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Screening and Molecular Charaterisation of Alpha Thalassemia among Eligible Blood Donors in University Tunku Abdul Rahman (UTAR)

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Background:
Thalassemia is a public health concern in Malaysia with α- and β-thalassemia as the two most common type of thalassemia with the prevalence rate up to 5%. Thalassemia is caused by either reduction or absence of globin chain synthesis due to mutation occurs at the globin gene cluster. As α-thalassemia carrier always presented with normal hematological readings with normal hemoglobin level (Hb), many donors donated their blood unaware they are α-thalassemia carrier. Thus, the objective of this study was to screen α-thalassemia among blood donor using both screening and molecular analysis.

Methods:
Comparison analysis was conducted to compare red blood cell indices including red blood cells, hemoglobin, and hematocrit, MCV, MCH, MCHC and RDW with their α-globin genotype. Ninety (n=90) eligible blood donors were recruited from the blood donation campaign held in UTAR Kampar, fulfilling the calculated sample size. Venipuncture blood was screened by full blood count using hematology analysis. After screening, all the blood samples were subjected for genomic DNA extraction for downstream molecular analysis. Alpha globin genotyping was conducted for seven deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{SEA}$,$--^{THAI}$, $--^{MED}$, $--^{FIL}$), six point mutations
[initiation codon (ATG→AGG), codon 30 (ΔGAG), codon 35 (TCC→CCC), codon 59 (GGC→GAC; Hb Adana), codon 125 (CTG→CCG; Hb QuongSze) and termination codon (TAA→CAA; Hb Constant Spring)] and two triplications ($\alpha\alpha\alpha^{anti3.7}$ and $\alpha\alpha\alpha^{anti4.2}$). Statistical analysis using one-way ANOVA was done to compare the different genotypes with red cell indices.

Results:
Out of ninety study subjects, five genotypes were demonstrated which consisting of 9 (9/90, 10%) $--^{SEA}/\alpha\alpha$, 9 (9/90, 10%) $-\alpha^{3.7}/\alpha\alpha$, 2 (2/90, 2%) $-\alpha^{4.2}/\alpha\alpha$, 1 (1/90, 1%) $\alpha\alpha^{CS}/\alpha\alpha$ and the remaining 69 cases with normal genotype (αα/αα; 69/90, 77%). All the ninety study subjects showed normal Hb level (12.5-16 g/dL). However, RBC, MCV, MCH, MCHC and RDW showed significant difference between genotypes. Thus, it indicates red cell indices except Hb are always relied on the extent of globin chain synthesis.

Conclusion:
Our study concluded that by using Hb level alone in screening for the eligibility of blood donors is not sufficient. Study concluded screening and molecular characterisation should be incorporated together to rule out α-thalassaemia carriers.

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AN AUTOPSY STUDY ON ASSOCIATION OF CARDIAC DISORDERS AND SUDDEN DEATH AT HOSPITAL CANSELOR TUANKU MUHRIZ

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Background:
Sudden death (SD) is an unexpected natural death within 1-hour onset of symptom or unwitnessed death occurs within 24 hours, most often used to describe death caused by cardiac failure. The aims of this study investigate the association between demographic factors and the morphological changes of myocardial tissue and coronary arteries in sudden death cases.

Methods:
Medico-legal cases were obtained from Department of Forensic Hospital Canselor Tuanku Muhriz (HCTM). Left Ventricle Myocardium tissue and Left Artery Descending (LAD) were collected during autopsy and the specimens were fixed in 10% formalin and undergo histopathology procedure with Haematoxylin and Eosin staining. Tissues were graded and classified respectively according to its artery occlusion, myocardial infarction and thrombus formation.

Results:
From medico-legal cases there were 4.6% cases of SD with majority involving male (96%). The range of age was 50 – 59 (36%) turned to be the highest and 70 -79 (4%) being the lowest. The distribution of ethnicity demonstrated majority cases found in Malay (40%), whereby the Indian was the minority (4%). Besides, the highest number of heart weight was 300g – 399g (44%) and the lowest heart weight was 600g – 699g (8%). In addition, from 25 cases there were 10 (40%) cases of coronary artery with atheroma and 15 (60%) cases of myocardial infarction based on histopathological examination. Atherosclerosis was determined by percentage of occlusion ranging zero (normal) to four. The highest number of cases was grade III with 11 patients (44%) followed by grade IV with 5 patients (20%), with 3 (12%) cases of grade I and II, whereby grade 0 was 2 (8%) cases. Whereas for myocardial infarction there were acute myocardial infarction with 14 (56%) cases, healing 9 (36%) and old infarction 1 (4%). Out of the 25 samples 21 (84%) showed negative for alcohol and drugs, whereby (12%) positive alcohol and 4 (16%) positive drugs.

Conclusion:
Based on the results, we can conclude that there is an association between of having cardiovascular disorders with sudden death. However further investigation need to be done to investigate any specific biomarker to predict SD in future.

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COMPARISON OF ANTIOXIDANT LEVELS AND ANTI-INFLAMMATORY ACTIVITIES OF KELULUT HONEY HARVESTED IN DIFFERENT TIME INTERVALS

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Background:
Previous studies showed that harvesting time greatly influence the total phenolic content (TPC), antioxidant levels and anti-inflammatory activities of the honey, particularly on Acacia honey. In Malaysia, the study regarding different harvesting time is still limited, particularly on Kelulut honey (KH). Thus, study of KH harvested in different time is important to maximize the antioxidant levels and antiinflammatory activities of KH. Hence, the main objective of this study is to evaluate the antioxidant levels and anti-inflammatory activities of KH harvested at different time intervals.

Methods:
In this study, KH that was harvested in three different time intervals (3, 6 and 9 months) was supplied by RH Bee Farm located in Sibu, Sarawak, Malaysia. The TPC for KH was determined using Folin-Ciocalteu reagents. The antioxidant level of KH was determined by DPPH, ABTS and FRAP assay. Gallic acid and Trolox were used as a standard for calibration curve construction in TPC and antioxidant assay, respectively. Prior to anti-inflammatory, percentages of cell viability of murine macrophage cells (RAW264.7) treated with different concentrations (0-3%) of KH was measured using MTT assay. Next, the highest concentration of KH that was non-toxic to RAW 264.7 cell was further selected for antiinflammatory assay using lipopolysaccharide (LPS)-induced RAW 264.7 cells. Dexamethasone (0.1µM) was included as a positive control. The inhibitory effect of KH on nitric oxide (NO) production was measured by Griess reagent.

Results:
The TPC and antioxidant activities (DPPH, ABTS and FRAP) of KH harvested in different time intervals varied from 630.7 ± 18.4 to 717.1 ± 30.0 µg GAE/g KH and 128.5 ± 9.1 to 156.5 ± 8.8 µg TEAC/g KH, respectively. The TPC and antioxidant activities showed no significant difference among all of the intervals. KH at 1% of concentration showed no cytotoxic activity (with more than 95% of viable cells) towards RAW 264.7 after 24 hours of incubation. However, the concentration (1% of KH) showed insignificant inhibition on NO production in LPS-induced RAW264.7 cells among all KH harvested in different time intervals.

Conclusion:
It is suggested that harvesting time does not influence the total phenolics, antioxidant and antiinflammatory activities of Kelulut honey.

Keywords: Kelulut honey, total phenolic content, antioxidant levels, anti-inflammatory activity

CYTOLOGICAL EVALUATION OF A NOVEL METHOD FOR ELECTRON MICROSCOPY OF PARAFFIN-EMBEDDED TISSUES EXPOSED TO ELECTROMAGNETIC FIELD (EMF)

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Background:
It is a normal procedure for sampled tissues to be formalin-fixed paraffin-embedded (FFPE) for diagnostic surgical pathology purposes. The FFPE block of tissues can survive long-term storage and has proven to be more cost effective. Recently there has been a renewed interest to find out if the integrity of the tissues were preserved during the long term storage thus allowing the tapping of valuable resources for a long term clinical follow up or simply revisiting the tissues with newer diagnostic techniques. There have reports that some of the FFPE tissues disintegrated after dewaxing and this has been an issue for developing procedures of upcycling FFPE block of tissues to be used for electron microscopy analysis. Thus in this study we propose alternative procedures to make use of FFPE tissues that have been in long term storage in scanning and transmission electron microscopy.

Methods:
The paraffin-embedded tissue blocks that have been stored for a year were used in this study. The tissues were exposed to EMF at different intensities *in vivo*: the Control group, T1: 0.5 mT and T2:1.0 mT. All tissue blocks were processed and H&E stained. The areas of interest were selected and later were *en bloc* for electron microscopy staining. The control stain was osmium tetroxide and comparisons were made on the quality of the staining according to the changes of tissue structure in the treatment groups. The study also evaluates the use and the absence of xylene, and the replacement of propylene oxide with acetone during the dewaxing process, prior to electron microscopy tissues preparation.

Results:
We observed the changes in the tissue structures due to the exposures to EMF at different intensities. The quality of the specimen image produce using post-fixation via osmium tetroxide managed to salvage the changes of tissue structure in the Control, T1:0.5 mT and T21.0 mT groups. Without the post-fixation in osmium tetroxide, the quality of the specimen has the approximately similar to the control.

Conclusion:

As there were no changes in the quality of the staining observed in the light and electron microscopies, this newly developed procedures proved that firstly we can make use of FFPE blocked tissues that has been in long term storage for electron microscopy studies and secondly we can opt for safer but effective histological procedures without employing carcinogenic xylene and acetone.

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EVALUATION OF GLIAPROTECTIVE EFFECT OF XANTHONES ISOLATED FROM Calophyllum spp. AND Garcinia parvifolia AGAINST GLUTAMATE-INDUCED TOXICITY IN BV2 MICROGLIA CELLS

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Background:
Excess extracellular glutamate can lead to glutamate-induced toxicity and eventually causes neurodegenerative diseases and stroke in both *in vitro* and *in vivo* settings. Xanthones with hydroxyl and prenyl groups have been shown to be neuroprotective against toxic insults. Therefore, the gliaprotective effect of euxanthone (EX) and ananixanthone (AX) isolated from *Calophyllum* spp. and rubraxanthone (RX) and 1,3,7 trihydroxy-2,4-bis (3-methylbut-2-enyl) (TX) isolated from *Garcinia parvifolia* against glutamate-induced toxicity were investigated in mouse microglia BV2 cells.

Methods:
The maximum non-toxic dose (MNTD) of four xanthones was determined prior to determining its gliaprotective effect. MTT assay was used to access the cell viability, and intracellular ROS generation was determined by DCFH-DA assay. Apoptosis was determined by observing nuclei morphological changes after staining with DAPI, followed by nuclei morphometric measurements by Image J software to obtain nuclear area factor.

Results:
The MNTD of EX, AX, RX, and TX were 15.85 µM, 10.23 µM, 0.89 µM, and 3.98 µM, respectively. Glutamate treatment alone in BV2 cells induced decrease in cell viability, with the high increment in intracellular ROS production, while the co-treatment of all four xanthones with glutamate significantly increased cell viabilities by 14-22 %, but with insignificant reduction in intracellular ROS production. In addition, the cell and apoptotic morphology analysis indicated mild membrane blebbing, less chromatin condensation, and higher nuclear area factor in BV2 cells co-treated with xanthones and glutamate, suggesting that these xanthones may inhibit glutamate-induced apoptosis in BV2 cells.

Conclusion:
The MNTDs of isolated xanthones confer gliaprotective effect against glutamate-induced toxicity in BV2 cells by increasing in cell viability and inhibiting apoptosis, but less intracellular ROS attenuation. Therefore, the detailed molecular mechanisms involved in the gliaprotective effect of xanthones warrants further investigation.

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SERUM BDNF LEVEL AND MENTAL HEALTH ASSESSMENT OF TRAFFIC POLICE OFFICERS IN THE FEDERAL TERRITORY OF KUALA LUMPUR

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Background:
Mental health status is listed by WHO as one of the criteria for one to be declared as 'healthy'. Stress and traumatic events are among the factors known to cause mental health disorders. This study aims 1) to assess the mental health among the traffic police officers in Kuala Lumpur and 2) to measure the level of brain-derived neurotrophic factor (BDNF) among them where BDNF level has been proved to be influenced by several mental disorders. With this study, the relationship between the level of BDNF and mental health such as depression, anxiety and stress can be further investigated.

Methods:
Seventy four traffic police officers and thirty two administrators from police station located at Jalan Tun H. S. Lee, Kuala Lumpur were recruited. A set of questionnaire including demographic information survey, Malay-version Depression Anxiety Stress Scale (DASS-42), Malayversion Police Stress Questionnaire (PSQ) and Malay-version Post-Traumatic Stress Disorder screening questionnaire (PTSD) was administered to them. Blood serum were collected to measure the BDNF level using enzyme-linked immunosorbent assay (ELISA) method.

Results:
From the results of DASS questionnaire, 24.3% of the traffic police officers were presented with depression, anxiety and stress symptoms. There was no significant difference shown in the depression, anxiety and stress level between the traffic police officers and the control subjects. The serum BDNF level was lower in traffic police officers with depression, anxiety and stress symptoms compared with the healthy control subjects.

Conclusion:
Malaysian traffic police officers seemed to be suffering from various work stressors that lead to the occurrence of mental health disorders among them. The serum BDNF level was lower and found to be related to the mental health disorders in this study. Future works shall focus on the mental health support implemented in traffic police officers.

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ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF FRACTIONATED *DURIO ZIBETHINUS* LINN (D197) LEAF EXTRACTS

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Background:
Durio zibethinus Linn (D197) or commonly known as durian is a tropical plant belonging to the Bombacacaea family which originated from the Malay Peninsular. Traditionally, the leaves of *D. zibethinus* Linn are used as a remedy for influenza, fever and jaundice. Over the years, there has been an increase in pharmacological screening of plants for their bioactive compounds which exhibit medicinal properties. Hence, the present study focused on investigating the antioxidant and antidiabetic properties of *D. zibethinus* Linn leaves.

Methods:
The ethyl acetate crude extract of *D. zibethinus* Linn leaves were fractionated via gravity column chromatography and thin layer chromatography. Subsequent fractions were then tested for their antioxidant activity using DPPH assay. The Folin-Ciocalteu assay and aluminium chloride colourimetric assay were used to quantify the total phenolic content and total flavonoid content of fractions respectively. Four fractions were then chosen based on their antioxidant activity to be tested for antidiabetic properties using alpha-amylase inhibition assay and alpha-glucosidase inhibition assay.

Results:
A total of 16 fractions were obtained from column chromatography which was subsequently combined to yield 7 fractions. DPPH assay showed that fraction 7 had the highest free radical scavenging activity with an EC_{50} value of 743.31 µg/mL. Fraction 7 also had the highest total phenolic content (132.456 µg GAE/mg) and the third highest total flavonoid content (82.07 µg GAE/mg). The highest alpha-amylase and alpha-glucosidase inhibition activity was exhibited by fraction 7 (IC_{50} value of 416.56 µg/mL) and fraction 2 (IC_{50} value of 36.48 µg/mL) respectively. The fractions tested for free radical scavenging activity, alphaamylase inhibition activity and alpha-glucosidase inhibition activity showed dose-dependent relationships.
Based on the results, fraction 7 showed the highest antioxidant and antidiabetic potential.

Conclusion:
D. zibethinus leaves showed potential antioxidant and antidiabetic properties. Overall, fraction 7 was proven to have the most potent antioxidant and antidiabetic properties hence it should be further studied to determine the bioactive compounds present in it.

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INTERLEUKIN-13 RS20541 (R130Q) SINGLE NUCLEOTIDE POLYMORPHISM INCREASES ADIPOGENESIS IN MOUSE ADIPOCYTES 3T3-L1

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Background:
Obesity is excessive fat accumulation that endangers individual's health and it involves proinflammatory cytokine-mediated chronic inflammation. Interleukin-13 (IL-13) is an anti-inflammatory cytokine that is mainly secreted by T-helper cells 2 (T_H2) cells and adipose tissue macrophages. Recently, transgene administration of *IL-13* gene in mice was found to have anti-obesity effects. *IL-13* has a common single nucleotide polymorphism (SNP) rs20541 at position 130, which involves from substitution of arginine (R) to glutamine (Q) (R130Q). This SNP results in enhanced effector mechanisms in allergic inflammation and also has been extensively associated with allergies and chronic inflammatory diseases in ethnically-diverse populations. However, its role in obesity is still unknown. Therefore, the objective of this study was to investigate the effect of IL-13 R130Q on adipogenesis in differentiated mouse adipocytes, 3T3-L1.

Methods:
Differentiated 3T3-L1 cells were transiently transfected or treated with recombinant proteins of human wildtype (wt) or R130Q IL-13. Adipogenesis was assessed by Oil Red O staining, gene expression of adipogenesis genes (*C/EBPα, PPARγ2* and *aP2*) and protein expression of phospho-acetyl-CoA carboxylase (pACC), an enzyme involved in the carboxylation of acetyl-CoA to malonyl-CoA, a substrate for the biosynthesis of fatty acids. Lipolysis activity was quantified by free glycerol concentration and levels of pro-inflammatory cytokine IL-1β were measured by ELISA.

Results:
R130Q IL-13 either by transient transfection or recombinant protein treatment significantly increased adipogenesis compared to wt IL-13, by elevating lipid droplet accumulation, increasing gene expression of *C/EBPα, PPARγ2* and *aP2* and decreasing protein expression of pACC. Lower concentration of free glycerol released from adipocytes transiently-transfected or treated with recombinant protein R130Q IL-13 signifies lower lipolysis activity, compared to wt IL-13. There was also decreased secretion of pro-inflammatory cytokine, IL-1β in adipocytes exposed to wt IL-13, while this effect was diminished in adipocytes exposed to R130Q IL-13.

Conclusion:
Taken together, R130Q IL-13 either by transgene overexpression or recombinant protein treatment increases adipogenesis in mouse adipocytes 3T3-L1. This suggests that this coding SNP may have functional effects in the pathogenesis of obesity.

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LOW DOSE OF MONOSODIUM GLUTAMATE INDUCED OXIDATIVE DAMAGE ON KIDNEY OF MALE SPRAGUE DAWLEY RATS

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Background:
Monosodium glutamate (MSG) is a flavour enhancer which commonly been used in food industries. MSG has been reported to cause oxidative stress that result in damage to male reproductive system, liver, red blood cells and bone marrow hence its usage is still controversial. However, up to date, there is no study done on the effects of low dose of MSG on kidney. Therefore this study was aimed to observe the effect of MSG with low dose on the kidney of male *Sprague Dawley* rats.

Methods:
A total of 18 male *Sprague Dawley* rats, weight between 250-300g were divided randomly into three groups (n=6) consists of control (received distilled water = 1 ml/kg), MSG 60 (received 60 mg/kg MSG) and MSG 120 (received 120 mg/kg MSG) groups. All the substances were given for 28 consecutive days. At the end of the study, all rats were sacrificed and kidneys were isolated for histology and biochemical test.

Results:
Malondialdehyde (MDA) level showed significantly increased ($p<0.05$) in MSG 60 group (3.687 ± 0.233) nmol/mg protein compared with control group (2.449 ± 0.389 unit) nmol/mg protein and MSG 120 group (2.541 ± 0.129) nmol/mg protein. MSG 120 group (10.805 ± 0.998) nmol/mg protein showed significantly increased ($p<0.05$) in protein carbonyl (PC) level compared to control group (1.528 ± 0.253) nmol/mg protein and MSG 60 group (4.932 ± 0.538) nmol/mg protein. Superoxide dismutase (SOD) activity showed significantly increased ($p<0.05$) in MSG 120 group (18.510 ± 0.620) nmol/mg protein compared to control group (3.481 ± 0.557) nmol/mg protein and MSG 60 group (11.032 ± 1.316) nmol/mg protein. However, no significant difference was found in Glutathione (GSH) level of all treated groups
($p>0.05$). Meanwhile, the histological observation showed vasodilation and glomerulus shrinkage in MSG 120 group.

Conclusion:
These findings show MSG can cause small adverse effects on kidney in repeated exposure for 28 consecutive days.

Keywords: Oxidative Damage, Glutamate Receptors, Antioxidant, Reactive Oxygen Species, Protein Oxidation, Lipid Peroxidation

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VALIDATING CYTOTOXCITY OF HYDROGEN PEROXIDE AND INSULIN IN SK-N-SH, NEURONAL CELLS.

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Background:
Neurodegenerative disease such as Alzheimer's disease (AD) has become world public health challenge due to increase in dementia cases. It also characterized by loss or dysfunction of neurons in the central nervous system. Two pathological hallmarks of AD named senile plaque and neurofibrillary tangles are contributed by mainly oxidative stress and mitochondrial dysfunction in the neurons triggered by hydrogen peroxide and insulin resistance respectively. Hydrogen peroxide is a product of Reactive Oxygen Species (ROS) where excess accumulation of ROS can damage proteins, DNA and cell membranes which then induce cell death via the mitochondrial apoptosis pathway. Besides, insulin resistant in brain interrupt the energy metabolism which consequently lead to cellular death via the increased oxidative stress as the brain is highly dependent upon glucose as a source of energy.

Methods:
The neuroblastoma (SK-N-SH) cells are cultured and seeded in 96-well plate before being exposed to 25µM, 50 µM, 100µM and 200µM of Hydrogen Peroxide (H_2O_2) and 62.5nM, 125nM, 250nM and 500nM of insulin for 24 hours. After incubation, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazoliumbromide] assay was performed to assess the toxicity level at 570nm spectrophotometrically. The immunocytochemistry (ICC) was done in 24-well plates to observe the morphological changes upon exposure to different concentrations of toxicant which symbolize the extent of injury from the toxicant induction. Fixation, permeabilization and immuno-blocking were conducted prior the incubation of primary antibody of class III beta tubulin (β-tubulin) in blocking solution. Subsequently, cells were incubated with diluted secondary antibody before being stained with nuclear counterstained DAPI. Morphological changes were analysed under fluorescent microscope attached with imaging software. The MTT results were analyzed using one way analysis of variance (ANOVA).

Result:
Cell viability assay conducted proved the presence of injury in the cells upon minimal concentration of toxicant induction which then validated by morphological analysis. Inhibitory Concentration (IC50) for hydrogen peroxide was achieved at 138µM. In contrast, cells treated with different concentrations of insulin showed no sign of toxicity upon exposure to high concentration of insulin.

Conclusion:
Determination of minimal cytotoxic concentration of hydrogen peroxide and insulin that allow cell recovery is rather crucial for prophylactic study. Therefore, minimal injury of both toxicants to SK-NSH, neuroblastoma cells lines were validated.

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PULMONARY FUNCTION ASSESSMENT ON TRAFFIC POLICE OFFICERS IN FEDERAL TERRITORY KUALA LUMPUR

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Background:
Traffic police officers are vulnerable to hazard occupational exposures which include air pollution, noise and stress. Traffic police officers work at busy traffic and get exposed to various chemicals, heavy metals and respirable hazardous particles (PM_{10}) of pollutions from vehicle emission which eventually could affect the pulmonary function status. However, there is still no study being conducted on their pulmonary status. Thus, the aim of this study is to assess the pulmonary function status by spirometer, collecting PM_{10} level during their 6 hours of working shift and to assess the biomarker of lead toxicity among traffic police officers in Federal Territory Kuala Lumpur.

Method:
Demographic data encompasses pulmonary function test (FEV_1, FVC and FEV_1/FVC%) was performed on office workers (control group) and traffic police officers by using Pony FX Dekstop Spirometer by following standard protocol from NIOSH. To measure the level of particulate matter which is less than 10µm (PM_{10}), a Gillian Personal Air Sampler Pump was attached to the traffic police officers from 0600 hours to 1400 hours by following standard protocol by NIOSH 0600. To determine the urinary delta-aminolaevulinic acid as the biomarker of lead toxicity, a simple rapid one-tube method was measured in urine.

Results:
Out of 39 respondents, 17 are smokers and 22 are non-smokers. Mean ± SD for the BMI for control (28.39 ± 4.40) was higher than traffic police officers (27.200 ± 4.65). The FEV_1 value of control was 2.87 ± 0.78 and FEV_1 value for traffic police officers was 2.90 ± 0.66. Meanwhile, the FVC value of control (3.23 ± 0.75) was lower than the traffic police officers (3.65 ± 0.87). The FEV1/FVC% value was 84.67 ± 5.83 and 82.97 ± 6.93 for control and traffic police officers respectively. Concentration of ALA in urine for control and traffic police officers was 0.53 ± 0.52 and 0.49 ± 0.39 respectively. However, there is no significant difference ($p>0.005$) between smoking, BMI, FEV1, FVC, FEV1/FVC and ALA level between control and traffic police officers.

Conclusion:
We concluded that the pulmonary function status of traffic police officers is normal. Further studies are needed to investigate the pulmonary function status of traffic police officers with a bigger size of sample.

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ASSOCIATION OF SEROTONIN TRANSPORTER SLC6A4 STIN2 VNTR POLYMORPHISM WITH OBESITY, EATING BEHAVIOR AND ALCOHOLISM RISK AMONG UTAR STUDENTS

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Background:
Abnormal eating behaviors such as uncontrolled and emotional eating, obesity and alcoholism had become global issues. Variable number tandem repeat (VNTR) polymorphism in the second intron of *SLC6A4* gene has been extensively associated various neuropsychiatric conditions. Therefore, the objective of this study was to investigate the association of this polymorphism with obesity and its related anthropometric parameters, eating behaviors and alcoholism risk among UTAR students.

Methods:
There are a total of 332 subjects who participated in this study. Eating behaviors namely Cognitive Restraint (CR), Uncontrolled Eating (UE) and Emotional Eating (EE) scores were assessed by the Three Factor Eating Questionnaire-R18, while alcoholism risk was assessed by Self-Rating Effects of Alcohol (SRE) questionnaire. Anthropometric measurements were taken and *SLC6A4* STin2 VNTR was genotyped by PCR using DNA extracted from mouthwash samples. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software.

Results:
Among 332 subjects, 265 of them carried 12/12 genotype, 46 subjects carried 12/10 genotype and 21 carried 10/10 genotype. The minor allele frequency was 0.133. Subjects carrying the 12 allele had significantly higher BMI and visceral fat/subcutaneous fat ratio than those with 10 allele (22.14 ± 0.16 *vs*. 21.14 ± 0.40; $p = 0.03$ and 0.28 ± 0.01 *vs*. 0.24 ± 0.02; $p = 0.008$, respectively). STin2 was associated with overweight, obesity and high overall and central adiposity; those with 10 repeat allele had lower risks [OR: 0.33 (CI: 0.18-0.61; $p < 0.001$), OR: 0.29 (CI: 0.13-0.67; $p = 0.003$), OR: 0.50 (CI: 0.28-0.93; $p = 0.03$) and OR: 0.30 (CI: 0.13-0.67; $p = 0.004$), respectively]. For eating behaviors and alcoholism risk, neither genotype nor allele was associated with them.

Conclusion:
In conclusion, STin2 10 repeat allele confers a protective effect against obesity and high adiposity, but not associated with eating behavior and alcoholism.

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LESSONS LEARNT: A TRANSWELL MIGRATION ASSAY FOR MICROGLIA

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Background:
Inflammation within the central nervous system (CNS) is initiated by microglia. Microglia are migratory cells and respond to various migratory cues including extracellular adenosine triphosphate (ATP). ATP is released when neurons die and is a sign of cellular stress. In this study, ATP was used to establish a transwell migration assay for the BV-2 microglia cell line. For this, various optimisations and considerations were made to develop this model.

Methods:
BV-2 microglia were seeded on a transwell insert with a membrane pore size of 8μm in a 24-well plate containing 5% FBS or 250μM ATP. After incubation, inserts were removed from the plate, rinsed, fixed and permeabilised prior to staining with crystal violet. Cells that had migrated were then viewed with a microscope for analysis and quantification.

Results:
A lesson learnt is that BV-2 migration occurs when serum is present in the culture media. As this may compound the data, migration assays were hereafter performed in the absence of serum. Next, ATP concentrations ranging from 100μM to 1000μM were tested. The concentration of 250μM was discovered to be ideal as it stimulated the highest number of cells to migrate at 24 hours. Later, 250μM ATP was tested in a range of time points from 1.5 hours to 24 hours. The time point of 6 hours showed the best model of BV-2 microglia migration with almost 3.2 to 3.7 times more cells migrated than the negative control. Lastly, a count of migrated cells in 10 random microscopic fields was shown to be representative of the data.

Conclusion:
A relatively simple assay such as the transwell migration assay still requires important considerations during its design and analysis. Here we demonstrated that (i) the transwell migration assay should be performed without serum, (ii) time points and concentration of the ATP chemoattractant were optimised and (iii), a random count of migrated cells was representative of the migration. An ATP-induced *in-vitro* migration assay model for BV-2 microglia represents a generic model of microglia migration that would be relevant in the context of various neuroinflammatory conditions.

Keywords: BV2 microglia, migration assay, transwell, ATP

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THE EFFECTS OF *NYPA FRUTICANS* SUPPLEMENTS ON THE HEALTH STATUS OF PESTICIDES SPRAYERS

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Background:
Pesticides have been used in agricultural sectors to protect the crops from insects and control the disease vectors. Besides giving good effects by increasing the world food production, the processes involved in pesticides handling comes with hazard. Exposure to pesticides may eventually lead to oxidative stress in the body which is associated with hypertension and diabetes mellitus. This problem highlights the importance of finding an alternative to protect the pesticide sprayers. *Nypa fruticans* supplement is suggested as it possessed antioxidant properties which may lower the oxidative stress.

Methods:
In this study, the effect of *Nypa fruticans* supplement on the health status of paddy farmers from Tanjung Karang, Selangor and Pekan, Pahang were assessed. A total of 80 paddy farmers that were exposed to pesticides were selected. Data on the glucose test, blood pressure, blood profile and neurobehavioral symptoms of the paddy farmers were collected upon intervention. The subjects were given 150ml of pure *Nypa fruticans* drink every day for two months. After two months, the blood will be collected again for post-intervention analysis. Asides from glucose test and blood pressure, other parameters that were investigated from the blood are the renal function test, liver function test and lipid profile.

Results:
Currently, the study is still in the intervention phase. However, based on the result from the pre intervention, it can be seen that most of the subject had shown results for renal function test, liver function test and lipid profile that are out of normal range. The intervention from *Nypa fruticans* supplement is expected to be able to lower the blood pressure and glucose level as well as normalizing the reading for renal function test, liver function test and lipid profile. It is also expected to improve the neurobehavioral symptoms among the pesticide sprayers due to the antioxidant effect of *Nypa fruticans.*

Conclusion:
The therapeutic properties of *Nypa fruticans* will show its potential as antioxidant in protecting the farmers against the toxicity of pesticides. *Nypa fruticans* supplement may significantly lower the level of blood pressure, blood glucose and neurobehavioral symptoms after two months' intervention. These findings may increase the understanding on the effect of *Nypa fruticans* on the health status of the farmers. The farmers will be suggested to consume *Nypa fruticans* consistently as they continue to work using pesticides on their paddy fields.