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CO01- EFFECT OF PROPOLIS AND ITS POLYPHENOLIC/FLAVONOIDS COMPOUNDS ON DNA DAMAGE INDUCED BY RADIATION TO MOUSE LYMPHOCYTES

Oršolić N¹, Benković V¹, Horvat-Knežević A¹, Kopjar N², and Ivan Bašić¹

¹Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia; ²Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR-10000 Zagreb, Croatia

This study assessed the antioxidant potencies of several widespread flavonoids present in propolis and propolis alone. CBA mice were injected intraperitoneally (ip) with two preparation of propolis (water and ethanolic extract of propolis; WSDP or EEP) and propolis polyphenolic compounds (caffeic acid, naringin, chrysin, quercetin) at dose of 100 mg kg⁻¹ body weight for 3 consecutive days before or after whole body γ -irradiation (WBI). Synthetic protector 2-aminoethylisothiuronium bromide hydrobromide (AET) was used as a positive control. Mice were exposed to WBI with 9 Gy of ⁶⁰Co γ -radiation source. Thirty min after irradiation and/or treatment with test components we examined DNA damage of lymphocytes using the single-cell gel electrophoresis assay (comet assay). The WBI of mice resulted in a significant elevation of DNA damage of lymphocytes as compared with unirradiated mice. Pretreatment of mice with WSDP or EEP and flavonoids produced the reduction in oxidative DNA damage of lymphocytes as compared with control and they were ranked in decreasing order of potency as follows: naringin (2.98%); chrysin (16.84%); quercetin



(33.67%); AET (48.52%); caffeic acid (49.51%); EEP (53.47%); and WSDP (54.46%), respectively. Also treatment with propolis and its polyphenolic/flavonoids compounds after irradiation resulted in a significant reduction of DNA damage as follows: caffeic acid (32.31%); AET (75.39%); naringin (78.46%); EEP (80%); chrysin (83.08%); quercetin (84.62%); and WSDP (89.24%). These data suggest that WSDP and EEP are more protective than flavonoids from propolis alone. Data are also consistent with the hypothesis that radioprotective activity of EEP and/or WSDP related to synergistic antioxidative effect of components present in EEP or WSDP.

CO02- CYTOTOXICITY OF PROPOLIS AND ITS POLYPHENOLIC COMPOUNDS ON PRIMARY CULTURE OF HUMAN URINARY BLADDER TRANSITIONAL CELL CARCINOMA

Nada Oršolić¹, Damir Štajcar², and Ivan Bašić¹

¹Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, Zagreb, Croatia; ²General Hospital, Urology Department, Meštrovićeva b.b. 42000 Varaždin, Croatia

This study was carried out to find whether propolis and its polyphenolic/flavonoids compounds may induce cytotoxicity in primary culture of human urinary bladder transitional cell carcinoma (TCC) cells as compared to normal urinary bladder epithelial cells. Pieces of TCC or normal epithelial tissue were collected by transurethral surgery from patients in different stages (grade G1, G2, G3) of TCC. Incubation of TCC cells for cytotoxicity testing were carried out with or without different concentration (50, 150, 300 µg/ml) of test components. The cytotoxicity of two preparations of propolis (water and ethanolic extract of propolis; WSDP or EEP) and its polyphenolic compounds (caffeic acid, naringin, chrysin, and quercetin) was determined using trypan blue exclusion assay. Findings suggest that EEP is the most effective in inhibition of urinary bladder TCC cell proliferation as compared to WSDP or single flavonoids derived from propolis. All test components showed no cytotoxicity to normal epithelial cells. The result of this study may provide great impact on the potential activity of EEP as an adjuvant to surgery, to suppress or prevent tumor recurrence in urinary bladder since only a few anti-cancer drugs have been effective in tumor control. Since immunomodulation by BCG has been used to improve the results of surgery it is likely that propolis preparation (EEP) as immunomodulating compound may be a substitute for mycobacterial treatment since propolis preparation or its polyphenolic components have expressed no side effect after treatment in animal models.

CO03- THERAPEUTICS EFFICACY OF THE ULCEPROL CREAM. RESULTS OF A STUDY IN TWO HOSPITALS

Bermúdez I^a, Frómata V^b and Suárez C^c

^aOriente University. Faculty of Natural Sciences. Department of Pharmacy. Patricio Lumumba s/n. Santiago of Cuba City. Cuba. email: isisb@cnt.uo.edu.cu ^b“Joaquín Castillo Duany” Military Hospital. Santiago of Cuba city. Cuba. ^c“Saturnino Lora” Provincial Hospital. Santiago of Cuba city. Cuba.

Introduction: Propolis (bee glue) is of interest to science due to its several therapeutics uses in traditional medicine which have not yet not been totally investigated. The use of propolis in traditional medicine and its demonstrated antioxidant and antimicrobial properties encouraged the Pharmacy Department of the Oriente University in 1992 to elaborate a cream with propolis collected in the Manzanillo area and Contramaestre area to make its clinical use easy and to study the effects of the propolis on the patients with diabetic foot. **Material and methods:** The investigation was carried out on 240 patients with diabetic foot ulcers and 60 healthy subjects with age, sex and race similar to the sample of the population. The clinic essay was carried out according to the Helsinki Declaration was double blind, controlled, stratified according to the size of the lesion with inter-individual or in parallel comparison and involved 2 hospitals of Santiago of Cuba city. Serum samples were collected, before and after of the treatment and the samples were assayed for the determination of serum susceptibility to lipid peroxidation and antioxidant activity. (Ozdemirler G y col. 1995). Wound sample was harvested and used for microbiological analysis and ulcer areas were measured by tracing and subsequent electronic planimetry. **Results:** The results indicated that the creams had a significant antimicrobial effect in ulcers infected with *Staphylococcus aureus* and *Staphylococcus epidermidis* and that the propolis has regulator effect over the redox status because these patients show a susceptibility to the lipid peroxidation under of 2460±6,22 nmol/L and an antioxidant capacity greater than 66,2±10,2%. **Conclusions:** These results suggest that Ulceprol creams elaborated with propolis of the different areas keep the antimicrobial and



antioxidant effects and help to the healing of the ulcers. Ulceprol cream is an alternative in the treatment of the diabetic foot.

CO04- PERIODONTITIS TREATMENT WITH BRAZILIAN GREEN PROPOLIS GEL

Amaral RRC, Rocha WMS, Abreu SRL, Santos VR

Department of Clinic Pathology and Surgery. Department of Restorative Dentistry, Dentistry School, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil. E-mail: vegneer2003@yahoo.com.br

Propolis has been exhibits *in vitro* antimicrobial properties against periodontal pathogens microorganisms. The aim of this study was to observe the Brazilian Green Propolis gel (BGP) efficacy treatment in patients with gingivitis and Chronic Periodontitis (CP). Four patients, 1 male and 3 females, 36, 42,46, 51 years old age, presented rooth calculus, gingivitis, oedema, bleeding, gingival recession, pocket depths, attachment loss, suppuration, tooth mobility and alveolar bone loss was submitted at BGP 15% treatment. The patients' mouths were divided in four quadrants. Superior Right (SD) - BGP irrigation; Superior Left (SL) – scraping/ smoothing dental root (RAR) and pocket depths BGP irrigation; Inferior Right (IR) - RAR; Inferior Left (IL)- was the control. Dental brushing with BGP and washing mouth with propolis solution daily was carried through during the treatment. BGP was applied in each periodontal pocket 1 time week, during 5 weeks, having used barren dismissable syringe. The results shown a regression of 95% gingivitis and suppuration in all the teeth irrigated with BGP, as well as a pocket depths reduction in all unsubmitted and submitted teeth previously to the RAR. Do not observe alveolar bone reorganization. It was observed an increase of gingival contraction and dental mobility reduction. In this clinic study, the patient treated with the BGP showed periodontitis/gingivitis regression. It means that the 10% BGP used, in the therapeutic method assigned in this research is effective in the treatment of Chronic Periodontitis. The propolis gel efficacy in periodontitis treatment is Public Health of great interest in Brazil. Other studies with significant number patients are necessary for statistical analysis confirmation of these results. **Acknowledgments:** Dentistry Studies Centre IPSEMG/ PharmaNéctar®

CO05- PRODUCTOS NATURALES EXISTENTES EN EL PROPOLEO BRASILEÑO Y SUS DERIVADOS SINTÉTICOS PRESENTAN PROPIEDADES ANTITUMORALES

Quincoces J¹, Rando DG¹, Augusto-Maria D², Galvonas M³, Hamilton G⁴, Estrada E⁵, Carvalho J⁶

¹Grupo de Síntese Orgânica, Universidade Bandeirante de São Paulo, Brasil. ²Instituto Butantan de São Paulo, Brasil. ³Universidade Federal de São Paulo, Brasil. ⁴Universidad de Viena, Austria. ⁵Universidad de Santiago de Compostela, España. ⁶Universidade de Campinas, Brasil. ¹Rua Maria Cândida, 1813. Vila Guilherme. CEP: 02071-013 São Paulo, Brasil Email: quincoco2002br@yahoo.com

El propóleo brasileño es un producto natural formado principalmente por fenoles poli-funcionales que han mostrado propiedades anti-neoplásicas, entre otras actividades biológicas relevantes. En el presente estudio fue realizada una predicción de las propiedades antitumorales de productos naturales y derivados existentes en el propóleo brasileño mediante los programas computacionales TOPS-MODE. Los productos con mayor probabilidad antitumoral potencial fueron sintetizados a partir de fenoles. Las actividades antiproliferativas *in vitro* fueron determinadas con ayuda de los métodos de Sulforodamina B, MTT y MTT modificado en diversos tipos de células tumorales humanas. Fueron empleados en los ensayos *in vivo* ratones C57BL/6J a los que se les implantaron tumores de melanoma determinándose posteriormente la actividad antitumoral de los compuestos. Los derivados de la vanilina mostraron una actividad antitumoral *in vivo* significativa. Los derivados fenólicos prenilados exhibieron una alta acción antiproliferativa contra células tumorales humanas de pulmón, mama, colon y páncreas sin afectar los fibroblastos. Se concluye que se obtuvieron resultados muy destacados en los ensayos anti-proliferativos *in vitro* e *in vivo* efectuados a compuestos pertenecientes a 6 familias de productos naturales y sus derivados con una baja toxicidad.

Referencias: 1. Quincoces, J. et al.(2003) Brazilian Patent PI 02007141-0 (PCT/Br 03/00177, 2003; USA N° PCT/Br 2003/000177; Japan N° 2004-554088; Europa N° 03773364.9. 2. Quincoces, J., et al.,Patente DE 102005044156.4, 2005. [3] Quincoces, J., et al.,Patente DE 102004032711.4-44, 2004.



CO06- THE BIOLOGICAL ACTIVITIES OF *Serenoa repens* EXTRACT IN HUMAN PROSTATE

Habib-Fouad K and Buck C

Prostate Research Group, Edinburgh Cancer Research Centre, School of Molecular and Clinical Medicine, the University of Edinburgh, 4th Floor, MRC Human Genetics Building, Western General Hospital, Crewe Road South, Edinburgh EH4 2XU

Medical therapies derived from natural sources have been used for centuries and many are recognised for being as effective as synthetically based medications. The use of plant derived medication for lower urinary tract symptoms associated with benign prostatic hyperplasia is no exception. In particular extracts of the fruit of the American Dwarf Palm (*Serenoa repens*, saw palmetto) are widely available and their use is rising throughout the world. The underlying basis for the popularity of *Serenoa repens* extracts (SrE) stems from their safety and tolerability profile. However, in spite of their extensive use, the mechanism of action of SrE has not been definitely clarified. In the present report, we will survey the scientific basis for the efficacy of this drug in the treatment of prostate diseases and explore the mechanism(s) by which SrE may induce its clinical benefits. In particular, we will concentrate on the action of Permixon®, a lipido-sterolic extract commercialised by Pierre Fabré Medicament. This brand has been subjected to greater scrutiny and involved in more clinical trials and pharmacological analyses than any other preparation of SrE. Permixon® is selective for prostate cells since cells derived from breast, skin, epididymis, testes and kidney appear not to be susceptible to the drug. Treatment of prostate cells with Permixon® damages the intracellular membrane of the cells and ultimately induces apoptosis. The compound has also been shown to be a non-competitive inhibitor of 5-alpha-reductase, the enzyme responsible for the conversion of testosterone to dihydrotestosterone in the prostate. Additionally, Permixon® has been found to exhibit anti-oestrogenic and anti-inflammatory properties; the latter mediated via the inhibition of the cyclooxygenase enzymes associated with the synthesis of prostaglandins in target cells. So far, we have been unable to identify the nature of the active ingredient in SrE responsible for the action of the drug. However, preliminary data suggests that this might be induced in part by free fatty acids alone or in combination with phyto-oestrogens present in the extracts. This talk will review some of these biological properties and explore the impact of phyto therapy in the treatment of prostatic diseases.

CO07- TREATMENT OF IRON DEFICIENCY ANAEMIA IN DIFFERENT POBLATIONAL GROUP. EFFICACY OF TWO PHARMACEUTICAL FORMULATIONS OF NATURAL IRON (TROFIN & NEOTROFIN)

Aznar-García E^a, González HR^a, González M^a, Díaz BY^a, Barquié M^b, González M^b, Morono M^b, Carrasco M^b and Leyva B^b

^aCentro Nacional de Biopreparados. BioCen. Apartado 6048. Habana 6. Ciudad de La Habana. Cuba. ^bPoliclínico Elpidio Berovides. La Lisa. Hospital Pediátrico "William Soler"; Centro Iberoamericano para la Tercera Edad. Hospital "Salvador Allende". Ciudad de La Habana. Cuba. Email: eaznar@biocen.cu; eaznar@infomed.sld.cu

Introduction: Iron deficiency anaemia is a common problem. It's commonly treated by giving iron salts, but the salts preparations cause adverse effects, and the treatment wasn't continued. These observations suggest that other new products with an other iron may be an alternative for obtain products with high absorption, efficacy and tolerability. We obtain an antianaemic and restorative product developed from natural raw materials, and its composition includes proteins, peptide, aminoacids and minerals (ferrous iron) and bee honey. The objective is the study the efficacy and tolerability of these products Neotrofin and Trofin in the treatment of iron deficiency anaemia. **Material and methods:** Two pharmaceutical formulas were elaborated: oral solutions (Trofin/Biotrofer) and tablets (Neotrofin) with an anti-anaemia action. In the Clinical trials studies follow the Good Clinical Practices of these protocols. Adults patients with anemia were enrolled into this group (610 patients; 1005 children's and 247 pregnant women. These one was administered with Trofin solution. Neotrofin tablets were evaluated in pregnant women. Hematological indicator was studied before and after the treatment administrations. **Results:** The increase in hemoglobin, with Trofin was in mean value 98g/L and 118 g/L before and after treatment. The efficacy was 90.5 %; In the children populations; 90.2 % in the adults and 85 % in pregnant women. In this group there is difference with Neotrofin (tablets) with 90 % efficacy. An increase in serum iron was demonstrated. The Comparative Clinical Trials between Trofin and iron salts showed in children the following advantages of this Trofin in these one none adverse reaction. These results showed that natural iron has high solubility and absorption. **Conclusions:** Trofin, Neotrofin are products with a high biological value to



prevent and treat iron deficiencies; for the efficacy and tolerance, it is a product of choice for all ages. The studies that allowed to obtain the Sanitary Register in Cuba, and others country.

CO08- CLINICAL TRIALS ON THE EFFICACY OF AN EXTRACT OF *Rhizophora mangle*, L. BARK EXTRACT IN SKIN AND MUCOSA WOUNDS HEALING

Fernández Limia O,^a de Armas E,^a Sarracent Y^b and Dalla G^c

^aCENSA, Carretera de Jamaica y Autopista Nacional, San José de las Lajas, La Habana, Cuba, ^bHospital Docente "Freire de Andrade", Ciudad de La Habana, Cuba. ^cHospital Militar Docente "Carlos J. Finlay" Ciudad de La Habana, Cuba. E-mail: octavio@censa.edu.cu

Introduction: The antiseptic and wound healing stimulating effects of *Rhizophora mangle*, L bark extract (RMBE) were evaluated in a model of skin open surgical wounds and in another of not induced oral wounds (aphthous ulcers). **Material and methods:** Thirty seven patients with open wounds of surgical interventions by pilonidal sinus (23, 62.2%) and cysts (14, 37.8%), were voluntarily recruited in a comparative, single blinded clinical study, which were random distributed in 3 groups of treatment: RMBE once a day, twice a day and mercurochrome (Merbromin) twice a day. The efficacy of the treatments was evaluated weekly since 10 to 12 days after surgery until 6 weeks through the reduction of the wounds' area by digital images planimetry and the security by the registration of adverse effects. The initial area was taken in consideration as covariable in the Lineal Generalized Model employed for ANOVA analysis. In order to evaluate the wound healing effect in oral mucosa, 32 patients with aphthous ulcers were recruited in a controlled single blinded, random clinical trial, 15 were treated with Placebo and 17 with RMBE from Monday to Friday, topically once to the day. The efficacy of treatment was evaluated through the aphthae evolution by clinical observation. **Results:** In all cases of skin wounds treated with RMBE a fine dark layer, covering the wounds, was observed. There were differences ($p < 0.05$) in the reduction of the wounds areas of the groups treated with RMBE once or twice per day compared with the control of mercurochrome since the fourth week of the operation, without finding differences between them. Cases of adverse reactions to the *R. mangle* formulation were not observed. In oral mucosa wounds group treated with RMBE showed a decrease of 11.67 ± 0.84 to 7.29 ± 0.39 days and of 7.55 ± 0.65 to 3.4 ± 0.27 days in order to reach the conditions of healed and enhanced, respectively ($p < 0.0001$), besides it was capable of diminish the duration of the erythema from 10.54 ± 1.24 to 4.94 ± 0.72 days ($p = 0.0003$), difficulty chewing from 7.43 ± 1.21 to 2.92 ± 0.23 days ($p = 0.0011$) and of soreness from 7.00 ± 0.76 to 2.93 ± 0.49 days ($p = 0.0001$). Adverse effects were not observed. **Conclusions:** With these studies the beneficial effect of the extract of *R. mangle* was demonstrated in skin and oral mucosa wounds reducing the time of healing and improving the life quality of patients. **Acknowledgements:** To Drs. Luis Espinosa ("Freire de Andrade" Hospital) and Jose Capdevila ("Carlos J Finlay" Hospital) for their helpful suggestions and support.

CO09- ECHINACEA AND GINSENG FOR IMMUNE ENHANCEMENT AND PREVENTION OF RESPIRATORY INFECTION

Barrett B

University of Wisconsin – Madison, Department of Family Medicine. 777 South Mills, Madison, Wisconsin, USA. Email: bruce.barrett@fammed.wisc.edu

Introduction: Many different phytomedicines are used to enhance the immune system and protect against acute respiratory infection. Here we focus on published evidence from human trials testing extracts of *Panax* (ginseng) and *Echinacea* species. **Methods:** We searched MedLine/PubMed and other databases to find reports of all randomized controlled trials (RCTs) testing ginseng or echinacea for immune enhancement and/or prevention against acute respiratory infection (common cold and influenza). **Results:** Several RCT reports suggest that *Panax* extracts can effect adaptive (specific) and innate (nonspecific) immune pathways. Double-blinded RCTs by Scaglione (1996), McElhaney (2004), and Predy (2005) suggest that ginseng may prevent respiratory infection and/or reduce symptoms. Scaglione also reports enhanced antibody response to influenza vaccination. Numerous studies report immunostimulating effects of various echinacea extracts, with enhanced macrophage and natural killer cell activity the best established. While at least 16 RCTs have tested echinacea as treatment for respiratory infection, only eight have looked for preventive effects. Of these, three reported significant benefits. The other five trend toward benefit without



reaching statistical significance. Pooling data from three “negative” induced cold (inoculated rhinovirus) RCTs, a meta-analysis by Schoop et al. (2006) reports that “the likelihood of experiencing a clinical cold was 55% higher with placebo than with echinacea (O.R. 1.55 [95% CI, 1.02 - 2.36]; $p < 0.043$.” We could not find reports of RCTs testing echinacea for ability to enhance immune response to vaccination. **Conclusions:** Randomized controlled trials suggest that ginseng and echinacea phytomedicines may act through immune pathways to protect against acute respiratory infection. **Plan:** We have proposed a double blind RCT in which 630 adults aged 50 and older would be randomized to ginseng, echinacea or placebo, then followed for eight months, with symptoms and biomarkers (nasal neutrophil, interleukin-8, PCR identification of viruses) assessed for all acute respiratory infections. Subjects would also receive influenza vaccination, with serum antibody level assessed three weeks after vaccination. This research proposal to the National Center for Complementary and Alternative Medicine at the U.S. National Institutes of Health was sent June 1, 2006, and not yet been reviewed.

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CO10- BOSWELLIA-GLYCYRRHIZIN-CURCUMIN PREPARATION FOR THE TREATMENT OF CHRONIC HEPATITIS C: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED PHASE I/II TRIAL

Badria-Farid A¹ and Said S²

¹Departments of Pharmacognosy and ²Pharmacology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

Background: Alpha-interferon monotherapy leads to hepatitis C virus (HCV)-RNA clearance in a minority of patients. Although combination therapy with Ribavirin increases efficacy, the sustained response rate is still below 50%. The aims of this study were to evaluate the effect of Boswellia-Glycyrrhizin-Curcumin preparation on serum alanine aminotransferase (ALT), hepatitis C virus (HCV)-RNA and its safety among Egyptian patients. **Methods:** 120 patients with chronic hepatitis C, non-responders or unlikely to respond to interferon therapy, were randomized to one of the two groups: Boswellia-Glycyrrhizin-Curcumin treated or placebo. Medication was administered orally thrice daily for 12, 24, and 36 weeks. **Results:** Within 2 weeks of start of therapy, serum ALT had dropped 15% below baseline ($P < 0.02$). The mean ALT decrease at the end of active treatment was 72%, significantly higher than the placebo group (6%). Normalization of ALT at the end of treatment occurred in 70% (four of 41). The effect on ALT sustained after cessation of therapy. During treatment, viral clearance was observed: the mean decrease in plasma HCV-RNA after active treatment was 4.1×10^6 genome equivalents/mL (95% confidence interval, $0-8.2 \times 10^6$; $P > 0.1$). No major side-effects were noted. None of the patients withdrew from the study because of intolerance. **Conclusions:** Boswellia-Glycyrrhizin-Curcumin thrice daily for 36 weeks, lowers serum ALT during treatment and cleared HCV-RNA levels between weeks 24 and 36. The preparation appears to be safe and is well tolerated.



CO11- MODULATION OF NF- κ B PATHWAY BY *Mangifera indica* L. EXTRACT (VIMANG®) AS THE MOLECULAR MECHANISM RESPONSIBLE OF ITS IMMUNOLOGICAL AND ANTI-INFLAMMATORY ACTIVITIES

García D¹, Garrido G¹, Delgado R¹, Muñoz E², Leiro JM³

¹Center of Pharmaceutical Chemistry. 200 and 21, Atabey, Playa, Havana. Cuba. ²Laboratory of Immunology, University of Córdoba, ³Faculty of Pharmacy, University of Santiago de Compostela, Spain.
Email: dagmar.garcia@infomed.sld.cu

The transcription factor, nuclear factor κ B (NF κ B), is believed to play a pivotal role in immune and inflammatory responses through the regulation of genes encoding proinflammatory cytokines, adhesion molecules, chemokines, enzymes and growth factors. In resting cells, NF- κ B is retained in the cytoplasm as an inactive complex with the inhibitor κ B (I κ B). Cellular stimulation with various agents leads to phosphorylation, ubiquitination, and subsequent degradation of I κ B. This leaves NF- κ B free to translocate to the nucleus, where it binds to κ B sites in specific target genes and induces the transcription of genes related with immune and inflammatory response. Vimang® is the brand name of an aqueous extract of the stem bark of *Mangifera indica* L. that contains a defined mixture of components including polyphenols (principally mangiferin), triterpenes, phytosteroids, fatty acids and microelements. This extract has reported anti-inflammatory, immunomodulatory and antioxidant activities. This study investigated the effects of *Mangifera indica* L. extract (Vimang®) on expression and activation mediated by TNF α of NF κ B. Western blot and EMSA on Jurkat cells were used to determine the I κ B degradation and NF- κ B in the nuclear extract, respectively. The levels of mRNA of NF- κ B and I κ B were determined by RT-PCR on activated peritoneal murine macrophages. The extract (25 μ g/mL) prevented TNF α -induced I κ B degradation and the binding of NF- κ B to the DNA. Also, the extract at 4-400 μ g/mL decreased mRNA levels of NF- κ B but did not affect expression of the NF κ B inhibitor I κ B. In previous studies, we demonstrated that *Mangifera indica* L. (40-400 μ g/mL) reduced levels of mRNA of NOS-2, COX-2, TNF α , IL-1 β and GM-CSF on activated macrophages with LPS and IFN γ . The gene transcription of all of these proteins is regulated by NF- κ B. These experimental evidences can be explained by the *Mangifera indica* L. extract modulation of NF- κ B. In conclusion, the *Mangifera indica* L. extract modulates the NF- κ B pathway by inhibition of the gene transcription of NF- κ B, I κ B degradation and the binding of NF- κ B to the DNA. This study may help to explain at the molecular level some of the biological activities attributed to the aqueous stem bark extract of *Mangifera indica* L. (Vimang®).

CO12- *Mangifera indica* L. EXTRACT MODULATES TCR-INDUCED NF- κ B SIGNALLING IN HUMAN T LYMPHOCYTES

Hernandez P¹, Rodriguez PC¹, Delgado R¹, Walczak H²

¹Department of Biomedical Research, Center of Pharmaceutical Chemistry, PO Box 16042, Havana, Cuba. ²Apoptosis Regulation, Tumor Immunology Program, German Cancer Research Center, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany. E-mail: patricia.hdez@infomed.sld.cu

A particular form of apoptosis induced by repeated T cell receptor (TCR) stimulation, known as activation-induced cell death (AICD), maintain the immune system homeostasis. The imbalance in this apoptotic process leads to severe diseases. CD95 ligand (CD95L) expression is crucial in the induction of AICD. TCR engagement results in the activation of several transcription factors including AP-1, NF- κ B and NF-AT that cooperatively act on the CD95L promoter to induce *de novo* transcription. It is well established that NF- κ B is activated by oxidative signals produced during TCR signalling. The stem bark extract from *Mangifera indica* L. (Vimang), rich in polyphenolic compounds, has probed *in vitro* and *in vivo* antioxidant activities. We have previously established *M. indica* extract protects T cell from *in vitro* AICD by a mechanism that involves AP-1 and NF-AT signalling. In the present study, we investigated the contribution of NF- κ B in the protective effect demonstrated by *M. indica* extract in T cells. The effects of *M. indica* extract on TCR-mediated activation of I κ B and NF- κ B (p65) proteins in the cytosol were examined by immunoblot kinetic analysis in human peripheral blood T lymphocytes. TCR activation was mimicked by anti-CD3 antibodies. Our results show, *M. indica* extract treatment caused a decrease in the constitutive protein expression of NF- κ B (p65). We also found, *M. indica* extract did not influence on I κ B degradation but reduced the increase of NF- κ B (p65) protein



expression induced upon TCR triggering. Our findings suggest that the T cell survival effect of *M. indica* extract on T cells is associated with its capacity to modulate NF- κ B signalling.

CO13- *Mangifera indica* L. EXTRACT (VIMANG) AND MANGIFERIN REDUCE NEURONAL LOSS AND OXIDATIVE DAMAGE AFTER EXCITOTOXIC INSULTS

Lemus-Molina Y^a, Sánchez MV^b, Delgado R^a, Matute C^b

^aLaboratorio de Farmacología, Dpto. Investigaciones Biomédicas. Centro de Química Farmacéutica, Ciudad Habana, Cuba. ^bLaboratorio de Neurobiología, Dpto de Neurociencias, Facultad de Medicina y Odontología, Universidad del País Vasco, Leioa, Vizcaya, España. E-mail: yeny.lemus@infomed.sld.cu .

Introduction: High intracellular level of Ca²⁺, oxidative stress and apoptosis play an important roll in degenerative diseases of Central Nervous System. They can be consequences of glutamatergic excitotoxicity. Due to the importance of oxidative stress on the neural dead evoked by glutamatergic excitotoxicity, some antioxidant products have been studied as therapeutic agents. **Material and methods:** *In vitro* antioxidant properties of Vimang and mangiferin were analyzed by 1,1 diphenil -2- picril hidrazil (DPPH). Absorbance was quantified at regular intervals of 10 min for a period of 1 h. Neuronal cultures were obtained from the cerebral cortex of rat embryos (18 days) and maintained for 8 days before the experiment. Neurons were exposed for 10 min to 50 μ M of glutamic acid and glicine to evoke excitotoxicity. Neuronal damage was analyzed 3 h after insult. Reactive oxygen species and neural survival were quantified 15, 30, 60 and 180 min after that time using H₂DCFDA and Calcein AM assay, respectively. To study the effect of Vimang and mangiferin on the mitochondrial membrane potential, neurons were exposed for 10 min to glutamic acid. 30 min after stimulation, neurons were incubated for 15 min at 37°C in TMRE o calcein AM and mitochondrial membrane potential values were measured. **Results:** Antioxidant properties of Vimang were obtained from 10 min after the incubation and it was maintained for 40 min. Its maximum of neural dead inhibition was 40.16 \pm 1.5% when it was used a concentration of 5 μ g/ml. Antioxidant properties of mangiferin were observed from 20 min after the beginning of the reaction. Maximum inhibition of DPPH radical production was obtained 20 min latter when a concentration of 6.25 μ g/ml was used. This inhibition had a value of 28.13 \pm 1.0181%. Both Vimang and mangiferin protected against glutamic acid- induced dead, 30% and 45% respectively. They also inhibited the oxidative stress evoked by the excitotoxic stimulus. Vimang was effective 15, 30 and 60 min post- stimulation and mangiferin was effective 15, 30 y 180 min after insult. On the other hand, neurons recovered the normal value of the mitochondrial membrane potential when they were treated with Vimang at concentrations of 5 μ g/ml and 2.5 μ g/ml. mangiferin had the same effect at concentrations of 25 μ g/ml and 6.25 μ g/ml. **Conclusions:** Early modulation of cellular mechanisms triggered by glutamatergic excitotoxicity makes Vimang and mangiferin important therapeutic candidates in the treatment of nervous diseases in which excitotoxicity and oxidative stress are present.

CO14- ANTI-ANGIOGENIC ACTIVITY OF *Mangifera indica* L. STEM BARK EXTRACT AND ITS GLUCOSYL XANTHONE MANGIFERIN

Delgado R^a, Rodríguez J^a, Herrera B^a, Rojas A^a, Beltran A^a, Sordo L^b, Nuevas L^b, Garrido G^a, Núñez A^b

^aPharmacology and ^bChemical Analysis Laboratories, Department of Biomedical Research. Center of Pharmaceutical Chemistry, P.O. Box 16042, Atabey, Playa, Havana, Cuba. E-mail: rdelgado@infomed.sld.cu

Angiogenesis, the development of new blood vessels, is an important process in tissue development and wound healing but becomes pathologic when associated with solid tumor growth, proliferative retinopathies and rheumatoid arthritis. Chemically characterized and standardized *Mangifera indica* L stem bark extract (MISBE) is used in Cuba for the ethnomedical treatment of cancer and others diseases as antioxidant supplement. Its main chemical ingredient, mangiferin (MF), is a glucosilxanthone which has been known for its immunomodulatory, antitumoral and anti-inflammatory actions. MISBE was assessed in three "*in vitro*" models for human angiogenesis: human placental blood vessel explants assay, the gel-over-gel and the matrigel assays. In addition, experimental *in vivo* models of angiogenesis were performed using matrigel and tumors cells. MISBE (12,5-100 μ g/mL), present in the culture medium, significantly exhibited an inhibitory effect on capillary tubes formation in the first two assays and did not modified the tubes-like formation of endothelial cells on matrigel. Mangiferin abolished the neovascularization in the sandwich assay. Both, MISBE and mangiferin, were capable of reduce the TNF α induced angiogenesis in mice into



matrigel and the tumor neovascularization induced by melanoma B16F1 cells. Others results suggest that the antiangiogenic mechanism could involve the inhibition of matrix metalloproteinases degradation. The results of the present investigation demonstrate that the extract possesses antiangiogenic properties *in vitro* and *in vivo* with mechanisms that involve the inhibition of the activity of the metalloproteinases, being able to represent a therapeutic alternative of natural sources that could be used after developing further preclinical and clinical studies for the treatment of some types of tumors.

CO15- FE(III) SHIFTS THE MITOCHONDRIA PERMEABILITY TRANSITION-ELICITING CAPACITY OF MANGIFERIN TO ORGANELLE'S PROTECTION. A POTENTIAL PROTECTIVE MECHANISM TOWARDS PROOXIDANT ACTION OF CATECHOL-CONTAINING ANTIOXIDANTS

Pardo-Andreu GL¹, Cavalheiro RA², Naal Z³, Delgado R¹, Vercesi AE², Curti C³

¹Departamento de Investigaciones Biomédicas, Centro de Química Farmacéutica, Calle 200, Esq. 21, Playa, Ciudad de La Habana, Cuba. Tel.: (53 7) 2715067, Fax: (53 7) 2736471. E-mail: g031071@yahoo.com.

²Departamento de Patología Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 13083-970 Campinas, São Paulo, Brazil. ³Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. Café s/n, 14040-903 Ribeirão Preto, São Paulo, Brazil.

Mangiferin displays an important antioxidant activity on mitochondria (*Eur.J.Pharmacol.* **513**:47-55, 2005), but in the presence of Ca²⁺ it elicits mitochondrial permeability transition – MPT (*Arch.Biochem.Biophys.* **439**:184-193, 2005), as evidenced by cyclosporine A-sensitive mitochondrial swelling. We now provide evidence by means of electrochemical and UV-Vis spectroscopical analysis that Fe(III) coordinates with mangiferin, both preventing it to display MPT-eliciting capacity, and enabling it to display MPT-inhibiting capacity *via* reactive oxygen species scavenging, in apparent association with the protection of mitochondrial glutathione (GSH) and/or membrane protein thiols from oxidation. Accordingly, Fe(III) significantly improved the capacity of mangiferin to scavenge the 2,2-diphenyl-1-picrylhydrazyl – DPPH radical, as well as to display antioxidant activity towards *t*-butyl hydroperoxide-induced H₂O₂ accumulation and membrane lipid peroxidation in mitochondria. We postulate therefore, that coordination with Fe(III) constitutes a potential mechanism protecting catechol-containing antioxidants from inducing MPT, as well as a possibly ideal approach for the use of these compounds in pathological iron overloading.

CO16- RESULTS OF RESEARCH WITH AN EXTRACT OF *Mangifera indica* L. (VIMANG®): FROM ETHNOMEDICINE TO CLINICAL TRIALS

Guevara M, Álvarez A, Riaño A, Pardo G, Reynaldo G, Garrido G, Delgado R

Biomedical Research Department. Centre of Pharmaceutical Chemistry. 200 str. and 21 Ave., Atabey, Postal Code 11600. Havana City, Cuba. E-mail: mariela.guevara@cqf.sld.cu, marielaguevara@infomed.sld.cu

For more than one hundred year the plants have been used as medicinal aims. Mango (*Mangifera indica* L.), which belongs to the Anacardiaceae family, is widely found in Cuba and in many other tropical and sub-tropical regions, Mango stem bark has been traditionally used like aqueous extract, obtained by decoction, in ethnomedical practice. The Centre of Pharmaceutical Chemistry has within its high-priority lines of work the introduction of this natural product for medical use. The extract has a lot of scientific evidences, related with its pharmacological properties. The first clinical investigation was made with the objective to know the traditional applications, the effectiveness and the potentialities of the extract of *Mangifera indica* L. in ours conditions. For approximately 10 years, we are working on preclinical demonstration of antioxidant, anti-inflammatory and immunomodulator effects. After that, our institution began the development of a strategy of clinical evaluation, for demonstrating the therapeutic effectiveness of the extract in diverse pharmaceutical forms through controlled clinical trials in diseases with an important inflammatory or/and oxidative stress component that guarantee the introduction of Vimang® like phytomedicine. The present work shows the results of clinical trials until the present time with Vimang® in patients with AIDS, in aging, intense physical exercise, bronchial asthma and its effect on blood coagulation. Besides, this work shows the strategies for the future.



CO17- PLANT MEDICINAL: COMPILATION OF CUBAN AUTHORS SCIENTIFIC ARTICLES

Castro R, Suárez A, González R, Tillán J, Díaz A, Domínguez MT, Vizoso F

Drug Research and Development Center (CIDEM). Pharmaceutical Information Center (CINFA). Ave 26 No. 1605 e/ Boyeros y Puentes Grandes. Plaza. Ciudad de La Habana. CP: 10600. Cuba.
E-Mail: cinfa@infomed.sld.cu; cinfa@cidem.quimefa.cu

An informative product is presented in format CD-ROM that contains 621 scientific articles published by Cuban authors between 1968 and 2006. This compilation, in its version 1.0, constitutes the first step in the effort to materialize a source of national information in digital format that contains the biggest volume possible of articles on the thematic one published in national journals and foreigners. For all the works it is available the complete text and you consents to the same one through five indexes: titles of articles, authors, years, publication and institutions. The product has an index of more than 970 Cuban authors and foreigners and the participation of more than 120 scientific and educational institutions. This valuable information resource, supported in multimedia format, also describes the fundamental antecedents that give him origin, as well as the perspectives for the inclusion of new scientific articles.

COMUNICACIONES ORALES / ORAL COMMUNICATIONS (CO)

MIÉRCOLES, 22 DE NOVIEMBRE / WEDNESDAY, NOVEMBER 22

CO18- EFFECTS OF RHYNCHOPHYLLINE ON RAT CORTICAL NEURONS STRESSED BY METHAMPHETAMINE

Mo ZX¹, Xu DD¹, Yung KKL²

¹School of Chinese Medicine, Southern Medical University, Guangzhou 510515, PR China. ²Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, PR China. E-mail: cherryimo@fimmu.com

Methamphetamine (MA), a psychostimulant, has been known to induce a psychologic dependence and damage in central nerve system. Rhynchophylline (Rhy) is a tetracyclic oxindole alkaloid isolated from *Uncaria rhynchophylla* (Miq.) Jacks., which has been long used as a medicinal herb in China. In the present study, the neurotoxicity of MA, the calcium changes involved in the MA dependence and the effects of Rhy on rat cortical neurons treated with MA were investigated. MTT assay were used and the free intracellular free calcium concentration ($[Ca^{2+}]$) were determined by the Fluo-3/AM method. The results showed that neurotoxicity of MA was in a dose-dependent manner within the concentration range of 10-150 μ mol/L. The level of $[Ca^{2+}]$ in the cultured cortical neurons was markedly elevated after chronic exposure of MA for 48 h. Rhy had a neuroprotective effect against MA in culture viability. Rhy, at the concentration of 20 μ mol/L, significantly decreased $[Ca^{2+}]$ of cortical neurons pre-treated with MA. The study indicated that NMDA receptors and calcium signalling play important roles in MA dependence. Rhy showed a neuroprotective action against MA *in vitro*, which resembles the effect of noncompetitive NMDA receptor antagonist ketamine. This property of Rhy may also contribute to the neural activity of the origin of *Uncaria* species plants. These results suggest Rhy may be of benefit to treatment for MA dependence. **Acknowledgements:** Supported by: the National Science Foundation of China, No.30371773.



CO19- THE DISCRIMINATIVE STIMULUS PROPERTIES OF *Mitragyna speciosa* EXTRACT IN RATS

Aziz Z, Latiff A Aishah

¹Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur Malaysia. ²Doping Control Center, University of Science, 11800 Minden, Penang, Malaysia

Introduction: *Mitragyna speciosa* KORTH (Rubiaceae) is a tree native to South East Asia and the abuse of the leaves is a growing problem in these countries. The abuse poses a potential drug abuse problem world-wide, as *Mitragyna speciosa* seeds are currently both sought after and advertised on the internet. **Objectives:** A drug discrimination procedure was used to determine whether the crude extract was associated with a specific stimulus effect and to characterise these effects in comparison to other psychoactive drugs. **Method:** Rats were trained in drug discrimination assays to discriminate between a drug (extract) state and a no drug (saline) state under FR10 schedule of reinforcement for glucose rewards in standard Skinner Boxes. Trainings were continued until response rates approximated to a "steady" state. To determine whether mitragynine (the main alkaloid) will generalise to the extract, various doses of mitragynine was substituted with the extract. In an attempt to characterise the discriminative cues of the extract, another four groups of ten rats each were trained to respond differentially to training drugs and saline. The four training drugs were morphine, d-amphetamine, cocaine and pentobarbital. When the rats met the learning criteria, various doses of the extract and mitragynine were administered. **Results:** Rats trained to discriminate the extract and saline required many training sessions to reach the performance criteria, indicating that the extract produced weak control over differential lever responding compared to the more readily discriminable drugs like d-amphetamine and pentobarbital. Mitragynine showed generalisation to the extract. However, both the extract and mitragynine did not generalise to the four psychoactive drugs tested indicating different discriminative control. **Conclusion:** The findings suggest that *Mitragyna speciosa* has weak but unique discriminative stimulus properties and mitragynine may be the psychoactive constituents of the leaves.

CO20- BEHAVIOURAL AND THERAPEUTIC EFFECTS OF *Ignatia amara* CONTAINING COMPLEX HOMEOPATHIC REMEDIES

Wasilewski BW

Medical Centre for Postgraduate Education, Psychosomatic Institute, Poleczki str. 49, 02-822 Warsaw, Poland.
E-mail: b.wasilewski@ips.pl

Objective: With the example of treatment of menopause-related vegetative and emotional disturbances, the author verifies the effectiveness of the use of *Ignatia amara* plant extracts containing complex homeopathic remedies (IACCHR) by 933 patients. Very low cost of production for IACCHR group treatments and the lack of patent limitation with respect to the composition and manner of manufacture of these preparations, improves importance of this communication. **Results:** Substantial improvement in psychological and psychosomatic symptoms was observed. Climacteric complaints including depression diminished or disappeared completely in the majority of women. Compared to standard pharmaceuticals, IACCHR treatment was tolerated better and lower risk of side effects was observed. Specific effects in pharmacological tests confirmed psychotropic activity of *Ignatia amara*. Spontaneous locomotor and exploratory activity was evaluated by rats in the open field test; the influence on acquisition, consolidation and retrieval of conditioned responses were assessed in the passive avoidance situation using the one-trial-learning method. The elevated "plus" maze was used to estimate effects of IACCHR on anxiety. Drug was administered in form of Ignatia Homaccord preparation once at a single dose of 5 drops, or 5 drops was given every 15 min during one hour directly into the stomach. Single or multiple administered Ignatia Homaccord did not change mobility of rats in the open field test, except diminished rearings in 10 min of observation in manifold given Ignatia Homaccord - group of rats; and did not influence on acquisition and consolidation in the passive avoidance situation. This drug used once significantly enhanced retrieval of passive avoidance but used at a multiple doses impaired this process. Ignatia Homaccord given only at the single dose produced significant anxiolytic-like effect in elevated "plus" maze. **Conclusions:** Main benefit arising from the research conducted for this work is the identification of IACCHR – a new group of treatments, previously unidentified in the psychopharmacological literature.

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CO21- SURFACEN AND SP-A: PRECLINICAL RESEARCH IN RESPIRATORY DISTRESS SYNDROME

Blanco-Hidalgo O

Chemistry, Pharmacology and Toxicology Group, National Centre for Animal and Plant Health (CENSA), Apdo. 10, San José de las Lajas, La Habana, Cuba. E-mail:oblanco@censa.edu.cu

Introduction: The pharmaceutical application of exogenous natural pulmonary surfactant preparations has shown its efficiency in the therapeutical treatment of neonates with Respiratory Distress Syndrome (RDS). In Cuba, the natural exogenous pulmonary surfactant SURFACEN has proved to be effective in RDS. There are evidences that this treatment and addition of protein SP-A might be effective in other lung disease for example Acute Respiratory Distress Syndrome (ARDS). The present work shows the biophysical, anti-inflammatory and bactericidal properties of SURFACEN and antioxidant properties of SP-A. **Material and methods:** SURFACEN from porcine lung lavage was produced and supplied by CENSA and SP-A is obtaining by purification from lung lavage, biophysical properties was measured by interfacial, morphology and thermotropic methods; anti-inflammatory properties by *in vitro* and animal models and antioxidants properties of SP-A by Fenton system. **Results:** The results showed that SURFACEN presents properties similar interfacial to the natural surfactant, including the presence of quick transitions bicapamonomocapa, low surface tensions and stability during repeated cycles of compression-expansion. These properties turned out to be superior to those of a commercial exogenous surfactant. From the structural point of view SURFACEN show processes of lateral separation of similar phases to those of the native surfactant and thermotropic properties consistent in transitions of complex phases. SURFACEN administrated intratracheal in rats challenge with LPS, showed the inhibitory effect on myeloperoxidase activity, malonaldehyde levels and total cell number. Also was able to reduce the TNF level produced in LPS-stimulated monocytes and inhibit the ICAM-1 in cell assays. SURFACEN was able to reduce of colony forming units in all types of bacteria tested, showing antibacterial effect on bacteria causing lung disease. Sp-A is able to reduce the TBARS in dexosiribose assays and protects SURFACEN from oxidative stresses. These results demonstrate that SURFACEN can be considering as adequate preparation to improve the physiological status of ARDS patients and its enrichment with SP-A it will allow to have a more efficient pharmaceutical preparation. **Acknowledgments:** I wish to express my gratitude to Professor Jesús Pérez Gil, Department of Biochemistry and Molecular Biology, Faculty of Biology, Complutense University of Madrid, Spain for the opportunity offered to collaborate with CENSA staff on lung surfactant research, also to Professor Angel Catala from National University, La Plata, Argentina and Dr Rene Delgado from Chemistry and Pharmaceutical Center. Thanks are due to The Cuban Minister of Public Health and The Cuban Minister of Higher Education for financial support of the projects.

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CO22- CURCUMIN, NORDIHYDRGUIARETIC ACID, QUERCETIN AND RESVERATROL INHIBIT INTERLEUKIN-1-INDUCED ADAMTS-4 (AGGRECANASE-1) GENE EXPRESSION BY IN ARTICULAR CHONDROCYTES: NATURAL PRODUCTS AS POTENTIAL ANTI-ARTHRITIC AGENTS

El Mabrouk M, Ahmad R, Sylvester J and Zafarullah M

Department of Medicine, University of Montreal and Research Centre of CHUM Notre-Dame Hospital, 1560 Sherbrooke E, Montreal Quebec Canada H2L 4M1

Introduction: Interleukin-1 (IL-1 β) is the main proinflammatory cytokine stimulant of cartilage degeneration in arthritis. Aggrecanases (ADAMTS or A Disintegrin And Metalloproteinase with ThromboSpondin motifs) are enzymes implicated in tissue remodeling and cleavage of aggrecan core protein between Glu³⁷³-Ala³⁷⁴, a major structural protein of cartilage extracellular matrix giving its characteristic compressive stiffness. This study screened for natural products



with the ability to inhibit IL-1-induced ADAMTS-4 gene expression in human articular chondrocytes. **Methods:** Confluent normal human knee articular chondrocytes maintained in serum-free medium were pretreated either with natural products at different doses and stimulated further for 24 h with IL-1 β (10 ng/ml). Total cellular RNA was extracted. ADAMTS-4 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA levels were analyzed by RT-PCR. **Results:** IL-1 β induced ADAMTS-4 RNA in high-density human chondrocyte monolayer cultures. Pretreatment with a leukotriene and c-Fos (component of activating protein or AP-1 transcription factor) inhibitor, nordihydroguaiaretic acid (NDGA) suppressed the ADAMTS-4 RNA induction. Quercetin and Resveratrol at 50-100 μ M partially reduced ADAMTS-4 RNA induction. Further AP-1 and nuclear factor kappa B (NF- κ B) transcription factors inhibitor, curcumin partially inhibited aggrecanase-1 induction. The levels of internal control, GAPDH RNA remained consistent. **Conclusions:** Several natural products can interfere with proinflammatory cytokine signal transduction pathways (such as MAPKs) or their target transcription factors and thus inhibit IL-1 induction of ADAMTS-4 in chondrocytes. Such inhibition warrants further studies on toxicology and potential for reducing ADAMTS-4-driven cartilage resorption in arthritis. **Support:** Canadian Institutes of Health Research, the Arthritis Society, Wyeth Canada and Canadian Arthritis Network.

CO23- A NOVEL, FULLY DISSOCIATED COMPOUND OF PLANT ORIGIN FOR INFLAMMATORY GENE REPRESSION

De Bosscher K¹, Vanden Berghe W¹, Beck I¹, Van Molle W², Hennuyer³, Haggood J⁴, Libert C², Staels B³, Louw A⁴ and Haegeman G¹

¹Laboratory of Eukaryotic Gene Expression and Signal Transduction (LEGEST), Department of Molecular Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium. E-mail: guy.haegeman@ugent.be ²Department for Molecular Biomedical Research, (V.I.B.) and UGent, Technologiepark 927, B-9052, Zwijnaarde, Belgium. ³Département d'Athérosclérose - U.545 Inserm Institut Pasteur de Lille, 1 rue Calmette BP245, 59019 Lille cedex, France. ⁴Department of Biochemistry, University of Stellenbosch, Matieland 7602, Stellenbosch, Rep. of South Africa

The identification of selective glucocorticoid receptor (GR) modifiers, which separate transactivation and transrepression properties, represents an important research goal for steroid pharmacology. Here we present that Compound A (CpdA), a plant-derived phenyl aziridine precursor, although not belonging to the steroidal class of GR-binding ligands, does mediate gene-inhibitory effects by activating GR. CpdA exerts an anti-inflammatory potential by downmodulating TNF-induced pro-inflammatory gene expression, such as IL-6 and E-selectin, but interestingly, does not enhance GRE-driven genes or induce GR binding to GRE-dependent genes *in vivo*. The anti-inflammatory mechanism involves both a reduction of the *in vivo* DNA-binding activity of p65 as well as an interference with the transactivation potential of NF- κ B. Finally, CpdA is as effective as DEX in counteracting acute inflammation *in vivo*, and does not cause a hyperglycemic side effect. Taken together, this compound may be a lead compound of a novel class of anti-inflammatory agents with fully dissociated properties and might thus hold great potential for therapeutic use.

CO24- ISOLATION, CHARACTERIZATION AND IMMUNOMODULATING EFFECT OF PECTIC POLYSACCHARIDES

Popov SV, Ovodova RG, Golovchenko VV, Koval OA, Markov PA, Nikitina IR, Popova GYu and Ovodov YuS

Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str., 167982, Syktyvkar, Russia, E-mail: popov@physiol.komisc.ru

Pectic polysaccharides have been shown to possess potent anti-infectious and anti-tumor activities due to activation of phagocytes (neutrophils and macrophages) as predominant immunopharmacological effect. The present study is devoted to isolation of pectins from plants of the European North of Russia and an elucidation of their capability to effect on the immunity in dependence of structural features. Pectins (Mw 100-300 kDa) of duckweed *Lemna minor* (lemnan LM), marsh cinquefoil *Comarum palustre* (comaruman CP), elephant ear *Bergenia crassifolia* (bergenan BC) and some other plants were shown to contain the linear α -1,4-D-galacturonan as the backbone and individual branched heterogalacturonans as the "hairy" regions. The following pectins: bergenan, lemnan and comaruman were found to belong to various types of pectic polysaccharides, namely, galacturonan, rhamnogalacturonan and apiogalacturonan, respectively. At oral administration (50-100 mg/kg), lemnan LM and bergenan BC were found to



enhance the immune response to oral antigen of laboratory mice. Comaruman CP was shown to exhibit anti-inflammatory action in relation to carrageenan-induced paw swelling and acetic acid-induced colitis. Fragmentation of the parent pectins was carried out using partial acidic (2M TFA, 100 °C for 5 h) and enzymic hydrolysis (pectinase "Fluka") and different fragments of pectic macromolecules were obtained. The galacturonic backbone was determined to be an active region of the macromolecule of comaruman CP. Galacturonic fragments of bergenin and lemnan were also found to possess anti-inflammatory effect. Galacturonans (50-200 mkg/ml) obtained were found to diminish adhesion to fibronectin of human neutrophils stimulated by phorbol 12-myristate 13-acetate (1.6 mkM) whereas the parent pectins except comaruman failed to influence on cell adhesion. Branched apiogalacturonic fragment of lemnan LM was shown to mediate immunostimulating effect of the pectin. Lemnan was found to increase a dose of ingested antigen. Thus, the linear galacturonic backbone and branched region of pectic macromolecule appeared to mediate anti-inflammatory and immunostimulating activity of pectins, respectively.

CO25- CHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF *Metaxya rostrata*

Virtbauer J^a, Krenn L^a, Kählig HP^b, Hühner A^c, Marian B^d

^a Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria. ^bInstitute of Organic Chemistry, University of Vienna, Währinger Strasse 38, A-1090 Vienna, Austria. ^cInstitute of Pharmaceutical Sciences, University of Graz, Schubertstrasse 1, A-8010 Graz, Austria. ^dDepartment of Internal Medicine 1, Institute of Cancer Research, Medical University Vienna, Borschkegasse 8a, A-1090 Vienna, Austria.

The development of new therapeutics for colorectal cancer is an important ongoing task for which tropical plants used in ethnomedicine provide a rich source (Cragg & Newman, 2005). *Metaxya rostrata* (Kunth) C. Presl is a tropical fern distributed widely throughout Middle America. Suspensions of the pulverized rhizome in water are administered orally in Costa Rican ethnic medicine in the therapy of intestinal tumors. Thus, in the presented investigation an aqueous and a methanolic extract of *Metaxya rostrata* roots were analysed phytochemically and several compounds were isolated by bio-assay guidance. The cytotoxic effects of the extracts and fractions thereof were determined in cultures of SW480 colorectal carcinoma cells. The extracts showed cytotoxicity in a concentration-dependent way. Cell loss was caused by apoptosis as shown by the characteristic morphology of the nuclei. In addition FACS analysis using JC1 as mitochondrial tracker showed loss of mitochondrial membrane polarization in about 25% of the population exposed to *Metaxya* extracts. In addition, nuclei with increased size suggested a cell cycle block in G2 which was also shown by FACS analysis. The extracts were fractionated by partition procedures with solvents of different polarity to avoid losses by absorption. By sub-sequent testing of the resulting fractions the most promising ones were selected for further fractionation by vacuum liquid chromatography (=VLC) and in the next step by gel permeation chromatography on Sephadex[®]LH 20. After this fractionation 2 main fractions contained cytostatic activity causing a cell cycle block and apoptosis. From one of those two trimeric proanthocyanidins, cinnamtannin B-1 and aesculitannin B were isolated and characterized by mass spectrometry, ¹H-, ¹³C-NMR spectroscopy as well as by 2D NMR techniques, thereby finding two interchangeable rotational isomers for cinnamtannin B-1 in solution. In addition saccharides and sterols such as sitosterol, stigmasterol and campesterol were identified.

Reference: Cragg GM, Newman DJ. (2005) *J Ethnopharmacology* 100: 72-79.

CO26- ASCORBATE AND HYPOXIC RESPIRATORY REACTIVITY

Pokorski M

Department of Respiratory Research, Medical Research Center, Polish Academy Sciences, Warsaw, Poland

The redox state of carotid chemoreceptor cells is influential in chemotransduction. Ascorbic acid (AA) is present in the cat carotid body where it gets depleted in hypoxia (Pokorski & Gonet, *Respir Physiol*, 107:213-218, 1997). Supplementation of AA also enhances the hypoxic ventilatory response (HVR) in older human subjects (Pokorski & Marczak, *J Int Med Res* 31: 448-457, 2003). The role of AA, a water-soluble compound, should be enhanced if it penetrated the lipid bilayers, the target sites of signal transduction. In the present study we addressed this issue by using the lipid-soluble ascorbyl-6-palmitate (AP), given by gavage (600 mg/kg daily for 6 days) to cats. The control group received the vehicle only. The animals were then anesthetized, paralyzed, vagotomized, ventilated, and subjected to isocapnic hypoxia (7% O₂ in N₂). The effect of AP on HVR was assessed from the phrenic nerve output.



We found that AP significantly augmented the peak stimulation and slowed down the roll-off of the biphasic HVR. The other objective of this research was to study the ability of AP to penetrate both central and peripheral neural tissues, assessed *in vitro* from HPLC measurements of the ascorbate moiety recovered from the cortex and the carotid body, dissected from the AP-treated or AA-treated cats. HPLC showed that the content of the ascorbate moiety was markedly higher in both cortical and carotid body tissues after AP than that after AA, with the predominance of the carotid body. We conclude that AP is biologically active with respect to respiration and interferes with the hypoxia-sensing mechanisms at the carotid body, the organ that seems to be a target for this reducing compound.

CO27- A PLANT POLYPHENOL EXTRACT AMELIORATES THE DISFUNCTIONS OF ALVEOLAR MACROPHAGES IN INFLUENZA VIRUS-INFECTED MICE

Toshkova R¹, Stefanova Ts², Nikolova N², Serkedjieva J²

¹Institute of Experimental Pathology and Parasitology, ²Institute of Microbiology, ³Institute of Botany, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria. Email: jserkedjieva@microbio.bas.bg

A semi-standardized polyphenol extract from the medicinal plant *Geranium sanguineum* L. (PC) protected mice from mortality in the experimental influenza virus infection (EIVI). At the same time it was found that PC stimulated *in vitro* the phagocytic activity of peritoneal macrophages and blood polymorphonuclears and suppressed NO production. The current study was undertaken to reveal the effect of PC on the number and functions of alveolar macrophages (aMØ) from PC-treated healthy and influenza virus-infected mice (VIM) and on the production of ROS and RNI. Mice were challenged intranasally (i.n.) with 10 LD₅₀ of influenza A/Aichi/2/68 (H3N2) virus. PC (10 mg/kg) was administered by i.n. instillation 3 h before infection. Influenza infection provoked a significant increase of H₂O₂, O₂⁻ and NO production, which peaked on day 9 post infection (p.i.). PC-treatment decreased the release of ROS and RNI; this resulted in reduced lung tissue damage. In the infected and healthy controls PC-treatment induced a continuous 2.5-4-fold rise of the number and migration of aMØ, the maximum being on day 9 p.i. Influenza infection impaired also the phagocyte functions of aMØ; PC-treatment restored them and on day 9 p.i. phagocyte indices reached control values. In healthy mice the phagocyte abilities of aMØ were enhanced markedly after the application of PC. Interestingly, *in vitro* in the dose of 25 µg/ml, PC did not affect the phagocyte activity and the migration of aMØ. The restoration of the compromised functions of aMØ in VIM was consistent with a prolongation of the mean survival time and reduction of the mortality rate and the infectious virus titer. The obtained results outlined the immunomodulatory properties of the plant preparation and demonstrated its beneficial effect on the oxidative stress response in influenza virus-induced pneumonia. These alternative mechanisms of action might contribute to the overall protective effect in the lethal murine experimental influenza infection.

CO28- OZONE OXIDATIVE PRECONDITIONING REDUCES MULTIORGAN DAMAGE AND STIMULATES ANTIOXIDANT SYSTEM IN PERITONEAL SEPSIS INDUCED IN RATS

Zamora Z^a, Guanche D^a, González R^a, Hernández F^a, Alonso Y^a and Schulz S^b

^aLaboratory of Biological Assays, Department of Biomedical Research, Ozone Research Center. P. O. Box 6414, Havana, Cuba. ^bVeterinary Services and Laboratory Animal Medicine, Philipps-University of Marburg, Marburg 35033, Germany. Email: zullyt.zamora@cnic.sld.cu

Sepsis and septic shock are the major cause of morbidity and mortality in patients admitted to the intensive care units in United States and many others countries (1). Recently, we demonstrated the protective effect of oxidative preconditioning with low doses of ozone in mixtures with oxygen (OOM) in the acute renal failure induced by cisplatin in rats. Therefore, taking into account the paramount important of septic shock and the multi-organ failure, which provokes its high mortality, we decided to investigate the potential protective effects of ozone oxidative preconditioning (OOP) in an experimental model of peritoneal sepsis induced in male Wistar rats. These animals were divided into seven groups of them each one: 1) non-treated control rats, 2) control group with sepsis induced by intraperitoneal injection of rat fecal material (0.55 mg/kg b.w); 3) OOM (0.8 mg/kg, i.p plus sepsis); 4) OOM (2.4 mg/kg, i.p plus sepsis); 5) OOM (4 mg/kg, i.p plus sepsis); 6) oxygen-treated, i.p control plus sepsis; 7) OOM (4 mg/kg, i.p without sepsis. 24 h after the last administration of OOM treatment septic shock was induced. Blood was taken from all the animals for biochemical determination in serum of ASAT, ALAT and creatinine, as well as other biochemical tests



indicators of oxidative stress such as GSH-Px, SOD, CAT and TBARS content. Additionally myeloperoxidase (MPO) was measured in rats' lung.

The results demonstrated that OOP with OOM is able to restore the endogen antioxidant system, because induced significant increased of the enzymatic activities of SOD, GSH-Px. Also ALAT and ASAT activities and creatinine levels in blood of rats were significant reduced by OOM which provides strong evidence in favor of renal and hepatic protective effects of ozone. Furthermore, in concordance with these findings MPO in rat lung was also decreased in 34 % with the lower dose of OOM used. In summary, OOP is able to decrease the multi-organ damage induced by septic shock and to increase the endogen antioxidant defense as well as several enzymes and markers of multi-organ.

References: 1. Angus, D.C., Linde-Zwirble, W.T., Lidicker, C.G., Carcillo, J and Pinsky, R.M. (2002) The epidemiology of severe sepsis in the United States: analyses of incidence, outcome and associated cost of care. *Crit Care Med* 29: 1303-1310. 2. Ramon, L. Añel., Dellinger, R.P. (2001) Sepsis and bacteremia. In: *Conn's Current Therapy*, Rakel RE, Bope ET (Eds.), WB Saunders Co: 56-62.

CO29- ANTICOCCIDIAL SCREENING OF *Azadirachta indica* (NEEM) IN BROILERS

Rao-Zahid A¹, Iqbal Z¹, Akhtar MS², Khan MN¹, Jabbar A¹, Sandhu Zia-U-din¹

¹Department of Veterinary Parasitology, ²Department of Physiology and Pharmacology, University of Agriculture, Faisalabad-38040, Pakistan. E-mail: raouaf@hotmail.com

Anticoccidial screening of *Azadirachta indica*, fruit (Neem) was carried out in broiler chickens naturally infected with a mixed *Eimeria* infection @ 50,000 per bird. Powdered *Azadirachta indica* fruits were administered orally in doses of 10, 15 and 20 mg/Kg body weight and their water and methanol extracts in amounts equivalent to 20mg/Kg of the powder were also administered. As a control drug Amprol plus (R) was administered orally. Faecal oocyst per gram (OPG) counts were made by the Mc Master Egg Counting technique. *Azadirachta indica* powder as well as its extracts in methanol and water significantly ($P < 0.05$) reduced the OPG counts at the dosage levels of 10, 15 and 20 mg/Kg. The percentage reduction in OPG counts on the 3rd, 10th and 15th days after administration of 10mg/Kg body weight of drug were 8.2 ± 5.5 , 13 ± 8.0 and 22 ± 4.1 , respectively. Treatment with 15 mg/Kg body weight of the *Azadirachta indica* powder produced 18 ± 5 , 29 ± 8 and 33 ± 11 percent reductions on days 3rd, 10th and 15th, respectively. The respectively OPG reductions in the group treated with 20mg/Kg were 22 ± 8 , 70 ± 14 and 85 ± 20 percent. The OPG count reductions at this dose were non significantly different from that of control drug on day 15th. The aqueous extract equivalent to 20mg/Kg body weight of the powder produced percentage reductions of 24 ± 6 , 61 ± 11 and 68 ± 16 on 3rd, 10th and 15th days post treatment, respectively. The OPG reduction produced by the methanol extract of *Azadirachta indica* were 12 ± 6 , 58 ± 13 and 78 ± 14 percent on days 3rd, 10th and 15th, respectively, showing a non significant ($P > 0.05$) difference in the OPG count reduction from the control drug on the 15th day. The data suggest that single oral administration of 20mg/Kg of *Azadirachta indica* fruits and their extracts in methanol and water in equivalent amounts are effective in controlling the *Eimeria* infection in chickens. However, since at higher doses mortality was observed they should not be used alone for therapeutic purposes in the poultry birds but may be recommended perhaps in smaller amounts along with other herbal ingredients.

CO30- COMMERCIALIZATION OF HARUAN *Channa striatus* BIOMEDICAL PRODUCTS

Manan-Mat JA

Department of Biomedical Sciences. Faculty of Medicine and Health Sciences. University Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia

Haruan *Channa striatus* is an indigenous fresh water carnivorous air breathing fish species, widely distributed within the country, both the West and East Malaysia, and other members of the same family Channidae are also found in neighboring countries in the region. The fish is known as natural remedy in traditional medicine, and popular among post-operative patients especially caesarian mothers and injuries due to road accidents to induce wound healing. Furthermore, with the quality of the biochemistry of both wild and cultured fishes are equally good, haruan which contained high level of the essential amino acids and good profile of fatty acids that could directly involves in tissue growth and healing is certainly a promising candidate for future nutraceutical and pharmaceutical products. Obviously this white boneless meaty and tender taste fish is providing a good source of protein, and is a popular restaurant menu in many countries in the region namely Vietnam, Cambodia, Thailand and Malaysia. As part of the interesting wound



healing physiological and pharmacological properties of the haruan extracts, to name a few are its anti-microbial, anti-inflammation, cell proliferation and inducing platelet aggregation. The latest, which is the platelet aggregation, is now the on-going collaborative works to establish the actual mechanism and the bioactive involves in the activity. The other interesting and important of the haruan's extract, is the anti-nociceptive properties that has been recognized by the Society of Anaesthesiologist as the most original Paper Commendation Award at their Annual Scientific Meeting, in Singapore 17 April 1997. Our group is almost identify the bioactive compound(s), and now working to commercialize our award winning Haruan Based Biomedical Products, while the clinical trials are still on-going activities. I am hoping here to share experiences, looking for outsourcing facilities, establish networking and collaboration especially in area of drug discovery, analytical chemistry and commercialization of biomedical products.

CO31- HISTORY OF LATIN-AMERICAN AND CARIBBEAN BULLETIN OF MEDICINAL AND AROMATIC PLANTS (BLACPMA)

Martínez JL¹, Medina ME², Garrido G³, Arenas P⁴, Prieto JM⁵

¹UNICIT, Santiago, Chile; ²TRAMIL Nicaragua, ³Centro de Química Farmacéutica, La Habana, Cuba, ⁴Universidad Nacional de La Plata, Argentina, ⁵Universidad de Londres, Reino Unido.

The first BLACPMA was issued five years ago -after a talk held by José L. Martínez (UNICIT, Chile) and Jorge Rodríguez Chanfreau (CIDEM, Cuba)- as a communication tool to keep informed the Latin-American community on the many events related with Medicinal and Aromatic Plants taking place around the World. This primary objective is still achieved by means of the so-called "supplements" issued in between each bulletin. The official launch of the bulletin took place in Buenos Aires, Argentina, during the First Latin-American Congress of Phytochemistry, and it was backed by an Editing Board composed by Arnaldo Bandoni, Argentina; Patrick Moyna, Uruguay; Francisco Morón, Cuba, and Lionel Robineau, Guadalupe, recently replaced by María E. Medina, Nicaragua. BLACPMA started to feature scientific contributions in its second year of life. Since then, the number of papers submitted has increased steadily as well as their quality, and some of them are signed by highly recognized authors. We still remember with pleasure the reception of the first European contribution and the surprising arrival of the first African and Asian contributions. Nowadays we are receiving papers from over the World in a regular basis. The complexity of the edition process was increasing accordingly, and it was necessary to engage people specially committed with its supervision. The first 'supervisor of edition' was Patricia Landazuri (Colombia), followed by Rita Zeichen (Argentina). Today, this critical task is performed by Patricia Arenas (Argentina) and Gabino Garrido (Cuba). The bulletin was soon indexed by LATINDEX, and recently by the more internationally known INDEX COPERNICUS. This "step-up" was possible after the radical change that BLACPMA underwent under the impulse of José María Prieto (University of London, UK), who, after January 2004, became fundamental in raising the standards of the bulletin, and putting it in the international scientific arena. Proof of this is the fact that articles published in BLACPMA has started to be cited by other articles published by major journals in the field like Journal of Ethnopharmacology and Economic Botany (1, 2). Finally, it is already a few years that under the initiative of Lionel Robineau, we started to work on the idea of organizing our own Symposia. The effort has been fruitful and we are now celebrating at Varadero the First Symposium of BLACPMA that hopefully will be followed by many more. With your help we very much expect to see how BLACPMA continues its evolution until becoming an important reference in the Medicinal and Aromatic Plants field.

References: 1. Journal of Ethnopharmacology, 106: 429-441, 2006. 2. Economic Botany, 60:159-181, 2006.

CO32- NATURAL ANTIPLATELET AGENTS

García Mesa M

144 Velásquez St., 10600 Cerro, Havana, Cuba. Angiology and Vascular Surgery National Institute. 1551 Calzada del Cerro, 12000 Cerro, Havana, Cuba. E mail: milagros.mesa@infomed.sld.cu

Introduction: Antiplatelet agents are useful as antithrombotic drugs. **Objectives:** To show that natural products could provide new alternatives for antiplatelet therapy. The analysis of the effects of the Policosanol, a by-product from sugar cane wax, in clinical assays is an example. **Methods:** The following experimental designs have been performed in order to assess the antiplatelet effect of: double blind randomized versus placebo clinical assay of Policosanol (1 mg/day x 12 weeks) in hypercholesterolemic patients. A crossed-over: double blind randomized versus placebo



clinical assay of Policosanol (10 mg/day x 7 days) in type II diabetic patients was the second experimental design. A descriptive study on platelet reactivity of atherosclerotic patients consuming Policosanol, aspirin or both was performed too. The decrease of platelet aggregation in platelet-rich plasma induced by physiologic stimuli (ADP, collagen or epinephrine) was the end point of the pharmacological effect. **Results:** Platelet aggregation was significantly lower in hypercholesterolemic and diabetic patients treated with Policosanol than in those in the placebo groups. Furthermore, there were not statistical difference among the groups of atherosclerotic patients who were taking Policosanol, aspirin or both with respect to the inhibition of ADP, collagen and epinephrine-induced platelet aggregation, suggesting the similarities of Policosanol and aspirin with regard to the antiplatelet efficacy and mechanism of action. **Conclusion:** These results suggest that Policosanol may be an alternative for antiplatelet therapy.

CO33- EVALUATION OF PLATELET ANTIAGGREGANT EFFECT OF *Hypericum perforatum* EXTRACTS AND ISOLATED COMPOUNDS

Costa D^a, Monteiro MC^b, Gonçalves MJ^c, Almeida-Dias A^b and Dias ACP^a

^aUniversity of Minho (a), Campus de Gualtar, 4710-057 Braga, Portugal. ^bPolytechnic Institute of Health - North (b), Rua Central de Gandra, 1317, 4585-160 Gandra PRD, Portugal. ^cSuperior Institute of Health Sciences - North (c), Rua Central de Gandra, 1317, 4585-160 Gandra PRD, Portugal. Email: dcosta@bio.uminho.pt; acpdias@bio.uminho.pt

Platelet activation plays an important role in cardiovascular diseases (CVD), and the enhancement of platelet aggregation is a known mechanism that increases the risk of CVD. *Hypericum perforatum* (St. Johns wort) is an important medicinal plant used for its biological activities such as antidepressive, anti-inflammatory and healing agent. Additionally, *H. perforatum* contains several compounds known for their antioxidant activity that could be relevant in the inhibition of platelet aggregation (PA). The aim of our study was to evaluate the inhibitory effect of different ethanolic extracts (TEE) and a pure compound (II3-III8 biapigenin) of *H. perforatum*, on PA induced by different physiological agonists. We tested three *H. perforatum* TEE: one of *in vivo* plants, other from *in vitro* shoots and another from cultured suspension cells; and the compound biapigenin. PA induced by collagen, epinephrine and ADP was studied in platelet rich plasma (PRP), from healthy volunteers. PRP was incubated with several concentrations of the TEE or biapigenin, for 15 minutes, and aggregation was performed using the turbidimetric technique. All tested extracts significantly inhibited PA induced by collagen (1µg/mL), and the inhibitory effect was dose dependent. Comparing the IC₅₀ of all extracts, the most effective was obtained from cultured cells (0,28mg/ml). A similar response was obtained for maximal aggregation rate, evaluated by slopes, which decreased as the tested dose increased for all extracts. In the same way, was observed inhibition in PA induced by ADP (5µM) and epinephrine (10µM). Results obtained with biapigenin indicated that this is an active compound of the plant, as platelet antiaggregant. Our results show that tested extracts of *Hypericum perforatum* as well as biapigenin, have platelet antiaggregant activity and emphasize the need for further studies. We believe, it could be recognized as a substance with anti-platelet properties and thus considered in the prevention of cardiovascular diseases.

CO34- SERUM SQUALENE AND NON-CHOLESTEROL STEROLS RELATED TO CHOLESTEROL SYNTHESIS AND ABSORPTION IN TYPE 2 DIABETES

Simonen P, Gylling H, Miettinen-Tatu A

Department of Medicine, Division of Internal Medicine, University of Helsinki, Helsinki, Finland, and Department of Clinical Nutrition, University of Kuopio, and Kuopio University Hospital, Kuopio, Finland

Serum non-cholesterol sterols, cholestanol and plant sterols, campesterol and sitosterol, are known to positively reflect cholesterol absorption and negatively cholesterol synthesis. Opposite associations are obtained for cholesterol precursors, including squalene, cholestenol and lathosterol. We compared non-cholesterols and squalene in serum with those in lipoproteins, and related the values to absolute cholesterol synthesis and absorption of patients (n=33) with type II diabetes, range of body mass index (BMI) being 21-40. Lipids in serum and ultracentrifuge fractions were measured with routine laboratory methods. Gas liquid chromatography was used to measure cholesterol, squalene and non-cholesterol sterols in serum and lipoproteins. Sterol balance technique was used to determine absolute cholesterol synthesis and a double label system for measurement of absorption percentage of dietary cholesterol.



Two-thirds of the non-cholesterol sterols were carried in LDL and one-fifth in HDL, whereas squalene was mainly in VLDL and LDL. The synthesis and absorption markers were interrelated in serum and all lipoproteins suggesting intact regulation of cholesterol metabolism. The absorption and synthesis marker ratios to cholesterol were mostly similar in serum and lipoproteins, even though absorption sterols accumulated to HDL and IDL and synthesis markers in VLDL and IDL. The ratios to cholesterol of absorption markers were negatively, those of synthesis markers mostly positively related to BMI not only in serum but also in most lipoprotein fractions, and also to respective absolute synthesis and absorption percentage of cholesterol. Also, the proportions of synthesis marker sterols to those of absorption markers (eg. lathosterol/sitosterol) were positively related to BMI and absolute synthesis and negatively to absorption percentage of cholesterol. The findings indicate that even in patients with type II diabetes, including markedly increased body weight and altered cholesterol metabolism, measurement of serum non-cholesterol sterols and squalene reveals information of cholesterol synthesis and absorption without complicated clinical and laboratory methods.

CO35- RED WINE POLYPHENOLS PREVENT CARDIOVASCULAR ALTERATIONS IN EXPERIMENTAL HYPERTENSION: MOLECULAR MECHANISM

Pechánová O^a, Bernátová I^a, Babál, P^b, Martínez MC^c, Cacányiová S^a, Stvrtina S^b, Andriantsitohaina R^c

^aInstitute of Normal and Pathological Physiology, Slovak Academy of Sciences, ^bDepartment of Pathology, Medical Faculty, Comenius Univ., Bratislava, Slovak Republic, ^cUniversité Louis Pasteur de Strasbourg, UMR CNRS 7034, Faculté de Pharmacie, Illkirch, France. Email: olga.pechanova@savba.sk

Introduction: Red wine polyphenols have been reported to possess beneficial properties for preventing cardiovascular diseases but their effects on hemodynamic and functional cardiovascular changes in association with their molecular mechanisms are studied much less. **Design:** The effects of the red wine polyphenols, Provinols™, on arterial hypertension as well as left ventricular (LV) hypertrophy, myocardial fibrosis and vascular remodeling were investigated in rats during chronic inhibition of nitric oxide synthase (NOS) activity. Rats were divided into four groups: a control group, a group treated with N^G-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day), a group receiving Provinols™ (40 mg/kg/day) alone or Provinols™ plus L-NAME. **Results:** Provinols™ markedly reduced the increase in both blood pressure and protein synthesis in the heart and aorta caused by chronic inhibition of NO synthesis. Provinols™ reduced myocardial fibrosis even though it did not affect LV hypertrophy. In addition, Provinols™ prevented aortic thickening and corrected the augmented reactivity of the aorta to norepinephrine and the attenuated endothelium-dependent relaxation to acetylcholine in NO- deficient rats. These alterations were associated with an increase of NOS activity, a moderate enhancement of eNOS protein expression, with reduction of oxidative stress and a decrease of redox-sensitive transcription factor NF-κB expression in the LV and aorta. **Conclusion:** Our results provide evidence that Provinols™ partially prevents L-NAME-induced hypertension, cardiovascular remodeling and vascular dysfunction via the increase of NO-synthase activity and prevention of oxidative stress. Thus, the beneficial effects of plant polyphenols in prevention of hypertension may result from their complex influence on the NO balance in the cardiovascular system. **Supported** by VEGA 2/6148/26, 1/3429/06 and APVV-51-018004.

CO36- VITAMIN E PREVENTS FOAM CELL FORMATION AND DEVELOPMENT OF ATHEROSCLEROSIS

Kartal-Özer N

Marmara University, Turkey.

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CO37- PRESENTATION OF CENTER OF TOXICOLOGICAL AND BIOMEDICAL INVESTIGATION OF SANTIAGO DE CUBA. DEVELOPMENT PERSPECTIVES IN THE INVESTIGATIONS WITH NATURAL PRODUCTS

TOXIMED

Santiago de Cuba, Cuba.

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CO38- IMMUNOLOGICAL EFFECTS OF AN *Echinacea purpurea* EXTRACT IN PATIENTS WITH BREAST CANCER

Elsässer-Beile U¹, Bühler P¹, Wetterauer U¹, Rostock M², and Bartsch H²

¹Department of Urology, University of Freiburg, Germany. ²Tumor Biology Center at the University of Freiburg, Germany. Email: ursula.elsaesser@uniklinik-freiburg.de

Introduction: *Echinacea purpurea* extracts have been applied for immunostimulation for many years. However, the clinical application is based mainly on delivered practical experiences rather than on the results of clinical studies. Since the cell-mediated immune response is controlled by a variety of soluble cytokines, measurements of these substances may be a means for determining the cellular immunological potential. The current report focuses on the measurement of *ex vivo* cytokine production of patients treated orally with an aqueous extract from *Echinacea purpurea* in order to evaluate whether these immunological parameters are changed during the treatment. **Materials and Methods:** 115 patients aged between 32 and 73 years, were entered into the study after curative treatment of breast cancer. These patients were randomly distributed in three groups: A) no therapy (n=35), B) therapy with 8 ml *Echinacea* extract daily for 3 weeks (n=43) and C) therapy with 24 ml *Echinacea* extract daily for 3 weeks (n=37). From all patients heparinized blood was taken at day 0, 14 and 21. For cell culture the blood was stimulated with 10 µg/ml PHA or 5 µg/ml PWM for 2 days. In the supernatants the levels of the cytokines IL1-beta, IL2, IL6, IFN-gamma and TNF-alpha and the soluble cytokine receptors for IL2 and TNF-alpha were determined by ELISA. **Results:** Comparison of *ex vivo* leukocyte cytokine production in the blood cell cultures of the patients without treatment (group A) revealed no significant change between day 1 and day 21. In the blood cell cultures of the patients of group B a slight, but not significant increase of IFN-gamma was found. In the blood cell cultures of the patients treated with 24 ml *Echinacea* extract daily (group C) a significant increase of IL-1-beta, IFN-gamma, TNF-alpha and TNF-receptor was seen between day 1 and day 21. **Conclusions:** *Echinacea* extract seems to have dose dependent immunostimulatory effects on monocytes and lymphocytes.

CO39- INVESTIGATION OF SOUTH AFRICAN PLANTS FOR ANTI-CANCER PROPERTIES

Fouche G, Khorombi E, Kolesnikova N, Maharaj VJ, Nthambeleni R and van der Merwe M

Bioprospecting, Biosciences, CSIR, PO Box 395, Pretoria, 0001, Republic of South Africa.
E-mail: gfouche@csir.co.za

A collaborative research programme between the Council for Scientific and Industrial Research (CSIR) in South Africa and the National Cancer Institute (NCI) in the USA aimed at the screening of plant extracts and identification of potentially new anti-cancer drug leads was initiated in 1999. Plant extracts that exhibited anti-cancer activity against a panel of three human cell lines (breast MCF7, renal TK10 and melanoma UACC62) at the CSIR were screened by the



NCI against sixty human cancer cell lines organized into sub panels representing leukaemia, melanoma and cancer of the lung, colon, kidney, ovary and central nervous system. To date 7500 randomly selected plant extracts representing 700 taxa were screened for anti-cancer activity at a single concentration of 100 ppm. Extracts which exhibited a growth inhibition of above 75% for two or more of the cell lines (GI₇₅) were advanced into the dose response assay at a concentration range of 6.25-100 ppm. Extracts which exhibited a total growth inhibition (TGI) of less than 6.25 ppm for at least two cell lines were regarded as potent. The results indicated that a hit rate of 3.4% was obtained based on the number of taxa screened. Although the extracts of these taxa were randomly selected during the screening programme, 88% of these taxa were reported to be used medicinally. The potent plant extracts were evaluated for efficacy at the NCI against a panel of sixty human cancer cell lines over a defined range of concentrations to determine the relative degree of growth inhibition against each cell line. Desktop literature investigations aimed at establishing information on the scientific validation of the plants demonstrating anti-cancer activity were conducted. The extracts of taxa with limited scientifically published information for their anti-cancer properties were subjected to bioassay-guided fractionation and the active constituents were isolated and identified. Results from this study led to the isolation and identification of known metabolites that have been described in the literature studies and were either patented or published for their use as anti-cancer agents. Anti-cancer activity was demonstrated for several metabolites and a few examples are discussed. These anti-cancer screening results mirrored the NCI experience where essentially, no new plant-derived clinical anti-cancer agents had been found from plants since the discovery of the taxanes and camptothecins in the early (1960-1980) program (Cragg et al, 2005). Based on the outcome of this screening programme, a strategy was employed to target endemic plant species as well as plant species containing selected classes of compounds. An evaluation of these plant species is ongoing.

Reference: Cragg G.M. and Newman D.J., Plants as a source of anti-cancer agents, *Journal of Ethnopharmacology*, 2005; 100: 72-79.

CO40- INTRAVESICAL MISTLETOE EXTRACT FOR ADJUVANT TREATMENT OF SUPERFICIAL URINARY BLADDER CANCER

Bühler P, Leiber C, Wetterauer U, Elsässer-Beile U

Department of Urology, University of Freiburg, Germany. Email: patrick.buehler@uniklinik-freiburg.de

Introduction: Patients with superficial bladder cancer are mostly treated by transurethral tumour resection and adjuvant intravesical Bacillus Calmette-Guerin (BCG), which was shown to reduce tumour recurrence significantly. However, serious side effects of this treatment promoted the search for other immunoactive substances, which to date have failed to show the equal efficacy as BCG. Therefore, the aim of the present study was to evaluate the effect of intravesical mistletoe extract (ME) with respect to tolerability and recurrence rate. **Materials and Methods:** In a phase I/II clinical trial an aqueous ME, standardised to mistletoe lectin, was administered intravesically to 24 patients with urinary bladder cancer of the stages pTa G2 (n=14) and pT1 G2 (n=10). Four to seven weeks after transurethral resection each patient received 6 instillations at weekly intervals with 50 ml of ME at lectin concentrations between 10 ng/ml and 5,000 ng/ml. Three patients per group received a dose, which was then doubled in the next group. Clinical follow-up consisted of cytology, cystectomy and random biopsies. For determination of cytokine secretion, venous blood and urine samples were taken before instillation, and 2, 6, and 20 hours later. The local historical control group consisted of 18 patients with pTa G2 (n=5) and pT1 G2 (n=13) tumours that were treated with 6 BCG instillations after transurethral resection. **Results:** The tolerability of the ME was very good at all applied concentrations. None of the patients had local or systemic side effects. Within the observation time of 12 months, the patients treated with ME showed a recurrence rate of 8/24 (33 %). In the BCG treated group the recurrence rate was 5/18 (28 %) and therefore similar in both groups. Comparison of blood and urine cytokine levels brought about no significant alterations before and after instillation of ME. **Conclusions:** Intravesically applied standardized ME could be a potential alternative adjuvant therapy for superficial bladder cancer.



CO41- ANTITUMORAL AND ANTIMETASTATIC EVALUATION OF THE BROMELINA OBTAINED FROM STEMS OF PINEAPPLE (*Ananas comosus* (L) MERRILL)

Báez R¹, Hernández M², Vásquez A¹

¹Carretera a Moron, CP: 65200, Ciego de Ávila. Cuba. Facultad de Ciencias Médicas de Ciego de Ávila. rosanna@trocha.cav.sld.cu ²Lab. Ingeniería Metabólica. Centro de Bioplasmas. UNICA. Ciego de Ávila. Cuba.

Introduction: The bromelain is a protease, isolated and purified from different organs from plants of the Bromeliaceae family. Its use is recognized in the vaccine formulation and to skin debridement of burns, stand out its potentialities anti-inflammatory, antitumoral, and antithrombotic. **Materials and methods:** The bromelain was obtained through a process of extraction and developed purification in Cuba, from stems of adult plants of pineapples (*Ananas comosus* (L) Merrill). The crude extract was purified by chromatography of ionic interchange in cellulose-52 carboxymetil (CMC). The employed tumor lines were: P-388 leukemia, sarcoma (S-37), ascitic tumor of Ehrlich (ATE), pulmonary carcinoma of Lewis (PCL), melanoma (M-B16F10) and adenocarcinoma mammary (ADC-755). The treatment with bromelain by intraperitoneal route (1, 5, 12.5 and 25 mg /kg) began to the 24 hours after the inoculation of a million of tumor cells by animals. The antitumoral activity became by means of the calculation of the index of increase of survival (A.S %), of the treated mice and survival average of the mice controls. The antimetastatic activity of the Bromelain on the CPL and ATE were evaluated. To the 21 days, the mice lungs were extracted and suspended in Bouin solution by 48 hours; later they told to the macrometastatics in Carl Zeiss stereoscopic. **Results:** In spite of being differences among them, demonstrated a remarkable antitumoral effect in Ip-388, s-37 and ATE. A diminution of the number of metastasis in carrying animals of PCL took place and free animals of metastasis in ATE registered themselves. **Conclusions:** This protease had a antitumoral and antimetastatic effect in the treated animals which indicates that the probably pharmacologic mechanism is not the direct action on the circulating cells but in one of the processes implied in the metastasis cascade where the immune system of the animal play a determining paper in the elimination of such cells.

CO42- ISOLATION AND CHARACTERIZATION OF CYTOTOXIC POLYPEPTIDES FROM MARINE ORGANISM GYRM-12S

RM Yu^a, LY Song^{a,b}, SF Ren^a, Y Zhao^b

^aDepartment of Natural Medicinal Chemistry and Pharmacognosy,, College of Pharmacy, Jinan University, 510632 Guangzhou, China. ^bDepartment of Traditional Chinese Medicine and Natural Drug Research, College of Pharmaceutical Sciences, Zhejiang University, 310031 Hangzhou, China. E-mail: tyrm@jnu.edu.cn

As a source of novel molecules, the potential of marine organism is immense and yet it has been barely investigated. Because of their longer evolutionary history, marine organisms likely possess a greater molecular diversity than do their terrestrial counterparts. Over the past decade, several new experimental anticancer agents derived from marine sources have entered preclinical and clinical trials. GYRM-12s, a marine animal native to the seas around China, has been utilized in the treatments of anemia and inflammation for centuries. During the course of our extensive screening program of traditional Chinese medicinal products for in vitro anti-tumor activity, marine organism GYRM-12s was selected because of its widespread geographic distribution in China, as well as its unique reputation among the folklore medicines.

In the present work, we investigated the protein component of marine organism GYRM-12s by a combination of chemical and biological approaches. The purified polypeptides G-3 and G-4-2 were obtained from marine organism GYRM-12s using the techniques of homogenization, salting-out with ammonium sulfate, ion-exchange chromatography and gel filtration chromatography. The purity of G-3 and G-4-2 was over 96% as measured by RP-HPLC. G-3 and G-4-2 were given by SDS-PAGE and IEF-PAGE with molecular weights of 8250.4 Da and 15970.8 Da, respectively and isoelectric points of 6.6 and 6.1, respectively. Amino acid constituents of G-3 and G-4-2 were also detected, and Phenol-vitriol test revealed that saccharides exist in G-3. G-3 and G-4-2 may both inhibit the proliferation of tumor cells in vitro. The IC₅₀ values of G-4-2 were 22.9 µg ml⁻¹ and 46.1 µg ml⁻¹ against Hela and HL-60 cell lines, respectively, as were measured by MTT assay and the IC₅₀ value of G-3 against HL-60 was measured to be 123.2 µg ml⁻¹.



CO43- CYTOTOXIC AND ANTITUMOUR ACTIVITIES OF VENEZUELAN PLANT EXTRACTS *in vitro* AND *in vivo*

Villasmil J^a, Abad MJ^a, Arsenak M^a, Michelangeli F^b, Fernández A^b, Ruiz MC^b, Williams B^b, Herrera F^c, Taylor P^a

^aCentro de Medicina Experimental, ^bCentro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 21827, Caracas 1020-A, Venezuela, ^cEscuela de Bioanálisis, Universidad Central de Venezuela. E-mail: ptaylor@ivic.ve

Venezuela is a country where indigenous and local communities use over 1,500 species of plants for medicinal purposes. Many drugs used in the treatment of cancer are of plant origin. From our databases of botanical collections and ethnobiological usage, we selected 10 species to screen for antitumour activity both *in vitro* and *in vivo*. Different parts of the plants were extracted in 95% ethanol. These crude extracts were screened for activity against a) four tumour cell lines, b) primary tumour growth and metastasis in the B16/BL6 melanoma / C57BL/6 mouse model, and c) NF- κ B inhibitory activity in HeLa cells transfected with an NF- κ B / luciferase reporter gene plasmid. Extracts from 4 species were cytotoxic at 100 μ g/ml or less on one or more cell lines, with all but two extracts showing varying degrees of growth inhibition. We identified four plants with inhibitory activity on the growth of subcutaneous primary tumours when injected i.p. every other day during the course of tumour growth, and four which significantly reduced lung metastases after i.v. inoculation of tumour cells. Two plants from the *Protium* genus showed activity in both models. The nuclear factor NF- κ B is a promising potential target for antitumour and anti-inflammation therapy. Four plants inhibited NF- κ B activity in the HeLa cell reporter gene assay when the cells were activated with TNF- α , but also showed cytotoxic activity at higher concentrations in short-term MTS assays with the same cells. Further studies are being undertaken to investigate the relationship between the antitumour and NF- κ B inhibitory activities shown by these extracts.

CO44- *Cimicifuga racemosa radix* HEPATIC SAFETY

Firenzuoli F^a, Gori L^a, Cinotti S^b

^aCentre of Natural Medicine, S.Giuseppe Hospital. Via Paladini 1, 50053 Az USL 11. Empoli. Italy. ^bDepartment of Emergency Medicine. S.Giuseppe Hospital. Az USL 11. Empoli, Italy. Email: f.firenzuoli@usl11.toscana.it

Cimicifuga racemosa radix extract is actually alleged to be a possible hepatotoxic medicinal herb especially after a statement of EMEA that collected 42 cases of possible hepatotoxicity and 4 considered possible, so that in Italy it is actually no more sold over the counter. In our department we administer *Cimicifuga racemosa* as special herbal extract together with soy isoflavones, *Trifolium pratense* and *Medicago sativa* at the dose of 500- 1000 daily mg daily for treatment of menopause related disorders. After the EMEA's official signal we have contacted all our patients assuming the herbal extract continuously from more than 12 months. We followed-up 98 women, and asked them by telephone and/or after anamnesis and clinical examination (72/98) to undergo a blood sample examination of hepatic transaminases, coagulation and gamma-glutamyl-transpeptidase. In all the patients (comprehending 4 patients suffering of benign hepatic disease and 1 suffering of chronic toxic hepatitis) there were no sign of hepatic disease neither alteration of plasma hepatic parameters, or worsening of already altered but stable parameters. We think on the base of these data and current literature *Cimicifuga racemosa radix* extract can be considered safe concerning liver diseases.

CO45- HERBS AND DIETARY SUPPLEMENTS AND HOW THEY ARE USED IN THE US. APPLICATIONS FOR ARTHRITIS, DEPRESSION, AND DRUG-HERB INTERACTIONS

Bauer B

Mayo Clinic, USA.

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CO46- DRUG-HERBAL/FOOD INTERACTIONS COUNSELLING CAN REDUCE THE ADVERSE EVENTS IN SENSITIVE PATIENT POPULATIONS

Buttar HS

Therapeutic Products Directorate, Health Canada, Ottawa, Ontario K1A 1B9

Simultaneous intake of drugs and herbal products or dietary supplements may cause additive, synergistic, or antagonistic interactions, which may complicate the dosing regimen of long-term medications, or lead to undesirable side effects. Major adverse events, including death, have been recorded in case reports and post-marketing surveillance when herbal and dietary products were used chronically alone or in combination with prescription drugs. Drug-herbal/food interactions may be significantly important for drugs with narrow therapeutic margin (e.g. warfarin, digoxin, antiarrhythmics), and for sensitive populations such as elderly patients, pregnant women and nursing mothers as well as children and very sick individuals (e.g. AIDS/HIV, cancer and organ transplant patients) who may be exposed to polypharmacy. Multiple ingredients in herbs and dietary supplements may modify the intestinal pH and motility, and inhibit/induce intestinal drug transporters or metabolising enzymes (e.g. PgP, CYP3A4), and thus change the rate and extent of absorption, metabolism and disposition of conventional drugs. Before prescribing a medication, physicians should enquire whether their patients are consuming an interacting food or herbal product and either instruct their patients to stop consuming such products or adjust drug dosage to compensate for drug-food or drug-herbal effects. Collaborative efforts are required from patients, physicians, and pharmacists as well as industry (drug manufacturers, herbal products and food suppliers) to minimize or possibly prevent any potential risks associated with the concomitant use of natural health products, dietary supplements and interacting pharmaceuticals. This communication would highlight the mechanisms of drug-herbal/food interactions, and the regulatory initiatives (post-market surveillance and labelling changes) being carried out within Health Canada to meet the opportunities and challenges associated with the rapidly emerging area of herbal remedies and dietary supplements.

CO47- THERAPEUTIC CONSULTATION SERVICE: AN INFORMATION SOURCE ABOUT NATURAL PRODUCTS

Calvo-Barbado D, Cires-Pujol M, and Delgado-Martinez I

Pharmacoepidemiology Development Center, 44 Str. and 5ta Ave., 11300, Havana. E-mail: dulce@mcdf.sld.cu

The need for the development and dissemination of objective and complete drug information for prescribers and consumers is an important aspect to reach in developed and developing countries. The information about any kind of drugs oriented to individualized patients is difficult to find, the same happen to scientific knowledge of the use of natural medicines in specific patients.¹⁻⁵ The appropriate use of drugs is a priority of our Ministry of Public Health. In 1996 a network encompassing 175 municipal primary health care centers and 14 province pharmacoepidemiology groups was set up. The whole network is coordinated by the National Pharmacoepidemiology Development Center (PDC). As a part of our national medicine politics, in 1997 the Cuban national health system adds the traditional and natural medicine program, like a new measure inside the national medicines program. Functions of pharmacoepidemiologists in research, teaching and health care request the provision of drug information with the main goal: the improvement of efficacy and safety in the clinical use of drugs. In order to reach this objective our therapeutic consultation service was established in 1999, as a section of PDC.⁶⁻⁷ It offers the possibility of complementing drug product information with problem-oriented drug information emerging from cases in the real world of prescribing. This service has work procedure established, who include the question reception, research and identification of the knowledge deal with the formulated question, the assessment about the information and the relationship with the specific patient, the discussion and the inclusion in our database. We analyzed our 5 years' database to find the number of them with relationship to natural and traditional medicine. The database of therapeutic consultations was designed by Microsoft office tool, ACCESS. We selected 2 of the most interesting consultation received during this period in our service. From the 963 consultations present in our database, around 5.6 % belong to issues related to natural medicines. The most consultant products were spirulina, PV2, policosanol and propolis. The questions was formulated in the majority of cases by patients. These consultations were classified as documentation and the related topics were general information. In province the questions about natural medicines occupied the first places. Besides



the importance of this alternative medicine there are not much questions of it, nevertheless in the last years this sort of consultation won a good position.

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CO48- THREE YEARS OF FOLLOW UP OF SUSPECT ADVERSE DRUG REACTIONS WITH HERBAL AND TRADITIONAL PRODUCTS IN CUBA

Jiménez G, Ávila J, González B

National Pharmacological Surveillance Coordinating Unit. Pharmacoepidemiology Development Centre. Calle 44 esq. 5ta Ave No 502 Miramar Playa CP 11300 Ciudad de La Habana. Cuba.
Email: giset@mcdf.sld.cu

Plants have been used for the treatment of illness in humans since prehistoric time. About 80% of the world's population currently depends on the traditional herbal medicines for managing their diseases. While information about adverse reactions to allopathic drugs has been recorded over several decades, it has become increasingly clear that the surveillance system must be extended to herbal remedies. Since 2001, the national coordinating unit has been working on natural products; the number of adverse drug reactions associated with herbal remedies and natural health products in our country reflects a growing awareness that these natural products may also cause harm. Based on spontaneous drug surveillance reports we made a retrospective and descriptive study, analyzing those reports since January 2003 until December 2005. Up to end of December 2005, the national pharmacological surveillance coordinating unit had received more than 680 reports of adverse drug reactions (ADR) in which products with one or more herbal ingredients were suspected to be the cause of the reaction. The top natural products were herbals (57.8%), acupuncture (37.0%) and apitherapy (2.1%). The causality assessment were probable for the majority of the reports (78.9%) and about the 72.0% of the reports were classified mild, there were not fatal reports and the low frequency reactions were reported in 21.5%. The collection of adverse drug reactions reports from herbals and similar products will lead to use herbal remedies in the same way that adverse drug reactions reports from allopathic medicines have done. In addition, reporting of ADR from herbals may lead to more knowledge about the plants used, which may, in turn, provide information that can be useful in the search for new allopathic medicines.

CO49- CQF: STRATEGIES OF RESEARCH & DEVELOPMENT IN NATURAL PRODUCTS

Jáuregui-Haza UJ, Delgado-Hernández R, Garrido-Garrido G, González-Lavaut JA, Núñez-Sellés AJ

Centro de Química Farmacéutica (CQF), 200 y 21, Atabey, Playa, Apdo. 16042, Ciudad de La Habana, Cuba.
Tel. (537)2717822, Fax: (537)2736471. Email: ulises.jauregui@cqf.sld.cu

The pharmaceutical industry consecrates their efforts in research and development of new formulations from natural products. Those processes have to be safe for human health and environment, renewable and sustainable. The Cuban government pays special attention to the rational exploration of natural resources, focusing an important part of the research and development processes to the search of health products.

The Center of Pharmaceutical Chemistry (CQF) is an institution of Ministry of Public Health devotes to generate and to assimilate technologies, services and knowledge through the development of scientific and technical investigation in order to obtain and commercialize pharmaceutical products and nutraceuticals. Here we present



the strategy and results obtained by the Center of Pharmaceutical Chemistry in the search of pharmaceuticals, intermediates and nutraceuticals from Cuban natural products.

A clean technology for producing an aqueous extract from stem bark of *Mangifera indica* L (VIMANG®) has been developed. VIMANG® has demonstrated anti-oxidant, anti-inflammatory and analgesic effects in different pharmacological preclinical models. Nowadays, VIMANG® is widely used as nutraceutical supplement, cosmetic and phytomedicine. Various galenic formulations are being developed in order to be used in clinical treatments.

Several prostaglandins and steroids from natural intermediates have been studied. The prostaglandin A_2 extracted from the gorgonia *Plexaura homomalla* var. *S* was the raw material for the synthesis of 15-methyl prostaglandin $F_{2\alpha}$ methyl ester (luteolytic and abortifacient) and prostacyclin (vasodilator for pulmonary hypertension). On the other hand, a full series of androgenic and/or anabolic derivatives were obtained from the natural hecogenine of *Agave furcroyde*.

Finally, the strategy of searching for new antiviral and anti-inflammatory drugs from Cuban endemic flora is presented. The preliminary results of the screening from some Cuban species of *Erythrin*, *Erythroxyllum*, *Hypericum* and *Zanthoxyllum* are shown.

CO50- DRUG RESEARCH AND DEVELOPMENT CENTER. SCIENTIFIC STRATEGY IN NATURAL PRODUCT

Rodríguez-Chanfrau JE

Drug Research and Development Center (CIDEM). Ave 26 #1605. Nuevo Vedado. Ciudad de la Habana. CP 10600. Cuba. E-mail: cidem.id@infomed.sld.cu

The Drug Research and Development Center was created to research and develop medicines, nutritional supplements and cosmetics. The center has always considered all ways possible to obtain and use the techniques and state of the art technologies in the creation, design, evaluation and production of a medicine, following all international requirements.

The center's research area includes researchers and technicians with a high profile of expertise backed with categories and scientific degrees. One of this important works in this center is a Natural Product.

The fields of research in this center are:

- Agro-technological and phytochemical studies.
- Technological development and obtaining of raw materials from natural sources.
- Technological development of finished pharmaceutical products from natural sources.
- Stability studies
- Pharmacology, toxicology and genetic toxicology studies.

The objective of this conference is show the scientific strategy of Drug Research and Development Center in natural product research for next years.