

HERBAL PROTECTION

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PEOPLE  
MARIE CURIE ACTIONS

**International Research Staff Exchange Scheme (IRSES)  
Call: FP7-PEOPLE-2012-IRSES**

## **PART B**

**“HERBAL PROTECTION”**

## HERBAL PROTECTION

### B1 QUALITY OF THE EXCHANGE PROGRAMME

#### B 1.1 OBJECTIVE AND RELEVANCE OF THE JOINT EXCHANGE PROGRAMME

The applicants have addressed to mycotoxins involved in spontaneous animal mycotoxicoses in all countries participated in this proposal and the possible protective effects of some herbal additives in feeds, because the course of mycotoxicoses are often accompanied with higher mortality due to secondary bacterial infections provoked by immunosuppressive effects of mycotoxins (Stoev et al, 2000b; Deshmukh et al, 2005). Such a problematic mycotoxicosis widely encountered in Bulgaria appears to be spontaneous porcine/chicken nephropathy (PN/CN) caused by alimentary ingestion of secondary fungal metabolites possessing nephrotoxic properties and encountered in feeds/foods made mainly from cereals (Stoev et al, 1998a,b,c, 2002a). There are however some variances in the manifestation of the disease, especially in the clinicomorphological picture, which in many cases is influenced by secondary intestinal bacterial diseases as a result of the pronounced immunosuppression in the affected animals (Stoev et al, 2000a,b, Stoev, 2008a,b). Some studies were undertaken under Framework 6, via Marie Curie fellowship of the main applicant, in order to clarify the nature of PN/CN widely ranged in Bulgaria and South Africa, which, was found to have multi-mycotoxic etiology involving several mycotoxins, mainly ochratoxin A (OTA) and fumonisin B1 (FB1) (Stoev et al, 2010a,b). On the other hand, there are some recent evidences that Balkan Endemic Nephropathy (BEN) in humans, which is a problematic disease, widely ranged in Bulgaria and other Balkan countries (Petkova-Bocharova and Castegnaro, 1991; Stoev and Petkova-Bocharova, 1994; Stoev, 1998) has similar multimycotoxin aetiology with PN/CN (Stoev, 2008a; Stoev et al, 2010a).

Both mycotoxins OTA and FB1 are widely encountered in animal feeds and human foods in all countries involved in this project. FB1 has been found to be associated with esophageal cancer in humans within the Transkei region of South Africa (Sydenham et al, 1990). In India, a high level of FB1 content has been detected in maize and poultry feeds (Shetty and Bhat, 1997; Jindal et al, 1999). Chatterjee and Mukherjee (1994) found FB1 levels up to 366 ppm in the maize samples. The most obvious effects of OTA-contaminated feed on chicks are reported to be reduced rates of weight gain (Gupta et al, 2005; Stoev et al., 2000a, 2002a,d), hepatotoxicity and nephrotoxicity (Gupta et al, 2008; Stoev et al, 2000a, 2002a,d; Khan, 2010; Thakur, 2010), decreased egg production (Haazele et al., 1993, Stoev, 2010b), immunosuppression (Stoev et al., 2000a,b, 2002d; Gupta, et al, 2008), carcinogenicity (Stoev, 2010a) and increased mortality (Stoev et al, 2002b; Khan, 2010). Similar toxic effects can be observed in animal exposed to FB1 (Stoev et al, 2011). In India, FB1 has been shown to have immunosuppressive effects with increase in susceptibility to *Salmonella Gallinarum* infections (Deshmukh et al, 2005; Gupta, et al, 2008), nervous signs in Japanese quail (Asrani et al, 2006) and hepatotoxic or nephrotoxic effects (Sharma et al, 2008).

The widely encountered renal disease in pigs/chicks may not only cause affected kidneys to be rejected, but it is also correlated with poor liveweight gains and carcass quality in affected animals (Stoev et al, 1998a,c, 2002a). In addition, a pronounced morbidity of secondary bacterial infections often accompanies animals suffering from such nephropathy, because of the immunotoxic effects of mycotoxins involved (Stoev et al, 2000a,b, 2002d). Degenerative and weight changes in kidneys, liver and lymphoid organs similar to spontaneous CN as well as a pronounced immunosuppression were observed in chickens at only 0.2 or 0.3 mg/kg OTA in combination with PA (Stoev et al., 1999, 2000a, 2004). In India, OTA was also found to be associated with heavy necrotic or degenerative changes in the kidneys/livers of Japanese quail at 2-3 mg/kg (Khan, 2010; Thakur, 2010). It is important to mention that FB1 may also contribute to the immunosuppression and the increase in secondary bacterial infections observed in pigs/chicks with spontaneous PN (Stoev et al., 2000b), as can be anticipated from the observed increase of intestinal colonization by pathogenic *E. coli* in FB1-treated pigs (Oswald et al., 2003) or the increased mortality among Japanese quail fed on diet contaminated with both OTA and FB1 (Khan, 2010).

**THE OBJECTIVES** of the present study is to explore some ways of safely utilizing chick/pig feeds contaminated with the nephrotoxic mycotoxins OTA or FB1 in order to reduce farm losses from a decrease of weight gain in stock chicks/pigs and to avoid the rejection or condemnation of such feed. This will be achieved by addition of some herbs to the diet of chicks/pigs, which are known to have protective effect on kidney/liver (the main damaged organs by OTA/FB1) or known to have an immunostimulating effect. Some of the same herbs, well known among Himalayan tribes living in higher ranges of mountains (Lahaul & Spiti, Kinnaur and Chamba districts of the state) as well as among some South African tribes to have strong healing effects on wounds or human health, will be further studied on their possible effects on wound granulation separately or in appropriate mixture.

In the proposed studies and staff exchange programme, we are going to measure the effect of some target herbs, which will be added to the chick feeds in appropriate means in order to combat some toxic or immunosuppressive effects of OTA and FB1. The potential power of these feed additives (herbs) will be compared with the power of known herb as Artichoke extract (Stoev, 2000; Stoev et al, 1999, 2000a, 2002d, 2004), which was found to has a strong protective effect on kidneys, partially neutralising the known toxic effects of OTA. The approach for collaboration in this project between the EU and partner countries will focus on the following: (A) Selection Criterion; (B) Bioactivity-guided screening and selective characterization of herbs or herbal products; (C) Standardization and joint patents of standardized processes; (D) Drug Formulation from lead natural products.

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**JUSTIFICATION FOR COLLABORATION AND EXCHANGE SCHEME.** The planned collaborations and exchange scheme will be invaluable for the sharing of methodologies and information between the collaborating countries, i.e., Bulgaria, Hungary, India and South Africa. In particular, Bulgarian collaborators need the expertise developed in India and South Africa on the use of various herbs and their healing potential to combat various diseases or to improve wound granulation, whereas South African & Bulgarian collaborators can provide expertise in mycotoxin production. Hungarian collaborators can ensure high quality conditions for a successful performance of various kinds of animal experiments in order to identify the potential of protective effects of various herbs on animal health and immunity as well as their possible antibacterial effects. Bulgarian and South African collaborators will also provide some help and expertise in identifying the biocontent of various herbs and could help in explaining the scientific mechanism of their action and developing some herbal products based on multiple herbs.

A plethora of herbs like *Boerhavia diffusa* and *Bergenia ligulata* (known as diuretic plants); *Andrographis paniculata*, *Phyllanthus niruri* and *Berberis* spp. *Picrorhiza kurroa* (having hepatoprotective effect), *Tinospora cordifolia*, *Embllica officinalis* and *Dioscorea bulbifera* (known as immunostimulants); *Moringa oleifera* and *Ricinus communis* (having anti-inflammatory effect); *Hippophae rhamnoides*, *Woodfordia fruticosa*, *Jasminum officinale* and *Rubia cordifolia* (known as wound healer), etc are typical mainly for Himalayan and some other regions of India or South Africa and Bulgarian/Hungarian collaborators need the help of their Indian/South African colleagues to supply with the same herbs. South Africa on its part has about a third (30,000 species) of world's medicinal plant species and some of these have been identified for their usefulness in the treatments of various ailments in humans. These indigenous plant species i.e. *Hypoxis hemerocallidea* L., *Ranunculus multifidus* Forssk, *Senecio serratuloides* DC., *Artemisia afra*, *Mentha longifolia* and *Leonotis leonurus*, *Ruta graveolens*, *Melianthus comosus*, *Syzygium cordatum* Hochst (antimicrobial) and several others are widely used by traditional healers in South Africa. On the other hand Indian collaborators need the help of South African and Bulgarian collaborators, which will supply with some mycotoxins necessary for the planned study, which commercial prices are very high and the necessary quantities of the same mycotoxins will be more than 100,000.00 Euro, if purchased from the market. In this regard, the efficacy of Picroliv (25 mg.kg-1.d-1 x 10 d), a standardized iridoid glycoside fraction of *Picrorhiza kurroa*, has been already studied and was found to have significant effect against the *Amanita phalloides*-induced hepatotoxicity in rats (Dwivedi et al, 1992).

**TABLE 1: LIST OF WORK PACKAGES:**

WP №	Work package title	Beneficiary/Partner organisation short name	Start month	End month
1	FB1 production (S. Africa) OTA production (Bulgaria)	TU (Bulgaria) UJ (South Africa)	10/2013 01/2014	12/2013 03/2014
2	Herbs collection from India and South Africa	TU (Bulgaria) UNIKAPOS (Hungary)	04/2013 07/2013	06/2013 09/2013
3	Selective characterization of some herbs from India and South Africa for their bioconstituents and preparing some target herbal extracts or fractions	DRDO (India) UJ (South Africa) UNIKAPOS (Hungary) TU (Bulgaria)	10/2013 10/2013 01/2014 01/2014	12/2013 12/2013 03/2014 03/2014
4	Elaboration of mixtures of target herbal extracts for preparing of some sprays/unguents designed for stimulation of wound granulation	DRDO (India) UJ (South Africa) TU (Bulgaria)	04/2014 07/2014 10/2014	06/2014 09/2014 12/2014
5	Investigation on stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation	DRDO (India)	04/2015	06/2015
6	Pig experiments (“in vivo” and “in vitro”) on protective effects of herbal additives against toxicity of OTA or/and FB1	UJ (South Africa)	10/2014	12/2014
7	Chick and rats experiments on protective effects of herbal additives against toxic and immunosuppressive effects of OTA or FB1	DRDO (India) TU (Bulgaria)	01/2016 07/2016	06/2016 12/2016

**TABLE 2: WORK PACKAGES:**

<b>Work package number</b>	1	<b>Start date or starting event:</b> 10/2013	6 Months
<b>Work package title</b>	OTA and FB1 production		
<b>Beneficiary/Partner Organisation short names</b>	TU (Bulgaria) – 3 months 2 persons UJ (South Africa) – 3 months 2 persons		
<b>Objectives:</b>	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.		

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<p><b>Description of work:</b> The commercial price of mycotoxins is rather high and therefore all mycotoxins used in animal experiments will be produced under this project and knowledge will be exchanged between collaborators:</p> <p><b>Fumonisin B1 production.</b> Some quantity of FB1 is produced in advance under Marie Curie Fellowship (MOIF-CT-2005-018674 – CAUSE KIDNEY DAMAGE) in University of Johannesburg (South Africa) as described by Stoev et al (2011) and we are going to produce some more using the strain <i>Fusarium verticillioides</i> (isolate MRC 826 - this strain was used under material transfer agreement with Medical Research Council of PROMEC Unit, Tygerberg, South Africa), which usually grows 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) as described by Alberts et al (1993). The moistened ground corn kernels will be directly inoculated with 1 ml of a spore suspension (lyophilized conidia of <i>F. verticillioides</i> MRC 826 in 100 ml of sterile distilled water) and incubated on a rotary shaker at 25°C in the dark for 2 weeks. 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.</p> <p><b>Ochratoxin A production.</b> <i>Aspergillus ochraceus</i> (isolate D2306, as used by Stoev et al., 2000b, 2002b,c) supplied by our UK collaborator from Imperial College (London) will be used for production of OTA in TU (Bulgaria) 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 will be used for growing of the same strain of <i>A. ochraceus</i>. Now, we have some quantity of the same mycotoxins OTA, produced previously under Marie Curie Fellowship (FP6), but we are going to produce some more. 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. The concentrations of the above-mentioned mycotoxins in experimental diets pre-enriched with OTA and/or FB1 will be determined by high performance liquid chromatographic (HPLC) technique following a simple extraction/ immune-affinity clean-up (for FB1) prior to the feeding trials.</p> <p>Task 1.1. Production of enough quantity of mycotoxin FB1 Task 1.2. Production of enough quantity of mycotoxin OTA</p>			
<p><b>Deliverables:</b> D 1.1.: Ensuring of enough quantity of mycotoxin FB1 for experimental work D 1.2.: Ensuring of enough quantity of mycotoxin OTA for experimental work</p>			
<p><b>Researchers involved:</b> Prof. Stoycho Stoev and Prof Stefan Denev (Bulgaria) as well as Dr Patrick Njobeh and Dr Mulunda Mwanza (South Africa) Transfer of knowledge to Prof. Stefan Denev and Prof. Stoycho Stoev (Bulgaria) Transfer of knowledge to Dr Patrick Njobeh and Dr Mulunda Mwanza (South Africa)</p>			
<b>Work package number</b>	<b>2</b>	<b>Start date or starting event: 04/2013</b>	<b>6 Months</b>
<b>Work package title</b>	Herbs collection from India and South Africa		
<b>Beneficiary/Partner</b>	TU (Bulgaria) – 3 months 2 persons in India + 3 months 1 person in S. Africa		
<b>Organisation short names</b>	UNIKAPOS (Hungary) – 3 months 1 person in S. Africa		
<p><b>Objectives:</b> To ensure suitable herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects</p>			
<p><b>Description of work:</b> Some of the mentioned above herbs or fruits are well known among Himalayan and South African tribes to have strong healing effects on wounds or human health. Such indigenous herbs from India with possible activity to reduce the deleterious effects of mycotoxins will be collected from different regions of India by Dr. Rajesh Arora and his team at suitable stage of their growth in order to be studied for possible effects on wound granulation or for protective effects on kidneys and liver via the respective studies. Some suitable mixtures of herbal extracts will be suggested for the same studies.</p> <p>Similar collection of herbs will be performed in South Africa under the guidance of Prof Krause and Dr Derek Tantoh Ndinteh. Some of the herbs include but are not limited to <i>Hypoxis hemerocallidea</i> L., <i>Ranunculus multifidus</i> Forssk, <i>Senecio serratuloides</i> DC., <i>Artemisia afra</i>, <i>Mentha longifolia</i> and <i>Leonotis leonurus</i>, <i>Ruta graveolens</i>, <i>Melianthus comosus</i> and <i>Syzgium cordatum</i> Hochst. Plant samples will be identified and characterized and voucher specimens deposited at the University of Johannesburg herbarium. This will be done with geographical- and seasonal factors taken into account. An ethno-botanical informed extraction will be carried out and extracts will be suggested for the planned experiments.</p> <p>A short training will be performed for Bulgarian and Hungarian collaborators in order to receive target skills on various herbs and their healing effects on animals/humans.</p> <p>Task 2. 1.:Collection of suitable herbs at appropriate time Task 2.2.: Biological activity screening of the same herbs</p>			
<p><b>Deliverables:</b> D 2.1.: Ensuring of enough quantity of target herbs for necessary experimental work D2.2.: Establishment of biological activity of the same herbs</p>			
<p><b>Researchers involved:</b> Dr. R Arora (India) as well as Prof R Krause and Dr Derek Ndinteh (South Africa) Transfer of knowledge to Prof D Pavlov, Prof I Zarkov and Prof S Stoev (Bulgaria), and Prof M Kovac (Hungary)</p>			

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<b>Work package number</b>	<b>3</b>	<b>Start date or starting event: 10/2013</b>	<b>6 Months</b>
<b>Work package title</b>	Selective characterization of some herbs from India and South Africa for their bioconstituents and preparing some target herbal extracts or fractions		
<b>Beneficiary/Partner Organisation short names</b>	DRDO (India) – 3 months 1 person UJ (South Africa) – 3 months 3 persons TU (Bulgaria) – 3 months 3 persons UNIKAPOS (Hungary) – 3 months 2 persons		
<b>Objectives:</b> 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.			
<p><b>Description of work:</b> The preparing of some herb extracts will be performed at DRDO (India) under the guidance of Dr Rajesh Arora and his team. There are all necessary equipment for this e.g., rotary evaporator, lyophilizer etc. The selective characterization of herbs and the preparation of some herbal extracts or fractions will be done in Dept of Plant science of Faculty of Agriculture of TU (Bulgaria) by Prof. D. Pavlov and Dept of Chemistry and Biochemistry of Faculty of Medicine of TU (Bulgaria) by Assoc Prof A. Zheleva. There are all necessary apparatuses, laboratories and chemicals for such investigations such as EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy). A short training will be performed of Indian and South African collaborators in order to receive target skills on selective biocharacterization of herbs using the mentioned above technologies as NIRS on “Fiber Optic Spectrometer” NIR Quest 512 (Ocean Optic, USA, 2009 scope 900-1700 nm Spectral region), and exchange of target knowledge between collaborators will be realized in this regard. Similar training and exchange of knowledge will be performed for Bulgarian and Hungarian scientists in Dept of Chemical Technology of Faculty of Science in UJ (South Africa) by Prof Rui Krause and his team. At the Applied Chemistry Dept (Nanotechnology Unit) of UJ, there is a setup to isolate and characterise different natural products and there is an existing expertise to investigate the bioprotection aspects. The extraction of plant materials and compound isolation will be carried out using various chromatographic techniques including low pressure column chromatography, preparative thin layer chromatography, high pressure liquid chromatography, high speed counter current chromatography. Metabolites isolated will be characterised using nuclear magnetic resonance (NMR), infrared spectroscopy (IR), ultraviolet (UV), elemental analyses (EA), Mass Spectroscopy (MS), Raman spectroscopy (RS), Mossbauer analyses (MA). This is a fairly routine process carried out in Prof Krause’s research labs by several post graduates and a number of postdoctoral fellows. There is also an electrospray apparatus recently bought with the aim to synthesize fine particles of active herbal components even down to nanoscale level, thereby increasing their surface area for increased bioactivity. This department also has a good network of both South African and Central African (including Cameroon) researchers who could help identify target plants, and there is a good Botany section that can authenticate such plants.</p> <p>Task 3.1: Selective characterization of some Indian and South African herbs for their bioconstituents (such as levels of flavonoids, carotenoids, etc)</p> <p>Task 3.2: Preparing of some herbal extracts or fractions.</p>			
<b>Deliverables:</b> D 3.1. Establishing the bioconstituents of some target Himalayan and South African herbs D 3.2. Elaboration of technology for preparing some target herbal extracts or fractions Preparing research patents and relative publications in this regard.			
<p><b>Researchers involved:</b> Prof. Dimitar Pavlov, Prof V. Gadjeva and Assoc. Prof. Antoaneta Zheleva (Bulgaria), Prof Rui Krause and Dr Derek Tantoh Ndinteh (UJ, South Africa), and Dr Rajesh Arora (India)</p> <p>Transfer of knowledge to Dr Rajesh Arora (India)</p> <p>Transfer of knowledge to Prof V Gadjeva, Assoc. Prof. A Zheleva and Assoc Prof T Mircheva (Bulgaria)</p> <p>Transfer of knowledge to András Bónai and Viola Vántus (Hungary)</p> <p>Transfer of knowledge to Prof R Krause, Dr Derek Ndinteh and Youmbi Thierry (South Africa)</p>			
<b>Work package number</b>	<b>4</b>	<b>Start date or starting event: 04/2014</b>	<b>9 Months</b>
<b>Work package title</b>	Elaboration of mixtures of target herbal extracts for preparing of some sprays/unguents designed for stimulation of wound granulation		
<b>Beneficiary/Partner Organisation short names</b>	DRDO (India) – 3 months 2 persons UJ (South Africa) – 3 months 1 person TU (Bulgaria) – 3 months 2 persons		
<b>Objectives:</b> 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.			
<b>Description of work:</b> Appropriate unguents or sprays from herbal extracts will be elaborated and suitably tested.			

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<p>This will be done in Dept of Pharmacology of Faculty of Veterinary medicine and Dept of Plant science of Faculty of Agriculture (TU, Bulgaria) under the guidance of Assoc Prof R Mutafchieva and Prof D. Pavlov. There are all necessary laboratories and chemicals for such studies as system for extraction and distillation of water extracts from medicinal plants and application in biological experiments with animals by appropriate treatment. Another part of this work will be done in DRDO (India) in regard to preparing of all suitable herbal extracts. There are all necessary laboratories and infrastructural facilities for such studies.</p> <p>Task 4.1: Preparing of some mixtures of suitable herbal extracts using appropriate constituents, which will be further tested for their stimulating effects on wound granulation</p> <p>Task 4.2: Elaborating appropriate unguents or sprays from herbal extracts designed for stimulation of wound granulation via exchange of knowledge between Indian and Bulgarian collaborators.</p>			
<p><b>Deliverables:</b> D 4.1. Establishing the best mixture of suitable herbal extracts using appropriate constituents, designed for stimulation of wound granulation.</p> <p>D 4.2. Elaboration of appropriate unguents or sprays from herbal extracts for stimulation of wound granulation. Preparing a research paper in this regard.</p>			
<p><b>Researchers involved:</b> Prof D. Pavlov and Assoc Prof R. Mutafchieva (Bulgaria) and Dr Rajesh Arora (India)          Transfer of knowledge to Dr Rajesh Arora and A Gupta (India)          Transfer of knowledge to Prof Mike Dutton (South Africa)          Transfer of knowledge to Assoc. Prof Rumiana Mutafchieva and Prof Ivan Borissov (Bulgaria)</p>			
<b>Work package number</b>	<b>5</b>	<b>Start date or starting event: 04/2015</b>	<b>3 Months</b>
<b>Work package title</b>	Investigation on stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation.		
<b>Beneficiary/Partner Organisation short names</b>	DRDO (India) – 3 months 1 person		
<p><b>Objectives:</b> To investigate the prepared herbal extracts (unguents or sprays) for their stimulating effects on wound granulation.</p>			
<p><b>Description of work:</b> The investigation on the stimulating effect of the prepared herbal extracts (in the form of unguents or sprays) on wound granulation will be done in the Dept of Surgery of Fac of Veterinary Medicine (TU, Bulgaria) under the guidance of Prof I. Borissov as described below. There are all necessary patients and surgery equipments for such studies. The incisional wounds created during regular surgical interventions in pigs/calves/canines/rats (or on the both sides of the back) will be treated by various herbal extracts/(unguents or sprays) for studying the stimulating effects on granulation tissue using parameters such as percent wound contraction including study of size, shape, colour of lesion and wound edges, computerized planimetry (colour digital image processing), extent of grossly visible exudation or granulation, cicatrisation followed by collection of tissues for sequential histopathology (inflammation, cell types, extent of revascularization and granulation, epithelialisation including morphometric studies, extent of collagen, and its texture whether loose/compact etc.) and histochemical determination of extent of collagen, elastin and other tissue elements at various time intervals.</p> <p>Task 5. Testing the stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation.</p>			
<p><b>Deliverables:</b> D 5. Establishing the best unguents or sprays from herbal extracts for stimulation of wound granulation. Preparing a research paper in this regard.</p>			
<p><b>Researchers involved:</b> Prof. Ivan Borisov (Bulgaria)          Transfer of knowledge to Dr Rajesh Arora (India)</p>			
<b>Work package number</b>	<b>6</b>	<b>Start date or starting event: 10/2014</b>	<b>3 Months</b>
<b>Work package title</b>	Pig experiments (“ <i>in vivo</i> ” and “ <i>in vitro</i> ”) on protective effects of herbal additives against toxicity of OTA or/and FB1		
<b>Beneficiary/Partner Organisation short names</b>	UJ (South Africa) – 3 months 3 persons		
<p><b>Objectives:</b> To investigate some target herbal extracts for their protective effects against toxicity of FB1 in pigs or rabbits (“<i>in vivo</i>”) 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 (“<i>in vitro</i>”).</p>			
<p><b>Description of work:</b> The investigations will be performed in Dept of Animal Physiology and Hygiene of UNIKAPOS (Hungary), whereas all necessary facilities are available to investigate metabolism, absorption, accumulation and elimination of mycotoxins. The effects of mycotoxin FB1 and/or OTA and target herbal extracts on gut microbiota of pigs will be studied. Herbal extract – mycotoxin – gut microbiota interaction will be also investigated both <i>in vivo</i> and <i>in vitro</i>. Some cytotoxicity studies (MTT assay &amp; Comet assay) will be performed in order to evaluate the role of plants extracts in protecting the cytotoxic effects of OTA or FB1 using pig Cac 40 (intestinal cell line) or peripheral blood cells as was previously described (Mwanza et al, 2009; Njobeh et al, 2009).</p> <p>Task 6. To study protective effects of some herbal additives against toxicity of OTA and/or FB1 in pigs/rabbits.</p>			

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<b>Deliverables:</b> D 6. Establishing the protective effects of some herbal additives against toxic effects of OTA and/or FB1 in pigs. Preparing a research paper in this regard.			
<b>Researchers involved:</b> Attila Zsolnai, PhD - will be responsible for the molecular determination of gut microbiota; András Bónai – will be responsible for culturing techniques; Katalin Horvatovich, PhD – will be responsible for in vitro testing (UNIKAPOK, Hungary) Transfer of knowledge to (Dr Patrick Njobeh, Dr Mwanza Mulunda and Fosso-Kankeu Elvis (South Africa)			
<b>Work package number</b>	<b>7</b>	<b>Start date or starting event: 01/2016</b>	<b>12 Months</b>
<b>Work package title</b>	Chick and rats experiments on protective effects of herbal additives against toxic and immunosuppressive effects of OTA and FB1		
<b>Beneficiary/Partner Organisation short names</b>	DRDO (India) – 6 months 1 person TU (Bulgaria) – 6 months 1 person		
<b>Objectives:</b> To investigate some target herbal extracts for their possible protection against toxic and immunosuppressive effects of mycotoxins OTA and FB1 in chicks/rats.			
<b>Description of work:</b> The chick experiments will be performed at experimental base of TU (Bulgaria) using the most encountered breed of chicks Plymouth Rock. The chicks will be purchased at a week of age and housed in wire floor cages with continuous infra-red lighting at a temperature suitable for their age. Commercially prepared complete standard feed containing defined concentrations of OTA and/or FB1 with or without some suitable herbal additives supposed to protect against these mycotoxins, will be available <i>ad libitum</i> . The feed levels of both mycotoxins, used in these experiments, will correspond to the levels of OTA and FB1 found in the farms with MP/MCN (Stoev et al, 2010a,b). Pathomorphological and biochemical changes as well as antioxidative or immunostimulating effects on humoral immune response will be studied in various groups of chicks at the end of these experiments in order to explore the possible protective effects of some herbs against the toxic or immunosuppressive effects of OTA and FB1. Blood and/or serum samples from chicks will be examined for various haematological and biochemical parameters as serum glucose, creatinine, urea, total protein, albumin, uric acid, etc, using appropriate tests or methods as previously described (Stoev et al, 1999, 2000a, 2002d). Materials for histological examination will be taken from kidneys, liver, heart, thymus, bursa Fabricii, spleen, intestine, cerebellum or brain and fixed in 10% neutral buffered formalin. The fixed tissues will be processed for paraffin embedding and will be sectioned and stained with haematoxylin-eosin or Periodic acid-Schiff (PAS) as previously described (Stoev et al, 2002d). Immunostimulating effects of the same herbs will be studied at the end of 21 <sup>st</sup> day after immunization against Newcastle disease via serological examination of the blood of experimental chicks as described by Stoev et al (2000a, 2002d). This will be done in Dept of Epizootology and Microbiology and Dept of Pathology of Fac of Veterinary medicine, TU (Bulgaria) under the guidance of Prof. I. Zarkov and Prof Stoev. A 5% total water-extract of artichoke ( <i>Cynara scolymus</i> L) will be prepared as an herbal feed additive with known effect against the toxic effect of OTA in order to ensure comparison with other presumable herbal additives. It will be prepared as steam infusion from dried leaves of artichoke as described by Stoev et al (1999, 2000a, 2002d, 2004). Artichoke-extract has been recommended as a diuretic agent in cardiac and renal insufficiency, and might accelerate the urinary route of excretion of OTA (Stoev et al., 1999). The other part of experimental work will be done in DRDO (India). Two months of age Swiss albino rats will be purchased from a commercial source and will be maintained under strict hygienic conditions throughout the period of experiment. Commercially prepared complete standard feed containing defined concentrations of OTA with or without some suitable herbal additives supposed to protect against these mycotoxins, will be available <i>ad libitum</i> . The studies will be carried out via the mentioned above parameters. This will be done in DRDO using the help of Defence Institute of Physiology and Allied Sciences, Delhi. Task 7. To investigate all possible protective effects of some herbal additives against toxic and immunosuppressive effects of OTA and/or FB1 in chicks/rats.			
<b>Deliverables:</b> D 7. Establishing the protective effects of some herbal additives against toxic and immunosuppressive effects of OTA and FB1 in chicks/rats. Preparing a research paper in this regard.			
<b>Researchers involved:</b> Prof. I. Zarkov and Prof. S. Stoev (Bulgaria) as well as A. Gupta (India) Transfer of knowledge to A Gupta (India) Transfer of knowledge to Prof S. Stoev (Bulgaria)			

**TABLE 3: LIST OF MILESTONES**

List and schedule of milestones					
Milestone No	Milestone name	WPs No	Lead Beneficiary/Partner organisation name	Delivery date	Comments
1	OTA and FB1 production	1	TU (Bulgaria) UJ (S. Africa)	03/2014	Production of enough mycotoxins for the planned

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					experiments
2	Herbs collection	2	TU (Bulgaria) UNIKAPOS (Hungary)	09/2013	Collection of necessary herbs for experimental work from India and South Africa
3	Identification of some herbal bioconstituents and preparation of target herbal extracts or fractions	3	DRDO (India) UJ (South Africa) UNIKAPOS (Hungary) TU (Bulgaria)	03/2014	Identification of herbal bioconstituents and preparation of target herbal extracts or fractions will be necessary for elaboration of suitable technology for preparing some herbal medicines against the toxic effects of target mycotoxins
4	Elaboration of herbal sprays/unguents designed for stimulation of wound granulation	4	DRDO (India) UJ (South Africa) TU (Bulgaria)	12/2014	The herbal sprays/unguents will be further tested as stimulants of wound granulation
5	Effectiveness of target herbal extracts (unguents or sprays) as stimulants of wound granulation	5	DRDO (India)	06/2015	The herbal sprays/unguents will be tested for their stimulating effects on granulation of postoperative or other wounds
6	Establishing of possible protective effects of herbal additives against toxic and immunosuppressive effects of OTA and FB1	6 & 7	DRDO (India) TU (Bulgaria) UJ (South Africa)	12/2016	Some herbal additives will be tested <i>in vivo</i> and <i>in vitro</i> for their protective effects against toxic and immunosuppressive effects of some target mycotoxins. Some herbal products based on multiple herbs will be developed

### B 1.2 RESEARCH QUALITY OF THE PARTNERS

**TRAKIA UNIVERSITY (BULGARIA):** Trakia University (TU) is founded in 1995 and has 4 faculties, 5 colleges and 1 Institute: Faculty of Veterinary Medicine; Faculty of Agriculture; Faculty of Medicine with University Hospital; Faculty of Pedagogics; Three College of Medicine (in Stara Zagora, Haskovo and Yambol); One Bulgarian-German College of Agriculture; one Technical College; One Institute for Professional Qualification. **Faculty of Veterinary Medicine**, which is now a part of Trakia University, is founded on May 11, 1923 as a faculty at the Sofia University. By that time, it was placed at the 14 place in the FAO list among the most distinguished veterinary faculties around the world. In 1974 the Faculty of Veterinary Medicine moved from Sofia to Stara Zagora and since 1995 the Faculty is a part of the Trakia University. The Faculty includes 12 departments; clinics of surgery, obstetrics, non-infectious, infectious and parasitic diseases; a polyclinic for small and decorative animals; lots of laboratories with contemporary equipment (including laboratory on electron microscopy) as well as a large experimental base for all kind of animals. During the last year the university has 127 scientific projects (27 International Projects and the rest projects are at national and university level).

The **international cooperation** has an important place in the scientific and educational activities of TU - Stara Zagora in the terms of global partnership and knowledge sharing. The International and European cooperation is crucial for the future of the university to develop new areas of teaching and research, exchange of experience and further development of the university to be an integral part of the European education and research area. The TU develops contacts with internationally recognized educational institutions through its membership in the European and World associations of universities and by signing agreements promising to develop academic links and long-term cooperation in higher education, exchange of professors, students exchange and support of research activity.

So far, TU has international contacts with more than 40 countries. Annually, the university is visited by many foreign representatives - participants in international events, professors and students under direct contracts. Every year many representatives of the university realize teaching and student mobility under the European Program for Lifelong Learning - "Erasmus" based on the successful work of more than 50 bilateral exchange agreements with its partner institutions. University professors participate in many educational and scientific European and global mobility programs, scholarships and fellowships, lectures delivery, exchange of experience, conducting research and participating in conferences and workshops - Fulbright, Norman Borlaug, EEA Grants and others. Priority in the development strategy of TU takes the international research activities in international programs. Scientists from



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the university work in international research teams and implement international projects, including: 1) Leonardo da Vinci - DUKOM, ADEM; 2) Grundtvig - "Toys of my grandparents"; 3) Comenius - Key TTT (Key Skills - Teamwork, Training & Technology); 4) Different COST actions for cooperation in science & technology research; 5) Projects under the Seventh Framework Program - Annual activities within the project Researchers Night - "Regae - Researchers as Guides to Glory of Ancient Europe, U \* Night - Sustainable Ecological Balance, STAR NIGHT - Astronomy and Challenges of New technologies, REACT-Researchers in Industry and Academia for Technology Development, RECSSES - Scientists and chemistry to support the economy and society.

The project activity of TU involves partnerships with countries from various regions of the world, including: Japan - Memorandum of Understanding for research in molecular and radiation photodynamic cancer therapy; the project "Practical Training Program of Young Researchers for Strategic Bioresource Utilization and Preservation Among of Match-Making between EU and Asia to avoid Food Crisis" under the program for funding Supporting Society for the Promotion of Science; USA - Long term exchange of Faculty staff. Programs relating to research, teaching and extension with Iowa State University; India - Studies on Antioxidants against Oxidative Stress Induced by Drugs and Radiation under the program for Bilateral Scientific and Technological Cooperation between Bulgaria and India; Russia - "Days of Russian Language and Culture: Cultural and educational rally"; Norway - "Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora region" under the Funding program Innovation Norway; Turkey - project "Network for open dialogue inter professional education and training" program for projects without borders and many others.

As a result of the participation in various projects in TU are designed and operate more than 20 research laboratories. In the context of the 2020 strategy TU is open to new partnerships with universities and research organizations worldwide for scientific cooperation, development of scientific infrastructure, implementation of promising, fundamental, practical research and national, regional and global application of results of research.

The main scientific research collaborations and International cooperations in TU: Germany: University of Veterinary Medicine (Hannover), Institute of Agrarian Development for Central and Eastern Europe (Halle), Technical University (Munich); France: Ecole Nationale Veterinaire (Nantes), Academie de Lyon; Italy: University of Udine; Portugal: University of Lisbon; Greece: Agricultural University of Athens, Aristotelian University Thessaloniki, University of Crete; Turkey: University of Bursa, Ankara University, Mustafa Kemal University, Canakkale University, Suleyman Demirel University; Romania: University of Cluj-Napoca; Makedonia: University of Skopje; Slovakia: University of Vet Medicine (Kosice); Czech Republic: Technical University of Agriculture (Prague); Slovenia: University of Ljubljana; Israel: Institute of Animal Science; Russia: Moscow Veterinary Academy; Ukraine: SouthSlav Institute of Nikolaev; India: Institute of nuclear medicine (New Delhi), Japan: University of Agriculture and Technology (Tokio), National Institute of Radiological Sciences; USA: State University of Mississippi, Minnesota and Iowa; South Africa: University of Johannesburg.

### Some relevant and recent publications of Bulgarian scientists involved in this project:

-G. Beev, S. Denev, Tz. Lalev, D. Pavlov. 2007. Comparison of Different Commercial Wheat Cultivars and New Selection Lines Durum and Soft Wheat to Fusarium infection in the Region of Stara Zagora. *Ecology and Future*, 3; 29-34.

-Zarkov, Iv., Ilian Bochev, Ruth Manvell, Wendy Shell. The First Isolation of Avian Influenza Virus in Bulgaria. *Veterinary Record*, 158, 3, 2006, 106-107 (IF=1.2)

-Zarkov, Iv., I. Bochev. Influence of inoculation dose of avian H6N2 influenza A virus on virus shedding and humoral immune response of chickens after artificial experimental intravenous infection, *Revue Medicin Veterinaire*, 2008, 159, 10, 489-493 (IF=0.26)

-Zarkov, Iv. Use of a commercial immunoassay test (Directigen™ Flu A) for rapid detection of Influenza A antigen in duck (*Anas platyrhynchos*). *Veterinary Record*, 2008, 163: 661-662 (IF=1.2)

-Koynarski V, S Stoev, N Grozeva, T Mirtcheva, H Daskalov, P Mantle, Experimental coccidiosis provoked by *E acervulina* in chicks simultaneously fed on OTA contaminated diet, *Res Vet Sci*, 2007, 82, 225-231 (IF=1.4)

-Andonova, M., Borissov, I., Sotirov, L., Changes in some factors of the innate immunity and in serum zinc and iron concentrations in pigs following intravenous administration of Escherichia coli lipopolysaccharide, *Onderstepoort Journal of Veterinary Research*, 2001, 68, 91-99 (IF=0.69)

-Chaprazov, Ts., Ceorgiev, D., Borissov, I., Surgical management and antibacterial therapy of bite wounds in a green iguana (*Iguana iguana*), *Bulgarian J Vet Med*, 2010, 13, № 4, 259-263.

-Zheleva, Didier Michelot, Zhivko D. Zhelev. Sensitivity of alpha amanitin to oxidation by a lactoperoxidase-hydrogen peroxide system. *Toxicol* 38, 1055-1063, 2000. (IF = 2.03)

-Gadjeva, V Grozdanka Lazarova, A. Zheleva. Spin labeled antioxidants protect bacteria against the toxicity of alkylating antitumor drug CCNU. *Toxicol. Lett.*, 144, 289-294, 2003 (IF = 2.437).

-Zheleva, A Tolekova, M Zhelev, V Uzunova, M Platikanova, V Gadzheva, Free radical reactions might contribute to severe alpha amanitin hepatotoxicity, *Medical Hypotheses*, 69, 361-367, 2007, (IF=1.30).

-Hristakieva E, V. Gadjeva,. Oxidative stress in patients with vitiligo. *J.Eur Academy of Dermatol and*

*Venerology*, (2001), v15, suppl. 2, P23-19. (IF 0,98)

-Rajesh Arora, AS Dhaker, J Sharma, M Adhikari, R Chawla, D Gupta, A. Zheleva, Y. Karamalakova, Raj Kumar, Raj K. Sharma, A Sharma, RK Sharma, V Gadjeva. Radiomodulatory Effects of Extracts of *Psoralea corylifolia* Linn. Evaluated by *in vitro* Assays and EPR Spectroscopy. *Z. Naturforschung* (in press). (IF=0.80).

-Yanka Karamalakova, A. Zheleva, Galina Nikolova, Raj Kumar, Krishna Chuttani, Anil K. Mishra, Rakesh K. Sharma, V Gadjeva. A NEW PROMISING HEPATOPROTECTOR AQGPD WITH NATURAL ORIGIN. *European Journal of Medical Research*, vol 15/ suppl I: 181-182, 2010. (IF=1.13).

-Gadjeva V, E. Hristakieva, S. Karamihov. Correlation between blood antioxidant levels and lipid peroxidation in patients with eruptive psoriasis. *J.Eur Academy of Dermatol and Venerology*, (2001), v15, suppl. 2, P17-9. (IF 0,98)

-Kumar R, Bansal DD, Patel DD, Mishra S, Karamalakova Y, Zheleva A, Gadjeva V, Sharma RK. Antioxidative and radioprotective activities of semiquinone glucoside derivative (SQGD) isolated from *Bacillus* sp. INM-1. *Mol Cell Biochem*. 2010 Nov 30. (IF=1,89)

-Adhikari M, Arora R, Chawla R, Sharma J, Dhaker AS, Gupta D, Dubey N, Kumar R, Ivanov V, Gadjeva V, Gevrenova R, Sharma RK. Evaluation of silymarin as a promising radioprotector. *Z Naturforsch C*. 2010;65(5-6):337-46 (IF=0,72)

-Zhelev Z, Bakalova R, Aoki I, Matsumoto K, Gadjeva V, Anzai K, Kanno I. Nitroxyl radicals as low toxic spin-labels for non-invasive magnetic resonance imaging of blood-brain barrier permeability for conventional therapeutics. *Chem Commun (Camb)*. 2009, 7;(1):53-5. (IF=5,14)

-Gadjeva V, Dimov A, Georgieva N. Influence of therapy on the antioxidant status in patients with melanoma. *J Clin Pharm Ther*. (2008), 33(2): (IF=1,16)

-Gadjeva V, Tolekova A, Vasileva M. (2007). Effect of spin labeled 1-ethyl-1-nitrosourea on CCNU – induced oxidative injury. *Die Pharmazie*. 62(8):608-13 (IF = 0, 69)

-Valcheva-Kuzmanova S., V. Gadjeva, D. Ivanova and A. Belcheva. Antioxidant activity of *Aronia melanocarpa* fruit juice *in vitro* *Acta Alimentaria*, Vol. 36 (4), pp. 425–428 (2007). (IF=0.27)

-Georgieva N., V. Gadjeva, A. Tolekova, D. Dimitrov, (2005). Hepatoprotective effect of isonicotinoylhydrazones SH7 against chronic isoniazid toxicity. *Die Pharmazie* 60, 138 - 141. (IF = 0, 69)

-Georgieva, N. and V. Gadjeva, (2005). Beneficial effect of isonicotinoylhydrazones on isoniazid induced disruption of ecological oxidative balance. *Medical Hypotheses* 64 (3), 662. (IF = 0,68)

#### **Current Research projects related to this proposal:**

1) Project title: “**Study on Antioxidants against Oxidative Stress induced by Drugs and Radiation**” with Coordinator from Bulgarian side: Prof. Veselina Gadjeva and PI: Antoaneta Zheleva

Quantum and source of funding: 50,000 lv, **Contract БИИ-7/08-2008, Indo-Bulgarian Inter-Governmental Program of Co-operation in Science and Technology**

2) Project title: “International Project “Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora region” with Coordinator: Prof. D. Pavlov (Leader of working group soils and plants)

Quantum and source of funding: 637 654 € Program Innovation Norway, Number 2008/115236

3) Project title: “**RIVERS**, Resistance of influenza viruses in environmental reservoirs and systems” with Coordinator: Prof. Angel Galabov and PI: Prof Ivan Zarkov

Quantum and source of funding: 80 000 € 7<sup>th</sup> Framework of EU

4) Project title: “Experimental studies on the sensitivity of domestic geese to low pathogenic avian influenza isolate / LPAIV / H6N2 in birds in Bulgaria” with Coordinator: Prof. Ivan Zarkov

Quantum and source of funding: National Science Fund of Ministry of Science and Education in Bulgaria. National Science Fund of Ministry of Science and Education in Bulgaria (TU project № 2/2010) – 3400 Lv.

5) Project title: Investigations on preserving infectious possibilities of pathogenic avian influenza isolate / LPAIV / H6N2 in the faeces of experimentally infected chicks and duckings (strain *Anas Platyrhynchos*) with Coordinator: Prof. Ivan Zarkov

Quantum and source of funding: National Science Fund of Ministry of Science and Education in Bulgaria. National Science Fund of Ministry of Science and Education in Bulgaria (TU project № 3/2011) – 4123 Lv.

6) Project title: “Solutions and interventions for the technological transfer and innovation of the agro-food sector in South East regions –TECH.FOOD” (2008) with Coordinator: Prof Vesselina Gadjeva

Source of funding: South East Europe Transnational Cooperation Programme

7) Project title: “Molecular imaging and radiation-induced photodynamic therapy of cancer using spin-labeled probes” - contract between National Institut of Radiological Sciences, Chiba, Japan and Medical faculty, Trakia University, Stara Zagora, Bulgaria (2008) with Coordinator: Prof Vesselina Gadjeva

#### **A SHORT INFORMATION ABOUT THE COORDINATOR OF THE PROJECT – PROF. S D STOEV**

**Date and place of birth:** 8<sup>th</sup> of September 1960, Sinapovo, Bulgaria.

**Titles/Qualifications/Year:** PhD – 1998, DSc – 2004, Assoc. Prof. – 1999, Prof. in Vet. Pathology – 2007.

**Research Experience closely related to the project proposal (institution, duration, year):**

1) Project leader of 4 research projects (regarding mycotoxic nephropathy in farm animals and humans): CC273 (1992-1995), L414 (1994-1997), CC449 (1994-1997) and CC1003 (2001-2003) funded by National Science Fund of Ministry of Science and Education in Bulgaria:

2) Bulgarian project leader of Joint Research Project between Bulgaria and UK funded by The Royal Society - London (Joint Project Grant - Reg. Ch. No 207043, Ref. FSU/CEE/JP) and titled: "Fungal nephrotoxins in porcine renal disease in Bulgaria" (1997-2000) aimed to clarify the cause of spontaneous nephropathies in Bulgaria

3) A specialisation in Imperial College of Science, Technology and Medicine - London, U.K (September, 1998) – it addressed receiving a first hand knowledge about the methodology of producing a high quantity of OTA

4) A specialisation in Laboratoire de Toxicologie et Sécurité Alimentaire, Toulouse, France, (4 months in 2000 - NATO grant) - it addressed some genotoxic investigations on tissue samples from spontaneous nephropathies.

5) Marie Curie Outgoing International Fellowship (6<sup>th</sup> Framework of EU – project 018674 "Cause Kidney Damage") for 2 years (2007-2008) in University of Johannesburg, titled: "An expected multicausal nature of spontaneous animal and human nephropathy in Bulgaria and South Africa (see <http://www.esastap.org.za/esastap/stories/stoev.php>). The project has addressed the cause of porcine/chicken/human nephropathies in Bulgaria and human nephropathy in South Africa. **The fellowship was selected as one of the seven success stories in the Worldwide Research featured in the publication of European Commission highlighting the most successful fellows** (see pp 264-267 of "Inspiring researchers": [http://ec.europa.eu/research/mariecurieactions/docs/inspiring\\_researchers\\_en.pdf](http://ec.europa.eu/research/mariecurieactions/docs/inspiring_researchers_en.pdf) and pp 104-105 of "eStrategies Projects": <http://viewer.zmags.com/publication/b802e4ab/#/b802e4ab/106> ).

6) Specializations in field of multi-mycotoxin screen and extraction, ELISA, TLC, HPLC, Fungal screening and identification, MTT bioassay (cytotoxicity testing), PCR (polymerise chain reaction) analysis of fungal species, etc, performed in Food, Environment and Health Research Group, University of Johannesburg (2 years - 2007-2008)

**Invited recent lectures and seminars closely related to the project proposal:**

-Invitation to Open Vet Africa 2007 Congress by The Board of Trustees of Animal Aid for Africa (AAA) with Opening Speech and talk with title "Food Safety and some foodborne mycotoxicoses" (27.07.2007, Johannesburg).

-Invitation to give a talk in a meeting with Director and Deputies Directors of Strategic Partnership and International Resources of SA Department of Science and Technology, Pretoria and the Manager Science and Technology Agreements Fund of National Research Foundation in South Africa (29.01.2007)

-Invitation to give a presentation for the Marie Curie instruments used to reach scientific goals of researchers in Information session on FP7 and the European-South African Science and Technology Advancement Programme (ESASTAP) in University of Johannesburg on 13.04.2007

-Invited lectures/seminars on "Mycotoxic nephropathy in farm animals and humans – preventive measures and risk assessment" in Faculty of Health Science, University of Johannesburg (04.05.2007 and 18.11.2009)

-Invitation to give a seminar/lecture in the Onderstepoort Veterinary Institute in Pretoria (14.04.2008) on the topic "Complex aetiology and pathology of mycotoxic nephropathy in Bulgarian pigs and chicks".

-Invitation to give seminars/lectures in Kaposvar University in Hungary (22-29.06.2010) on the topic "Mycotoxic nephropathy in Bulgarian and S. African pigs: complex etiology and similarity with Balkan Endemic Nephropathy"

-Invited lecture at *International Symposium* "Power of Fungi and Mycotoxins in Health and Disease", Primošten, Croatia (19-22.10.2011) on the topic "Mycotoxic nephropathy in animals: complex etiology and similarity to Endemic Nephropathy" (<http://www.hmd-cms.hr/power-of-fungi/stoycho-dimitrov-stoev.php>)

**Major achievements and results closely related to the project proposal:**

The pathology, frequency, duration, occurrence and spreading of mycotoxic nephropathy in various farm animals and chicks in Bulgaria (Stoev et al, 1998a; Stoev et al, 2002a) and South Africa were established (Stoev et al, 2010a,b); a strong immunosuppression and appearance of secondary bacterial infections were seen in pigs and chicks fed on OTA-contaminated diet (Stoev et al, 2000a,b); carcinogenic and teratogenic effects were found in mice, rats and chickens fed on OTA-contaminated diet (Stoev, 2010a); some protocols for the safe utilization of OTA-contaminated feeds were established in order to reduce farm losses from a decrease of weight gain and egg production in stock chicks - a real protective effect was found for some feed additives (water extract of artichoke, sesame seed, Roxazyme-G and phenylalanine) against the suppressive effect of OTA on egg production of laying hens as well as against the carcinogenic effects of OTA; (Stoev et al, 2002d, Stoev, 2010a); some preventive measures and risk evaluation were recommended as high effective to prevent human/animal exposure to OTA (Stoev, 1998, 2008a,b; Stoev et al, 1998c), etc.

**More than 100 original papers in peer reviewed international journals related to the project proposal**

**The principal publications in authoritative peer-reviewed international journals during the last years:**

-Stoev, S. D., D. Gundasheva, I. Zarkov, T. Mircheva, D. Zapryanova, S. Denev, Y. Mitev, H. Daskalov, M. Dutton, M. Mwanza, Y-J. Schneider, Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and fumonisin B1, *Exp Toxicol Pathol*, 2012 (DOI: 10.1016/j.etp.2011.01.008) **IF=1,2**

-Stoev, S. D. Food safety and increasing hazard of mycotoxin occurrence in foods and feeds, *Critical Reviews in Food Science and Nutrition* 2012 (in press). **IF=4.1**

-Stoev, S. D., Studies on carcinogenic and toxic effects of ochratoxin A in chicks, **Special issue "Ochratoxins"**, *Toxins*, 2010a, 2, 649-664 (<http://www.mdpi.com/2072-6651/2/4/649/pdf>)

-Stoev, S. D. Studies on some feed additives and materials giving partial protection against the suppressive effect of ochratoxin A on egg production of laying hens, *Research in Veterinary Science*, 2010b, 88, 486-491 (<http://dx.doi.org/10.1016/j.rvsc.2009.12.007>). **IF=1.4**

-Stoev, S. D., M. F. Dutton, P. B. Njobeh, J. S. Mosonik, P.A. Steenkamp, Mycotoxic nephropathy in Bulgarian pigs and chickens: complex aetiology and similarity to Balkan Endemic Nephropathy, *Food Additives and Contaminants Part A*, 2010a, 27 (1), 72-88. **IF=1.8**

-Stoev, S. D., S. Denev, M. F. Dutton, P. B. Njobeh, J. S. Mosonik, P.A. Steenkamp, I. Petkov. Complex etiology and pathology of mycotoxic nephropathy in South African pigs, *Mycotox Research*, 2010b, 26 (1), 31-46

-Stoev, S. D., S. Denev, M. F. Dutton, B. Nkosi, Cytotoxic effect of some mycotoxins and their combinations on human peripheral blood mononuclear cells as measured by MTT assay, *The Open Toxinology Journal*, 2009, 2, 1-8 (<http://www.benthamscience.com/open/totnj/articles/V002/1TOTNJ.pdf>).

-Stoev S. D., Complex Etiology, Prophylaxis and Hygiene Control in Mycotoxic Nephropathies in Farm Animals and Humans, **Special Issue "Mycotoxins: Mechanisms of Toxicological Activity - Treatment and Prevention"**, Section "Molecular Pathology", *International Journal of Molecular Sciences*, 2008, 9, 578-605, (<http://www.mdpi.org/ijms/papers/i9040578.pdf>). **IF=1.0**

-Stoev, S. D., M. Stefanov, S. Denev, B. Radic, A-M. Domijan, M. Peraica, Experimental mycotoxicosis in chickens induced by ochratoxin A and penicillic acid and intervention by natural plant extracts, *Veterinary Research Communications*, 2004, 28, 8, 727-746. **IF=0.6**

-Stoev, S. D., N. Grozeva, R. Simeonov, I. Borisov, H. Hubenov, Y. Nikolov, M. Tsaneva, S. Lazarova, Experimental cadmium poisoning in sheep, *Exp Toxicol Pathol*, 2003, 55, 4, 309-314. **IF=1.2**

-Stoev, S. D., H. Daskalov, B. Radic, A. Domijan, M. Peraica, Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology, *Veterinary Research*, 2002a, 33, 1, 83-94 ([http://www.edpsciences.org/articles/vetres/pdf/2002/01/EDPS-VR1-2002\\_8.pdf](http://www.edpsciences.org/articles/vetres/pdf/2002/01/EDPS-VR1-2002_8.pdf)). **IF=3.1**

-Stoev, S. D., V. Koynarsky, P. G. Mantle, Clinicomorphological studies in chicks fed ochratoxin A while simultaneously developing coccidiosis, *Veterinary Research Communications*, 2002b, 26, 189-204. **IF=0.6**

-Stoev, S. D., M. Paskalev, S. MacDonald, P.G. Mantle, Experimental one year ochratoxin A toxicosis in pigs, *Experimental and Toxicologic Pathology*, 2002c, 53, 481-487. **IF=1.2**

-Stoev, S. D., Djuvinov D., Mirtcheva T., Pavlov D., Mantle P., Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks, *Toxicology Letters*, 2002d, 135, 1-2, 33-50. **IF=3.2**

-Stoev, S.D., Vitanov, S., Anguelov, G., Petkova-Bocharova, T., Creppy, E. E. Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and penicillic acid, *Veterinary Research Communications*, 2001, 25, 3, 205-223. **IF=0.6**

-Stoev, S. D., G. Anguelov, I. Ivanov, D. Pavlov, Influence of ochratoxin A and an extract of artichoke on the vaccinal immunity and health in broiler chicks, *Exp Toxicol Pathol*, 2000a, 52, 43-55. **IF=1.2**

-Stoev, S. D., D. Goundasheva, T. Mirtcheva, P. G. Mantle, Susceptibility to secondary bacterial infections in growing pigs as an early response in ochratoxicosis, *Exp Toxicol Pathol*, 2000b, 52, 287-296. **IF=1.2**

**Recent monographs, chapters in books closely related to the project proposal:**

-Stoev, S. D., *Special pathology and diagnostics of mycoses, mycotoxicoses, parasitoses, intoxications and avitaminoses* (Eds: L. Diakov, V. Koynarski, A. Angelov), Publ House CD Contrast, St Zagora, 2010, 1-239.

-Stoev, S. D., Mycotoxic nephropathies in farm animals – diagnostics, risk assessment and preventive measures, **In: Mycotoxins in Farm Animals, Chapter 8**, I Oswald, I Taranu (Eds), SG Pandalai (Managing Editor), 2008, pp. 155-195, Transworld Research Network, 37/661 (2), Fort P.O., Trivandrum-695 023, India.

-Stoev, S., *Mycotoxic nephropathies in farm animals and humans – diagnostics, risk assessment and preventive measures* (Eds: S. Bozhkov, L. Diakov), Publishing House Contrast, Stara Zagora, 2002, pp. 1-172

**Membership in Scientific and Professional Institutions, Bodies and Organizations; Scientific awards:**

Editorial Board Member of "The Open Toxinology Journal" and "ISRN Veterinary Science". Reviewer/member in International Reviewers Panel of many peer reviewed international journals. Full member in "International Society on Toxinology", "Euroscience Association" and "Science Advisory Board". Earned a place among the most important contributors in field of Medicine and Healthcare (included in the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> editions of "Who's Who in Medicine and Healthcare"), in field of Science and Engineering (included in the 7<sup>th</sup>, 8<sup>th</sup>, 9<sup>th</sup>, 10<sup>th</sup> and 11<sup>th</sup> editions of "Who's Who in Science and Engineering"), and in the World (included in 21<sup>st</sup> -2004 and 22<sup>nd</sup> – 2009 editions of "Who's Who in the World" and in the 61<sup>st</sup> edition "Who's Who in America" - 2007), which chronicle the most accomplished professionals in these areas from across the world. Included in the issues: "Cambridge Blue Book – 2004-2007" as a "Laureate of the Cambridge Blue Book", "2000 Outstanding Scientists of the 21<sup>st</sup> Century

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– 2004, 2007, 2009, “IBC Leading Scientists of the World – 2005, 2006 and 2008“ and “**IBC Top 100 Scientists – 2005**”, published by International Biographical Center - IBC, Cambridge. Nominated for “International Scientist of the year 2004”, “Laureate of the Da Vinci Diamond” (2004 and 2006) and “The Archimedes Award” (2006) by IBC, Cambridge. Inducted into “**American Hall of Fame**” (2009) and selected to be a recipient of prestigious “**Albert Einstein Award of Excellence for 2010**” for Distinguished Accomplishments in “Toxicologic Pathology”. Included in “**Great Minds of the 21<sup>st</sup> Century** – 2004, 2005, 2007 editions” and in “World Book of Knowledge – 2005 edition” published by ABI, and nominated for “Noble Laureate 2004” and “Gold Medal for Bulgaria” (2006, 2007 and 2009) by the Governing Board of Editors of ABI. Included in “**eStrategies Projects**” (pp 104-105) and “**Inspiring researchers**” (pp 264-267) **published by European Commission of EU.**

**VIP Number: 30016596**

### **KAPOSVAR UNIVERSITY (UNIKAPOS, HUNGARY)**

**Expertise in the field tackled by the project:** UNIKAPOS is the largest institution engaged in animal sciences in Hungary and carries out basic, applied and developing research in the field of living natural sciences. At the Department of Animal Physiology and Hygiene of Kaposvár University metabolism, absorption, accumulation and elimination of FB1 have been studied in pigs both *in vivo* and *in vitro*. The acute and chronic, dose-dependent effect of FB1 has been also intensively studied in order to determine the NOAEL (no observed adverse effect level) in pigs. FB1 was found to be foetotoxic in pigs and rabbits, but less immunosuppressive in pigs. Several studies on the ochratoxin A exposure of humans and the pathological effect (immunosuppression, reproductive toxicity) of T-2 toxin were also carried out. Non invasive digital imaging techniques (CT and MRI) have been introduced for monitoring pathological changes. The studies in digestive physiology focus on nutrition – gut ecosystem – immune response interaction. Several natural feed additives (as possible alternatives for antibiotic growth promoters) have been examined for their mode of action. Most recently pro/prebiotics – mycotoxins – ecosystem interaction investigations have been started.

### **Participation in international projects/cooperations:**

-Participation in EU FP6 Young Train Programme (‘Training and mentoring young scientists.....in a whole food chain approach to quality and safety’) 2005-2007.

-Participation in EU FP5 (‘Standardisation of pig carcass classification in the EU through improved statistical procedures and new technological developments’) 2000-2003.

-Participation in a COST 848 Action ‘Multi-facetted research in rabbits: a model to develop a healthy and safe production in respect with animal welfare’. Workgroup: Nutrition and feeding strategies for improving the health of the doe and the young rabbit (2000-2005).

### **-Current Intergovernmental cooper S&T cooperation programmes; partners and topics** in what follows:

a) INRA, Centre de Toulouse, UMR 1289 TANDEM - New approach in investigating the rabbit caecal ecosystem to develop new strategies for improving digestive health, production and food safety.

b) Food, Environment & Health Research Group, Univ. of Johannesburg - Investigation into the mechanism of action of selected mycotoxins on specified specie cell and tissue cultures with reference to known mycotoxicoses.

c) Institute of Animal Physiology Slovak Academy of Sciences, Laboratory of Animal Microbiology - New bacteriocinogenic probiotics and their use in rabbit production.

d) Technical University of Munich, Department of Animal Science, Academic Chair of Animal Hygiene - Studies on the analysis and metabolism of Fusarium toxins in biological samples (blood, organs, urine and faeces).

### **Current participation in national projects/cooperations:**

Two projects funded by the Hungarian Scientific Research Fund (funding basic research in Hungary):

a) ‘Study of the possible interaction between selected respiratory pathogens and fumonisin B1 toxin in pigs using modern imaging techniques’ and ‘In vitro evaluation of the single and combined effect of mycotoxins’.

b) Scientific leader of a TÁMOP project (Social Renewer Operational Programme of the National Development Agency) the topic of which is ‘Interaction between nutrition – gut ecosystem – immune response’.

### **A short information about Hungarian Scientist in charge of this project – Prof. Melinda Kovács**

**Date and place of birth:** Budapest, 25th April, 1959

**Titles/Qualifications/Year:** 2005 - Doctor of the Hungarian Academy of Sciences (DSc); 1997 - Dr. habil; 1988 – PhD in Veterinary Science; 1983 - Pedagogical training; 1982 - Veterinary doctor;

**Titles of positions:** 2007-2010: vice-rector, professor, head of department in University of Kaposvár; 2000-2006: vice-dean, professor, head of department; 1992-1999: associate professor, head of department; 1987-1990: research worker; 1983-1987: postgraduate state scholarship in Univ. Vet. Med. Budapest)

**Teaching activity:** Animal physiology and anatomy (graduate); Digestive physiology, production physiology, Nutritional immunology (postgraduate); Physiology of animal production (PhD students)

**Research:** A) Digestive physiology of pig and rabbits (development of the GI tract, composition of the microflora of the hind gut, development of brush border enzyme activities, physiology of early weaning, physiological effects of different pro/prebiotics). B) Animal and human health aspects of certain mycotoxins

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(determination of tolerable limit values of FB1 in pigs and T-2 in rabbits, examination of foetotoxic effect, residue formation in different organs, elimination and metabolism of FB1 in pigs, monitoring human exposure to OTA in Hungary, immunosuppressive and cytotoxic effect of FB1 and T-2, effect of T-2 and FB1 on male reproduction).

**PhD student supervised:** 1) 1997-2000: Tóth Á: Time and dose dependent effect of FB1 on pig production; 2) 1999-2005: Tornyos G: Early diagnosis of fumonisin toxicosis in pig by computer tomographic examination; 3) 2000-2005: Donkó T: Effect of atrophic rhinitis on pig production; 4) 2002-2006: Fodor J: Investigation of the FB1 kinetics in pigs; 5) 2005-2007: Szabó M: Analysis of public health risk of mycotoxins getting into the food chain

**Current PhD students:** 1) 2006- : Bónai A: Investigation of factors influencing caecal fermentation processes in rabbits; 2) 2006-: Pósa R: Study of porcine respiratory disease complex by non invasive digital imaging techniques; 3) 2009-: Rajli V: Cytotoxicity of T-2 toxin measured in vivo and in vitro; 4) 2010-: Hafner D: Combined effect of T-2 and FB1 measured in vivo and in vitro; 5) 2011-: Vántus V: Detection of changes in the caecal microbiota of rabbits by molecular genetics tools

**Project management (in the previous 5 years):** project leader in 7, deputy leader of 5, participant in 5 projects. Member of Working Group for Nutrition in a COST project (COST Action 848) on rabbit research (Multi-facetted research in rabbits: a model to develop a healthy and safe production in respect with animal welfare).

**Publications:** chapters in 17 textbooks, 45 refereed papers in foreign language, 39 refereed papers in Hungarian, 30 proceedings, 22 abstracts, **IF: 26.491**, indep.cit: 262

### **Some relevant and recent publications of the Hungarian scientists involved in this project:**

-Zomborszky-Kovács, M., Bárdos, L., Bíró, H., Tuboly, S., Wolf-Táskai, E., Tóth, Á., Soós, P.: Effect of beta-carotene and nucleotide base supplementation on blood composition and immune response in weaned pigs. *Acta Vet. Hung.*, 2000. 48. (3). 301-311 (**IF = 0,511**)

-Zomborszky-Kovács, M., Vetési, F., Kovács, F., Bata, Á., Tóth, Á., Tornyos, G.: Examination of the harmful effect to foetuses of fumonisin B<sub>1</sub> in pregnant sows. *Teratogen Carcin Mut*, 2000. 20. 293-299 (**IF = 1,106**)

-Zomborszky-Kovács, M., Vetési, F., Repa, I., Kovács, F., Bata, Á., Horn, P., Tóth, Á., Romvári, R.: Experiment to determine limits of tolerance for fumonisin B<sub>1</sub> in weaned piglets. *J. Vet. Med. B* 47. 2000. 277-286. (**IF = 0,59**)

-Zomborszky-Kovács, M., Vetési, F., Horn, P., Repa, I., Kovács, F.: Effects of prolonged exposure to low-dose fumonisin B<sub>1</sub> in pigs. *J. Vet. Med. B.*, 2002. 49. 197-201 (**IF = 0,67**)

-Fazekas, B., Tar, A.K., Zomborszky-Kovács, M.: Ochratoxin contamination of cereal grains and coffee in Hungary in the year 2001. *Acta Veterinaria Hungarica*, 2002. 50. (2), 177-188 (**IF = 0,33**)

-Zomborszky-Kovács, M., Kovács, F., Horn, P., Vetési, F., Repa, I., Tornyos, G.: Investigations into the time- and dose-dependent effect of fumonisin B<sub>1</sub> in order to determine tolerable limit values. *Livestock Production Science*, 2002. 76. 251-256 (**IF = 1,117**)

-Tornyos, G., Kovács, M., Rusvai, M., Fodor, J., Horn, P., Kovács, F.: Effect of dietary fumonisin B<sub>1</sub> on certain immune parameters of weaned pigs. *Acta Veterinaria Hungarica*, 2003. 51. 2. 171-179 (**IF = 0,535**)

-Meyer, K., Mohr, K., Bauer, J., Horn, P., Kovács, M.: Residue formation of fumonisin B<sub>1</sub> in porcine tissues. *Food Addit Contam.* 2003. 20. (7) 639-647 (**IF = 1,46**)

-Fazekas, B., Tar, A., Kovács, M.: Ochratoxin A content of urine samples of healthy humans in Hungary. *Acta Veterinaria Hungarica*, 2005. 53 (1) 35-44 (**IF = 0,53**)

-Fazekas, B., Tar, A., Kovács, M.: Aflatoxin and ochratoxin-A content of spices in Hungary. *Food Addit Contam.* 2005. 22(9). 856-863 (**IF = 1,61**)

-Fodor, J., Bauer, J., Horn, P., Kovács, F., Kovács M.: The distribution and elimination of fumonisin analogues in weaned piglets after oral administration of *Fusarium verticillioides* fungal culture. *Food Addit Contam.* 2006. 23 (5). 492-501 (**IF = 1,78**)

-Kovács, M., Szendrő, Zs., Milisits, G., Bóta, B., Bíró-Németh, E., Radnai, I., Pósa, R., Bónai, A., Kovács, F., Horn, P.: Effect of nursing methods and faeces consumption on the development of the bacteroides, lactobacillus and coliform flora in the caecum of the newborn rabbits. *Reprod Nutr Dev*, 2006. 46. 205-210 (**IF = 1,81**)

-Fodor, J., Meyer, K., Gottschalk, K., Mamet, R., Kametler, L., Bauer, J., Horn, P., Kovács, F., Kovács, M.: In vitro microbial metabolism of fumonisin B<sub>1</sub>. *Food Addit Contam.* 2007. 24. (4). 416-420 (**IF = 2,04**)

-Fodor, J., Balogh, K., Weber, M., Mézes, M., Kametler, L., Pósa, R., Mamet, R., Bauer, J., Horn, P., Kovács, F., Kovács, M.: Absorption, distribution and elimination of fumonisin B<sub>1</sub> metabolites in weaned piglets. *Food Additives and Contaminants*, 2008. 25(1):88-96 (**IF = 1,81**)

-Kovács, M., Kósa, E., Horn, P., Szendrő, Zs., Milisits, G.: Effect of a grain extract on certain digestive physiological parameters in early weaned rabbits. *Acta Vet. Brno*, 2009, 78: 379-386 (**IF = 0.40**)

-Pósa R, Kovács M, Donkó T, Szabó-Fodor J, Mondok J, Bogner P, Repa I, Magyar T: Effect of Mycoplasma hyopneumoniae and fumonisin B<sub>1</sub> toxin on the lung in pigs. *Ital J Anim Sci*, 2009. 8 (Suppl 3) 172-174 (**IF = 0.13**)

-Bónai, A., Szendrő, Zs., Matics, Zs., Fébel, H., Kametler, L., Tornyos, G., Horn, P., Kovács, F., Kovács, M.: Effect of inulin supplementation and age on growth performance and digestive physiological parameters in weaned rabbits. *World Rabbit Science*, 2010. 121-129 (**IF = 0.66**)

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-Mwanza M, Kametler, L., Bonai A., Rajli, V., Kovacs, M., Dutton, M.F.: The cytotoxic effects of fumonisin B<sub>1</sub> and ochratoxin A on human and pig lymphocytes using MTT assay. *Mycotoxin Research*, 2009. 25. 4. 233-238.

-Pósa, R., Donló, T., Bogner, P., Kovács, M., Repa, I., Magyar, T.: Interaction of *Bordetella bronchiseptica*, *Pasteurella multocida* and fumonisin B1 in the porcine respiratory tract followed up by computed tomography. *Can. J. Vet. Res.* 2011. 75. 176-183 (IF = 1.29)

-Kovács, M., Tornyos, G., Matics, Zs., Kametler, L., Rajli, V., Bodnár, Zs., Kulcsár, M., Huszenicza, Gy., Keresztes, Zs., Cseh, S.: Subsequent effect of subacute T-2 toxicosis on spermatozoa, seminal plasma and testosterone production in rabbits. *Animal*, 2011.10.1563-1569 (IF = 1.45)

**UNIVERSITY OF JOHANNESBURG.** The University of Johannesburg – UJ (the former Technikon Witwatersrand) traces its roots back to the beginning of the last century to the Transvaal Technical Institute, which was established in 1903 to serve the needs of the gold-mining industry. In 1923, an Act of Parliament made provision for technical training, which led to the establishment of the Witwatersrand Technical Institute in 1925. This was the founding date of the Technikon Witwatersrand. The Witwatersrand Technical Institute underwent a series of changes to become Technikon Witwatersrand (TWR) in 1979. Today, it is a large and complex institution. Above 12000 students and 1200 staff members from all spheres of multifaceted community make up a vibrant educational centre extending across the east-west axis of Johannesburg. The TWR has a well-established track record. Currently it is merged with the Rands Afrikaans University (RAU) and two Vista campuses formed the University of Johannesburg (UJ). The mission of UJ is to train and educate under- and post-graduate to a high degree of excellence in their chosen professions. As the technological arm of the newly constituted UJ its role will be to provide South Africa with applied scientists and technologists of the highest caliber. Within the present project, Food Environment and Health Research Group (FEHRG) and the departments of Environmental Health, Biotechnology and Food Science in UJ will be involved. Faculty of Health Sciences in partnership with the Department of Applied Chemistry (Faculty of Science) will be responsible for the general coordination of the project and will play a central function through the collaboration links with other units within UJ.

### **The experience in field of research.**

The current research situation at UJ can be illustrated by the high number of postgraduate (master's and doctoral) students (342), the high number of postgraduate research students (84) and the high number of active research and development projects (41). Various funds were obtained for equipment for the niche research area *Evaluation of environmental and occupational mutagens* from the Fuchs Foundation, Anglovaal Mining, Anglo American Chairman's Fund, the Goldfields Foundation, and the NRF, to all of whom we are grateful for their generous contributions. The acquisition of a Soft Image Analyser System will obviate tedious, manual data analyses by rapidly calculating results, and has the advantage that can be used by various research programmes across the faculties. The main current research is divided in the following scientific fields: *Nanomaterials technology* – focused on the synthesis of carbon-based nanomaterials and the search for new uses of these materials; *Minerals processing and technology* – researches a range of physico-chemical processing methods for minerals and new materials; *Evaluation of environmental and occupational mutagens* – which examines the effect of a range of mutagens at the cellular and epidemiological levels; *Design, artefactual production and economic development* – projects focus on the establishment of sustainable SMEs based on the manufacture of products emerging from the fine arts; *The technology of cereal, tuber and legume foods* – investigates the biotechnology and macro food properties of this range of food products. The above niche areas encompass about 75% of the research activities in UJ. Most of the remaining activities are encompassed in three further niche areas whose formal establishment is at various stages of development: *Management challenges in the technikon sector: Enhancing equity, quality and relevance through transformation* – examines possible interventions in transforming higher education institutions; *Knowledge management in public institutions* – will explore the use of Information and Communication Technologies in the public sector; *Culture and identity in South African art and design* – explores the interaction of these two aspects and its impact on the production of artefacts in the fine and creative arts.

The strategic partnership with the National Laser Centre (NLC), a new research body formed from an amalgamation of the laser divisions of the CSIR, Denel, and the Atomic Energy Corporation was strengthened through the establishment of a collaborative research programme that will explore the effect of Low Level Laser Therapy (LLLT) on living tissue. The project is part of the *Environmental and Occupational Mutagens* programme.

**Faculty of Health Sciences:** The faculty supports several research niches, including Food – cereals and legumes; water and health; mycotoxins and health; and environmental mutagens including laser technology. These niches have fully equipped laboratories to support their activities and supply postgraduate students. Current postgraduate student statistics include - M.Tech (51) and D.Tech (18). Faculty is made up of several departments that include Physics chemistry, Chemical Engineering and Applied Chemistry. At Applied Chemistry Dept where part of this project will be carried out, there is a centre of excellence in string materials and nanoscience as well as in water research. This department also has a strong analytical chemistry focus in improving detection limits for very low concentration compounds and elaborating new methods for sample analyses using HPLC LC-MS

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GCxGCTOF units, etc. It also has a natural products, drug discovery and drug delivery focus. Currently there are 47 Postgraduates (Master and PhD) studying within this department in addition to 7 post doctoral fellows.

### **Current Research projects related to this proposal:**

1) Project title: “Quantification of FUM1 gene of *Fusarium* spp., NOR-1 of *Aspergillus* spp. and mycotoxins in animal feeds from South Africa associated with animal health disorders” with Leader: Dr PB Njobeh  
National Research Foundation/University of Johannesburg two year post doctoral project

2) Project title: “Identification and characterisation of secondary metabolites with anti- tuberculosis activities from plant sources” with Leader: Dr Ndinteh Derek

Source of funding: National Research Foundation/University of Johannesburg three year post doctoral project

3) Project title: “Anti-bacterial secondary metabolites from alchronea species” with Leaders: Prof R Krause and Dr Ndinteh Derek

Source of funding: Univeristy of Johannesburg Research Council funding

4) Project title: “Synergistic effects of herbal samples from ethnobotanicval preparations” with Leaders: Prof R Krause and Dr Ndinteh Derek

Source of funding: Faculty of Science Research Council funding

5) Project title: “Acetylcholinestrase inhibitory compounds from selected African medicinal plants.” with Leaders: Prof R Krause and Dr Ndinteh Derek

Source of funding: Faculty of Science Research Council funding

6) Project title: “Anti-diabetic metabolites from erythrina species.” with Leaders: Prof R Krause and Dr N Derek  
Source of funding: Faculty of Science Research Council funding and Publications funds

7) Project title: “The prevention of *Candida* oral infection in HIV positive patients by selected South African herbal plant extracts.” with Leaders: Prof M F Dutton (UJ) and Professor M Patel (University of Witwatersrand)

Source of funding: South African MRC research funding

8) Project title: “Chemoprotective properties of imfino (herbal) plant extracts against aflatoxin B<sub>1</sub> carcinogenicity” with Project leader: Prof M F Dutton

Source of funding: University of Johannesburg URC funding

### **Some relevant and recent publications of the South African scientists involved in this project (see also the main common publications of the coordinator of the project – S. Stoev):**

-Njobeh PB, Dutton MF, Makun, HA, 2010. Chapter 6. Mycotoxins and human health: Significance, prevention and control. In: *Smart Biomolecules in Medicine*. Research Signpost/Transworld Research Network (Ed). VBRI Press, India. Pp 132-177 (IF=0.73).

-Mishra AK, Mishra SB, Tiwari AD, Mamba BB, Krause RW, Njobeh PB, Dutton MF and Kankeu EF, Synthesis, characterization and in-vitro antibacterial and antifungal studies of tin (IV) thiohydrazide complexes. *Journal of Coordination Chemistry*, 2011. 64 (20): 3622-3636 (IF=0.73).

-Reiter EV, Dutton MF, Mwanza M, Agus A, Prawano D, Häggblom P, Razzazi-Fazeli E, Zentek J, Andersson G, Njobeh PB, Quality Control of Sampling for Aflatoxins in Animal Feedingstuffs: Application of the Eurachem/CITAC guidelines. *Analyst*, 2011, 136 (19): 4059-4069 (IF=3.76).

-Makun HA, Dutton MF, Njobeh PB, Mulunda M, Kabiru AY, Natural multi- mycotoxin occurrence in rice from Niger State, Nigeria. *Mycotoxin Research*, 2011, 27: 97-104

-Njobeh, P.B., M.F. Dutton, A.A. Chuturgoon, S.H. Koch, P.A. Steenkamp, S.D. Stoev, Identification of novel metabolite and its cytotoxic effect on human lymphocyte cells in comparison to other mycotoxins. *International Journal of Biological and Chemical Sciences*, 2009, 3 (3), 524-531.

-Mwanza M, L. Kametler, A. Bonais, V. Rajli, M. Kovacs, and M. Dutton., The cytotoxic effect of fumonisin B<sub>1</sub> and ochratoxin A on human and pig lymphocytes using MTT assay. *Mycotoxin Research*, 2009, 25 (4) 233-238

-Njobeh, P. B., M. F. Dutton, S. H. Koch, A. A. Chuturgoon, S. D. Stoev, S. J. Mosonik, Simultaneous occurrence of mycotoxins in human food commodities from Cameroon, *Mycotoxin Research*, 2010, 26: 47-57.

Njobeh, P. B., M. F. Dutton, S. H. Koch, A. Chuturgoon, S. D. Stoev, K. Seifert. Contamination with storage fungi of human food from Cameroon. *International Journal of Food Microbiology*, 2009, 135, 193-198 **IF=2.8**

-Chelule P, Gqaleni N, Chuturgoon AA and Dutton MF. 2001. A study of the occurrence fumonisin B<sub>1</sub> in a rural community in Kwazulu Natal, South Africa. *Environmental Health Perspectives*. 109 253-256 (IF=6.12).

-Palanee, T, Dutton, M and Chuturgoon, A 2001. Cytotoxicity of aflatoxin B<sub>1</sub> and its chemically synthesised epoxide derivative on the A549 human epitheloid lung cell line. *Mycopathologia*. 150 155-159 (IF=1.65).

-Myburg RB, Dutton MF, and Chuturgoon AA. 2002. Cytotoxicity of Fumonisin B<sub>1</sub>, Diethylnitrosamine and Catechol on the SNO oesophageal cancer cell line. *Environmental Health Perspectives*. 110, 813-815 (IF=6.12).

-Dutton, M.F. 2003. Mycotoxins in South Africa. *Advances in Applied Microbiology* 53: 213-241 (IF=1.65).

-Olivier O.K., E.A. Shikanga, S. Combrinck, R.W.M. Krause, T. Regnier and T.P. Dlamini, South African Lippia herbal infusions: Total phenolic content, antioxidant and antibacterial activities, *South African Journal of Botany*, 2010, Volume 76(1), 58-63



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-Durbach SH, RWM Krause, MJ Witcomb, and NJ Coville, Synthesis of branched carbon nanotubes using copper catalysts in a hydrogen-filled DC arc-discharger. *Carbon* (2009), 47, 635–644 (IF = 4.515)

-Poumale H, Siwe X, Ntede H, Yoshihito Shiono, Ngadjui B, Krause R, Ndinteh D and Mbafor JT. Two New Fatty Acid Derivatives from the Stem Bark of *Alchornea laxiflora* (Euphorbiaceae) *J. Amer. Oil Chem. Soc.*, 2011.

-Nguyen P, Na M, Dao T, Ndinteh D, Mbafor J, Park J, Cheong H, Oh W. New stilbenoid with inhibitory activity on viral neuraminidases from *Erythrina addisoniae*. *Bioorg Med Chem Lett*. 2010 15:6430-4 (IF = 2.53)

-Nguyen PH, Nguyen TN, Kang KW, Ndinteh DT, Mbafor JT, Kim YR, Oh WK. Prenylated pterocarpanes as bacterial neuraminidase inhibitors. *Bioorg Med Chem*. 2010 May 1;18(9):3335-44 (IF = 3.07).

-Nguyen PH, Nguyen TN, Dao TT, Kang HW, Ndinteh DT, Mbafor JT, Oh WK. AMP-activated protein kinase (AMPK) activation by benzofurans and coumestans isolated from *Erythrina abyssinica*. *J Nat Prod*. 2010 Apr 23;73(4):598-602 (IF = 2.84).

-Cui L, Lee HS, Ndinteh DT, Mbafor JT, Kim YH, Le TV, Nguyen PH, Oh WK. New prenylated flavanones from *Erythrina abyssinica* with protein tyrosine phosphatase 1B (PTP1B) inhibitory activity. *Planta Med*. 2010 May;76(7):713-8 (IF = 1.96).

-Nguyen PH, Le TV, Thuong PT, Dao TT, Ndinteh DT, Mbafor JT, Kang KW, Oh WK. Cytotoxic and PTP1B inhibitory activities from *Erythrina abyssinica*. *Bioorg Med Chem Lett*. 2009 1;19(23):6745-9 (IF = 2.53)

-Dao TT, Nguyen PH, Thuong PT, Kang KW, Na M, Ndinteh DT, Mbafor JT, Oh WK. Pterocarpanes with inhibitory effects on protein tyrosine phosphatase 1B from *Erythrina lysistemon* Hutch. *Phytochemistry*. 2009 Dec;70(17-18):2053-7 (IF = 2.94)

-Cui L, Thuong PT, Lee HS, Ndinteh DT, Mbafor JT, Fomum ZT, Oh WK. Flavanones from the stem bark of *Erythrina abyssinica*. *Bioorg Med Chem*. 2008 Dec 15;16(24):10356-62 (IF = 3.07).

-Cui L, Ndinteh DT, Na M, Thuong PT, Silike-Muruumu J, Njamen D, Mbafor J, Fomum Z, Ahn JS, Oh WK. Isoprenylated flavonoids from the stem bark of *Erythrina abyssinica*. *J Nat Prod*. 2007;70(6):1039-42 (IF = 2.84).

### Some other research outputs:

-Njobeh PB, Dutton MF, Iheanacho H, Obiajili E. FUM1, NOR~1 transcript genes and mycotoxins in animal feeds from South Africa associated with animal health disorders. A paper presented at the 33<sup>rd</sup> Mycotoxin Workshop, Freising, Germany. 30<sup>th</sup> May-1<sup>st</sup> June, 2011.

-Njobeh PB. Mycotoxins Problem in South Africa: An updated overview. A lecture presented at the National Veterinary Institute (SVA), Uppsala, Sweden, May 20, 2011.

-Reiter EV, Dutton M, Njobeh P, Andersson G, Häggblom P, Zentek J, Dockner A, Razzazi-Fazeli E. Occurrence of aflatoxins in extracted cotton seed cake originating from South Africa. 31st Mycotoxin Workshop, Münster, GERMANY. June 15–17, 2009.

-Njobeh PB, Dutton MF, Koch SH, Stoev SD, Chuturgoon AA. Studies on mycotoxins in human food commodities from Cameroon. A presentation at the Annual Genetic Toxicology Association meeting. University of Delaware, Newark, USA. 10<sup>th</sup> to 11<sup>th</sup> of September, 2008. *Genetic Toxicology Association Newsletter* 32 (1) 14.

**DRDO (INDIA):** The Defence Research and Development Organisation (DRDO) is an agency of the Republic of India, responsible for the development of technology for use by the defence and civil population and is headquartered in New Delhi, India. It was formed in 1958 by the merger of Technical Development Establishment and the Directorate of Technical Development and Production with the Defence Science Organisation. DRDO has a network of 52 laboratories which are deeply engaged in developing defence technologies covering various fields, like aeronautics, armaments, electronic and computer sciences, human resource development, life sciences, materials, missiles, combat vehicles development and naval research and development. The organization includes more than 7,000 scientists and about 25,000 other scientific, technical and supporting personnel. The Annual Budget is approximately US\$ 2.0 Billion (2011-12). The area of interest in Life Sciences includes development of life support technologies to improve physiological efficiency. To enhance health and well-being of the soldiers even in extremes of environmental and operational conditions, building the human capital for the Armed Forces, development of novel food technologies (fresh and processed) to provide appropriate nutrition, strengthening man-machine interface through human factors engineering, enhancing the moral and motivation of troops.

### Experience of the Partner in International Collaboration

DRDO presently has MOU (Memorandum of understanding) with 33 countries, but the major partners are Russia, USA, UK, France, Germany, Hungary, Belarus, Singapore, Kazakhstan, Kyrgyzstan, etc. At Institute of Nuclear Medicine and Allied Sciences, we have had successful collaborations with Trakia University, Stara Zagora (Bulgaria) as a part of the Indo-Bulgarian Joint Research Project jointly funded by the Department of Science and Technology, Government of India and the Ministry of science and education in Republic of Bulgaria.

Currently we have **5 PhD students** who are working in the field related to the protective effects of herbs:

-Mr. Manish Adhikari, (Joined 25th August, 2008; Bharathiar University, Coimbatore); Title: Evaluation of Antioxidant and Radioprotective Efficacy of Some Novel Natural Products and Nature Identical Compounds/Extracts/Products.

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-Ms. Jyoti Sharma, (Joined 27th August, 2008; Bharathiar University, Coimbatore); Title: Ionizing Radiation-induced Biological and Behavioural Alterations and its Modulation by Natural Products.

-Mr. Atlar Singh Dhaker, Registered with Jamia Hamdard, New Delhi (DOJ: 25 October, 2007; Date of Registration: 11 Feb, 2009); Title: Bioprospection for Radioprotective Compounds from Some Natural Products.

-Mr. Ravinder Kumar Sagar, Currently: Lecturer, Acharya Narendra Dev College, University of Delhi, New Delhi, Registered at MAMC, Delhi (Jan, 2005 onwards); Title: Modulation of Gamma radiation-induced Damage to Mammalian Hemopoietic System by certain Herbal Preparations.

-Ms. Shikha Singh, Registered with Jamia Hamdard University, New Delhi in the Department of Medical Elementology and Toxicology (May, 2005 onwards); Title: In vitro/In vivo Studies on Some Effective and Non-toxic Herbal Radioprotectors.

In addition we also have **many M.Tech/ M.Sc/ B.Tech students** who are working in the same field.

Also we have a **product patent** (Goel, H.C., Arora R, J. Prasad, AK Sharma, S Singh, TL Mathew, OP Chaurasia, B Singh, A Radioprotective Herbal Extract. Indian Patent no. 192177 dated 11.7. 2005) in the field of herbal protection in addition to some more **process patents** as follow:

-Goel, H.C., Arora R, J. Prasad, AK Sharma, S Singh, TL Mathew, OP Chaurasia, B Singh, A Process for Preparation of a Radioprotective Herbal Extract-I. Indian Patents (no. 192177 and 191821) dated 11.7. 2005

-Goel, H.C., Arora R, V Shobi, TL Mathew A Process for Preparation of a Behavioural Radioprotective Herbal Extract. Indian Patent (no. 194325) dated 30.1. 2006

-Goel, H.C., Arora R, A.K. Sinha, B.P. Joshi, J. Prasad, S Singh, I Prem Kumar, R Sagar, CA Salin, PK Agarwala, H Prakash, D Gupta, AK Sharma, A Mittal, V Pathania, TL Mathew, B Singh A Process for Preparation of a Radioprotective Herbal Extract from *Hippophae rhamnoides*. Indian Patent (no. 192060) dated 07.4.2006

-Goel, H.C., IP Kumar, N Samanta, Arora R A Process for Preparation of an Anticancer and Radioprotective Herbal Extract. Patent application filed with The Controller of Patents, Indian Patents Office, Delhi, India. Indian Patent (no. 191747) dated 17.10.2006

-Goel, H.C., Arora R, A.K. Sinha, R Dogra, J. Prasad, S Singh, R Sagar, I Prem Kumar, PK Agarwala, AK Sharma, N Samanta, D Gupta, H Prakash, CA Salin, S Saji Kumar, A Mittal, V Pathania A Process for Preparation of a Radioprotective Herbal Extract from *Tinospora*. Indian Patent (no. 195062) dated 17.3.2006

-Goel HC, Arora R, Prasad J, Singh S, Sharma AK, Bala M, Sawhney RC. A process for preparation of a radioprotective herbal extract from *Rhodiola species*. Indian Patent dated 17.3.2006

### **Some relevant and recent publications of the Indian scientists involved in this project:**

-Karamalakova Y, Ionkova, Nikolova G, Hadjibojeva P, Tolekova A, Gadjeva V, Arora R, Sharma R and Zheleva. (2010) Comparative EPR in vitro and ex vivo spectroscopy study of the levels of lipid peroxidation processes in livers and kidneys of mice after treatment by naturally isolated antioxidants. *Trakia J Sci* 8: 137-143.

-Arora R, Dhaker A, Sharma J, Adhikari M, Chawla R, Gupta D, Zheleva A, Karamalakova Y, Kumar R, Sharma A, S Sultana, Sharma R, Gadjeva V. (2011) Radiomodulatory Effects of Extract of *Psoralea corylifolia* Linn. Evaluated by *in vitro* Assays and EPR Spectroscopy. *Z Naturforschung C* 66(1-2), 35-46 (**IF=0,72**)

-Singh P, Kumar R, Sharma A, Arora R, Chawla R, Jain S and R Sharma (2009) *Podophyllum hexandrum* fraction (REC-2006) shows higher radioprotective efficacy in p53 carrying hepatoma cell line: A role of cell cycle regulatory proteins. *Integrative Cancer Therapies*, 8(3), 261-272. (**IF = 2.26**)

-Arora R, Sagar RK, Singh S, Kumar R, Singh S, Prasad J, Puri SC, Qazi GN, Krishan Bal, Sharma RK, Tripathi RP (2007) Hemoprotective effect of *Podophyllum hexandrum* against radiation: Role of heme oxygenase-1 and BCL-2 *Integrative Cancer Therapies* 6 (1): 54-65. (**IF = 2.26**)

-Chawla R, Arora R, Singh S, Sagar R K, Sharma R K, Kumar R, Sharma A, Gupta M L, Singh S, J Prasad, Khan H A, Swaroop A, Sinha AK, Gupta AK, Tripathi R P, Ahuja PS (2007) Radioprotective and Antioxidant Activity of Fractionated Extracts of Berries of *Hippophae rhamnoides*. *J Med Food* 10(1):101-109 (**IF = 1.28**).

-Chawla R, Arora R, Singh S, Sagar RK, Sharma RK, Kumar R, Sharma A, Tripathi RP, Puri SC, Khan HA, Shawl AS, Sultan PS, Krishen T, Qazi GN (2006) *Podophyllum hexandrum* offers radioprotection by modulating free radical flux: Role of aryl-tetralin lignans. *Evid-Based Compl Alt Med* 3(4)503-511. (**IF = 1.954**)

-Arora R, Gupta D, Chawla R, Prasad J, Singh S, Sharma AK, Kumar R, Sagar RK, Samanta N, Sharma RK (2005) Radioprotection by Plant Products: Present Status and Future Prospects. *Phytother Res* 19: 1-22 (**IF = 1.77**)

-Arora R, Chawla R, Prasad J, Singh S, Kumar R, Sharma A, Sagar, Singh S, (2005) Radioprotective activity of *Rhodiola imbricata*-a high altitude medicinal plant. *Mol Cell Biochem* 273: 209-223 (**IF = 2.16**)

-Arora R, Chawla R, Puri SC, Sagar RK, Singh S, Kumar R, Sharma AK, Prasad J, Singh S, Kaur G, Chaudhary P, Qazi GN, Sharma RK (2005) Radioprotective activity of Low altitude *Podophyllum hexandrum*. *J Environ Pathol Tox* 24 (4): 299-314 (**IF = 1.56**).

-Chawla R, Arora R, Kumar R, Sharma AK, Prasad J, Singh S, Sagar RK, Chaudhary P, Shukla S, Kaur G, Sharma RK, Puri SC, Handa G, Gupta VK, Qazi GN (2005) Antioxidant activities of fractionated extracts of High Altitude *Podophyllum hexandrum* in radiation protection. *Mol Cell Biochem* 273: 193-208. (**IF = 2.16**)

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- Gupta D, Arora R, Garg AP and Goel HC (2003) Radiation Protection of HepG2 cells by *Podophyllum hexandrum* Royle, **Mol Cell Biochem** 247: 25-40 (IF = 2.16)
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- Arora R, Malhotra P, Sundriyal S, Yashavanth HS, Pai RJ and Baliga MS (2011) Medicinal plants as remedies for gastrointestinal ailments and diseases: a review. **Bioactive Foods and Chronic Disease States**. (ed. R Watson), Elsevier, The Netherlands
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- Karamalakova Y, Sharma J, Zheleva A, Arora R, Sharma R, Gadjeva V. (2010) Comparative evaluation on antioxidant properties of Bulgarian and Indian Essential Oils by two different methods. **Adv BioTech** 10(5), 124.

### **Current Research projects related to this proposal:**

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- 1) Project title: “Development of Herbal Radioprotectors for Human Applications” with Group leader: R Arora  
Source of funding: Rs. 11.2 million; Department of Defence Research and Development
- 2) Project title: “EPR Studies on Some Bulgarian and Indian Medicinal Plants with Anticancer and Radioprotective Activity” with Co-investigator: Rajesh Arora  
Source of funding: Trakia University / Bulgarian Ministry of science
- 3) Project title: “Evaluation of Some Bulgarian and Indian Medicinal Plants for Alleviation of Anticancer Drug and Radiation-induced Hepatotoxicity” with Co-investigator: Rajesh Arora  
Source of funding: Trakia University / Bulgarian Ministry of science
- 4) Project title: “Studies on Antioxidants against Oxidative Stress induced by Drugs and Radiation” with Coordinator from Indian side: Rajesh Arora  
Source of funding: Indo-Bulgarian Inter-Governmental Program of Co-operation in Science and Technology

### **B 1.3 COMPLEMENTARITIES/SYNERGIES BETWEEN THE PARTNERS**

We hope the project will finally make it possible to specify some suitable and cheap preventive measures, which could be undertaken in order to **reduce economic loss due to the morbidity from secondary bacterial infections** (Stoev et al, 2000b) **as a consequence of the increased susceptibility to infection and inflammation in the intestine accompanying mycotoxic nephropathies in Bulgaria** (Stoev et al, 2000a,b) **or similar mycotoxicoses in India** (Shetty and Bhat, 1997; Jindal et al, 1999; Chatterjee and Mukherjee 1994), **South Africa** (Stoev et al, 2010b) **or Hungary** (Sandor et al, 1982).

By using the present collaboration some new important knowledge will be received on the required subjects: in the field of **mycotoxin action and its prevention**, in the field of **undertaking some preventive measures against immunosuppressive or cytotoxic effects** (Stoev et al, 2009) **of mycotoxins** as well as in the field of **practical use of some herbal mixtures in appropriate constituents to improve wound granulation**. The further **introduction of the transferred methods/knowledge through the applicants in their institutions** will subsequently **improve their research experience and increase their levels of education**. It is worth to mention the fact that those to be involved in the characterization of active herbal plant extracts at the University of Johannesburg are postgraduates from S Africa and Hungary and as such, **transfer of knowledge to these future researchers** is a major attribute of the projects highlighted herein as **set-out of future joint research projects**.

The required transfer of knowledge to be carried out in the framework of this proposal will bring valuable benefits at all participating institutions/countries. By allowing Indian researchers to **exchange some target knowledge and expertise in the field of mycotoxicoses and their prevention by using cheap local herbs**, in addition to the new knowledge about the possible bioconstituents of some well known Indian herbs, a **significant progress will be made in prevention of some problematic diseases (mycotoxicoses)** as well as in a **safely utilization of mycotoxin-contaminated feeds**. Moreover, by using the acquired new knowledge about the possible mechanisms of protective effects of some known Himalayan or South African herbs, a further explanation of their healing effect on various animal or human will be achieved.

By extending their expertise on the creation of research labs for biocharacterization of herbs and by sharing the technology for preparation and stabilization of herbal extracts, Bulgarian collaborators will prepare the Indian collaborators to manage with the growing demands of the herbal medicine.

The expected results will not only address the issues on the **prevention of farm losses due to animal/poultry mycotoxicoses in India, South Africa, Hungary or Bulgaria**, but will also provide a scope for development of herbal products aimed at **partial protection against human ailments as a result of kidney or liver diseases or at improving human immune response**. These valuable results only could be achieved by **exchange of some local knowledge as follow**: 1.) the knowledge of Indian and South African collaborators of various known healing or protective effects against renal or kidney diseases of some local herbs in India or South Africa and their specific use by Himalayan or South African tribes as immunostimulants or in stimulation of wound granulation as well as some knowledge about the adverse effects of some target mycotoxins; 2) the knowledge of Bulgarian and South African collaborators about technology for preparation and stabilization of herbal extracts and identification of herbal bioconstituents and their mechanism of action; 3.) the knowledge of Bulgarian collaborators about the technology of preparing of some herbal sprays or unguents which are going to be used in animal experiments and subsequently for commercial purposes as well as the knowledge of various adverse and immunosuppressive effects of some target mycotoxins in animals/humans; 4) the knowledge of South African collaborators about the technology of mycotoxin production by various fungal species and the specific conditions and laboratory equipment for such mycotoxin production in large quantity; 5) the knowledge and experimental base of Hungarian collaborators necessary for production of various animal experiments/studies, in addition to the knowledge and facilities for both *in vitro* or *in vivo* testing of the interaction between herbal extract – mycotoxin – gut microbiota, the knowledge and facilities about metabolism, absorption and elimination of mycotoxins, cytotoxicity tests as MTT assay or Comet assay, etc.

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By using the skills and knowledge acquired during the implementation of this project, a further **increasing of feed/food safety and appropriate means of food utilization of mycotoxin-contaminated feeds/foods will be achieved and some new herbal products/medicines will be elaborated via mutual efforts and exchange of some target knowledge between the participants.**

### B 2 TRANSFER OF KNOWLEDGE

#### B 2.1 QUALITY AND MUTUAL BENEFIT OF THE TRANSFER OF KNOWLEDGE

##### PROGRAMME FOR TRANSFER OF KNOWLEDGE BETWEEN PARTNERS

<b>1<sup>st</sup> year Time Schedule (04.2013-03.2014)-</b>	
<b>Bulgarian programme</b>	<p>-To receive some knowledge from Indian and South African scientists in regard to <b>identifying, collecting and preserving all necessary herbs</b> (leaves, barks, seeds, fruits or roots) in different stages of their growth known to have strong diuretic or protective effects on liver and kidneys as well as immunostimulating or antibacterial effects, etc.</p> <p>-To transfer some knowledge to Indian and South African scientists about <b>selective characterization of some Himalayan herbs for their bioconstituents</b> (as the levels of flavonoids, carotenoids, etc) using EPR or NIRS in order to explain the mechanism of their antioxidative and protective effects on kidneys and liver or their immunostimulating or antibacterial effects and to <b>prepare some extracts or fractions</b> with the aim of exploring their protective abilities on chicks and rats in this regard.</p> <p>-To receive some knowledge from South African scientists in regard to <b>producing enough quantity of fumonisin B1 (FB1)</b> using some producers of <i>Fusarium verticillioides</i>.</p> <p>-To transfer some knowledge to South African scientists for <b>producing of enough quantity of ochratoxin A (OTA)</b> using some appropriate fungal species as various strains of <i>A. ochraceus</i></p> <p>-To transfer some knowledge to Indian scientists in regard to exploring <b>in vitro antioxidant properties</b> or <b>ex vivo protective abilities</b> of some herbal extracts or fractions <b>against oxidative stress</b> caused by proper xenobiotics using EPR spectroscopy.</p> <p>-To receive some knowledge from South African scientists about <b>selective characterization of some South African herbs or herbal products for their bioconstituents using some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc.</b></p> <p>-Some <b>educational courses or workshop</b> will be organized in Bulgaria in this regard.</p>
<b>Hungarian programme</b>	<p>-To receive some knowledge from South African scientists in regard to <b>identifying, collecting and preserving all necessary herbs</b> (leaves, barks, seeds, fruits or roots) in different stages of their growth known to have strong diuretic or protective effects on liver and kidneys as well as immunostimulating or antibacterial effects, etc.</p> <p>-To receive some knowledge from South African scientists about <b>selective characterization of some South African herbs or herbal products for their bioconstituents using some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc.</b></p> <p>-A <b>presentation/workshop</b> will be attended in South Africa in this regard.</p>
<b>South African programme</b>	<p>-To transfer some knowledge to Bulgarian and Hungarian scientists in regard to <b>identifying, collecting and preserving all necessary herbs</b> (leaves, barks, seeds, fruits or roots) in different stages of their growth known to have strong diuretic or protective effects on liver and kidneys as well as immunostimulating or antibacterial effects, etc.</p> <p>-To receive some knowledge from Bulgarian scientists about <b>selective characterization of some Himalayan herbs for their bioconstituents</b> (as the levels of flavonoids, carotenoids, etc) using EPR or NIRS in order to explain the mechanism of their antioxidative and protective effects on kidneys and liver or their immunostimulating or antibacterial effects.</p> <p>-To transfer some knowledge to Bulgarian scientists in regard to <b>producing enough quantity of fumonisin B1 (FB1)</b> using some appropriate producers of <i>Fusarium verticillioides</i></p> <p>-To transfer some knowledge to Bulgarian and Hungarian scientists about <b>selective characterization of some South African herbs or herbal products for their bioconstituents using some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc., and to prepare some extracts or fractions</b> with the aim of exploring their protective abilities on pigs in this regard</p> <p>-To receive some knowledge from Bulgarian scientists in regard to <b>producing of enough quantity of ochratoxin A (OTA)</b> using some appropriate fungal species of <i>A. ochraceus</i>.</p> <p>-Some <b>educational courses or workshop</b> will be organized in South Africa in this regard</p>
<b>Indian programme</b>	<p>-To transfer some knowledge to Bulgarian scientists in regard to <b>identifying, collecting and preserving all necessary herbs</b> (leaves, barks, seeds, fruits or roots) in different stages of their growth known to have strong diuretic or protective effects on liver and kidneys as well as</p>

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	<p>immunostimulating or antibacterial effects, etc.</p> <p>-To receive some knowledge from Bulgarian scientists about <b>selective characterization of some Himalayan herbs for their bioconstituents</b> (as the levels of flavonoids, carotenoids, etc) using EPR or NIRS in order to explain the mechanism of their antioxidative and protective effects on kidneys and liver or their immunostimulating or antibacterial effects and to help in <b>preparing of some extracts or fractions</b> with the aim of exploring their protective abilities on chicks and rats.</p> <p>-To receive some knowledge from Bulgarian scientists in regard to exploring <b>in vitro antioxidant properties</b> or <b>ex vivo protective abilities</b> of some herbal extracts or fractions <b>against oxidative stress</b> caused by proper xenobiotics using EPR spectroscopy.</p> <p>-A <b>presentation/workshop</b> will be given by Indian scientists in this regard.</p>
<b>2<sup>nd</sup> year Time Schedule (04.2014-03.2015)-</b>	
<b>Bulgarian programme</b>	<p>-To exchange some knowledge with Indian scientists via exchanging of target scientists from both countries in regard to preparing some <b>unguents or sprays</b> using appropriate constituents and some target herbs or extract of herbs (or herbal mixtures) in order to facilitate the using of the same herbs in the target studies <b>for stimulation of wound granulation</b>.</p> <p>-To receive some knowledge from Indian scientists about some target herbs as <i>Hippophae rhamnoides</i> (<i>Seabuckthorn leaf</i> extracts) in appropriate stage of their growth, known to have stimulating effects on wound granulation.</p> <p>-A <b>presentation/workshop</b> will be given by Bulgarian scientists in this regard.</p>
<b>Hungarian programme</b>	<p>-To transfer some knowledge to S African scientists in regard to exploring the <b>protective effects of some herbs or herbal extracts against toxicity of FB1/OTA in pigs or rabbits via in vivo and in vitro</b> experiments on the interaction between mycotoxins – herbal extract – gut microbiota.</p> <p>-To transfer some knowledge to South African scientists in regard to <b>protective role of target plant extracts against the cytotoxic effects of FB1 and/or OTA</b> on pig lymphocyte and/or intestinal cells <b>using some in vitro cytotoxicity tests as MTT assay and Comet assay</b>.</p> <p>-Some <b>educational courses or workshop</b> will be organized in Hungary in this regard.</p>
<b>South African programme</b>	<p>-To receive some knowledge from Bulgarian scientists in regard to preparing some <b>herbal unguents or sprays</b> using appropriate constituents and some target herbs or extract of herbs (or herbal mixtures), which could be used <b>for stimulation of wound granulation</b>.</p> <p>-To receive some knowledge from Hungarian scientists in regard to exploring <b>protective effects of some herbs or herbal extracts against toxicity of FB1/OTA in pigs or rabbits via in vivo and in vitro</b> experiments on the interaction between mycotoxins – herbal extract – gut microbiota.</p> <p>-To receive some knowledge from Hungarian scientists in regard to <b>protective role of target plant extracts against the cytotoxic effects of FB1 and/or OTA</b> on pig lymphocyte and/or intestinal cells <b>using various in vitro cytotoxicity tests as MTT assay and Comet assay</b>.</p> <p>-A <b>presentation/workshops</b> will be attended in Hungary or Bulgaria in this regard</p>
<b>Indian programme</b>	<p>-To exchange some knowledge with Bulgarian scientists via exchanging of target scientists from both countries in regard to preparing some <b>unguents or sprays</b> using appropriate constituents and some target herbs or extract of herbs (or herbal mixtures) in order to facilitate the using of the same herbs in the target studies <b>for stimulation of wound granulation</b>.</p> <p>-To transfer some knowledge to Bulgarian scientists and to supply with some target herbs as <i>Hippophae rhamnoides</i> (<i>Seabuckthorn leaf</i> extracts) in appropriate stage of their growth, known to have stimulating effects on wound granulation.</p> <p>-A <b>presentation/workshop</b> will be given/attended in Bulgaria/India in this regard.</p>
<b>3<sup>rd</sup> year Time Schedule (04.2015-03.2016)-</b>	
<b>Bulgarian programme</b>	<p>-To transfer some knowledge to Indian scientists in regard to exploring the stimulating effects of various herbal mixtures on <b>wound granulation in animals</b> at Department of Surgery in Faculty of Veterinary Medicine of Trakia University via using some herbal sprays or unguents prepared in advance via exchanging of knowledge between the scientists from both countries.</p> <p>-A <b>presentation/workshop</b> will be given by Bulgarian scientists in this regard.</p>
<b>Indian programme</b>	<p>-To receive some knowledge from Bulgarian scientists in regard to exploring the stimulating effects of various herbal mixtures on <b>wound granulation in animals</b> at Department of Surgery in Faculty of Veterinary Medicine of Trakia University via using some herbal sprays or unguents prepared in advance via exchanging of knowledge between the scientists from both countries.</p> <p>-A <b>presentation/workshop</b> will be attended by Indian scientists in this regard.</p>
<b>4<sup>th</sup> year Time Schedule (04.2016-12.2016)-</b>	
<b>Bulgarian programme</b>	<p>-To transfer some knowledge to Indian scientists in regard to exploring the <b>protective effects of some herbs or herbal extracts given as feed additives against toxic effects of some target</b></p>

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	<p><b>mycotoxins (OTA and/or FB1) on various internal organs, body weight gain and humoral immune response against Newcastle disease in Plymouth Rock chicks in Bulgaria.</b></p> <p>-To transfer some knowledge to Indian scientists in regard to <b>pathomorphological and biochemical investigations</b> performed in the same experiments in order to appreciate the real protective effects of various herbs or herbal extracts on kidney, liver, brain, cerebellum, lung and immunocompetent organs as bursa of Fabricius, thymus and spleen of the respective chicks.</p> <p>-To receive some knowledge from Indian scientists in regard to exploring the <b>protective effects of some herbs or herbal extracts given as feed additives against toxic effects of target mycotoxins (OTA or FB1)</b> on kidney, liver, brain, lung and spleen <b>in Swiss albino rats</b> in India</p> <p>-To receive some knowledge from Indian scientists in regard to <b>pathomorphological and biochemical investigations</b> performed in the above experiment in order to appreciate the real protective effects of various herbs or herbal extracts on internal organs of rats.</p> <p>-A <b>presentation/workshop</b> will be given/attended by Bulgarian/Indian scientists in this regard.</p>
<p><b>Indian programme</b></p>	<p>-To transfer some knowledge to Bulgarian scientists in regard to exploring the <b>protective effects of some herbs or herbal extracts given as feed additives against toxic effects of some target mycotoxins (OTA or FB1)</b> on kidney, liver, brain, lung and spleen <b>in Swiss albino rats</b> in India.</p> <p>-To transfer some knowledge to Bulgarian scientists in regard to <b>pathomorphological and biochemical investigations</b> performed in the above experiment in order to appreciate the real protective effects of various herbs or herbal extracts on internal organs of rats.</p> <p>-To receive some knowledge from Bulgarian scientists in regard to exploring the <b>protective effects of some herbs or herbal extracts given as feed additives against toxic effects of some target mycotoxins (OTA and/or FB1) on various internal organs, body weight gain and humoral immune response against Newcastle disease in Plymouth Rock chicks in Bulgaria.</b></p> <p>-To receive some knowledge from Bulgarian scientists in regard to <b>pathomorphological and biochemical investigations</b> performed in the same experiments in order to appreciate the real protective effects of various herbs or herbal extracts on kidney, liver, brain, cerebellum, lung and immunocompetent organs as bursa of Fabricius, thymus and spleen of the respective chicks.</p> <p>-A <b>presentation/workshop</b> will be given/attended by Bulgarian/Indian scientists in this regard.</p>

**THE MAIN ADDED VALUE (IN TERMS OF GAINED KNOWLEDGE) FOR VARIOUS PARTNERS.** The present exchange programme will help the partner institutions to achieve their own objectives via collaborative efforts and transfer of target information and knowledge. In such a way, new knowledge will be achieved in the field of **the appropriate preventive measures against the adverse (toxic or immunosuppressive) effects of mycotoxins** as well as in **the possible manners of practical use of some cheap herbal mixtures supporting wound granulation.** In addition, a later introduction of the new methods/knowledge through the applicants in their institutions (Trakia University in Bulgaria; DRDO in India, Kaposvar University in Hungary and University of Johannesburg in South Africa) **by including them in the teaching process for students (including PhD students), will strengthen their levels of education and research experience.**

In conclusion, it is worth mentioning that the transfer of knowledge to be carried out in the framework of this proposal will bring benefits at all participating countries: Bulgaria, India, Hungary and South Africa. By giving to the Indian and South African applicants the opportunity to exchange some knowledge and expertise in the field of **mycotoxin action and possible cheap preventive measures using the local herbs** and by **identifying the biocontent of the same herbs**, a significant contribution will be made to the respective country's efforts to **combat various mycotoxicoses and safely use mycotoxin-contaminated feeds** (Stoey, 2007), in addition to better understanding the mechanism of protection of various known Himalayan or South African herbs and their healing effect on various animal or human wounds.

In addition to **extending the expertise on the establishment of research labs for biocharacterization of herbs**, Bulgarian and South African collaborators will **share the technology for preparation and stabilization of herbal extracts.** On the other hand, Bulgarian and Hungarian collaborators will also share **the applied knowledge on designing the experimental work plans** via many *in vivo* or/and *in vitro* experiments (including the experiments on the interaction between mycotoxins – herbal extract – gut microbiota) in order to prepare the Indian and South African collaborators to meet the challenges of the growing demands of the herbal medicine industry. **The acquired knowledge will also provide a scope for development of herbal products aimed at partial protection against human ailments as a result of kidney or liver diseases or at improving human immune response in addition to wound granulation.**

On the other hand, this project could further contribute to **increasing of feed/food safety and finding appropriate means to utilize mycotoxin-contaminated feeds/foods.**

Finally, some new manners of herbal protection (in the form of feed supplements to the feeds) against the action of target mycotoxins will be elaborated, in addition to developing some herbal products useful for

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wound granulation, immunostimulation, kidney or liver diseases. Some joint scientific papers will be also elaborated and published in peer reviewed international journals as well as some presentations/seminars will be given by target leading scientists in the respective countries in this regard.

Any outcome of the project if comes out in the form of commercializable product (s) or joint patent technology in the international market, generated in Bulgaria, Hungary, South Africa or India, will be shared with each other country's partners using the principle of equality in order to make it a successful and mutually profitable research network.

### B 2.2 ADEQUACY AND ROLE OF STAFF EXCHANGED WITH RESPECT TO THE TRANSFER OF KNOWLEDGE THE ROLE OF THE EXCHANGED RESEARCHERS AND GOALS TO BE ACHIEVED:

WP №	Work package title	Beneficiary/Partner organisation short name	Exchanged researchers and their specific role in the Work package	Goals to be achieved through exchange of researchers (see also chapter B1.2 Research quality of the partners)
1	FB1 production  OTA production	TU (Bulgaria)  UJ (South Africa)	Prof S Stoev (Bg) Prof S Denev (Bg)  Dr P. Njobeh (SA) Dr M Mulunda (SA)	The same researchers have vast knowledge in the production of target mycotoxins OTA (BG researchers and FB1 (SA researcher) – see chapter B1.2 Research quality of the partners. This will allow a fruitful exchange of their knowledge in this regard and performing some target experiments.
2	Herbs collection from India and South Africa	TU (Bulgaria)  UNIKAPOS (Hungary)	Prof D Pavlov (Bg) Prof I Zarkov (Bg) Prof. S Stoev (Bg)  Prof M Kovac (Hg)	Some suitable herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects will be collected via the help of Dr R Arora (DRDO, India) and Prof R Krause (S. Africa). A short training will be performed of Bulgarian and Hungarian collaborators in order to receive target skills on various herbs, their identification and healing effects on animals and humans.
3	Selective characterization of some herbs from India and South Africa for their bioconstituents and preparing some target herbal extracts or fractions	DRDO (India)  UJ (South Africa)  TU (Bulgaria)  UNIKAPOS (Hungary)	Dr R Arora (Ind)  Prof R Krause (SA) Dr D Ndinteh (SA) Youmbi Thierry (SA)  Prof V Gadjeva (Bg) Doc A Zheleva (Bg) Doc T Mircheva (Bg)  András Bónai (Hg) Viola Vántus (Hg)	Most of the same researchers have target knowledge in this field and additional transfer of knowledge will be realized in Bulgaria via the help of Prof D Pavlov and Assoc Prof A Zheleva in the field of selective characterization of some Himalayan herbs for their bioconstituents (as the levels of flavonoids, carotenoids, etc) using some specific techniques as EPR and NIRS 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. Similar research activities and knowledge exchange will be performed in UJ (S Africa) by the guidance of Prof Krause using some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc



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4	Elaboration of mixtures of target herbal extracts for preparing of some sprays or unguents designed for stimulation of wound granulation	DRDO (India)  TU (Bulgaria)  UJ (South Africa)	Dr R Arora (Ind) Dr A Gupta (Ind)  Doc Mutafcieva (Bg) Prof I Borissov (Bg)  Prof M Dutton (SA)	These researchers have some specific knowledge in this field and additional transfer of knowledge will be realized in Bulgaria and India in order to prepare suitable herbal extracts using appropriate constituents, which will be further tested for their stimulating effects on wound granulation and appropriate unguents or sprays will be further elaborated.
5	Investigation on stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation	DRDO (India)	Dr R Arora (Ind)	The same researcher has some target knowledge in this field and additional transfer or exchange of knowledge will be realized in Bulgaria under the guidance of Prof I Borissov in Dept of Surgery via exploring the previously prepared herbal extracts (unguents or sprays) for their stimulating effects on wound granulation in animals.
6	Pig experiment ( <i>in vivo</i> and <i>in vitro</i> ) on protective effects of herbal additives against toxicity of OTA or/and FB1	UJ (South Africa)	Dr P Njobeh (SA) Dr M Mulunda (SA) Fosso-Kankeu (SA)	These researchers have been working in the same field since a long time and some additional knowledge in the designed field of investigation ( <i>in vivo</i> and <i>in vitro</i> experiments, including MTT assay or Comet assay, on the protective effects of some target herbal extract against mycotoxins and the respective interaction between mycotoxins – target herbal extract – gut microbiota) in pigs/rabbits will further strengthen their professional competences. The target investigations will be done in UNIKAPOS (Hungary) using the help of Dr Attila Zsolnai (responsible for the molecular determination of gut microbiota), András Bónai (responsible for culturing techniques), Dr Katalin Horvatovich, (responsible for <i>in vitro</i> testing
7	Chick and rats experiment on protective effects of herbal additives against toxic and immunosuppressive effects of OTA or/and FB1	DRDO (India)  TU (Bulgaria)	Dr. A Gupta, (Ind)  Prof S Stoev (Bg)	The same researchers have many publications in the same field and further experience in regard to the studies of target herbal extracts for their protective effects against toxic and immunosuppressive effects of mycotoxins OTA and FB1 in chicks or rats, and respective exchange of knowledge will strengthen significantly their professional competencies in the desired fields.

The planned transfer or exchange of knowledge will make possible **defining some cheap preventive measures against immunosuppressive or toxic effects of mycotoxins and practical use of some herbal mixtures (elaborated during the project) in healing of wound, which will be introduced by the respective researchers in their own countries of origin.**

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By allowing Indian and South African applicants the opportunity to exchange some target knowledge and expertise in the field of mycotoxicoses and their prevention using some widely spread local herbs and by identifying the biocontent of the same herbs, a significant help will be made to the career development of all researchers and the leading world experts involved in this program.

By exchanging some target knowledge on the establishment of research labs for biocharacterization of herbs and sharing the leading technologies for preparation and stabilization of herbal extracts Indian, Bulgarian, Hungarian and South African collaborators will be able to meet the recent challenges of the growing demands of the herbal medicine industry.

This project will also improve the existing knowledge on feed/food safety in regard to mycotoxicoses and will allow finding of appropriate means to utilize mycotoxin-contaminated feeds/foods.

The acquired competencies and skills (via a series of *in vivo* and *in vitro* studies) by the scientists from involved countries will allow them to undertake some new measures in regard to prevention of farm losses due to animal/poultry mycotoxicoses in India, South Africa, Hungary or Bulgaria as well as to develop of some new herbal products aimed at partial protection against human ailments as a result of kidney or liver diseases or at improving human immune response as a whole.

### B 3 IMPLEMENTATION

#### B 3.1 CAPACITIES (EXPERTISE/HUMAN RESOURCES/FACILITIES/INFRASTRUCTURE) TO ACHIEVE THE OBJECTIVES OF THE PLANNED COOPERATION

THE HUMAN RESOURCES and HOST EXPERTISE of each collaborator in training experienced researchers in the designed field and its capacity to provide mentoring/tutoring is vast and well developed as can be seen in chapter B 1.2 RESEARCH QUALITY OF THE PARTNERS and chapter B3.2 – see Practical and administrative.....

#### INFRASTRUCTURE/FACILITY NECESSARY FOR PROJECT IMPLEMENTATION IN TU (BULGARIA)

Within the present project, several departments of Faculty of Veterinary Medicine, Faculty of Agriculture and Faculty of Medicine of TU are involved in addition to the experimental base of the same faculties.

a) *Experimental base.* A suitable experimental base for all kind of animals (pigs, chickens, mice) is available for all kind of experiments planned by applicants.

b) *Department of General and clinical pathology.* This unit is fully equipped for pathological studies and slides preparing for microscopic investigations. Also, laboratory staff is available for the needs of applicant. The visiting researchers will be associated to the work carried out in the department under the supervision of Prof. Stoev.

c) *Laboratory of Electron microscopy.* Two laboratories of Electron microscopies are available in Faculty of Veterinary Medicine and in Faculty of Medicine for the needs of applicant. All necessary chemicals are available.

d) *Department of Veterinary surgery.* This unit is fully equipped for surgical interventions/manipulations and for investigation on stimulating effect of the prepared herbal extracts (unguents or sprays) on wound granulation. The visiting researchers will be associated to the work carried out under the supervision of Prof. I. Borisov

e) *Department of Veterinary Biochemistry of Faculty of Vet Medicine.* This unit is fully equipped for biochemical investigations of serum sample from animals/chicks. A biochemical laboratory of this unit is fully equipped for performing such investigations. The visiting researchers will be associated to the work carried out in the department under the supervision of Assoc Prof. T. Mircheva

f) *Department of Chemistry and Biochemistry of Faculty of Medicine.* This unit is fully equipped for selective characterization of some Himalayan herbs for their bioconstituents and preparing some target herbal extracts or fractions. There are all necessary apparatuses, laboratories and chemicals for such investigations such as EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy) using “Fiber Optic Spectrometer” NIR Quest 512 (Ocean Optic, USA, 2009 scope 900-1700 nm Spectral region), etc. The visiting researchers will be associated to the work carried out in the dept under the supervision of Assoc Prof A Zheleva

g) *Department of Plant science.* This unit will be also used for preparing of some herbal extracts and all herbal feed additives needed for the chick experiment. Homogenization of feed additives and mycotoxins with the forages will be also made there. The visiting researchers will be associated to the work carried out in the department under the supervision of Prof. D Pavlov

h) *Department of Pharmacology.* This unit is fully equipped for preparing of some unguents or sprays using appropriate constituents and some target herbs (or herbal mixtures) designed for stimulation of wound granulation. The visiting researchers will be associated to the work carried out under the supervision of Assoc Prof Mutafchieva

i) *Department of Epizootology.* This unit is fully equipped for investigation of protective effects of herbal additives against toxic and immunosuppressive effects of OTA and FB1 in chicks. The visiting researchers will be associated to the work carried out in the department under the supervision of Prof. I Zarkov

#### INFRASTRUCTURE/FACILITY NECESSARY FOR PROJECT IMPLEMENTATION IN UJ (SOUTH AFRICA)

The Mycotoxin Research Group (Food Environment and health Research Group – FEHRG in UJ) will be responsible for the general coordination of the project. In addition, within the project, the departments of Applied Chemistry, Environmental Health, Biotechnology, Food Science and Biomedical Technology, UJ (TWR) will be

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involved. The Mycotoxin Research Group will ensure facilities for the production of necessary quantity of mycotoxins. A fully equipped mycotoxicological laboratory, access to a cell cultures facility, fermenter room, and advanced chromatography facility (HPLC) are available.

For the characterization of herbal plants which will be done at the Applied Chemistry Dept (Nanotechnology Unit), there exist well equipped laboratories with nuclear magnetic resonance (NMR), infrared spectroscopy (IR), ultraviolet (UV), elemental analyses (EA), Mass Spectroscopy (MS), Raman spectroscopy (RS), Mossbauer analyses (MA), etc., and the unit has a host of postgraduates and postdoctoral fellows that routinely characterize and elucidate structures of various medicinal plants under the leadership of Prof Krause. These young researchers are not only citizens of South Africa, but originated also from other African countries including Cameroon, Malawi Zimbabwe and Swaziland, all of them with experience in chromatography, spectroscopy and structure elucidation. These students will play a very key role in the proposed project and as such, their involvement would be ideal in skills development and transfer. One of the postdoctoral fellows working on these aspects is very good at natural products isolation and characterisation. In other words, a good network is available within UJ of both South African and Central African researchers. In addition, a fruitful collaboration is available between the Botany section and Dept of Applied Chemistry which will be very helpful in regard to successful characterization of plants.

Aside from these, FEHRG and the Nanotechnology unit of UJ have established a very fruitful collaboration since 2007, which does not only focus solely on research, but is extended to training of postgraduates. In this regard, an Electrospray technique is available to produce fine particles of active herbal components to nanoscale level, thereby increasing their surface area and bioactivity.

### **INFRASTRUCTURE/FACILITY NECESSARY FOR PROJECT IMPLEMENTATION IN DRDO (INDIA)**

Facilities for collection of herbs, extraction, fractionation and chemical characterization as well as facilities for biological activity testing at *in vitro* and *in vivo* level are available. A fully approved (approved by the Institutional Animal Ethics Committee) animal house facility is available. In addition, nude mice facility also exists. A multi-laser flow cytometer (LSR-II from M/s Beckton Dickinson, USA) has been installed at INMAS (Institute of Nuclear Medicine & Allied Sciences). The instrument offers UV laser (350 nm), Blue line laser (488 nm) and a Diode laser (630 nm) with excitations and supports analysis up to 10 parameters (2 scatter and 8 colors). Other basic research facilities include Gamma irradiators, Elisa readers, Gamma counters, Beta counter, Confocal microscope, Fluorescence microscope and Comet assay analysis facility, DIC microscope, PCR, HPLC, etc.

### **INFRASTRUCTURE/FACILITY NECESSARY FOR PROJECT IMPLEMENTATION IN UNIKAPOS (HUNGARY)**

Kaposvar University (UNIKAPOS) is the largest institution engaged in animal sciences in Hungary and carries out basic, applied and developing research in the field of living natural sciences (see [www.ke.hu](http://www.ke.hu)). Its Study- and Experimental farm is offering practical training closely related to education as well as R+D+I and professional consulting. The training facility provides the conditions for carrying out farm experiments in the case of all significant livestock (cattle, horse, sheep, pig, poultry, rabbit, fish etc.). The Experimental Animal House offers the conditions for experiments with cattle, sheep, horse, pig with calf, separated piglet, young piglet and porker, poultry, rabbit, non-traditional animal species (such as deer) and laboratory white rat. The Faculty contains infrastructure facilitating non-invasive digital diagnostics processes: computerised X-ray tomographs (CT) and other, valuable equipment, such as the magnetic resonance imaging (MRI), NMR spectrometers, UH scanner and special operating facilities that allow for the examination of physiological, genetic and other problems on animal models. The University has well equipped laboratories, highly qualified scientists and laboratory staff in the field of feed and food analytics, molecular biology, digestive physiology, microbiology, and animal product quality estimation. In the research activity the guidelines of our quality management system are followed, which is certified according to ISO 9001:2008. The highest technology for mycotoxin analytics is provided by LC-MS and LC/MS-IT-TOF. All facilities for the animal experiments and laboratory tools for MTT assay and Comet assay are available. A complete microbiology lab and molecular genetics lab (qPCR, PCRs – polymerize chain reaction, fragment-analyser, chip reader) provides the infrastructure for microbiota determination.

### **B 3.2 APPROPRIATENESS OF THE PLANS FOR THE OVERALL MANAGEMENT OF THE EXCHANGE PROGRAMME**

**THE MANAGEMENT PLAN, Proposed work sharing and exchange visits for the entire duration of the project including a general view of complementarities and synergies between partners, and their specific contributions:** (Number of exchange visits, their duration and purpose of the visit, from each side given as a table)

<b>Tentative Date</b>	<b>№ of persons × Duration</b>	<b>Purpose of respective visits and specific contribution to the implementation of overall plan and management of the project</b>
<i>Bulgaria to S Africa</i> <b>07/2013</b>	1 person × 3 months	-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 stages of their growth, etc.

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<p><b>10/2013</b></p> <p><b>01/2014</b></p>	<p>2 persons × 3 months</p> <p>3 persons × 3 months</p>	<p>-Production and supply with mycotoxin FB1 for experimental studies in Bulgaria and Hungary, and exchange of knowledge on this subject via participation in target experimental work with target FB1-producers and via presentations.</p> <p>-Receiving some knowledge in regard to selective characterization of South African herbs for their bioconstituents using some nanotechnologies, NMR, IR, 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.</p> <p>-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 &amp; workshops</p>
<p><u>S Africa to Bulgaria</u></p> <p><b>10/2013</b></p> <p><b>01/2014</b></p> <p><b>07/2014</b></p>	<p>3 persons × 3 months</p> <p>2 persons × 3 months</p> <p>1 person × 3 months</p>	<p>-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)</p> <p>-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 &amp; workshops</p> <p>-Production and supply with mycotoxin OTA for experimental studies in Bulgaria and Hungary, and exchange of knowledge on this subject via participation in target experimental work with target OTA-producers and via presentations.</p> <p>-Exchange of some target knowledge in regard to elaboration of some mixtures of target herbal extracts, which will be further tested for their stimulating effects on wound granulation and preparing of some appropriate unguents/sprays in this regard</p> <p>-Some educational courses or workshop will be organized in Bulgaria in this regard.</p> <p>-Exploring a joint venture and preparing common research papers.</p>
<p><u>Hungary to S Africa</u></p> <p><b>07/2013</b></p> <p><b>01/2014</b></p>	<p>1 persons × 3 months</p> <p>2 persons × 3 months</p>	<p>-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 stages of their growth, etc.</p> <p>-Receiving some knowledge in regard to selective characterization of South African herbs for their bioconstituents using some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc., and way of preparing various herbal extracts or fractions via attending various presentations and workshops</p> <p>-Supply with some target herbs (leaves, barks, seeds, fruits or roots) to be used in the pig experiments on protective effects of herbs in Hungary and exchange of knowledge in regard to the way of action of target herbs and the way of collection and preservation of the same herbs</p> <p>-Exchange of some target knowledge via presentations in order to receive target skills on selective characterization of herbs and their healing effects on animals and humans.</p>
<p><u>S Africa to Hungary</u></p> <p><b>10/2014</b></p>	<p>3 persons × 3 months</p>	<p>-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 <i>in vivo</i> and <i>in vitro</i> experiments on the interaction between mycotoxins – herbal extract – gut microbiota.</p> <p>-Receiving some knowledge in regard to protective role of target plant extracts against the cytotoxic effects of mycotoxins FB1 and/or OTA on pig lymphocyte and/or intestinal cells via participation in various <i>in vitro</i> cytotoxicity tests as MTT assay and Comet assay.</p> <p>-Exchange of some experimental data with the aim of preparing common research papers and elucidation of various protective effects of target</p>

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		<p>herbs with a view of their further using in the practice and exchange of some target knowledge on this subject.</p> <p>-Exploring a joint venture with the aim of developing some herbal products based on multiple herbs.</p>
<p><u>Bulgaria to India</u> <b>04/2013</b></p>	<p>2 persons × 3 months</p>	<p>-Collection of some target Himalayan 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 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.</p>
<p><b>10/2014</b></p>	<p>2 persons × 3 months</p>	<p>-Elaboration of mixtures of target herbal extracts, which will be further tested for their stimulating effects on wound granulation and preparing of some appropriate unguents in this regard</p>
<p><b>07/2016</b></p>	<p>1 person × 6 months</p>	<p>-Exchange of some target knowledge via presentations</p> <p>-Supply with mycotoxins OTA and FB1 as well as with the found bioconstituents of some herbs necessary for experimental studies with rats in India and exchange of knowledge on this subject via participation in target experimental work.</p> <p>-Supply with Artichoke leaves necessary for rats experiment in India.</p> <p>-Exploration of protective effects of herbal additives against toxicity of OTA and FB1 via participation in rats experiment and performing some pathomorphological studies.</p> <p>-Exploring a joint venture with the aim of developing some herbal products based on multiple herbs and preparing common publications.</p> <p>-Exchange of some target knowledge via presentations.</p>
<p><u>India to Bulgaria</u> <b>10/2013</b></p>	<p>1 person × 3 months</p>	<p>-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) 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.</p>
<p><b>04/2014</b></p>	<p>2 persons × 3 months</p>	<p>-Exchange of knowledge between Bulgarian and Indian ways of preparing various herbal extracts or fractions via mutual presentations &amp; workshops</p> <p>-Exploration of <i>in vitro</i> antioxidant properties or <i>ex vivo</i> protective abilities of some herbal extracts or fractions against oxidative stress caused by proper xenobiotics using EPR spectroscopy.</p>
<p><b>04/2015</b></p>	<p>1 person × 3 months</p>	<p>-Elaboration of mixtures of target herbal extracts, which will be further 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.</p>
<p><b>01/2016</b></p>	<p>1 person × 6 months</p>	<p>-Supply with some target herbs to be used in the study on wound granulation and chick experiments on protective effects of herbs in Bulgaria and exchange of knowledge on the way of action of target herbs.</p> <p>-Investigation on stimulating effect of the prepared herbal extracts (unguents or sprays prepared in advance) on wound granulation in animals at Dept of Surgery in Faculty of Vet Medicine of TU.</p> <p>-Exchange of some target knowledge via presentations</p> <p>-Exploration of protective effects of target herbal additives against toxic and/or immunosuppressive effects of OTA and FB1 via chick experiments with Plymouth Rock</p> <p>-Exchange of some blood and pathomorphological samples from chicks and rats experiments performed in Bulgaria and India with the aim of elucidation of hepatoprotective or nephroprotective, antioxidative and diuretic effects of target herbs and exchange of knowledge on this subject.</p> <p>-Exchange of some target knowledge via presentations</p>

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		-Exploring a joint venture with the aim of developing some herbal products based on multiple herbs. -Preparing common research papers on various protective and immunostimulating or antioxidative effects of target herbs with a view of their further using in the practice.
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The host expertise of involved partner universities in training experienced or early stage researchers in the respective field and its capacity to provide mentoring/tutoring is well developed and proved as can be seen in the **CHAPTER B 1.2 RESEARCH QUALITY OF THE PARTNERS**.

**Practical and administrative arrangements and support for the hosting of the scientists** from the partner countries will be made by administration of each of the partner universities. Every effort will be made by the **respective human resources** to accommodate the requirements of the incoming scientists from the other partner universities/institutions whilst residing in the host institution/university. All partner universities have a wide range of accommodation and the surrounding suburbs also have suitable rented accommodation for academics. The English language courses for persons who do not have English as a first language are also available in each partner university/institution. All partner institutions have also international offices that deal with both internal and external administration, accreditation of foreign qualifications, permits and visas, child care and local schools, etc.

### **DETAILS OF AVAILABLE MATCHING FUNDS AND RESEARCH PROJECTS WITH THE PROJECT COORDINATORS:**

A total 25 international and national projects in TU, Bulgaria (7 projects), UNIKAPOS, Hungary (6 projects), UJ, South Africa (8 projects) and DRDO, India (4 projects) are available for performing all planned studies (see the chapter **B 1.2 RESEARCH QUALITY OF THE PARTNERS - Current Research projects related to this proposal**)

## **B 4 IMPACT**

### **B 4.1 RELEVANCE OF THE PROPOSED PARTNERSHIP TO THE AREA OF COLLABORATION AND FOR THE EUROPEAN RESEARCH AREA**

The training activities and Transfer of knowledge will be very important to allow the researchers from partner institutions to make further progress in their target research in regard to establishment of possible preventive measures against various toxic and immunosuppressive effects of some mycotoxins.

**The training activities and transfer of knowledge will be achieved by applicants from different participating countries in the field of biocharacterization of various herbs for their bioconstituents, in the field of possible protective properties of some target herbs and their mixtures against the toxic effects of target mycotoxins, against kidney or liver diseases, in the field of better understanding the possible interaction of mycotoxins with herbal extract and gut microbiota or in regard to improving animal/human immune response and wound granulation as well as in the field of elaboration of appropriate herbal medicines as spray or unguents to be used for stimulation of wound granulation, etc.**

The mentioned above selective characterization of some target herbs and the preparation of some herbal extracts or fractions or sprays/unguents can be done only with collaborative efforts of all partner institutions via exchanging target knowledge in regard to the possible way of action of each target herb, establishment of bioconstituents of some herbs using various techniques available in some of the partner countries as EPR (Electron Paramagnetic Resonance) or NIRS (Near Infrared Reflectance Spectroscopy), some nanotechnologies, NMR, IR, UV, EA, MS, RS, MA, etc. The training activities and transfer of knowledge in this regard will be an important part of this project, which will allow **the applicants from different countries to receive target skills on selective biocharacterization of herbs using the mentioned above technologies as EPR, NIRS on “Fiber Optic Spectrometer” NIR Quest 512 (Ocean Optic, USA, 2009 scope 900-1700 nm Spectral region), nanotechnologies, nuclear magnetic resonance (NMR), infrared spectroscopy (IR), ultraviolet (UV), elemental analyses (EA), Mass Spectroscopy (MS), Raman spectroscopy (RS), Mossbauer analyses (MA), etc.**

In addition, some new experience will be acquired by the researchers from the partner countries in the production of defined quantity of OTA and FB<sub>1</sub> in order to serve some future experimental work in the respective countries. So far, the production of mycotoxins have been done mainly by some foreign partners (Imperial college in London, University of Johannesburg), working in collaboration with the applicants.

**Such techniques and methods will be a good supplement to the skills of researchers from India and Hungary, in addition to the skills which will be acquired via exchange of target knowledge between the collaborators from Bulgaria and South Africa in regard to biocharacterization of herbs.** All these newly acquired skills and knowledge will allow the same researchers to become independent in their further study in target fields. After acquiring all these new up-to-date skills, which match very well with the experience of various participants, they will be in a position to reach a new level in their career as research workers and experts.

All participants (exchanged researchers) will be involved in regular workshops or meetings and conferences in the respective (host) universities.

**After exchanging of some target knowledge some preventive measures could be undertaken in order to reduce the economic loss, which appeared as a consequence of the increased susceptibility to infections in**

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animals/chicks exposed to the action of various mycotoxins via spontaneously contaminated feeds in the field (Stoev et al, 2002a). This is mentioned **to explain the benefit that will be gained from undertaking this project.**

**The present project can be also a good prelude to the successful carrying out of some future experiments** already mentioned above (by using the defined amount of target mycotoxins, which will be produced under the present project and by receiving the target skills and knowledge) with the aim of experimental reproduction the real situation in the practice and defining of some suitable preventive measures to protect animals and chicks. In addition, **a later introduction of the new methods/knowledge through the participants in their countries** (Trakia University, Bulgaria; Kaposvar University, Hungary; DRDO, India; University of Johannesburg, South Africa) **by including them in the teaching process for students** (including PhD students), will strengthen their educational, research and diagnostic capacity. Moreover, **worldwide dissemination of acquired knowledge** on possible protective effects of various herbs or herbal extracts on kidneys, liver and immune system and or on possible improvement of wound granulation **will bring a significant benefit in European Research Area of EU.**

In conclusion, it is worth mentioning that **the transfer of knowledge** to be carried out in the framework of this proposal will bring **benefits at the Community level.** By giving to the participating countries **the opportunity to gain expertise in the field of possible preventive measures against toxicity of some target mycotoxins, a significant contribution will be made to their efforts to combat some problematic diseases provoked by mycotoxins and to pay more attention to the quality and safety of the food chain (including human prevention of exposure to such mixtures of mycotoxins from pork) with important implications on human health at the Community level.** It is obvious, that only integrated approach to food safety, which includes systematic identification and assessment of hazards in foods/feeds and various means to prevent their toxic effects, could resolve the existing problems in this field.

**EACH PARTNERSHIP CONTRIBUTION TO THE AREA OF COLLABORATION and detailed information about the respective researchers involved in the management and transfer of knowledge can be seen clear in the chapter B3.2. MANAGEMENT PLAN, "TABLE 2 WORK PACKAGES" and chapter "B 2.2 ADEQUACY AND ROLE OF STAFF EXCHANGED WITH RESPECT TO THE TRANSFER OF KNOWLEDGE - The Role of the exchanged researchers and goals to be achieved"**

### **B 4.2 POTENTIAL TO DEVELOP LASTING COLLABORATION WITH THE ELIGIBLE THIRD COUNTRY PARTNERS, IN PARTICULAR IN VIEW OF SETTING-UP JOINT RESEARCH PROJECTS**

The overall programme and planned collaborations will be invaluable for the **sharing of methodologies and information between the collaborators**, i.e., Bulgaria, India, Hungary and South Africa. In particular, Bulgarian research workers need the expertise developed in India and South Africa in regard to the knowledge on protective effects of target herbs used by local Indian or South African tribes, whereas Indian and Hungarian collaborators can benefit from the expertise in mycotoxin production or selective biocharacterization of some target herbs and their bioconstituents, which can be provided by Bulgarian and South African researchers, etc. This **interdisciplinary and international cooperation** between the mentioned above partner institutions **will undoubtedly help each partner country to achieve the own objectives via the present project.** This would be achieved only **through collaborative efforts and by exchanging some target knowledge in very specific research areas mentioned above, which will further strengthen the scientific base of ERA and will increase European competitiveness.** This project will be a natural extension and a complement to some recent joint collaborative projects between Bulgarian and South African participants (**6<sup>th</sup> Framework EU project 018674 "Cause Kidney Damage"**) as well as between Bulgarian and Indian participants (**Contract БИИ-7/08-2008, Indo-Bulgarian Inter-Governmental Program of Co-operation in Science and Technology**).

The present project matches very well with the main objective of FP7 to contribute to the **creation and strengthening of the European Research Area (ERA) by improving integration and coordination of research in Europe through international co-operation.** By the same project, **undertaking a training process through research, it will be possible to develop further the natural and informal collaboration made recently between the researchers from all partner countries.** Moreover, some important information and target knowledge will be exchanged between the partner institutions in order to achieve **their own objectives**, which are in a point of contact within the present project. In such a way this collaboration may develop into **successful- and mutually profitable research network** with identical or at least **similar goals (establishing some natural and safe sources as herbs to protect against toxic effects of various mycotoxins as well as against kidney or liver diseases or to improve immune system of animals/humans, identifying the potential of target herbs to combat some mycotoxicoses or to improve wound granulation or to improve immune response of animals and humans, etc.).** Via this joint collaborative project and newly created cooperation and exchange of target knowledge between the leading countries in herbal industry, some new herbal products (having protective effects on kidneys and liver diseases or having immunostimulating effects or improving wound granulation) **will be elaborated, which undoubtedly will benefit not only ERA, but also animal/human health in all participating countries and all over the world.** This will give a sound grounding in the planned future joint research projects and lasting collaborations.

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### B 5 ETHICAL ISSUES

All animals (chicks, rats or pigs) will be fed *ad libitum* on standard complete commercial feed suitable for their species and their age. Fresh drinking water will be available *ad libitum*. The chickens will be housed in wire-floor cages with continuous infrared lighting at a temperature and ventilation suitable for their age (ensuring the respective minimum area per bird, minimum enclosure size and height or length of feed trough per bird as given in Table 8.1 of **Directive 2010/63/EU**). The pigs will be housed in standard pig boxes in a pig farm with the respective ventilation and the temperature (incl. infrared lighting) suitable for their age and floor area and minimum enclosure size and living space per animal suitable for their body weight according to the Table 7.3 of **Directive 2010/63/EU**. The rats will be also housed in standard cages with 12 hour light cycle and floor area (above 450 cm<sup>2</sup>) and minimum enclosure size (above 800 cm<sup>2</sup>) or height (above 18 cm) per animal suitable for their body weight according to the rule of the respective Institutional Animal Ethics Committee and the requirements given in Table 1.2 of adopted **Directive 2010/63/EU**. The ongoing monitoring of animal-welfare needs, appropriate veterinary care will be available at all times and a staff members will be responsible for the care and welfare of animals. The contamination levels of mycotoxins used (OTA and FB1) will be the same as in the spontaneously contaminated feeds with these mycotoxins encountered in all countries participated in this project (about 0,5 ppm OTA and 4-5 ppm FB1). In such a way the real situation in the practice will be reproduced experimentally and the real protective effects of herbs or herbal extract will be evaluated. The number of experimental chicks, pigs and rats will be the minimum required for such experiments – 10 chicks, 10 rats and 6 pigs in each group. At the end of the experiments on the protective effects of various herbal additives, the respective animals or chicks will be slaughtered (via exsanguinations) at the respective slaughterhouses according to the rule accepted in each slaughterhouse (electrical stunning for pigs, chickens, etc.) in each country or via using CO<sub>2</sub> and euthanasia for rats in compliance with the table 3 of methods for animal killing of adopted **Directive 2010/63/EU**.

Blood for clinical biochemistry and immunological investigations will be taken after respective premedication and narcosis from v. cava cranialis (in pigs) or from the wing vein (in chicks), whereas tissues for pathomorphological investigations will be taken at slaughter time in the respective slaughterhouses or after the euthanasia of rats. The incisional wounds in animals (pigs/calves/canines) created during the regular surgical interventions (such as castrations or other routine operations in the Department of Surgery) after the respective regular narcosis (premedication) and the local anesthesia as well as after the respective regular treatments will be additionally treated by newly created herbal extracts/(unguents or sprays) for studying their stimulating effects on granulation tissue.

All “in vivo” or “in vitro” experiments are already funded under the given sources of funding (7 research projects in TU – see page 10; 8 research projects in UJ – see page 15 and 4 research projects in DRDO – see page 20) and therefore all Ethical issues required by the respective funding bodies and countries are already answered and some of the experiments already performed. No experiments will be funded by this IRSES project and therefore the respective ethical issues were not given in details in the previous Ethical issues subchapter. The respective Animal Care Ethic Committees in each country have approved the study protocol and the pigs/chicks/rats in all future experiments will be housed, maintained and slaughtered in accordance to the relevant international rules and recommendations (including adopted **Directive 2010/63/EU** from 22 of September which updates and replaces the 1986 **Directive 86/609/EEC** on the protection of animals used for scientific purposes and is directed to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the Three Rs, to Replace, Reduce and Refine the use of animals) as well as to the Welfare Regulations in each country, where the experiments will be performed. The pig experiment in Hungary has been also authorised by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office. The 3 Rs principle (Replacement, Reduction and Refinement) will be rigorously applied in each country and “in vitro” studies such as MTT assay or Comet assay (using Cac-40 intestinal cell line or lymphocytes - see page 6) will be performed where it is possible to evaluate the role of plants extracts in protecting the cytotoxic effects of OTA or FB1 and to avoid the “in vivo” studies in which the minimum number of respective animals will be also used. No animals will be exposed to any pain or suffer, because the surgical interventions are not additional or different than the planed daily routine or regular surgical operations (as castrations, etc.) in the Department of Surgery and only some additional herbal unguents or sprays will be used and tested for improving postoperative wound granulation. The pigs/chicks/rats will be fed by the same commercial feed and exposed to the same contamination levels of FB1 and/or OTA as the farm animals in the practice as described above and will be slaughtered in the same way as farm animals or euthanased (using CO<sub>2</sub> for rats) and therefore will not be exposed to any pain, suffering or stress. In such a way **no sensitive ethics issues are raised in this proposal and in all experiments funded by the respective sources/bodies mentioned in this project.**



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### ETHICAL ISSUES TABLE - No sensitive ethics issues are raised in this proposal

(Note: Research involving activities marked with an asterisk \* in the left column in the table below will be referred automatically to Ethical Review)

<b>Research on Human Embryo/ Foetus</b>		<b>YES</b>	<b>Page</b>
*	Does the proposed research involve human Embryos?		
*	Does the proposed research involve human Foetal Tissues/ Cells?		
*	Does the proposed research involve human Embryonic Stem Cells (hESCs)?		
*	Does the proposed research on human Embryonic Stem Cells involve cells in culture?		
*	Does the proposed research on Human Embryonic Stem Cells involve the derivation of cells from Embryos?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	yes	

<b>Research on Humans</b>		<b>YES</b>	<b>Page</b>
*	Does the proposed research involve children?		
*	Does the proposed research involve patients?		
*	Does the proposed research involve persons not able to give consent?		
*	Does the proposed research involve adult healthy volunteers?		
	Does the proposed research involve Human genetic material?		
	Does the proposed research involve Human biological samples?		
	Does the proposed research involve Human data collection?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	yes	

<b>Privacy</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
	Does the proposed research involve tracking the location or observation of people?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	yes	

<b>Research on Animals</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve research on animals?	yes	6,7,32
	Are those animals transgenic small laboratory animals?		no
	Are those animals transgenic farm animals?		no
*	Are those animals non-human primates?		no
	Are those animals cloned farm animals?		no
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		

<b>Research Involving Developing Countries</b>		<b>YES</b>	<b>Page</b>
	Does the proposed research involve the use of local resources (genetic, animal, plant, etc)?		
	Is the proposed research of benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	yes	

<b>Dual Use</b>		<b>YES</b>	<b>Page</b>
	Research having direct military use		
	Research having the potential for terrorist abuse		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	yes	

**MOST OFTEN ABBREVIATIONS IN THIS PROPOSAL:** EPR - Electron Paramagnetic Resonance; EA - elemental analyses; FB<sub>1</sub> – fumonisin B<sub>1</sub>; HPLC - high performance liquid chromatographic; IR - infrared spectroscopy; LC-MS - liquid chromatography/mass spectrophotometry; MA - Mossbauer analyses; MN – mycotoxic nephropathy; MPN/MCN – mycotoxic porcine/chicken nephropathy; NIRS - Near Infrared Reflectance Spectroscopy; NMR - nuclear magnetic resonance; OTA – ochratoxin A; PA – penicillic acid; RS - Raman spectroscopy; UV – ultraviolet

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## **ENDPAGE**

PEOPLE  
MARIE CURIE ACTIONS

**International Research Staff Exchange Scheme (IRSES)**  
**Call: FP7-PEOPLE-2012-IRSES**

## **PART B**

**“HERBAL PROTECTION”**