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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 52nd Annual Meeting of the Society of Toxicology, held at the Henry B. Gonzalez Convention Center, March 10–14, 2013.

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The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 561.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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effect of its flavonolignans. Because of the importance of oxidative stress and mitochondrial dysfunction in causing neuronal death, prompted us to investigate the effects of SM against an in vitro model of reactive oxygen species (ROS) production in the human neuroblastoma cell line SH-SY5Y. We selected two cytotoxic stimuli, for one hand, hydrogen peroxide (H2O2)(500 μM), and on the other hand the combination of 30 μM rotenone plus 10 μM oligomycin-A (R/O) that inhibit mitochondrial respiration complexes I and V, respectively. Cell viability, measured as MTT reduction, was decreased to 70% in cells treated with H2O2 and to 60% in cells exposed to R/O. Cell incubation with increasing concentrations of SM (1-1000 μM) for 24 hr, followed by a 24-hr period with H2O2 (extracellular ROS) or R/O (intracellular ROS). Maximum protection was achieved with 300 μM SM (30% protection). Our results showed that R/O and H2O2-induced cytotoxicity in SH-SY5Y cells was suppressed by treatment with SM. Because, it is recently reported that SM crosses the blood–brain barrier and enters the CNS and it is non-toxic even at higher doses, this flavonoid may be useful in diseases known to be aggravated by reactive oxygen species and in the development of novel treatments for neurodegenerative disorders. This work was supported by projects Ref. BSCHGR58/08(UCM), Ref. No. S2009/AGR-1469(CAM) and Consolider-Ingenio 2010 No.CSD2007-063(MEC), Spain.

PS 1782 Thymoquinone, a Bioactive Component of *Nigella sativa*, Modulates Redox Status and Insulin Secretion from Pancreatic Beta Cells.

J. P. Gray[1, 3], R. Follmer[1], R. Rebar[1], N. Seeram[2] and E. Heart[3]. [1]*Science, US Coast Guard Academy, New London, CT;* [2]*Bioactive Botanical Research Laboratory, University of Rhode Island, Kingston, RI;* [3]*Cellular Dynamics, Marine Biological Laboratory, Woods Hole, MA.*

Nigella Sativa is a traditional medicine that has been used in the Mediterranean to treat a variety of disorders, including type 2 diabetes. A primary component of Nigella sativa extract is thymoquinone which, like Nigella sativa extract, attenuates diabetes symptoms. The molecular targets and interactions of thymoquinone with metabolic pathways relevant to glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells have not yet been identified. Our laboratory previously demonstrated that low (nM-μM) doses of various quinones such as menadione stimulate insulin secretion from β-cells, and this action was coupled to the generation of low levels of H_2O_2, a putative mediator of GSIS. Here we compared the mechanism of action of thymoquinone to that of menadione in β-cells. Like menadione, thymoquinone induced a dose-dependent increase in the production of H_2O_2. Unlike menadione, the redox cycling of thymoquinone was not dependent on the glucose concentration. Both NADPH and NADH supported the redox cycling of thymoquinone in cytosolic and mitochondrial fractions. This was consistent with the ability of thymoquinone to decrease NADH/NAD^+ and NADPH/$NADP^+$ ratios, thus reducing intracellular redox poise. Thymoquinone-dependent redox cycling activities were inhibited by diphenylene iodonium, an inhibitor of flavin-containing oxidoreductases. Dicoumarol and MAC220, NQO1 inhibitors previously shown to inhibit menadione-dependent redox cycling, failed to inhibit thymoquinone-dependent redox cycling. Unlike menadione, thymoquinone was found to potentiate GSIS in a dose-dependent manner at stimulatory glucose (concentrations which potently stimulate insulin secretion). These data suggest that while the mechanisms of thymoquinone redox cycling are different than those of menadione, thymoquinone retains the ability to regulate both redox status and insulin secretion from β-cells.

PS 1783 Antioxidant and DPPH-Scavenging Activities of Compounds and Ethanolic Extract of the Leaf and Twigs of *Caesalpinia bonduc L.* Roxb.

O. O. Olubanke[1], O. E. Ogunlana[2], A. T. Lawa[1], J. O. Olagunju[3], A. A. Akindahunsi[4] and N. H. Tan[5]. [1]*Biological Sciences, Covenant University, Ota, Nigeria;* [2]*Biological Sciences, Crawford University, Igbesa, Nigeria;* [3]*Medical Biochemistry, College of Medicine, Lagos State University, Ikeja, Nigeria;* [4]*Biochemistry, Federal University of Technology, Akure, Nigeria;* [5]*State Key Laboratory of Phytochemistry and Plant Resources, Kunming Institute of Botany, Kunming, China.*

Antioxidant effects of ethanolic extract of *Caesalpinia bonduc* and its isolated bioactive compounds were evaluated *in vitro*. The compounds included two new cassane diterpenes, 1α,7α-diacetoxy-5α,6β-dihydroxyl-cass-14(15)-epoxy-16,12-olide (1) and 12α-ethoxyl-1α,14β-diacetoxy-2α,5α-dihydroxyl-cass-13(15)-en-16,12-olide (2); and others, bonducellin (3), 7,4'-dihydroxy-3,11-dehydrohomoisoflavanone (4), daucosterol (5), luteolin (6), quercetin-3-methyl ether (7) and kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (8). The antioxidant properties of the extract and compounds were assessed by the measurement of the total phenolic content, ascorbic acid content, total antioxidant capacity and 1-1-diphenyl-2-picryl hydrazyl (DPPH) and hydrogen peroxide radicals scavenging activities. Compounds 3, 6, 7 and ethanolic extract had DPPH scavenging activities with IC_{50} values of 186, 75, 17 and 102 μg/ml respectively when compared to vitamin C with 15 μg/ml. On the other hand, no significant results were obtained for hydrogen peroxide radical. In addition, compound 7 has the highest phenolic content of 0.81±0.01 mg/ml of gallic acid equivalent while compound 8 showed the highest total antioxidant capacity with 254.31±3.54 and 199.82±2.78 μg/ml gallic and ascorbic acid equivalent respectively. Compound 4 and ethanolic extract showed a high ascorbic acid content of 2.26±0.01 and 6.78±0.03 mg/ml respectively. The results obtained showed the antioxidant activity of the ethanolic extract of *C. bonduc* and deduced that this activity was mediated by its isolated bioactive compounds.

PS 1784 Selective Elimination of Malignant Melanomas through Autophagic and Mitochondria-Based Mechanisms by the Antitumor Agent Osw-1.

K. Riaz Ahmed, C. Garcia-Prieto, L. Feng and P. Huang. *Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX.* Sponsor: M. Smith.

Drug resistance and lack of therapeutic selectivity are two of the biggest challenges to successful melanoma therapy. Constitutive activation of Extracellular Signal Regulated Kinase 1/2 (ERK1/2) and subsequent chemoresistance has been reported in malignant melanomas. ERK1/2 has also been implicated in activation of mitochondrial gene expression and regulation of autophagy thus making it an important therapeutic target.

The natural product, OSW-1, isolated from the bulbs of ivory coast lily, has been shown to be highly cytotoxic in numerous cancer cell lines with yet undefined mechanisms of action. Herein, we report our results on the anticancer activity and selectivity of OSW-1 in malignant melanoma cells and its potential mechanisms of action.

Our preliminary results demonstrated that OSW-1 was highly effective in killing tumor cells that are resistant to most of the currently available anticancer drugs, with IC50 values in sub-nM concentrations. Importantly, OSW-1 preferentially killed melanoma cells and exerted much lower toxicity to normal melanocytes in culture. Biochemical analysis revealed that OSW-1 treatment caused damage of the mitochondrial membrane integrity, leading to a decrease in transmembrane potential and subsequently initiating cell death, apparently through autophagy. Further study demonstrated that OSW-1 inhibited ERK1/2 expression in melanoma cells and caused a significant disturbance of cellular calcium homeostasis, leading to aberrant calcium-mediated processes including mitochondrial impairment. Based on these results, we postulate that OSW-1 inhibits ERK1/2 mediated signaling and triggers mitochondrial damage in cells leading to a significant disturbance of cellular calcium and cell death through autophagy. This study is of great significance since ERK1/2 signaling is important to melanoma cell survival and inhibition of ERK1/2 expression and induction of autophagic cell death by OSW-1 will be critical to combat therapeutic resistance and enhance drug selectivity.

PS 1785 3-Caffeoyl, 4-Dihydrocaffeoylquinic Acid from *Salicornia herbacea* Attenuates High Glucose-Induced Hepatic Lipogenesis in Human HepG2 Cells.

H. Chun[2], Y. Hwang[3, 1], J. Choi[1], H. Kim[1], Y. Chung[3] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea;* [2]*Korea Research Institute of Bioscience and Biotechnology, Jeongeup, Republic of Korea;* [3]*International University of Korea, Jinju, Republic of Korea.*

3-Caffeoyl, 4-dihydrocaffeoylquinic acid (CDCQ) from Salicornia herbacea has a variety of pharmacological properties, including antioxidant and anti-inflammatory and hepatoprotective properties. The aims of our study were to provide new data on the molecular mechanisms underlying the role of CDCQ in prevention of high glucose-induced lipid accumulation in human HepG2 cells. We found that CDCQ suppressed high glucose-induced lipid accumulation in HepG2 cells. CDCQ strongly inhibited the high glucose-induced FAS expression by modulating SREBP-1c activation. Moreover, the use of a specific inhibitor or liver kinase B1 (LKB1)-siRNA transfected HepG2 cells showed that CDCQ activated AMP-activated protein kinase (AMPK) via silent information regulator T1 (SIRT1) or LKB1 in HepG2 cells. These results indicate that CDCQ prevents lipid accumulation by

blocking the expression of SREBP-1c and FAS through LKB1/SIRT1 and AMPK activation in HepG2 cells, suggesting that CDCQ is a novel AMPK activator with a potential role in the prevention obesity.

PS 1786 Effects of Saponins from the Roots of *Platycodon grandiflorum* on TGF-β1-Induced Epithelial-Mesenchymal Transition in in A549 Cells.

C. Ho[1], H. Kim[1], Y. Hwang[2], Y. Chung[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea; [2]International University of Korea, Jinju, Republic of Korea.*

Epithelial to mesenchymal transition (EMT) is a key event in the progression of cancer. EMT is characterized by the loss of epithelial and the gain of mesenchymal features. Previous studies have revealed that treatment with CKS, saponins from the roots of Platycodon grandiflorum, significantly reduces metastasis and tumorigenesis, but the underlying mode of action has not been elucidated. In this study, we investigated the inhibitory effect of CKS on transforming growth factor (TGF)-β1-induced alterations characteristic of EMT in human lung carcinoma cells. We found that CKS-treated cells displayed inhibited TGF-β1-mediated E-cadherin down-regulation and Vimentin up-regulation and also retained epithelial morphology. Furthermore, TGF-β1-increased Snail expression was reduced by CKS. Pretreatment of cells with CKS blocked TGF-β1-induced Smad2/3 phosphorylation and Smad7 down-regulation. CKS inhibited TGF-β1-induced phosphorylation of Akt, ERK1/2, and glycogen synthase kinase-3β (GSK-3β). Furthermore, TGF-β1-increased Snail expression was reduced by pharmacology inhibitors of Akt, ERK1/2, and GSK-3β. These results indicate that pretreatment with CKS inhibits the TGF-β1-induced EMT process and prevents TGF-β1-induced transdifferentiation via activation of Akt and ERK1/2 and inactivation of GSK-3β in A549 cells.

PS 1787 Platycodin D Regulates Hepatic Lipogenesis via an AMP-Activated Protein Kinase Dependent Signaling Pathway in Human HepG2 Cells.

H. Lee[2], Y. Hwang[3, 1], J. Choi[1], H. Kim[1], Y. Chung[3] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea; [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; [3]International University of Korea, Jinju, Republic of Korea.*

Platycodin D, the saponins from the roots of Platycodon grandiflorum (CKS), has a variety of pharmacological properties, including anti-hyperlipidemic, antioxidant and hepatoprotective properties. This study was conducted to suggest the role of AMP-activated protein kinase (AMPK) pathway in the anti-obesity effect of platycodin D. We characterized the underlying mechanism platycodin D's effects in HepG2 cells by Western blot and RT-PCR analysis. Platycodin D increased the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) in HepG2 cells. Use of a specific inhibitor showed that platycodin D activated AMPK via SIRT1/calcium/calmodulin-dependent kinase kinase (CaMKK) in HepG2 cells. Taken together, these data suggest that platycodin D exert improving effects on high glucose-induced lipogenesis by reducing of SREBP-1c and FAS expression via AMPK activation.

PS 1788 Rutaecarpine Suppresses LPS-Induced Inflammation in Mouse Macrophages: A Possible Pathway through the Induction of Heme Oxygenase-1 Expression.

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Rutaecarpine, a quinazolinocarboline alkaloidal compound, is a natural product isolated from Evodia rutaecarpa and has various biological and pharmacological effects, including anti-inflammatory and anti-oxidative properties. In the present study, we investigated the effect of rutaecarpine against lipopolysaccharide (LPS)-induced inflammation in RAW264.7 macrophages. Treatment with rutaecarpine suppressed inducible nitric oxide synthase expression and nitric oxide (NO) production by downregulating NF-κB activity in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Rutaecarpine acts by inducing the expression of heme oxygenase-1 (HO-1) in a dose- and time-dependent manner. The signaling pathway involved in rutaecarpine-mediated HO-1 induction included Ca2+/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal regulated kinase 1/2 (ERK1/2). Furthermore, the CaMKII-ERK1/2 cascade targets the transcription factor, NF-E2-related factor-2 (Nrf2). Taken together, our results demonstrate that rutaecarpine-induced expression of HO-1 is mediated by the Ca2+-CaMKII-Nrf-2-HO-1 pathway and inhibits LPS-induced inflammation in RAW264.7 macrophages.

PS 1789 Cultivated Ginseng Inhibits Tarc Expression by Suppressing the Activation of NF-κB and STAT1 in Human Keratinocyte Cells.

B. Park[1], J. Choi[1], J. Choi[2], Y. Chung[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea; [2]International University of Korea, Jinju, Republic of Korea.*

Atopic dermatitis (AD) is associated with the paradigm of an allergic T helper (Th) 2-mediated disease, characterized by abnormal IgE production, peripheral eosinophilia, mast cell activation, as well as Th2 and Th1 cytokine up-regulation in chronic skin lesions. This study examined the inhibitory effects of cultivated ginseng (CG) (Panax ginseng C.A. Meyer) on atopic dermatitis in HaCaT cells. CG, an herb used in Korean herbal medicine, has been widely used in China and Japan to treat fatigue and to enhance resistance to many diseases. CG suppressed TNF-α/IFN-γ-induced TARC, CTACK, and MDC mRNA expression in HaCaT cells. Additionally, CG inhibited TNF-α/IFN-γ-induced activation of NF-κB and STAT1. These results indicate that CG inhibits chemokine expression in keratinocytes by suppressing of NF-κB and STAT1 activation.

PS 1790 Mollugin Inhibits Proliferation and Induces Apoptosis by Inhibiting HER2 Expression in HER2-Overexpressing Breast and Ovarian Cancer Cells.

M. Do, T. Tran, M. Na and H. Jeong. *Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

Overexpression of the HER2 gene causes many cancer types and has been reported to enhance cell proliferation, tumor growth, angiogenesis, and metastasis. This study investigated the molecular mechanisms by which mollugin exerts anti-tumor effect in HER2-overexpressing breast and ovarian cancer cells. Mollugin exhibited potent inhibitory effects on cancer cell proliferation, especially in HER2-overexpressing SK-BR-3 human breast cancer cells and SK-OV-3 human ovarian cancer cells in a dose- and time-dependent manner. Additionally, caspase-3 activity and PARP cleavage were significantly upregulated in HER2-overexpressing SK-BR-3 and SK-OV-3 cells treated with mollugin. Mollugin treatment caused a dose-dependent inhibition of HER2 gene expression at the transcriptional level, potentially in part through suppression of NF-κB activation. Moreover, mollugin inhibits cyclin D1 expression by downregulating HER2 activation and consequently reducing PI3K/Akt signaling. These results suggest that mollugin is a novel modulator of the HER2 pathway in HER2-overexpressing cancer cells with a potential role in the treatment and prevention of human breast and ovarian cancer with HER2 overexpression.

PS 1791 Multiple Signaling Pathways Involved in Suppression of MDR1 by Mollugin from *Rubica cordifolia.*

T. Tran, H. Kim, M. Do, S. Jin, E. Shim, H. Han, M. Na and H. Jeong. *Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

Multidrug resistance (MDR) is known to be a serious problem in cancer treatment and has been identifed as a negative prognostic factor in malignancies. This study investigated mollugin, purified from roots of Rubica cordifolia L., down-regulated MDR1 expression in MCF-7/adriamycin (MCF-7/adr) cells, human breast multidrug-resistant cancer cell. Mollugin treatment significantly inhibited MDR1 expression by blocking MDR1 gene transcription. Mollugin treatment also significantly increased intracellular accumulation of the fluorescently-tagged P-gp substrate, rhodamine-123. The suppression of MDR1 promoter activity and protein expression was mediated through mollugin-induced activation of AMP-activated protein kinase (AMPK) and inhibited NF-κB as well as CREB activation.