Basic Medical Sciences

Chairs: P Singh and S Brown

(O – 05)

Assessing the cytotoxic effect of fungal extracts against Pc-3 cell line

M Jackson[1], P Facey[2], S Badal[1]
[1]*Department of Basic Medical Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica and* [2]*Department of Chemistry*, *The University of the West Indies, Mona, Kingston 7, Jamaica*

Background: Prostate cancer is the leading cause of cancer mortality among Jamaican men. Due to the insufferable side-effects associated with current chemotherapy treatments, the search for safer and more cost-effective lead molecules is ongoing. Endophytic fungi have demonstrated potential anti-cancer properties using cancer cell lines, with some comparable to and others more potent than known chemotherapy drugs. This makes them ideal candidates for further research towards the development of anti-cancer drugs.
Methods: Extracts from six endophytic fungi (LR-L01-PM, LR-L01-R, LR-S06-PM, LR-S06-R, LR-S08-PM and LR-S08-R) isolated from *Laguncularia racemosa* (white mangrove) were screened against the prostate cancer, PC-3 cell line. PC-3 cells were cultured in complete medium (90% RPMI + 10% FBS + 1% Pen/Strep). When the concentration of cells reached 1.5 x 10^6 cells/ml, they were resuspended in 15 ml of complete media. A total of 100 µl of the cell suspension was transferred to each well of a 96 well plate and incubated for 24 hours in the presence and absence of fungal extracts ranging in concentration from 1 µg/ml to 1600 µg/ml. The percentage cell viability was then measured using the MTS Assay and readings taken using a spectrophotometer at 490 nm.
Results: Of the six extracts screened, four showed significant activity against PC-3 cells. Extracts LR-S08-PM and LR-S08-R showed no activity against PC-3 (at low concentrations) and as such were excluded from further studies. Extracts LR-L01-PM and LR-L01-R showed promising activity against PC-3 cells at concentrations 96 µg/ml and 200 µg/ml respectively, inhibiting cell viability by more than 50%. Extracts LR-S06-PM and LR-S06-R showed cell inhibition greater than 50% at concentrations 400 µg/ml and 64 µg/ml respectively.
Conclusion: From these results, the endophytes from the white mangrove exhibited promising anti-cancer activity against the prostate cancer PC-3 cell line. Further work is needed to determine the identity of the endophytes and the individual ingredients responsible for the observed cytotoxic effect.
Keywords: Anti-cancer, cytotoxic, endophytes, *Laguncularia racemosa*, prostate cancer

(O – 06)

Sedative/hypnotic effects of an aqueous extract of *Arachis hypogaea* testa in mice

S Francis, T Clarke, J Howden, M Mckoy
Department of Basic Medical Sciences, Pharmacology Section, The University of the West Indies, Mona, Kingston 7, Jamaica

Background: *Arachis hypogaea* (peanut) testa has been used in Jamaican folklore to produce a 'calming effect'. The leaves of the plant possess sedative activity. However, there is no scientific evidence to support the sedative/hypnotic effects of the testa. This study sought to investigate the sedative/hypnotic potential of an aqueous extract of *Arachis hypogaea* testa in mice.
Methods: The Hippocratic screen test, balance beam and pentobarbital-induced sleep time test were used to evaluate sedative/hypnotic effects of an aqueous extract of *Arachis hypogaea* testa in mice. Male Swiss albino mice (20–25 g) were randomly placed into groups (n = 6). Groups of mice were administered the aqueous plant extract (90–420 mg/kg), saline (10 ml/kg) as the normal control, or diazepam (2 mg/kg) as the positive control intraperitoneally. Flumazenil, a benzodiazepine site antagonist at the GABA receptor, was administered 15 minutes prior to the administration of diazepam or the plant extract in order to investigate the involvement of the GABAergic system.
Results: The aqueous extract (90, 120 and 240 mg/kg) significantly ($p < 0.05$) decreased motor activity, motor coordination and respiratory rate in mice in the Hippocratic screen test. Additionally, the extract (90 and 240 mg/kg) significantly increased the time taken by mice to traverse a balance beam. The extract (300 and 360 mg/kg) also significantly decreased the onset and increased the duration of

pentobarbital-induced sleep. Flumazenil (2 mg/kg) inhibited the sedative effects of *Arachis hypogaea* extract.
Conclusion: The study showed that an aqueous extract of *Arachis hypogaea* testa possessed dose-dependent sedative/hypnotic effects mediated by GABA receptors.

(O – 07)
[Retracted]

(O – 08)
Vitamin D deficiency enhances antiphospholipid antibody-mediated thrombosis in a passive immunization mouse model

K Roye-Green[1], *R Willis*[2], *Z Romay-Penabad*[2], *E Papalardo*[2], *A Schleh*[2], *EB Gonzalez*[2], *M Smikle*[1]
[1]*Department of Microbiology, The University of the West Indies, Mona, Kingston 7, Jamaica and* [2]*University of Texas Medical Branch, Internal Medicine, Rheumatology, Galveston, Texas, United States of America*

Background: Several studies indicate that vitamin D deficiency occurs frequently in antiphospholipid (APS) patients and is correlated with thrombosis, although conflicting data have been reported. However, there are limited mechanistic data on the immunomodulatory effect of vitamin D in antiphospholipid antibody (aPL)-mediated thrombosis.
Methods: CD1 male mice (n = 5 per group) were fed either a vitamin D normal (VDnorm) or deficient (VDdef) diet for six weeks until 25-hydroxy-vitamin-D (25OHVD) levels were determined to be stable in phase I of the study. In phase II, mice were fed the appropriate diet and then inoculated (two doses) with either purified IgG from an APS patient (IgG-APS) or from normal human serum (IgG-NHS) prior to thrombosis induction surgery in the femoral vein. Blood was collected weekly after start of diet, immediately prior to IgG treatment and at the time of surgery for analysis.
Results: In phase I, mice developed vitamin D deficiency (< 20 ng/ml) by week three, which continued until week six. Phase II experiments were done at week four, and the mean 25OHVD was significantly less in IgG-APS-VDdef *versus* IgG-APS-VDnorm mice (18.8 ± 2.9 ng/ml *versus* 41.3 ± 7.8 ng/ml; $p < 0.0001$). Mean anti-β2GPI IgG levels in IgG-NHS treated mice, IgG-APS-VDnorm and IgG-APS-VDdef mice were 2.4 ± 0.7, 75.8 ± 5.1 and 75.9 ± 1.7 G units respectively at the time of surgery. Thrombus sizes (cross-sectional area) in these three groups were 555.9 ± 82.8, 1195.6 ± 242.3 and 2327.8 ± 657.2 μm^2 (p-values < 0.001 for all comparisons).
Conclusion: We provided the first mechanistic data indicating that vitamin D deficiency amplified the thrombogenic effect of IgG aPL. Future studies will focus on the immunomodulatory changes relevant to the effect of vitamin D on aPL activity in this context.

Keywords: Antiphospholid, thrombosis, Vitamin D

(O – 09)
A bioassay-guided study into the hypoglycaemic effect of fatty acids isolated from the rhizomes of *Smilax balbisiana* (Chainy Root)

DA Peddie, *RL Lindo*
Department of Basic Medical Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica

Objective: To investigate the hypoglycaemic effect of *Smilax balbisiana* rhizomes (SBH) and identify the active compounds through bioassay-guided purification methods.
Methods: Administration of the crude hexane extract of SBH (at 50 mg/kg body weight (BW)) and its fractions, SBH1–SBH4 (at 50 mg/kg BW), was observed to display a significant hypoglycaemic effect when compared with their respective controls in normal, healthy Sprague-Dawley (S-D) rats *via* intravenous (iv) route. Through bioassay-guided fractionation, SBH4 was further purified using silica gel chromatography to produce six main sub-fractions (SBH4.1–SBH4.6). The sub-fractions SBH4.1 and SBH4.2 were bioassayed *via* the Oral Glucose Tolerance Test (OGTT) using iv administration of these naturally isolated compounds at 10 mg/kg BW. The identified compounds from the active sub-fraction were used to perform a dose-dependent study in the ratio revealed by Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and compared with metformin.
Results: Intravenous administration of SBH4.1 and SBH 4.2 were able to lower the blood glucose concentration significantly at the 120-minute interval compared with the control (p = 0.003 and 0.007 respectively). However, only SBH4.2 was able to significantly lower both fasted and post-prandial blood glucose levels and was therefore subjected to compound analysis using GC-MS. Retention time and MS library comparisons indicated that the major constituents of SBH4.2 were palmitic acid, oleic acid and stearic acid. Bioassays (OGTTs *via* oral route) of these compounds were carried out at 600, 750 and 900 mg/kg BW in normal S-D rats using a combination of these commercially available fatty acids. Administration at 900 mg/kg BW showed significant lowering of the blood glucose concentration throughout the post-prandial region when compared with the control ($p \leq 0.05$), while when compared with metformin, a similar hypoglycaemic effect was observed in the postprandial region except at the 90-minute interval (6.27 ± 0.22 mmol/L *versus* 7.41 ± 0.12 mmol/L; p = 0.004) where the combination was significantly lower.
Conclusion: The results indicated that *Smilax balbisiana* (Chainy Root) rhizomes contained a mixture of fatty acids showing a comparable hypoglycaemic effect to metformin. The synergistic action of these fatty acids might be beneficial for the management of diabetes.

Keywords: Fatty acids, hypoglycaemia, *Smilax balbisiana*

(O – 10)

Jamaican actinomycetes: new weapons against antibiotic resistance

E Terrelonge, S Brown
Department of Basic Medical Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica

Background: New antibiotic-resistant pathogens pose a significant risk to the medical community worldwide. Therefore, the development of new antibiotics has become a matter of necessity and urgency. Over two-thirds of clinically useful antimicrobial compounds have been isolated from actinomycetes, a ubiquitous group of soil-dwelling bacteria. However, no research has been conducted on actinomycetes found in Jamaica or the wider Caribbean. It is highly probable that these unexplored Jamaican actinomycetes will produce novel antimicrobial compounds, which may prove useful in the battle against multi-drug resistant pathogens.
Methods: Jamaican actinomycete strains from soil samples in five parishes were analysed in order to identify potential antimicrobial producers. Antibiotic-resistance screening, 16S rRNA sequencing and rep-PCR were conducted to characterize morphologically and molecularly the actinomycete strains isolated. The isolates were then assayed for antimicrobial activity against Gram-positive and Gram-negative pathogens using the agar well-diffusion protocol.
Results: While 225 microbes were initially isolated, these were narrowed down to 88 distinct strains using rep-PCR. These include actinomycetes such as *Streptomyces* and *Arthrobacter,* as well as other soil-dwelling genera like *Novosphingobium.* Thirty-five isolates showed antimicrobial activity against Gram-positive or Gram-negative bacteria, including an ampicillin-resistant strain of *E coli* from the University Hospital of the West Indies. This indicates the likelihood of Jamaican actinomycetes being broad-spectrum antibiotic producers.
Conclusion: This research highlights the potential of Jamaican actinomycetes as a source of novel antimicrobial compounds and illustrates the need for further exploration of actinomycete strains in Jamaica.
Keywords: Actinomycete, antibiotic resistance, antimicrobial

(O – 11)

Renal vascular protein targeting by cadmium*

G McCalla[1], PD Brown[1], WC Cole[2], C Campbell[2], CR Nwokocha[1]
[1]Department of Basic Medical Sciences, Faculty of Medical Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica and [2]The Smooth Muscle Research Group, Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 4N1

Objective: Exposure to cadmium (Cd), a heavy metal, is implicated in vascular damage reportedly mitigated by potassium supplementation. Target organs for Cd include the heart, liver, kidney and spleen. The objective was to investigate the tissue specificity of Cd on vascular proteins and the ameliorative role of potassium supplementation.
Methods: Six cohorts of control, Cd-fed, and potassium-supplemented male Sprague-Dawley rats were selected. Cadmium chloride (2.5 or 5 mg/kg body weight) was fed *via* gavage three times weekly for eight weeks. Weights were measured three times weekly. Western blots of aorta and mesenteric and renal arteries were analysed after eight weeks *via* densitometry for total myosin light chain phosphatase targeting subunit 1 (MYPT1), eNOS and MAPK (p38 and p44/42) levels, and phosphorylation of MYPT1-Thr697, MYPT1-Thr855, and myosin light chain. Statistical analysis was done using One-Way ANOVA. The results were reported as mean ± standard error of the mean. Games-Howell post hoc test was used for multiple comparisons.
Results: Cadmium significantly ($p < 0.05$) elevated total renal MYPT1, its Thr697 phosphorylation site, and p44 MAPK. Potassium supplementation ameliorated these effects and also significantly ($p < 0.05$) attenuated pT697 and pT855 levels in Cd-exposed aorta.
Conclusion: Cadmium exposure demonstrated renal artery specificity. Inhibition of MLCP *via* MYPT1-Thr697 and oxidative stress *via* the p44 MAPK pathway are Cd targets. These results warrant further investigation into the mechanism of Cd, and since Thr697 is a Rho-kinase binding site, small arteries and Rho-kinase/MYPT1 interactions are of interest. The study underscores the importance of renal intervention in Cd-related conditions.
* presented as ‘Tissue specificity of cadmium on vascular proteins’ in the Conference