ABSTRACT OF

Proceedings of the 21st Scientific Meeting of the Malaysian Society of Pharmacology and Physiology (MSPP)

24–25 April 2006

School of Medical Sciences,
Universiti Sains Malaysia, Health Campus,
16150, Kota Bharu,
Kelantan

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Malaysian Journal of Pharmaceutical Sciences,
Vol. 5, Supplement 1 (2007)

PHYSIOLOGY/PHARMACOLOGY

FUNCTIONAL CONTRIBUTION OF α_{1A}-ADRENOCEPTORS IN RENAL HEMODYNAMICS OF RATS WITH A COMBINED STATE OF HYPERTENSION AND RENAL FAILURE

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α_1-adrenoceptors are considered the major functional subtypes of α-adrenoceptors in rat renal vasculature. This study investigated the involvement of α_1-adrenoceptors in the renal resistance vessels of rats with a combined state of acute renal failure and hypertension. Acute renal failure was induced by a single injection of cisplatin (5 mg kg^{-1}, i.p.) and confirmed from renal functions and histological observations of the kidney. The study was carried out on twenty-four (n = 24) male spontaneously hypertensive (SHR) rats (250–300 g) with or without renal failure. Animals were anesthetised with sodium pentobarbitone, 60 mg kg^{-1}, i.p. tracheostomy followed by cannulation of right carotid artery and right jugular vein was done. The kidney was exposed by midline abdominal incision followed by cannulation of left iliac artery for the close intrarenal administration of adrenergic agonists and antagonist. The renal artery was isolated and cleared for renal blood flow (RBF) measurement by electromagnetic flowmetry. The changes in RBF was determined in response to graded frequencies of RNS and graded doses of adrenergic agonists viz. noradrenaline, phenylephrine and methoxamine in the absence and presence of specific α_{1A}-adrenoceptor antagonist 5-methylurapidil (MeU) and Ca^{+2} channel blocker amlodipine (AMP). Data obtained indicated a significant (all $p<0.05$) attenuation of adrenergically induced renal vasoconstrictor responses by both MeU and AMP in both groups of animal except that MeU caused a significant accentuation ($p<0.05$) in the phenylephrine induced responses in renal failure SHR rats. Results derived showed the presence of α_{1A}-adrenoceptors as the major functional subtype in the renal resistance vessel of renal failure SHR rats with possible involvement of other α_1-adrenoceptors subtypes.

METHOD OPTIMISATION ON THE USE OF POSTOCCLUSIVE REACTIVE HYPEREMIA MODEL TO ASSESS MICROVASCULAR FUNCTION

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Recent developments had allowed non-invasive assessment of microvascular function *in vivo,* however, the method has not been fully optimised. In this study, we aimed to assess the "effective" occlusion duration needed to elicit a sufficient post-occlusive reactive hyperemia (PORH) response in forearm skin using laser doppler fluximetry (LDF) in subjects with differing ages, gender and menstrual phases. 120 healthy subjects were studied (20 subjects each in the age ranges of 21–30, 31–40, 41–50 years for both genders). Male subjects were randomised to receive 1, 2 or 3 min occlusion on three different study days. Female subjects attended six study days: the first three days (with different occlusion times) were performed during the low estrogenic phase of menstrual cycle (days 2–5) and subsequent three visits were performed during high estrogenic phase of menstrual cycle (days 10–13). Skin perfusion was measured before, during and after occlusion using LDF. The magnitude and temporal responses to the process of reactive hyperemia were expressed as $PORH_{max}$ (maximal increase in hyperemia perfusion) and T_p (time-to-peak), respectively. For $PORH_{max}$ analysis, optimum occlusion duration should be applied based on one's age, gender and menstrual phase. The $PORH_{max}$ responses were more homogenous during high estrogenic phase with 2 min found as the "effective" occlusion duration in all female groups. For T_p analysis, 3 min occlusion produced significant change in all age range for both genders irrespective of menstrual phase. This study revealed that, for assessment of microvascular function using LDF and reactive hyperemia, the occlusion duration for the parameter $PORH_{max}$ is affected by age, gender and menstrual phase. Measurement based on T_p is however independent of these factors.

EFFECT OF A SELF EMULSIFYING FORMULATION OF PALM VITAMIN E ON ARTERIAL COMPLIANCE AND VITAMIN E BLOOD LEVEL IN HEALTHY SUBJECTS

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Tocotrienol (T3) is the predominant vitamin E in palm oil. This study assessed the effect of a self emulsifying preparation of tocotrienol rich vitamin E (SF-TRE) on arterial compliance (index of vascular health) and plasma concentration in healthy subjects. This randomised, placebo controlled, blinded end point clinical trial with a parallel design involved 36 male subjects divided into four groups. Each group took either placebo, or SF-TRE at doses of 50, 100 or 200 mg T3 daily for two months. The SF-TRE contained 23.54%, 43.16%, 9.83% and 23.5% of α, γ, δ-T3 and α-tocopherol, respectively. Measures of arterial compliance, pulse wave velocity (PWV) and augmentation index (AI), and other parameters that were plasma total antioxidant status, plasma T3 and tocopherol concentrations, serum total cholesterol and low density lipoprotein were measured before and two months after treatment. Baseline T3 levels were low, however, after treatment, all treated groups had significantly higher plasma α, δ, γ-T3 concentrations compared to placebo. There was borderline significance between placebo and 100 mg ($p = 0.076$) for their change in AI from baseline to end of treatment; treated groups showed significant improvement in AI after treatment, change for groups placebo, 50, 100 and 200 mg being 2.22 ± 1.54, –6.59 ± 2.84, –8.72 ± 3.77 and –6.27 ± 2.67, respectively. Change in PWV with treatment was not significantly different between groups, although groups 100 and 200 mg showed improvement from baseline to end of treatment. There was no effect of TRE on other study parameters. There was a trend towards improvement in arterial compliance with two months of SF-TRE, further studies on patients with vasculopathy is suggested.

EFFECTS OF HIGH AND LOW SODIUM CONCENTRATIONS ON ACETYLCHOLINE-INDUCED RELAXATION IN AORTIC RINGS OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Previous studies using aortic rings from normotensive male rats fed on a high-salt diet for over three days displayed impaired acetylcholine (ACh)-induced relaxation. In the present study, we investigate further the effects of low- and high-NaCl concentrations on vascular

responsiveness to ACh in streptozotocin (STZ)-induced diabetic male and female rats. Rat aortic rings obtained at 3–7 days, 3–4 weeks, 11–12 weeks, and 16–17 weeks after STZ or vehicle (control rings) injection were suspended in a 10 mL tissue bath containing low-, normal- or high-NaCl Krebs solution at 37°C. Isometric tension recordings of cumulative concentration-responses to ACh were measured. Control aortic rings produced a significantly lower ACh-induced relaxation in high-NaCl solution, but a similar degree of relaxation to ACh in low-NaCl solution, when compared to that in normal-NaCl solution. In normal-NaCl solution, ACh-induced relaxation was significantly impaired in aortic rings from diabetic female rats of 3–7 days ($p<0.001$), 3–4 weeks ($p<0.001$) and 16–17 weeks ($p<0.05$) when compared to control rings. On the other hand, aortic rings from diabetic male rats exhibited a significant impaired ACh-induced relaxation ($p<0.01$) only after 11–12 weeks when compared to control rings. Low-NaCl solution completely restored the impaired ACh-induced relaxation in aortic rings of male and female diabetic rats of 11–12 weeks and 16–17 weeks, respectively, when compared to control rings. High-NaCl solution had no effect on the ACh-induced relaxation of aortic rings from male and female diabetic rats of 11–12 weeks and 16–17 weeks when compared to control rings. Our data show that ACh-induced relaxation in diabetic vessels is preserved in low extracellular sodium concentration at the later stages of diabetes and is not altered in high extracellular sodium concentration.

LIPID-LOWERING AND ANTI-INFLAMMATORY ACTIVITIES OF *CHANNA MICROPELTES* (TOMAN) IN CHOLESTEROL-FED RABBITS

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C. micropeltes possess anti-transudative and anti-proliferative activities in chronic inflammation that was comparable with non-steroidal anti-inflammatory drugs (NSAIDs). This study was carried out to evaluate the lipid-lowering and anti-inflammatory effects of *C. micropeltes* extract in diet-induced hypercholesterolemic rabbit. The serum lipid profile, i.e. total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride (TG), as well as inflammatory markers, fibrinogen and C-reactive protein (CRP), were measured every fortnightly for six weeks in 24 male New Zealand White rabbits (n = 6 per group). All the groups except normal group (N) were fed cholesterol-enriched diet to induce hypercholesterolemia. One of the groups was treated concurrently with 5 mg/kg atorvastatin (A) and the other with 300 mg/kg aqueous extract of *C. micropeltes* (T). One group acted as cholesterol-control (CC) with no treatment given. TC, LDL and TG were significantly lowered in rabbits treated with 300 mg/kg *C. micropeltes* compared to CC group ($p<0.05$). Moreover, *C. micropeltes* increased the HDL cholesterol level from week 0 ($p<0.05$). Besides that, 300 mg/kg extract also exhibited anti-inflammatory effect by significantly reducing CRP and fibrinogen level ($p<0.05$). Atherogenic index (AI) at week 6 showed decreased athrogenesis in groups given the extract where the values were lower than atorvastatin-treated group ($p<0.05$ vs

CC & A). As a whole, it can be concluded that 300 mg/kg *C. micropeltes* extract is an excellent lipid-lowering and anti-atherogenic as well as anti-inflammatory agent.

EFFECTS OF A SUPERVISED EXERCISE PROGRAMME ON AEROBIC FITNESS OF ELDERLY ADULTS IN HULU LANGAT DISTRICT

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Participation in a regular exercise programme is an effective intervention to reduce/prevent a number of functional declines associated with aging. Apart from improving muscular strength and offsetting loss of muscle bulk, it also improves cardiorespiratory or aerobic fitness provided the exercise regimen prescribed is effective. The objective of this study was to investigate the effects of eight weeks of supervised exercise programme on the aerobic fitness of elderly adults in Hulu Langat district. Fifty-eight healthy elderly adults (mean age 60.1 years (SD = 6.3)) 21 males and 37 females were allocated into two groups: an intervention group (n = 30) and a control group (n = 28). They have been conveniently sampled from a larger cohort of elderly people who were participants of the MediPro-HUKM programme in Hulu Langat district. The exercise group underwent a moderately intense exercise programme based on the FITT principle for eight weeks supervised by a qualified instructor. The frequency is three times per week (Mondays, Wednesdays and Saturdays) with exercise intensity of between 60% to 80% of maximum heart rate (depending on estimated initial fitness level), type of exercise included low impact aerobics (LIA) and calisthenics for a total of one hour per session. Aerobic fitness was evaluated using the two minute step test before and after the intervention period. Although estimated aerobic fitness improved in both groups, the improvement seen in the exercise group was significantly higher. Eight weeks of regular, moderately intense exercise has a positive impact on estimated aerobic fitness in this group of elderly subjects.

EFFECTS OF REGULAR EXERCISE ON SYTEMIC BLOOD PRESSURE AND LIPID PROFILE OF ELDERLY ADULTS IN HULU LANGAT DISTRICT

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Age related changes normally bring about an increase in systemic blood pressure and is among the factors frequently implicated in increasing the incidence of cardiovascular diseases among this age group. Regular exercise has been recommended in lowering the

systemic blood pressure and improving lipid profiles. The purpose of this study is to determine whether the selected exercise regimen was able to induce a change in both the blood pressure and lipid profile among an elderly population. Fifty eight healthy subjects (21 males and 37 females, mean age = 60.1 years (SD = 6.3)) were conveniently divided into an intervention (n = 30) and control group (n = 28). The intervention group underwent eight weeks of exercise programme (three times a week (Mondays, Wednesdays and Saturdays)), at an intensity of between 60% to 80% of maximum heart rate, total exercise time of one hour per session, consisting of low impact aerobics and calisthenics). Resting systemic blood pressure was measured and fasting venous blood samples were taken for lipid profile assessment pre and post intervention. After eight weeks, there was a significant decrease in mean diastolic blood pressure (DBP) of 7.5 mmHg and systolic blood pressure (SBP) of 2.2 mmHg in the intervention group ($p<0.05$) while in the control group there was a decrease of 3.6 mmHg in DBP and an increase in SBP of 2.3 mmHg. Total cholesterol levels (TC) showed a mean decrease of 0.33 mmol/L in the intervention group and 1.06 mmol/L in the control group. Triglyceride levels showed a significant decrease of 0.42 mmol/L in the intervention group ($p<0.05$) while there was an increase of 0.23 mmol/L in the control group. Low density lipoprotein cholesterol (LDLC) levels decreased 0.67 mmol/L in the intervention group and 2.02 mmol/L in the control group. High density lipoprotein cholesterol (HDLC) increased 0.54 mmol/L in the intervention group and 0.82 mmol/L in the control group. After eight weeks of an exercise programme, a decrease in systemic blood pressure was seen in the intervention group, however the lipid profile changes between the two groups were non conclusive. These variable results may have been affected by dietary changes among the subjects.

TGF-β1 mRNA EXPRESSION IN KIDNEY OF DIABETIC RATS

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Studies have shown that TGF-β1 may in part play a role in the pathogenesis of glomerulosclerosis that is associated with chronic renal failure and diabetic nephropathy. The present study sets out to analyse the expression level of TGF-β1 mRNA in diabetic rats. Seven-week-old male Sprague Dawley (SD) rats were divided into two groups. One group was made diabetic via intraperitoneal injection of streptozotocin (60 mg/kg), the other was control. Hemodynamic studies were performed on anaesthetised animals (sodium pentobarbitone 60 mg/kg, i.p.) on the ninth day after diabetic induction. The kidney was subsequently isolated at the final stage and RNA was extracted to be measured quantitatively by real-time PCR. Diabetic induced rats showed 12.5% increase in GFR, however it was not significantly different from that of control. The body weight of diabetic rats declined throughout the experimental duration and the reverse was true for control rats ($p<0.01$). Meanwhile the blood glucose levels in diabetic rats increased throughout the nine day duration and the normal rats showed a stable reading ($p<0.01$). Haemodynamic data showed significant increase in blood pressure of diabetic rats as compared to control rats ($p<0.01$) whereas the renal blood flow showed no significant

difference between the two groups. The TGF-β1 mRNA expression was significantly elevated in diabetic rats as compared to control rats ($p<0.01$). This study shows that TGF-β1 mRNA expression is elevated in diabetic rats. Since TGF-β1 is implicated in renal diseases, therapeutic interventions could target on this specific gene expression as a treatment approach.

IN VITRO CYTOTOXICITY OF ANTIFUNGAL DRUGS ITRACONAZOLE AND FLUCONAZOLE: INFLUENCE OF CYTOCHROME P450 INHIBITORS

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Itraconazole and fluconazole are triazole antifungal drugs, which have been reported to induce hepatotoxicity in patients. The mechanism underlying the hepatotoxicity is unknown. The present study was design to investigate the role of cytochrome P450 inhibitors, SKF 525A and curcumin pretreatment on the cytotoxicity of anti-fungal drugs fluconazole and itraconazole. For three consecutive days, female rats were administered daily with SKF 525A or curcumin (5 and 25 mg/kg). Control rats received equivalent amount of dosed vehicle. The animals were anaesthetised 24 h after receiving the last dose for liver perfusion and hepatocytes were harvested. Hepatocytes (1×10^6 viable cells/mL) were then exposed to various concentrations of anti-fungal drugs (0.001, 0.01, 0.1 and 1.0 mM). *In vitro* incubation of hepatocytes with itraconazole revealed significantly lower viability when compared to fluconazole assessed by lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase activities. The cytotoxicity of itraconazole was enhanced when incubated with hepatocytes pretreated with SKF 525A. SKF 525A had no effects on the cytotoxicity of fluconazole. Curcumin failed to either increase or decrease the cytotoxicity of both anti-fungal drugs. ATP levels also showed significant decrease in both itraconazole and fluconazole incubated hepatocytes. However, SKF 525A pretreated hepatocytes had significantly lower ATP levels after itraconazole incubations. Collectively, these results suggest the involvement of cytochrome P450 in the cytoprotection of itraconazole induced hepatocyte toxicity.

EFFECTS OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* ON MALE SEXUAL BEHAVIOUR IN SPRAGUE DAWLEY RATS

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Traditional medicinal are widely utilized in the treatment of various ailments on an empirical basis. A variety of plants have been used for the treatment of ulcer, hypertension, diabetes and male reproductive dysfunction. In folk medicine, *Andrographis paniculata* (AP) have been used extensively to treat diabetes. Whilst, AP was believed to have anti-fertility effects, its effect on sexual behaviour is not known. The male sexual behaviour in Sprague Dawley (SD) rats are complicated, post significant challenges when utilized for studying of drugs effect. No published information are found in the literature on the effect of ethanolic extract of AP on the sexual behaviour of male SD rats. The main objective of this study is to evaluate the effects of ethanolic extract of AP on male sexual behaviour of SD rats. The design of the study involves the use of adult male SD rats weighing 170 to 210 g (8–10 week of age) administered of the extract of AP (APE) at five doses: 0.5, 1.0, 10, 100 and 100 mg/kg for more than 77 days by oral gavaging. The control group received distilled water for the same duration. All animals undergo male reproductive performance study test before being used for the experiments. The male sexual behaviour study involved the experimental animals undertaking a 30 minutes exposure to estrus female rats. The mating activities were videotaped and subsequently analysed. Parameters observed include mount latency (ML), intromission latency (IL), ejaculation latency (EL), number of intromission (NI), post ejaculatory interval (PEI), intromission frequency (IF), inter intromission interval (III) and copulatory rate (CR). Data derived from groups that achieved second ejaculation or more were used for calculation. APE treated groups shown no significant differences as compared to control groups. All APE treated groups showed similar pattern with regards to male sexual parameters as that of control groups. However, male rats receiving 10 mg/kg of APE did not achieve second ejaculation. The male sexual behaviour of APE treated rats were similar to that of control groups. These provide evidence that APE did not induce any changes to male sexual behaviour in SD rats.

MORPHOLOGICAL ALTERATIONS IN MITOCHONDRIA FOLLOWING DICLOFENAC AND IBUPROFEN ADMINISTRATION IN RATS

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Diclofenac is a commonly used non-steroidal anti-inflammatory drug (NSAID) known to cause hepatotoxicity, while ibuprofen is considered as one of the safest NSAID available. Recent *in vitro* studies indicate that these drugs may trigger apoptosis probably by inducing mitochondrial permeability transition. This study was conducted to identify and to compare the mitochondrial morphological alterations in livers of rats treated with diclofenac and ibuprofen. Male Sprague Dawley (SD) rats were dosed with 3, 5 and 10 mg/kg diclofenac and ibuprofen in saline via intraperitoneal injection for 15 days. The control group was administered with saline in a similar manner. Livers were removed, cleaned and processed for ultrastructural study. Mitochondria were observed to be enlarged with some membranes found to be ruptured in liver sections obtained from rats treated with 10 mg/kg diclofenac and ibuprofen after day 15. Chromatin structure found to be condensed in the nucleus of the same groups. These changes were not observed in animals after three and nine days of treatment or those receiving lower doses. The presence of apoptotic cells following microscopic observation was confirmed by TUNEL assay conducted in all groups. Apoptotic cells were found in perivenular regions in rats treated with diclofenac and ibuprofen dosed at 10 mg/kg on day 15. This observation may indicate the possibility of the changes in mitochondria structure by both diclofenac and ibuprofen.

THE REM SLEEP DEPRIVED RATS EXHIBIT CHANGES IN THE PAIN BEHAVIOURS IN FORMALIN-INDUCED PAIN: PRELIMINARY DATA

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REM sleep deprivation in rats has been shown to affect the pain modality after subjected to various pain stimuli including the chemical (formalin) noxious stimulus. Nevertheless, the pattern of pain behavioural responses of the formalin-induced pain in the REM sleep-deprived rats is poorly investigated. The purpose of this preliminary study is to determine whether the REM sleep-deprived rats exhibit certain pattern of pain behavioural responses after subjected to formalin test. Ten adults male Spraque Dawley (SD) rats, weighing 250 to 300 g were randomly assigned to free moving control group (FMC, n = 5) and REM sleep-

deprived group (REMsd, n = 5). REMsd was elicited for 48 hours using an "inverted flower pot" technique. After 48 hours, formalin test was performed by injecting 2.5% formalin subcutaneously to the plantar surface of the rat's right hindpaw. Following the formalin injection, the frequency of flinching or time spend licking the injected paw was recorded for the phase 1 response (0–5 min after injection) and phase 2 responses (20–60 min). For phase 1 response, both flinching and licking of the injected paw showed no significant differences between FMC and REMsd. For flinching, in phase 2 responses showed significantly higher frequency in FMC than REMsd ($p<0.05$). For phase 2 responses, cumulative time of licking the injected paw FMC showed significantly longer duration than REMsd ($p<0.05$). Overall, the data showed a significant reduction in the pain behavioural responses in the phase 2 of the formalin-induced pain after subjected to REM sleep deprivation.

PREVALENCE OF CYP11B2 GENE (T344C) POLYMORPHISM IN DIABETES MELLITUS TYPE II AMONG MALAYS

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Aldosterone synthase encoded by CYP11B2 gene has steroids 11 β-hydroxylase activity that is essential to synthesise aldosterone. Aldosterone is known regulator of vascular tone. Excess of aldosterone initiates the collagen deposition in blood vessels. The point mutation T344C of CYP11B2 gene is associated with increase aldosterone plasma level and alteration in large arteries in hypertension. The cardiovascular complications are more common in diabetes mellitus therefore objective of the present study is to determine the prevalence of T344C polymorphism in type II diabetes mellitus. A cross-sectional study was done including age and sex matched 180 uncomplicated diabetic, normotensive and 180 normoglycemic, normotensive subjects. All subjects gave informed written consent. Anthropometric measurements and blood pressure was measured, blood samples were taken for routine laboratory investigations and genotyping. Genotyping was done by PCR-RFLP technique with the help of enzyme restriction Hae III. Allele frequencies were determined according to the Garcia-Barcelo et al. (2000). Frequency of –344T allele is 71.38% in diabetic group and 71.16% in control group whereas frequency of –344C allele is 28.61% in diabetic group and 28.33% in control group. Between the two groups no differences were observed in both alleles. Similar frequency in both groups suggests that –344C allele is not associated with diabetes mellitus type II.

ANGIOTENSIN II TYPE 1 RECEPTOR A1166C GENE POLYMORPHISM AND ISCHAEMIC STROKE IN MULTIETHNIC MALAYSIAN POPULATION

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The angiotensin II type 1 receptor (AT_1R) A1166C gene polymorphism has been associated with ischaemic stroke in some population studies. We investigated the incidence of the AT_1R gene polymorphism in multiethnic Malaysian ischaemic stroke patients. Ethics approval and informed consent were obtained for the study. A number of 117 ischaemic stroke patients and 156 controls were recruited from Universiti Malaya Medical Centre, Kuala Lumpur. They were of Chinese, Indian and Malay ethnicity. The AT_1R genotype was analysed by PCR and RFLP. The genotype frequency for the stroke group was 0.31, 0.42, and 0.27 for the AA, AC and CC genotypes, respectively, while the control group genotype, frequency was 0.63, 0.28 and 0.1, respectively. The genotype distribution was significantly different ($p<0.001$). When broken down into different ethnic groups, the distribution was also significantly different in the Chinese and Malays, but not the Indians. The AT1R A1166C gene polymorphism may be associated with increased risk for ischaemic stroke.

CRUDE EXTRACTS OF *CATHARANTHUS ROSEUS* (PERIWINKLE) HAVE STRONG ANTIOXIDANT PROPERTIES

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Natural resources offer an unlimited source of new agents for the cosmeceutical, nutraceutical, pharmaceutical and agrochemical industries. *Catharanthus roseus* (CR) has been shown to contain components, such as vinblastine and vincristine, that have anti neoplastic properties for cancers of the breast, bladder, lung, lymphomas, leukemias and various other neoplastic diseases. In this study, we investigated whether the crude extracts of CR have any antioxidant properties using DPPH free radical scavenging method. For this study, the crude extract from the CR white flower (CRW), water (CRW-W) and ethanol (CRW-E) extracts were analysed. The total protein and total polyphenol contents of the extracts were evaluated using a modification of Lowry and Schalbert methods. CRW-W extract showed high radical scavenging activities with EC_{50} of 25.7 µg/mL and total protein of 44.5%. These values are higher compared to CRW-E that showed EC_{50} 64.7 µg/mL and total protein 12.1%. However, the total polyphenol of

CRW-E extract showed higher content than the CRW-W extract. The results from this study indicate that CR crude extracts may have components with strong antioxidant properties and this property may be due to the protein content of the extract.

EFFECTS OF *ANDROGRAPHIS PANICULATA* (HEMPEDU BUMI) ETHANOL EXTRACT AND ANDROGRAPHOLIDE ON GLUTATHIONE–S-TRANSFERASE ACTIVITY (*IN VITRO)*

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The effects of ethanol extract of *Andrographis paniculata* (AP) and andrographolide (AND) were studied on glutathione-S-transferase (GST) enzyme activity. AP (Hempedu bumi) from the *Acanthaceae* family is a medicinal plant in the traditional treatment of diseases such as fever, malaria, asthma, bronchitis and hepatitis. AND is the bioactive compound of AP. An *in vitro* study was conducted on the AP extract at concentrations of 5, 50 and 500 µg/mg protein; AND at concentrations of 0.5, 5 and 50 µg/mg protein. Post-mitochondria fractions from liver of normal male Sprague Dawley (SD) rats were prepared according to calcium precipitation method. The protein levels were measured according to Lowry method and total GST activity was determined with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. In the *in vitro* study of AP on GST, there was a significant decrease ($p < 0.05$) in enzyme activity of GST at AP concentration of 500 µg/mg protein (0.10 µmol/min/mg protein) compared to control (0.17 µmol/min/mg protein). There were no significant differences in GST activity at lower levels (5 and 50 µg/mg protein) compared to control. Study on AND at concentrations of 0.5, 5 and 50 µg/mg protein showed no significant differences compared to control. In conclusion, the results showed that the ethanol extract of AP modulate the activity of GST depending on the doses. AND at concentrations of 0.5, 5 and 50 µg/mg protein had no effect on the activity of GST enzyme.

DOES HONEY IMPROVE *IN VITRO* EMBRYO DEVELOPMENT IN MICE INDUCED WITH STRESS?

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Psychosocial stress has been reported to have a negative effect on pregnancy such as abortion or preterm delivery. The detrimental effect of psychosocial stress is also related to increased oxidation. Honey contains a variety of phytochemicals that act as antioxidant. This study was undertaken to determine the effect of honey on the development of preimplanted embryos in mice immersed in water. Fourty female mice were divided equally into four groups. The first group was the control group. The second group was immersed in water for half an hour while the third group was immersed in water and given honey. The last group was given only honey. After one month of treatment, the female mice were superovulated and mated with the male mice. Once fertilization has occured, the female mice were killed, the oviducts were removed and the embryos were flushed out for *in vitro* culture. The number of normal and abnormal embryos were determined using an inverted microscope. The normal embryos were then cultured in Whitten medium at 5, 24, 48, 72 and 96 hours. The number of embryos are determined and observed at the specified hours. The results showed that at 0 hour, the group that received only honey showed the highest percentage of normal embryos (39.4%) followed by the control group with 30.1%, the stress plus honey group had 22.3% normal embryos and the least number of normal embryos is in the stress group (8.2%). The same pattern was seen 96 hours later. At this stage the *in vitro* culture of the group given stress showed the lowest number of embryos (1.3 ± 0.7) which was significant compared to the other three groups (control: 6.1 ± 1.9, honey: 5.7 ± 1.0 and honey+stress: 4.3 ± 0.9) at $p<0.05$. The honey plus stress group is significantly higher than the stress group ($p<0.05$) but did not differ significantly with the other two groups. In conclusion, this study indicated that stress induced by immersion in water reduced *in vitro* embryo development and that supplementation of honey protects against this negative effect.

LABISIA PUMILA VAR *ALATA* INCREASED 11 BETA HYDROXYSTEROID DEHYDROGENASE TYPE 1 ACTIVITY AND INDUCED FAT DEPOSITION IN THE LIVER OF OVARIECTOMISED SPRAGUE DAWLEY RATS

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*Labisia pumila v*ar *alata* or as locally known Kacip Fatimah (KF) is a small plant that is presumed to have numerous uses. It is a traditional herb used by Malay women to induce and facilitate childbirth, postpartum medicine as well as to increase a woman's libido. It is usually prepared by boiling over low heat and the water decoction taken as a drink. This study investigates mainly the effect of *Labisia pumila* consumption on the enzyme 11β-Hydroxysteroid Dehydrogenase type 1 (11β-HSD1) in the liver and adipose tissue. Thirty five adult female (Sprague Dawley SD) rats, six months old were used in the experiment. Rats were divided into normal (NOR) and ovariectomised (OVXC, KF and ERT). Bilateral ovariectomy was performed under anesthesia using the ventral approach. Body weight was checked and recorded monthly. At the end of the study period, rats were sacrificed by cervical dislocation and tissue samples from liver and abdominal fat was collected and either transferred into –70°C environment for bioactivity assay or fixed in paraformaldehyde for electronmicroscopy. Liver samples were also fixed in 10% formalin for light microscopy. *Labisia pumila*-treatment induced a significant increase ($p<0.05$) in mean hepatic 11β-HSD1 when compared to the OVXC group. There was 21.4% increase in mean enzyme activity when comparing KF and OVXC compared to only 5.6% increase when comparing ERT and OVXC. In adipose tissue, there was no significant difference in the percentages recorded for the groups. This study showed that *Labisia pumila*-treatment increased hepatic 11β-HSD1 activity and contributed in fatty liver induction in OVX rats. Further study is needed to confirm the observation.

ANTIOXIDANT AND HEPATOPROTECTIVE PROPERTIES OF *ORTHOSIPHON STAMINEUS* BENTH WATER/METHANOL STANDARDIZED EXTRACT

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The aim of the present study was to evaluate the *in vitro* antioxidant and free radical scavenging activities and *in vivo* hepatoprotective activity of *Orthosiphon stamineus* leaves methanol/water (50:50) extract (SEOS). The DPPH radicals scavenging and Fe^{3+}-induced lipid peroxidation inhibiting activities and trolox equivalent antioxidant capacity (TEAC)

of SEOS were determined. The results indicated that SEOS exhibited antioxidant, lipid peroxidation inhibition and free radical scavenging activities. The hepatoprotective activity of the SEOS was studied using CCl_4-induced liver toxicity in rats. The activity was assessed by monitoring liver function tests through the measurement of alanine transaminase (ALT) and aspartate transaminase (AST). Furthermore, hepatic tissues were also subjected to histopathological studies. Pretreatment of SEOS (125, 250, 500 and 1000 mg/kg p.o.) dose-dependently reduced the necrotic changes in rat liver and inhibited the increase of serum ALT and AST activities. The results of the present study indicated that the hepatoprotective effect of *O. stamineus* might be ascribable to its antioxidant and free radical scavenging property.

ANTIOXIDANT ACTIVITY OF *GYNURA PROCUMBENS* LEAVES

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The aim of the present study was to evaluate the *in vitro* antioxidant and free radical scavenging activities of methanol extract of *Gynura procumbens* leaves and its fraction: chloroform, ethyl acetate, butanol and water fractions. The antioxidant activity of the extract and fractions were assessed by means of four different *in vitro* assays: (1) Trolox Equivalent Antioxidant Capacity (TEAC), (2) 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging, (3) β-carotene linoleate, and (4) xanthine oxidase inhibition methods. The total phenolic content of methanol extract and its fractions were also determined using Folin-Ciocalteu method. The results obtained show that the extract and all its fractions possess antioxidant activity in all the *in vitro* models used with ethyl acetate and butanol fractions being the strongest. The total antioxidant activity of 2.5 mg/mL ethyl acetate and butanol fractions were 12.6 mM and 4.8 mM Trolox equivalents, respectively. Ethyl acetate and butanol fractions (100 ppm) caused 82.2% and 80.9% inhibition of xanthine oxidase activity respectively, which was greater than caused by the same concentration of allopurinol (75.5%). The total of phenolic content in ethyl acetate and butanol fractions were 23.58% and 8.36%, respectively. The results obtained in the present study indicated that *G. procumbens* leaf is a good source of natural antioxidant.

THE EFFECT OF *TINOSPORA CRISPA* EXTRACT ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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Atherosclerosis refers to the "hardening of the arteries" in which the wall of the arteries thickens due to lipid deposition on the intimal layer. The aim of this experiment is to study the effect of *Tinospora crispa* (TC) extract on the development of experimental atherosclerosis. The duration of this study was eight weeks. Fifteen male New Zealand White rabbits with body weight of 1 to 1.5 kg were randomly divided into five groups: group A (normal pellet), group B (normal pellet + 10% TC aqueous extract), group C (pellet coated with 20% ghee), group D (pellet coated with 20% ghee +10% TC aqueous extract) and group E (pellet coated with 20% ghee + 5 mg simvastatin). Body weights were recorded at week 0 (w0), week 4 (w4) and week 8 (w8). The blood was collected from ear vein at w0, w4, w8 and the serum was analysed for total cholesterol and lipid peroxidation index measured using TBARS method. The aortas were excised and examined macroscopically by Sudan IV staining for atherosclerotic plaque and microscopically by H&E staining to observe formation of foam cells. All the results were analysed by one-way ANOVA and Tukey HSD with significant difference at $p<0.05$. The results showed that TC and simvastatin treatment do not influence the body weight of rabbits throughout the experimental period. The treatment of TC slightly reduced 23% of total cholesterol level and significantly lowered ($p<0.05$) the MDA level. No atherosclerotic areas were observed in the macroscopic examination. In H&E satined sections, foam cells were observed in group C and group D. TC possesses lipid lowering and antioxidant properties as indicated by lowered total cholesterol level and lipid peroxidation index.

BUTANOLIC FRACTION OF *GYNURA PROCUMBENS* LOWERS BLOOD PRESSURE OF THE RAT BY VASORELAXATION

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In Malaysia, the leaves of *Gynura procumbens* (*G. procumbens*) are used in traditional medicine as an antihypertensive remedy. In the previous studies, we have shown that both the final aqueous fraction (FA) obtained from the crude ethanolic extract of the

leaves and its purer subfraction (FA-I) have significant hypotensive activities in rats. In the present study, another fraction that is extracted in butanol (the butanolic fraction, BU) is evaluated for a possible hypotensive activity. In addition, attempts are made to elucidate the underlying mechanisms involved. Direct blood pressure measurement was monitored from a cannulated carotid artery connected to a pressure transducer using the Maclab recording system. Intravenous administrations of BU (0–20 mg/kg) produced a dose-dependent and significant ($p<0.001$) fall in the mean arterial blood pressure of anaesthetised spontaneously hypertensive rats, as well as their normotensive controls, the Wistar-Kyoto rats. In rat isolated thoracic aorta preparations, BU ($1x10^{-6}$–$1x10^{-1}$ g/mL) induced a concentration dependent relaxation of endothelium-intact and -denuded aortic rings precontracted with phenylephrine (PE, $1x10^{-6}$ M) or KCl (80 mM). The BU fraction concentration dependently inhibited the contraction induced by PE ($1x10^{-9}$–$3x10^{-5}$ M) or KCl (10–80 mM) in both endothelium-intact and -denuded aortic rings. The Ca^{2+}-induced vasocontractions (0.1–10 mM) were antagonised by BU concentration dependently in Ca^{2+}-free and high K^{+} (60 mM) medium, as well as in Ca^{2+}- and K^{+}-free medium containing $1x10^{-6}$ M PE. However, contractions induced by noradrenaline ($1x10^{-6}$ M) and caffeine (45 mM) were not affected by BU. The results suggest that BU has a hypotensive effect and the blood pressure-lowering action is probably due to vasorelaxation caused by the inhibition of Ca^{2+} influx via receptor-operated and/or voltage-dependent calcium channels.

POSSIBLE SYNERGISTIC EFFECTS OF AN OXIDATIVE COMPOUND, PHYTIC ACID (IP-6) ON COLON CANCER CELLS, *IN VITRO* AND *IN VIVO*

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Colon cancer is a serious health problem in most developed countries and according to Ministry of Health (MOH, 2002), colon cancer is the third cause of death in Malaysia which contributes 9.23% of total death cases. Phytic acid (myoinositol hexa-phosphoric acid, IP6) has been extensively studied for its potential chemopreventive activity against colon carcinogenesis. It is found in high concentration especially in wheat bran, rice bran and wheat germ. Therefore, the purpose of this study is to investigate the effect of phytic acid extracted from rice bran to colon cancer cell through *in vivo* and *in vitro* experiments. For *in vitro* study, the phytic acid extract is tested to colon cancer cell line, HT29. The cytotoxic effect of the IP6 were quantified using MTS [3-(4,5-dimethyiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-3(4-sultophenyl)-2H-tetrazolium] inner salt assay. The cell was treated with the IP6 in different range of concentration ($1x10^{-6}$ to 10 mM). This is to screen the anti-proliferation effect of the IP6 on cell growth in dose-dependent manner. For *in vivo* study, an aberrant crypt foci was used as a marker for pre-neoplastic lesions in rats. Rats received two intraperitoneal injection of colon-specific carcinogen, azoxymethane (AOM), at 15 mg/kg body weight. The IP6 was administrated at 0.2% and 0.5% (w/v) in the drinking water during post-initiation phase of carcinogenesis. Results indicate that the

aberrant crypt formation in rats treated with azoxymethane (AOM), compared with rats treated with azoxymethane (AOM) was significantly inhibited alone (26.00 ± 4.55 and 40.5 ± 10.15 vs 59.5 ± 3.87). In conclusion, IP6 has the possibility to be used as a chemotherapeutic compound for the treatment of colon cancer.

THE EFFECT OF MENADIONE ON APOPTOSIS IN PANCREATIC ACINAR AR42J CELLS

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The understanding of the regulation of apoptosis is becoming exceedingly important in investigations of the pathogenesis and treatment of acute pancreatitis. The decision of stressed cells to die or to survive is made by integrating signals at different levels through multiple check points. However, initiation and continued progression toward apoptosis in pancreatic cells may be blocked by the overexpression of members of the Bcl-2 family proteins. Therefore, the activation of the apoptosis appears as a major therapeutic target in acute pancreatitis. Apoptosis removes injured acinar cells without provoking inflammation; prior induction of apoptosis reduces the severity of acute pancreatitis. In this study, we investigated the effect of menadione or vitamin K_3 as an apoptosis inducer and potential chemotherapeutic agent at specific doses and time of treatment. The study was carried out on the rat AR42J cell line for the occurance of DNA damage and activation of caspase 3 as analysed by the flow cytometer. Western blot was performed to identify the protein of interest, antiapoptotic Bcl-2 and proapoptotic Bax. Flow cytometry result shows that menadione at lower concentration, 20 μM induced apoptosis within 24 hours drug treatment, while at the concentration of 50 μM and 30 minutes treatment, a slight caspase 3 activation was observed. There is potential for menadione to be further investigated as possible chemotherapeutic approach for acute pancreatitis. By defining conditions in which calcium induces apoptosis and the role of mitochondrial membrane potential in acinar cells in the future, it is hoped that a new therapy can be developed.

THE PERFORMANCE OF MONOCLONAL ANTIBODIES RAISED AGAINST THE SAME STEROID IS AFFECTED DIFFERENTLY IN BUFFERS OF VARYING PH

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In immunoassays, buffers and pH conditions are often set empirically based on "previous experience" with other assays. However this may lead to suboptimal performance. The purpose of this study is to study the effect of buffer and pH on the binding of a panel of monoclonal antibodies (Mab) that have been raised against 17α hydroxyprogesterone (17OHP) is studied. The binding activity of three of the Mabs to 17OHP that had been generated in our laboratory was studied using buffers of different pH, ranging from 3.3 to

12.0. EIA plates were coated with 17OHP-BSA and Mab's were incubated in the wells at 37°C for 1.5 hours. Bound Mab were detected with a secondary antibody tagged with horseradish peroxidase and read on an EIA plate reader at 405 nm after colour development with ABTS. Results indicate that although all antibodies were IgG's raised against the same steroid and possess high affinity and specificity, the binding of each Mab to antigen varied over the range of buffers and pH used. Thus, in setting an assay procedure the conditions must be reoptimized if a different antibody is used. It cannot be assumed that the same conditions will apply with every antibody used.

ACUTE TOXICITY OF 1-ISONICOTINYL-2TETRADECANOYL HYDRAZINE, AN ISONIAZID DERIVATIVE

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Several derivatives of isoniazid (INH) were synthesized in an attempt to find new more active and safer antituberculosis drugs. One of the derivatives synthesized was 1-isonicotinyl-2-tetradecanoyl hydrazine (INH-C14), which was found to be five times more active than isoniazid itself. The minimum inhibitory concentration (MIC) against *Mycobacterium tuberculosis* of INH-C_{14} was 0.01 μg/mL as compared to 0.05 μg/mL for isoniazid. Therefore the acute toxicity of the derivative on Sprague Dawley (SD) rats was studied to determine the safety of the newly synthesized drug. Five groups of six male rats and six female rats weighing 180 to 200 g were fasted overnight. Four groups were treated orally with 300, 600, 1200 and 2400 mg/kg INH-C14, respectively and one more group with distilled water as control. All animals were observed closely for sign and symptom of toxicity and the incidence and times to onset of toxic effect were recorded. The weight of each animal was also followed every week. At the end of the experiment the animal were sacrificed, cut open and the organs were isolated and weight. All the animals survived to the end of two weeks experiment except three male rats treated with 300 mg/kg, 1200 mg/kg and 2400 mg/kg and two female rats treated with 600 mg/kg and 2400 mg/kg INH-C14 and therefore, the 50% lethal dose (LD50) could not be determined. There was no significant different in the increase of body weight in treated male rats with control group ($p<0.05$). Similarly, on post mortem there was no significant different in internal organs weight between treatment groups and control ($p<0.05$). The weekly increase of body weight in the female rats treated with 2400 mg/kg INH-C14 was significantly less than the control group ($p>0.05$). On post mortem it was found that the weight of gut full, gut empty, gut content, spleen, right and left kidneys were significantly different from control group ($p>0.05$). The only different between the 1200 mg/kg treated rats with control was in the weight of gut full ($p>0.05$). It suggests that the LD_{50} of INH-C14 is higher than 2400 mg/kg, which is safer than INH (LD 50 = 600 mg/kg). It also suggests that INH-C14 is more toxic to female than male rats.

EVALUATION OF ANTINOCICEPTIVE ACTIVITIES OF *STROBILANTHES CRISPUS* ETHANOLIC EXTRACT IN MICE

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Strobilanthes crispus was first recorded by Thomas Anderson (1832–1870) who classified the plant under spermatophyta (flowering plants and gymnosperma). The Javanese commonly called it "daun pecah beling" and "pokok pecah kaca" by the Malay. This bush-like plant grows well in secondary forest with good water supply. Its usually grows using its stacks. The leaves are oblong-lanceolate, rather obtuse and shallowly crenate-crispate with either side covered with short hairs. The upper surfaces are darker compared to the below surfaces whereas the flower appears in short, dense cone-shape. The study was intended to evaluate the analgesic activities of *S. crispus* leaves ethanolic extract. The analgesic investigations were carried out against two types of noxious stimuli: chemical (formalin-induced pain and acetic acid-induced test) and thermal (hot plate test). The effects following aspirin and naloxone pretreatment were also studied. The antinociceptive studied using hot plate test which use to measure response latencies when the mice placed onto a metal surface maintain at 55 ± 0.2°C, writhing or acetic acid-induced abdominal constriction test and also formalin test. The extract (30, 100 and 300 mg/kg) is a significantly in a dose-dependent manner, reduced the nociception induced by the acetic acid i.p. injection and also significantly reduced painful stimulus in both phases of the formalin test. The result showed that the plant had both central and peripheral acting effects but with similar response as aspirin which have less effect on central stage. In conclusion, *S. crispus* has central and peripheral analgesic properties and suggest both involvement of opioid receptors and arachidonic acid pathways to inhibit nociception.

VITAMIN E REVERSED ADVERSE EFFECTS OF NICOTINE ON BONE METABOLISM BIOMARKERS

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Nicotine, which is an oxidant, has been shown to be detrimental to bone metabolism. This study aimed to determine the effects of vitamin E supplementation in nicotine-treated rats on bone metabolism biomarkers. Sprague Dawley (SD) rats were divided into five groups: (1) control group (C), (2) nicotine treated group (N), (3) α-tocopherol supplemented group (ATF), (4) γ-tocotrienol supplemented group (GTT), and (5) tocotrienol rich fraction

supplemented group (TRF). Treatment period was for four months. C group only received normal saline while N group received nicotine 7 mg/kg intraperitoneally daily. For ATF, GTT and TRF groups, the rats were injected with nicotine 7 mg/kg for two months and then for the two consecutive months nicotine treatment was stopped and replaced with the respective vitamin E preparations (60 mg/kg) given orally daily. Parameters measured were serum levels of osteocalcin, pyridinoline (PYD), interleukin-1 (IL-1) and interleukin-6 (IL-6). Osteocalcin and PYD are the markers for bone formation and resorption, respectively while IL-1 and IL-6 are the bone resorbing cytokines. Nicotine caused a reduction in osteocalcin and an increase in PYD, IL-1 and IL-6. This implied a reduction in bone formation and an increase in bone resorption. Both GTT and TRF reversed nicotine effects on osteocalcin, PYD and IL-1 and normalized IL-6 values. ATF was able to reverse the effects on osteocalcin only but not the other parameters. Nicotine impaired bone metabolism. GTT and TRF groups were superior than ATF in improving bone metabolism markers in nicotine treated rats.

THE EFFECT OF *SMILAX CALOPHYLLA* AND ESTRADIOL ON PLASMA TESTOSTERONE LEVELS IN MALE RATS

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Elevated plasma estradiol (E_2) levels in male rats reduced plasma testosterone (T) levels by acting centrally at the hypothalamic level, as well as locally at the Leydig cell level. Our previous study demonstrated that *Smilax calophylla* (SC) could counteract the inhibitory effect of corticosterone (B) on plasma T levels. The present study aims to determine whether lyophilized water extract of SC counteracts the adverse effects of E_2 on plasma T levels in normal as well as B-treated male rats. Control rats were given the vehicle while the treated rats were given SC, B or E_2 alone, SC in combination with B or E_2, respectively. All treatments were given for seven consecutive days. Plasma T and E_2 levels were determined using Coat-A-Count Diagnostic Product and the data were expressed as mean ± confidence interval. Corticosterone significantly increased plasma E_2 levels and lower plasma T levels, compared to that of control. Plasma T levels in E_2-treated rats was markedly reduced compared to the control. Conversely, SC administration in B-treated rats brings all parameters towards control value. Administration of SC in E_2-treated rats significantly increased plasma T levels compared to E_2-treated rats alone. Elevated plasma E_2 levels in B-treated rats could be a contributing factor in reducing plasma T levels, an effect that was counteracted by SC. The data

suggest that the effect of SC in increasing the plasma T levels in B-treated rats is mediated via a glucocorticoid receptor. However, SC could not fully overcome the inhibitory effect of E_2 treatment on plasma T levels. This suggests that SC does not act on estradiol receptors. In conclusion, we found that SC could partially overcome the inhibitory effect of high E_2 levels on T production.

ECG CHANGES IN AORTIC STENOSIS – A RAT MODEL OF PRESSURE OVERLOAD HYPERTROPHY

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Left ventricular hypertrophy (LVH) is a potential risk factor for cardiovascular disease related to high mortality and morbidity. The present study investigated electrocardiographical changes in experimental LVH in rats with pressure overload induced by aortic stenosis. Age and weight matched male Sprague Dawley (SD) rats (n = 18) were randomized into three different groups viz. sham operated, banded for 30 days and banded for 90 days. Metabolic data of all groups was collected in terms of water intake, urine output and change in body weight. Urinary and plasma Na^+ was also measured. ECG was recorded and analysed for any changes in QRS amplitude, QT interval, R-R interval and the heart rate, pre- and post-operative and on the day of euthanising. Carotid blood pressures were recorded before the animals were euthanised and heart and kidney were collected for kidney to body-weight (Kw/Bw) and heart to body-weight (Hw/Bw) ratios. There was no significant (all $p>0.05$) change in urine-output in sham operated rats on day 0, 7, 15 and 30 but small changes were observed in the banded rats on these days with an apparent diuresis at day 7. There was no meaningful change in water intake in any group of rats. There was a decrease in serum and urine Na^+ in banded animals (both 30 and 90 days) as compared to sham operated rats but statistically insignificant. ECG analysis revealed significant ($p<0.05$) increase in the Q-T interval between sham operated and banded animals on day 90. QRS amplitude decreased on both day 30 (significant, $p<0.05$) and day 90 of aortic banding as compared to the sham operated group. But there was no significant difference in R-R interval. Marginal decrease was also observed in the heart rate of banded rats as compared to sham operated animals. However, there was significant ($p<0.05$) increase in the carotid blood pressure in the banded animals. Furthermore a significant ($p<0.05$) increase was observed in Hw/Bw and Kw/Bw ratios in banded animals on day 30 (significant, $p<0.05$) but not significant on day 90 as compared to sham operated animals. The results derived lead us to speculate that this animal model of pressure overload caused by aortic stenosis is leading to heart failure. Moreover, change in renal function in terms of renal handling of Na^+ and diuresis was also observed in the pressure overloaded animal group.

HYPOGLYCEMIC AND ANTIDIABETIC EFFECT OF AQUEOUS AND ETHANOL EXTRACT OF *OXALIS BARRELIERI* IN STREPTOZOTOCIN-INDUCED DIABETIC RAT MODELS

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Oxalis barrelieri also known as "belimbing tanah" in Malaysia is believed to be a herbal remedy for diabetes mellitus. The objective of this study is to investigate the hypoglycemic properties of the aqueous (AQ) and ethanolic (EtOH) extract of *O. barrelieri.* The administration of both the extracts of the plant on Sprague Dawley (SD) rats produced significant reductions of glycemia in both non-diabetic and diabetic rats. Dosages used were 500 mg/kg and 1000 mg/kg. Results of the OGTT showed a significant decrease ($p<0.05$) in blood glucose levels in both non-diabetic treated and diabetic treated groups. Long-term plasma glucose studies for eight weeks also revealed significant decrease ($p<0.001$) in blood glucose levels among the treated animals compared to the untreated. Comparisons were made between the actions of various dosages of both the aqueous and ethanol extract of *O. barrelieri* and glibenclamide. A qualitative HPLC method was developed to compare the chemical profiles of the AQ and EtOH extract of *O. barrelieri.* The AQ extract revealed one major peak (ERS01) at a mean retention time of (2.862) minutes and the EtOH extract with two major peaks (ERS11 and ERS12) at mean retention time of 2.938 and 3.295 minutes. The HPLC method developed in this study can be used for the identification and evaluation of *O. barrelieri* for further drug development. As a conclusion, our data suggest that the aqueous and ethanol extract of *O. barrelieri* is a potent antidiabetic. This study confirms the promising hypoglycemic activity of *O. barrelieri* and its potential source for the isolation of new active agents for antidiabetic therapy.

THE COMPARISON OF BLOOD PRESSURE VARIABILITY AND ARTERIAL COMPLIANCE IN SUBJECTS WITH FAMILY HISTORY OF HYPERTENSION

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Normotensive subjects with FHT have been reported to have increased left ventricular mass index, reduced ventricular and arterial compliance even before the onset of

hypertension (HT). Of interest is whether blood pressure variability (BPV), which has been associated with target organ damage, is then part of this complex inherited syndrome? Manuck et al. found that there was no difference with regards to blood pressure (BP) reactivity due to mental stress test in subjects with FHT and control. This contradicted the study by Lemne et al. who showed that even a mild level of heredity for HT led to increased BPV. The objectives of this study are to find out whether there is any significant difference in BPV in subjects with FHT as compared to controls and its possible correlation with arterial compliance. There were 35 subjects with self reported FHT and 35 matched controls. None of the subjects were hypertensive or diabetic. Noninvasive 24-hour ambulatory BP monitoring was recorded with BR-102 monitor (Schiller Inc. Germany). Arterial compliances were measured following the 24-hour BP measurement using the HDI/Pulsewave Cardiovascular Profiling Instrument (Hypertension Diagnostic Inc. USA). There were no significant differences in biochemical profiles in both groups. Subjects with FHT have higher night time systolic, diastolic and mean arterial BPV as compared to the control subjects. There was no significant difference in arterial compliances between both groups but there was an inverse relationship between small arterial compliance and night time systolic BPV in subjects with FHT.

CARDIOVASCULAR REACTIVITY TO MENTAL ARITHMETIC TEST IN NORMOTENSIVE YOUNG ADULTS WITH FAMILY HISTORY OF HYPERTENSION

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Exaggerated cardiovascular reactivity to mental test is correlated to future hypertension. However, whether normotensives with family history of hypertension have greater cardiovascular reactivity to mental test than those without is still controversial. The objective of this study is to compare cardiovascular reactivity to mental arithmetic test in normotensive young adults with and without family history of hypertension. Normotensive undergraduate students of normotensive parents (n = 40) and of hypertensive father/mother/both (n = 40), aged 20–30 years, performed serial subtraction test in a sitting position for three minutes. Blood pressure and pulse rate were recorded in pre-test, test, and post-test period by using an automated oscillometric device. Change scores rather than absolute scores were analysed. There were no significantly differences in age, body mass index, fasting blood sugar, and plasma creatinine between the two groups. Normotensives of hypertensive parents had higher office systolic blood pressure than those of normotensive parents. There was no significant difference in office diastolic blood pressure between the two groups. Normotensives of hypertensive parents demonstrated greater reactivity in diastolic blood pressure ($p = 0.05$), mean blood pressure and rate pressure product ($p<0.05$) than those of normotensive parents. These differences in cardiovascular reactivity were still seen among female subjects but disappeared among male subjects. In conclusion, normotensive young adults with family history of hypertension had greater cardiovascular reactivity to mental arithmetic test than those without. However, the differences were gender specific.

ISOLATION OF HUMAN MESENCHYMAL STEM CELLS: UMBILICAL CORD VERSUS BONE MARROW

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Stem cells can be found in the bone marrow, umbilical vein, cord blood and also the umbilical cord. We attempted the isolation and characterization of mesenchymal stem cells (MSCs) from umbilical cord matrix (UCM) or also known as Wharton's jelly and bone marrow aspirates using optimized isolation and culture conditions. The umbilical cord samples showed characteristics of mesenchymal morphology similar to MSCs from adult bone marrow. Human cord matrix produced a clump of heterogeneous cells with two types of cells observed: spindle fibroblast-like cells and large flat and big cells with seemingly jagged ends. But morphology changed to spherical colonies when cultures reached confluence. Human UCM cells formed an adherent layer within 24 hr of culture and reached confluent within two weeks of primary culture. Using immuno-cytochemical staining, we found that both the cells isolated from the umbilical cord matrix and bone marrow expressed matrix receptors markers (CD105, CD166) whose coexpression defines mesenchymal stem cells in umbilical cord matrix and bone marrow. In summary, cells from the human umbilical cord matrix are easily attainable and expanded *in vitro* and these cells may therefore be another new source of cells for cellular therapies and also will avoid the ethical and technical issues involved in the use of cells from other origins.

MESENCHYMAL STEM CELL IN CELL RICH ZONE

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There are many ways in which human stem cells can be used in basic research and in clinical research. Cancer cell lines for example, are used to screen potential anti-tumor drugs. Previous study showed dental pulp cells were highly proliferative cells. This study aims to isolate and characterize mesenchymal stem cell from cell rich zone in deciduous pulp cell *in vitro*. Extracted deciduous molars were collected from 4–5 years old children. One-step RT-PCR was performed for RNA isolation. The PCR primers included insulin-like growth factor-2 IGF-2) sense (5'-ctctccgtgctgttctctcc-3') and antisense 5'-cgggccagatgttgtacttt-3'), Discordin domain tyrosine kinase 2 (DDR2) sense (5'-caagaacagccctattccca-3') and antisense (5'-caggcactgacagcatcact-3'). Antibody against human antigen: CD105 and CD166 were used. Progenitor cells were found positive for CD 105 and CD 166. RT-PCR analysis revealed expression of DDR2 and IGF-2 on mesenchymal stem cell surface marker. Collectively, these data revealed the presence of distinct mesenchymal stem cell populations which have the capability to regenerate living human tissues.

INTERACTION OF α_1-ADRENOCEPTOR AND ANGIOTENSIN II RECEPTOR IN DIABETES AND HYPERTENSION WITH DIABETES IN REGULATING RENAL HAEMODYNAMIC

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This study investigated the functional roles of subtypes of α_1-adrenergic and angiotensin II receptors, and their interactions at the level of renal resistance vessels in the regulation of renal haemodynamics in diabetic WKY and SHR rats. The animals were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.) and prepared for blood pressure measurements and fluid administration, respectively. An electromagnetic flow probe was placed on the left renal artery for RBF measurement. The left iliac artery was cannulated to allow infusion of all drugs closed intra renally. Reductions in RBF to electrical stimulation, phenylephrine, methoxamine and angiotensin II were determined before and after bolus doses of 5-methylurapidil, chloroethylclonidine, BMY 7378 or amlodipine. Perindopril (0.2 mg/kg) and losartan (10 mg/kg) were given orally 48-hours post-STZ for seven days daily. Data, means $\pm$ s.e.m. were compared with one- and two-way ANOVA followed by Bonferroni-post hoc with the significance level of 5%. Although there were significant drops in the basal mean arterial pressure in some of the treatment groups in both WKY and SHR, the renal blood flow remained unaltered. Significant attenuation in renal vasoconstrictor effects were observed in diabetic WKY and SHR treated with 5-methylurapidil and amlodipine. Chloroethylclonidine significantly reduced the response to AngII in the WKY group. Perindopril-treated WKY produced a greater constriction to RNS whereas in the SHR, significant attenuated responses were observed to all pressor agents. In losartan treated groups, only responses to AngII were significantly blocked in WKY whereas in SHR, responses to all pressor agents but RNS yielded significant attenuation. Collectively, α_{1A}-adrenoceptors and angiotensin type 1 receptors are predominantly involved in the vasoconstriction with the involvement of extracellular calcium ions in both diabetes and hypertension with diabetes. Circulating AngII does play a role in α_1-adrenoceptor-mediated vasoconstriction in hypertension with diabetes. The interaction between these two systems is possibly via the α_{1B}-adrenoceptor in diabetes and α_{1A}-adrenoceptor in hypertension with diabetes.

EVIDENCES OF MULTIPLE FUNCTIONAL α_1-ADRENOCEPTOR SUBTYPES IN THE KIDNEY OF RATS WITH A COMBINED STATE OF HYPERTENSION AND RENAL FAILURE

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The kidney has sensory innervations responsive to external stimulae that pass afferent information to the brain and reflexly determines sympathetic outflow to influence hemodynamics and functionality of organs. The present study investigated whether the functional α_1-adrenoceptor subtype(s) mediating the vasoconstrictor actions of the renal sympathetic nerves are altered in spontaneously hypertensive (SHR) rats with cisplatin induced renal failure (RF). Twenty-four (n = 24) male SHR rats (250–300 g) either normal or induced with RF by cisplatin (5mg kg^{-1} i.p.) were used. The RF state was confirmed from renal functions and histological observations of the kidney. Seven days post-cisplatin the rats were anesthetised (sodium pentobarbitone, 60 mg kg^{-1} i.p.) for acute study. The reductions in renal blood flow (RBF) caused by electrical renal nerve stimulation (RNS) and close intrarenal administration of noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) were determined before and after chloroethylclonidine (CEC) or BMY 7378, selective antagonists of α_{1B}- and α_{1D}-adrenoceptor, respectively. BMY 7378 caused significant attenuation (all $p<0.05$) of the adrenergically induced renal vasoconstrictor responses in rats of both RF and normal groups. CEC produced interesting results with a non-significant ($p>0.05$) attenuation of the RNS induced renal vasoconstrictor response by its low dose followed by a significant ($p<0.05$) accentuation by its high dose in RF SHR rats. However, in these rats CEC did not cause any shift in NA and PE induced changes but enhanced the ME induced changes. In normal rats, CEC did not cause any shift in renal vasoconstrictor responses caused by any of the adrenergic stimuli used. Data obtained showed that α_{1D} was one of the functional α_1-adrenoceptor subtypes present in the renal resistance vessels of either group of SHR rats along with a possible minor involvement of pre-synaptic α_{1B}-subtype in RF animals. Further, in RF rats a complex interaction between the α_1-adrenoceptor subtypes was apparent from a biphasic action of CEC in the case of RNS vasoconstrictor responses. This indicated that there is a possibility that in RF rats, occupation of α_{1B}-subtypes might lead to an alteration in the properties of α_{1A}-subtype that the normal agonist and antagonists interaction could not happened. Another possibility could be that in the RF rats the blockade of α_{1B}-subtype enhanced the sensitivity of other α_1-adrenoceptor subtypes (α_{1A}-and α_{1D}-subtypes).

DIMENSIONAL ANALYSIS OF ISOKINETIC STRENGTH

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The purpose of this study was to model isokinetic strength in adult recreational athletes. Subjects (9 men, 23.02 ± 3.86 years, 175.59 ± 8.82 cm, 70.47 ± 9.80 kg and 10 women, 24.22 ± 3.70 years, 163.54 ± 6.27 cm, 60.82 ± 10.38 kg) were recruited from taekwondo clubs in London, UK. Strength was assessed on a Cybex Pro at 60°/sec and 90°/sec during leg extension and flexion. Lean body mass (LBM) was derived from regression equations for body density. Gender* Movement* Angular velocity ANOVAs with repeated measures on the second and third factors were used to determine differences between men and women in peak torque. Regression diagnostics were run to test the model specifications. The men were stronger in absolute terms (177.94 ± 38.00 Nm vs. 114.75 ± 22.19 Nm, $p<0.001$, $eta^2 = 0.560$) and relative to LBM per ratio standard (3.00 ± 0.39 Nm/kg LBM vs. 2.55 ± 0.25 Nm/kg LBM, $p = 0.001$, $eta^2 = 0.470$; $r = 0.13$, $p = 0.732$ for the men and $r = –0.09$ $p = 0.803$ for the women). Regression diagnostics showed a quadratic relationship ($R^2 = 0.6612$, SE = 1.8406, $p = 0.039$) in the men for peak torque/body mass. There was a tendency for a second-order polynomial fit for LBM and relative peak torque in the men ($R^2 = 0.5808$, SE = 2.0475, $p = 0.074$). In the women, more variance was explained by a cubic relationship ($R^2 = 0.0151$, SE = 3.6801), although it was not significant ($p = 0.992$). The assumption of a linear relationship between the predictor and performance variables could not be unequivocally confirmed when using the ratio standard.

REPEATEDLY HEATED PALM OIL IS LESS DETRIMENTAL TO OVARIECTOMISED-RAT BONE COMPARED TO SOY OIL

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Heating frying oils repeatedly may oxidize their lipid content and destroy their vitamin E. Ingestion of heated frying oils have been associated with an increase in oxidative stress condition. This would be unhealthy for post-menopausal women as free-radicals have been linked to the pathogenesis of post-menopausal osteoporosis. Therefore, we aimed to look at the effects of ingestion of palm oil or soy oil, which was either heated once or five times on bone histomorphometric parameters in ovariectomised rats. Sixty four female Sprague Dawley (SD) rats weighing 180 to 250 g were divided into eight groups. The first group was not ovariectomised and acted as a normal control group (NC). The second group is the ovariectomised control group (Ovx), while the rest of the groups were ovariectomised and given oral gavage of either fresh soy oil (SOF), fresh palm oil (PO), oils heated once (SO1, PO1) or oils heated five times (SO5, PO5). As expected,

ovariectomy caused negative effects on almost all the histomorphometric parameters as seen on Ovx group. In some of the parameters, supplementation of SOF, POF, SO1 or PO1 were able to protect bone from ovariectomised-induced changes on bone histomorphometric parameters. The other significant findings were seen in the SO5 and PO5 groups, where both SO5 and PO5 were detrimental to bone. However the effects caused by SO5 were worse than PO5. This study shows that the effects of palm oil or soy oil on bone metabolism is the same when they were fresh or heated once, but when they were heated five times, palm oil was less detrimental to bone compared to soy oil. This may be related to the high saturated fatty acids and tocotrienol content of palm oil which enables it to withstand repeated thermal oxidation. Therefore for repeated use of frying oil, it is better to use palm oil than soy oil.

EFFECT OF MALATHION ON LUNG SURFACTANT PHOSPHOLIPIDS

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Inactive or inadequate phospholipids in surfactant can affect its physiological function in the lungs. Malathion, a well-known organophosphate insecticide, is widely used in agriculture. Its effects on the lung surfactant system are not well known. The objectives of this study were therefore to investigate the effect of inhalation of malathion on lung surfactant phospholipids (LSP), in particular, on (i) phosphatidylcholine (PC) and (ii) phosphatidylglycerol (PG) in the rat. Thirty-two Sprague Dawley (SD) rats, weighing about 220 g, were divided into four equal groups: (i) control, (ii) malathion single dose (21.67 µL), (iii) malathion double dose (43.34 µL), and (iv) recovery following a single dose. Malathion was administered via a nebuliser attached to a perspex box, measuring 26 x 24 x 30.5 cm in height, width and breadth, respectively, for a period of one hour and in a concentration recommended by the manufacturer. Control animals were given deionized water for the same period of time. Immediately after treatment, rats were anesthetised with thiopental sodium, and the lungs and the trachea were removed. Broncho-alveolar Lavage (BAL) was performed with normal saline. The presence of PC and PG in the bronchial lavage was identified using thin layer chromatography (TLC). PG and PC were absent in 50% of the rats administered a single dose of malathion and was completely absent in the bronchial lavage of rats (n = 8) when the dose was doubled. Both PG and PC were present in animals allowed to recover for two weeks after single dose exposure of malathion. In conclusion, it appears that malathion reversibly affects the presence of PG and PC in the lung surfactant in a dose dependent manner.

SHORT TERM LOW INTENSITY VERSUS HIGH INTENSITY RESISTANCE TRAINING ON BONE MINERAL DENSITY AND SERUM HUMAN OSTEOCALCIN IN YOUNG MEN

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The purpose of this study was to determine the effectiveness of a short term, low (LI) and high intensity (HI) resistance training programme on bone mineral density (BMD) and serum human osteocalcin in young men. Forty five subjects with a mean age of 23 years were divided into three groups, i.e. low intensity, 50% of 1RM , high intensity, 70% of 1RM, and control group (CG). Both the LI and HI groups underwent exercises utilizing Nautilus weight machines at ten repetitions per set for three sets, training thrice a week over six weeks. Measurements of BMD ($g.cm^{-2}$) utilizing dual energy x-ray absorptiometry (DEXA) were obtained for the lumbar spine (L1-L4) and the left femoral neck. Blood samples obtained were analysed for serum human osteocalcin after a 12 hour fast. Training at LI and HI resulted in significant increase ($p<0.05$) of 1.9% and 1.3%, respectively in BMD of the lumbar spine. All the other variables showed no significant changes. These data indicate that short term resistance training was successful in increasing BMD of the lumbar spine in both training groups with a greater response from LI. Serum human osteocalcin appears to have no influence in the increase in BMD of the lumbar spine and was unaffected by the intensity of resistance training.

THE EFFECTS OF ACUTE SWIM STRESS ON C-*FOS* EXPRESSION IN RATS SPINAL CORD

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C-*fos* is a proto-oncogene which is extensively used as a specific marker for pain processes corresponding to noxious stimulation. Studies have demonstrated induction of c-*fos* expression in rats spinal cord due to noxious stimulation and chronic swim stress. The aim of this study was to determine the effects of acute swim stress on Fos-like immunoreactivity (FLI), the protein product of c-*fos*, in rats spinal cord. Male Sprague Dawley (SD) rats weighing between 250 to 300 g were divided into four groups consisting of rats subjected to acute swim stress with formalin injection (n = 6) and without formalin injection (n = 6), and rats not subjected to acute swim stress with formalin injection (n = 6) and without formalin injection (n = 6), respectively. Acute swim stress was performed for three minutes in water at 21°C and 50 µl of 1% formalin was subcutaneously injected into the plantar surface of the right hind paw of the rats. The rats were then sacrificed and the lumbar L4 and L5 segments of spinal cords were removed for c-*fos* immunohistochemistry. Data were analysed using one-way ANOVA. FLI was

significantly higher on both ipsilateral (injected) and contralateral (not injected) sides of the spinal cord in rats given formalin injection ($p<0.01$) compared with rats not given formalin injection regardless of whether they were subjected to acute swim stress or not. In rats given formalin injection, acute swim stress significantly reduced FLI in laminae I-II and laminae V-VI ($p<0.01$) on the ipsilateral side compared with rats not subjected to acute swim stress. In contrast, on the contralateral side, acute swim stress caused an increase in FLI in laminae I-II and laminae V-VI ($p<0.01$) compared with rats not subjected to acute swim stress. In conclusion, stress alone does not induce FLI expression in rats spinal cord but FLI expression due to noxious stimulation can be modulated by acute stress.

INFLUENCE OF DIABETES ON THE mRNA EXPRESSION OF IGF-2 AND IGF-2R IN MOUSE FALLOPIAN TUBE AND UTERUS

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Alteration of the expression of IGFs and their receptors has been found in many tissues of diabetic animals, raising the question of their involvement in the occurrence of diabetic embryopathy. The aim of this study is to determine the mRNA expression of IGF-2 and IGF-2 receptor in the fallopian tube and uterus at various stages of preimplantation embryo development in normal and diabetic mice. Fallopian tubes and uterine tissues were obtained from superovulated streptozotocin-induced diabetic and normal mice at 48, 72 and 96 hours post-hCG treatment. Total RNA was extracted from the tissues and cDNA prepared. The mRNA expression of IGF-2 and IGF-2 receptor was quantified using real-time Polymerase Chain Reaction (PCR). The mRNA expression IGF-2 in the fallopian tube of diabetic mice was significantly increased at 48 and 96 hours post-hCG treatment. Similarly, the mRNA expression of IGF-2R in the fallopian tube and uterus of diabetic mice was significantly increased at 48 and 96 hours, and at 48 hours post-hCG treatments, respectively. The increased expression of IGF-2 was consistent with previous reports on the elevated levels of IGF-2 observed in infants of diabetic mothers as well as adolescents with insulin-dependent diabetes mellitus and hyperglycemia. As the main function of IGF-2R is to cause IGF-2 degradation, the increase in both IGF-2 and IGF-2R will minimize the detrimental effect of excessive IGF-2.

EFFECT OF LEPTIN ON SPERM COUNT AND MORPHOLOGY IN SPRAGUE DAWLEY RATS

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Leptin, a 16-kD obese gene (ob) product, has been shown to influence reproductive function. It is known to inhibit testosterone secretion in the rat. However, its effects on sperm morphology and count remain unclear. This study therefore investigated the effect of leptin administration on sperm count and morphology in rats. Sprague Dawley (SD) rats, aged 10 weeks and with a mean body weight of 200 ± 1.44 g, were treated daily with a single intraperitoneal injection of either 5, 10 or 30 μg/kg body weight of leptin for either 7, 15 or 42 days (n = 10 for each group). At the end of each treatment, rats were mildly anesthetised with ether and immediately killed by cervical dislocation. Laparotomy was performed and blood samples were collected from the inferior vena cava. Right epididymis was removed and sperm count was conducted and the percentage of abnormal sperm was also calculated, as per the standard procedure. Leptin in the serum was measured using ELISA technique (Cayman Chemical). Although mean serum leptin concentration was slightly higher in rats injected with leptin compared to the controls, the difference however was not significant statistically. Sperm counts were significantly lower in rats given leptin when compared to their respective controls ($p<0.05$). In addition, the fraction of abnormal sperms was significantly higher in rats given 30 μg/kg body weight of leptin for 42 days ($p<0.05$). In conclusion, it appears that exogenous leptin administration to rats has a negative effect on sperm count and morphology.

ROLE OF VASOCONSTRICTOR PROSTAGLANDIN ON ACETYLCHOLINE-INDUCED RELAXATION IN AORTIC RINGS FROM MALE AND FEMALE STREPTOZOTOCIN INDUCED-DIABETIC RATS

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Previous work has demonstrated that vascular prostanoid production was altered in streptozotocin (STZ)-induced diabetic male rats. The following experiments were performed to further study the possible role of prostanoids in vascular responsiveness to acetylcholine in STZ-induced diabetic male rats and also to extend this study to the female rats. Rat aortic rings obtained at 3–4 weeks and 20–21 weeks after STZ or vehicle (control rings) injection were suspended in a 10 mL tissue-bath containing Krebs solution at 37°C. Isometric tension recordings of cumulative concentration-responses to acetylcholine

(ACh) and phenylephrine (PE) were measured. ACh-induced relaxation was significantly impaired in aortic rings from diabetic female rats at 3–4 weeks ($p<0.001$) and 20–21 weeks ($p<0.01$) when compared to control rings. By contrast, significant enhanced and impaired relaxations to ACh were observed in aortic rings from male rats of 3–4 weeks ($p<0.001$) and 20–21 weeks ($p<0.001$), respectively, when compared to control rings. GR 32191 (a PGH_2-thromboxane A_2 receptor inhibitor) was found to restored ACh-induced relaxation in aortic rings from male diabetic rats of 3–4 weeks and 20–21 weeks, and from female diabetic rats of 3–4 weeks. On the other hand, significant increases in PE-induced contractions to nitro-L-arginine methyl ester ($p<0.001$) were observed in aortic rings of both diabetic male and female rats at 3–4 weeks and 20–21 weeks compared with their respective control rings indicating an enhanced basal nitric oxide released in chronic diabetic vessels. These data suggest that an impaired endothelium-dependent relaxation of aortic rings in chronic diabetic rats is not due to a decrease in basal nitric oxide production, but may, at least in part, due to generations of vasoconstrictor prostaglandin(s).

THE ROLE OF CHEMICAL SYMPATHECTOMY ON RENAL HAEMODYNAMICS OF RATS WITH NEPHROTOXIC RENAL FAILURE

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This study examined the role of renin-angiotensin (RAS) and sympathetic nervous (SNS) systems in renal haemodynamics of rats with renal failure in an attempt to examine any alterations in renal haemodynamics after sympathectomy. Sympathectomy was carried out chemically by the administration of multiple dosages of 6-OHDA (50–100 mg/kg i.p.). In acute study, the animals were anesthetised (60 mg/kg i.p., sodium pentobarbitone), blood pressure and renal blood flow (RBF) were measured using a pressure transducer and electromagnetic flowmeter, respectively. Data were recorded in a computerized data acquisition system, expressed as mean ± s.e.m. and compared by two-way ANOVA followed by Bonferronni post-hoc test with a significance level at 5%. Less reduction of percentage drop of RBF was observed in sympathectomised renal failure rats (all $p<0.05$) as compared to normal in response to renal nerve stimulation (RNS) and adrenergic agonists, noradrenaline (NA) and phenylephrine (PE). Interestingly, a significant ($p<0.05$) increase in percentage drop of RBF is observed in sympathectomised renal failure rats in response to intrarenally administered ME. However, intrarenal administration of angiotensin II also caused decrease percentage drop of RBF in sympathectomised renal failure rat but was not significant ($p>0.05$). The results suggested that an intact SNS is important in mediating adrenergically induced renal haemodynamic changes in sympathectomised renal failure rats and perhaps RAS is not playing any important role in

mediating such changes. In sympathectomised renal failure rats, it further appeared that there is a biphasic action of RNS and adrenergic agonists in controlling the RBF in terms of down-regulation and/or up-regulation or hypersensitivity of multiple α_1-adrenoceptor subtypes and probably there are greater participation of α_{1A}-and α_{1D}-adrenoceptors.

ANTIINFLAMMATORY AND ANALGESIC EFFECTS FROM COMPOUNDS IN *ARDISIA CRISPA*

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The genus *Ardisia* is the largest in family Myrsinaceae and approximately 500 species of evergreen shrubs and trees are found throughout the subtropical and tropical regions of the world. There were a few of *Ardisia* species that have been studied and reported for its medicinal properties. It has also been reported to produce several groups of biologically active phytochemicals including saponins, coumarins and quinones. Exhaustive extraction and isolation of the dried root (ACR) yielded two compounds from the n-hexane fraction (ACRH) namely AC1 (viminalol) and AC2 identified as 2-methoxy-6-undecyl-1,4-benzoquinone. The fraction, ACRH and its compounds, AC1 and AC2 were shown to be pharmacologically active for its antiinflammatory and antinociceptive effects. The pure compounds, AC1 and AC2 have been tested on carageenan induced oedema for its antiiflammatory properties and carrageenan induced hyperalgesia by using Plantar test for its analgesic properties. We found AC1 and AC2 to possess both activities as its ED50 of antiinflammatory effect is 5-fold and 2.5-fold less potent compared to ED50 of ACRH respectively, while for the antinociceptive effect, ED50 of AC1 is 1.5-fold less potent compared to ED50 of ACRH and ED50 of AC2 is 3.4-fold more potent than ED50 of ACRH. Based on the skeleton structure of AC2, we postulated that both its anti-inflammatory and analgesic effects might be due to its 5-lipoxygenase (5-LOX) inhibiting action. Therefore, further investigation should be done to confirm whether AC2 might be possible to act as a 5-lipoxygenase inhibitor in the near future.

EFFECTS OF *TINOSPORA CRISPA* ON LIPID PROFILES AND ATHEROMATOUS PLAQUE FORMATION IN RABBITS FED WITH HIGH FAT DIET

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Atherosclerosis refers to the "hardening of the arteries" in which the wall of the arteries thickens due to lipid deposition on the intimal layer. The aim of this experiment is to study the effect of *Tinospora crispa* (TC) extract on the development of experimental atherosclerosis. The duration of this study was eight weeks. Fifteen male New Zealand White rabbits with body weight of 1 to 1.5 kg were randomly divided into five groups. Groups A and B were used as a negative and positive (1% cholesterol) control. Groups C was given high fat diet (HFD) with 5 mg/kg simvastatin. Groups D, E and F acted as treatment groups by giving HFD with supplementation of 150, 300 and 450 mg/kg of TC extract, respectively. Body weights were recorded at week 0 (w0), week 4 (w4) and week 8 (w8). Blood was collected from ear vein at w0, w4, w8 and the serum was analysed for lipid profiles index. The aortas were excised and examined microscopically by H&E staining to observe formation of foam cells. All the results were analysed by one-way ANOVA and Tukey HSD with significant difference at $p<0.05$. The results showed that TC treatment do not have the effect of decreasing the level of total cholesterol, low-density lipoprotein (LDL) and triglyceride (TG) throughout the experimental period. Meanwhile high-density lipoprotein (HDL) level increased from week 0 to week 10 in all treatment groups. The atheromatous plaque formation in group given HFD is significantly higher than group given treatment. In the group that was given 450 mg/kg of TC which is the highest dose, as well as negative group and group given simvastatin, no atheromatous plaque formation was found. TC does not possess lipid lowering but have anti atherosclerotis effect as indicated by absence of atheromatous plaque formation.

RELATIONSHIP OF DIHYDROTESTOSTERONE (DHT) WITH HUMAN SPERM QUALITY

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The role of hormones in male fertility is a long established area of research. Apart from testosterone, dihydrotestosterone (DHT) has also been found to have potential to aid in male fertility. Nevertheless, extensive research on the role of this particular androgen has been uncommon with contradicting results, especially when pertaining to specific seminal analysis parameters like sperm motility and sperm concentration. The objective of this study is to determine the relationship between DHT and parameters of semen quality, i.e. total sperm motility, sperm concentration, good and poor sperm activity. Semen samples

were collected from Sarawak General Hospital. There were a total of 21 samples, 15 from patients who attended the infertility clinic and 6 control samples (from proven fathers). The levels of DHT were then measured for every sample using DHT ELISA kit. This was followed by correlating the parameters for each sample with their corresponding level of DHT concentration through Pearson's Correlation statistical analysis to determine the significance of the correlations. The results of this research showed that there was a significant ($p<0.01$) positive correlation between DHT and good sperm activity ($r = 0.7848$). There was also a significant ($p<0.01$) positive correlation between DHT and total sperm motility ($r = 0.3667$) and between DHT and sperm absence of DHT in seminal plasma with reference to sperm quality may indicate possible defects in testicular or epidydimal tissues.

CONTRIBUTION OF EXPERIMENTAL EARLY DIABETIC NEPHROPATHY ON THE FUNCTIONAL POPULATION OF α_1-ADRENOCEPTORS IN RENAL HEMODYNAMICS OF RAT

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Diabetic nephropathy is a leading cause of end-stage renal failure which is frequently accompanied by exaggerated sympathetic nervous system activity. It has been reported that streptozotocin induced diabetic in rats can cause shift in the functional α_1–adrenoceptors in the kidney. This study investigated the impact of experimental early diabetic nephropathy (EDN) on the functional α_1–adrenoceptors populations in the renal vasculature of rats. EDN was induced by single injection of streptozocin (55 mg kg^{-1}, i.p.) and confirmed from marked hyperglycemia, weight loss, marked polyuria, reduction in creatinine clearance and impaired renal handling of sodium as observed weekly over a period of four weeks. The study was carried out on twenty-four ($n = 24$) male spontaneously hypertensive rats (250–300 g) with or without EDN. On day 29 of the metabolic study, animals were anesthetised (sodium pentobarbitone, 60 mg kg^{-1}, i.p.), tracheostomy followed by cannulation of right carotid artery and right jugular vein was done. The kidney was exposed by midline abdominal incision followed by cannulation of left iliac artery for the close intrarenal administration of adrenergic agonists and antagonists. The renal artery was isolated for renal blood flow (RBF) measurement by electromagnetic flowmetry. The percentage change in RBF was determined in response to graded frequencies of renal nerve stimulation (RNS) and graded doses of adrenergic agonists (noradrenaline, phenylephrine and methoxamine) in the absence and presence of specific α_{1A}-adrenoceptor antagonist 5-methylurapidil (MeU) and L-type Ca^{+2} channel blocker amlodipine (AMP). Results obtained showed significant (all $p<0.05$) attenuation of adrenergically induced renal vasoconstrictor responses by both MeU and AMP in both

groups of rat. Data obtained indicated the presence of α_{1A}-adrenoceptors as the major functional subtype in the renal vasculature of EDN along with its control rats and at this point it can be said that EDN did not influence the functional population of α_{1A}-adrenoceptors in this vasculature. However, further study is underway to investigate the contribution of EDN in relation to other subtypes of α_1-adrenoceptors in renal vasculature.

CLINICAL STUDIES

PARAOXONASE ACTIVITIES AND LIPID PROFILE IN THE HEALTHY POPULATION OF MAJOR ETHNIC GROUPS IN MALAYSIA

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Paraoxonase (PON1), a HDL-associated enzyme is likely to give protection against atherosclerosis by preventing LDL-oxidation. Variation in susceptibility to cardiovascular diseases and mortality rates of ischemic heart diseases (IHD) has been observed among ethnic groups. PON1 polymorphism and activities have been proposed as a marker for the risk of IHD and is suspected to vary among ethnic groups. Dyslipidemia is also a risk factor for CHD. The present study deals with the PON1 activities, lipid profile and correlation between them among the three major ethnic groups in Malaysia. A prospective study was carried out on a total number of 150 healthy Malay, Chinese and Indian volunteers. Fasting serum samples from volunteers were analysed for paraoxonase activity towards different substrates and lipid profile. There were no significant differences ($p>0.05$) observed in the levels of any of the lipid profile studied among the three major ethnic groups. There were no significant correlation found between the PON1 activities and lipid profile except a fair correlation with total cholesterol. Our study suggested that there were no ethnic differences in the lipid profile as well as PON1 activities and therefore the difference in the susceptibility to IHD among these three major ethnic groups in Malaysia cannot be explained by it. However, a large population study has to be carried out to confirm it.

FAMILY PLANNING PRACTICE AMONG MARRIED WOMEN IN MUKIM JAYA SETIA, KOTA BHARU, KELANTAN

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In Malaysia, family planning expanded tremendously since it was introduced in 1930s. In 2001, new acceptors of family planning were 58,704, increased by 15.7% compared to

2000. Among the new acceptors, 75.2% chose pills as their methods of contraceptive. Other methods such as condom, IUD and sterilization were not very popular. In 2002, 75.8% acceptors were from government agencies. Non-government agencies, and private clinic and hospital also offer such services. There are still discrepancies in the distribution of family planning as acceptors in Malaysia. The aim of this study was to determine the prevalence of family planning practice and its associated factors among married women in Mukim Jaya Setia, Kota Bharu. A cross sectional study on the prevalence and associated factors of family planning among married women aged between 15 to 49 years old in Mukim Jaya Setia was conducted in January 2005. Structured questionnaire was used as research instrument by trained interviewers to interview 201 married consented women. The prevalence of family planning practice was 44.3%. Most of the women used pills (64.0%) followed by injectables (14.6%) and other methods (21.4%). The criteria of chosen family planning methods were safe, easy to use, availability, cheap and others. Most of the women (94%) perceived the family planning as effective and 81.8% knew about family planning. Most of the women get the knowledge from government's clinic. There were significant associations between practice of family planning and number of children ($p<0.001$), educational level ($p = 0.006$) and monthly household income ($p = 0.010$). Other factors such as age and occupation status were not significant. The family planning practice among married women in Mukim Jaya Setia, Kota Bharu was low. Number of children, educational level and household income were significant associated factors in these women.

THE PRACTICE OF EXCLUSIVE BREASTFEEDING AMONG MALAY POST NATAL MOTHERS AND ITS ASSOCIATION WITH SHORT PREGNANCY SPACING

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Pregnancy spacing is the interval between delivery of one child and the conception of the next child. Too short or too long spacing is associated with maternal and child morbidity and mortality. Exclusive breastfeeding is known to be beneficial to the baby and is also able to protect the mother against pregnancy. The aim of this study was to determine the prevalence of short pregnancy spacing and its association with exclusive breastfeeding practice. A cross sectional study was conducted involving 388 Malay postnatal mothers admitted at Hospital Universiti Sains Malaysia (HUSM). They were interviewed and antenatal cards were reviewed. Short pregnancy spacing was defined as pregnancy spacing of less than 24 months. Giving the baby breast milk only with no other food for the first six months of life was accepted as exclusive breastfeeding. Analysis of data was done using SPSS Version 11. Median pregnancy spacing for 388 Malay postnatal mothers at HUSM was 27.0 (29.0) months. The prevalence of short pregnancy spacing was 44.3%. Majority of the respondents with short pregnancy spacing were housewives (64.2%) compared to working mothers (35.8%). Only 22.4% of the respondents had practiced exclusive breastfeeding to their babies prior to the current deliveries. However, there was no significant association between exclusive breastfeeding and short pregnancy spacing.

In conclusion, the prevalence of short pregnancy spacing among Malay mothers was still high. Even though no association was detected between short pregnancy spacing and exclusive breastfeeding in this study, strategies to improve exclusive breastfeeding practices should be strengthened since its prevalence was still low.

KNOWLEDGE ON DANGERS OF HERBAL MEDICINES BETWEEN USERS AND NON USERS DURING PREGNANCY IN TUMPAT, KELANTAN

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About 7.0% to 55.0% of women consume herbal medicines during pregnancy. Some herbs consumed during pregnancy can adversely affect the foetus. Knowledge on dangers of herbal medicines is a factor influencing their use during pregnancy. This is a comparative cross sectional study to compare the level of knowledge on dangers of herbal medicines between users and non users during pregnancy. Women who gave birth in June 2002 to June 2005 were selected randomly and interviewed. Those who used herbal medicines during pregnancy were identified as users. Non users were women who did not use any herbal medicines during pregnancy. Their level of knowledge on dangers of herbal medicines was assessed using a guided and structured questionnaire. A total of 316 women (153 users, 163 non users) were studied. The knowledge score between users and non users was not much different. The estimated marginal mean (EMM) of knowledge score among users was 18.84 (95% CI = 17.58, 20.10), while the EMM for non users was 19.80 (95% CI = 18.63, 20.98). Only 33.90% of non users and 26.90% of users obtained acceptable marks (18 marks and above). Besides knowledge, other important factors affect use of herbal medicines during pregnancy, which were not studied in this preliminary study such as family influence and beliefs that herbal medicines give health benefits. Health education on dangers of herbal medicines during pregnancy is still very important to improve the women's knowledge and increase their awareness on the adverse effects of herbal medicines to the unborn child.

INTERVENTION STUDY ON THE EFFECTIVENESS OF HEALTH EDUCATION PROGRAMME ON KNOWLEDGE, ATTITUDE AND PRACTICE AMONG FOOD HANDLERS IN MACHANG, KELANTAN

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The incidence of food-water borne diseases continues to be a challenge to the health staffs. A significant portion of cases are related to consumption of food outside of home. Therefore, food handlers have the major roles in preventing these illnesses. They may cross-contaminate raw and processed foodstuffs as well as acts as carriers. The aim of the study was to evaluate the effectiveness of health education programme on food hygiene knowledge, attitude and practice of food handlers in Machang, Kelantan. This is an intervention study involving 31 food handlers, who attended food hygienic and safety course by Food Safety and Quality Unit, Machang District Health Office on January 12, 2006. The participants were asked to answer a set of questionnaire prior the beginning of the course. They were then answering the same set of questionnaire upon completion of the course. Paired t-test was used to analyse the difference in pre-and post-intervention score. There was significant increase in knowledge and practice score ($p = 0.002$ and $p<0.001$) respectively. The difference in attitude score were not significant ($p = 0.238$). There was no significant correlation between the difference in knowledge and practice score by single linear regression test. In conclusion, health education programme regarding food hygienic and safety by the Food Safety and Quality Unit does improved the course participant's knowledge and practice score. Therefore this programme should be made compulsory by law to all the food handlers before they obtained the certificate.

ORAL HEALTH PROMOTION ACTIVITIES AMONG PRIMARY SCHOOL CHILDREN IN MUKIM TASIK, KOTA BHARU, KELANTAN

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Appropriate oral health education is vital to create an impact in behavioural changes. Children require a comprehensive and supportive approach to oral health education to enable positive changes in their oral health related behaviours. The objective of this study is to assess the oral health related knowledge, attitude and practices of primary school children before and after exposure to various oral health promotion activities. This is an experimental study involving 138 school children aged 9–11 years old. They were divided into intervention and control groups in a ratio of 1:1. Pre-intervention knowledge, attitude

and practices were assessed by using a set of questionnaire. Subsequently the intervention group was exposed to a series of oral health promotion activities such as dental health talks, quizzes, poster presentations, small group discussions and tooth brushing demonstrations. The control group was not exposed to these activities. At three weeks post intervention, a similar set of questionnaire was distributed to both groups to assess knowledge, attitude and practices of the children. Data were analysed using SPSS Version 12. The results at three weeks post-intervention, the children in the case group showed significant improvement in the scores compared to the control group. In conclusion, comprehensive oral health promotion activities are vital to ensure a significant impact in the knowledge, attitude and practices of children. These impacts lead to positive behaviour changes. Creative oral health promotion activities in schools are a powerful tool to educate children about oral health and its importance.

FACTORS ASSOCIATED WITH POORLY CONTROLLED TYPE 2 DIABETIC PATIENTS IN KELANTAN

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Diabetes mellitus is a common chronic disease with high morbidity and mortality. The United Kingdom Prospective Diabetic Study (UKPDS) showed that complications of diabetic patients strongly associated with poor glycaemic control. The objective of this study was to determine common factors associated with poorly controlled type 2 diabetic patients in Kelantan. Two hundred and eight subjects were selected randomly from health centres in Kelantan in a cross-sectional study. Sociodemographic data, physical examination and blood samples were taken from the subjects to determine the related associated factors. Results showed that the level of HbA1c was significantly associated with fasting blood glucose (FBS) and educational level, marital status and the body mass index (BMI). Every one mmol/L increased in FBS and one unit increase in BMI was associated with 0.23 unit (95% CI of 0.18 to 0.28 unit) and 0.05 unit (95% CI of 0.01 to 0.09 unit) increase in HbA1c, respectively. Those who were married had HbA1c of 0.59 unit (95% CI of 0.05 to 1.13 unit), which is higher compared to those who were not married. Those with primary and secondary education had 0.84 unit (95% CI of 0.22 to 1.45 unit) and 0.67 unit (0.03 to 1.31 unit), which is lower level of HbA1c compared to those without formal education, respectively. In conclusion, FBS, BMI and marital status were associated with higher level of HbA1c and formal education was associated with lower level of HbA1c in poorly controlled type 2 diabetic patients in Kelantan.

CHARACTERISTICS OF TYPE 2 DIABETIC PATIENTS AND GLYCAEMIC CONTROL ON FOLLOW-UP AT KKB MACHANG, KELANTAN

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A cross-sectional study was conducted with aim to determine the characteristics and glycaemic control of type 2 diabetic patients in Machang, Kelantan. One hundred type 2 diabetic patients who were on follow-up in KKB Machang between August 2005 to February 2006 were selected. Data were collected by using a questionnaire, medical record and biochemistry for HbA1c analysis. Of the total hundred patients, all of them were Malays, only 1% on insulin alone, 88% on oral hypoglycaemic agent and 11% on combination therapies. Only 11% (11) having good glycaemic control (HbA1c ≤ 7.0%). Good glycaemic control group mean ± SD for age was 56.0 ± 7.48 years, weight 61.4 ± 7.90 kg, height 153.1 ± 5.25 cm, body mass index (BMI) 26.3 ± 3.78 kg/m^2, HbA1c 6.4 ± 5.60%, random blood sugar 8.6 ± 1.72 mmol/L, systolic 132.1 ± 30.65 mmHg, diastolic 83.8 ± 13.00 mmHg and numbers of visit 7.0 ± 1.22. Median for family income was RM918.1 ± 780, duration of diagnosis 3.8 ± 4.00 years and distance to health clinic 5.6 ± 5.50 km. Poor glycaemic control group, mean age was 54.1 ± 7.72 years, weight 64.7 ± 10.26 kg, height 154.7 ± 8.39 cm, BMI 27.0 ± 3.58 kg/m^2, HbA1c 9.4 ± 1.64%, random blood sugar 11.2 ± 3.06 mmol/L, systolic blood pressure 137.6 ± 19.18 mmHg, diastolic blood pressure 85.4 ± 10.28 mmHg, visit 7.3 ± 1.66. Median family income was RM1108.2 ± 970, duration 7.0 ± 5.99 years and distance 6.2 ± 4.75 km. Good glycaemic control group were newly diagnosed (disease duration less than five year) and better random blood sugar reading during follow-up. Majority of diabetic patients seen in primary care had poor glycaemic control. Appropriate remedial measures should be taken in management of type 2 diabetes in primary care which includes both patients and also primary health care provider for a better glycaemic control and furthermore reduce diabetes complications.

SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF AMPUTATED AND NON-AMPUTATED DIABETIC FOOT IN HUSM

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A cross-sectional study was conducted with the aim to evaluate sociodemographics and clinical characteristics of amputated and non-amputated diabetic foot who were admitted to HUSM from May to December 2005. Universal sampling was applied whereby 97 diabetic foots were interviewed and their medical record were reviewed. Results revealed 23.7% (23) were amputated and 76.3% (74) non-amputated. In amputated group, 65.2% (15) were male, 34.8% (8) female, 100% (23) Malay, 91.3% (21) married, 8.7% (2) divorce, 26.1% (6) had no education, 30.4% (7) primary, 43.5% (10) secondary, 56.5% (13) self-referral, 43.5% (10) referred by clinic or hospital, 26.1% (6) Wagner's stage 1, 8.7% (2) stage 2, 8.7% (2) stage 3, 47.8% (11) stage 4, 17.4% (4) stage 5, 82.6% (19) on oral hypoglycaemic agent, 13.0% (3) insulin, 4.3% (1) combination therapies, 43.5% (10) had hypertension, 8.7% (2) coronary heart disease, 4.3% (1) chronic renal failure, no stroke, 30.4% (7) preceded by minor trauma. The mean ± SD of age was 57.5 ± 9.16 years, hospitalization 20.1 ± 10.28 days, blood sugar on admission years 15.2 ± 5.71 mmol/L, duration of diabetes 10.9 ± 8.07 years, haemoglobin 11.1 ± 2.64 g/dL, TWDC 15166.1 ± 7049.91 per cu. mm, haematocrite 32.7 ± 7.53%, urea 7.8 ± 4.77 mmol/L, creatinine 132.4 ± 143.83 mmol/L, systolic blood pressure 139.7 ± 22.15 mmHg, diastolic blood pressure 80.1 ± 12.15 mmHg. Only 34.8% (8 out of 23) had HbA1c being done with mean ± SD was 10.6 ± 1.37%. Independent *t* test revealed that hospitalization ($p = 0.006$), blood sugar ($p = 0.013$) and HbA1c ($p = 0.010$) was significant. Chi-Square test showed Wagner's classification was the only significant variable. Good blood glucose control with reduction in hospitalization duration and early detection of diabetic foot is important to prevent the amputation in diabetic foot.

CONTRACEPTION USE AMONG MALAY POSTNATAL MOTHERS AT HUSM PRIOR TO THE CURRENT PREGNANCY

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More than one-third of all pregnancies each year are unwanted or mistimed. Contraception is important for birth spacing to ensure optimum health and well-being of the women, children and the family. The aim of this study was to determine the prevalence of contraception use among Malay postnatal mothers prior to the current pregnancy. A cross-sectional study was conducted involving 388 subjects. They were interviewed using a validated questionnaire. Contraception use was defined as history of using any form of contraceptive methods in between the second last and the last pregnancy. Data were analysed using SPSS version 11. The prevalence of contraception use prior to the current pregnancy was 35.3%. Majority of the subjects with history of contraception use had been using oral contraceptive pills (82.5%), followed by injectable contraception (9.5%). 67.9% of them took contraception from the government clinics, hospitals and LPPKN clinics. 23.4% took from private hospitals and clinics, while 12.4% bought at the pharmacies. The main reason for taking contraception was spacing of childbirths (98.5%). Planning to have another baby was the most common reason for discontinuing contraception (61.3%), followed by side effects (16.8%) and forgetting the pills or schedule (12.4%). The main reasons for not using any contraceptive methods were still wishing to get pregnant (17.5%), disapproved by husbands (15.9%) and believed that contraception had many side effects (15.9%). As a conclusion, the prevalence of contraception use among Malay postnatal mothers who delivered at HUSM was still low. Efforts should be made to increase awareness and access to the various methods of contraception.

CHANGES IN THE KNOWLEDGE AND ATTITUDE ON THE FAMILY PLANNING FOLLOWING INTERVENTION AMONG MARRIED COUPLES IN MUKIM JAYA SETIA, KOTA BHARU, KELANTAN

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In Malaysia, family planning expanded tremendously since it was introduced in 1930s. Family planning enables a family to systematically plan the future of the children's

welfare, to achieve desired birth spacing and family size as well as to safeguard the health of the mother and her offspring. Smaller families and longer birth intervals have contributed to the better health of the infants, children, and the women, and have improved social economic role of women. In order to ensure their continuous practice, the community must have adequate knowledge about the family planning. The objective of this study was to determine changes in the knowledge and attitude towards family planning among married couples after an intervention programme in Mukim Jaya Setia, Kota Bharu. The study was conducted in August 2005. We randomly selected 50 households from 285 households. Trained interviewers used structured questionnaires to interview 50 consented pairs of husband and wives in the selected houses. Pre-intervention assessments were done during the promotion prior to the intervention programmes. Intervention programmes were done for the respondent for three days and post-intervention assessment were done using the same sets of questionnaires on the following weekend. Data was analysed using paired t-test to determine changes in knowledge and attitude in the same group and independent t-test for determination of changes in knowledge and attitude between groups. There were significant changes in knowledge and attitude after intervention programme among the married couples ($p<0.001$, mean difference = 15.3 and $p<0.001$, mean difference = 3.8). There was significant difference in knowledge between husbands and wives ($p = 0.034$). However, the difference in attitude between husbands and wives was not significant. Knowledge and attitude can be changed by specific intervention programmes on the specific subjects and target groups.

PATTERN OF HERBAL MEDICINES USE DURING PREGNANCY AMONG WOMEN IN TUMPAT, KELANTAN

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Herbal medicines have traditionally been used to promote a healthy pregnancy. The objective of this cross-sectional study is to describe the pattern of use of herbal medicines during pregnancy among local reproductive age women. Women who gave birth in June 2002 to June 2005 were interviewed. A total of 316 women were studied. There were 153 women (48.4%) who reported using herbal medicines during pregnancy of their babies born in June 2002 to June 2005. The most commonly used herbal medicines were coconut oil, orang asli herbal products, kacip fatimah, homeopathy herbal products, getam guri, manjakani and celaka. Some of the common indications were to facilitate labour, to abort the pregnancy, to prevent retained placenta and to promote the baby's health. Among the users, 103 (67.3%) women took herbal medicines during the third trimester only, 15 (9.8%) women took during the first trimester only, 18 (11.8%) women used during both the first and third trimester and 11 (7.2%) women used throughout the three pregnancy trimesters. Use of herbal medicines is common among pregnant women. Further research is needed to clarify the safety and effectiveness of herbal medicines during pregnancy.

PHARMACEUTICAL ANALYSIS

METHOD DEVELOPMENT FOR ISOLATION AND IDENTIFICATION OF PROTEINS EXTRACTED FROM BREAST CARCINOMA

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Female breast cancer is the leading cause of mortality and morbility among women worldwide. In Malaysia, female breast cancer was reported to be the highest incidence of cancers. In this study, a protein analysis method was developed according to proteomics approach. The procedures of the developed method include the extraction, separation and identification of the proteins. A sequential extraction buffer made up of three buffers was used to extract total cellular proteins from the homogenized breast cancer and normal tissues. The extracted proteins were then separated according to their molecular masses using SDS-PAGE, the gel was then Coomassie blue stained and the image was capture and analysed by an imaging system. The proteins that were differentially expressed between cancer and normal were targeted and excised from the gel followed by digested in-gel by trypsin. The tryptic peptides were then eluted from the gel and analysed with LC/MS/MS which allows the sequencing of their amino acid sequences. Protein identification was determined using the product ion spectrum in MS/MS scan. The resulted MS/MS data were then search against the MASCOT protein database search engine and from which the identity of target proteins were determined. Using such technique, a number of differentially expressed proteins from the cancer and normal tissues were identified.

X-RAY CRYSTALLOGRAPHY AND BIOLOGICAL PROPERTIES OF INDOLE ACETIC BENZOYLHYDRAZONE-SALICYLALDEHYDE AND THEIR METAL COMPLEXES

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Several new indole-hydrazones had been prepared in acidic ethanol by condensation of either indole-3-acetic acid hydrazide with either substituted salicylaldehyde. All the ligands were characterized by elemental analysis, IR, ^{1}H, ^{13}C NMR, X-ray studies and

fluorescence studies. Indole-hydrazones of indole-3-acetic acid hydrazide with substituted salicylaldehyde are fluores except for nitro-substituted indole-hydrazones. The Schiff base ligands were also screened for antioxidant activities using FTC method. Generally, indole-hydrazones of indole-3-acetic acid hydrazide with substituted salicylaldehyde shows better antioxidant activity than vitamin E. Electron-withdrawing substituted ligands exhibit higher antioxidant activity compared to the electron-donating ligands.

A SENSITIVE ASSAY FOR SIMULTANEOUS DETERMINATION OF NICOTINE AND COTININE IN HAIR BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Nicotine, a major addictive compound in cigarette smoke is rapidly and extensively metabolized to several metabolites in human. Cotinine, a major metabolite found in human biological fluids has been used as a biochemical marker to determine the status of active or passive smokers. However, cotinine is not a suitable marker for monitoring chronic exposure due to its short half life (< 48 hours). Nicotine is known to be preserved in hair shaft throughout the life of the hair. The amount accumulated depends on its plasma levels. Each centimetre of hair reflects approximately one month of exposure, thus nicotine in hair provides more information on long-term exposure to tobacco smoke. There were several published methods for measurement of nicotine in hair but the detection and quantitation limits were higher. The procedures were also lengthy as compared to our present method. The aim of this study was to develop a simple, sensitive, rapid and high throughput GC-MS assay for simultaneous quantification of nicotine and cotinine in hair. The analytes and internal standard were first digested followed by dilution and liquid-liquid extraction. The clear extract obtained was directly injected into GC-MS where selective ion monitoring (SIM) mode was used. Calibration curves for both nicotine and cotinine ranged from 0.2 to 1000 ng or 0.04 to 200 ng/mg hair were established with linear correlation coefficients (R^2) greater than 0.998. The limit of detection and limit of quantitation for both compounds were 0.20 ng or 0.04 ng/mg hair. The recovery of the assay for nicotine and cotinine were in the range of 106.1% to 117.0% and 95.4% to 103.2%, respectively. The within and between assays accuracies were between 0.8% to 14.4% for nicotine and 0.2% to 11.9% for cotinine. The good within and between assay precisions were also achieved for Nicotine (6.0%–16.3%) and cotinine (3.0%–16.0%). This assay can be used for routine monitoring to assess the active smoking and exposure to environmental tobacco smoke. The applicability of the assay was demonstrated in a comparison study between smokers and non-smokers.

FT-IR, UV-VISIBLE AND ATOMIC ABSORPTION SPECTROSCOPIC STUDIES OF *GANODERMA LUCIDUM*

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A rapid and efficient method for the extraction of bioactive molecules in *Ganoderma lucidum* is described. This method is based on extraction of *G. lucidum* with hot water, methanol and a 1:1 v/v mixture of hot water-methanol. The FT-IR spectra of *G. lucidum* and its extracts were recorded in KBr over the range of 4000–400 cm^{-1}. The UV-Visible spectrophotometric measurements were performed in water and methanol over the range of 200–800nm. Bioactive elements in the acid-digested *G. lucidum* samples were determined by flame atomic absorption spectroscopy. The FT-IR spectra of *G.* and its extracts exhibited characteristic absorption bands with significant difference in their number and position. The infrared spectral bands indicated presence of functional groups attributed to polysaccharides, amino acids, vitamins, terpenes and enzyme moieties. The UV-Visible spectra, however, could not give much information on the structure of the compounds extracted, due to very complex nature of biological molecules. The concentration of elements determined in *G. lucidum* by flame AAS was found for Na 12.5 ± 0.001 mg/L, K 210.4 ± 0.003 mg/L, Ca 23.9 ± 0.001 mg/L, Mg 7.1 ± 0.005 mg/L and Zn 1.9 ± 0.001 mg/L.

ENANTOMERIC SEPARATION OF D, L-TRYPTOHPAN BY CAPILLARY ELECTROPHORESIS WITH NEUTRAL CYCLODEXTRINS

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In the pharmaceutical field, analysis of chiral drug is of utmost importance given the pharmacological differences existing between enantiomers. Capillary electrophoresis (CE) is recognized as a technique of choice for enantiomers separation thanks to its high efficiency, low sample and reagent consumption and versatility. By adding a chiral selector to the background electrolyte (BGE), it is possible to modify enantiomers mobility by forming labile diastereoisomeric complexes. D,L-Tryptophan was enantioseparated in CE by use of a running background electrolyte containing chiral crown ether (18C6H4) as chiral solvating agent. However, chiral crown ether is not easily commercially available and quiet expensive. The aim of the present work was to investigate the enantioseparation of D,L-Tryptophan using neutral cyclodextrins, namely α-, β- and γ-cyclodextrin as chiral selector. Furthermore the effect of cyclodextrin concentration and the pH of the buffer on the resolution and migration time were investigated. The chiral resolution was performed in an untreated fused-silica capillary (75 μm inner diameter, 53 cm total length) by using Tris-phosphate buffer in pH range 2.0–3.0 supplemented with cyclodextrins. Successful

separation of D,L-Tryptophan was achieved with α-cyclodextrin. Resolution was strongly influenced by the concentration of α-cyclodextrin added to the background electrolyte.

X-RAY CRYSTALLOGRAPHY AND BIOLOGICAL PROPERTIES OF INDOLE-AROMATICHYDRAZONE AND THEIR METAL COMPLEXES

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The indole-aromatichydrazone series have been prepared from either substituted benzhydrazide or 2-thiophenecarboxylic hydrazide with substituted indole in acidified ethanol. The new metal complexes have been prepared by condensation of indole-aromatichydrazone and metal acetate in basic medium. The x-ray crystallographic structures of Ni(II), Cu(II) and Zn(II) complexes have been obtained. The Schiff behaves as tridentate ligands and coordinate to metal in a tridentate manner via O, N and O atoms. The IR, ^{1}H and ^{13}C NMR data also indicate coordination of the hydrazone ligands to the metal centers. The Schiff bases and Zn(II) complexes showed higher antioxidant activities than the quercetin or vitamin E and the data is comparable with butylated hydroxytoluene (BHT), a commercially used synthetic antioxidant. Other biological properties, antidiabetic and antihypertensive will be discussed.

DETERMINATION OF PARACETAMOL AND CAFFEINE IN DOSAGE FORMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTO DIODE ARRAY DETECTION

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A simple, accurate and reproducible high performance liquid chromatographic method for the separation and simultaneous determination of paracetamol and caffeine in dosage forms is described. The HPLC system consisted of Waters Alliance Series 2475 with photo diode array detection. The separations were carried out at room temperature on a μBondapack C-8 (5 μm, 250 x 4.6 mm i.d., Waters Milford, MA, USA) analytical column. A mixture of 0.01 M KH_2PO_4, methanol, acetonitrile and isopropyl alcohol (120:30:20:30 v/v/v/v) was used as a mobile phase at a flow rate of 0.5 mL/min. Mobile phase solvents were degassed for 30 min using ultrasonic bath and filtered through a 0.45 μm Millipore filter. The validation parameters: linearity (r> 0.999), precision (RSD = 0.11%–0.26%), sensitivity (LOD, S/N ratio 3 = 0.05–0.15 μg/mL and LOQ, S/N ratio 10 = 0.09–

0.40 µg/mL), accuracy (recoveries = 98.6%–99.3%). The calculations of the concentration of paracetamol and caffeine were based on peak areas using calibration plots. This method was successfully applied to the analysis of commercial pharmaceutical preparations, yielding better resolution, sensitivity and ease of operation as compared to the existing methods.

MISCELLANEOUS

INVESTIGATION OF THE CHARACTERISTICS OF DIFFERENT MOLECULAR WEIGHTS OF CHITOSAN AS MATRIX FOR ENZYME IMMOBILIZATION IN GLUCOSE BIOSENSOR

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Different molecular weight of chitosan membranes were investigated as matrix for enzyme immobilization in biosensor using glucose oxidase as a model enzyme. The average molecular weight values of chitosan samples, FCHIT and SCHIT determined using Mark-Houwink equation were 107470.59 and 79388.63 Da, respectively. The fabricated amperometric glucose biosensors were highly sensitive to glucose. FCHIT- biosensor was linear in the range of 9.99×10^{-6}–1.08×10^{-2} M glucose while SCHIT-biosensor was 1.05×10^{-5}–1.14×10^{-2} M glucose. The apparent Michaelis-Menten constants $\left(K_m^{app} \right)$ determined for FCHIT- and SCHIT-biosensors from Lineweaver-Burk plot were 4.2292 and 3.2841 mM, respectively. The small K_m^{app} values indicated a high affinity of the immobilized enzyme for the substrate. The sensitivity levels and detection limits for FCHIT and SCHIT-biosensors were 47.4995 and 32.4857 nA/mM as well as 9.93×10^{-5} and 1.04×10^{-4} M, respectively at S/N > 3. The biosensors coated with Nafion could minimize the effect of interferents. The FCHIT- and SCHIT-biosensors retained their initial activity of 30% and 20%, respectively over a two-month storage period. Good repeatability (n = 20) was observed for FCHIT and SCHIT-biosensors in their catalytic response to 3.98 mM glucose with a relative standard deviation of 6.23% and 5.33%, respectively. In conclusion, the use of different molecular weight of chitosan did not influence the characteristics of biosensor, although there was a slight difference in sensitivity level.

NANOTECHNOLOGY: AIDS FOR RESEARCHERS

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The objectives of this paper is to address several applications of nanotechnology in our clean room laboratory which utilizable by biomedical as well as pharmaceutical researchers. Our clean room laboratory is equipped with various type of deposition methods which suitable for both organic and inorganic materials. They are consisted of thermal evaporation, electron beam evaporation, DC and RF (magnetron) sputtering and molecular beam epitaxy is the most adequate methods as the thickness of deposited films can be controlled in few atomic layers dimension. This is the required working scale in bio-physiological and pharmaceutical research areas. With the help of ultraviolet lithography and electron beam lithography fabricates patterns in micron dimension while electron beam lithography, tissue and cell implantation is achievable in our lab as well. Ultraviolet lithography fabricates patterns in micron dimension while electron beam lithography can reach 20 nm patterning on tissues in its optimized conditions. Thermal treatment for all kinds of samples is available in our clean room NOR laboratory, such as oxidation, nitridation, hydridation and sample doping. Those processes can work from room temperature to 1500 degrees at either atmospheric pressure or controlled vacuum environment. The electrical and optical properties as well as the morphology of fabricated samples can be characterized under the same clean room environment. The characterization methods are thickness measurement (thickness of few atomic layers with ellipsometry techniques), X-ray diffraction (2-theta), Raman spectroscopy, energy dispersive x-ray analysis, scanning electron microscopy, atomic force microscopy (for detection and mapping the surface roughness by a 3-D surface mapping with nanometers resolution), Hall effects measurements, and photo-spectrometer. In this article we aim to convey the message that our laboratory is well equipped with the technology which can fulfill your research experimental needs and measurements. The goal is to use nanotechnology as a tool to help in generating the real benefit for our country and the world and it is available just next door.

SECONDHAND SMOKE: INDOOR AIR MONITORING OF $PM_{2.5}$ IN KUALA LUMPUR INTERNATIONAL AIRPORT (KLIA)

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Malaysia has introduced smoke-free areas policy in most indoor buildings. The Kuala Lumpur International Airport (KLIA) is designated as smoke-free, however, smoking is allowed in a smoking room located in the premises. Question has been raised to whether

smoking in the smoking room, pose significant problems to the air quality in designated smoke-free airport. Because cigarettes smokes can produce a fine respirable suspended particle, measuring $PM_{2.5}$ was recently introduced as a mean to quantify the extent of exposure to cigarette smokes in indoor building. This study was carried out to determine $PM_{2.5}$ in various locations and spots including smoking room in the KLIA as well as surrounding areas within the building. A real time aerosol monitor (TSI SidePak) was used to sample and record the level of $PM_{2.5}$ and below continuously for every minute. For every spot, three readings were determined over a period of 30 minutes. Dimensions of room were also taken to quantify the volume of the room together with some observational data including average number of people and cigarettes burnt. All data were then downloaded and then analysed using TrakPro software. Detail findings of the study will be presented and should be able to provide us with an indication as to whether the KLIA can be considered "smoke free" and whether smoking room does pose a risk to non-smokers in the airport.

ANTIMICROBIAL EFFECT OF *TINOSPORA CRISPA* ON BOTH GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Antimicrobial effect of *Tinospora crispa* (Akar Patawali) was evaluated for its abilities to inhibit or kill bacteria. Despite of many studies on antimicrobials that has been conducted previously, no experimental design has addressed the effect of *T. crispa* as an antimicrobial agent. This study was evaluating antimicrobial properties of aqueous and organic extracts of *T. crispa* on both gram positive and gram negative bacteria. Both aqueous and organic extracts of stem, leaf and Borapet® capsule (from Thailand) of *T. crispa* were used to determine antimicirobial effect to 2 strain *Staphylococcus aureus* (ATCC 29247, 700698) bacteria from gram positive and *Escherichia coli* (ATCC 23519, 25922) bacteria from gram negative. Dish diffusion method was used to determine the potential of all extract (both aqueous and organic) to the bacteria that were used. A serial dilution of the extract was carried out to evaluate the lowest concentration of extract to inhibit bacteria growth considered to be the minimal inhibitory concentration (MIC). Additionally, the minimal bactericidal concentration (MBC) can be determined by subculturing the contents of the MIC tubes and examining for bacterial growth. As for the result, stem-aqueous extract, leaf-organic and Borapet®-organic extract are effective against both strains of *S. aureus* (ATCC 29247, 700698) as shown in disk diffusion. Subsequently, the serial dilutions showed that leaf-organic caused minimal inhibitory/bactericidal at lower concentration, followed by stem-aqueous and Borapet®-organic. For *E. coli* ATCC 23519, stem-aqueous, leaf-organic and Borapet®-organic gives positive result in disk diffusion. However only stem-aqueous is effective against *E. coli* ATCC 25922. In serial dilutions, stem-aqueous gives MIC/MBC value at the lowest point

followed by leaf-organic. In conclusion, this finding suggested that *T. crispa'*s stem, leaves and Borapet® capsule extraction have antimicrobial effect to inhibit or kill bacteria.