secondment from TU was postponed in order to collect some more Himalayan herbs appeared to have a pronounced protective or healing effect on animals.

-Work package 3 – without changes and all the tasks and scientific objectives were achieved. Some secondment from each of the partners were postponed for the last year in order to prepare some additional selective characterizations of some Himalayan herbs appeared to have a pronounced protective or healing effect on animals.

-Work package 4 – with minor changes, but all the tasks and scientific objectives were achieved. Only one secondment from the partner DRDO (3 months) was not realized, because of some organizational changes in this partner. The same secondment were transferred to the beneficiary TU in order to achieve the objectives planned.

-Work package 5 – The secondment belonging to the partner DRDO (3 months) was transferred to the beneficiary TU, because of some organizational changes in this partner. The same secondment was transferred to the beneficiary TU in order to achieve the objectives planned.

-Work package 6 – without changes and all the tasks and scientific objectives were achieved. Part of the designed secondments (only 3 months) were not realized and transferred to the other WPs, because the planned objectives on this WP were achieved earlier.

-Work package 7 – this work package was fulfilled in advance and only some secondments (belonging to the partner DRDO – 6 months) were transferred to the beneficiary TU, because of some organizational changes in this partner. The same secondment was transferred to the beneficiary TU in order to achieve the objectives planned.

In regard to the tasks, scientific objectives, deliverables and milestones planned in the project for this reporting period – all of them (and even more) are achieved according to the plan and only some additional publications have to be published on this matter.

The reason for these small amendments, incl. fulfillment of some of the tasks in advance (related to WP7) and the transfer of some other tasks and secondments (related to WP5) from the partner DRDO to the beneficiary TU, is due to some personal and organization changes - some of the researchers included in this project leaved their institutions and we had to find some other researchers to take their place in the planned experimental work. Some health problems of the seconded researchers to DRDO-India also appeared. A replacement of the host institution (from DRDO-India to TU-Bulgaria) for the mice experiment is also done, because of the reconstruction of the vivarium (experimental animal base) in INMAS-DRDO-India. Independently of the circumstance that we fulfilled all the tasks (even some of the tasks were fulfilled in advance) and achieved all deliverables and/or milestones within the time-frame of the planed work, we have still unused several months secondments under this project. We like to use these secondments to develop some new topics and area of common interests (use of nanoparticles along with some new plant extracts, etc) within the same work packages on which we will be able to work with our partners during the next year in order to achieve the best results and to create the most profitable network.

Objective of the research

Objective 1: Production of enough quantity of ochratoxin A (OTA) and fumonisin B1 (FB1), which will be used in the planned experiments on the herbal protection against mycotoxins using various kinds of animals/chicks.

-WORK PACKAGE 1 - Production of mycotoxins Fumonisin B1 and Ochratoxin A for experimental studies – completely done according to the plan – The produced mycotoxins were used in the planned experiments on the herbal protection against mycotoxins using various kinds of animals/chicks.

-Involved researchers so far: Prof. S. Denev (ER from TU moved to UJ), Prof. S. Stoev (ER from TU moved to UJ), Dr Patrick Njobeh (ER from UJ moved to TU), Ms Judith Phoku (ESR from UJ moved to TU), Prof. M. Dutton (ER from UJ moved to TU), Ms Kh. Ndleve (ESR from UJ moved to TU)

-Used full-time equivalent months – 11.84 months, Planned – 12 months

-Deliverables – completely done:

D1.1. Ensuring of enough quantity of mycotoxin FB1 for experimental work is done

D1.2. Ensuring of enough quantity of mycotoxin OTA for experimental work is done

-Milestone – M1. Production of enough quantity of OTA and FB is done:

-Description of work finished in Work package 1:

• FB1 is produced using the strain Fusarium verticillioides (isolate MRC 826 - this strain was used under material transfer agreement with Medical Research Council of PROMEC Unit, Tygerberg, South Africa). The strain was grown on moistened ground corn kernels (50 g ground maize kernels in 1000 ml conical flasks moistened by addition of 70 ml sterile water and then autoclaved 30 min at 121°C and 120 kPa). The moistened ground corn kernels were inoculated with 1 ml of a spore suspension (lyophilized conidia of F. verticillioides MRC 826 in 100 ml of sterile distilled water) and incubated on a rotary shaker at 25°C in the dark for 2 weeks or 3 weeks. The medium was then frozen and freeze-dried and analyzed by HPLC for FB1 content. The FB1-rich moulded ground maize kernels will be then homogenised into chick/pig/rat ration to give the required concentration of FB1 in diet. Part of the FB1 was extracted and purified for in vitro studies according to Shephard and

Sewram, (2004) as follows: A finely ground maize sample (20 g) was mixed with 100 ml methanol/water (75:25 v/v), placed on a mechanical shaker for 60 mins. The mixture was then centrifuged at 500g for 10 min at 4oC. The pH of the supernatant was adjusted to 5.8 using 1 M sodium hydroxide or 0.1 M glacial acetic acid, wherever necessary. An aliquot (10 ml) of extract was passed through a previously conditioned solid phase strong anion exchange (SAX) column at a flow rate of 2 ml/min, while allowing the column not to dry out. The column was then sequentially washed with 5 ml methanol/water (75:25, v/v) and 3 ml methanol. Fumonisin was eluted with 10 ml methanol/glacial acetic acid (99:1, v/v) at flow rate of 1ml/min and the elution solvent dried under a stream of nitrogen gas at 60oC.

• Aspergillus ochraceus (isolate D2306, as used by Stoev et al., 2000b, 2002b,c) supplied by our UK collaborator from Imperial College (London) was used for production of OTA as described in our previous studies (Stoev et al, 2000b, 2002b,c). The sterilised shredded wheat (40 g) in 500 ml conical flasks, moistened by a 40% (v/w) addition of sterile water and incubated on a rotary shaker at 27 ◦C for 2 weeks was used for growing of the same strain of A. ochraceus. The final product was sterilised at 80 ◦C for 1 h (yield 2 kg) and stored at −20 ◦C. The ochratoxin-rich moulded shredded wheat will be then homogenised into chick/pig/rat ration (diluted more than 2000-fold) to give the required concentration of OTA in diet.

-----Staff secondments and transfer of knowledge

Transfer of knowledge was realized between researchers from TU and UJ in regard to various way of mycotoxin production

Objective 2: To ensure suitable herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects.

-WORK PACKAGE 2 - Collection of target herbs with known protective, immunostimulating or antimicrobial effect from South Africa and India and receiving some herbal extracts – completely done according to the plan – The collected herbs were used in the planned experimental work on the herbal protection using various kinds of animals/chicks.

-Involved researchers so far: Prof. R. Zheleva (ER from TU moved to DRDO), Prof. V. Gadjeva (ER from TU moved to DRDO), Prof. S. Stoev (ER from TU moved to UJ), Prof. Miroslav Stefanov (ER from TU moved to UJ and DRDO), Prof. Vesselin Ivanov (ER from TU moved to UJ), Ms Ch. Carmen Celia (ESR from UNIKAPOS moved to UJ)

-Involved researchers during the second 2-years Period: Prof. Vesselin Ivanov (ER from TU moved to UJ), Prof. Miroslav Stefanov (ER from TU moved to UJ and DRDO).

-Used full-time equivalent months – 11,33 months, Planned – 12 months, Remaining – 0,67 months

Deliverables - done:

D2.1. Ensuring of enough quantity of target herbs for necessary experimental work is done

D2.2. Establishment of biological activity of the same herbs is done

-Milestone – M2. Herb collection is done, and some additional herbs having immunostimulating or antimicrobial activity were collected and explored during the second period:

-Description of work finished in work package 2 - .

Some indigenous herbs from India with possible activity to reduce the deleterious effects of mycotoxins were collected from different regions with the help of Dr. Rajesh Arora and his team in order to be studied for possible effects on wound granulation or for protective effects on kidneys and liver via the respective studies. Similar collection of herbs was done in S. Africa under the guidance of Prof. Krause (RU), Dr. P. Njobeh and Dr D. Ndinteh (UJ). Some of the herbs currently supplied via the SA firm Parceval (Ulrich Feiter) include:

1) Centella asiatica – It is known by traditional healers in Kwazulu-Natal to heal wounds, cuts,

grazes and burns. It contains triterpenoid saponoids that help to promote cell replication. It increases the reproduction of peripheral blood vessels and connective tissue, improves circulation and helps to retain/restore elasticity of the skin. It speeds up collagen formation and increases antioxidant levels within the wound in the early stages of tissue repair. It was suggested as spindle poison for skin operation (as it stops cell division). Now, we found that the herb has anti-bacterial, anti-fungal and anti-inflammatory and antioxidative activities and is currently tested for wound-healing and nephroprotective effects. It was found to contain some flavonoids.

2) Withania somnifera (leaves used for wound healing) – It is described as "Indian ginseng, having positive influence on the endocrine, cardiopulmonary and central nervous systems. It contains flavonoids and we found that it has antioxidative effect. It is currently tested for anti-inflammatory/antibacterial, immunostimulating or wound healing effect as well as for antioxidative and hepatoprotective properties.

3) Silybum marianum – It contains flavonoids and we found that it has antioxidative and radioprotective effects. Silymarin, 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 was found to ameliorate gamma-radiation-induced genotoxicity and is suggested to be implicated for possible chemical, biological, radiological or nuclear defence, because of mitigating the deleterious effects of radiation and therefore being an effective radiation countermeasure. Silymarin tends to reduce the effects of radiation in living system by many ways i.e., antioxidant activity, it was found to have a good in vivo and in vitro protection against lethal gamma-rays, in addition to its strong hepatoprotective effect, etc. Silymarin nanoemulsion was found to have a better efficacy in the radioprotection and in the protection against deleterious effects of some mycotoxins than the parent silymarin compound. It is currently tested additionally for nephroprotective or immunostimulating effects and for improving wound granulation.

4) Stem bark of the trees Piptadenastrium africanum – It was found to have antioxidative effect and protective effect against UV irradiation in some in vitro and in vivo studies

5) Haberlea rhodopensis extracts - It was found to have antioxidative effect and protective effect against UV irradiation in some in vitro and in vivo studies.

6) Tinospora cordifolia – It was tested for anti-inflammatory, immunostimulating or antioxidative effects and hepatoprotective effect (extract of fresh stems or powder materials were tested)

7) Glycyrrhiza glabra Linn. (Fabaceae). Sanskrit/Indian Name: Yashti-madhu, Yashti-madhuka, Mulhathi, Jethi-madh – it was tested for antioxidative, anti-inflammatory, immunostimulating or hepatoprotective effects

Fresh stems of Tinospora cordifolia Miers. (Family Menispermaceae) and roots of Glycyrrhiza glabra L. (Family: Fabaceae) were collected from the central plains/plateau region of India with a prevalence of humid subtropical climate, or the comparatively less hotter lower Himalayan tract (250-530 m altitude). The plants were identified at the Institute of Nuclear Medicine and Allied Sciences, Delhi by a qualified Botanist and voucher specimens deposited. The stem portions of Tinospora cordifolia and roots, rhizomes and stolons of were cut into small pieces (ca. 2.5 5-3.0 cm in length) and were shade dried for 72-120 h with the precaution of contamination from the dust. The plant material was further mechanically dried in a hot air oven between 55-60°C for 72 h to remove any left over moisture content. The plant material was then converted into a fine powder form in a grinder and stored in air tight containers preventing it from moisture until the start of the respective experimental work.

-----Staff secondments and transfer of knowledge - Knowledge regarding Identification of some Himalayan and/or South African herb was exchanged between the Indian, South African, Hungarian and Bulgarian researchers.

Objective 3: To perform selective characterization of some Indian or South African herbs for their bioconstituents (as the levels of flavonoids, carotenoids, etc) in order to explain the mechanism of their antioxidative and protective effects on kidneys and liver or their immunostimulating or antibacterial effects and to prepare some extracts or fractions with the aim of exploring their protective abilities in this regard

WORK PACKAGE 3 - Selective characterisation of some South African and Indian herbs for their bioconstituents (flavanoids, etc) via EPR (Electron Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), NMR, FTIR, UV, EA, MS, RS, MA, etc. in order to explain the mechanism of their antioxidative and protective effects and preparing of target herbal fractions or extracts. In vitro study on antioxidative effect of some herbal extract against oxidative stress via EPR spectroscopy – all necessary work is done

-Involved researchers so far: D. T. Ndinteh (ESR from RU moved to TU and UNIKAPOS), Xavier Siwe Noundou (ER from RU moved to TU and UNIKAPOS), Hilary Ihesinaulo Ezuruike (ESR from RU moved to TU), Bertha Chitambo (ESR from RU moved to TU), G. Beev (ER from TU moved to RU), V. Ivanov (ER from TU moved to RU), I. Dinev (ER from TU moved to UJ), M. Kachleck (ESR from UNIKAPOS moved to UJ), N. Gabor (ER from UNIKAPOS moved to UJ), Manish Adhikari (ESR from DRDO moved to TU)

-Involved researchers during the second 2-years Period: D. T. Ndinteh (ESR from RU moved to UNIKAPOS), Xavier Siwe Noundou (ER from RU moved to UNIKAPOS and TU), Hilary Ihesinaulo Ezuruike (ESR from RU moved to TU), Bertha Chitambo (ESR from RU moved to TU, V. Ivanov (ER from TU moved to RU), I. Dinev (ER from TU moved to UJ), N. Gabor (ER from UNIKAPOS moved to UJ), Manish Adhikari (ESR from DRDO moved to TU)

-Used full-time equivalent months – 29,63 months, Planned – 27 months,

Deliverables - done:

D 3.1. Establishing the bioconstituents of some target Himalayan and South African herbs – the work is done according to the plan

D 3.2. Elaboration of technology for preparing some target herbal extracts or fractions and preparing relative publications in this regard. – the work is done according to the plan.

-Milestone – M3. Identification of some herbal bioconstituents and preparation of target herbal extracts or fractions is done according to the plan

-Description of work finished in work package 3:

A) Antioxidant screening of active components was done in TU. Ten different extracts and fractions from medical plants (including Silybum marianum) and trees were studied in TU for their free radical scavenging capacity against the stable free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) by EPR (electron paramagnetic resonance) spectroscopy and for superoxide dismutase enzyme (SOD) activity by spectrophotometry (in vitro before and after 2 hrs UV irradiation). The ABTS diamonium salt radical cation decolorization test is also used as a radical scavenging test.

-Among all studied South African samples, the methanol extracts isolated from stem bark of the trees Piptadenastrium africanum and Millettia Laurentii showed the highest DPPH scavenging capacity and highest SOD like activity.

-The data of free radical scavenging activities of silymarin against DPPH (>65%) and ABTS (22%) showed the strong antioxidant activity of silymarin. Reduction in micronucleus count was seen at 25 μg/ml with 2 Gy gamma-irradiation in HEK cells and therefore silymarin was found to have the capacity of minimizing the deleterious effects of radiation.

-For the first time Electron Paramagnetic Resonance (EPR) spectroscopy methods have been used in comparative studies on water solutions of extracts isolated from stem bark of Piptadenastrium africanum and leaves of Haberlea rhodopensis. The radical scavenging capacity of these extracts towards the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was investigated and

compared, before and after UV irradiation. A twofold DPPH scavenging capacity was found for UV irradiated Haberlea rhodopensis sample as compared to that of untreated (non-irradiated) sample, while radical scavenging abilities of the Piptadenastrium africanum extract was not affected. EPR spectra of Piptadeniastrum africanum and Haberlea rhodopensis water solutions extracts were also studied before and after 2h of UV irradiation by direct EPR spectroscopy. After alkalization both non UV irradiated samples give rise to EPR singlet signals with equal g values of 2.00564 ± 0.00001 G that were ascribed to ortho-semiquinone free radical structures. We consider that further more detailed EPR in vitro and in vivo studies on experimental animal models have to be carried out for possible application of those extracts as potential radical scavengers and UV protectors.

-Further these both extracts were investigated by EPR spectroscopy for their in vivo effects on liver and kidney oxidative status of healthy mice treated by them and were compared to that effect of the leaves methanol extract isolated from Bulgarian medicinal plant Haberlea Rodopensis. Moreover, by EPR methods in vivo protective effects of the above extracts against oxidative stress induced by xenobiotics (antitumor drugs) were evaluated and compared in mice pretreated by them.

-Structure characterization/elucidation of active components/fractions was done via UV-VIS spectrum, FT-IR spectrum, NMR spectrum, GCXGC spectrum, but the structure elucidation confirmation is still pending. Chromatographic separation of active components was also done via TLC and column chromatography

-Cytotoxicity screening of active components and effects in reducing cytotoxicity against OTA and FB1 is also currently studied – it was found that the above tested compounds are not toxic for human lymphocytes.

B) Selective characterization of 10 Indian herbs including herbs from Himalayan region for their bioconstituents profile was also done in DRDO. The plants belonging to the following families: Fabaceae, Menispermaceae, Zingiberaceae, Leguminiaceae were identified, characterized and screened on the basis of bioprospection analysis with bioactivity characterstics like antioxidative, diuretic, protective effects on kidneys and liver, immuno-stimulating or antibacterial effects etc.

a) The phytochemical fingerprint analysis of these herbs revealed following:

Phenolic content of identified herbs ranges from 60 to 85% with respect to gallic acid used as standard equivalence

Flavonoid content of identified herbals ranges from 30 to 70% with quercetin used as standard equivalence

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)

b) The bioactivity analysis of selected herbals provided 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%)

c) Selected Herbs from Himalayan region were prepared in various solvent systems i.e., water-100%, water : alcohol (50:50); alcohol – 100% targeting specific pre-dominant phytochemicals):

Glycyrrhiza glabra (Family: Fabaceae)
Tinospora cordifolia (Family: Menispermaceae)
Zingiber officinale(Family: Zingiberaceae)

Curcuma longa (Family: Zingiberaceae)
Macrotylomauniflorum(Family: Leguminiaceae)

C) Preparation of some herbal extracts or fractions

-Herbal extracts for selected herbs were prepared with the process of hot maceration. Process has been standardized for preparation of extract(s) from various plant species of the Himalayan region. Selected Herbs from Himalayan region were prepared in various solvent systems i.e., water (100%), water alcohol (50:50); alcohol (100%) targeting specific pre-dominant phytochemicals.

-The yield (%) of the herbs ranged from 10-20%.

-Biofingerprint profile of the selected herbs was done for qualitative analysis of classes of phyto-chemicals presents in the extracts and for obtaining the fingerprints in order to avoid batch to batch variation among herbal fractions.

The table describes the characteristics of the extracts fractionated from the herbs mentioned above. The following extracts were prepared in INMAS, DRDO and the process has been standardized.

-10 plants identified using bioprospection

-5 selected herbs were developed as extracts (range of bioactivity and phyto-chemical fingerprint developed)

D) Additional work on this work package done in UJ

a) Chitosan nanoparticles functionalized with plant extracts for the inhibition of the toxic effects of aflatoxin B1 and ochratoxin A. The study was to characterize chitosan nanoparticles synthesized with methanol medicinal plant extracts (green nanotechnology) with possible applications in preventing damages caused by target mycotoxins such as aflatoxin B1 (AFB1) and/or ochratoxin A (OTA) to improve food safety and boost human and animal health. In order to achieve that, chitosan nanoparticles with extracts from medicinal plants (Menta Longifolia and Leonotis leonurus) were synthesized using an ionic gelation method with sodium triphosphate as the crosslinker and characterized using transmission electron microscopy, x-ray diffraction, zetasizer and fourier-transfom infrared transmission spectroscopy. The antioxidant ability of extracts was evaluated before being incorporated into chitosan using 2, 2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging assay. The cytotoxic effect of the synthesized nanoparticles on Vero cell lines was also performed. The nanoparticles were successfully synthesized and showed the presence of different functional groups as expected. Under TEM, we found that plain chitosan nanoparticles were roughly spherical in shape with smooth surfaces, but nanoparticles containing extracts were spherical in shape, with fairly rough surfaces. It was found that all synthesized nanoparticles had positive zeta potentials between 26 – 28 mV and average particle sizes ranged between 31 – 65 nm as measured using TEM while average particle sizes obtained using zetasiser was 78 – 190 nm. In the presence of OTA and AFB1, at a concentration of 125 µg/ml, the antioxidant ability of M. longifolia and L. leonurus extracts was 97 and 70%. The cytotoxicity data on nanoparticles with or without the extract showed that the synthesized nanomaterials were not toxic even when concentrations were increased to 500 µg/ml with less than 20% of loss in cell viability observed under these conditions.

b) The binding ability of ochratoxin A using nano-enabled materials to mitigate exposure. Silica nanoparticles (SiNP's) stabilized with methyl acrylate polyethylene glycol (MPEG) and hexamethylenediamine (HED) were synthesized by the microemulsion method. Zeolite Nu-6(2) nanoparticles were synthesized by the hydrothermal method and together with purchased zeolite, pure silica gel and molecular sieves, were then functionalized with 3-aminopropyltriethoxysilane (APTS) at 120 ºC in hexane. Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XR) and laser Raman spectroscopy were used to characterize the materials and evaluate for their binding potentials on

OTA in buffered water solutions. This was done by mixing 100 mg of each powdered materials in buffered water and dosing different OTA concentrations (1, 5 and 10 µg/mL) (in duplicates). After centrifugation, the supernatant was obtained and OTA content determined using high performance liquid chromatography (HPLC). Accordingly, HPLC analysis showed a dose depending absorption of OTA for the analyzed samples. In fact, all tested sample materials exhibited strong binding affinity toward OTA in solution, with a 76% uptake as the lowest absorption capacity that was noted for unmodified silica gel. In general, our in-house made nanomaterial and modified particles adsorbed better than untreated materials with a 100% adsorption recorded for synthetic silica nanoparticles stabilized with hexamethylenediamine (S-HED). It was found that surface modified materials also increased OTA uptake in the solution. Following this evaluation, a methyl thiazol tetrazolium (MTT) assay was performed to investigate the cytotoxic effect of the produced materials on human lymphocyte cells. To achieve this, cells were exposed to each powdered material at different levels (0.01, 0.1 and 100 mg/mL) in triplicates under standard conditions (37ºC, 5% CO2, 95% humidified atmosphere) for 24, 48 and 72 hours. Data obtained revealed that human lymphocyte cells were able to maintain their integrity during exposure even at high concentrations of the produced materials as it was found that no human lymphocyte cell loses were recorded. It was however, noted that these nanomaterials interact with MTT salts after 72 hours of exposure resulting in an increase in percentage cell viability. It can thus, be concluded that the use of these nanoparticles as feed additives in ameliorating the toxicity of OTA in animals and humans seemed promising. However, further studies to test the toxicity of these nanoparticles by other means i.e. Xcelligence testing followed by experiments using some animal models are still required to ascertain the potentials of these materials for use as OTA binders.

I) DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenger antioxidant assay of herbal extracts

The aim of this study was to evaluate how efficient and how much of our samples (six different extracts of both leaves and stem bark of Erythrina caffra and three marine natural products extracts) is necessary to trap the free radicals.

DPPH● scavenging activity was determined by Sánchez-Moreno's (1998) method, modified into a microplate format. The stable free radical's (DPPH●) purple color in methanolic solution (λmax=517) disappears when it reacts with antioxidants (AH) as in (1) and (2) were described. The product's yellow colour has ignorable absorbance at 517 nm.

DPPH● + AH → DPPH2 + A● (1)

DPPH● + A● → DPPH-A (2)

After half an hour incubation time, the absorbance can be measured with a photometer around λmax. Using different concentrations of the AH, and always the same of DPPH●, EC50 value can be calculated, which means how many antioxidant needs to ignore the half of the free radical.

(_"rem" ^"%" )"D" □"PPH" □^"●" "= " □"[DPPH]" □^"●" /□□"[DPPH" □^"●" "]" □_"t=0" "·10" (3)

DPPH● scavenging activity with half-maximal effective concentration (EC50) by using different concentrations of the AH, and always the same of DPPH●, can be calculated. EC50 means how many antioxidant needs to defuse the half of the free radical. An exponential correlation is expected between the remaining DPPH● (3) and the antioxidant concentration, therefore after plotting the ln(%remDPPH●) vs. AH concentration one can read the EC50 value at ln(50) in weight/volume (w/v) terms which can be converted to the more suitable weight/weight (w/w) unit by dividing with the initial concentration of the free radical [DPPH●]t=0. Until the final calculation step everything was expressed as µg/ml which was determined from a calibration curve. Determinations were performed in triplicate and reported on a dry matter. Results are expressed as mean values ± standard deviation.

The results revealed that all of our samples possess antioxidant activity but not with the same efficacy. The medium polar of our samples exhibited the best radical scavenging activity. Some

co-publications drafts between Prof Dr Gadjeva, Prof. Stoev, Prof. Dr. Kovacs and Prof Krause groups from Bulgaria, Hungary and South Africa are ongoing for this particular project. Some problems were encountered due to difficulty in using the same conditions for the solubility of the dye and the samples. But after many trials, we managed to overcome that issue by finding the right solvent mixture.

II) Spectrophotometrical analisys of Bulgarian natural and synthetic antioxydants before and after gamma- irradiation – the following tasks were performed:
-Estimation of total polyphenolic content
-Estimation of total flavonoid content
-DPPH radical scavenging activity
-ABTS radical decolourization assay
-Reducing Power Assay
-Linoleic acid degradation assay
-Hydroxyl radical scavenging activity
-Nitric oxide ion scavenging potential
-Antioxidant activity (aqueous phase)
-Protection of membrane against radiation damage (membrane protection index)

III) Cell protection and activation of Bulgarian natural and synthetic antioxydants before and after gamma- irradiation – the following tasks were performed in Indian INMAS lab:
-Radiao-sensitization ability of different natural and synthetic antioxidants, at different doses
-Effects of antioxidants on normal and kidney cells were investigated for toxic/ nontoxic effect at different concentrations. Investigation of radio-sensitization activity was performed.
-Determination of ROS levels in kidney cells. ROS was quantified by monitoring dichlorofluorescein (DCF) fluorescence in flow cytometer.
-Cell micriscopic analysis.

IV) EPR investigations
-Ex vivo assay ascorbate radicals levels, the levels of ROS production, the nitric oxide levels in the tissue homogenates of mice, treated with 80 mg / kg indian natural products by EPR spectroscopy; Biodistribution investigations in organ of 2 Indian antioxidants. Ex vivo of ascorbate radicals levels, the levels of ROS production, the nitric oxide levels in the tissue homogenates of mice, treated with OTA and 80 mg / kg Indian natural products (by EPR spectroscopy; (10 days)
-Direct EPR spectroscopy study on root extracts of Indian antioxydants in powdered and aqueous solution form before and after UV irradiation; In vitro EPR spectroscopy study on DPPH radical scavenging capacity of aqueous root extracts; In vitro spectrophotometry study on DPPH scavenging activity of aqueous root extracts; In vitro EPR spectroscopy study on the effect of incubation time on DPPH radical scavenging capacity of extracts in solution form before and after UV irradiation and gamma irradiation.

Summary
Stable radical 1, 1-dipheny-2-picrylhydrazyl (DPPH) is widely used at in vitro models to investigate antioxidant and radical scavenging abilities of natural extracts. This work presents comparative study on DPPH radical scavenging capacity before and after UV irradiation of aqueous extract of Glycyrrhiza Glabra, a plant species belonging to the Indian flora. DPPH scavenging activities of different extract concentrations (at different incubation time intervals) were analyzed and compared by in vitro spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy. 9.93% and 16.79%, DPPH scavenging activities before and after UV irradiation respectively were found by spectrophotometry. By the EPR spectroscopy study statistical significant increase in DPPH radical scavenging for the Glycyrrhiza Glabra extracts was established after UV irradiation (78.39±

0.001%) comparing to the non irradiated samples (14.02± 0.02).
----Staff secondments and transfer of knowledge
Knowledge regarding Characterization and Extract Preparation of Himalayan and/or South African herb was exchanged between the Indian, South African and Bulgarian researchers using the above described methods/assays.

Objective 4: To prepare some mixtures of suitable herbal extracts using appropriate constituents, which will be further tested for their stimulating effects on wound granulation and elaborating appropriate unguents or sprays designed for stimulation of wound granulation.

WORK PACKAGE 4 - Elaboration of mixtures of target herbal extracts for preparing of some sprays/unguents designed for stimulation of wound granulation - is done according to the plan

-Involved researchers: M. L. Dlamini (ESR from UJ moved to TU), T. Fonkui (ESR from UJ moved to TU), R. Changwa (ESR from UJ moved to TU), Prerna Agarwal (ESR from DRDO moved to TU), S. Stoev (ER from TU moved to DRDO), Prof. Miroslav Stefanov (ER from TU moved to RU)

-Involved researchers during the second 2-years Period: Prerna Agarwal (ESR from DRDO moved to TU), Prof. Miroslav Stefanov (ER from TU moved to RU)

-Used full-time equivalent months – 15,67 months, Planned – 15 months,

Deliverables - done:

D 4.1. Establishing the best mixture of suitable herbal extracts using appropriate constituents, designed for stimulation of wound granulation – is done.

D 4.2. Elaboration of appropriate unguents or sprays from herbal extracts for testing the stimulation of wound granulation and preparing a research paper in this regard – is done.

-Milestone – M4. Elaboration of herbal sprays/unguents designed for stimulation of wound granulation is already done

-Description of work finished in work package 4.

-Antifungal screening of plant extracts against 5 mycotoxin producing fungal species via “MIC of highest activity measurement” and “preparing of TLC-Bioautogram of antifungal activity via TLC-Bioautography” showed that most activity recorded for P. africanum, and less activity for M. longifolio and L. leonorus. Chromatographic separation of active components was done via TLC and column chromatography.

-Extracts or whole powder from South African herbs Centella asiatica, Withania somnifera, Silybum marianum and/or having well defined wound-healing activity and/or antiinflammatory activity and/or antibacterial or antifungal activities were mixed in different proportions with appropriate constituents in order to prepare different unguents, which were tested for improving wound granulation.

-Selective characterization of Indian herb Curcuma Longa (Turmeric) for various bioconstituents was also done in DRDO. Turmeric has been used in traditional medicine for the treatment of jaundice and other liver ailments, ulcers, parasitic infections, various skin diseases, sprains, inflam-mation of the joints, cold and flu symptoms. It is also used for preserving food as antimicrobial agent. Chemical constituents of turmeric rhizomes include volatiles and non-volatiles and preliminary investigations showed the following biological activities: Antioxidant activity; Anti-protozoal activity; Wound-healing activity; Antiinflammatory activity; Antibacterial and Antifungal activities; Antiviral activity; and therefore is useful for wound healing and protection.

-Selective characterization of Indian herb Ginger (the rhizome of the Zingiber officinale) for various bioconstituents was also done in DRDO. It is commonly consumed dietary condiments, generally considered to be safe and used to cure various diseases and preliminary investigations showed the following biological activities: Antioxidant activity; Anti-inflammatory and

anti-analgesic activity; Anti-microbial activity, and therefore is useful for wound healing and protection.

(I). Bioactivity linked standardization of extracts targeted for analyzing the stimulating effects on wound granulations.

10 herbal extracts, as mentioned above in the table were selected for screening on the basis of their antioxidant potential. The primary screening methodology was adopted for screening of herbal extracts based on their free radical scavenging activity (DPPH), modulation capacity of extracts in terms of its ability to act as Superoxide dismutase and reducing potential.

-10-70% reduction of DPPH by selected herbal extracts was observed. The analysis of DPPH scavenging activity of extracts revealed the following order: RDP06> RDP03> RDP09> RDP010> RDP07> RDP08> RDP014> RDP> RDP011.

-SOD like activity of the herbal extracts ranged from 5-35 Units/mg. The analysis of 'SOD like activity' revealed the following order: RDP03> RDP06> RDP09> RDP08> RDP014> RDP07> RDP010> RDP> RDP011.

-ABTS radical scavenging activity of the above mentioned herbal extracts was also analysed. It ranged from 20-80%. The order of scavenging was in accordance with of DPPH radical scavenging activity.

Qualitative and quantitative estimation of the plant constituents known for high antioxidant potential such as flavanoids and polyphenols was done.

-Phenolic content of identified herbals ranges from 80-800 mg/gm of extract, which is 60 to 85% with respect to gallic acid used as standard equivalence

-Flavonoid content of identified herbals ranges from 30 to 70% with quercetin used as standard equivalence. Quantified amount was estimate to be around 25-100 mg/gm of extract.

-The Unit Absorbance Value for these herbals in terms of their total reducing ability was calculated and it ranged from .001- 0.009.

-Correlation analysis with dielectric constant of the solvent systems revealed increase in antioxidant power with respect to decreasing dielectric constant of selected herbal extracts

The extracts RDP-03. 06 and 10 were selected for further investigating their stimulating effects on wound granulation.

(II). Various extracts or whole powder from South African herbs, e.g. Centella asiatica, Withania somnifera, Silybum marianum and Indian herbs Glycyrrhiza glabra, Tinospora cordifolia, Ginger (the rhizome of the Zingiber officinale) and Curcuma Longa (Turmeric) having well defined wound-healing activity and/or antiinflammatory activity and/or antibacterial or antifungal activities were mixed in different proportions with various constituents in order to prepare different unguents, which were tested for improving wound granulation.

Objective 5: To investigate the prepared herbal extracts (unguents or sprays) for their stimulating effects on wound granulation

. WORK PACKAGE 5 - Investigation on stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation - done.

-Involved researchers: G. Terziev (ESR from TU moved to UJ), K. Dimitrov (ESR from TU moved to UJ)

-Used full-time equivalent months – 3,37 months, Planned – 3 months,

Deliverables - done:

D 5. Establishing the best unguents or sprays from herbal extracts for stimulation of wound granulation.

-Milestone – M5 The effect of target herbal extracts (unguents or sprays) as stimulants of wound granulation was established.

-Description of work finished (some additional investigations on antimicrobial activity of herbs were done):

The herb Liquorice (Glycyrrhiza glabra) was found to possess natural antibacterial, antiviral, antifungal, and anti-inflammatory properties in addition to its antioxidative and immunostimulating effects. Some Liquorice constituents, e.g. glycyrrhizin or glycyrrhetic acid possess steroid-like anti-inflammatory activity, similar to the action of hydrocortisone, which is partially due to inhibition of phospholipase A2 and cyclooxygenase activity, and prostaglandin formation.

The herb Tinospora cordifolia was also found to have similar anti-oxidative, imunostimulating and anti-inflamatory effects and was found to be a suitable herb for wound treatment. The anti-inflammatory effect of this herb was found to be comparable with indomethacin and its mode of action appeared to resemble that of nonsteroidal anti-inflammatory agent and to be effective in both acute and subacute models of inflammation and/or wound granulation - both alcoholic and aqueous extracts of Tinospora cordifolia were found to have such effects being mixed with appropriate constituents. A comparatively potent antibacterial activity of the above herbal extracts were found against some bacterial agents, e.g. Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aeruginosa, etc.

Apart from wound healing the herb Centella asiatica was found to combat oxidative damages, e.g. oxidative stress. It was found to have similar anti-inflammatory and anti-bacterial or anti-viral, and immunostimulating properties as the above two herbs.

Some other herbs, which were seen to have a potent anti-inflamatory, anti-oxidative and especially immunostimulating effect for humoral immune response and to be appropriate for using to teat various wounds were found to be Silybum marianum and Withania somnifera.

Some Additional Target antimicrobial studies of herbs:

A) Tetracera potatoria Afzel. Exg. Don (Dilleniaceae) is a medicinal plant used traditionally in Africa for the treatment of tuberculosis related ailments and respiratory infections. The antibacterial activity of the medium polar extracts of T. potatoria leaves and stem bark was recently reported against Mycobacterium smegmatis (MIC 25 µg/mL) and M. aurum (65 µg/mL), two fast-growing Mycobacterium strains used as model micro-organisms for the more pathogenic strain Mycobacterium tuberculosis (Fomogne-Fodjo et al., 2014). The aim of this study was consequently to isolate 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.

Materials and methods: T. potatoria medium polar extract [MeOH/DCM (1:1, v/v)] was fractionated sequentially with petroleum ether to which EtOAC and MeOH were gradually added to increase the polarity. The examination of T. potatoria extract and its fractions was guided by bioassays for anti-mycobacterial activity against M. smegmatis (ATCC 23246) and M. aurum (NCTC 10437) using the minimum inhibitory concentration (MIC) method. All the isolated compounds were structurally elucidated using spectroscopic techniques and evaluated for their anti-mycobacterial activity.

Results: Two novel secondary metabolites (1, 2) named tetraceranoate and N-hydroxy imidate-tetracerane, together with five known compounds [β-stigmasterol (3), stigmast-5-en-3β-yl acetate (4), betulinic acid (5), betulin (6) and lupeol (7)] 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 anti-mycobacterial

activity against both strains (MIC 15 µg/mL).

Conclusion: Seven compounds were isolated from the medium polar extract [MeOH/DCM (1:1, v/v)] of T. potatoria stem bark. Only tetraceranoate one of the isolated compounds showed antibacterial activity against M. smegmatis having efficacy 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 research supports the use of T. potatoria in African traditional medicine for the treatment of tuberculosis related symptoms.

B) The leaves, stems and roots of Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg. are used as traditional medicine in many African countries for the management of gastrointestinal, respiratory and urinary tract infections as well as for the treatment of wounds.

Aim of the study: To determine the in vitro antibacterial activity of the crude extracts of leaves and stems of A. cordifolia on gastrointestinal, skin, respiratory and urinary tractpathogens and to identify the compounds in the extracts that may be responsible for this activity.

Materials and methods: The antibacterial activities of crude extracts [hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), ethanol (EtOH), methanol (MeOH) and water ($H_2O$)] as well as pure compounds isolated from these extracts were evaluated by means of the micro-dilution assay 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 coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, Moraxella catarrhalis ATCC 23246 and Proteus mirabilis ATCC 43071. The isolation of the active constituents was undertaken by bio-autographic assays in conjunction with chromatographic techniques. The identification and characterization of the isolated compounds were done using mass spectrometry (MS) and Fourier transformed infrared spectrometry (FTIR) as well as 1D- and 2D-nuclear magnetic resonanc e(NMR) analyses.

Results: The leaves and stems of A. cordifolia exhibited varied antibacterial activity against all eight pathogens. Most of the MIC values ranged between 63 and 2000 mg/ml. The highest activities for the crude extracts (63 mg/ml) were observed against S. saprophyticus [stem (EtOAc, $CHCl_3$ and hexane), leaves (MeOH, EtOH, EtOAc and $CHCl_3$)], E. coli [stem (MeOH and EtOH), leaves (MeOH, EtOH, EtOAc and $CHCl_3$)], M. catarrhalis [leaves (EtOAc and $CHCl_3$)], K. pneumoniae [stem ($CHCl_3$), leaves ($CHCl_3$)] and S. aureus [leaves($CHCl_3$)]. 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) andmethyl-3,4,5- trihydroxybenzoate(methylgallate) (7)] were isolated from the stem MeOH extract. All these compounds displayed some antibacterial activity against the eight pathogens with highest activity against S. saprophyticus (2 mg/ml). Furthermore, this is the first report of compounds 1, 2, 3, 4, 6 and 7 isolated from A. cordifolia and where a complete set of 2D-NMR data for fridelane-3-one-28-al (4) is presented.

Conclusion: 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.

Staff secondments and transfer of knowledge:

The supply with the above target herbs to be used in the study on microbial activity of herbs, e.g. wound granulation in Bulgaria or South Africa and exchange of knowledge on the way of action of these herbs were realized between both countries. Some exchange of knowledge on the stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation on rats or farm animals was realized between the researchers from TU, DRDO, RU and UJ via the respective presentations.

Objective 6: To investigate some target herbal extracts for their protective effects against toxicity of FB1 in pigs or rabbits ("in vivo") and to study the protective role of such plant extracts against the cytotoxic effects of mycotoxins FB1 and/or OTA on pig lymphocyte cells via MTT assay and Comet assay ("in vitro").

WORK PACKAGE 6 – Pig/rabbits experiments ("in vivo" and "in vitro") on protective effects of herbal additives against toxicity of OTA or/and FB1 – done according to the plan

-Involved researchers: Dr Patrick Njobeh (ER from UJ moved to UNIKAPOS), Prof. M. Dutton (ER from UJ moved to UNIKAPOS), M Web (ESR from UJ moved to UNIKAPOS).

-Involved researchers during the second 2-years Period: M Web (ESR from UJ moved to UNIKAPOS).

-Used full-time equivalent months – 4,81 months, Planned – 9 months.

-Deliverables:

D 6. Establishing the protective effects of some herbal additives against toxic effects of OTA and/or FB1 in some in vivo and/or in vitro experiments – the experiment is done and the research paper is under elaboration.

-Milestone – M6. Establishing of possible protective effects of herbal additives against toxic and immunosuppressive effects of OTA and/or FB – the experimental work is done and a paper is prepared and will be published soon.

-Description of work finished in Work package 6.

A) The milk thistle Silybum marianum (L). seed extract, called silymarin, has been found to be an useful remedy for liver diseases. Silymarin consists of more than ten identified flavonoids, but the main component is the silibinin, which is a racemic mixture of two diastereomers, silybin A and silybin B. It was proved, that silymarin, as therapeutic agent affects positively the toxin-induced liver damage

The aim of this study was the investigation of any potential positive effect(s) of Carduus marianus on growing rabbits consuming dietary DON in high dosage.

Materials and methods

90 weaned (at the age of 35 days) Pannon White rabbits were housed in metal mesh wired cages (3 rabbits per cage). The duration of this experiment was 6 weeks (between 5-11 weeks of rabbits' age). The rabbits were bred at the farm of Kaposvár University. The experimental trial consisted of two periods. During the first 3 weeks the rabbits were separated in three groups (control, herb 0,5% and herb 1%) and on the 4th week the 3 groups were subdivided into toxin groups as well. Feed was provided ad libitum and the animals had free access to drinking water provided by pacifiers. The animals were checked daily for mortality and morbidity; weight and feed intake were recorded weekly. At the end of the experimental period the rabbits were euthanized by cervical dislocation and were exsanguinated.

DON was produced using Fusarium graminearum strain number IFA 77 (from "Das Interuniversitäre Department für Agrarbiotechnologie", Tulln, Austria) fungal culture (7 days old), grown on Potato Dextrose Agar (PDA; Chemika-Biochemica, Basil, Switzerland). The homogenized fungal cultures contained DON at concentration of 7140 mg/kg (or around 10 ppm DON).

Diets were prepared at the Department of Nutrition of Kaposvár University and formulated to meet the nutritional needs of weaned rabbits. Six different batches were prepared: control (C), control with DON (CT), 0,5% of Carduus marianus (H1), 0,5% of Carduus marianus and DON (H1T), 1% of Carduus marianus (H2) and 1% of Carduus marianus and DON (H2T).

Silibinin (SBN) concentration was determined by LC-MS. SBN was used in two concentrations: H1 (71.1±2.5 mg/kg) and H2 (143.6±6.1 mg/kg).

Blood sampling was performed on days 0, 14 and 39 for full blood count, clinical chemistry,

immunological parameters' determination and antioxidant parameters. Blood collection was also performed at the day of slaughtering for antioxidant parameters' investigation. After the exsanguination, body weight, weight of various organs (liver, kidney, spleen, heart) and caecum were recorded. The pH of the contents of stomach and caecum was measured as well.

Results. Productive performance and slaughtering parameters were not affected significantly by the consumption of DON. The caecal microbiota were affected by an increase in the number of aerobic bacteria when the toxin was consumed (independently of the plant treatment) which is an undesirable incident since in caecum anaerobic conditions occur. C. marianus had no significant effect on antioxidant parameters in rabbits. Despite the high concentration of the toxin rabbits were not affected severly. There was not established any significant interaction of the medicinal plant C. marianus and DON in rabbits.

B) Studies on the cytotoxic effects of extracts of the plants Gunnera perpensa Hydnora abyssinica and their possible protective effect against mycotoxin T-2

The aim of this study was the investigation of the cytotoxic effect of the extracts derived from the plants Gunnera perpensa and Hydnora abyssinica and subsequently to assess any protective effect against the mycotoxin T-2 using as endpoints cyto- and genotoxicity.

To assess the cytotoxic effect CCK-8 kit was used. CCK-8 is a water tetrazolium salt (derivative of the well-known MTT; Mossman, 1983) which is less toxic than MTT and does not require the additional step of solubilisation. The cells were treated for 48h and five replicates were used for each sample. Five equidistant concentrations of each plant were used (6.25, 12.5, 25, 50 and 100 ug/ml).

Based on the results of the preliminary experiments specific concentrations of each plant extract were chosen to be tested for their possible protective effect against the mycotoxin T-2.

Regarding the extracts derived from Gunnera perpensa there was a clear dose-effect observed whereas in the case of the extracts of Hydnora abyssinica there is a decrease from 6.25 to 25 ug/ml and 50ug/ml but for the 100ug/ml there was an increase (as the student stated this is in accordance with previous antibacterial assays performed at the University of Johannesburg).

Gunnera perpensa viability rates range was 28.5-80.9% whereas for Hydnora abyssinica was 40.3-70.6%.

T-2, the most toxic compound of type A trichothecenes was used at a concentration of 0.5 μM (which corresponds to 0.23 μg/ml) to assess the possible protective effect of the selected extracts. None of the extracts showed any protective effect since the viabilities of the combinations were similar to the viability of the T-2 treated cells.

Genotoxic effects were investigated with the help of Comet assay after exposure to the plant extracts for 24 and 48h. Gunnera perpensa was not genotoxic at all the tested concentrations resulting in a percentage of tail intensity less than 10% (scoring of comets with the Comet IV software). In the case of Hydnora abyssinica regarding the concentrations of 12.5 and 25 ug/ml were not genotoxic but the concentrations of 50 and 100ug/ml there were not enough cells to be scored but from the investigation under the fluorescence microscope it could be concluded that there was a DNA damage corresponding to 2-3 score (estimation-visual scoring).

C) MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

MTT assay is a colorimetric assay to assess the metabolic activity of the cells. In the case of our extracts, the objective of the investigation was to evaluate the protective effect of samples (samples from various herbs, e.g. leaves and stem bark of Erythrina caffra) on the lymphocyte cells in the presence of T-2 toxin by cell viability method. The blood was collected from pigs and the lymphocytes were isolated from the blood. The lymphocytes, T-2 toxin and extracts were mixed together and incubated for 24 hours and the MTT was added thereafter, etc.

To assess the cytotoxic effect CCK-8 kit was used. CCK-8 is a water tetrazolium salt (derivative of the well-known MTT; Mossman, 1983) which is less toxic than MTT and does not require the

additional step of solubilisation. The cells were treated for 24, 48 and 72h and five replicates were used for each sample. Three different extracts were used-aqueous, methanolic and ethanolic- in three different concentrations (20, 50 and 100 ug/ml).

Based on the results of the preliminary experiments specific concentrations of each plant extract were chosen to be tested for their possible protective effect against the mycotoxin T-2.

Regarding the extracts derived from the leaves ethanolic extract, it was shown to exert the lowest viability in comparison to control with a minimum viability of 52%. In all cases (aqueous, methanolic and ethanolic extract) the highest concentration (i.e. 100 ug/ml) exhibited the lowest viability (dose-effect). A time effect was also observed especially regarding the highest concentration.

In the case of stem derived extracts the aqueous had similar tendency but the methanolic and ethanolic extracts were quite cytotoxic with a cell viability of approximately 20% being observed after 24h.

T-2, the most toxic compound of type A trichothecenes was used at a concentration of 0.5 μM (which corresponds to 0.23 μg/ml) to assess the possible protective effect of the selected extracts. Some extracts didn't show any protective effect since the viabilities of the combinations were similar to the viability of the T-2 treated cells.

Due to the promising results observed in the MTT assay, a comet assay was subsequently performed on the extracts to investigate whether there is a genotoxicity relationship on the mechanism of cells toxicity.

4) Comet assay

The comet assay was performed to evaluate the DNA damage of the lymphocytes cells. In general for this assay a high damage of the DNA is expressed by a large tail of the comet observed. This assay uses almost the same protocol as the MTT (using the same extracts, e.g. E. caffra) where the cells are mixed with the T-2 toxin and the extracts and incubated for overnight. After the incubation, an electrophoresis step was performed to line up the damaged DNA before the analysis on a microscope connected to a computer.

Objective 7: To investigate some target herbal extracts for their possible protection against toxic and immunosuppressive effects of mycotoxins OTA and FB1 in chicks/rats

WORK PACKAGE 7 – Chick/mice experiments on protective effects of herbal additives against toxic and immunosuppressive effects of OTA and FB1 – done according to the plan

-Involved researchers: Dr Galina Dimitrova Nikolova (ER from TU moved to DRDO), Dr Yanka Dimitrova Karamalakova (ER from TU moved to DRDO), I. Dinev (ER from TU moved to UJ and North-West University)

-Involved researchers during the second 2-years Period: I. Dinev (ER from TU moved to UJ and North-West University)

-Used full-time equivalent months – 7,07 months, Planned – 12 months.

-Deliverables:

Establishing the protective effects of some herbal additives against toxic and immuno-suppressive effects of OTA and FB1 in chicks/rats. Preparing a research paper in this regard.– The experimental work is done – some papers and conference reports were done and some others research paper are under elaboration.

-Milestone – M6. Establishing of possible protective effects of herbal additives against toxic and immunosuppressive effects of OTA and/or FB – the experimental work is done and several publications are prepared – some of them already published.

-Description of work finished in Work package 7:

A) Development of Nanoformulation from herbal extracts (incl. Silymarin)

SNEDDS (Self NanoEmulsifying Drug Delivery Systems) was prepared to increase the solubility and oral absorption for achieving better bioavailability and therapeutic activity of silymarin. This was attained by dissolving silymarin in oil phase (Labrafac Lipophile WL1349) and then surfactant (Solutol HS 15) and co-surfactant (Transcutol HS) were added as per optimized procedure. It was characterized by determining globule size distribution and Transmission electron microscopy.

Evaluation of efficacy at in vivo level. Various in vivo enzymatic and non-enzymatic experiments e.g., Catalase, Glutathione Reductase, Glutathione Peroxidase and Superoxide dismutase were performed in liver and intestine tissues to reduce mycotoxin-induced toxicity.

Deliverable. Nanoformulation of Silymarin with higher efficacy has been developed.

a) Comparative toxicity analysis with 4 herbal extracts on 4 different mammalian cell lines (HepG2, NKE, ACHN and A498) was performed. Ethanolic Extract of Glycyrrhiza glabra and Tinospora cordifolia at a concentration of 50 µg/ml and 100 µg/ml were found to protect NKE cells, whereas 40-60% cell death was observed in case of transformed cells.

b) Studies on cytotoxicity by Ochratoxin A and Fumonisin B1 were performed on mammalian cell line. Incubation of HepG2 cells with increasing concentration of Ochratoxin A resulted in a dose and time dependent cytotoxicity as measured by SRB uptake assay. Half lethal concentration (LC50) of OTA was 10 µM and for Fumonisin B1 350 µM after 72 hrs of incubation with OTA.

B) Several ex vivo, in vivo and in vitro experiments were done to evaluate possible protective effects of various herbs against the toxic effects and oxidative stress provoked by ochratoxin A

a) Studies on in-vitro efficacy of herbal extracts against Ochratoxin A

-Cyto-toxic studies with 05 screened extracts against Ochratoxin A on Normal Kidney Epithelial cells (NKE) and tumorigenic Kidney cell line (ACHN) were performed.

-Cyto-toxic studies with 05 Bulgarian extracts with respect to radiation on Normal Kidney Epithelial cells (NKE) was performed in order to validate their efficacy against oxidative stress.

-To validate the antioxidant potential of the extracts, NF-κB activation ability of 6 herbal extracts with radiation was determined on Lac-Z reporter cells. Extract E1 and E2 were found to have highest activity. Studies on changes in cellular physiology (ROS, MMP) with radiation was determined on NKE cells and the results were found to be in corroboration with the above mentioned results.

b) Studies on ex-vivo or in vivo efficacy of herbal extracts [RDP-03, RDP- 06 and; RDP-10] against Ochratoxin A

The studies were performed with the help of EPR in collaboration with Trakia University, Stara Zagora, Bulgaria. 05 herbal extracts (RDP03, RDP06, RDP010, RDP09 and RDP011) were selected for further analysis.

-The presence of free electron in the plant extracts responsible for antioxidant property was determined with the help of EPR.

-The presence of free electron in the plant extract responsible for antioxidant property was determined with the help of g-factor. The g-factor for free electron is ~2.0023. g-factor for different plant extracts was calculated with the help of EPR. The mean deviation for each sample was less than 2%.

-It is verified whether the radical structures registered in the studied samples of RDP 06, RDP03, RDP09, RDP010 and RDP011 extracts belong to a semiquinone free radical; their EPR spectra were evaluated after alkalization.

-The antioxidant capability of the resulting plant extracts against a stable radical DPPH was evaluated and EPR spectroscopy was performed. Plant extracts have shown high efficiency in the DPPH test.

-DPPH free radical scavenging capacities of plant extracts with concentration 1 mg/ml were determined at different time intervals before and after UV irradiation. The results were determined and compared. Statistically significant increase was observed in the DPPH scavenging activity of herbal extracts after 10 min of incubation. Whereas, it started decreasing after 10 min on incubation with DPPH.

-Ex-vivo studies were performed using electron paramagnetic resonance spectroscopy (EPR) methods to elucidate the radical scavenging activity of herbal extracts by following out the levels of some "real time" oxidative stress biomarkers in tissue homogenates and blood sample of experimental animals treated with Ochratoxin A, RDP03 and in combinations of both. 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.

Ex vivo studies with other extracts is in progress.

Animals.

Male white non-inbred mice weighting 25-35 g were used. The mice were housed in polycarbonate cages in controlled conditions (12 h light/ dark cycles), temperature of 18–23oC and humidity of 40–60%, with free access to tap water and standard laboratory chow.

1 group: The animals (6/per group) were injected by Zingiber officinale, Tinospora cordifolia, Curcuma longa, Glycyrrhiza glabra extract and bacterial sample 006.9 G, 80 mg/kg/ given every day for period of 10 days.

2 group: The animals (6/per group) were injected by Zingiber officinale, Tinospora cordifolia, Curcuma longa, Glycyrrhiza glabra extract and bacterial sample 006.9 G, 80 mg/kg/ given every day + 3 times OTA for period of 10 days. Experiments were carried out in accordance with national regulations and the European directive 210/63/EU from 22.09.2010, concerning the protection of animals used for scientific and experimental purposes. After 10th day the mice were dissected and tissues from liver, kidneys, spleen and heart were homogenates in cold PBS solution and studied by direct and spin trapping EPR spectroscopy and results were compared to those of non treated controls (6 mice).

Electron Paramagnetic Resonance measurements:

Ex vivo assay the levels of ROS production in the tissue homogenates of mice by EPR spectroscopy: Briefly, about 0.1 g of liver, kidneys, pancreas, heart, brain and blood samples were homogenized after addition 1.0 ml of 50 mM solution of the spin-trapping agent PBN dissolved in DMSO. EPR settings were as follow: center field 3503 G; sweep width 10.0 G; microwave power 12.83 mW; receiver gain 1 x 106; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 81.92 s, 5 scans per sample.

Ex vivo assay of the ascorbate radicals levels in the tissue homogenates of mice by EPR spectroscopy: Tissues from liver, kidneys, pancreas, heart, brain and blood n were collected in cold saline and processed immediately. Tissue samples were weighed and homogenized in DMSO (10% w/v) and centrifuged at 4000 g, at 40C for 10 min. Supernatants were collected and the level of Asc. was evaluated by EPR spectroscopy. EPR settings were as follows: center field 3505 G; sweep width 30 G; microwave power 12.70 mW; receiver gain 1 x 104; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 82.94 s; 1 scans per sample.

Ex vivo assay of the nitric oxide levels in the tissue homogenates of mice by EPR spectroscopy: Briefly, to 50μM solution of Carboxy PTIO.K dissolved in a mixture of 50 mMTris (pH 7.5) and DMSO in a ratio 9:1. To 100μl tissues of liver, kidneys, pancreas, heart, brain and blood was added 900μl Tris buffer dissolved in DMSO (9:1) after that the mixture was centrifuged at 4000 rpm for 10 min at 4ºC. 100 μL of sample and 100 μL 50 mM solution of Carboxy PTIO were mixed and EPR spectrum of the spin adduct formed between Carboxy PTIO spin trap and generated •NO radicals was recorded. The EPR settings were as follows: 3505 G centerfield, 6.42mW microwave power, 5G

modulation amplitude, 75 G sweep widh, 2.5x102 gain, 40.96 ms time constant, 60.42 s sweep time, 1 scan per sample.

Ex vivo assay of the biodistribution levels in the tissue homogenates of mice by EPR spectroscopy:

EPR study on the biodistribution of Curcuma longa, Glycyrrhiza glabra extract 80 mg/kg/ given every day and bacterial sample 006.9 G 40mg / kg and 80 mg/kg/ administratedafter 1hr and 2hrs.

The drug was administrated i.p. and weighing tissue samples of liver, kidneys, pancreas, heart, brain and blood were homogenized in PBS (10% w/v) and centrifuged at 2000 g for 15 min. Supernatants were collected and placed in EPR cavity and EPR spectra of the drug in the different samples were recorded. EPR settings were as follow: center field 3505 G; sweep width 70 G; microwave power 13.02 mW; receiver gain 2 x 104; mod. amplitude 10 G; time constant 327.68 ms; sweep time 327.68 s, 1 scan. The concentration of Psoralea corylifolia Linn. in each sample was determined by double integration of the corresponding EPR spectrum and expressed in arbitrary units.

Results: All of natural compounds acting as a typical antioxidants and reduce ascorbate radicals levels ROS production and nitrite oxide.

Curcuma longa

| | Ascorbate radicals | ROS production | NO- radicals |
|---|---|---|---|
| Controls | 0.134+-0.00 | 0.492+-0.01 | 3.4+-0.74 |
| Liver | 0.151+-0.02 | 0.199+-0.002 | 0.63+-0.09 |
| Controls | 0.43+-0.00 | 0.369+-0.11 | 5.48+-0.89 |
| heart | 0.013+-0.00 | 0.278+-0.03 | 4.77+-0.91 |

All of natural compounds acting as a typical antioxidants and reduce OTA levels in a combinations and combination OTA+ antioxidant reduce ascorbate radicals, levels ROS production and nitrite oxide.

c) Studies on influence of ochratoxin-A and an extract of Tinospora cordifolia against biochemical and oxidative changes in mice spleen

Collection of the plant material and preparation of Tinospora cordifolia extract:

The stem part of TC for experiment purpose was collected from authenticated Ayurvedic store from a local market at New Delhi, during the month of November, 2013 and was confirmed by expert botanist. Dried stems of TC were cleaned to separate unwanted material and then grinded into coarse powder using mortar and pestle. Fine powder was obtained after grinding in grinder and then passing through sieve. Powdered stems were extracted by Kinetic maceration for 48 hrs using 100% ethanol as solvent. The whole mixture then underwent a coarse filtration by a piece of clean, white muslin cloth which was followed by filtered through whatman no.1 filter paper. The total filtrate was dried till semi-liquid using rotary evaporator (Buchi B-480, India) at 400C and was further lyophilized using lyophilizer (IIshin Lab Co. Ltd, USA) to get the crude extract, and was used as a practical approach to supply the mice with described compounds presumed to protect against OTA intoxication in spleen.

Experimental animals:

Specific pathogen-free male Balb/c mice (second line, non- inbred, weighted 25-35 g mice, 5-6 weeks old) were purchased at two-weeks of age, housed in polycarbonate wire floor cages in controlled conditions (12 h light/ dark cycles), temperature of 18–23oC suitable for their age and humidity of 40–60%, with free access to tap water and standard laboratory chow were maintained. Mice were grouped in 3 experimental groups and 1control group (6 animals in each one/ oral pretreatment/ 11 days experiment) and fed respectively: control group - OTA free, standard diet; group I - TC extract, 80 mg/kg, given 3 times for period of 11 days; group II - 15 ppm OTA, daily in diet; group III – 15 ppm OTA and 80 mg/kg extract of TC. All mice were carefully examined and weighed at 3th, 7th and 11th day (Table 1), and the consumed feed was measured at the end of

experiment. For following up the weight and the changes of spleen and internal organs, organs of all treated groups and controls were compared. Experiments were carried out in accordance with national regulations and the European directive 210/63/EU from 22.09.2010, concerning the protection of animals used for scientific and experimental purposes. After 11th day the mice were dissected and spleen tissues were homogenase in cold PBS solution and studied by direct and spin trapping EPR spectroscopy and spectrophotometric biochemical analyses. Results were analyzed and compared to those of non treated controls.

Electron Paramagnetic Resonance measurements:

For all EPR measurements an X-band EMXmicro, EPR spectrometer (Bruker, Germany) equipped with standard Resonator was used. Spectral processing was performed using Bruker WIN-EPR and SimFonia software. The levels of the Asc., NO. radicals and ROS production in experimental mice were calculated by double integration of the corresponding EPR spectra registered in the spleen (arbitrary units).

Ex vivo assay the levels of ROS production in the spleen homogenates of mice by EPR spectroscopy:

The level of ROS productions was studied according to Shi et al., 2005 with some modifications by Zheleva et al., 2011. Briefly, about 0.1 g of spleen samples were homogenized after addition 1.0 ml of 50 mM solution of the spin-trapping agent PBN dissolved in DMSO. EPR settings were as follow: center field 3503 G; sweep width 10.0 G; microwave power 12.83 mW; receiver gain 1 x 106; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 81.92s, 5 scans per sample.

Ex vivo assay of the ascorbate radicals levels in the spleen tissue homogenates of mice by EPR spectroscopy:

The Asc. levels in organ homogenates were studied according to Buettner & Jurkiewicz, 1993 with slight modifications. Tissues from spleen were collected in cold saline and processed immediately. Tissue samples were weighed and homogenized in DMSO (10% w/v) and centrifuged at 4000 g, at 40C for 10 min. Supernatants were collected and the level of Asc. was evaluated by EPR spectroscopy. EPR settings were as follows: center field 3505 G; sweep width 30 G; microwave power 12.70 mW; receiver gain 1 x 104; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 82.94 s; 1 scans per sample.

Ex vivo assay of the nitric oxide levels in the spleen tissue homogenates of mice by EPR spectroscopy:

The levels of •NO radicals were studied according to methods of Yoshioka et al.,1994 and Yokoyama et al.,2004 with some modification. Briefly, to 50μM solution of Carboxy PTIO.K dissolved in a mixture of 50 mMTris (pH 7.5) and DMSO in a ratio 9:1. To 100μl tissues was added 900μl Tris buffer dissolved in DMSO (9:1) after that the mixture was centrifuged at 4000 rpm for 10 min at 4ºC. 100 μL of sample and 100 μL 50 mM solution of Carboxy PTIO were mixed and EPR spectrum of the spin adduct formed between Carboxy PTIO spin trap and generated •NO radicals was recorded. The EPR settings were as follows: 3505 G centerfield, 6.42mW microwave power, 5G modulation amplitude, 75G sweep widh, 2.5x102 gain, 40.96 ms time constant, 60.42 s sweep time, 1 scan per sample.

Ex vivo biochemical analyses of MDA measured specrtophotometrically:

The method estimation of lipid peroxidation of thiobarbituric acid (TBA), which measures Malondialdehyde (MDA)-reactive products, was used (Plaser et al. 1966). In brief, 0.5 mg fresh spleen-tissues, 1 ml physiological solution, and 1 ml 25% trichloroacetic acid were mixed and centrifuged at 7,000 rpm for 20 min. 2 ml protein-free supernatant with 0.5 ml 1% TBA (prepared in 0.025 M NaOH) were added in the reaction mixture. The resultant mixture was then subjected to 95°C for 1 h in a water bath. A pink coloured chromogen complex was formed, readable at 532 nm.

Conclusion

In conclusion, using EPR spectroscopy we have demonstrated increased levels of some “real time” biomarkers of oxidative stress such as Asc., NO. radicals and ROS products in the spleen of

mice after treatment by OTA. Moreover combination of OTA with oral administration of TC extract led to significant improvement in the levels of oxidative stress biomarkers in mice spleen.

In the light of these results, TC extract behaves as a good scavenger of ROS and RNS and might find application in further studies in order to find application in the pharmaceutical and food industry as a protector against various mycoses

……d) 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

Animals

Male non-inbred albino mice (25-40 g) were used. The mice were housed in polycarbonate cages in controlled conditions (12 h light/dark cycles), the temperature of 18-23 C and humidity of 40-70%, with free access to tap water and standard laboratory chow. Experiments were carried out in accordance with European directive 86/609/EEC of 24.11.1986 for the protection of animals used for scientific and experimental purposes. Mice were divided into six groups (6 animals in each group). The control group of mice was inoculated two i.p. injections with solvent, only). The second injection was administered 45 min after the first. To study the Ldopa effect we used the acute model of Bottiglieri et al., 2012. The mice from all tested groups (except controls) received either two i.p. injections of L-dopa (100 mg/kg) followed by benserazide (10 mg/kg). The second injectionwas administered 45 min after the first. The groups undergoing combination therapy were pre-treated first for one hour with i.p. injections in doses of 400 mg/kg of Ascorbic acid, Trolox, Rose oil or Lavender oils according to Umezu et al., 2006 and after that received L-dopa and benserazide. 30 min after the last injection all mice were sacrificed by light anesthesia. Blood was obtained by cardiac puncture and collected in tubes with 10% EDTA (ethylene diaminetetraacetic acid), centrifuged at 3000 rpm for 15 min and plasma samples were carefully separated. The brain was immediately washed in cool saline and was prepared homogenates and centrifuged at 3000 rpm for 15 min. After centrifugation, the samples were immediately studied by EPR spectroscopy for their radical scavenging abilities.

Spectrophotometric methods

All spectrophotometric measurements were performed on a Thermo Scientific spectrophotometer.

Ex vivo spectrophotometric DNPH assay

Quantification of protein carbonyl content (PCC) as final products of protein oxidation was carried out using the spectrophotometric DNPH method described by Dalle-Donne et al. (2003).

Ex vivo spectrophotometry assay for evaluation the levels of MDA

To evaluate the levels of lipid peroxidation, Thiobarbituric Acid Reactive Substances (TBARS) assay was used, which measures MDA reactive substances (Plaser et al., 1966).

Electron paramagnetic resonance (EPR) spectroscopy

EPR measurements of all tested samples were conducted at room temperature (18-23 C) on an X-band EMXmicro, spectrometer Bruker, Germany, equipped with standard Resonator. Quartz capillaries were used as sample tubes. The sample tube was sealed and placed in a standard EPR quartz tube (i.d. 3 mm) which was fixed in the EPR cavity. All EPR experiments were carried out in triplicate and repeated. Spectral processing was performed using Bruker WIN-EPR and SimFonia software.

Ex vivo EPR evaluation the levels of +NO radicals

Based on the previously methods (Yoshioka et al., 1996; Yokoyama et al., 2004) we developed and adapted the EPR method for evaluation the levels of +NO radicals. Briefly, the solution of Carboxy. PTIO.K (50 mM) was prepared after dissolving in a mixture of Tris buffer (50 mM, pH 7.5) and DMSO in a ratio 9:1. To 100 ml plasma/brain homogenateswas added 900 ml Tris buffer plus DMSO (9:1) and centrifuged at 4000 rpm for 10 min at 4 C. The tested sample (100 mL) and

100 mL 50 mM solution of Carboxy. PTIO were mixed. The EPR spectrum of the spin adduct formed between the spin trap Carboxy. PTIO and generated +NO radicals was recorded. The levels of +NO radicals were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units. The EPR settings were: 3505 G centerfield, 6.42 mW microwave power, 5 G modulation amplitude, 75 G sweep width, 2.5 x 102 gain, 40.96 ms time constant, 60.42 s sweep time, 1 scan per sample.

Summary of results

Levodopa (L-dopa) is a "gold standard" and most effective symptomatic agent in the Parkinson's disease (PD) treatment. The several treatments have been developed in an attempt to improve PD treatment, but most patients were still levodopa dependent. The issue of toxicity was raised in vitro studies, and suggests that L-dopa can be toxic to dopaminergic neurons, but it is not yet entirely proven. L-dopa prolonged treatment is associated with motor complications and some limitations. Combining the L-dopa therapy 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 Ldopa can reduce oxidative toxicity, and may play a key role in ROS/RNS disarm.

e) Studies on ex vivo effect of Glycyrrhiza glabra root extract on some "real time" biomarkers of oxidative stress – an EPR spectroscopy study

Plant extract and Chemicals

The air dried roots of Glycyrrhiza glabra were made into a coarse powder and after dissolved in 2l/ distilled water was subjected to hot maceration process, with continuous stirring for 48h. The water extract was filtered through muslin cloth and the filtrate was concentrated with evaporation on water bath and then lyophilized. The extract was made and providing from INMAS, India as reference. Dimethyl sulfoxide (DMSO), N-tert-butyl-alpha-phenylnitrone (PBN), 2-(4-carboxyphenyl)-4,4,5,5-tetra-methylimidazoline-1oxyl-3-oxide (Carboxy-PTIO.K) and and PBS were purchased from Sigma Chemical Co, St. Louis, USA. All the other chemicals used in this study were with analytical grade.

Animals

Male white non-inbred mice weighting 25-35 g were used. The mice were housed in polycarbonate cages in controlled conditions (12 h light/ dark cycles), temperature of 18–23oC and humidity of 40–60%, with free access to tap water and standard laboratory chow. The animals (6/per group) were injected by Glycyrrhiza glabra extract, 80 mg/kg/ given 3 times for period of 10 days. Experiments were carried out in accordance with national regulations and the European directive 210/63/EU from 22.09.2010, concerning the protection of animals used for scientific and experimental purposes. After 10th day the mice were dissected and tissues from liver, kidneys, spleen and heart were homogenates in cold PBS solution and studied by direct and spin trapping EPR spectroscopy and results were compared to those of non treated controls (6 mice).

Electron Paramagnetic Resonance measurements

For all EPR measurements an X-band EMXmicro, EPR spectrometer (Bruker, Germany) equipped with standard Resonator was used. Spectral processing was performed using Bruker WIN-EPR and SimFonia software. The levels of the Asc., NO. radicals and ROS production of the plant extract were calculated by double integration of the corresponding EPR spectra registered in the different organs (arbitrary units).

Ex vivo assay the levels of ROS production in the tissue homogenates of mice by EPR spectroscopy: The level of ROS productions was studied according to Shi et al., 2005 with some modifications by Zheleva et al. (2011). Briefly, about 0.1 g of liver, kidneys and pancreas samples were homogenized after addition 1.0 ml of 50 mM solution of the spin-trapping agent PBN dissolved

in DMSO. EPR settings were as follow: center field 3503 G; sweep width 10.0 G; microwave power 12.83 mW; receiver gain 1 x 106; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 81.92 s, 5 scans per sample.

Ex vivo assay of the ascorbate radicals levels in the tissue homogenates of mice by EPR spectroscopy: The Asc. levels in organ homogenates were studied according to Buettner & Jurkiewicz, 1993 with slight modifications. Tissues from liver, kidneys, spleen and heart were collected in cold saline and processed immediately. Tissue samples were weighed and homogenized in DMSO (10% w/v) and centrifuged at 4000 g, at 40C for 10 min. Supernatants were collected and the level of Asc. was evaluated by EPR spectroscopy. EPR settings were as follows: center field 3505 G; sweep width 30 G; microwave power 12.70 mW; receiver gain 1 x 104; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 82.94 s; 1 scans per sample.

Ex vivo assay of the nitric oxide levels in the tissue homogenates of mice by EPR spectroscopy: The levels of •NO radicals were studied according to methods of Yoshioka et al., and Yokoyama et al., with some modification. Briefly, to 50μM solution of Carboxy PTIO.K dissolved in a mixture of 50 mMTris (pH 7.5) and DMSO in a ratio 9:1. To 100μl tissues was added 900μl Tris buffer dissolved in DMSO (9:1) after that the mixture was centrifuged at 4000 rpm for 10 min at 4ºC. 100 μL of sample and 100 μL 50 mM solution of Carboxy PTIO were mixed and EPR spectrum of the spin adduct formed between Carboxy PTIO spin trap and generated •NO radicals was recorded. The EPR settings were as follows: 3505 G centerfield, 6.42mW microwave power, 5G modulation amplitude, 75 G sweep widh, 2.5x102 gain, 40.96 ms time constant, 60.42 s sweep time, 1 scan per sample.

Summary of results

Glycyrrhiza glabra (Licoric), Indian medicinal plant exhibited good antiinflammatory, antiviral, antimicrobial, antioxidative, anticancer, immunomodulatory, hepatoprotective and cardio- protective properties and excellent ex vivo radical scavenging capacity, which are relevant to radioprotection and uses in medicine. By the present research for the first time using ex vivo Electron Paramagnetic Resonance (EPR) spectroscopy methods to investigate changes in “real time” levels of Ascorbate radicals (Asc.), NO. radicals and ROS production in organs isolated from healthy mice (6 non-inbreed mice) treated with Glycyrrhiza glabra extract (80mg/kg/ given 3 times for 10 days). The mice were dissected and tissues from liver, kidneys, spleen and heart were homogenates in cold PBS solution and studied by direct and spin trapping EPR spectroscopy and results were compared to those of non treated controls (6 mice). Tissue homogenates in DMSO, DMSO solution of the spin trap Carboxy PTIO and DMSO solution of the spin trap N-tert-butyl-alpha-phenylnitrone (PBN) were prepared for determination of the Asc., NO. radicals and for ROS products, correspondingly. 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. Present EPR ex vivo study characterizes Glycyrrhiza glabra water extract as a good antioxidant.

f) Crateva adansonii DC is a plant traditionally used in Cameroon to treat constipation, asthma, snake bites, post menopausal complaints and cancers.

Aim: The anticancer potential of the dichloromethane / methanolextract of C. adansonii stem barks was investigated using human breast cancer cell and 7,12 dimethylbenz(a) anththracene (DMBA)-induced mammary tumorigenesis model in rats.

Material and methods: The cytotoxicity of C. adansonii extract was assessed in vitro towards breast carcinoma (MCF-7 and MDA-MB-231) and non-tumoral cell lines (NIH/3T3 and HUVEC) by Alamar Blue assay. Furthermore, in vivo studies were performed on female Wistar rats treated either with C. adansonii extract at a dose of 75 or 300 mg/kg body weight or with tamoxifen (3.3 mg/kg body weight), starting 1 week prior DMBA treatment and lasted 12 weeks. The investigation focused on tumour burden, tumour DNA fingerprint, morphological, histological, hematological, and

biochemical parameters.

Results: CC50 values for the in vitro assays were 289 mg/mL against MCF-7cells and >500 mg/mL in others cells, leading to a selectivity index >1.73. 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. A weak effect was also observed at 30 0mg/kg. 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, phenylpropanoïds, sesquiterpene derivatives, gallotannins and lignans. The LD50 of C. adansonii was estimated to be greater than 5000mg/kg.

Conclusions: These aforementioned results suggest that the C. adansonii extract may possess antitumor constituents, which could combat breast cancer and prevent chemically-induced breast cancer in rats.

g) Studies on estrogenic potential of Millettia macrophylla. This herb was previously reported to have estrogenic effects and to prevent postmenopausal osteoporosis in Wistar rats. So, the study deals with the identification of its secondary metabolites and the evaluation of their estrogenicity and cytotoxicity toward tumoural cells. Thus, 13 known compounds were obtained from successive chromatographic columns and identified by NMR data compared to those previously reported.

Methods: In vitro estrogenicity of the isolates and the phenolic fraction (PF) of M. macrophylla were performed by E-screen and reporter gene assays, while their cytotoxicity was evaluated by Alamar Blue (resazurin) assay. A 3-days uterotrophic assay and the ability of PF to alleviate hot flushes in ovariectomized adult rats were tested in vivo.

Results: Seven of the 13 secondary metabolites turned to be estrogenic. Only two exhibited cytotoxic effects on MCF-7 and MDA-MB-231 with CC50 values of 110 μM and 160 μM, respectively. PF induced a significant ($p < 0.01$) MCF-7 cells proliferation and transactivated both ERα and ERβ in the reported gene assay at 10−2 μg/mL. In vivo, PF acted more efficiently than the methanol crude extract, resulting to a significant ($p < 0.01$) increase in the uterine wet weight, uterine protein level, uterine and vaginal epithelial height at the dose of 10 mg/kg BW. In addition, PF reduced the average duration and frequency of hot flushes induced in rat.

Conclusion: These aforementioned results indicate that PF is a good candidate for the preparation of an improved traditional medicine able to alleviate some menopausal complaints such as vaginal dryness and hot flushes.

C) Chick experiments were done to evaluate possible protective effects of various herbs against the toxic effects of mycotoxin ochratoxin A.

The experiment with female chicks (broiler ROSS 308) was started on 07/10/2015. Based on the data available with us and also conversion of dose (based on body w. and dose conversion factors available in literature (WHO norms and other standard toxicological manuals) from one animal to another animal/ birds), we have calculated the following dose, which might be the nearest effective dose for alleviating the effects of toxicants, incl. mycotoxins. Some variations and adjustments might be required based on age, sex, breed, and health status of the chicks as well:

I-1. Tinospora cordifolia: 300 mg/kg bw in chicks (oral) – or 4000 ppm via the feed
I-2. Glycyrrhiza glabra: 400-600 mg/kg bw in chicks (oral) - or 6600 ppm via the feed
SA-1. Centella asiatica: 300-400 mg/kg bw in chicks (oral) - or = 4600 ppm via the feed
SA-2. Withania somnifera: 200-400 mg/kg bw in chicks (oral) – or 4000 ppm via the feed
SA-3. Silybum marianum: 80 mg/kg bw in chicks (orally) – or 1100 ppm via the feed

Fresh stems of Tinospora cordifolia Miers. (Family Menispermaceae) and roots of Glycyrrhiza glabra L. (Family: Fabaceae) were collected from the central plains/plateau region of India with a prevalence of humid subtropical climate, or the comparatively less hotter lower Himalayan tract (250-530 m altitude). The plants were identified at the Institute of Nuclear Medicine and Allied Sciences, Delhi by a qualified Botanist and voucher specimens deposited. The stem portions of Tinospora cordifolia and roots, rhizomes and stolons of were cut into small pieces (ca. 2.5 5-3.0 cm in length) and were shade dried for 72-120 h with the precaution of contamination from the dust. The plant material was further mechanically dried in a hot air oven between 55-60°C for 72 h to remove any left over moisture content. The plant material was then converted into a fine powder form in a grinder and stored in air tight containers preventing it from moisture until the start of experiment.

All broiler chicks breed ROSS 308 were fed on Starter feed during the first 10 days (07-16/10/2015). Coli-terravet ® (1g/l) was given to the drinking water during the days 1-3 (07-09/10/2015), EGG BOOSTER (1g/l) was given to the drinking water during the days 4-7 (10-13/10/2015), Bioselet ® E (0,1 ml/l) was given to the drinking water during the days 7-10 (13-16/10/2015). The same scheme/design of medicine was repeated during the next days, etc.

At day 16 (6 days after beginning of experiment - 22/10/2015) SB3 was given in the drinking water to protect against coccidiosis. All chicks were immunized against Newcastle disease at day 14 (or day 4 after beginning of experiment - 20/10/2015) and reimmunization was done at day 28 (or day 18th after beginning of experiment - 03/11/2015) with vaccine 2510C3U3H

The experiment starts at day 10th after incubation (hatch) of chicks.

11-40 day – all chicks were fed as follow:

-C-1 group - Control 1 - standard grower

-3 ppm group - 3 ppm OTA and standard grower

-5 ppm group - 5 ppm OTA and standard grower

-I-1. Tinospora cordifolia given at 4000 ppm + 3 ppm OTA via the feed

-I-2. Glycyrrhiza glabra given at 6600 ppm + 3 ppm OTA via the feed

-SA-1. Centella asiatica given at 4600 ppm + 5 ppm OTA via the feed

-SA-2. Withania somnifera given at 4000 ppm + 5 ppm OTA via the feed

-SA-3. Silybum marianum given at 1100 ppm + 5 ppm OTA via the feed

Immunization

The control and experimental chicks were immunized at an age of 14 days (4 days after beginning of the experiment) against Newcastle disease with commercial vaccine B1 No 2510C3U3H. A re-immunization was also done at an age of 28 days (18th day after beginning of the experiment). The immunization was realized per os via the drinking water according to the respective prescriptions (2 ml vaccine per 100 chicks).

Assessment of immune response and serological examinations

The blood for serological investigations was taken from the wing vein at an age of 42 days (14 days after re-immunization). The vaccinal immune response was measured by the haemagglutination inhibition test. The viral antigen (strain B1) was used in 8 HU (haemagglutination units). β procedure (Diluted Serum-Constant virus) was performed in 96 well round bottomed microtiter plates. Serial dilutions of the tested serum were made and the antigen was subsequently added. After that, the serum was incubated for 30 minutes and 1% (v/v) chicken erythrocyte suspension was added. The plates were left at room temperature until the known HI- positive wells showed a tight, well-circumscribed button of unagglutinated, sedimented erythrocytes. The haemagglutination inhibiting antibody titer was calculated as the reciprocal value of the highest dilution of serum at which there was complete inhibition of haemagglutination. The results were validated by using negative control serum, which doesn't give a titre >1/8, and a positive control serum with the titre 1: 256 (Hitchner, 1979).

Measurements

The weight of the chicks was measured at day 1 (b.w. was between 40 and 50 g) and at day 10,

just before the beginning of the experiment (b.w. was ranged between 190 and 245 g). The chicks were distributed randomly in various experimental or control groups immediately after the last measurement. The quantity of the feed utilized by the chicks in each group, the body weight and the absolute- or relative weight of liver, kidneys, heart, bursa Fabricii, spleen, thymus and carcass were measured at the end of the experiment at day 42 (32 days after beginning of the experiment). Blood/serum for clinical biochemistry and tissues samples for pathomorphological investigations were also taken that time from all experimental and control chicks.

Histological examination

Materials for histological examination were taken from kidneys, liver, lung, heart, spleen, thymus, bursa Fabricii, intestine, brain, cerebellum, medulla and bone marrow. The same were subsequently fixed in 10% neutral buffered formalin or processed for freezing microtome. For proving the fat, the freezing materials were stained with Sudan III. All fixed tissues were subsequently embedded in paraffin, sectioned at 6 µm and stained with haematoxylin-eosin. Periodic acid - Schiff (PAS) staining was also performed for proving of glycoproteid, mucoproteid or lipoproteid substances in various tissues or cell components. Part of the embedded tissues were stained according to Weigert iron haematoxylin to prove the presence or absence of fibrin.

Clinical biochemistry

Various blood and/or serum samples were investigated for various haematological and/or biochemical parameters within 1-2 h of their collection and immediately after separation of the serum. The blood sugar, serum cholesterol and serum creatinine were measured by test of Boehringer Mannheim (Mannheim, Germany); the serum total protein by Bio-La-Test (Lachema Diagnostica, Brno, Czech Republic); the uric acid by EnzUric-FT-test (Labordiagnostica, Gopecke, Germany); the serum albumin according to Kolb and Kamushnikov (1982); the serum enzyme activity of ASAT and ALAT was measured by Cormay test (Smolenskiego, Warsaw, Poland).

Summary of results

Protective effects of herbal feed additives Glycyrrhiza glabra and Tinospora cordifolia against the growth inhibitory effect of ochratoxin A and associated immunosuppression and biochemical or pathomorphological changes were seen. The intensity of pathological and biochemical changes, the changes in relative organs' weight and body weight, and the decrease of antibody titer were strongest in OTA-treated chicks without antidote-supplementation, but the same were less pronounced in chicks treated additionally with Glycyrrhiza glabra and especially in those supplemented with Tinospora cordifolia. The higher relative weight of lymphoid organs of chicks supplemented with both herbs revealed their beneficial effects on immune system. A hepatoprotective effect was seen for both herbs being stronger for chicks additionally supplemented with Glycyrrhiza glabra as can be seen from the pathomorphological findings and the lower levels of ASAT and ALAT. A protective effect on bone marrow and kidneys was found for Tinospora cordifolia, whereas Glycyrrhiza glabra was seen to increase additionally the serum levels of uric acid. It seems, that both herbs could be used as a practical approach for ensuring a better utilization of OTA-contaminated feed as can be seen from the better feed performance and higher body weight of chicks treated with both herbs.

Protective effects of herbal feed additives Silybum marianum or Withania somnifera and slightly of Centella asiatica against the growth inhibitory effect of ochratoxin A and associated immunosuppression and biochemical or pathomorphological changes were seen. The intensity macroscopical and histopathological changes, the deviations in relative organs' weight and body weight, biochemical changes and the decrease of antibody titer were strongest in OTA-exposed chicks without antidote-treatment, followed by chicks treated additionally with Centella asiatica, whereas the same changes were significantly slighter or not seen in chicks additionally treated with the herbal additives Withania somnifera or Silybum marianum. The slighter increase in the serum levels of uric acid and the enzyme activity of ASAT and ALAT also supported the protective effects of the both herbs on the kidneys and/or liver. The strong immunesuppressive effect of OTA on humoral immune response against Newcastle disease was completely prevented in chicks given the

herbal additives Withania somnifera or Silybum marianum, which was additionally supported by the higher relative weight of immunocompetent (lymphoid) organs in the same chicks. A hepatoprotective effect was found in OTA-exposed chicks treated additionally with Withania somnifera and Silybum marianum, whereas a nephroprotective effect was only found in chicks additionally treated with Silybum marianum as has been seen from the relative organs' weight, macroscopic, biochemical and pathomorphological findings. The same herbs or appropriate mixture between them could be used as a practical approach for safely utilizing of OTA-contaminated feed.

Staff secondments and transfer of knowledge

-Bulgarian Researchers exchange some knowledge with Indian and South African researchers regarding animal experimentation.

-Literature regarding mycotoxin and herbal experiments was shared.

-Lecture and presentation on targeted mycotoxins toxicity and herbal protection were delivered by the Bulgarian researchers in the leading Universities in South Africa or India.

-The leads identified have been shared with respective laboratories i.e., Department of Chemistry and Biochemistry, Trakia University, Department of Physiology, Trakia University, Stara Zagora, Bulgaria, and DRDO-India, and Rhodes University or University of Johannesburg in South Africa, etc.

-Exchange of knowledge between Bulgarian researchers and researchers from the Nanotechnology Innovation Centre, under the direction of Prof Nyokong. These include Dr John Mack and Dr Jonathan Britton, Dr Pierre Kempgens on various characterisation techniques, including EPR, NMR, FTIR, UV, Raman, ToF-SIMs, XRD, and XPS.

PROJECT RESEARCH ACHIEVEMENTS

Selective characterization of 10 Indian and South African herbs including herbs from Himalayan region for their bioconstituents profile

More than 10 Plants originated from Himalayan region or from South Africa, belonging to following families: Fabaceae, Menispermaceae, Zingiberaceae were identified and characterized in order to be screened against targeted mycotoxins (Ochratoxin A and Fumonisin B1). The herbs were selected on the basis of their bioactivity characteristics such as antioxidative, diuretic, protective effects on kidneys and liver, immuno-stimulating or antibacterial effects etc and validated by in silico bioprospection.

In total 16 herbal extracts were analyzed for the presence of bio-active constituents and antioxidant potential. The bioactivity analysis of selected herbals provided 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)

On the basis of the above results 7 herbals were further screened in order to test their efficacy against targeted mycotoxins. Herbals selected belongs to following families:

Glycyrrhiza glabra (Family: Fabaceae)
Tinospora cordifolia (Family: Menispermaceae)
Zingiber officinale (Family: Zingiberaceae)
Curcuma longa (Family: Zingiberaceae)
Centella asiatica
Silybum marianum
Withania somnifera

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 ROSS) against various toxic effects of mycotoxin ochratoxin A on the body weight, relative organ weight, biochemical indices and humoral immune response. A hepatoprotective effect was seen for Tinospora cordifolia and Glycyrrhiza glabra being stronger for chicks additionally supplemented with Glycyrrhiza glabra as can be seen from the pathomorphological findings and the lower levels of ASAT and ALAT. Protective effects of herbal feed additives Silybum marianum or Withania somnifera and slightly of Centella asiatica against the growth inhibitory effect of ochratoxin A and associated immunosuppression and biochemical or pathomorphological changes were seen, e.g. protective effects on the kidneys (strongest for Silybum marianum) and/or liver. The strong immunesuppressive effect of OTA on humoral immune response against Newcastle disease was completely prevented in chicks given the herbal additives Withania somnifera or Silybum marianum, which was additionally supported by the higher relative weight of immunocompetent (lymphoid) organs in the same chicks. The same herbs or appropriate mixture between them could be used as a practical approach for safely utilizing of OTA-contaminated feed.

New research in our laboratories has pointed towards the use of natural compounds as universal protectors/mitigators against radiation and mycotoxin toxicity. This property has been exhibited to be present in naturally occurring dietary ingredients, mainly containing flavonoids.

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.

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 mycotoxin toxicity reduction studies revealed that Silymarin nanoemulsion has promising results better than the parent silymarin compound. The silymarin nanoemulsion-pretreated

(10µg/ml) irradiated group (Balb/c mice) showed lower frequency of apoptotic bodies and blebbing 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 more 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 antiinflammatory 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 (1, 2) 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 anti-mycobacterial activity against both strains (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 coli 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) andmethyl-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 extract E1 and E2 were found to have highest activity.

Studies on ex-vivo or in vivo efficacy of herbal extracts [RDP03, RDP06, RDP010, 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.

mycotoxicoses.

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 dimethylbenz(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, phenylpropanoïds, sesquiterpene derivatives, gallotannins and lignans. The LD50 of C. adansonii was estimated to be greater than 5000 mg/kg.

Transfer of knowledge and Training activities (workshops):

Staff secondments and transfer of knowledge in work package 1.

-ER2 moved from Trakia University (Bulgaria) to University of Johannesburg (South Africa) for nearly 6 months in order to perform the following objectives: Production and supply with mycotoxin FB1 for experimental studies in Trakia University (Bulgaria) and Kaposvar University (Hungary) and exchange of knowledge on this subject via participation in target experimental work with target FB1-producers and via presentations.

-ER2 and ESR2 moved from University of Johannesburg (South Africa) to Trakia University (Bulgaria) for nearly 6 months in order to perform the following objectives: Production and supply with mycotoxin OTA for experimental studies in Trakia University (Bulgaria) and Kaposvar University (Hungary) and exchange of knowledge on this subject via participation in target experimental work with target OTA-producers and via presentations.

Staff secondments and transfer of knowledge in work package 2.

-ER3 moved from Trakia University (Bulgaria) to DRDO (India) in order to perform the following objectives: Collection of some target Hymalayan herbs with known protective effects on human/animal health, liver and kidneys or known to have a potent immunostimulating and/or antibacterial effects via visits of some tribal areas in higher ranges of mountains and exchange of knowledge in regard to collecting and preserving all necessary herbs (leaves, barks, seeds, fruits or roots) in different stages of their growth, etc.

-ER2 moved from Trakia University (Bulgaria) to University of Johannesburg (South Africa) in order to perform the following objectives: Collection of some target South African herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects via visits of some tribal areas and exchange of knowledge in regard to collecting and preserving all necessary herbs (leaves, barks, seeds, fruits or roots) in different stage of their growth, etc.

-ER1 moved from Kaposvar University (Hungary) to University of Johannesburg (South Africa) in order to perform the following objectives: Collection of some target South African herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects via visits of some tribal areas and exchange of knowledge in regard to collecting and preserving all necessary herbs (leaves, barks, seeds, fruits or roots) in different stage of their growth, etc.

-ER1 moved from TU to RU in order to perform the following objectives:

A) Receiving some knowledge from South African scientists from the south regions of South Africa in regard to identifying, collecting and preserving some target herbs (leaves, barks, seeds, fruits or roots) in different stages of their growth known to have strong protective effects on liver and kidneys as well as immunostimulating or antibacterial effects, etc.

B) Exchange of knowledge between Bulgarian, South African and Indian researchers in regards to identifying, collecting and preserving some target herbs in different stages of their growth.

Staff secondments and transfer of knowledge in work package 3.

-ER1 moved from DRDO (India) to Trakia University (Bulgaria) in order to perform the following objectives:

A) Receiving some knowledge in regard to selective characterization of some Himalayan herbs for their bioconstituents (as the levels of flavonoids, carotenoids, etc) using EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy) on “Fiber Optic Spectrometer” in order to explain the mechanism of their antioxidative and protective effects on kidneys and liver or their immunostimulating or antibacterial effects and to prepare some extracts or fractions with the aim of exploring their protective abilities.

B) Exchange of knowledge between Bulgarian and Indian ways of preparing various herbal extracts or fractions via mutual presentations & workshops

C) Exploration of in vitro antioxidant properties or ex vivo protective abilities of some herbal extracts or fractions against oxidative stress caused by proper xenobiotics using EPR spectroscopy.

-ESR4 moved from RU (South Africa) to Trakia University (Bulgaria) in order to perform the following objectives:

A) Receiving some knowledge in regard to selective characterization of some South African herbs for their bioconstituents (as the levels of flavonoids, carotenoids, etc) using EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy) on “Fiber Optic Spectrometer”

B) Exchange of knowledge between Bulgarian and South African ways of selective

characterization of herbs and the respective ways of preparing various herbal extracts or fractions via mutual presentations & workshops

C) Exploration of in vitro antioxidant properties or ex vivo protective abilities of some herbal extracts or fractions against oxidative stress caused by proper xenobiotics using EPR spectroscopy.

-ER2 moved from RU (South Africa) to University of Kaposvar (Hungary) in order to perform the following objectives:

A) Transfer of some knowledge in regard to selective characterization of South African herbs for their bioconstituents using some nanotechnologies, NMR, FTIR, UV, EA, MS, RS, MA, etc., and in regard to the way of preparing various herbal extracts or fractions via attending various presentations and workshops.

B) Exchange of knowledge between Hungarian and South African ways of selective characterization of herbs and the respective ways of preparing various herbal extracts or fractions via mutual presentations & workshops

-ER3 moved from Trakia University (Bulgaria) to RU (South Africa) in order to perform the following objectives:

A) Receiving some knowledge in regard to selective characterization of South African herbs for their bioconstituents using some nanotechnologies, NMR, FTIR, UV, EA, MS, RS, MA, etc., and in regard to the way of preparing various herbal extracts or fractions via attending various presentations and workshops.

B) Exchange of knowledge between Bulgarian and South African ways of selective characterization of herbs and the respective ways of preparing various herbal extracts or fractions via mutual presentations & workshops

…..-ER1 and ESR1 moved from University of Kaposvar (Hungary) to University of Johannesburg and Rhodes University (South Africa) in order to perform the following objectives:

A) Receiving some knowledge from South African scientists about selective characterization of some South African herbs or herbal products for their bioconstituents using some nanotechnologies, NMR, FTIR, UV, EA, MS, RS, MA, etc, and in regard to the way of preparing various herbal extracts or fractions via attending various presentations and workshops

B) Exchange of knowledge between Hungarian and South African ways of selective characterization of herbs and the respective ways of preparing various herbal extracts or fractions via mutual presentations & workshops

Staff secondments and transfer of knowledge in work package 4.

-ER1 moved from DRDO (India) to Trakia University (Bulgaria) in order to perform the following objectives: Elaboration of mixtures of target herbal extracts, which will be tested for their stimulating effects on wound granulation and preparing of some appropriate unguents/sprays using appropriate constituents in this regard via exchanging of some knowledge between Bulgarian and Indian scientists.

-ER1 moved from Trakia University (Bulgaria) to Rhodes University (South Africa) in order to perform the following objectives: Elaboration of appropriate sprays or unguents of mixtures of target herbal extracts, which will be tested for their stimulating effects on wound granulation and exchanging of some knowledge between Bulgarian and South African scientists.

Exchange of some target knowledge via presentations

-ESR3 moved from University of Johannesburg (South Africa) to Trakia University (Bulgaria) in order to perform the following objectives:

A) Exchange of some target knowledge in regard to elaboration of some mixtures of target herbal extracts, which will be tested for their stimulating effects on wound granulation and preparing of some appropriate unguents/sprays in this regard

B) Exploring a joint venture and preparing common research papers.

-ER1 moved from Trakia University (Bulgaria) to DRDO (India) in order to perform the following objectives:

A) Elaboration of mixtures of target herbal extracts, which will be tested for their stimulating effects on wound granulation and preparing of some appropriate unguents in this regard

B) Exchange of some target knowledge via presentations

Staff secondments and transfer of knowledge in work package 5.

-ESR2 moved from Trakia University (Bulgaria) to University of Johannesburg (South Africa) in order to perform the following objectives: To transfer some knowledge to South African scientists in regard to exploring the stimulating effects of various herbal mixtures on wound granulation in animals at Department of Surgery in Faculty of Veterinary Medicine of Trakia University and to exchange some knowledge between the scientists from both countries.

Exchange of some target knowledge via presentations.

Staff secondments and transfer of knowledge in work package 6.

-ER2 moved from University of Johannesburg (South Africa) to Kaposvar University (Hungary) in order to perform the following objectives:

A) Receiving some knowledge in regard to exploring the protective effects of some herbs or herbal extracts against toxicity of FB1/OTA in pigs or rabbits via participation in some in vivo and in vitro experiments on the interaction between mycotoxins – herbal extract – gut microbiota.

B) Receiving some knowledge in regard to protective role of target plant extracts against the cytotoxic effects of mycotoxins FB1 and/or OTA on pig/rabbits lymphocyte and/or intestinal cells via participation in various in vitro cytotoxicity tests as MTT assay and Comet assay.

C) Exchange of some experimental data with the aim of preparing common research papers and elucidation of various protective effects of target herbs with a view of their further using in the practice and exchange of some target knowledge on this subject.

D) Receiving some knowledge how animal experiments are designed and equally took part in some of the animal experiments in University of Kaposvar. Receiving some knowledge how mycotoxins i.e., fumonisins, ochratoxins, aflatoxins and the trichothecenes are produced for animal experiments. Receiving some experience in magnetic resonance imaging and spectroscopy and positron emission tomography when collecting data from live animals as well as their interpretations.

E) Exploring a joint venture with the aim of developing some herbal products based on multiple herbs. Some discussions were consolidated with regards to areas of future joint research.

-ESR1 moved from University of Johannesburg (South Africa) to University of Kaposvar (Hungary) to in order to perform the following objectives:

A) To receive some knowledge from Hungarian scientists in regard to exploring protective effects of some herbs or herbal extracts against toxicity of FB1/DON in rabbits and their possible protective effects on the intestine via the interaction between mycotoxins – herbal extract – gut microbiota.

B) Transfer of some knowledge to South African scientists in regard to protective role of target plant extracts against the cytotoxic effects of FB1 and/or OTA on pig lymphocyte and/or intestinal cells using various in vitro cytotoxicity tests as MTT assay and Comet assay.

D) Receiving some knowledge about the results of animal experiments produced in University of Kaposvar. Receiving some experience in magnetic resonance imaging and spectroscopy and positron emission tomography when collecting data from live animals as well as their interpretations.

E) Exploring a joint venture with the aim of developing some herbal products based on multiple herbs. Some discussions were consolidated with regards to areas of future joint research.

Staff secondments and transfer of knowledge in work package 7.

-ER2 moved from Trakia University (Bulgaria) to DRDO (India) in order to perform the following objectives: Transfer of some knowledge between Bulgarian and Indian scientists in regard to exploring the protective effects of some herbs or herbal extracts given as feed additives against the OTA-toxicity on various internal organs, the OTA-induced changes in biochemical indices, the OTA-induced decrease in body weight, the OTA-induced immunosuppression and against the oxidative stress provoked by OTA in mice/chicks.

-ER1 moved from Trakia University (Bulgaria) to University of Johannesburg in order to perform the following objectives: Transfer of some knowledge from Bulgarian to South African scientists in regard to exploring the protective effects of some herbs or herbal extracts given as feed additives against the OTA-toxicity on various internal organs, the OTA-induced changes in biochemical indices, the OTA-induced decrease in body weight, the OTA-induced immunosuppression and the oxidative stress provoked by OTA in mice/chicks.

A presentation/workshop was given/attended by Bulgarian scientists in this regard.

THE TRANSFER OF KNOWLEDGE AND TRAINING ACTIVITIES (WORKSHOPS) WERE DONE IN VARIOUS WORK PACKAGES VIA THE FOLLOWING ACTIVITIES:

Screening of Herbal extracts for their anti-toxin efficacy. Toxicological studies were performed on both normal and transformed cells in relation to efficacy of herbal extracts.

more than 20 extracts of herbal materials were studied for their efficacy.

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 imparted.

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 thermolabile 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 chromatograhopy.

The extraction and characterization of plant materials and compound isolation in Trakia University (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.

Training course of Hungarian Marie Curie ESR fellows Mariam Kachlek and Chiara-Carmen Celia on the subject „Animal Resarch leader” organized between 10 and 14 March 2014 in Kaposvar, Hungary by SZIE University Faculty of Veterinary Science (Budaoest) within the frame of the Marie Curie project. This course meets the requirements as indicated in the 63/2010 EU Directive, on the approximation of laws, regulation and administrative provision of the Member States regarding the protection of animals used for experimental purposes. The course included 80 hours lectures, written exercises, hand-on works with animals and written examination and the fellows were awarded the respective certificates.

Training course of Hungarian Marie Curie fellow Nagy Gabor in the Department of Biotechnology and Food Technology at Faculty of Science in University of Johannesburg (UJ) and

participation in some research within the frame of Marie Curie IRSES project. Acquire skills in the extraction of active medicinal plant components and characterizing them using various chromatographic techniques including TLC and GC-MS/MS under the guidance of, Dr Nginteh Derick, MTT assay and Comet assay.

Participation of Hungarian Marie Curie ESR fellows in a training course on mycotoxin detection techniques in Bari (Italy) at 6-10 October, 2014 within the frame of the Marie Curie project. It was organised by the International Society for Mycotoxicology. The course elucidated some new information about the major problems in mycotoxin analysis and contamination along the whole food chain. Lectures and laboratory training were provided, incl. sample preparation, chemical analyses, multi-mycotoxin analysis and rapid detection techniques.

Specialization of Bulgarian Marie Curie ESR fellow Dr Georgi Beev in the Department of Biotechnology and Food Technology, Faculty of Science at the University of Johannesburg (UJ) within the frame of Marie Curie IRSES project and acquiring some additional skills in chromatographic instrumentations that include HPLC, TLC, LC-MS and GC-MS under the guidance of Prof's Titus Msagati, Dr PB Njobeh, Mr Hlengilizwe Nyoni and Dr Dereck Ndinteh. He also performed plant extraction and gained experience in the use of Rotavapor equipment under the supervision of Dr. Nicolette Niemann and some microbial analysis on bee pollen samples from Bulgaria.

Co-supervision of Ms Pamela Ndlovu, an UJ on a project entitled 'Assessment of fungal contamination in food commodities from rural settlements in South Africa' by Bulgarian Marie Curie ESR fellow Dr Georgi Beev

Training course of Hungarian Marie Curie ESR fellows Mariam Kachlek in regard to the Microbiology aspects of her research, incl. antifungal activity, under the supervision of Dr Patrick Njobeh and Ms Judith Phoku from UJ and Dr George Beev from TU (Bulgaria.)

Training course of Hungarian Marie Curie ESR fellow Ms Chiara-Carmen Celia in the Department of Biotechnology and Food Technology at Faculty of Science in University of Johannesburg (UJ) and participation in some research within the frame of Marie Curie IRSES project. During her tenure in the Department, Ms. Celia had the opportunity to acquire skills in the extraction of active medicinal plant components and characterizing them using various chromatographic techniques including TLC and GC-MS/MS under the guidance of Prof Titus Msagati, Dr Nginteh Derick and Dr Nicolette Nieman. She equally evaluated the antifungal activity of these plant components. Her experience at UJ led her to propose the use of an alternative extraction method which needs to be followed up.

Training course of South African Marie Curie ESR fellow M. Dlamini, T. Fonkui and R. Changwa in the Dept of Chemistry, Faculty of Medicine, Trakia University and participation in some research within the frame of Marie Curie IRSES project via using EPR (Electron Paramagnetic Resonance) or NIRS (Near Infrared Reflectance Spectroscopy), etc.

Participation of South African Marie Curie ER fellows in animal experiments with rabbits in Kaposvar University in Hungary

Participation of Indian Marie Curie ESR fellows in animal experiments with mice in Trakia University in Bulgaria.

Training courses or specializations in different areas of research organized by various participants in different countries for receiving target skills.

Participation of Bulgarian fellows Miroslav Stefanov and Vesselin Ivanov in training activities in regard to characterization of plant metabolites in RU 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 course of South African Marie Curie ESR fellow Hilary Ihesinaulo Ezuruike, Xavier Noundou, Derek Ndinteh and Bertha Chitambo in the Dept of Chemistry, Faculty of Medicine, Trakia University and participation in some research within the frame of Marie Curie

IRSES project via using EPR (Electron Paramagnetic Resonance) or NIRS (Near Infrared Reflectance Spectroscopy), etc.

Training course of Indian Marie Curie ESR fellows Prerna Agarwal and Manish Adhikari in the Dept of Chemistry, Faculty of Medicine, Trakia University and participation in some research within the frame of Marie Curie IRSES project via using EPR (Electron Paramagnetic Resonance) or NIRS (Near Infrared Reflectance Spectroscopy), etc.

Participation of Indian Marie Curie ESR fellows in animal experiments with mice in Trakia University in Bulgaria.

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.

Specialization of Bulgarian Marie Curie ESR fellows Miroslav Stefanov, Vesselin Ivanov, Georgi Terziev and Ivan Dinev in the Department of Biotechnology and Food Technology, Faculty of Science at the University of Johannesburg (UJ) within the frame of Marie Curie IRSES project and acquiring some additional skills in chromatographic instrumentations that include HPLC, TLC, LC-MS and GC-MS under the guidance of Dr PB Njobeh and Dr Dereck Ndinteh.

Work of master students from UJ involved in our IRSES project:

In the University of Johannesburg, there are several students projects within the frame of the Marie Curie IRSES staff exchange programme HERBAL PROTECTION with several postgraduate and academic staff members within the Department of Biotechnology and Food Technology and the Department of Applied Chemistry, which are involved in this IRSES programme. Some of these projects are completed that have led to awards of Master's degree and also generated research outputs, while others are in the final stages of completion. Some of these are outlined below:

1) PhD project by the Marie Curie IRSES fellow Fonkui Youmbi Thierry, "Mitigating the occurrence of mycotoxins and their effects thereof using nano-enabled binders.", Supervisor Dr Patrick Njobeh and Co-supervisor Prof R. Krause (all of them participated in our IRSES project)

2) Masters student project by the Marie Curie IRSES fellow in the new MSC (Nanoscience Programme), Kulani J, "Chitosan nano-particles functionalized with protective plant extracts for the inhibition of aflatoxin B1 and Ochratoxin A activity", Supervisor Dr Patrick Njobeh and Co-supervisors Prof. A. Mishra and Prof Rui Krause (some involved in our IRSES project).

3) Masters student project by the Marie Curie IRSES fellow Dlamini ML, "The application of some target formulations of active herbal plant components in reducing animal exposure to mycotoxins and their possible effects", Supervisor Dr Patrick Njobeh and Co-supervisor Prof R. Krause (all of them participated in our IRSES project).

4) Masters student project by the Marie Curie IRSES fellow Khanyisa Ndleve, "Mycotoxin dietary exposure levels in humans in the rural areas of KZN", Supervisor Dr Patrick Njobeh, participant in our IRSES project.

5) Masters student project by the Marie Curie IRSES fellow Mr Veli Thipe Clement, "Optimization of the antifungal activity of several antifungal agents using gold nanoparticles (AuNPs) synthesized through green chemistry." Supervisor Dr Patrick Njobeh and Co-supervisor Prof Sabelo Mhlanga (participated in our IRSES project).

Current Projects of students from Rhodes university partly connected with our IRSES project

1) Synthesis of iron nanoparticles coated with plant exudates for mycotoxin concentration and extraction.

2) Coated nanoparticles for mycotoxin destruction in food and feed

3) Coated Nanoparticles for pre-concentration of secondary metabolites from marine bacteria and fungi

Two of these projects are developing at the postdoc level and the other as an Honours project (4th-year BSc)

Some of the mentioned above students and/or supervisors travelled to Bulgaria or Hungary for some training and exchange of knowledge.

# MANAGEMENT REPORT

Please describe the management activities relative to the initial financial planning of the project

Management Report:

REALIZED VISITS AND MEETINGS AND AGREEMENTS RELATED TO IRSES PROJECT

1) 19.07.2013 – Meeting of Prof. Stoycho Stoev - coordinator of IRSES project and UJ coordinator P. Njobeh with the Dean of Faculty of Science in University of Johannesburg Prof. Bhekie Mamba at Auckland Park Kingsway Campus on the advance of our Marie Curie IRSES project.

2) 26.07.2013 – Visiting of Prof. Stoycho Stoev - coordinator of IRSES project, Marie Curie fellow Prof. S. Denev and UJ coordinator P. Njobeh a Zulu Open Herbs Market in Johannesburg for collecting more information from the source about the healing or protective effects of some local herbs used by traditional healers.

3) 29.07.2013 – Meeting of Prof. Stoycho Stoev - coordinator of IRSES project with the Vice Chancellor and Principal of UJ and Chairperson of Higher Education South Africa Prof. Ihron Rensburg as well as with the Dean of Faculty of Science in UJ Prof. Bhekie Mamba, the Dean of Faculty of Health Science in UJ Prof. Andre Swart and the Executive Director Internationalisation at Auckland Park Kingsway Campus on the advance of our Marie Curie IRSES project.

4) 30.07.2013 – Meeting of Prof. Stoycho Stoev, coordinator of IRSES project and UJ coordinator P. Njobeh with the Registrar of UJ at Auckland Park Kingsway Campus on the needs of our Marie Curie IRSES project.

5) 04.08.2013-03.09.2013 – Visit to Rhodes University and Meeting of Prof. Stoycho Stoev - coordinator of IRSES project, Marie Curie fellow Prof S Denev and RU coordinator Prof R. Krause with the Head of Department of Chemistry of RU in Grahamstown on the possibility to visit some rural areas in order to collect some information about the medicinal plants and the way of their use by traditional healer in the tribes in the rural areas.

6) 08.08.2013 – Meeting of Prof. Stoycho Stoev - coordinator of IRSES project, Marie Curie fellow Prof S Denev and RU coordinator Prof R. Krause with the Dean of Faculty of Science of Rhodes University Professor Ric Bernard on the advance of our Marie Curie IRSES project.

7) 14.08.2013 – Meeting of Prof. Stoycho Stoev - coordinator of IRSES project, Marie Curie fellow Prof S Denev and RU coordinator Prof R. Krause with the Deputy Vice Chancellor on Research and Development of Rhodes University Dr Peter Clayton on the advance of our Marie Curie IRSES project and the measure to be taken to facilitate the successful performance of the project tasks.

8) 15.08.2013 – Introducing Prof. Stoycho Stoev - coordinator of IRSES project and Marie Curie fellow Prof S Denev in Nuclear Magnetic Resonance (NMR) technics using the help and guidance of Dr Xavier in Rhodes University

9) 23.08.2013 – Visit of Prof. Stoycho Stoev - coordinator of IRSES project, Marie Curie fellow Prof S Denev and RU coordinator Prof R. Krause to Nelson Mandela Metropolitan University (NMMU) in Port Elizabeth and meeting with the Head of Chemistry and the Head of Biochemistry Departments. Meeting with the Chief of CSIR (Council of Science and Industrial Research) Biosciences in Pretoria - Dr Rajesh Lalloo and the Chief Researchers of CSIR Materials Science in Port Elizabeth – Rajesh Alandjiwala on the possible ways to collect some herbs and to introduce herbs products in the market in South Africa.

10) 11.09.2013 – Meeting of Prof. Stoycho Stoev - coordinator of IRSES project and Marie Curie fellow Prof S Denev with the Chief of Scientist-Bioprocess of CSIR (Council of Science and Industrial Research) Biosciences in Pretoria - Dr Rajesh Lalloo and Chief Researcher and Research Group Leader of CSIR Materials Science and Manifacturing Dr Rajesh Anandjiwala and the

Commercialisation Manager of CSIR Biosciences Dr Stephanus Francois Maraisthe and the Technology Manager of CSIR Biosciences Dr Vinesh Maharaj in regard to the measure to be taken to facilitate the successful performance of the project tasks and the possibility for introducing new medicinal plant extracts in the trade market.

11) 7-9 October 2013 Mycotoxin Workshop in Department of Animal Health in Mafikeng Campus of North West University

-7 October - 9:20-10:20AM - Prof. M. F. Dutton presentation: Mycotoxins research in Africa: challenges (Mycotoxin analysis: sampling, modern rapid test methods, data validations

-8 October 09:00-10:30 AM Session 1: Chair: Professor D. Stoev

-9:00-9:50AM - Prof. M. F. Dutton presentation: Mycotoxins in Africa: perspectives, publications in peer reviewed mycotoxins Journals

-11:00-11:50AM- Prof. Stoev presentation: The specific multi-mycotoxic nature of some foodborne mycotoxicoses and the hazard for animals or humans.

12) 9 October 2013 - Meeting of Prof. Stoycho Stoev - coordinator of IRSES project and Marie Curie fellow Prof M. Dutton with the Vice rector in North-West University Prof Mashudu Davhana-Maselesele on the current progress of our project.

13) 16.10.2013 – Meeting of Prof. Stoycho Stoev, coordinator of IRSES project and UJ coordinator P. Njobeh with Prof. Ben-Eric van Wyk (Chairman of the Aloe Council in SA, Chairman of the Indigenous Plant Use Forum in SA, Member of the Association for African Medicinal Plant Standards – AAMPS, Member of the Presidential Task Team on African Traditional Medicine) from Dept of Botany, Fac of Science UJ - about the top 10 herbs, which would be useful for our study and experiments

14) 12.12.2013 – Meeting of Prof. Stoycho Stoev, coordinator of IRSES project and UJ coordinator P. Njobeh with Prof. J. van Staden, Director of Research Centre for Plant Growth and Development, School of Life Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa - about the top 10 herbs, which would be useful for study and experiments under Marie Curie International Research Staff Exchange Project of European Union PIRSES-GA-2012-316067.

15) 13.12.2013 – Meeting of Prof. Stoycho Stoev, coordinator of IRSES project and UJ coordinator P. Njobeh with Prof. JN (Kobus) Eloff, Leader Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa - about the top 10 herbs, which would be useful for study and experiments under Marie Curie International Research Staff Exchange Project of European Union PIRSES-GA-2012-316067.

16) 07.04.2014 – Meeting between Project coordinator Prof Stoev and the UJ Marie Curie fellows: Prof. Dutton, Dr. P. Njobeh and 3 PhD students from UJ with Prof. Gadjeva and her team in Dept. of Chemistry in Fac. of Medicine in Trakia University (Bulgaria) in regard to some future studies and students qualifications to be undertaken as EPR (Electron Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), etc., under the IRSES project.

17) -Review Monitoring Meeting with REA-EC (European Commission) for the advance of IRSES project. Project presentation from the coordinator prof S. Stoev - 10-11.04.2014, Bulgarian Academy of Sciences, Institute for Nuclear Research and Nuclear Energy, Sofia.

18) 19 June, 2014 - Meeting of Prof. Stoycho Stoev, coordinator of this project and Dr. Rajesh Arora, Indian coordinator of this project and Dr. Damodar Gupta, participant in the project with the Deputy Director of Institute of Nuclear Medicine and Alied Science (INMAS) in New Delhi in regard to the measure to be taken to facilitate the successful performance of the project tasks and the experimental work planned in INMAS.

19) 19 June, 2014 - Meeting of Prof. Stoycho Stoev, coordinator of this project and Dr. Rajesh Arora, Indian coordinator of this project and Dr. Damodar Gupta, participant in the project with the Director of Institute of Nuclear Medicine and Alied Science (INMAS) in New Delhi, Dr. Rajendra Prashad Tripathi in regard to the measure to be taken to facilitate the successful performance of the project tasks and experimental work planned in INMAS and in regard to receiving a clearance to

perform some experiments with rats in INMAS.

20) 25 June, 2014 - Meeting of Prof. Stoycho Stoev, coordinator of this project and Dr. Rajesh Arora, Indian coordinator of this project and Dr. Damodar Gupta, participant in the project with Dr. K. K. Appu Kuttan, Director of Mauluna Azad National Institute of Technology in Bhopal (India) in regard to the measure to be taken to facilitate the successful performance of the project tasks and the public dissemination of the knowledge received under the IRSES project among the regular and postgraduated students.

21) 26 June, 2014 – Delivering an expert lecture by Project coordinator Prof. Stoycho Stoev on "Mycotoxic Nephropathy in Animals: Complex Etiology and Possible Preventive Measures" in the Short term training program on "Synthesis, Characterization and Applications of Biomaterials" under the Technical Education Quality Improvement Programme (TEQIP-II) in Mauluna Azad National Institute of Technology, Bhopal, India.

22) 26 June, 2014 – Delivering an expert lecture by Dr Damodar Gupta on "Radioprotectors" in the Short term training program on "Synthesis, Characterization and Applications of Biomaterials" under the Technical Education Quality Improvement Programme (TEQIP-II) in Mauluna Azad National Institute of Technology, Bhopal, India.

23) 26 June, 2014 – Delivering an expert lecture by Dr Damodar Gupta on "Low-cost Health and CBRN Mitigation Technologies: Spin-off Applications for Societal Benefits from Defence R&D" in the Short term training program on "Synthesis, Characterization and Applications of Biomaterials" under the Technical Education Quality Improvement Programme (TEQIP-II) in Mauluna Azad National Institute of Technology, Bhopal, India.

24) 16 July, 2014 - Meeting of Prof. Stoycho Stoev, coordinator of this project and Dr. Rajesh Arora, Indian coordinator of this project and Dr. Damodar Gupta, Indian participant in the project with the Joint Director International Cooperation of DRDO – Ministry of Defence, Indian Government in the Headquarter of DRDO, New Delhi in regard to the measure to be taken to facilitate the successful performance of the project tasks and experimental work planned in INMAS and in regard to concluding new collaborations with Bulgarian Research Institutions or Universities.

REALIZED PRESENTATIONS, CONFERENCES OR WORKSHOPS RELATED TO IRSES PROJECT

1) Presentation of Indian Marie Curie ER fellow Rajesh Arora (Indian Coordinator of IRSES project) on Marie Curie IRSES project 316067 "HERBAL PROTECTION" and experience within the IRSES scheme, EURAXESS Share Workshop, 03 April 2013 (Wednesday), New Delhi, India.

2) Presentations of Marie Curie IRSES fellows Arora R, Adhikari M, Agarwal P, Chawla R, Gupta D, Karamalakova Y, Zheleva A, Gadjeva V and Stoev S on Marie Curie IRSES project: "A FlavanolignanNanoFormulation as an Effective Radiation and Biothreat Countermeasure Agent: Evidence from In vitro and In vivo Studies " presented at Trakia University conference under Marie Curie actions (2013)

3) Presentation of Bulgarian Marie Curie ER fellow S. D. Stoev (coordinator of IRSES project) of the Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 "HERBAL PROTECTION": Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation (2013-2016), Scientific seminar "Community of science and success", 09 May 2013 (Thursday), Hotel Meridian Palace, Stara Zagora, Bulgaria.

4) Presentation and address on Marie Curie IRSES project of Bulgarian Marie Curie ER fellow Prof. V. Gadjeva: "Oxidative stress and related diseases" at Indo-Bulgarian International Conference "Recent Advances in Herbal Technology", organized on 7th September, 2013, ISF College of Pharmacy, Moga, Punjab, India.

5) Presentation on Marie Curie IRSES project of Bulgarian Marie Curie ER fellow Prof. A.

Zheleva: “Electron Paramagnetic Resonance Spectroscopy – Application for study of free radical scavenging capacity of compounds, extracts and fractions with different origin” at Indo-Bulgarian International Conference “Recent Advances in Herbal Technology”, organized on 7th September, 2013, ISF College of Pharmacy, Moga, Punjab, India.

6) Presentation of Bulgarian Marie Curie ER fellow and coordinator of IRSES project S. D. Stoev, Mycotoxic nephropathy in Bulgarian and South African pigs: complex etiology and similarity with Balkan Endemic Nephropathy, Mycotoxin Workshop 2013 “Mycotoxins research in food: challenges and perspectives”, 7-9 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa.

7) Presentation of Bulgarian Marie Curie ER fellow and coordinator of IRSES project S. D. Stoev,, The specific multi-mycotoxic nature of some foodborne mycotoxicoses and the hazard for animals or humans. Mycotoxin Workshop 2013 “Mycotoxins research in food: challenges and perspectives”, 7-9 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa.

8) Presentation of South African Marie Curie ER fellow M.F. Dutton, Mycotoxins research in Africa: challenges - mycotoxin analysis: sampling, modern rapid test methods, data validations, Mycotoxin Workshop 2013 “Mycotoxins research in food: challenges and perspectives”, 7-9 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa.

9) Presentation of South African Marie Curie ER fellow M.F. Dutton, Mycotoxins in Africa: perspectives, publications in peer reviewed mycotoxins Journals, Mycotoxin Workshop 2013 “Mycotoxins research in food: challenges and perspectives”, 7-9 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa.

10) Workshop on Comet assay was organised by the Hungarian partner at Kaposvár University on 21-23 May 2013 in Kaposhvar, Hungary within the frame of Marie Curie IRSES project. The aim of the workshop was to invite experts (Maria Disinska and Anrew Collins) to train young researchers the principles of the in vitro genotoxicity test, its possible application in toxicological studies, and also to give practical experience performing it in the laboratory. The genotoxicity test is used in those experiments, where the effect of mycotoxins combined with herbal extract is investigated, to see if the herbal extracts have any protective effect against the genotoxicity of certain mycotoxins.

11) Workshop on fumonisins was organised by the Hungarian partner at Kaposvár University on 10 June 2013 in Kaposhvar, Hungary within the frame of Marie Curie IRSES project. Within the frame of the workshop experts of the analytical techniques for mycotoxins were invited (Tibor Bartók, Chiara Dall’Asta, Claudia Falavigna) to discuss and train young researchers for analytical methodologies, focused also on the occurrence, formation and detection of hidden mycotoxins.

12) Presentation of Hungarian Marie Curie ESR fellow Mariam Kachlek on the „PhD” Round Table Discussion” event in Kaposvar University about the job performed in University of Johannesburg, South Africa within the frame of the Marie Curie project, 6 October, 2014, Kaposvar, Hungary. The title of her presentation: The single and combined cyto- and genotoxic effect of certain Fusarium toxins – may certain herbal extracts have any protective effect?

13) Presentation of Hungarian Marie Curie ESR fellow at the 8th World Mycotoxin Forum held on 10-12 November 2014 in Vienna, Austria with two poster presentations, titled: “Determination of the proportion of matrix-associated fumonisin B1 in different, animal feeding experiment-aided matrices after in vitro digestion”, and “N-deoxyfructosyl-fumonisin B1 may cause DNA damage in porcine mononuclear cells”

14) Presentation of Bulgarian Marie Curie ESR fellow Georgi Beev in the Department of Biotechnology and Food Technology, Faculty of Science at the University of Johannesburg (UJ) within the frame of Marie Curie IRSES project, 18 July, 2014.

15) Presentation of Bulgarian Marie Curie ER fellow S. D. Stoev (coordinator of IRSES project) of the new achievements and current progress in Marie Curie IRSES (International Research Staff

Exchange Scheme) project 316067 “HERBAL PROTECTION”: “Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation (2013-2016)”, Scientific seminar “Community of science and success”, 14 May 2014, Hotel Verea, Stara Zagora, Bulgaria.

16) Presentation of South African Marie Curie ER fellow M.F. Dutton of the SA work on Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 “HERBAL PROTECTION”: "Mycotoxins in South African foods: a case study on aflatoxin M1 in milk", Scientific seminar “Community of science and success”, 14 May 2014, Hotel Verea, Stara Zagora, Bulgaria.

17) Presentation of Bulgarian Marie Curie ER fellow S. D. Stoev (coordinator of IRSES project) of the new achievements and current progress of the Marie Curie IRSES project 316067 “HERBAL PROTECTION” (2013-2016): “Mycotoxic Nephropathy in Animals: Complex Etiology and Possible Preventive Measures”, Short Training Program on “Synthesis, Characterization and Applications of Biomaterials”, 25-29 June, 2014, Maulana Azad National Institute of Technology, Bhopal, India.

18) Presentation of Indian Marie Curie ER fellow Damodar Gupta on some achievement related to Marie Curie IRSES project 316067 “HERBAL PROTECTION” (2013-2016): “Radioprotectors”, Short Training Program on “Synthesis, Characterization and Applications of Biomaterials”, 25-29 June, 2014, Maulana Azad National Institute of Technology, Bhopal, India.

19) Presentation of Indian Marie Curie ER fellow Rajesh Arora (Indian Coordinator of IRSES project) on some achievement related to Marie Curie IRSES project 316067 “HERBAL PROTECTION” and experience within the IRSES scheme (2013-2016): “Low-cost Health and CBRN Mitigation Technologies: Spin-off Applications for Societal Benefits from Defence R&D”, Short Training Program on “Synthesis, Characterization and Applications of Biomaterials”, 25-29 June, 2014, Maulana Azad National Institute of Technology, Bhopal, India.

• 20) Organizing of press conference given in the Breefing Press Center in Stara Zagora on 06th of February 2015 for Presentation of achievements of Marie Curie IRSES project 316067 “HERBAL PROTECTION”. Various achievements and profits derived from this project were presented in the mass media via by the coordinator of the project Prof. S. Stoev and the Bulgarian coordinator Prof V. Gadjeva - representative of Trakia University and Dr Manish Adhikari – representative of DRDO, INMAS - India.

• 21) Participating in Marie Curie Researcher Night Workshop on 25 September 2015 and presentation of project achievements by the coordinator Prof. Stoycho Stoev, incl. poster presentation of the Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 “HERBAL PROTECTION” on the topic: “Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins” (2013-2016)

• 22) Organizing of International Conference “New Challenges in Mycotoxin Research” and Workshop by Prof. Melinda Kovac (beneficiary 2) at University of Kaposvar on 09th of November 2015. Invited lectures in the International Conference were given by Prof. S. Stoev (coordinator of IRSES project) on the topic: “New challenges related to animal health aspects of mycotoxins and by Prof. Mike Dutton (representative of the partner UJ – South Africa) on the topic “New challenges related to human health aspects of mycotoxins.

• 23) Participating in 42nd National Convention of the South African Chemical Institute (SACI) on 29th November up to 4th December 2015 in Durban, South Africa and Poster presentation by Siwe Noundou on the topic: “Erythrina caffra: A broad spectrum of biological activities”.

• 24) Participating in National Conference of Young Researchers “Biological Science for better future” on 30-31 October, 2015 in University of Plovdiv, and delivering a presentations by Prerna Agarwal, Yanka Karamalakova, Manish Adhikari on the topic: “Aqueous root extract of Glycyrrhiza Glabra: An comparative study of the reaction with DPPH”.

• 25) Participating in National Conference of Young Researchers “Biological Science for better future” on 30-31 October, 2015 in University of Plovdiv, and delivering a presentations by Prerna

Agarwal, Yanka Karamalakova, Manish Adhikari on the topic: “Glycyrrhiza Glabra: “Real Time” oxidative status of animals”
• 26) Participating in National Conference of Young Researchers “Biological Science for better future” on 30-31 October, 2015 in University of Plovdiv, and delivering a presentations by Prerna Agarwal and Yanka Karamalakova on the topic: “Investigation The Influence Of Natural Antioxidants On "Real Time" Oxidative Status Of Animals”
• 27) Participating in National Conference of Young Researchers “Biological Science for better future” on 30-31 October, 2015 in University of Plovdiv, and delivering a presentations by Manish Adhikari on the topic: “Y-radiation induced DNA damage attenuation by Nano-silymarin:”
• 28) 20.01.2015 – Meeting between Project coordinator Prof Stoev and the DRDO visitors Prerna Agarwal and Manish Adhikari with Prof. Gadjeva and her team in Dept. of Chemistry in Fac. of Medicine in Trakia University (Bulgaria) in regard to some future studies and students qualifications to be undertaken as EPR (Electron Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), etc., under the IRSES project.
• 29) 10.10.2015 – Meeting between Project coordinator Prof Stoev and the RU visitor Hilary Ihesinaulo Ezuruike with Prof. Gadjeva and her team in Dept. of Chemistry in Fac. of Medicine in Trakia University (Bulgaria) in regard to some future studies and students qualifications to be undertaken as EPR (Electron Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), etc., under the IRSES project.
• 30) Presentation of IRSES project in National Seminar on “Challenges of Climate Change and Green Environmental Solutions” on 10th December, 2016, organized by Chaudhary Charan Singh University, Meerut, India. Via presenting the paper “Impact of climate change on mycotoxins in food: management interventions by herbs of Indian, European & South African origin" by Dr Rajesh Arora from DRDO.
• 31) Invited lecture of prof. Ivan Dinev under the WP7 as part of a research collaboration programme on mitigation of mycotoxins on the topic “Review on the incidences of some major pathologies of leg skeleton in broiler chickens and broiler breeders related to poor animal welfare” in Department of Animal Health at the Mafikeng Campus of the North-West University (NWU)
• 32) Invited visits and meetings of Prof Ivanov and prof Stefanov in Rhodes University with Prof William Froneman (Zoology), Prof Alan Hutchinson (Electron Microscopy Unit), Prof Martin Hill (Entomology), Prof Adrian Craig (Zoology), Prof Susanne Vetter (Botany), Prof Rosmary Dorrington (Microbiology), Dr Caroline Knox (Microbiology), Prof Joanne Dames (Biochemistry), Prof Brett Pleschke (Biochemistry), Dr Leonie Goosen (Pharmacy), and Dr Fanchesca Porri (South African Institute for Aquatic Biology – SAIAB) in regard to the advance of the IRSES project and future collaborations on some particular topics (November-December, 2016).
• 33) Invited discussions of Prof Ivanov and prof Stefanov with researchers in the Department of Chemistry, incl. with Dr Xavier Siwe Noundou (Natural Products Chemistry), Prof Tebello Nyokong (Nanotechnology Innovation Centre), Dr Vincent Smith (Crystallography), and Dr David Khanye (Inorganic and Medicinal Chemistry) on the achieved results under the IRSES project and elaboration of mixtures herbal extracts, having a protective effect against toxicity of target mycotoxins or improving wound granulation and immune response of animals as well as in regard to elaboration of target publications (1st week of December).

• DRAWBACKS AND PROBLEMS WE FACED

-We faced some problems with Bulgarian Embassy giving to some SA researchers only 1 month visa instead 2 or 3 months visa without giving us any reasonable explanation for that – I already informed REA and the Head of Marie Curie unit Alessandra Luchetti about this problem, which was subsequently resolved.

-Most of my colleagues, participated in our IRSES project have possibilities to realize only 1

month or shorter visits to our partners or beneficiaries – unfortunately IRSES project is not suitable for short time visits and the money available for 1 month can only cover the ticket price. Therefore, my proposal is to suggest some amendments in the financial rules of IRSES projects – such as some additional money for ticket to be ensured for short-time visits.

-Another problem we faced is the customs taxes and duties which we had to pay for the herbs received from South Africa for our experiments in Bulgaria and Hungary (more than 400 Euro were paid by our Hungarian colleagues for customs duties and taxes, etc).

-We also had a PROBLEM with the Regional Service of Phytosanitary Control and the Customs Service in Sofia, where our imported herbs from S. Africa via DHL were retained (consignment 6078281145) and we were asked to make a registration and to pay some taxes as importer of herbs, etc.

-There was a problem with realizing the secondments of Bulgarian researchers to India and part of them had to be postponed, because of some health problems of the seconded researchers to DRDO-India. This happened because the food in India is very spiced and hot and all of our visitors from Bulgaria had some stomach or gut health problems during their visits - they are not used to eat such hot food as the local people there. This problem was partially resolved via some agreements with the owner of the private Persona Hotel International in New Delhi (the food which is usually prepared in this hotel is not extremely hot and spiced).

-ALL MENTIONED ABOVE PROBLEMS WERE SUCCESSFULLY RESOLVED.

# 2. USE AND DISSEMINATION OF FOREGROUND

## Section A (public) – DISSEMINATION MEASURES

This section should describe the dissemination measures, including any scientific publications relating to foreground and specify any applications for patents etc. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union.

## Dissemination activities

This section must include a list of planned dissemination activities (publications, conferences, workshops, web, press releases, flyers, etc) in free text format. Where Articles have been published in the popular press, please provide a list as well.

THE PRINCIPAL PUBLICATIONS IN AUTHORITATIVE PEER-REVIEWED INTERNATIONAL JOURNALS AND CONFERENCES/POSTERS RESULTING FROM THE PROJECT:

1’’’’) Arora, R., Adhikari M., Agarwal P., Chawla R., Gupta D., Sharma R.K., Ivanov V., Karamalakova Y., Zheleva A., Gadjeva V., Stoev S. Amelioration of γ-radiation-induced genotoxicity by nanosilimarin: a comparative study indicates possible implications for chemical, biological, radiological and nuclear (CBRN) defence. Trakia Journal of Sciences, Vol. 12, Suppl. 1, 2014, 1-10.

2’) Adhikari M., Dhaker A., Adhikari J., Ivanov V., Singh V., Chawla R., Kumar R., Sharma R., Karamalakova Y., Gadjeva V. and Arora R. In vitro studies on radioprotective efficacy of silymarin against γ-irradiation. International Journal of Radiation Biology, 2013, 89 (3), 200-211.

3’) Stoev, S. D., S. A. Denev, Porcine/Chicken or Human Nephropathy as the Result of Joint Mycotoxins Interaction, Special issue “Recent Advances in Ochratoxins Research”, Toxins, 2013, 5 (9), 1503-1530, doi:10.3390/toxins5091503 (http://www.mdpi.com/2072-6651/5/9/1503). IF=2,12.

4’’’’) Grigorov B., Y. Karamalakova, G. Nikolova, B. Popov, D.T. Ndinteh, V. Gadjeva and A. Zheleva First Electron Paramagnetic Resonance Spectroscopy Studies on Extracts Isolated from Piptadeniastrum Africanum and Haberlea Rhodopensis, Journal of Chemical Biological and Physical Sciences Sec. B, 2014; Vol.4, No.3; 2216-2226. IF=0.723

5’’) Adhikary M., Karamalakova Y., Ivanov V., Zheleva A., Gadgeva V., Arora R. Silymarin as a potent mitigator for handling radiological emergencies. XX International congress of medical sciences. Sofia, 9-12 May 2013, Supplement to issue, volume LXV

6’’) Adhikary M., Ivanov V., Dhaker A., Singh K., Baboota S., Gadjeva V., Karamalakova Y., Khar RK., Chavla R., Arora R., Sharma RK. Development, characterization and evaluation of silymarin nanoemulsion as radioprotector. Biologically active compounds and materials: basic и applied problems of production and application. May 27 – June 1, 2013, Novy svet, AR Crimea, Ukraine, Volume 2, p. 437

7’’) Gadjeva, V. Oxidative stress and related diseases, Indo-Bulgarian International Conference “Recent Advances in Herbal Technology”, 7 September, 2013, ISF, College of Pharmacy, Moga, Punjab, India.

8’’) Zheleva, A. Electron Paramagnetic Resonance Spectroscopy – Application for study of free radical scavenging capacity of compounds, extracts and fractions with different origin. Indo-Bulgarian Conference” Recent advances in Herbal Technology”, 7 September, 2013 ISF, College of Pharmacy, Moga, Punjab, India.

9’’) Nicheva S., Zhelev K., Ivanov V., Atanasoff A., Nikolov G. Effect of flavonoid on the growth

performance of juvenile rainbow trout (Oncorhynchus mykiss) cultived in recirculation system. XVI International Veterinary Medicine Students Scientific Research Congress, 8-10 May, 2014, Istanbul
10'') Popov B., Georgieva Sv., Petrova-Tacheva V., Ivanov V., Todorova K., Tsokeva Zh., Grigorov B., Alekova S. Evaluation of radioprotective potential of plant extracts and natural products. University Scientific Conference, 3th-4th of July, 2014, Veliko Tarnovo, Bulgaria.
11'') Grigorov B., Y. Karamalakova, G. Nikolova, B. Popov, D.T. Ndinteh, V. Gadjeva and A. Zheleva, Comparative study on extracts isolated from Piptadenastrium africanum and Haberlea rhodopensis by Electron Paramagnetic Resonance spectroscopy, First Trakia Medical Days International Scientific Conference, May 22-23, 2014, Stara Zagora, Bulgaria.
12'') Thipe VC, Njobeh PB, Mhlanga SD, RNAi in fungi using gold nanoparticles (AuNPs) synthesized through green chemistry to efficiently deliver and release the siRNA into A. flavus and A. parasiticus to facilitate gene silencing of the aflD (nor-1) gene. V International Conference on Environmental, Industrial and Applied Microbiology- BioMicroWorld2013, Madrid, Spain, 2-4 October, 2013, http://www.biomicrowo rld2013.org. Oral presentation.
13'') Thipe VC, Njobeh PB, Mhlanga SD, Optimization of the antifungal activity of several antifungal agents using gold nanoparticles (AuNPs) synthesized through green chemistry. MAM-14 7th International Symposium On Macro- and Supramolecular Architectures and Materials, Johannesburg, South Africa, 23 - 27 November, 2014, http://www.mam-14.com/. Oral presentation.
14'') Thipe VC, Njobeh PB, Mhlanga SD, Synthesis, characterization and antifungal properties of eco-friendly gold nanoparticles (AuNPs) using Aspalathus linearis. Nanoscience Workshop, Centre of Green Nanotechnology, University of the Western Cape, South Africa, 14-15 July, 2014. Oral presentation.
15'') Arora R, Adhikari M, Agarwal P, Chawla R, Gupta D, Karamalakova Y, Zheleva A, Gadjeva V and Stoev S, A Flavanolignan NanoFormulation as an Effective Radiation and Biothreat Countermeasure Agent: Evidence from In vitro and In vivo Studies, First Trakia Medical Days" International Scientific Conference, May 22-23, 2014, Stara Zagora, Bulgaria. (Poster)
16'') Arora R, Adhikari M, Agarwal P, Chawla R, Gupta D, Karamalakova Y, Zheleva A, Gadjeva V and Stoev S. NanoSilymarin as an Effective Radiation and Biothreat Countermeasure Agent: Evidence from In vitro and In vivo Studies. PB-18, Firs Trakia Medical Days" International Scientific Conference, May 22-23, 2014, Stara Zagora, Bulgaria. (Short Communication).
17') Stoev, S. D. Food safety and increasing hazard of mycotoxin occurrence in foods and feeds, Critical Reviews in Food Science and Nutrition, 2013, 53 (9), 887-901. IF=5.78
• 18') Nikolova G, Karamalakova Y, Kovacheva N, Stanev S, Zheleva A, Gadjeva V, 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, Regulatory Toxicology and Pharmacology 81 (2016) 1-7. IF=2.22; http://dx.doi.org/10.1016/j.yrtph.2016.06.024
• 19'''') Agarwal P, Karamalakova YD, Adhikari M, Gupta D, Nikolova GD, Hadzhibozheva PV, Gadjeva VG, Stoev S, Arora R, Zheleva A, Investigations on DPPH scavenging capacity before and after UV-irradiation of aqueous root extract of Glycyrrhiza Glabra, ISSN: 1314-6246, J. BioSci. Biotechnol., 2015, SE/ONLINE: 183-188.
• 20'''') Agarwal P, M Adhikari, G Nikolova, Dr Gupta, T Georgiev, V Gadjeva, S Stoev, R Arora, Y Karamalakova and A Zheleva, Ex vivo effect of Glycyrrhiza Glabra root extract on some "real time" biomarkers of oxidative stress – an EPR spectroscopy study, J. BioSci. Biotechnol.
• 21'''') Karamalakova Y, P Agarwal, G Nikolova, M Adhikari, D Gupta, S Stoev, T Georgiev, P Hadzhibozheva, R Arora, Z Zhelev, S. Raisuddin, V Gadjeva and A Zheleva, Influence of ochratoxin-A and an extract of Tinospora cordifolia against biochemical and oxidative changes in mice spleen, Science & Technologies, Medical Biology Studies, Clinical Studies, Social Medicine And Health Care, Volume 6 (1), 2016, 242-251.
• 22'') Karamalakova Y, Stoev S, Gadjeva V, Nikolova G, Indian ayurvedic plants with potentially protective activities against ochratoxin A induced-toxicity, Proceeding of reports from annual

university scientific conference, 20-21 October, 2016. Veliko Turnovo, ISSN 2367-7481.
• 23') Fomogne-Fodjo MCY, DT Ndinteh, DK Olivier, P Kempgens, S van Vuuren, RWM Krause, Secondary metabolites from Tetracera potatoria stem bark with anti-mycobacterial activity, Journal of Ethnopharmacology, 195 (2017) 238–245, DOI: http://dx.doi.org/10.1016/j.jep.2016.11.027. IF=3.05
• 24') Noundou X.S., R.W.M. Krause, S.F. van Vuuren, D. T. Ndinteh, D.K. Olivier, Antibacterial effects of Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens, Journal of Ethnopharmacology, 179 (2016) 76–82. IF=3.05; DOI: http://dx.doi.org/10.1016/j.jep.2015.12.043
• 25') Zingue S, J Tchoumtchoua, DM Ntsa, LP Sandjo, J Cisilotto, CBM Nde, E Winter, CF Awounfack, D Tantoh Ndinteh, C Clyne, D Njamen, M Halabalaki, TB Creczynski-Pasa, Estrogenic and cytotoxic potentials of compounds isolated from Millettia macrophylla Benth (Fabaceae): towards a better understanding of its underlying mechanisms, BMC Complementary and Alternative Medicine (2016) 16:421, DOI 10.1186/s12906-016-1385-5. IF=1.98; DOI: 10.1186/s12906-016-1385-5
• 26') Zingue S, J Cisilotto, AB Tueche, A Bishayee, FA Mefegue, LP Sandjo, CBM Nde, E Winter, T Michel, D Tantoh Ndinteh, CF Awounfack, KK Silihe, TTM Tanekou, TB Creczynski-Pasa, D Njamen, Crateva adansonii DC, an African ethnomedicinal plant, exerts cytotoxicity in vitro and prevents experimental mammary tumorigenesis in vivo, Journal of Ethnopharmacology 190 (2016) 183–199. IF=3.05; DOI: http://dx.doi.org/10.1016/j.jep.2016.06.004
• 27'') Agarwal P, Yanka Karamalakova, Manish Adhikari, Damodar Gupta, Galina Nikolova, Raman Chawlal, Veselina Gadjeva, Stoycho Stoev, Rajesh Arora, and Antoaneta Zheleva, Aqueous root extract of Glycyrrhiza Glabra: An comparative study of the reaction with DPPH, National Conference of Young Researchers "Biological Science for better future", 30-31 October, 2015, University of Plovdiv, Biological Faculty, Plovdiv, pp 48-49
• 28'') Agarwal P, Rajesh Arora, Manish Adhikari, Damodar Gupta, Galina Nikolova, Raman Chawlal, Veselina Gadjeva, Stoycho Stoev, Yanka Karamalakova and Antoaneta Zheleva, Glycyrrhiza Glabra: "Real Time" oxidative status of animals, National Conference of Young Researchers "Biological Science for better future", 30-31 October, 2015, University of Plovdiv, Biological Faculty, Plovdiv, pp 52-53.
• 29'') Adhikari M, Karamalakova Y, Nikolova G, Gupta D, Chawia R, Ivanov V, Kumar R, Zheleva A, Gadjeva V, Arora R, Stoev S, Nanosilymarin as an antioxidant agent: Comparative in vitro studies. XIV International Congress of Medical Sciences, Sofia, Bulgaria, 7-10 May, 2015, Suppl 1, pp 93.
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• 36'') Stoev, S.D. (2015) New challenges related to animal health aspects of mycotoxins, International Conference on „New Challenges in Mycotoxin Research, 9 November, 2015, University of Kaposvar, Hungary
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• 40'') C C Celia, M L Kachlek, Zs Gerencsér, Zs Matics, Zs Szendrő, A Dalle Zotte, V Giaccone, M Kovács: Effect of Carduus marianum herb on the productive performances of growing rabbits. In: Steffen Hoy (szerk.), 19. Internationale Tagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere [19th International Symposium on housing and diseases of rabbits, furproviding animals and pet animals]. 320 p. Celle, Germany, 2015.05.27-2015.05.28. Giessen: Justus Liebig Universität, 2015. pp. 145-152.
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• 45''') Agarwal, P., R, Arora, R. Chawla, D. Gupta, A. Zheleva, V. Gadjeva, S. Stoev, Mycotoxins: Novel Approaches for Biological Threat Mitigation, In: Toxigological Problems, Chapter 60, Christophor Dishovsky, Julia Radenkova (Eds), Military Publishing House Ltd, Bulgarian Toxicological Society, Sofia, Bulgaria, ISBN 978-954-509-509-2, 2014, pp. 433-444
• 46''') Stoev, S. D., Food Security and Foodborne Mycotoxicoses, Risk Assessment, Preventive Measures, and Underestimated Hazard of Masked Mycotoxins or Joint Mycotoxin Interaction, In:

Food Toxicology, Chapter 9, Debasis Bagchi, Anand Swaroop (Eds), CRC Press, Taylor & Francis Group, 1st December, 2016, ISBN 9781498708746, pp 169-199

# Publications

With regard to scientific publications published before or after the final report, such details/references and an abstract of the publication must be provided to the REA or the Commission at the latest two months following publication.

Furthermore, an electronic copy of the published version or the final manuscript accepted for publication must also be provided to the REA or the Commission at the same time for the purpose of publication by the REA or the Commission if this does not infringe any rights of third parties.

| LIST OF SCIENTIFIC PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | Title / DOI | Main author | Title of the periodical or the series | Number, date or frequency | Publisher | Place of publication | Date of publication | Relevant pages | Is open access provided to this publication ? | Type |
| 1 | In vitro studies on radioprotective efficacy of silymarin against #-irradiation | Manish Adhikari , Atlar Dhaker , Jawahar Adhikari , Veselin Ivanov , Vijay Singh , Raman Chawla , Raj Kumar , Rakesh Sharma , Yana Karamalakova , Veselina Gadjeva , Rajesh Arora | International Journal of Radiation Biology | Vol. 89/Issue 3 | Informa Healthcare | United Kingdom | 01/03/2013 | 200-211 | Yes | Peer reviewed |
| 2 | Crateva adansonii DC, an African ethnomedicinal plant, exerts cytotoxicity in vitro and prevents experimental mammary tumorigenesis in vivo | Stéphane Zingue , Julia Cisilotto , Alain Brice Tueche , Anupam Bishayee , Francine | Journal of Ethnopharmacolo gy | Vol. 190 | Elsevier Ireland Ltd | Ireland | 01/08/2016 | 183-199 | Yes | Peer reviewed |

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| | | Azegha Mefegue , Louis Pergaud Sandjo , Chantal Beatrice Magne Nde , Evelyn Winter , Thomas Michel , Derek Tantoh Ndinteh , Charline Florence Awounfack , Kevine Kamga Silihe , Tito Tresor Melachio Tanekou , Tânia Beatriz Creczynski-Pasa , Dieudonné Njamen | | | | | | | | |
| 3 | Protective effect of two essential oils isolated from Rosa damascena Mill. and Lavandula angustifolia Mill, and two classic antioxidants against L-dopa oxidative toxicity induced in healthy mice | Galina Nikolova , Yanka Karamalakova , Natasha Kovacheva , Stanko Stanev , Antoaneta Zheleva , Veselina Gadjeva | Regulatory Toxicology and Pharmacology | Vol. 81 | Academic Press Inc. | United States | 01/11/2016 | 1-7 | Yes | Peer reviewed |
| 4 | Secondary metabolites from Tetracera potatoria stem bark with anti-mycobacterial activity | M.C.Y. Fomogne-Fodjo , D.T. Ndinteh , D.K. Olivier , P. Kempgens , S. van Vuuren , R.W.M. Krause | Journal of Ethnopharmacolo gy | Vol. 195 | Elsevier Ireland Ltd | Ireland | 01/01/2017 | 238-245 | Yes | Peer reviewed |
| 5 | Antibacterial effects of Alchornea cordifolia (Schumach. and Thonn.) Müll. | X. Siwe Noundou , | Journal of Ethnopharmacolo gy | Vol. 179 | Elsevier Ireland Ltd | Ireland | 01/02/2016 | 76-82 | Yes | Peer reviewed |

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| | Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens | R.W.M. Krause , S.F. van Vuuren , D. Tantoh Ndinteh , D.K. Olivier | | | | | | | | |
| 6 | Porcine/Chicken or Human Nephropathy as the Result of Joint Mycotoxins Interaction | Stoycho Stoev , Stefan Denev | Toxins | Vol. 5/Issue 9 | Toxins Editorial Office | Switzerland | 01/09/2013 | 1503-1530 | Yes | Peer reviewed |
| 7 | Estrogenic and cytotoxic potentials of compounds isolated from Millettia macrophylla Benth (Fabaceae): towards a better understanding of its underlying mechanisms<br>10.1186/s1290 6-016-1385-5 | Stéphane Zingue , Job Tchoumtchoua , Dieudonnée Mireille Ntsa , Louis Pergaud Sandjo , Julia Cisilotto , Chantal Beatrice Magne Nde , Evelyn Winter , Charline Florence Awounfack , Derek Tantoh Ndinteh , Colin Clyne , Dieudonné Njamen , Maria Halabalaki , Tânia Beatriz Creczynski-Pasa | BMC Complementary and Alternative Medicine | Vol. 16/Issue 1 | BioMed Central | United Kingdom | 01/12/2016 | 2-17 | Yes | Peer reviewed |
| 8 | Foodborne mycotoxicoses, risk assessment and underestimated hazard of masked mycotoxins and joint mycotoxin effects or interaction<br>http://dx.doi .org/10.1016/j.etap.2015. 01 .022 | Stoycho D. Stoev | Environmental Toxicology and Pharmacology | Vol. 39/Issue 2 | Elsevier | Netherlands | 01/03/2015 | 794-809 | | Peer reviewed |
| 9 | Balkan Endemic Nephropathy – Still continuing enigma, risk assessment and underestimated hazard of joint mycotoxin exposure of animals or humans<br>http://dx.doi .org/10.1016/j.cbi.2016.1 1. | Stoycho D. Stoev | Chemico-Biologi cal Interactions | Vol. 261 | Elsevier Ireland Ltd | Ireland | 01/01/2017 | 63-79 | | Peer reviewed |

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| | 018 | | | | | | | | | |
| 10 | Food Safety and Increasing Hazard of Mycotoxin Occurrence in Foods and Feeds<br><br>10.1080/10408 398.2011.571800 | Stoycho D. Stoev | Critical Reviews in Food Science and Nutrition | Vol. 53/Issue 9 | Taylor and Francis Inc. | United Kingdom | 14/06/2013 | 887-901 | | Peer reviewed |
| | Toxigological Problems, Chapter 60 | Agarwal, P., R, Arora, R. Chawla, D. Gupta, A. Zheleva, V. Gadjeva, S. Stoev | Mycotoxins: Novel Approaches for Biological Threat Mitigation | 60 | | | 17/09/2014 | 433-444 | No | Article |
| | Food Security and Foodborne Mycotoxicoses, Risk Assessment, Preventive Measures, and Underestimated Hazard of Masked Mycotoxins or Joint Mycotoxin Interaction | Stoev, S. D | Food Toxicology, Chapter 9 | | CRC Press, Taylor & Francis Group | | 01/12/2016 | 169-199 | Yes | Article |
| | Silymarin as a potent mitigator for handling radiological emergencies | Adhikary M., Karamalakova Y., Ivanov V., Zheleva A., Gadgeva V., Arora R | XX International congress of medical sciences | | Supplement to issue, volume LXV | | 12/05/2013 | | No | Conference |
| | Development, characterization and evaluation of silymarin nanoemulsion as radioprotector | Adhikary M., Ivanov V., Dhaker A., Singh K., Baboota S., Gadjeva V., Karamalakova Y., Khar RK., Chavla R., Arora R., Sharma RK | Biologically active compounds and materials: basic # applied problems of production and application | | Novy svet, #R Crimea, Ukraine, Volume 2, p. 437 | | 01/06/2013 | | No | Conference |
| | Oxidative stress and related diseases | Gadjeva, V | Indo-Bulgarian International Conference ?Recent Advances in Herbal Technology | | ISF, College of Pharmacy, Moga, Punjab, India | | 08/09/2013 | | | Conference |
| | Electron Paramagnetic Resonance Spectroscopy ? Application for study of free radical scavenging capacity of compounds, extracts and fractions with different origin. | Zheleva, A. | Indo-Bulgarian Conference? Recent advances in Herbal Technology?, | | ISF, College of Pharmacy, Moga, Punjab, India. | | 08/09/2013 | | | Conference |

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| | Effect of flavonoid on the growth performance of juvenile rainbow trout (Oncorhynchus mykiss) cultived in recirculation system | Nicheva S., Zhelev K., Ivanov V., Atanasoff A., Nikolov G. | XVI International Veterinary Medicine Students Scientific Research Congress | | Istanbul | | 10/05/2014 | | | Conference |
| | Evaluation of radioprotective potential of plant extracts and natural products. | Popov B., Georgieva Sv., Petrova-Tacheva V., Ivanov V., Todorova K., Tsokeva Zh., Grigorov B., Alekova S | University Scientific Conference | | Veliko Tarnovo, Bulgaria | | 04/07/2014 | | | Conference |
| | Comparative study on extracts isolated from Piptadenastrium africanum and Haberlea rhodopensis by Electron Paramagnetic Resonance spectroscopy | Grigorov B., Y. Karamalakova, G. Nikolova, B. Popov, D.T. Ndinteh, V. Gadjeva and A. Zheleva | First Trakia Medical Days International Scientific Conference, | | Star# Zagora, Bulgaria | | 23/05/2014 | | | Conference |
| | RNAi in fungi using gold nanoparticles (AuNPs) synthesized through green chemistry to efficiently deliver and release the siRNA into A. flavus and A. parasiticus to facilitate gene silencing of the aflD (nor-1) gene | Thipe VC, Njobeh PB, Mhlanga SD | V International Conference on Environmental, Industrial and Applied Microbiology- BioMicroWorld20 13 | | Madrid, Spain | | 04/10/2013 | | | Conference |
| | Optimization of the antifungal activity of several antifungal agents using gold nanoparticles (AuNPs) synthesized through green chemistry | Thipe VC, Njobeh PB, Mhlanga SD | MAM-14 7th International Symposium On Macro- and Supramolecular Architectures and Materials | | Johannesburg, South Africa | | 27/11/2014 | | | Conference |
| | Synthesis, characterization and antifungal properties of eco-friendly gold nanoparticles (AuNPs) using Aspalathus linearis | Thipe VC, Njobeh PB, Mhlanga SD | Nanoscience Workshop | | Centre of Green Nanotechnology, University of the Western Cape, South Africa | | 15/07/2014 | | | Conference |
| | Flavanolignan NanoFormulation as an Effective Radiation and Biothreat Countermeasure Agent: Evidence from In vitro and In vivo Studies | Arora R, Adhikari M, Agarwal P, Chawla R, | First Trakia Medical Days" International Scientific Conference | | Stara Zagora, Bulgaria | | 23/05/2014 | | | Conference |

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| | | Gupta D, Karamalakova Y, Zheleva A, Gadjeva V and Stoev S | | | | | | | | |
| | NanoSilymarin as an Effective Radiation and Biothreat Countermeasure Agent: Evidence from In vitro and In vivo Studies. PB-18 | Arora R, Adhikari M, Agarwal P, Chawla R, Gupta D, Karamalakova Y, Zheleva A, Gadjeva V and Stoev S | Firs Trakia Medical Days" International Scientific Conference | | Stara Zagora, Bulgaria | | 23/05/2014 | | | Conference |
| | Indian ayurvedic plants with potentially protective activities against ochratoxin A induced-toxicity | Karamalakova Y, Stoev S, Gadjeva V, Nikolova G | Proceeding of reports from annual university scientific conference | | Vassil Levski, Veliko Tarnovo | | 21/10/2016 | | | Conference |
| | Aqueous root extract of Glycyrrhiza Glabra: An comparative study of the reaction with DPPH | Agarwal P, Yanka Karamalakova, Manish Adhikari, Damodar Gupta, Galina Nikolova, Raman Chawlal, Veselina Gadjeva, Stoycho Stoev, Rajesh Arora, and Antoaneta Zheleva, | National Conference of Young Researchers “Biological Science for better future” | | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 25/11/2015 | 48-49 | Yes | Conference |
| | Glycyrrhiza Glabra: “Real Time” oxidative status of animals | Agarwal P, Rajesh Arora, Manish Adhikari, Damodar Gupta, Galina Nikolova, Raman Chawlal, Veselina | National Conference of Young Researchers “Biological Science for better future” | | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 25/11/2015 | 52-53 | | Conference |

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| | | Gadjeva, Stoycho Stoev, Yanka Karamalakova and Antoaneta Zheleva | | | | | | | | |
| | Nanosilymarin as an antioxidant agent: Comparative in vitro studies. | Adhikari M, Karamalakova Y, Nikolova G, Gupta D, Chawia R, Ivanov V, Kumar R, Zheleva A, Gadjeva V, Arora R, Stoev S | XIV International Congress of Medical Sciences | | Tribuna Medica, Sofia, Bulgaria | | 01/06/2015 | suppl 1, pp93 | Yes | Conference |
| | Impact of climate change on mycotoxins in food: management interventions by herbs of Indian, European & South African origin | Arora R, D. Gupta, R. Chawla, P. Agarwal, M. Adhikari, Yana Karamalakova, G. Nikolova, V. Ivanov, M. Stefanov, M. Kovács, V. Gadjeva, Stoycho Stoev | National Seminar on “Challenges of Climate Change and Green Environmental Solutions” | | Chaudhary Charan Singh University, Meerut, India | | 10/12/2016 | 37-38 | | Conference |
| | Indian Ayurvedic Plants with Potentially Protective Activities against Ochratoxin A induced toxicity | Karamalakova Y, S. Stoev, V Gadjeva, G Nikolova | Annual University Scientific Conference | | Military University, Vassil Levski, Veliko Turnovo | | 21/10/2016 | 26 | | Conference |
| | Effect of Tinospora cordifolia on Ochratoxin A-induced oxidative stress in mice spleen: Electron Paramagnetic Resonance and Biochemical Study | Agarwal P, G. Nikolova, M. Adhikari, D. Gupta, S. Stoev, T Georgiev, P. Hadzhibozheva, V. Gadjeva, R. Arora, A. Zheleva, Y. Karamalakova | XXVI International Scientific Conference | | Trakia University | | 03/06/2016 | 35 | | Conference |

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| | Ochratoxin A: Effects of plant antioxidants on metabolic oxidative transformation and nephrotoxicity in mice. | Agarwal P, Nikolova G, Adhikari M, Arora R, Stoev S, Zheleva A, Gadjeva V, Karamalakova Y, | XV International Congress of Medical Sciences | | Biochemistry, Sofia, Bulgaria | | 15/05/2016 | Suppl 1, pp 86 | | Conference |
| | Protective effect of Tinospora cordifolia against ochratoxin A-induced oxidative stress in mice | Karamalakova Y, Prerna Agarwal, Galina Nikolova, Manish Adhikari, Petia Hadzhibozheva, Tsvetelin Georgiev, Damodar Gupta | Competition “Science and Youth” | | Auditorium complex Plovdiv | | 14/05/2016 | | | Conference |
| | Cardioprotective activity of extract from Tinospora cordifolia against chronic ochratoxin A induced oxidative dysfunctions | Karamalakova Y, Prerna Agarwal, Galina Nikolova, Manish Adhikari, Petia Hadzhibozheva, Tsvetelin Georgiev, Damodar Gupta | Competition “Science and Youth” | | Auditorium complex Plovdiv | | 14/05/2016 | 17 | | Conference |
| | New challenges related to animal health aspects of mycotoxins | Stoev, S.D | International Conference on „New Challenges in Mycotoxin Research | | University of Kaposvar, Hungary | | 10/11/2015 | | | Conference |
| | Investigation the influence of natural antioxidants on "Real Time" oxidative status of animals | Yanka Karamalakova, Raj Kumar; Rakesh K. Sharma; Rajesh Arora, Galina | 70 years anniversary Scientific conference | | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 31/10/2015 | | | Conference |

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| | | Nikolova, Ekaterina Georgieva, Antoaneta Zheleva, Veselina Gadjeva | | | | | | | | |
| | Y-radiation induced DNA damage attenuation by Nano-silymarin: An in vitro Approach | Manish Adhikari, Rajesh Arora, Yana Karamalakova, Raj Kumar, Veselin Ivanov, Aatoaneta Zheleva, Veselina Gadjeva and Stoycho Stoev | 70 years anniversary Scientific conference | | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 31/10/2015 | | | Conference |
| | Effect of Carduus marianum herb on the productive performances of growing rabbits. | C C Celia, M L Kachlek, Zs Gerencsér, Zs Matics, Zs Szendr#, A Dalle Zotte, V Giaccone, M Kovács | 19th International Symposium on housing and diseases of rabbits, furproviding animals and pet animals | | Giessen: Justus Liebig Universität, Germany | | 28/05/2015 | 145-152 | | Conference |
| | Erythrina caffra: A broad spectrum of biological activities. | Siwe Noundou X., Chitambo B., Bors I., Zsuzsanna H.K., Zsófia B.B., Kachlek M., Krause R.W.M., van Vuuren S. and Kovács M. | Poster presentation, 42nd National Convention of the South African Chemical Institute (SACI) | | Durban, South Africa | | 04/12/2015 | | | Conference |
| | Trakia Journal of Sciences | Arora, R., Adhikari M., Agarwal P., Chawla R., Gupta D., Sharma R.K., | Amelioration of #-radiation-ind uced genotoxicity by nanosilimarin: a comparative study indicates possible implications for chemical, biological, radiological and nuclear (CBRN) defence | Vol. 12, Suppl. 1 | Trakia University | | 03/12/2014 | | No | Monogram |

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| | | Ivanov V., Karamalakova Y., Zheleva A., Gadjeva V., Stoev S. | | | | | | | | |
| | Journal of Bioscience and Biotechnology | Agarwal P, Karamalakova YD, Adhikari M, Gupta D, Nikolova GD, Hadzhibozheva PV, Gadjeva VG, Stoev S, Arora R, Zheleva A | Investigations on DPPH scavenging capacity before and after UV-irradiation of aqueous root extract of Glycyrrhiza Glabra | 5, 183-188 | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 07/01/2015 | | | Monogram |
| | Journal of Bioscience and Biotechnology | Agarwal P, M Adhikari, G Nikolova, Dr Gupta, T Georgiev, V Gadjeva, S Stoev, R Arora, Y Karamalakova and A Zheleva | Ex vivo effect of Glycyrrhiza Glabra root extract on some “real time” biomarkers of oxidative stress – an EPR spectroscopy study | 6 | PLOVDIV UNIVERSITY PRESS "PAISII HILENDARSKI" | | 04/04/2017 | | | Monogram |
| | Science & Technologies, Medical Biology Studies, Clinical Studies, Social Medicine And Health Care | Karamalakova Y, P Agarwal, G Nikolova, M Adhikari, D Gupta, S Stoev, T Georgiev, P Hadzhibozheva, R Arora, Z Zhelev, S. Raisuddin, V Gadjeva and A Zheleva | Influence of ochratoxin-A and an extract of Tinospora cordifolia against biochemical and oxidative changes in mice spleen | 6 | Researchers' Union, Stara Zagora | | 24/08/2016 | | | Monogram |
| | Folia medica | Adhikari M., Arora R., Karamalakova Y., Kumar R., Ivanov V., Zheleva A., | Gamma radiation reduced DNA damage attenuation by nano-silymarin: an in vitro approach. | 1, 62 | Medical University, Plovdiv | | 30/11/2015 | | | Monogram |

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| | | Gadjeva V., Stoev S. | | | | | | | | |
| | Poljoprivreda (osijek) | Kachlek M, Szabó-Fodor J, Bonai A, Bors I, Celia C, Gerencsér Zs, Matics Zs, Szendr# Zs, Dalle Zotte A, Kovács M | Assessing the possible interaction between Carduus Marianus and dietary deoxynivalenol on caecal microbiota and fermentation of growing rabbits | 21, suppl 1, 186-189 | Sveu#ilište Josipa Jurja Strossmayera u Osijeku | | 01/10/2015 | | | Monogram |
| | Food Toxicology, Chapter 9 | Stoev, S. D. | Food Security and Foodborne Mycotoxicoses, Risk Assessment, Preventive Measures, and Underestimated Hazard of Masked Mycotoxins or Joint Mycotoxin Interaction | Chapter 9 | CRC Press, Taylor & Francis Group | | 01/12/2016 | | | Monogram |
| | Journal of Chemical Biological and Physical Sciences Sec. B | Grigorov B., Y. Karamalakova, G. Nikolova, B. Popov, D.T. Ndinteh, V. Gadjeva and A. Zheleva | First Electron Paramagnetic Resonance Spectroscopy Studies on Extracts Isolated from Piptadeniastrum Africanum and Haberlea Rhodopensis | Vol.4, No.3 | CODEN (USA): JCBPAT | | 01/05/2014 | | | Monogram |

# Section B (confidential) - EXPLOITABLE FOREGROUND AND PLANS FOR EXPLOITATION

This section should specify the exploitable foreground and provide the plans for exploitation. It will be kept confidential and will be treated as such by the REA.

The applications for patents, trademarks, registered designs, etc. must be listed according to the template provided below.

The list should specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified.

| LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS,UTILITY MODELS, ETC. | | | | | |
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| Type of IP Rights | Confidential | Foreseen embargo date dd/mm/yyyy | Application reference(s) (e.g. EP123456) | Subject or title of application | Applicant(s) (as on the application) |

| OVERVIEW TABLE OF EXPLOITABLE FOREGROUND | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Type of Exploitable Foreground | Description of Exploitable Foreground | Confidential | Foreseen embargo date dd/mm/yyyy | Exploitable product(s) or measure(s) | Sector(s) of application | Timetable for commercial use or any other use | Patents or other IPR exploitation (licences) | Owner and Other Beneficiary(s) involved |

| ADDITIONAL TEMPLATE B2: OVERVIEW TABLE OF EXPLOITABLE FOREGROUND | |
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| Description of Exploitable Foreground | Explain of the Exploitable Foreground |

# 3. PERSON IN CHARGE QUESTIONNAIRE

**EXCHANGE MOBILITY ASSESSMENT:**

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| **What is the size of the hosting research group?** | 20 |

| **How many researchers have you supervised, within the past 10 years? Of which funded by:** | |
|---|---|
| **EC/Marie Curie actions** | 0 |
| **EC Other Funding** | 0 |
| **University fellowships** | 2 |
| **National public bodies** | 0 |
| **Industry** | 0 |
| **Other** | 0 |
| Other, please specify: | |
| | |

| | |
|---|---|
| **How many researchers have you supervised within this project?** | 4 |
| **Corresponding to how many person months?** | 10 |

| **Number of publications resulting directly from the research project:** | |
|---|---|
| **Selected researcher(s) and yourself** | 6 |
| **Selected researcher(s) alone** | 0 |
| **Selected researcher(s) with authors other than yourself** | 10 |

| **Participation of the selected researcher(s) in conferences (number):** | |
|---|---|
| **Passive** | 105 |
| **Active** | 30 |
| **How do you rate the overall success of the research training?** | Good |

| General assessment: |
|---|
| Good |

**RESEARCHER ASSESSMENT:**

| **Rate the overall level of the recruited researcher(s) integration in the research team and the host organisation with regards to:** | |
|---|---|
| **participation in meetings/seminars** | Very good |
| **discussions of results and project-related topics** | Very good |
| **co-operation with other team members** | Very good |
| **co-operation with other researchers of the** | Good |

| | |
|---|---|
| **host institution** | |
| **co-operation with other researchers of the partnership** | Good |

| **Rate the overall performance of the selected researcher(s) with regard to:** | |
|---|---|
| **originality of researchers' approach towards research (initiative/independent thinking)** | Good |
| **capacity to develop new skills and to benefit from training** | Good |
| **productivity (research results/ publications/ international conference attendance)** | Very good |
| **communication skills** | Fair |
| **group leader skills (collaboration with other groups/project management)** | Good |
| **training and/or teaching skills** | Good |

Comment:

**RESEARCH NETWORKING OUTCOMES:**

| | |
|---|---|
| **Do you intend to continue the collaboration and networking activities after the end of the project?** | Yes |

If no, please specify:

| | |
|---|---|
| **Has this project provided additional links with other research groups or institutions?** | Yes |
| **If yes, do you plan to submit a joint proposal?** | Yes |

| **If yes, indicate the number of contacts in each case** | |
|---|---|
| **Universities** | 3 |
| **Research Centres** | 1 |
| **Industry/private companies** | 0 |
| **Others** | 0 |

If Other, please specify:

| **Rate the importance of the following outcomes of the research training:** | |
|---|---|
| **results of the research** | Good |
| **number of publications** | Very good |
| **development of research** | Very good |
| **establishment of international collaborations** | Very good |
| **transfer of knowledge/technology** | Good |
| **training of students/researchers** | Good |

| | |
|---|---|
| **further academic qualifications (PhD, habilitation etc.) for fellows** | Good |
| Comments: | |
| | |

**YOUR OPINION ABOUT THE MARIE CURIE ACTIONS:**

| |
|---|
| Do you have any other comments or suggestions of how to improve the Marie Curie actions concerned? |
| 1)Most of my colleagues, participated in our IRSES project have possibilities to realize only 1 month or shorter visits to our partners or beneficiaries – unfortunately IRSES project is not suitable for short time visits and the money available for 1 month can only cover the ticket price. Therefore, my proposal is to suggest some amendments in the financial rules of IRSES projects and some additional money for ticket to be ensured for short-time visits.<br>2) Under the current IRSES project we had no money for research and, therefore had to ensure some money via some additional projects. This is a big disadvantage of this Marie Curie IRSES project. However, under the current RISE projects under the Horizon 2020, this problem is already resolved. |

| | |
|---|---|
| **Did you have previous knowledge of the Marie Curie actions?** | Yes |

| |
|---|
| If yes, what sort of image do you think that the Marie Curie actions have among the scientific community in your research area? |
| Good |

## Attachments

Date:

Person in charge of the project for the beneficiary(ies):