Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Antihypertensive effect of fractions derived from an aqueous extract of *Syzygium polyanthum* on anaesthetized spontaneously hypertensive rats

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Abstract

Syzygium polyanthum leaf has been used traditionally for treating hypertension. An aqueous extract of *S. polyanthum* leaf (ASP) has an antihypertensive effect; however, there is no further evaluation on its fractions yet. This study aims to identify the most promising fraction derived from ASP in terms of antihypertensive effect. Dried leaves of *S. polyanthum* were extracted using an ultrasound-assisted extraction method to produce ASP and then it was fractionated using column chromatography to yield F1ASP, F2ASP and F3ASP. Spontaneously hypertensive rats (SHR) was anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*), followed by left jugular vein cannulation for extract administration. Right carotid artery was then cannulated and connected to BIOPAC Blood Pressure System Version 3.6.7 for recordings of mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR). One-way and two-way ANOVA with Sidak post-hoc tests were performed using GraphPad Prism Version 6.0. F1ASP (30 to 60 mg/kg), F2ASP (20 to 60 mg/kg) and F3ASP (30 to 40 mg/kg) showed significant antihypertensive effect in comparison to negative control (normal saline). F2ASP caused more reduction ($p < 0.05$) in MAP of SHR as compared to ASP at doses of 20 and 30 mg/kg. The antihypertensive effect by F2ASP at doses of 20 to 60 mg/kg was comparable to positive control drug, captopril at 5 mg/kg. No significant effect observed on HR. F2ASP has the most promising antihypertensive effect in comparison to F1ASP and F3ASP.

Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Investigation of the origin and evolution of *DEFB* copy number variable gene using a multigenerational family pedigree

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Abstract

DEFB is known as a multiallelic gene including *DEFB4, DEFB103 DEFB104, DEFB105, DEFB106, DEFB107, DEFB108, DEFB109,* and *SPAG11* that cluster on chromosome 8p23.1 with common copy number varies between two and seven copies per diploid genome. For an individual with four copies of *DEFB*, the haploid copies contributed from each parent is questionable, thus as *DEFB* is multiallelic and variable in copy number, an analysis using family pedigree is one of the best methods to follow the segregation of *DEFB* copies. Therefore, this study aimed to quantify *DEFB* copy number and investigate the transmission pattern of *DEFB* alleles in family pedigree. In this study, a total of 53 individuals from eight multigenerational families was selected. Diploid copy number of *DEFB* was quantified using two Paralogue Ratio Tests (PRTs) and one indel (rs5889219). Additionally, two microsatellite analysis; EPEV1 and EPEV3 were used to validate the diploid copy number and to follow the segregation of haploid copies. Copy number obtained was in range from two to ten per diploid genome with five copies as the most common. While in haploid form, copy number was found to vary from one to five copies with two and three copies being the most common. Further analysis with EPEV1 and EPEV3 gave an alleles size range from 171 bp to 191 bp and 135 bp to 145 bp respectively. Interestingly segregation analysis for families of three-generations showed some alleles were not been passing down from first-generation to next generation. In addition, recombinant events potentially have occurred in two families and did not change the *DEFB* copy number. In this study, family pedigree analyses have allowed us to infer the haploid copy number of *DEFB* through the transmission of microsatellites alleles.

Detection and phylogenetic analysis of Torque teno virus among healthy blood donors in Malaysia

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Abstract

Torque teno virus (TTV) is a highly heterogeneous virus, which consists of numerous number of variants that are classified into seven related groups. TTV detection rate varies from region to region. TTV detection rate among Asian's general population was high but it is still insufficiently characterized. Our intense interest was to investigate the TTV prevalence among healthy Malaysian population and determine its phylogenetic relationship with the existing isolates. Polymerase chain reaction (PCR) of UTR A and UTR B was employed to detect the presence of TTV DNA in 137 plasma samples of healthy blood donors. PCR of N22 and phylogenetic analysis were then carried out to further characterize the Malaysian's isolate. Overall, the results report a TTV detection rate of 78.83% (108/137) and of 50.36% (69/137) as detected using PCR of UTR A and UTR B, respectively. Furthermore, molecular characterization of Malaysian isolates reveal that only 23.01% (18/78) of UTR A-positive samples could be amplified by N22-derived primer. Based on the phylogenetic reconstruction, Malaysian isolates were closely related with TTV genogroup 1, 2 and 3. As a conclusion, further investigation on large scale study and characterization of whole TTV genome are required in order to draw a comprehensive conclusion about TTV epidemiology in Malaysia and its genetic variability, respectively.

Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Renal histological changes in diabetic rats

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Abstract

Diabetes mellitus is the most common cause of kidney disease and chronic renal disorders which contribute to a major reason for dialysis and kidney transplantation. Diabetes results in a wide range of alteration in renal tissue, particularly the glomerulus. The effects of diabetes on glomerular and non-glomerular alterations in the kidney have been less attended. Thus, the present study was conducted to observe both glomerular and non-glomerular histological changes in diabetic renal tissue. Diabetes was induced in Sprague-Dawley rats by single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight. The study was conducted for 60 days, and the induced-diabetic rats were left untreated. At the end of 60 days, rats were euthanised and kidneys were harvested. Paraffin-embedded blocks of kidney tissue were prepared. Histological analysis with Hematoxylin and Eosin (H&E) demonstrates that glomerulus appeared large with expanded tuft, capillary lumens were small and some of mesangial cell proliferation were observed. Non-glomerular changes included loss of tubular brush border, tubular dilatation and mild eosinophilic deposit or protein cast in renal tubule were also seen. Furthermore, thickening of blood vessels wall was also identified. Findings of the study demonstrate some important renal histological changes in diabetes-induced rats with consistent with diabetic nephropathy. Diabetic nephropathy is a progressive disease and early identification of renal changes will provide better prognosis achievement.

Characterization of a pomegranate (*Punica granatum*) extract towards development of anthocyanin based on a non-opioid substitution therapy in *in vitro* and *in vivo* studies

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Abstract

Pomegranate is one of the oldest tropical and subtropical edible fruits. Keeping in view of its rich in constituents and antioxidants, it has been studied scientifically and its uses have been verified. Currently, some compounds in pomegranate have been proven to induce cognitive improvements, related signaling in managing withdrawal symptoms. The present study investigated the characterization of anthocyanin from the pomegranate extract towards non-opioid substitution therapy in *in vitro* and *in vivo* studies. Anthocyanin was analyzed from pomegranate extract using high-performance liquid chromatography. The MORs and cAMP levels in the cells were determined using ELISA Kit from Cusabio Biotech and Cell Biolabs. The memory impairment in male Sprague-Dawley rats was determined using Morris water maze method. Then, the CREB and BDNF levels in serum rats were determined using ELISA Kit Abcam and Cell Signaling Technology. The water extract resulted in the highest contained of anthocyanin. The extract then was used for co-treatment for *in vitro* and *in vivo* studies. Co-treatment of morphine and pomegranate extract significantly reduced the production of cAMP level at lower concentration ($p < 0.05$). In memory impairment test, treatment group significantly reduced the distance and time latency compared to morphine group ($p < 0.05$). This is in correlation with levels of CREB and BDNF that were also significantly higher than morphine group ($p < 0.05$). These findings suggest that anthocyanin had potency as non-opioid substitution therapy that could reduce the dependence and withdrawal effects on chronic morphine treatment. As per knowledge, this is the first finding of using pomegranate extract in opioid treatment.

Characterization of a pomegranate (*Punica granatum*) extract towards development of phenolic acids based on a non-opioid substitution therapy: *in vitro* and *in vivo* studies

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Abstract

Pomegranate is a nutrient dense fruit rich in phytochemical compounds, which phenolic acids are the main compounds that attribute for most of the functional properties of many fruits. The aim of this study was to characterize the pomegranate extract towards development of phenolic acids based on a non-opioid substitution therapy for *in vivo* and *in vitro* studies. Pomegranate was extracted using two different solvents with three different methods of extraction. The quantification of phenolic acids was performed using High-Performance Liquid Chromatography (HPLC). The level of Mμ Opioid Receptor (MOR) and cyclic Adenosine Monophosphate (cAMP) was analysed by using an *in vitro* assay to determine the morphine dependence. Meanwhile, the morphine withdrawal effect was investigated through an *in vivo* study by analysing the memory impairment of rats using Morris Water Maze (MWM) and by determining the level of Brain Derived Neurotropic Family (BDNF) and cAMP response element binding protein (CREB). The result reveals that the pomegranate extract with water had the highest phenolic acid composition of gallic acid, ferulic acid, and ellagic acid. In the *in vitro* study, it shows that treatment of morphine with extract significantly reduced the level of cAMP and increased the level of MOR as compared to morphine and methadone treatments. While in the *in vivo* study, rats were divided into normal, morphine, morphine + methadone, and morphine + pomegranate groups. Data obtained from MWM show memory impairment of morphine group for time escape from pool was significantly higher than other groups of treatment. Meanwhile, BDNF and CREB levels in serum from morphine + pomegranate group were significantly higher than morphine and morphine + methadone groups. As conclusion, this study suggests that the phenolic acids in the pomegranate extract has a potential to be implemented as a non-substitution therapy for morphine-dependent treatment.

Geometric morphometric assessment of manubrium by age and sex

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Abstract

Various studies have established sex and age assessment from different parts of the skeleton based on metrical qualitative morphological characteristics. The aim of this study is to use geometric morphometrics to study the shape of the manubrium and its sexual dimorphism. The sample size consists of 126 manubrium bone images, which were obtained from digital radiograph images (CT-scam images) in record. The samples were provided by the Radiology Department, Hospital Universiti Sains Malaysia (Hospital USM), which were equal in the number based on genders and classified into 10 groups based on range of age. Eleven landmark points were selected on the manubrium sterni area. Principal Analysis Component (PCA) and Procrustes ANOVA were used to obtain the shape and size variables for statistical analysis. The results show that there was a significance change in size and shape of the manubrium with the age. It is hoped that the results obtained will be beneficial for those assessing the age of the manubrium.

Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Comparison of quadratic dose protocols scan duration for the whole-body PET imaging

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Abstract

Recent studies show that the ability of quadratic dose protocol in the whole-body PET imaging to maintain a good quality images for overweight and obese patients is well presented. However, a practical approach on the implementation of such protocol in the whole-body imaging in Malaysia is currently lacking. Implementation of quadratic dose protocol using the PET system lengthens the scanning time to 226 s, as compared to 150 s used in current protocol. Hence, the aim of this study was to compare the PET image quality obtained by adhering to the two scan durations using the quadratic dose protocol. Comparison between these two different scanning times was performed using a commercial PET phantom, NEMA2012/IEC2008 located at Institut Kanser Negara (IKN). A PET Discovery ST, of which a BGO-based PET system was used in this study. This study was guided by a technical guidelines published by Koopman *et al*. (2016). Three consecutive slices of image were selected for the analyses. Quantitative analyses on the PET images show that, the minimal scan time (T_{min}) suggested by the quadratic dose protocol yielded better quantification of the image. Comparable signal-to-noise ratio was obtained by both scan durations (46.66 $\pm$ 2.13 and 45.92 $\pm$ 10.23). However, the T_{min} scan duration gave better tumour contrast (9.19 $\pm$ 0.37 compares to 7.23 $\pm$ 1.49) with greater noise on the image. The large standard deviation measured on the 150 s scan duration shows large fluctuation of each of the image slices. As a conclusion, the longer acquisition time suggested by quadratic protocol on our PET system gave better image quality.

Synergistic effect of FGF-2 and PDGF-BB on a co-culture of human gingival fibroblasts and umbilical vein endothelial cells

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Abstract

Numerous types of single cells in *in vitro* cultures have been used in tissue engineering, but the study on direct paracrine interactions between heterotypic cell populations is lacking. Co-culture approach establishes excellent atmosphere to study these interactions. The objective of this *in vitro* experimental study was to determine the effects of fibroblast growth factor (FGF-2) and platelet-derived growth factor (PDGF-BB) in a co-culture of human gingival fibroblasts (HGFs) and human umbilical vein endothelial cells (HUVECs). Cell viability was performed using MTT assay to determine the optimal concentrations of these growths factors. Next, the stimulatory effect of these growth factors in a co-culture of HGF and HUVECs was analyzed using real-time PCR (qRT-PCR) for HGF and HUVEC gene biomarkers (*Fibronectin, COL1A1, Vimentin, CD-31, Von Willebrand factor,* and *VE-Cadherin*). The results were evaluated statistically using one-way ANOVA and Kruskal Wallis test with $p < 0.05$ was considered statistically significant. Results of cell viability assay showed that the effect of FGF-2 on HGF was dose-dependent and optimum at a concentration of 5 ng/ml, while that of PDGF-BB on HUVEC was optimum at a concentration of 20 ng/ml. Co-culture result showed that both growth factors significantly upregulated the expression of gene biomarkers in the treatment groups after three days of co-culture compared to control. This study indicates that there is a synergistic effect of FGF-2 and PDGF-BB on the gene expressions of HGF and HUVEC in a co-culture, which is suggestive of a proangiogenic activity.

The inhibition of MAPKs and astrocytes effects the behavioural testing-pain stimulus in painful diabetic neuropathy rat model

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Abstract

Painful diabetic neuropathy (PDN) is a common complication of diabetes mellitus and one of the neuropathic pains that affects diabetic patients. Recently, glial cells such as astrocytes are reported to be a possible player in the development and maintenance of neuropathic pain through activation of mitogen-activated protein kinase (MAPK). The main aim of the present study is to determine the effects of MAPKs inhibitor and astrocytes inhibitor on behavioural testing-pain stimulus in streptozotocin (STZ)-induced diabetic neuropathy rats. Fifteen adult male Sprague-Dawley rats were used in this study. They were randomised into five groups: control saline (no STZ-induced) (CB+S), STZ-induced and treated with saline (STZ+S), STZ-induced and treated with MAPKs inhibitor (STZ+U) and astrocytes inhibitor (STZ+L) and non-painful diabetic neuropathy (NPDN). The rats were examined for behavioural testing: tactile allodynia, thermal hyperalgesia and formalin test. The results of tactile allodynia test showed the NPDN group was significant ($p < 0.05$) when compared between control (CB+S), STZ-induced diabetic groups at Day 14 and Day 22. Thermal hyperalgesia indicated by the percentage of thermal threshold gain revealed a significant reduction in STZ+L and STZ+U groups at Day 14 (pre-intervention) whilst a significant increase in NPDN group at Day 22 (post-intervention). Formalin test depicted as pain behaviour score showed that STZ+U group significantly increased in chemical pain in tonic phase compared to other groups. Our data showed the inhibition of MAPKs and astrocytes effects the behavioural pain stimulus in painful diabetic neuropathy rat model. Further studies will be carried out to investigate the correlation between the behavioural pain assessments and bio molecular parameters.

Musculoskeletal disorder among cashiers in selected stores in Kota Bharu, Kelantan

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Abstract

Cases of musculoskeletal disorder (MSD) have been increasing particularly to working populations including cashiers. Working condition is one of the causes triggering the musculoskeletal discomfort. This study aimed to determine musculoskeletal disorder signs and symptoms in different body regions among cashiers (n=122) in selected stores in Kota Bharu, Kelantan in association with demographic, ergonomic and psychosocial risk factors that act as the catalyst of the problems. This study used purposive sampling methods. Participants that suited the inclusion criteria were recruited in the study. Subjects were given a questionnaire after the consent was granted. Subjects completed the adopted standardized Nordic Musculoskeletal Questionnaire (NMQ) based on cashiers' socio-demographic, work characteristics and psychosocial variables. The results indicate that there were three body areas, which were neck (53.3%) and lower back (57.4%) followed by shoulders (32%), which showed the most MSD signs and symptoms experienced by the respondents. Based on Pearson Chi Square test, a demographic factor such as gender has a significant association with lower back (p = 0.014) and neck pain (p = 0.044), while working hours are related to wrists pain (p = 0.021). An ergonomic risk factor such as neck twisting was associated with shoulder pain (p = 0.013). A psychosocial factor such as low support from management was associated with neck (p = 0.011) and ankle pain (p = 0.040). This study concludes that the retail store cashiers had moderate rate of discomfort and pain developed in lower back, neck and shoulders. Therefore, it is recommended to implement intervention strategies in order to control MSD among working population.

Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Molecular characterization of α-enolase and endonuclease A in *Streptococcus pneumoniae* towards the molecular interaction in netosis

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Abstract

Streptococcus pneumonia is a causative agent for pneumococcal disease and a public concern due to high rate of mortality and morbidity reported each year. This Gram-positive bacteria can transmit from one person to another via inhalation and colonise at nasopharyngeal. Molecular characterization of genes of *S. pneumonia* is crucial in order to study the properties of virulent factor against human defense mechanism. This study aimed to analyse the sequence of α-enolase and endonuclease A genes of *S. pneumoniae* towards interaction in netosis. Netosis is one of the host defense mechanisms produced by neutrophils to kill any foreign antigens through a set of molecular proteins. In this study, the molecular characterization of these genes was performed and the associated elements were related with the netosis. Thus, the mechanism of interaction between proteins encoded by both genes with netosis will be further studied towards the development of *S. pneumoniae* vaccine candidates.

Association of beta-defensin (*DEFB*) gene copy number variables with HIV susceptibility

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Abstract

Recent findings have indicated that host genes can influence the outcome of HIV infection and progression. DEFB, a gene that encodes for innate immune system, has been found to have influence HIV progression. DEFB gene with copy number variable, which may alter the gene expression and subsequently, affects susceptibility towards diseases. Research in sub-Saharan Africa showed that higher DEFB copy number was associated with increased HIV load prior treatment and poor immune reconstitution following treatment initiation. In Malaysia, there is still insufficient information on the influence of host genetics particularly DEFB in HIV infection. Thus, this study aimed to investigate the association of DEFB copy number with HIV progression among three major ethnic groups in Malaysia. Two Paralogue Ratio Test system; PRT107a and HSPD21 were used to quantify the DEFB copy number. Then, two microsatellites; EPEV1 and EPEV3 were used to validate the copy number. Results show that DEFB in healthy and HIV groups varied between 2 and 8 copies. Mean of DEFB copy number for healthy group is 4.039 $\pm$ 1.151 and HIV group is 4.385 ± 1.009, with p = 0.034. Mean of DEFB copy number between Malay, Chinese and Indian HIV patients are 4.656 ± 1.181, 4.278 ± 0.815 and 3.900 ± 0.876, respectively, with p = 0.07. HIV patients had significantly higher DEFB copy compared to healthy participants. However, there is no association found between DEFB copy number with HIV infection among three major ethnic groups in Malaysia.

Session B | pp: 14 - 27
Health and the Environment Journal, 2018, Vol. 9, (Supplement 1)

Knowledge, attitude and practice on dengue fever among undergraduate health science students at Universiti Sains Malaysia, Health Campus

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Abstract

Nowadays, dengue is an ordinary and rapid spreading mosquito-borne viral disease in the world. Knowledge, attitude and practice regarding dengue fever are among the factors determine prevalence of dengue outbreak. Dengue fever affects all communities including university students. Until now, there is no specific statistics of university students that have been experienced dengue fever. This study aimed to assess the knowledge, attitude and practice regarding dengue fever among under graduate health science students of Universiti Sains Malaysia, Health Campus. A total of 250 health sciences students from seven different courses involved in this study. All participants need to answer the questionnaire regarding knowledge, attitude and practice of dengue fever. Total score of knowledge, attitude and practice of dengue fever among the students has been analyzed and it showed that dietetics student have higher (90.6%) total score compare to the other courses in this study. Apart from that, this study also showed that there was a significant correlation (p = 0.031) between knowledge and practice of dengue fever among the students. In conclusion, majority of the participants have high total score of knowledge, attitude and practice regarding dengue fever. However, improvement of the knowledge, attitude and practice regarding dengue fever among students must be initiated and implemented as soon as possible. It will help students and other community of the Universiti Sains Malaysia lives in good health environment and free from dengue fever.