

PHYSIOLOGY/PHARMACOLOGY

DISTRIBUTION OF α -, γ - AND δ -TOCOTRIENOLS IN RAT'S TISSUES

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The growing interest in tocotrienols has led to many studies on their potential therapeutic benefits. However, there is still a paucity of information on the disposition and distribution of tocotrienols in different tissues. Therefore, the objective of the present study was to investigate the distribution of α -, γ - and δ -tocotrienols in the skin, muscle, heart, liver, brain and eyeballs after oral administration of mixed tocotrienols using rat model. 10 Sprague-Dawley male rats were administered orally with 18.8 mg of mixed tocotrienols containing 4.5, 11.3 and 3.0 mg of α -, γ - and δ -tocotrienols respectively once daily for 15 days and subsequently sacrificed at the end of the study. The tissues namely, skin, muscle, heart, liver, brain and eyeballs were harvested, cleaned with saline and kept frozen in separate bottles at -20°C until analysis. Each tissue was homogenised and the three isomers of tocotrienols were extracted and analysed using a normal phase high performance liquid chromatography method. It was observed that the tocotrienols were distributed into all the tissues studied, with highest accumulation of total tocotrienols occurring in the heart, followed by the skin, liver, muscle, eyeballs, and lowest in the brain. This indicated that the tocotrienols are not distributed evenly into the various tissues studied. Moreover, it was observed that α -tocotrienol was preferentially accumulated in all six tissues investigated.

VASCULAR RESPONSIVENESS OF ACETYLCHOLINE AND PHENYLEPHRINE IN AORTIC RINGS FROM STZ-INDUCED DIABETIC RATS: EFFECTS OF GENDER AND DURATION OF DIABETES

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Several epidemiology studies revealed that the vascular protective effects of oestrogen are diminished after the onset of diabetes mellitus. To my knowledge, only two reports of vascular responsiveness to acetylcholine (ACh) and other vasoactive substances using female diabetes rats, one or six weeks after induction with streptozotocin (STZ), have been published. In this study, we examined the effects of duration of diabetes on the vascular responsiveness to ACh and phenylephrine (PE) in STZ-induced diabetic male and female rats. Aortic rings (2–2.5 mm wide transverse sections) from diabetic and non-diabetic rats

(controls) were suspended in a 10 ml organ-bath for isometric tension recordings of cumulative dose-responses of ACh and PE. Elevated glucose concentration in bath solution (22.2 mmol/l) was employed in assessing the diabetic vessels in order to mimic *in vivo* diabetic condition. Our results showed that ACh-induced relaxation was significantly impaired ($p < 0.001$) in female rats as early as 3–7 days after STZ injection, whereas, in male rats, significant impairment ($p < 0.01$) was seen only after 11–12 weeks. At 11–12 weeks and 16–17 weeks after diabetic induction, PE contractions were significantly increased in female rats ($p < 0.05$ and $p < 0.001$, respectively), but remained significantly attenuated ($p < 0.001$) in male rats when compared to their respective controls. Our results suggest that in female diabetic rats, aortic endothelial dysfunction occurred early and that a decreased nitric oxide activity may contribute to the increased PE-induced contractile tone at later stages of diabetes.

SYNAPTIC CONNECTIONS BETWEEN THE NUCLEUS INTERMEDIUS AND NTS IN RAT

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It has been reported that injections of retrograde tracers into the NTS of the medulla oblongata resulted in labelled neurones in the Nucleus Intermedius (InM). This study was undertaken to provide electrophysiological data on these connections. Male Wistar rats (15–21 days) were terminally anaesthetised with sodium pentobarbitone (120 mg/kg i.p.) and 300 μm coronal sections of medulla oblongata prepared. Whole cell patch clamp recordings were made from 15 neurones located within dorsal and dorsomedial ($n = 12$) or medial ($n = 3$) regions of the NTS, at room temperature. These NTS neurones had an action potential amplitude of 61.5 ± 1.70 mV (mean \pm s.e.m), an action potential duration of 4.9 ± 0.36 ms and an AHP amplitude of 15.6 ± 1.6 mV. Electrical stimulation of InM, using bipolar stimulating electrodes (voltage 10–20 V, duration 100 μs), elicited excitatory and/or inhibitory synaptic potentials in 12/15 NTS neurones. EPSPs had an amplitude (AMP) of 6.0 ± 0.6 mV and width at half-amplitude (HW) of 51.2 ± 5.1 ms ($n = 10$) and were blocked by 1 mM kynurenic acid indicating that they were mediated by excitatory amino acids. IPSPs [AMP = 6.01 ± 0.41 mV and HW = 51.3 ± 1.88 ms ($n = 9$)] in seven NTS neurones were GABAergic as they were blocked by bicuculline (10 μM) while the remaining two were mediated by both GABA and glycine as they were abolished by co-application of bicuculline (10 μM) and strychnine (2 μM).

**THE INTERACTION BETWEEN RENIN ANGIOTENSIN- AND
SYMPATHETIC NERVOUS SYSTEM ON RENAL
HAEMODYNAMICS IN NORMO- AND HYPERTENSION**

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The renin angiotensin- (RAS) and sympathetic nervous system (SNS) are closely interrelated and are involved in the maintenance of blood pressure and body fluid homeostasis. This study examined the role of RAS and SNS at peripheral levels in normo- and hypertension in the control of renal haemodynamics and to assess any alterations in renal haemodynamics after chemical sympathectomy. For this purpose, Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were utilized. Chemical sympathectomy was carried out by the administration of 6-OHDA intraperitoneally at doses of 50 mg/kg on day 1, 100 mg/kg on day 2, and 50 mg/kg on days 5 and 8. Perindopril was given orally at a dose of 0.2 mg/kg for seven days. In haemodynamics study, the animal was anaesthetised (60 mg/kg i.p., sodium pentobarbitone) followed by cannulation of carotid artery and jugular vein and isolation of renal artery. Renal blood flow (RBF) was measured using electromagnetic flow probe. Arterial blood pressure (BP) was measured using pressure transducer. All data were recorded in computerised data acquisition system and analysed by two-way ANOVA followed by Bonferroni post-hoc with the significance level of 5%. A substantial change was observed in the sympathectomised rats in term of haemodynamics. There was a marked reduction of BP in sympathectomised hypertensive rats. Noradrenaline was found to exert a significant difference ($p < 0.05$) in peripheral and renal haemodynamics in normotensive rats when administered intrarenally. Phenylephrine did not cause any significant change in peripheral and haemodynamics in both normo- and hypertensive animals. But a meaningful change ($p < 0.05$) was observed in RBF to methoxamine when administered peripherally and intrarenally in sympathectomised SHR. Angiotensin II peripherally and intrarenally did not cause major changes in pressor responses in both sympathectomised WKY and SHR. However there was attenuation in renal vasoconstrictor responses to peripheral angiotensin II in sympathectomised SHR. These results suggested that SNS plays an important role in the peripheral and renal haemodynamics in normo- and hypertension. Furthermore, RAS may interact with sympathetic nerve activity in the control of renal haemodynamics.

**MODULATION OF ANGIOTENSIN II INDUCED CONTRACTION
BY HYDROGEN PEROXIDE IN THE STREPTOZOTOCIN (STZ)-
INDUCED DIABETIC RAT MESENTERIC ARTERY**

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Hydrogen peroxide (H₂O₂) plays an important role in the regulation of vascular tone, especially in pathological states, including diabetes where it is suggested to be increased in production. Recently, H₂O₂ has also been associated with angiotensin II (Ang II) as one of the oxygen derived radicals, which contribute to Ang II-induced contraction in the rat aorta. Therefore, we investigated the involvement of H₂O₂ in the modulation of Ang II-induced contraction of the small resistance mesenteric artery from streptozotocin (STZ) -induced diabetic rats. Isometric tension of the Ang II-induced contraction was measured using the Mulvany-Halpern myograph and all preparations are endothelium intact. Incubation with the reactive oxygen species (ROS) scavengers and other inhibitors were carried out 30 min prior to Ang II-induced contraction. The results show that treatment with catalase, a H₂O₂ scavenger (CAT, 800 U/ml) significantly increased Ang II-induced contraction in diabetic (but not normal) tissues while superoxide dismutase (SOD, 150 U/ml), a superoxide anions scavenger significantly attenuated the Ang II-induced contraction in both groups. Treatment with L-NAME (0.1 mM) significantly enhanced the Ang II-induced contraction to the levels observed with catalase treatment. This suggests that H₂O₂ and NO inhibited Ang II contraction in diabetic tissues. Combined catalase and L-NAME treatment further enhanced the contraction implying that these agents may not be acting on a common pathway. The cyclooxygenase inhibitor, indomethacin (0.01 mM) did not alter the Ang II contraction but reduced catalase-induced increase contraction in the diabetic tissues. This suggests that the increased response to Ang II-induced contraction by catalase is mediated by cyclooxygenase byproduct e.g. vasoactive prostaglandins. In conclusion, in diabetic mesenteric artery, there is increased production of hydrogen peroxide, which acts as a vasodilator, attenuating the Ang II induced contraction via several mechanism(s).

**INVOLVEMENT OF SYMPATHETIC AND RENIN-ANGIOTENSIN
SYSTEMS IN THE REGULATION OF
RENAL HAEMODYNAMICS IN DIABETES**

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One of the complications in diabetes mellitus is the development of hypertension. This study was designed to investigate the interaction of adrenergic and renin-angiotensin systems in the regulation of renal haemodynamics in diabetes. Diabetes was induced using streptozotocin (STZ) (55 mg/kg i.p.). Acute effects of electrical stimulation, adrenergic agonists and angiotensin II on renal blood flow (RBF) without or in the presence of adrenergic receptor blockers, a calcium channel blocker, an angiotensin-converting enzyme inhibitor (ACEi) and an angiotensin II receptor blocker were studied in non-diabetic and diabetic Wistar-Kyoto (WKY) rats. The animals were anaesthetised and prepared for blood pressure measurements and fluid administration respectively. The left kidney was exposed by mid-abdominal incision and an electromagnetic flow probe was placed on the renal artery for RBF measurement. The left iliac artery was cannulated such that the beveled tip of cannula faced the renal artery to allow infusion of all drugs closed intrarenally. Reductions in RBF to electrical stimulation (1-0 Hz), bolus doses of phenylephrine (0.25-2.0 µg), methoxamine (0.5-4 µg) and angiotensin II (2.5-20 ng) were determined before and after bolus doses of 5-methylurapidil (5 and 10 µg/kg), chloroethylclonidine (5 and 10 µg/kg), BMY 7378 (100 and 200 µg/kg) or amlodipine (200 and 400 µg/kg). Perindopril (0.2 mg/kg) and losartan (10 mg/kg) were given orally for seven days daily prior to the acute studies and 48-h post-STZ for seven days daily in diabetic groups. Data, means \pm s.e.m. were compared with two-way ANOVA followed by Bonferroni post-hoc with the significance level of 5%. 5-methylurapidil and amlodipine caused significant reductions in the vasoconstrictor responses to all adrenergic agonists and angiotensin II in both non-diabetic and diabetic WKY. In chloroethylclonidine-treated groups, there were significant attenuation in responses to methoxamine and angiotensin II in non-diabetic WKY, but significant accentuation in responses to phenylephrine, methoxamine and angiotensin II were obtained in the diabetic WKY. BMY 7378 produced significant reductions in response to all but angiotensin II in non-diabetic WKY. However, there was no significant change in BMY 7378-treated diabetic WKY except to phenylephrine. In perindopril and losartan-treated non-diabetic WKY, there were significant reductions in all adrenergic agonists and angiotensin II. Interestingly, in the diabetic WKY treated with perindopril and losartan, there was no significant change in the vasoconstrictor response to any of the adrenergic agonists and angiotensin II, except a significant attenuation in response to angiotensin II in losartan-treated group. It can be suggested that a

complex interaction exists between the α_{1A} , α_{1B} , α_{1D} and AT1 receptors in the mediation of renal haemodynamics in diabetes.

EVALUATION OF α_1 -ADRENERGIC RECEPTORS INVOLVEMENT IN RENAL HEAMODYNAMICS OF ACUTE RENAL FAILURE RATS

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Amongst several factors involved in the pathogenesis of hypertension in renal diseases, a potential role of sympathetic overactivity has not yet been investigated, in particularly to the involvement of α -adrenergic receptors. It is known that renal chemo- and mechano-receptors are stimulated in the damaged kidney and may excite renal nerves. This study evaluated the involvement of α_1 -adrenoceptors in the renal resistance vessel of acute renal failure rats *in vivo*. In the rat renal resistance vessels, α_1 -adrenoceptors mediate the sympathetic activation and utilise changes in intracellular calcium as their primary signal transduction mechanism. In the present study we therefore chose to challenge this process using a calcium channel blocker, amlodopine (AMP) to get insights into the possible involvement of α_1 -adrenoceptors in the renal resistance vessel of the animals. Acute renal failure was induced by a single injection of cisplatin (5 mg/kg, i.p.). Metabolic data were collected for six days followed by acute renal haemodynamics study on day 7 of post cisplatin injection. The study was undertaken in normotensive male Wistar rats (250–275 g) with or without renal failure. In acute study, animals were anaesthetised (sodium pentobarbitone, 60 mg/kg, i.p.) and a tracheostomy was done followed by cannulation of a carotid artery and jugular vein. The mean arterial pressure (MAP) was recorded from carotid artery using a pressure transducer. The kidney was exposed by midline abdominal incision followed by cannulation of left iliac artery for the continuous infusion of saline and administration of adrenergic antagonist and agonists close renal arterially. The renal artery was isolated and cleared for renal blood flow (RBF) measurement by electromagnetic flowmetry. All data were recorded in a computerised data acquisition system. The renal nerves were identified and cleared for electrical stimulation (RNS). The change in RBF was determined in response to RNS followed by adrenergic agonists viz. noradrenaline, phenylephrine and methoxamine in absence and presence of AMP (200 μ g/kg and 400 μ g/kg). The data obtained was analysed by two-way ANOVA followed by Bonferroni post-hoc test taking significance at 5% level. Significant ($p < 0.05$) oligourea, change in water intake, reduction of creatinine clearance (> 50%) and reduction of urinary sodium excretion were observed in

the renal failure animals as compared to normal. The haemodynamics data showed a significant ($p < 0.05$) attenuation of renal vasoconstrictor responses by AMP in both groups of animal but however, was greater in renal failure rats. The data obtained thus indicated the presence of Ca^{++} influx dependent α_1 -adrenoceptors which is a characteristic of the α_{1A} -adrenoceptors and that these are the functional subtypes in the renal vasculature of these animals.

PHARMACOLOGICAL CHARACTERISATION OF THE HYPOGLYCAEMIC PROPERTIES OF *GYNURA PROCUMBENS* EXTRACT

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We have previously reported that the aqueous extract of *Gynura procumbens* at a single dose and repetitive doses for 14 days of 1 g/kg body weight possess hypoglycaemic properties in streptozotocin (STZ)-induced diabetic rats ($p < 0.05$) but not in normal rats. The *in vivo* studies also indicated that the mechanism by which this plant decreased blood glucose levels in diabetic rats is independent of insulin secretion. The present study was undertaken to further elucidate its mechanism of action. Exposure of clonal pancreatic β cells, RIN-5F cell line to the various concentration of aqueous extract (1-10 mg/ml) showed no stimulation in insulin secretion. Glibenclamide was shown to evoke a dosage-dependent stimulation of basal insulin release from the RIN-5F cell line. At the dose 10 mg/ml, glibenclamide was found to stimulate insulin secretion. Studies on the effects of the extract and acarbose, an alpha-glucosidase inhibitor, on intestinal glucose absorption showed that the hypoglycaemic effect is not due to the inhibitory action of the extract at the intestinal level ($P > 0.05$). Experiments using abdominal muscle of rats showed that the extract enhanced insulin-stimulated glucose transport across membranes similar to metformin either alone or as an insulin adjuvant. Taken together, the results suggest that a possible mechanism of the hypoglycaemic action of the extract is exerted at the peripheral level whereby the extract improves the uptake of glucose by peripheral tissues, such as the muscle cells.

METHANOL EXTRACTS OF *ORTHOSIPHON STAMINEUS* INHIBIT ETHYLENE GLYCOL-INDUCED STONE FORMATION IN RATS

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Orthosiphon stamineus is among the most popular plant used for the treatment of urolithiasis in Malaysian folk medicines. Therefore, an effort was made to study the effect of the plant extracts on ethylene glycol induced hyperoxaluria in rats. Rats were divided into four groups of eight animals each. The first group served as a negative control and received regular diet and water throughout the study.

The second, third and fourth groups were induced to be hyperoxaluric by giving 1% ethylene glycol in the drinking water for eight weeks. Moreover, the second, third and fourth groups were also administered with water (as positive control), 50% and 100% methanol extracts of *O. stamineus* 0.5 gm/kg daily respectively. Twenty four hours urine samples were collected from each rat at day 0 and weeks 2, 4, 6 and 8 for analysis. The results obtained show that ethylene glycol treatment had increased the total calcium ($P < 0.05$) in urine of the second group but not in the third and fourth groups. Similarly, aggregated calcium oxalate crystals were observed in the urine of the second group but not in the third and fourth groups at the end of the experiment. Methanol extracts of *O. stamineus* lowered ($P < 0.05$) the urinary calcium level and increased the total urinary magnesium, sodium and potassium levels of the rats in the third and fourth group. The results indicate that treatment with *O. stamineus* extracts may inhibit the stone formation by inhibiting crystal aggregation and decreasing the urinary calcium excretion. The inhibition of crystal aggregation and decrease in calcium and increase in magnesium levels are among the prominent mechanisms of action of antiurolithiatic drugs.

MOLECULAR MECHANISM OF INDUCTION OF PHASE I METABOLISM BY *MORINDA CITRIFOLIA* L. IN HEPATOCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS

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The objective of this study is to investigate the *in vitro* and *in vivo* molecular mechanisms of induction of phase I metabolism of aqueous extract of the Mengkudu or Noni fruit (*Morinda citrifolia* L.: family: *Rubiaceae*) in young female spontaneously hypertensive rat (SHR) hepatocytes. Aminopyrine was used as a model drug. Previously it was demonstrated that aqueous extract of the Mengkudu or Noni fruit increased aminopyrine metabolism in young female SHR rat hepatocytes. The hepatocytes were treated with IC_{50} or EC_{50} (inhibitor or effective concentration) of some cellular inducers/inhibitors namely IBMX, trifluoperazine, KT 5720, KT 5823, Gpp(NH)p, genistein, PMA, okadaic acid, and L-NI10. Our *in vitro* results did not show any significant alteration of the effect of aqueous extract of Mengkudu in the presence of all the cellular inducers/inhibitors. However, only KT5720, a protein kinase A inhibitor (PK_A) significantly affects the effect of aqueous extract of the Mengkudu at 6 g/kg concentration. The result indicates that the PK_A pathway may be involved in modulating the effect of aqueous extract of Mengkudu in SHR rat hepatocytes.

**CHARACTERISATION OF THE ANTI-HYPERTENSIVE
MECHANISM(S) OF THE CALYX EXTRACT
OF *HIBISCUS SABDARRIFFA* L.**

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Chronic treatment with *Hibiscus sabdarriffa* L. (HS) has been shown previously to exhibit anti-hypertensive effects in humans and experimental animals. The objective at the present study was to examine the mechanism(s) of the anti-hypertensive effects of HS in aortas isolated from spontaneously hypertensive rats (SHR). The freeze-dried methanolic extract of the HS was re-constituted in distilled water and serially diluted to obtain the final concentrations required. Thoracic aortic rings were isolated and suspended in tissue baths for isometric tension recordings. The results show that in high KCl (high K⁺, 80 mM) and phenylephrine (PE, 1 μM) pre-contracted SHR aortas, HS caused a dose-dependent relaxation, with a higher sensitivity towards the later. At the highest concentration tested (1 mg/ml) HS inhibited high K⁺ and PE-induced contractions by 27.9 ± 4.3% and 86.0 ± 4.8%, respectively. Endothelial nitric oxide synthase inhibitor, N^ω-L-nitro arginine methyl ester (L-NAME, 10 μM), but not indomethacin (10 μM), significantly inhibited the relaxant activity of HS against PE-induced contractions in SHR aortas, suggesting that the relaxant effects of HS were mediated in part by release of nitric oxide (NO) from the endothelium. The pre-treatment of SHR aortas with HS (0.3 mg/ml, 20 min) significantly improved acetylcholine (ACh) mediated endothelial nitric oxide (EDNO)-dependent relaxations (ACh/EDNO/cGMP-pathway), and NO donor sodium nitroprusside mediated EDNO-independent relaxations (SNP/NO/cGMP-pathway). In addition, presence of indomethacin in the tissue bath medium improved the relaxation responses to ACh, but had no effect on the effects of HS. From these findings it can be concluded that 1) the vasodilation induced by HS may involved EDNO/cGMP-pathway and to a certain extent inhibition of Ca⁺²-influx, and 2) the improvement in endothelial function and direct vasodilator effects of HS may contribute to the blood pressure lowering effects of HS *in vivo*.

**CONTRIBUTION OF α_{1A} -ADRENERGIC RECEPTORS IN RENAL
HAEMODYNAMICS OF CISPLATIN INDUCED
ACUTE RENAL FAILURE RATS**

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Renal diseases are closely associated with hypertension. It has been reported that kidney diseases provoke hypertension and renal injury raises sympathetic tone. Sympathetic overactivity has recently been implicated as an important predisposing factor in the pathogenesis of hypertension in renal diseases, however, among several possible factors involved this has not been investigated in-depth. The present study sets out to examine the involvement of α_1 -adrenoceptor subtypes in the renal resistance vessel of cisplatin induced acute renal failure rats *in vivo*. Acute renal failure was induced by a single injection of cisplatin (5 mg/kg, i.p.). Metabolic data was collected for seven days followed by hemodynamic studies on day seven of post cisplatin injection. The study was carried out in normotensive male Wistar rats (250–270 g) with or without renal failure. In hemodynamic studies animals were anaesthetised (sodium pentobarbitone, 60 mg/kg, i.p.), a tracheostomy was done followed by cannulation of a carotid artery and jugular vein. The kidney was exposed by midline abdominal incision followed by cannulation of left iliac artery for the administration of adrenergic agonists and antagonist close renal arterially. The renal artery was isolated and cleared for renal blood flow (RBF) measurement by electromagnetic flowmetry. The mean arterial pressure (MAP) was recorded from carotid artery using a pressure transducer. A computerised data acquisition system was used for data recording. The renal nerves were identified and cleared for electrical stimulation (RNS). The changes in RBF were determined in response to graded frequencies of RNS and graded doses of several adrenergic agonists viz. noradrenaline, phenylephrine and methoxamine in the absence and presence of a specific α_{1A} -adrenoceptors antagonists, 5-methylurapidil (5 μ g/kg and 10 μ g/kg). The data obtained was analysed by two-way ANOVA followed by Bonferroni post-hoc test taking significance at 5% level. The hemodynamic data showed a significant ($p < 0.05$) attenuation of renal vasoconstrictor responses by 5-methylurapidil in both groups of animal. The data obtained thus showed that the α_{1A} -adrenoceptors in the renal resistance vessel of renal failure rats were still functional and not compromised in this disease state.

HUMAN BODY COMPOSITION MEASUREMENTS- A LOOK INTO THE IDEAL METHOD

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Body composition estimations are very essential in nutritional and metabolic studies. It is known as the 'bloodless dissection' of the human body. There are both simple and expensive methods. The usage of the 'gold standard, technique hydro-densitometry is limited. Similarly, other methods also have limitations. The objectives of this study were: 1) To enlist all the available major methods of body composition measurements and to identify its merits and demerits, principle of working. 2) To figure out the most 'ideal method'. Several reviews and research publications have been referred to gather the relevant information. A few methods such as anthropometry, though simple and cost effective, do not fit into all types of studies. A few others are very expensive, laborious and time consuming. The method of choice depends on the aim of the study, type of study, component of interest to be measured, available know-how, time, cost involved and the subjects' burden. The ideal method seems to be one which is relatively inexpensive, maintenance free, little inconvenience to the subjects easy to operate, reproducible and accurate. In conclusion no known method is 'an ideal method' for body composition estimations. If better precision is required, measurements of more compartments of body composition or overlapping methods should be used. For population studies doubly indirect methods and for individual studies indirect methods appear to be reasonable and acceptable.

SUBACUTE TOXICITY STUDIES OF THE MISAI KUCING ON SPRAGUE DAWLEY RAT

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Misai Kucing (*Orthosiphon stamineus*, Benth.) has been widely used in Malaysia for treating kidney problems, gout, and diabetes. However, very few toxicity studies on Misai Kucing have been reported. This study aims to examine the possible subacute toxicity (1 day and 14 days-treatment) of the methanol extract of Misai Kucing on normal young male and female Sprague Dawley (SD) rat (7 weeks old \pm 1 week old). Control groups were treated orally with distilled water (vehicle) whilst a single dose or repeated doses of 0.5, 1, 3 and 5 g/kg body weight of methanol extract of Misai Kucing were fed orally to male and female SD rats respectively (10 rats/sex/day). Blood was taken via cardiac puncture and serum was used to determine several clinical biochemical tests, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine. No lethality incident and adverse effect on body weight gained, food and water consumption and necropsy findings was observed in any treatment group. However, a significant decrease in some serum biochemical tests was observed in both sexes of normal young SD rats after treatment with a single

dose or 14 doses of 1.0 to 5 g/kg body weight of methanol extract of Misai Kucing as compared to control group. Methanol extract of Misai Kucing within tested range and treatment duration would not cause any toxicity and adverse effects in young SD rats.

**EVALUATION OF ANTIHYPERTENSIVE POTENTIAL OF
AQUEOUS EXTRACT OF THE FLOWERS OF *GOSSAMPINUS
MALABARICA* IN RATS**

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Flowers of *Gossampinus malabarica* (Fam: Bombacaceae) are common locally and known as 'Bunga Ratu Pari'. The decoction is widely used traditionally to treat hypertension, diabetes, migraine, kidney stones, asthma, slimming, back ache, libido etc. A preliminary study was carried out to evaluate its antihypertensive activity and its possible mechanism of action. The commercially marketed flowers were ground, an aqueous extract was prepared by maceration and freeze drying. Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) weighing between 250 to 300 g of either sex were selected for the study and made into two groups. The animals were fasted overnight prior to the study and anaesthetised with pentobarbitone. The trachea, left jugular vein and right carotid artery were cannulated and the blood pressure was recorded on a polygraph via Gould Satham transducer. The aqueous extract (100 mg/kg bolus dose followed by 10 mg/kg IV, infusion) decreased the blood pressure in hypertensive rats from 139.1 to 90 mmHg but not in normal in acute studies suggesting its antihypertensive but not hypotensive activity. The extract was further evaluated for a possible adrenergic mechanism. Noradrenaline increased carotid blood pressure in SHR but not in WKY, but no meaningful changes to phenylephrine were seen. Hence it is hypothesised that there is a possible involvement of α_2 adrenoceptors in the blood pressure lowering effect. Extensive research on other possible mechanisms is required to confirm the exact mechanism of action. This preliminary work in rats supports the traditional usage of the flowers of *Gossampinus malabarica* in the treatment of hypertension.

EFFECT OF *VITEX TRIFOLIA* ON PREGNANT RATS

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Some species of *vitex* were used as contraceptive agents by local folks in Asia and some were used to restore pregnancy. No authentic evidence is available for these. Hence, this study was undertaken to evaluate the effect of the *Vitex trifolia* on pregnant Sprague Dawley rats. Pregnant rats were administered with the aqueous extract of the plant leaves orally from 1st day till 7th day of pregnancy. The doses of the extract given were 10 mg/kg and 100 mg/kg. One set of rats was laparotomised under ether anesthesia on 10th day and on 19th day of pregnancy to observe the foetuses and resorption sites. Another set of rats was sacrificed on 10th day of pregnancy. After observing the number of foetuses, blood was collected to estimate the level of sex hormones. Then ovaries and uterus were isolated for histological studies. The effect of the extract was determined by observations during laparotomy, histological studies and hormonal assays. The rats treated with 100 mg/kg of the extract had late resorption of 13.8% of the foetuses on 19th day. However the extract showed no significant effect at 10 mg/kg dose. Thus, results of the study indicate the very poor efficacy rate of *Vitex trifolia* as a contraceptive agent. The late resorptions caused by 100 mg/kg of extract suggest that it is not safe to use this plant to restore pregnancy.

ANTI-INFLAMMATORY STUDY OF *ORTHOSIPHON STAMINEUS*

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Orthosiphon stamineus or 'pokok misai kucing' has been widely used as folk medicine in South East Asia to treat many ailments including inflammation. Therefore, the aim of the present study is to verify the anti-inflammatory activity of the plant and attempt to identify the active compound contributing to the activity. The powdered dried leaves of the plant were extracted serially with petroleum ether, chloroform and methanol. The extracts were then concentrated under reduced pressure using rotavapour and freeze-dried. The anti-inflammatory activity was examined using carrageenan-induced hind paw oedema in mice. Among the extracts, chloroform extract 1.0 gm/kg p.o. was shown to be the most effective in inhibiting the oedema. ($P < 0.05$). Chloroform extracts were then fractionated into three fractions (CF1, CF2 and CF3). However, only fraction CF2 1.0 gm/kg caused significant inhibition ($P < 0.05$) on the mice hind paw oedema. Histological study of the hind paws demonstrated that CF2 caused less oedema, less polymonuclear (PMN) cells accumulation and migration

to dermis region and without any spongy like features as observed in the dermis layer of the oedematous hind paws. Fraction CF2 0, 5 and 1.0 gm/kg also was found to inhibit peritoneal capillary permeability. The results suggest that the anti-inflammatory activity of fraction CF2 may be due to inhibition of capillary permeability and migration of PMN's to the oedematous regions. Chemical studies showed that CF2 contains 4.0% phenolic compounds and flavonoids eupatorin, sinensetin and 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone.

LATERALISATION OF SYMPATHETIC ACTIVITY IN EYE AND EYE DOMINANCE

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Lateralisation of sympathetic activity in paired structures may contribute to bilateral asymmetries such as handedness. The present study intended to relate bilateral sympathetic asymmetry in eyes to the eye dominance. The present study involved 300 young subjects (females: n = 176, age 20.6 years \pm 1.6, mean \pm SD; males: n = 124, age = 21.2 years \pm 2.7, mean \pm SD). Eye dominance was determined by peep hole method. The size of palpebral fissure (measure of sympathetic activity to levator palpebrae superioris muscle) was measured from digital photographs. Pupillary diameter (measure of autonomic balance) was measured under controlled illumination by paired pin hole method. Left and right differences were analysed by paired t-test. In the right eye dominant group, the palpebral fissure did not differ between two sides but pupillary diameter in the right eye was significantly ($p < 0.001$) bigger (right eye = 3.4 ± 1.2 mm, left eye = 3.1 ± 1.1 mm; mean \pm SD). In the left eye dominant group, the palpebral fissure in the left eye was significantly ($p < 0.001$) bigger (right eye = 13.0 ± 2.0 mm, left eye = 13.2 ± 2.0 mm; mean \pm SD) but pupillary diameter did not differ. In the right eye dominant group, highest number of subjects possessed bigger palpebral fissure and bigger pupillary diameter in the right eye whereas in the left eye dominant group, highest number of subjects possessed bigger palpebral fissure in the left eye. The results suggested the possibility of higher sympathetic activity in the dominant eye.

INVESTIGATION OF UMF-078 METABOLIC PATHWAY IN RATS: A CANDIDATE DRUG FOR FILARIASIS TREATMENT

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Benzimidazole carbamate is a class of compound commonly used in the chemotherapy of lymphatic filariasis. UMF-078, an antifilarial candidate drug of benzimidazole derivatives with broader filaricidal activity and improved solubility has been developed. To date, pharmacokinetic properties and metabolic pattern of UMF-078 in animals have not been reported. In accordance

with this, an experiment has been conducted in rats. Male Wistar rats were administered with a single oral dose of 129 mg/kg of UMF-078 salt solution. In another study, rats were given radiolabeled flubendazole (FBZ) via parenteral route. All blood samples were collected at appropriate time points and analysed by validated HPLC methods using putative standards. UMF-078 was found to undergo extensive metabolism in rats to UMF-060, FBZ, decarbomethoxy-UMF-078 (D-UMF-078), decarbomethoxy-UMF-060 (D-UMF-060) and decarbomethoxyflubendazole (D-FBZ). After a single dose administration, UMF-078 peaked in the range of 45.0 to 55.0 µg/ml at 4.0 to 8.0 h and eliminated from the blood with a mean half-life of 12.1 h. The metabolic ratios of D-UMF-078, D-UMF-060, FBZ, UMF-060 and D-FBZ were 10.5%, 6.4%, 1.9%, 1.1% and 0.4%, respectively, with a net total percentage of about 20.3% only. However, the blood concentrations versus time curve of these metabolites is indicative of D-UMF-078 as an important biotransformation product of UMF-078 metabolism, followed by D-UMF-060, FBZ, UMF-060 and D-FBZ in decreasing order. The radiolabeled FBZ metabolism study suggests that UMF-060, D-UMF-060 and D-FBZ may not be important biotransformation products of FBZ in blood. In this study, the metabolic pathway of UMF-078 was proposed for better understanding of UMF-078 drug metabolism.

UNILATERAL LEFT NOSTRIL BREATHING FOR RELAXATION

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The autonomic balance tilts to lower sympathetic tone and higher parasympathetic tone by unilateral left nostril breathing and vice versa. Present study examined the possibility of voluntarily improving relaxation by unilateral left nostril breathing. Young, adult (21.8 ± 1.8 years, mean \pm SD) human subjects ($n = 72$) participated in the study. The level of sympathetic tone as an indicator for relaxation was assessed by recording the volar skin conductance (SC) from the fingers of both hands. The nostril dominance was assessed by comparing the airflow through left and right nostrils. The recordings were made using the PowerLab/410 hardware and software for Windows, ML116 GSR amplifier and ML/40 Spirometer front-end. Paired t-test was used in comparing the data. The P value of < 0.05 was accepted as significant. The SC was significantly decreased by the unilateral left nostril breathing (ULNB) on right and left hands in both the left nostril dominant (LND) group (n : males = 23; females = 16 and total = 39) and the right nostril dominant group (RND) group (n : males = 18; females = 15 and total = 33). Unilateral right nostril breathing (URNB) increased the SC significantly in the LND group but not in RND group. Reduced SC indicates decrease in sympathetic tone. Thus, the decrease in the sympathetic tone clearly by ULNB demonstrates improved relaxation. The increase in the sympathetic tone by URNB in LND group and no change in RND group served as controls. Possibly URNB enhances alertness.

**ABDOMINAL OBESITY AND SERUM HOMOCYSTEIN LEVEL
AMONG INDIAN COMMUNITY IN MALAYSIA -
DOES THE ETHNIC PREDISPOSITION MATTERS?**

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The aim of this study was to understand the association between abdominal obesity and serum homocysteine level (Hcy), a putative risk factor for cardiovascular morbidity, in a representative sample in a semi urban population in Kedah State of Malaysia. A cross sectional data from adult men (n = 39) and women (n = 51) in the age group of 23 to 65 years old were used, out of which large percentage were Indians and rest were the Chinese and native Malays. Standard questionnaires were used to collect detailed medical, personal and family history. Anthropometric measurement were used to classify the population into obese, on obese and centrally obese (CO) and centrally non obese group (CNO). Biochemical data involved plasma Hcy level, total cholesterol, triglycerides (TG) and fasting plasma glucose. Clinical data involved blood pressure, WHR, BMI and family history of cardiovascular diseases. In our study, subjects evaluated were consistently characterised by well-known markers of insulin resistance i.e., high TG, low HDL cholesterol, high BMI and hypertension. Differences between groups were analysed using ANOVA (SPSS software version 11.0 for windows). Plasma Hcy was higher among Indians and Malays (median: 10.2 vs. 12.1 $\mu\text{mol/l}$, $p < 0.01$, compared to Chinese) and Chinese showed a lower level of Hcy level. HDL cholesterol was significantly different between the three groups ($p < 0.001$), while fasting glucose was found to be higher among Indians. The Chinese population recorded least hypertensive cases (6.6%), and had no apparent risk factors which were significant compared to other communities. Among Indians and Malays, the mean fasting glucose was higher than the Chinese. Higher levels of fasting glucose were characteristics of the Indian group. Important observation was that the CO category, as defined by the WHR values shoed clearly higher levels of Hcy, compared to CNO group. We conclude that hyperlipidaemia and hyperhomocysteinemia are widely prevalent among Indian and Malay community, particularly in those subjects with abdominal obesity. Further research is needed on the associations of serum Hcy concentration in persons with varying ethnicity.

ANTIDEPRESSANT EFFECTS OF *CARISSA CARANDAS* SYRUP AND JUICE IN THE FORCED SWIMMING AND TAIL SUSPENSION TESTS IN MICE

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The forced swimming test (FST) and tail suspension test (TST) are non-escapable stressful situations and are widely used for screening substances for potential antidepressant effects. Briefly, when mice are forced to swim or hung upside down by the tail in an inescapable situation, they tend to become immobile after initial vigorous activity. It is well demonstrated that drugs with antidepressant activity reduce the time during which the animals remain immobile. Thus, the aim of the present study was to evaluate antidepressant effects of *Carissa carandas* syrup (1.7 g/ml), ripe and unripe fruit juices. The samples were administered intraperitoneally at 0.01 ml/g (body weight) in male mice 60 min prior testing. The results revealed that the immobility time in both FST and TST was significantly reduced by all the samples tested comparable to that of the tricyclic antidepressant imipramine (1.7 g/ml) indicating possible antidepressant activities. The unripe fruit juice showed the best antidepressant effect. Taken together, these results demonstrated that *Carissa carandas* syrup, ripe and unripe fruit juices possess antidepressant effects in mice and merit further research in order to search for a new antidepressant drug.

ANTICANCER AND ANTIOXIDANT PROPERTIES OF THE EXTRACTS OF *PHYLLANTHUS PULCHER*

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The use of herbs as a medicine for diseases seems to be globalised and established by scientific investigations throughout the world. *Phyllanthus pulcher* (*P. pulcher*) as one of the species of genus *Phyllanthus* is believed as a cancer-cure traditional herb. In this study, the properties of anticancer and antioxidant of chloroform, ethyl acetate and aqueous extract of *P. pulcher* were investigated. Cytotoxicity of these extracts has been tested on human hepatocellular carcinoma cell, HepG2, by using Methylene Blue Assay (MBA). Evaluation of antioxidant activity of the extracts has been conducted using scavenging free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Extract chloroform showed the best results in cytotoxicity and free radical scavenging with the EC₅₀ 0.75 µg/ml and 171.5 µg/ml, respectively. The results strongly suggested that the synergistic effects of bioactive compounds in the chloroform extract have the ability to combat cancer and to scavenge free radical. The correlation between both activities will be evaluated.

NATURAL INGREDIENTS FOR PROTECTION AGAINST FREE RADICALS AND UV DAMAGE

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Natural resources offer an unlimited source of new agents for the cosmeceutical, nutraceutical, pharmaceutical and agrochemical industries. Plant extracts, in particular, can be assessed for its ability to act as free radical scavengers and UV blockers, and thus be appropriately used in cosmetic formulations. Free radicals are incredibly destructive, they damage our cells and cause premature aging, reduced immune function, inflammation and ultimately degenerative disease. Our primary defense is antioxidant nutrients, of which the most well-known are vitamins C and E. Over exposure to sunlight, particularly to UV rays have harmful effects on human skin. The most damaging is UVB irradiation (290–320 nm), that can cause erythemas and skin burns. Compounds which have the property of absorbing ultraviolet radiation in the erythermal region make excellent "sunscreens" agents in cosmetic formulations. The anti-oxidant activity and UV absorbing properties of aqueous, ethanolic and propylene glycol (PG) extracts of *Zingiber zerumbet*, *Curcuma xanthorrhiza*, its mixtures and pure compounds were assessed. The anti-oxidant methods used included free radical scavenging using DPPH, superoxide anion scavenging using NBT/xanthine and lipid peroxidation assay. The various anti-oxidant activities were compared with standard antioxidants such as BHT, α -tocopherol, L-ascorbic acid and bearberry, a well-known cosmetic antioxidant. It was observed that curcumin, one of the major components in *Curcuma xanthorrhiza* displayed the highest EC₅₀ value of $1.3 \times 10^{-4}\%$. On the other hand, zerumbone (major component of *Zingiber zerumbet*) and Xanthorrhizol (another major component of *Curcuma xanthorrhiza*) did not display effective scavenging activity. In addition, the extracts from PG:H₂O (80:20) were the most effective in scavenging the free radicals. The absorbance/transmittance of these extracts in the regions of 290–320 nm (UVB) and 320–400 nm (UVA) were compared against some commercial UV blockers.

INTERACTION OF SYMPATHETIC AND RENIN-ANGIOTENSIN SYSTEMS IN THE REGULATION OF RENAL HAEMODYNAMICS IN HYPERTENSION

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Previously, we had shown that a close relationship at the level of renal haemodynamics between the sympathetic and local renin-angiotensin systems exists in the normotensive and hypertensive rats. This study was designed to

further investigate the interaction of adrenergic and renin-angiotensin systems in the regulation of renal haemodynamics in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Acute effects of electrical stimulation, adrenergic agonists and angiotensin II on renal blood flow (RBF) without or in the presence of adrenergic receptor blockers, a calcium channel blocker, an angiotensin-converting enzyme inhibitor (ACEi) and an angiotensin II receptor blocker were studied. The animals were anaesthetised and prepared for blood pressure measurements and fluid administration respectively. The left kidney was exposed by mid-abdominal incision and an electromagnetic flow probe was placed on the renal artery for RBF measurement. The left iliac artery was cannulated such that the beveled tip of cannula faced the renal artery to allow infusion of all drugs closed intrarenally. Reductions in RBF to electrical stimulation (1–10 Hz), bolus doses of phenylephrine (0.25–2.0 μ g), methoxamine (0.5–4 μ g) and angiotensin II (2.5–20 ng) were determined before and after bolus doses of 5-methylurapidil, chloroethylclonidine, BMY 7378 or amlodipine. Perindopril and losartan were given orally for seven days daily prior to the acute studies. Data, means \pm s.e.m. were compared with two-way ANOVA followed by Bonferroni post-hoc with the significance level of 5%. The renal vasoconstrictor effects were significantly attenuated by 5-methylurapidil, amlodipine and losartan in both WKY and SHR when subjected to all adrenergic agonists and angiotensin II. In BMY 7378-treated groups, the pressor responses to all adrenergic agonists were significantly reduced, but not to angiotensin II. In chloroethylclonidine-treated group, there were significant reductions only in WKY rats subjected to methoxamine and angiotensin II. Perindopril-treated groups yielded significant attenuated responses to all adrenergic agonists and angiotensin II in exception to angiotensin II in SHR. These data collectively suggested that there was a dynamic interaction between the adrenoceptor subtypes themselves (α_{1A} , α_{1B} and α_{1D}) and the co-existence and interaction of α and angiotensin II receptors at the level of the renal resistance vessels. The interaction was mediated mainly through the α_{1A} , α_{1B} and angiotensin type 1 (AT₁) receptors, but not α_{1D} . Though α_{1A} -subtype still predominantly involves in the vasoconstriction, upregulation of α_{1B} and angiotensin II receptors in spontaneous hypertension was also noted. Finally, influx of extracellular calcium ions played an important role in the regulation of vasoconstriction in these vessels.

EFFECTS OF NICOTINE ADMINISTRATION ON STRUCTURAL BONE HISTOMORPHOMETRIC PARAMETERS IN SPRAGUE-DAWLEY MALE RATS

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Nicotine, the major addictive component of tobacco, has been associated with various diseases such as cardiovascular disease, cancer and osteoporosis. Histomorphometric analysis is the recognised standard for the quantification of

the structural properties of bone. The effects of nicotine on structural parameter of bone histomorphometry analysis were studied in 16 Sprague-Dawley male rats. Rats aged three months and weighing between 250-300 g were divided into three groups. Group 1 was the baseline control (BC), which was killed without treatment. The other 2 groups were the control group (C) and the nicotine-treated group (N). The N group was treated with nicotine 7 mg/kg body weight and the C group was treated with normal saline only. Treatment was given by intraperitoneum injection, six days a week for four months. Histomorphometric analysis was done on the metaphyseal region of the trabecular bone of the left femur by using image analyser. The parameters measured were bone volume (BV/TV), trabecular thickness (TbTh), trabecular number (TbN) and trabecular separation (TbSp). Nicotine significantly resulted in a decrease in the BV/TV and TbN while an increase in TbSp in the N group compared to the BC group was seen. TbTh remained unchanged in N group as compared to the BC group. However, the TbTh and BV/TV were decreased in the N group as compared to the C group. The C group showed no significant changes in all four parameters as compared to the BC group. In conclusion, this shows that treatment with nicotine 7 mg/kg for four months had affected the trabecular bone structural parameters.

28-DAY REPEATED DOSE ORAL TOXICITY STUDY OF d-ALLETHRIN ON SPRAGUE-DAWLEY RATS

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d-Allethrin is a synthetic pyrethroid used as an insecticide for more than 60 years. As there has been lack of toxicological evaluation reports, a 28 days subacute toxicity study was performed in Sprague-Dawley rats. Doses of 150, 300, 600, and 30, 60, 120 mg/kg body weight per day d-allethrin were administered by gavage to male and female rats respectively (10 rats/sex/day). The control group received only the vehicle. Blood was taken via cardiac puncture at termination for clinical biochemistry and haematology tests. Neurotoxic symptoms such as hyperexcitation, tremor, ataxia, clonic and fasciculation were observed after administration of d-allethrin. No adverse effects on body weight, body weight gain, food consumption and necropsy findings were observed in all treatment groups. However, a significant increase in liver relative weight was observed at the highest dose as well as kidney relative weight at 150 mg/kg/day for male rats. As for clinical biochemistry parameters, only significant elevation of plasma creatinine level was observed in female treatment group rats at 60 and 120 mg/kg/day. However, no significant change on the haematology parameters was observed related to repeated dose administration of d-allethrin. The results obtained from this study is in agreement with previous studies which shows that d-allethrin is neurotoxic and T-syndrome was observed in animals treated with d-allethrin. Also, d-allethrin is categorised as a moderate toxic substance. Based on these results, it may be

concluded that the target organs involved are the liver and kidney. The no-observed-adverse-effect level (NOAEL) was considered to be 30 mg/kg/day for female rats and could not be determined in this examination for male rats.

5'-HYDROXY-OMEPRAZOLE FORMATION PREDOMINANTLY BUT NOT EXCLUSIVELY INVOLVES CYP2C19

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Previous studies have shown that the two main metabolites of omeprazole metabolism are 5'-hydroxy-omeprazole and omeprazole sulphone. It has also been suggested that 5' hydroxyl-omeprazole and omeprazole sulphone is formed predominantly via the CYP2C19 and CYP3A4 pathways, respectively. Omeprazole has therefore commonly become the substrate of choice for estimating the activity of CYP2C19 in *in vitro* and *in vivo* models. However, it has not been shown that CYP2C19 is the only CYP enzyme involved in 5'-hydroxylation of omeprazole. The main aim of the present study is to determine whether 5'-hydroxy-omeprazole formation is largely formed by CYP2C19 to the exclusion of CYP3A4. This was carried out by comparing the omeprazole sulphone formation *in vitro* using both the rat microsomal enzymes with its formation using pure recombinant CYP3A4 enzyme. Both metabolites of omeprazole were analysed using HPLC. The stationary phase was Purospher®STAR RP-18 *encapped* analytical column (particle size 5 µM; length; 250mm; inner diameter: 4.6 mm) and the mobile phase was made-up of acetonitrile: phosphate buffer pH 8.5 (25:75, v/v), at a flow rate 1.5 ml/min. The results showed that omeprazole sulphone formed 4.9% to 6.0% of the total metabolites when microsomal enzymes were used and 73.6% to 85% when pure recombinant CYP3A4 was used. A higher proportion of 5'-hydroxy omeprazole (94%-95.1%) was formed when rat microsomes were used. This study indicates that CYP 2C19 is the predominant enzyme involved in 5'-hydroxy-omeprazole but CYP3A4 is also minimally involved. The sulphone formation on the other hand, involves predominantly CYP3A4.

EXPRESSION OF EPHRIN B2 IN PRE-ECLAMPTIC PLACENTAL TISSUES - A PRELIMINARY OBSERVATION

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Pre-eclampsia is a major cause of maternal morbidity and mortality. Although the exact cause remains unknown, poor cytotrophoblast invasion of the uterine spiral arteries is suspected. ephrins, in particular ephrin B2, have been proposed to regulate the formation of vascular networks during embryonic development

but their role in trophoblast invasion is unknown although a defective expression of these molecules in pre-eclampsia is hypothesised. We therefore determined the expression of ephrin B2, in placentae from normal (n = 6) and pre-eclamptic (n = 6) pregnant women. An informed consent was received from all women. Small sections of freshly delivered placentae were washed with PBS and after fixation in 3% paraformaldehyde, were dehydrated by passage through graded concentrations of glucose. They were then embedded in OCT and frozen in liquid nitrogen. Expression of ephrin B2 was determined using *in situ* hybridisation with a ³⁵S] UTP labeled riboprobe on 5 µm thick sections. Mean age of gestation in weeks was 38.17 ± 1.33 (SD) and 32.17 ± 4.58 (SD) in normal and pre-eclamptic women, respectively. Signals indicating the expression of ephrin B2 were evident in basal plate and villous cytotrophoblast in five of the six normal placentae but were absent in all the placentae from women with pre-eclampsia. Our preliminary finding of absent ephrin B2 expression in pre-eclamptic supports the view that poor communication between the invading cytotrophoblast and maternal spiral arteries may be responsible for the poor cytotrophoblast invasion and the need for more detailed studies to investigate the role of eph and ephrins in this disease entity.

EFFECTS OF ALPHA LIPOIC ACID (ALA) AND TOCOTRIENOL RICH FRACTION (TRF) ON RENAL AND LIVER MICROSOMAL OXIDATIVE STRESS IN DIABETES MELLITUS-INDUCED RATS

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Hyperglycaemia in diabetes mellitus is said to increase free radical production and reduce antioxidant properties leading to oxidative stress causing disease complications. This study evaluated the effects of antioxidant treatments- 100 mg/ml alpha lipoic acid (ALA), 200 mg/ml tocotrienol rich fraction (TRF) and combination of both antioxidants on malondialdehyde (MDA) concentration and superoxide dismutase (SOD) enzyme activities in renal and liver microsomal diabetes mellitus-induced rats. Antioxidants were administered to Sprague-Dawley rats by force feeding throughout four weeks. In the renal study, MDA level in untreated diabetic rats was significantly higher ($p < 0.05$) compared to non-diabetic group. MDA level in diabetic rats treated with TRF was found to be the highest ($p < 0.05$) compared to all groups. Treatment with ALA and combined antioxidants showed improvements with lower MDA levels (insignificantly, $p > 0.05$) compared to untreated diabetic group. In contrast, the highest SOD activities were seen in diabetic rats treated with TRF ($p < 0.05$), followed by combined antioxidants and ALA alone, untreated diabetic rats and non-diabetic rats. In the liver study, MDA level in untreated diabetic rats was significantly higher ($p < 0.05$) compared to the non-diabetic group. MDA levels in

all treated diabetic groups were significantly lower ($p < 0.05$) compared to untreated diabetic group. SOD activities were lower ($p < 0.05$) in untreated diabetic group compared to non-diabetic. All treated groups showed higher SOD activities compared to untreated diabetic group, significantly in TRF and combination treatment groups ($p < 0.05$). This study suggests the potential of ALA and TRF in reducing oxidative stress to a certain extent, in which combined antioxidants showed good results, probably due to synergistic reaction among antioxidants.

INTERLEUKIN-18 EXPRESSION IN THE PLASMA OF MALARIA-INFECTED MICE

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Interleukin-18 (IL-18) is a potent inducer of interferon- γ production in mice following endotoxic shock. It has been demonstrated that IL-18 plays multiple roles in immune responses and inflammatory activity. The aim of this study was to investigate the expression of IL-18 in the systemic circulation of malarial mice infected with *Plasmodium berghei* ANKA. Male ICR mice were inoculated intravenously with 0.2 ml of 2×10^7 parasitised red blood cells (PRBC). Controls received an equivalent volume and dilution of normal RBC. Parasitaemia levels in the animals were monitored throughout the infection. Blood samples for plasma were collected from the animals via cardiac puncture on day 1, 2, 3, 4 and 5 following infection. IL-18 concentrations in the plasma were determined by means of Enzyme-Linked Immunosorbent Assay (ELISA). Results showed that the percentage parasitaemia of infected mice increased significantly from day 1 to day 5 ($5.77 \pm 0.61\%$, $8.28 \pm 0.39\%$, $26.29 \pm 1.54\%$, $52.09 \pm 4.17\%$ and $83.22 \pm 3.03\%$, respectively) as compared to that of normal mice on each respective day ($P < 0.05$). IL-18 concentrations in the plasma of infected mice were also increased significantly ($P < 0.05$) from day 3 to day 4 (40.48 ± 5.60 and 55.04 ± 9.26 ng/ml plasma, respectively) as compared to the controls on each respective day and then decreased slightly on day 5 (43.32 ± 7.55 ng/ml plasma). There was also a positive correlation between the increase in IL-18 concentrations and the increase in percentage parasitaemia measured during the infection. Results from this study suggest that malaria infection in mice is associated with an increase in IL-18 expression in their circulation. Positive correlation between IL-18 concentrations and percentage parasitaemia may also suggest that enhanced production of this cytokine may be related to the pathogenesis of the infection and IL-18 may play a key role(s) in mediating the severity of the disease.

**CONCURRENT ADMINISTRATION OF INSULIN AND
ANTISENSE TGF- β 1 ODN DELAYS DIABETIC NEPHROPATHY IN
STZ-INDUCED DIABETES**

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We have previously shown that the administration of antisense TGF-B1 oligodeoxynucleotide (ODN) in diabetic Sprague Dawley rats attenuated the progression of nephropathy even in an uncontrolled hyperglycaemic condition. As the hyperglycaemic condition itself may enhance the harmful effects of TGF-B1 in diabetes, we were interested to study the effects of antisense TGF-B1 ODN in insulin (INS) receiving animals (INS+ODN) in comparison to insulin and insulin-captopril (CAP) (INS+CAP) combination. Healthy Sprague Dawley rats were injected with a single dose of streptozocin (STZ), 60 mg/kg intraperitoneally to induce diabetes. The animals were given subcutaneous insulin daily at IU/day to maintain blood glucose level below 15 mmol/l from the third day after diabetes induction. CAP and ODN were administered at 50 mg/kg/day and 2 mg/kg/week, respectively from the fourth week after diabetes induction. Renal physiological assessments were carried out on the fourth, eighth and twelfth week after diabetes induction. The INS+CAP and INS+ODN group showed a delay in the onset of hyperfunction of the kidney in early nephropathy. Proteinuria was attenuated by 39.4% and 39.9% in INS+CAP and INS+ODN groups respectively, compared to INS group. Total renal mass was markedly raised in the INS group (3.21 ± 0.06 g, n = 6) compared to INS+CAP (2.52 ± 0.02 g, n = 6) and INS+ODN (2.40 ± 0.03 g, n = 6). Urine filtration rates in INS were elevated by more than 60% from the other two groups. We could conclude that weekly administration of antisense TGF-B1 ODN was comparably as effective as the daily administration of captopril with concurrent administration of insulin. We postulate that the enhanced targeted entry of the ODN may increase the therapeutic potential of this ODN in diabetic nephropathy in future.

**CARDIOVASCULAR PROTECTIVE EFFECTS OF KHOLESCAIR™
IN EXPERIMENTAL HYPERTENSION**

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Herbal medicines are gaining much importance in the treatment of various disease conditions, including hypertension. However, scientific data on the

effectiveness of such treatments are very much lacking. The objective of the present study was to examine the effects of a herbal preparation, Kholescair™, on various cardiovascular parameters including endothelial function in experimental hypertension. Male spontaneously hypertensive rats (SHR, 14-15 weeks old) were randomly divided into two groups (n = 8) and one group were fed orally with Kholescair™ (10 mg/kg body weight (BW)) for 7 weeks. The control group of SHR's was treated similarly with equal volume of tap water. The effects on BW, mean systolic blood pressure (SBP, non-invasive), vascular responsiveness of aortas, total antioxidant capacity of plasma (TAC, FRAP assay), lipid profile, and aortic hypertrophy were measured. The results show that at the end of the treatment period the BW among two groups remains essentially similar, but the SBP was significantly lower in the Kholescair™ treated SHR animals. The relaxation responses to endothelium-dependent vasodilator acetylcholine (ACh) were significantly greater in aortas from Kholescair™ treated SHR's. However, no significant differences were observed in responses to the endothelium-independent relaxant, sodium nitroprusside (SNP) between the two groups of animals. Similarly, no significant changes were observed in the plasma lipid profile, TAC, and PE-induced contractions. Chronic treatment with Kholescair™ was however found to significantly reduce the thickness of the aortic wall. From these findings, it can be concluded that chronic treatment with Kholescair™ attenuated the development of high blood pressure (anti-hypertensive) and reduced the thickness of the aortic wall in this animal model of hypertension.

EFFECT OF ANGIOTENSIN 1-7 ON THE VASOPRESSOR RESPONSES TO ANGIOTENSIN II IN ISOLATED PERFUSED KIDNEY OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Angiotensin 1-7 (Ang 1-7) has been identified as a potentially important modulator of various angiotensin II (Ang II) functions in both animal and human studies. Ang II plays an important role in the pathogenesis of the vascular complications of diabetes mellitus. We therefore, investigated the modulatory effect of Ang 1-7 on Ang II-induced vasoconstriction in the isolated perfused kidney of normoglycemic Wistar-Kyoto (WKY) rats and Streptozotocin (STZ)-induced diabetic rats. Male rats, aged 12 weeks were injected with STZ (75 mg/kg, i.p) to induce diabetes. After eight weeks, the right kidney was excised from pentobarbitone-anaesthetised rats and perfused through the renal artery with oxygenated Krebs at the rate 5 ml/min. The tissues were initially primed with 10 µM phenylephrine (PE). Changes in perfusion pressure to bolus injection of Ang II (10^{-13} M- 10^{-6} M) were observed before, and 30 min after pre-

treatment with Ang 1-7 (10^{-7} M- 10^{-15} M). The results show that Ang II-induced vasoconstriction was decreased in the diabetic rat kidney. Pre-treatment with Ang 1-7 (10^{-7} M) attenuated the Ang II (10^{-10} M- 10^{-6} M) responses in the normoglycaemic rats but not in the diabetic rats. PD 123319, an AT₂ receptor antagonist, D-ALA, an Ang 1-7 receptor antagonist and indomethacin, a cyclo-oxygenase inhibitor each did not affect Ang II-induced contraction, indicating that the contraction is not via AT₂ or Ang 1-7 receptor and is independent of the cyclo-oxygenase pathway. While D-ALA, indomethacin and L-NAME reversed the attenuating effect of Ang 1-7, PD123319 had no effect. In conclusion, the current data suggests that Ang 1-7 has a reno-vascular modulatory property which includes the attenuation of the contractile effect of Ang II. This action is possibly modulated by Ang 1-7 receptor and involves a cyclo-oxygenase pathway and nitric oxide release. This apparent protective effect of Ang 1-7 appears to be compromised in diabetes.

CHARACTERISING THE MECHANISM OF INSULIN-INDUCED VASODILATION IN NORMAL AND STREPTOZOTOCIN (STZ) - INDUCED DIABETIC RAT AORTA

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Insulin has been shown to exert vasodilatation in isolated vascular preparations from normal and diabetic animals. However the exact mechanism(s) involved are unclear. In the present study, therefore, we investigated the possible mechanism(s) involved in insulin-induced vasodilatation. Thoracic aortas from age-matched normal Wistar-Kyoto (WKY) and streptozotocin (STZ)-induced diabetic WKY rats were isolated and mounted in tissue baths for isometric tension recordings. The relaxation responses to insulin were recorded in endothelium-intact and-denuded aortic rings pre-contracted with phenylephrine (PE) in the presence or absence of various drug treatments. The results show that in either normal or diabetic tissues, insulin exerted a concentration-dependent vasodilatation of tissues with or without endothelium. In general the insulin effect was higher in the diabetic than the normal tissues and was significantly lesser in endothelium-denuded aortas when compared with the corresponding endothelium-intact tissues. This is indicative of an increased vascular sensitivity to insulin in this diabetic model and also that vascular endothelium is necessary for full insulin effect. In both normal and diabetic endothelium-intact tissues the relaxant activity of insulin was significantly inhibited by L-NAME (an endothelial nitric oxide synthase inhibitor), but not by indomethacin (a cyclo-oxygenase inhibitor) or glibenclamide (an ATP-sensitive potassium channels blocker). In addition, tetraethylammonium (TEA), a selective calcium-activated potassium channel blocker, significantly inhibited the insulin action in normal but not in diabetic aortas. In conclusion, these data suggest that the vasodilatory action of insulin is neither dependent on the cyclooxygenase pathway nor on the

ATP-sensitive potassium channels. The data however, indicate that NO mediates insulin vasodilatation in both normal and diabetic tissues. While the calcium-activated potassium channel is involved in the action of insulin in normal tissues, this mechanism is probably inactivated in diabetes.

ALPHA TOCOPHEROL PROTECTS BONE BETTER THAN TOCOTRIENOL AGAINST ADVERSE EFFECTS OF NICOTINE

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Nicotine has been found to delay bone healing and reduce bone mineral density. Our previous study showed that nicotine caused a loss in bone calcium content. This study is aimed at determining whether vitamin E supplementation (α -tocopherol or tocotrienol) is able to prevent the adverse effects on bone due to nicotine. Male Sprague-Dawley rats were divided into two groups of eight rats each and treated for three months. For the first month, the rats were given α -tocopherol or tocotrienol orally. Treatment was continued with nicotine, intraperitoneally, for the following two months. One group of eight rats was killed without any treatment and act as the baseline control. Parameters measured were bone mineral density, bone calcium content and serum calcium levels. Bone mineral density for both groups was significantly increased after three months compared to before treatment. However, the increment in the lumbar bones for TF-N was significantly greater compared to TT-N. Bone calcium content of the lumbar bones for the TT-N group was lower than the baseline group. Both groups showed lower serum calcium after treatment compared to before treatment. Alpha-tocopherol, but not tocotrienol, is able to maintain bone calcium content in rats exposed to nicotine. Alpha-tocopherol also showed better protection than tocotrienol against the effect of nicotine on bone mineral density.

A COMPARATIVE STUDY OF THE INVOLVEMENT OF α_1 -ADRENOCEPTOR SUBTYPES IN THE CARDIAC FAILURE INDUCED SPRAGUE DAWLEY AND SPONTANEOUSLY HYPERTENSIVE RATS

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The function of kidney deteriorates during the development of cardiac failure because of changes in renal haemodynamics and neurohormonal activity. This study aimed to examine the α_1 -adrenoceptor subtypes involved in mediating adrenergically induced renal vasoconstriction in a rat model of cardiac failure and hypertension. Normal Sprague Dawley (SD) and spontaneously hypertensive rats (SHR) were used in the study. Cardiac failure was induced by the combined treatment of caffeine (40 mg/kg) and isoprenaline (5 mg/kg) for seven days. On day eight, the rats were used for acute study. The left kidney was exposed, the renal artery cleared and an electromagnetic flow probe on it for renal blood flow (RBF) measurements. The reduction in RBF induced by increasing frequencies of electrical renal nerve stimulation, close intrarenal bolus doses of noradrenaline, phenylephrine or methoxamine were determined before and after administration of amlodipine, 5-methylurapidil, chloroethylclonidine and BMY 7378. Data, means \pm s.e.m were compared with two-way ANOVA followed by Bonferroni post-hoc with the significance level of 5%. The results obtained indicated that the renal vasoconstrictor responses in this model were attenuated mainly by amlodipine, 5 methylurapidil and BMY7378 but not by chloroethylclonidine. Furthermore, administration of chloroethylclonidine did not show a significant reduction in methoxamine induced renal vasoconstriction in cardiac failure SD and SHR. This supported the view that α_{1A} -adrenoceptors are involved in renal vasculature SD and SHR regardless of its pathophysiological state. The findings from this study further suggested that besides the α_{1A} , the α_{1D} -adrenoceptors contribute to the adrenergically induced renal vasoconstrictor responses in cardiac failure SD and SHR.

**IN VIVO EFFECT OF AQUEOUS EXTRACT OF MENGKUDU
(MORINDA CITRIFOLIA L.) ON DRUG METABOLISING ENZYMES
IN SPONTANEOUSLY HYPERTENSIVE RAT LIVER**

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The objective of this study is to investigate the *in vivo* effect of aqueous extract of the Mengkudu or Noni fruit (*Morinda citrifolia* L.; family: Rubiaceae) in spontaneously hypertensive rat (SHR) hepatocytes and liver microsomes on phase I and phase II metabolism respectively. Aminopyrine and *p*-nitrophenol were used as drug model for phase I and phase II, respectively. Previously, *in-vitro* study indicated that aqueous extract of the Mengkudu or Noni fruit increased aminopyrine metabolism in young female SHR hepatocytes. In this study, we found that single oral dose (6 g/kg) of aqueous extract of the Mengkudu increased aminopyrine metabolism whereas single oral dose (0.6 g/kg) of the same extract decreased *p*-nitrophenol metabolism. These results indicated that high dose of aqueous extract of the Mengkudu increased phase I aminopyrine N-demethylase activity in SHR hepatocytes but not phase II uridine diphosphate- glucuronosyltransferase (UDP-GT) activity which is responsible for *p*-nitrophenol conjugation. In reverse, it is observed that low dose (0.6 g/kg) of

aqueous extract of Mengkudu significantly decreased phase II UDP-GT activity but not phase I aminopyrine N-demethylase activity.

**ANTAGONISTIC EFFECT OF *EURYCOMA LONGIFOLIA*
ROOT AND CORTICOSTERONE ON TESTICULAR
HISTOLOGICAL CHANGES**

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Root extract of *Eurycoma longifolia* (Tongkat Ali) has been reported to increase testosterone level and libido in male. Exposure to high dose of corticosterone induced apoptosis of Leydig cells in rats and thereby reduced testosterone level. Acute withdrawal of testosterone causes degeneration of spermatogenic cells. Thus, the aim of the present study was to determine if *Eurycoma longifolia* could counteract the suppressive effect of corticosterone on the spermatogenic function of the testis. In this study, adrenalectomised male rats were given either *Eurycoma longifolia* root extract (800 mg/kg body weight) orally, corticosterone (25 mg/kg body weight) intramuscularly or combination of both. The treatments were given daily for seven consecutive days. Control rats received control vehicles without the tested chemicals. The rats were sacrificed one hour after the last dose. The testes were taken for histological study. Results showed that *Eurycoma longifolia* increased spermatogenic cell populations in adrenalectomised rats compared to normal control rats. Corticosterone given to adrenalectomised rats revealed a reduction in spermatogenic cells. Whereas, seminiferous tubules of adrenalectomised rats that received both *Eurycoma longifolia* and corticosterone did not differ from those of normal control rats. In conclusion, *Eurycoma longifolia* root extract is capable to counteract the suppressive effects of high corticosterone on spermatogenic cell populations of the testis.

**POTENTIAL OF *CURCUMA XANTHORRHIZA*
AND *ZINGIBER ZERUMBET* AS SKIN WHITENING AND
ANTI-INFLAMMATORY AGENT**

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The increasing demands on skin whitening and anti-inflammatory agents in Asia Pacific market have created vast excitement among the manufacturers of the cosmetic and pharmaceutical industry to introduce robust and effective, yet safe invention of topical and oral skincare products. The emergence of various herbal-based products stimulates comprehensive study to evaluate the potential of various edible tropical plants. Globally, it is estimated that out of 500,000 plant

species, 250,000 are known plants, nevertheless only 1% have been thoroughly study. Our study shows that *Curcuma xanthorrhiza* and *Zingiber zerumbet* possess potent anti-inflammatory and skin whitening activity. It is observed that the combined extract of *Curcuma xanthorrhiza* and *Zingiber zerumbet* displays synergistic activity in inhibiting tyrosinase activity in melanin production pathway, as well as inhibiting trypsin activity, which is the mediator of inflammation. IC₅₀ of the combined extract is almost 10 times lower than the IC₅₀ of the individual extract. This bioactivity trend was observed for extracts prepared in aqueous, ethanol and mixture of 80% propylene glycol in water, as well as via supercritical fluid extraction. Amongst these extracts, supercritical fluid extract was observed to be the most potent, followed by ethanol extract, 80% propylene glycol extract and finally the aqueous extract. These extracts display lower IC₅₀ value (IC₅₀ between 0.01%–0.5%) than the commercial skin whitening agent, arbutin (IC₅₀ = 2.21%).

**THE ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND
ANTIPYRETIC PROPERTIES OF *CORCHORUS
OLITORIUS* AQUEOUS EXTRACT IN MICE**

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The leaves and roots of *Corchorus olitorius* (*C. olitorius*) (senaung betina), an annual herb from the family Tiliaceae, are eaten as herbal medicine and as vegetable by local people in India, Egypt and the Philippines. Its leaves are used as demulcent, diuretic, febrifuge and tonic, and are used in the treatment of chronic cystitis, gonorrhoea, fever, pain and tumours. The objective of this study was to elucidate the effects of *C. olitorius* as agent for relieving pain, inflammation and fever. The aqueous extract of *C. olitorius* (AECO), in the concentration of 10%, 25%, 50%, 75% and 100%, was used throughout the study. The abdominal constriction test (ACT) and hot plate test (HPT) were used as the peripheral and central antinociceptive assays while the carrageenan-induced paw oedema and brewer's yeast-induced pyrexia were used as the anti-inflammatory and anti-pyretic assays, respectively. The AECO showed a significant ($P < 0.001$) antinociception in both tests with concentration-dependent activity observed only in HPT. The 100% concentration AECO exhibited significant lost of antinociception in ACT. The AECO, in the concentrations ranging from 25% to 100% and 10% to 100%, also exhibited significant ($P < 0.05$) anti-inflammatory and anti-pyretic activities that lasted until the end of the experiments,

respectively. The present studies have scientifically proven the folklore medicinal used of *C. olitorius* as agent in the treatment of pain, inflammation and fever.

EFFECTS OF VARIOUS RECEPTOR ANTAGONISTS, ENZYMES, TEMPERATURE AND pH ON THE ANTINOCICEPTIVE ACTIVITY OF AQUEOUS EXTRACT OF *CORCHORUS OLITORIUS* IN MICE

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Corchorus olitorius (*C. olitorius*), locally known as Senaung betina, is a herb that belongs to the family Tiliace. Traditionally, its leaves are eaten as herbal medicine by local people in India, Egypt and the Phillipines as demulcent, diuretic and tonic for the treatment of pain, fever and tumours. Recently, we have demonstrated that this plant posses antinociceptive, anti-inflammatory and antipyretic properties. The objectives of this study were: 1) To elucidate the involvement of opioid receptor in *C. olitorius*-induced antinociception. 2) To determine the effects of temperature, pH and enzymes on *C. olitorius*-induced antinociception. Acetic acid-induced abdominal constriction test (ACT) in mice was used as the antinociceptive assay. The male ICR mice were pre-challenged subcutaneously (*sc*) with opioid-, γ -aminobutyric acid (GABA)-, α - and β -adrenergic-, muscarinic- and nicotinic-receptor antagonists or enzymes (α -amylase, protease and lipase) followed by the 50% concentration aqueous extract of *C. olitorius* (AECO) *sc* administered or directly administered (*sc*) with the AECO pre-treated against a series of temperature (40°C, 60°C 80°C and 100°C) or pH (3, 5, 6.5, 9, 11 and 13) prior to subjection to ACT, respectively. The AECO antinociception was significantly reversed by the antagonists naloxone, bicuculine, phenoxybenzamine, pindolol and mecamlamine and the enzyme α -amylase. The AECO activity was also found to be stable against the effect of temperature and acidic pH (3, 5 and 6.5). Interestingly, the activity improved significantly ($P < 0.001$) after heating at 80°C and 100°C but decreased significantly ($P < 0.001$) under basic pH. The present study demonstrated the involvement of opioid, GABA, α - and β -adrenergic and nicotinic receptors in the *C. olitorius* antinociception and the bioactive compound are thought to contain some carbohydrate properties. Furthermore, this heat-stable activity was enhanced at high temperatures and decreased in the alkaline conditions.

**THE EFFECT OF ETHYL ACETATE FRACTION OF GYNURA
PROCUMBENS ON GLUCOSE ABSORPTION
IN AVERTED RAT JEJUNUM**

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Our previous study has shown that methanol extract of *Gynura procumbens* (*G. procumbens*) did not lower the blood glucose level of normal rats but reduced the blood glucose level of glucose loaded rats in glucose tolerance test and streptozotocin-induced diabetic rats. The methanol extract was then fractionated to chloroform, ethyl acetate, butanol and water fractions and subjected to glucose tolerance test. It was found that only ethyl acetate fraction significantly inhibited the rise in blood glucose level ($P < 0.05$). Therefore, it is of interest to find out how the ethyl acetate fraction influences the glucose absorption in the gastrointestinal tract, one of the mechanisms of actions of antidiabetic drugs. Jejunum was isolated from Sprague-Dawley rats, averted and cut into 5.0 cm pieces. It was filled with 1.0 ml glucose-Ringer solution and tied at both ends to form a sac. The sac was then incubated in 15 ml glucose-Ringer solution in the presence of test substances and aerated with 95% O₂, 5% CO₂ at 37°C for 60 min. The concentration of glucose outside the sac before and after the experiment was determined so that the amount of glucose transported into the sac could be calculated. The result showed that similar to acarbose, an α -glucosidase inhibitor, ethyl acetate fraction (1.0 and 2.0 mg/ml; n = 6) significantly inhibited the glucose absorption of the jejunum ($P < 0.05$). α -glucosidase inhibitors is known to reduce intestinal absorption of starch, dextrin and disaccharides by inhibiting the action of intestinal brush border α -glucosidase. The result suggested that one of the antidiabetic mechanisms of ethyl acetate fraction of *G. procumbens* is by inhibiting glucose and possibly starch and disaccharides absorption in the intestinal tract.

**THE EVALUATION OF THE LEAVES EXTRACT OF ANGSANA
OINTMENT PREPARATIONS ON THE RECOVERY OF
ARTIFICIALLY MADE INFECTED
WOUND IN GUINEA PIG**

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A study has been conducted on the effect of hydrophylic and hydrophobic ointment preparations of ethanol extract of Angsana leaves (*Pterocarpus indicus* Willd.) on the wound recovery of the artificially made wound of guinea pigs skin previously infected with *Staphylococcus aureus*. A decrease in the wound diameter of guinea pigs skin previously infected with *Staphylococcus aureus* was used as a

measure of the healing effectiveness. The data was analysed statistically by multivariate analysis of variance and repeated measures method. The results showed that the ethanol extract of *Angsana* leaves ointment had a better healing capacity against *Staphylococcus aureus* compared to that of ethanol extract of *Angsana* leaves only ($P < 0.05$). The hydrophylic ointment produced faster wound recovery compared to those of hydrophobic ointment and commercial gentamycin preparations ($P < 0.05$). In conclusion, the ethanol extract of *Angsana* leaves hydrophylic ointment preparations are useful as topical agents in the wound recovery of the artificially made wound of skin of guinea pigs previously infected with *Staphylococcus aureus*.

THE EFFECT OF *GARCINIA ATROVIRIDIS* ON *IN VITRO* EMBRYO DEVELOPMENT IN NICOTINE-TREATED MICE

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Nicotine, one of the chemicals found in tobacco products, has detrimental effects on many organs of the body including the embryological development and it causes damage to the tissues via free radicals. New evidence has shown that even maternal exposure to second-hand smoke may harm the foetus. *Garcinia atroviridis* (*G. atroviridis*) is a plant from Gultiferea family and is locally known as 'asam gelugur'. It has been reported that *G. atroviridis* acts as an antioxidant and probably could prevent the negative effect of nicotine by reducing the oxidative stress. This study was done to determine the effect of *G. atroviridis* on the development of embryos in female mice treated with nicotine. Forty female mice were divided equally into four groups. The first group was the control group. The second group was treated with subcutaneous nicotine (5 mg/kg) and the third group was given nicotine together with ethyl acetate extracts of *G. atroviridis*, given orally at a dose of 50 mg/kg. The last group was given only the extract. After one month of treatment the female mice were superovulated and mated with the male mice. Once fertilization has occurred, the female mice were killed, the oviducts were removed and the embryos were flushed out for *in vitro* culture. The numbers 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 h. The number of embryos are determined and observed at the specified hours. The results showed that at 0 h, the *G. atroviridis* extract showed a higher percentage of normal embryos (13.5%) than their respective control (8.1%) and nicotine treated mice (0.5%). After 96 h of *in vitro* culture the ethyl acetate extract of *G. atroviridis* given together with nicotine showed high percentage of embryo (99.52%) in the form of hatched

blastocyst compared to the control group. The embryos in the nicotine group did not develop any further after 24 h of *in vitro* culture. This study showed that nicotine retarded *in-vitro* embryo development and that *G. atroviridis* extract, suppressed this detrimental effect of nicotine-induced stress.

EXTRACT OF *HYDROCOTYLE JAVANICA* THUMB LEAVES AS A CONTRACEPTIVE: ITS EFFECTS ON HISTOLOGICAL APPEARANCE OF OVARY AND UTERUS OF FEMALE MICE

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The leaves of *Hydrocotyle javanica* Thumb have been used traditionally as contraceptive by drinking the water infusion of the leaves twice. The aim of this study is to investigate the antifertility effect of the extract of the leaves and its effect on histologic appearance of the ovary and uterus of mice (*Mus musculus*). Healthy, fertile female mice (*Mus musculus*) were used. The female mice were given orally either water or methanol extract of *Hydrocotyle javanica* Thumb leaves in various doses: 20, 40, 60 and 80 g/kg body weight (BW), everyday. On day 20, the mice were mated and when pregnancy occurred, treatment was stopped. On day 17 of pregnancy, the mice were sacrificed; uterus and ovaries were examined macroscopically and histologically. Thread of implantation, the quantity and condition of foetuses and the rate of pregnancy were observed and counted. Results and data were analysed using ANOVA and considered significant different when $P < 0.05$. The results showed that water and methanol extract of the leaves of the plant reduced the thread of implantation, quantity of the foetuses and the rate of pregnancy significantly ($P < 0.01$), but not the quantity of resorbtion. Histologic examination showed corpus luteum and follicles in various stadiums in ovary, and endometrium thickening in the uterus. In conclusion, water and methanol extract of *Hydrocotyle javanicum* Thumb showed contraceptive activity, probably by preventing implantation.

EVALUATION OF ANTINOCICEPTIVE ACTIVITIES OF *ALPINIA CONCHIGERA* ETHANOLIC EXTRACT IN MICE

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Alpinia conchigera (*A. conchigera*) or 'lengkuas genting' was used in traditional Thai medicines to relieve gastro-intestinal disorders and in the preparation of

Thai food dishes. Little is known about its chemical constituents but it has been reported that the phenylpropanoid derivatives, chavicol acetate and eugenol acetate are present in the fruit which have anti-inflammatory activity. This study was performed to evaluate the analgesic activities of ethanolic extract of *A. conchigera* leaves. The analgesic investigations were carried out against two types of noxious stimuli, namely, chemical (Formalin-induced pain and Acetic acid-induced test) and thermal (hot plate test). The effects following aspirin and naloxone pre-treatment were also studied. The antinociceptive studies used hot plate test which measured response latencies when the mice were placed on a metal surface maintained at $55 \pm 0.2^\circ\text{C}$, Writhing or Acetic acid-induced abdominal constriction test and formalin test. The extracts (30, 100 and 300 mg/kg) acted in a dose-dependent manner, reducing the nociception induced by the *i.p* injection of acetic acid and significantly reduced painful stimulus in both phases of the formalin test. The Hot plate test also shows increased response latencies of thermal stimuli. The results thus showed that the plant had both central and peripheral effects and this was confirmed by its effect on both phases of formalin-induced pain. In conclusion, *A. conchigera* has central and peripheral analgesic properties.

EVALUATION OF ANTINOCICEPTIVE ACTIVITIES OF *CANTHOPANAX TRIFOLIATUS* IN MICE

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Acanthopanax trifoliatum or "Daun Cina" is an evergreen bush or creeping plant. Its root can help invigorate stasis and assuage pain, whereas the leaves can help eliminate edema and relieve itching. In addition, it has a rather good curative effect on treating common cold, jaundice, gastric pain, diarrhoea and ulcer. The aim of this study is to evaluate the antinociceptive effect of *Acanthopanax trifoliatum* extract at spinal and supraspinal levels in mice. Two test models were used to study the effects of the extracts on nociception, namely, the Writhing test and hot plate test. This was carried out by injecting male ICR Balb/c strain mice with the extract at doses of 1.0 ml/10 g mice (concentration; dH₂O as a control, 30, 100, 300 mg/kg) intraperitoneally (*i.p*) prior to being subjected to both methods. Distilled water (aqueous extraction) was used to isolate the biologically active compound in the herb. In comparison to the control group, this study showed that the number of writhings produced by acetic acid (0.6% v/v) was reduced and the latency on the hot plate was increased with increasing concentrations of extracts administered. Results of the study revealed the extract to have significant ($P < 0.05$) antinociceptive effects at all doses in mice in both models except 30 mg/kg in hot plate test. The results suggest that the extract contain a pharmacologically active principle that contributes to the herb's antinociceptive activity.

**IN VITRO INHIBITION STUDIES ON CALCIUM OXALIC
CRYSTAL GROWTH AND TOTAL PHENOLIC
OF *SONCHUS ARVENSIS* EXTRACTS**

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Sonchus arvensis (Asteraceae) is one of the medicinal herbs has been used in traditional medicine for kidney stone and related diseases, diuretic, lithotriptic and antiurolithiasis. The plant is native from Eurasia and easily grows in the rainy areas, in the area of 50–1650 m above sea level. The plant is known as 'tempuyung' in Java, 'lempung', 'rayana', 'jombang' and 'galibug' 'lalakina' in Sunda (West Java). *Sonchus arvensis* contains flavonoid compounds. Flavonoid may be responsible for inhibiting the growth of calcium oxalic (CaOx) crystals. Flavonoid is a phenolic compound. The ability of this substance to inhibit CaOx crystals growth may be attributed to the phenolic compounds in these plants. The aim of this study is to determine the inhibitory properties to CaOx crystal growth and total phenolic content of *Sonchus arvensis* extracts. Inhibition of CaOx crystal growth was carried out by using the modified slide Gel Schneider method, 1983. Total phenolic content was estimated by using the modified Singleton, 1999 and Kosar, 2004 methods. All these extracts (five different solvents) showed positive inhibitory effect on CaOx crystal growth. The methanol extract by using soxhlet is the better than others, but less than sodium citric control, in its inhibitory action. All extracts contained phenolic compounds ranging from 0.95% to 13.99% to crude extracts. The methanol extract by using soxhlet has the highest phenolic compounds in percentage

**IN VITRO INHIBITION STUDIES ON CALCIUM OXALIC CRYSTAL
GROWTH AND TOTAL PHENOLIC OF
STROBILANTHES CRISPUS EXTRACTS**

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Strobilanthes crispus (L.) Bremek (Acanthaceae) has been used locally in traditional medicine for kidney stone and related diseases. The plant is native to countries from Madagaskar to Indonesia, and is commonly known as 'daun picah beling' in Jakarta, 'enyoh kelo', 'kecibeling' or 'kejibeling' in Java. Soediro (1983, 1988) isolated and identified verbacoside, glycosidic ester of caffeic acid and seven phenolics in the leaves. Phenolic acid may be responsible for inhibiting growth of calcium oxalic (CaOx) crystals. The ability of these substances to inhibit CaOx crystals growth may be attributed to the phenolic compounds in the plants. The aim of this study is to determine the inhibitory properties on CaOx crystal growth and total phenolic of *Strobilanthes crispus* extracts. Inhibition of the extracts on CaOx crystal growth was carried out by using the modified slide Gel Schneider method, 1983. Total phenolic were estimated by using the modified Singleton, 1999 and Kosar, 2004 methods. All of the extracts (five different

solvents) showed positive inhibitory effect on CaOx crystal growth. The methanol extract by using soxhlet is the better than others, but less than sodium citric control, in its inhibitory action. All the extracts contained phenolic compounds ranging from 1.23% to 2.42% to crude extracts. The methanol extract by using soxhlet has the highest phenolic compounds in percentage.

**THE EFFECT OF CHRONIC TREATMENT OF ETHANOL
EXTRACTS OF *ANDROGRAPHIS PANICULATA*
ON GLUCOSE LEVEL, INSULIN AND β -CELLS OF
STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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The antidiabetic activity of 50% and 95% ethanolic extracts of *Andrographis paniculata* (*A. paniculata*) have been studied. The extracts were found to lower the blood glucose level of streptozotocin-induced diabetic rats but did not inhibit the rise of blood glucose level of glucose loaded normal rats. Therefore it is of interest to find the explanation for the blood glucose lowering effect of the extracts and its correlation with insulin level and number of viable β -cell in the streptozotocin-induced diabetic rats. Four groups of streptozotocin-induced diabetic rats ($n = 6$) were treated orally either with water, metformin, 50% and 95% ethanol extracts of *A. paniculata* (0.5 g/kg) twice daily for 14 days respectively. The fasting blood glucose and insulin levels were determined before and after 14 days of treatment. The number of β -cells in the islets of Langerhans of the pancreas of each rat was determined histologically using modified Gomori's method. The study showed that at the dose employed, streptozotocin (65 mg/kg i.p.) destroyed around 87.49% to 89.46% of β -cells. The study also showed that 14 days treatment of ethanol extracts (50% and 95%) of *A. paniculata* lower the blood glucose ($P < 0.05$) and insulin ($p < 0.05$) levels but increased the recovery of β -cells population of streptozotocin-induced diabetic rats ($p < 0.01$). The results suggest that the antidiabetic effect of *A. paniculata* in the streptozotocin-induced diabetic rats may have been contributed by the increase in number of viable β -cells.

EFFECT OF ZEA MAYS HAIR EXTRACT ON HEART RATE AND BLOOD PRESSURE

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Zea mays hairs, commonly known as corn hairs or corn silks are discarded by humans and eaten by the cattle. The decoction of these hairs is believed to have hypotensive action. But research documentation is lacking. Hence this work had been taken up. Aqueous extract of the *Zea mays* hairs (corn silk) was screened for its effect on heart rate (HR) and blood pressure (BP) of Wistar rats. Twelve rats were divided into two groups with six animals in each group and were treated with 50 mg/kg and 100 mg/kg body weight orally by gavage. Treatment was given for 28 days and HR and BP were determined on the 8th day, 15th day and 29th day. BP and HR were recorded by using 'Blood Pressure Analyzer. 50 mg and 100 mg treatment for 7 days and 14 days did not show any significant change in HR. But 28 days' treatment with both the doses decreased HR significantly. The decrease was more significant in 100 mg dose ($p < 0.002$) than in 50 mg dose ($p < 0.018$). 50 mg treatment increased systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) significantly after 14 days and 28 days but not after 7 days ($p < 0.034$: $p < 0.002$). 100 mg dose showed significant increase in SBP, DBP and MAP after 7 days, 14 days and 28 days treatment. The significance was more in 100mg treatment ($p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively) than in 50 mg treatment. Thus the results show that the *Zea mays* hairs may not be safe as hypotensive agent.

INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME ACTIVITY BY A PARTIALLY PURIFIED FRACTION OF *GYNURA PROCUMBENS* IN SPONTANEOUSLY HYPERTENSIVE RATS

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Previous studies indicate that ethanolic extracts (FA) of the leaves of *Gynura procumbens* (*G. procumbens*) have significant hypotensive activity in rats. In the present study, further purification and analysis of FA were performed, and the effect of the purer extract (FA-I) on the angiotensin-converting enzyme (ACE) examined. The FA-I, obtained by subjecting FA to Sephadex LH-20 gel chromatography, was investigated for hypotensive activity, and analysed by a qualitative phytochemical method for unknown compounds. Spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats were used in the experiments. All blood pressure (BP) measurements were monitored by the Macintosh MacLab set-up. Plasma ACE activity was assayed by a colourimetric

method. Intravenous administration of FA-I (0 to 10 mg/kg) produced a marked ($p < 0.01$) dose-dependent reduction in the BP in SHR and WKY rats, with an ED_{50} of 1.09 and 1.05 mg/kg, respectively, and a significant ($p < 0.05$) inhibition of ACE activity ($IC_{50} = 0.8$ mg/ml). Furthermore, FA-I at 10 mg/kg strongly ($p < 0.01$) inhibited the angiotensin I-induced rise in BP. This response was comparable to that of captopril at 20 μ g/kg. The qualitative phytochemical analysis indicated the presence of glycoconjugates and peptides in FA-I. These results suggest that the hypotensive effect of *G. procumbens* may be due, in part, to the glycoconjugated or peptidal substances found in the FA-I that exhibit an inhibitory effect on ACE.

CLINICAL STUDIES

THERAPEUTIC WONDERS OF *CENTELLA ASIATICA*: BOOSTER TO THE BRAIN, HEART AND LIBIDO

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In traditional practices of Ayurvedic and Chinese medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease (AD). An ethnopharmacological approach has provided leads to identifying potential new drugs from plant sources, including those for cognitive disorders. The efficacy of *Centella asiatica* (*C. asiatica*), pennywort, 'gotu' cola or 'pegaga' on cognitive performance and biochemical markers was studied. Twenty-two male and female, middle-age human subjects were supplemented on *C. asiatica* for 60 days: 10 males were on *C. asiatica* supplementation (CM), and 12 females on *C. asiatica* supplementation (CF). Then, the same subjects were given a placebo (cross study design) for 60 days: 9 males were on placebo (PM) and 10 females were on placebo (PF). Baseline data was taken at 0, and also after 40 days, 60 days and 90 days (wash-out period of 30 days). Significant high differences ($p < 0.05$) were seen in CM and CF between controls (placebo) on long-term retrieval (glr), executive processes (gep), cognitive efficiency-extended (gce), and delayed recall (gdr). Significant differences ($p < 0.05$) were seen in CF on glr, visual spatial thinking (gv), and working memory (gwm) across time. Significant differences ($p < 0.05$) were also seen in CM on glr, gce, and gep across time. Females had higher speed processing (gs) and glr than males in the supplemented group. Cholesterol, low density lipoprotein (LDL) and triglycerides were significantly reduced ($p < 0.05$) in supplemented groups (CF and CM). High density lipoprotein (HDL) also dropped especially in CM but increased back after the wash out period. 70% of CM group claimed that their libido and energy level increased after one month of supplementation, while 50% of CF group claimed to have the same effect.

IN VIVO STUDY OF ENCAPSULATED FUSIDIC ACID FROM CREAM PREPARATION IN HEALTHY HUMAN VOLUNTEERS

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Various techniques which include skin extraction measurement, horizontal sectioning of strata, use of induced follicle-free skin, autoradiography, and non-invasive spectroscopic methods have been used to quantify the amount of drug within the skin in dermatological studies. The objective of this study was to explore the feasibility of employing a tape-stripping technique to compare the release of encapsulated fusidic acid and fusidic acid powder from cream preparations in healthy human volunteers. The effects of type of adhesive tape, application sites and subject variation were also investigated. Four healthy adult male volunteers participated in this study. Predetermined amount of cream sample containing either encapsulated fusidic acid (test cream) or fusidic acid (reference cream) were packed into cylindrical PVC rings and placed at multiple sites on the ventral forearms of each volunteer. At appropriate time intervals, excess cream samples were removed and the skin was stripped with adhesive tape strips. The results were expressed as the amount of drug absorbed per square cm of application area of the adhesive tape strip. The results of the study verified the feasibility of measuring drug concentration in the stratum corneum from cream preparations by tape-stripping method. TESA tape was found to be more suitable than 3M Transpore™ tape by giving lesser interference peaks in the HPLC chromatogram. In addition, inter-subject variation was a more prominent factor affecting percutaneous drug release than intra-subject variation. Encapsulated fusidic acid in cream achieved similar stratum corneum drug concentration as the non-encapsulated fusidic acid in cream.

MISUNDERSTANDING OF INSULIN SELF-ADMINISTRATION RESULTING IN HYPOGLYCAEMIC ATTACK

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In this era of health promotion, every health service agency should aim patients and their family members to be able to care for themselves properly at home according to their needs and capabilities. The objective is to discuss a case of misunderstanding of insulin self-administration resulting in hypoglycaemic attack. Mrs. SBA was a 54 years old diabetic patient with a history of uncontrolled blood glucose level manifested on several admissions and numerous toe amputations. On her last admission she complained of diabetic footpathy and was given insulin to be used at home for three days at the time of her discharge. Unfortunately she was re-admitted the next day because of hypoglycaemic attack due to high dose of insulin given by a family member. The

hypoglycaemic attack was due to two reasons; communication gap or inappropriate educational methodology. The physician claimed that because of time limitation, he did not give the proper instructions regarding administration of insulin. He assumed that the nurse would do that. It was reported that lack of communication, coordination among health care providers and patient education are related to high-cost care and re-admission in the hospital. This suggests that the structured and systematic health care staff-patient communication and sufficient patient education are necessary to achieve optimal health care.

PRESCRIBING TREND OF ANTI-EPILEPTICS AND HEALTH-RELATED QUALITY OF LIFE STATUS IN SABAH EPILEPSY POPULATION

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Despite the evolution of numerous treatments, epilepsy control generally remains insufficient. This is also complicated by impact on health-related quality of life (HRQoL) due to potential adverse drug reactions and disease-related psychosocial implications. This study aimed to examine the prescribing trend of anti-epileptic medications and HRQoL status in Sabah epilepsy sufferers. Fifty-two patients from seven Sabah government hospitals (Kota Kinabalu, KK = 22; Others = 30) consented participation whereby they completed the Malay Quality of Life In Epilepsy-30 (QOLIE-30) instrument (domains: *seizure worry, overall QoL, emotional well-being, energy/fatigue, cognitive functioning, medication effects and social functioning*). Respondents ranged from 18-76 years (mean age = 35; male = 31; unmarried = 27, jobless = 22). Thirty patients were prescribed one medication (KK=9, Others=21), eleven were on polytherapy (KK = 5, Others = 6) and three in KK were having no medication (missing = 8). The most commonly-prescribed epilepsy medications were sodium valproate (n = 21), carbamazepine (n = 14) and phenytoin (n = 13). Results showed that KK patients generally scored worse in all HRQoL domains (except *overall QoL* and *energy/fatigue*) compared to those outside KK who significantly reported better *cognitive functioning* (p = 0.037) and *social functioning* (p = 0.020). Such outcomes demonstrate that the majority of anti-epileptics are singly-prescribed especially in hospitals outside KK. Patients attending these hospitals were also experiencing better HRQoL compared to their KK counterparts. Prescribing pattern and HRQoL information could be of great value to health care providers in the management of epilepsy in order to deliver the best pharmaceutical service to the public.

**A CASE REPORT OF MYASTHENIC CRISIS SECONDARY
TO ABRUPT REDUCTION IN PYRIDOSTIGMINE
AND STEROID DOSES**

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Myasthenic crisis (MC) is myasthenia gravis (MG) with life-threatening symptoms of respiratory failure. The objective is to report a case of MG who developed MC secondary to abrupt reduction in the doses of pyridostigmine and steroids. A 30 year old female patient, diagnosed with MG, exhibited generalised body weakness, difficulty in swallowing, nasal regurgitation, drooling of saliva, ocular motor disturbances, ptosis, diplopia, bulbar palsy, and shortness of breath. She was treated with large doses of pyridostigmine and steroid for less than 48 h. Thinking of possible drug adverse effects, the physician planned to reduce the doses of these drugs. Unfortunately she developed MC secondary to abrupt reduction in pyridostigmine and steroid doses. However the patient responded well to the increased doses of pyridostigmine and steroid. The precipitants of MC include infection, stress, surgery, thyroid diseases, abrupt change in the dose of pyridostigmine and steroid. Based on reports, if the MG patient is stable with certain dose of these drugs, this dose should be maintained for at least 48 h to 72 h before changing the dose. Increasing or decreasing dose of these drugs should be done gradually.

**RASH ASSOCIATED WITH C-PENICILLIN ADMINISTRATION
IN LEPTOSPIROSIS**

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We report a case of C-Penicillin induced rash in a patient with leptospirosis. A 53-year-old Chinese man with no history of drug allergy developed a symmetrical rash while receiving C-Penicillin 2MU intravenously every 6 h. The rashes developed after day-6 of drug administration. The progression and worsening of rash following subsequent doses confirmed the allergy reactions. Fortunately, the rash resolved with the discontinuation of C-Penicillin. Patient's antimicrobial regimen was then changed to meropenem. The renal function, liver transaminase, bilirubin, coagulopathy, acidosis, and other clinical markers of infections improved significantly over a period of 20-day hospitalisation in the ICU. C-Penicillin is the drug of choice in treating severe leptospirosis. The rash is generally self-limiting and usually resolves within days after discontinuing the causative antimicrobial agent. The suggested mechanism of rash is a delayed type immune-mediated process. According to the Naranjo probability scale, the

association of C-Penicillin with the rash was classified as probable. Test dose should be considered before initiation of this drug. Drug that causes allergy should be stopped to avoid further severe consequences of drug allergy.

DRUG-INDUCED RHABDOMYOLYSIS IN PATIENT WITH RENAL IMPAIRMENT

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Treatment of hypercholesterolaemia has been proven to reduce mortality in patients with coronary artery disease. Patients with severe lipid abnormalities may require high-dose statin therapy, or combination with other lipid-lowering agents. We report a case of rhabdomyolysis induced by a combination of simvastatin and gemfibrozil therapy in a renal-impaired patient. A 51 year-old Malay male was admitted to the nephrology ward for haemodialysis. He developed acute renal failure secondary to rhabdomyolysis. Past medical history includes diabetes mellitus, renal impairment and hypercholesterolaemia. The patient was on simvastatin 20 mg/day and recently was put on combination with gemfibrozil 300 mg BD. The maximum level of creatinine kinase during hospitalisation was 35,410 U/l. Until 2001, there have been four reported cases of rhabdomyolysis associated with a combination of simvastatin and gemfibrozil. A combination therapy with statin and fibrate does increase the risk of rhabdomyolysis. Other alternatives such as alternate day statin-fibrate dosing may be considered in patients requiring such combination. Health care professionals play an important role in counselling and monitoring the warning signs of myopathy associated with these agents.

HYPERSENSITIVITY REACTIONS TO RIFAMPICIN: A CASE REPORT

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Decrease in blood pressure and shock have been reported with intermittent dosage regimes of rifampicin. The objective is to report a case of hypersensitivity reactions to rifampicin. A 28-year old Malay female was presented to the casualty unit with severe hypotension. The patient has started with anti-TB regime two weeks prior to admission and complained of poor appetite. She also had bilateral periorbital oedema. Allergy to anti-TB drugs was suspected. Anti-TB drugs rechallenge was planned. She was challenged with isoniazid 100 mg stat but no hypersensitivity reactions were noted. She was later challenged with rifampicin

150 mg stat and few hours later patient became hypotensive, febrile and had shortness of breath. Her periorbital oedema worsened as well. When challenged with pyrazinamide and ethambutol, she showed no hypersensitivity reactions. Hypersensitivity reactions in this patient was caused by rifampicin because when rifampicin was rechallenged, patient became hypotensive, had shortness of breath and her bilateral periorbital oedema worsened. The likelihood that this incident was drug-related could be classified as “definite” based on the Naranjo’s causal relationship algorithm. In conclusion close monitoring should be given in patient receiving rifampicin to avoid complication of hypersensitivity reaction.

THE COMPLEXITY OF PHENYTOIN TOXICITY AND POSSIBLY CONTRIBUTED METABOLIC ACIDOSIS IN STATUS EPILEPTICUS PATIENT: A CASE REPORT

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To present a case of the complexity of phenytoin toxicity and possibly induced metabolic acidosis in status epilepticus patient. A 20 year old male presented with fitting, characterised with generalised tonic clonic movement of all four limbs and up rolling of eye ball. Status epilepticus was diagnosed and aborted by IV diazepam, followed by IV phenytoin 750 mg loading dose and 100 mg maintenance dose. On day (D) 2 of admission patient developed nystagmus and the serum level of phenytoin was 40.60 mg/l on D5. On the same day, patient developed severe metabolic acidosis. Phenytoin was withheld for seven days and no fit was developed. Phenytoin at 100 mg BD restarted on D12 when the serum level of phenytoin was 7.5 mg/l, fit was developed. The dose of phenytoin was increased to 100 mg TDS with addition of IV sodium valproate 200 mg BD and seizure was then controlled. Phenytoin toxic level may be contributed by significant drug-drug interactions. Neurotoxic symptoms of phenytoin such as nystagmus and ataxia occurred when plasma concentration exceeds 20 mg/l. Phenytoin diluent and thiamine concentration possibly contributed metabolic acidosis. The judicious use of serum monitoring coupled with clinical evaluations enables the optimal anticonvulsant effects and minimal related toxicity.

LIVER TOXICITY ASSOCIATED WITH FLUCONAZOLE THERAPY: A CASE REPORT

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Fluconazole is considered a safe drug that occasionally has been associated with slight alterations of liver function tests that usually do not require interruption of treatment. The objective is to report a case with unusual liver toxicity associated with fluconazole, in whom discontinuation of the drug was required. A 58 yearold Malay man presented to the intensive care unit with acute exacerbation of bronchial asthma and pneumonia. IV fluconazole 200 mg daily was given on the fourth day of admission due to presence of *candida albicans* which was isolated from tracheal aspirate. Liver function tests at admission were 26 units/l alaninine aminotransferase (ALT), 92 units/l alkaline phosphatase (ALP), and 8 µmol/l bilirubin. A progressive increase was observed a week after fluconazole was started and they reached a peak after two weeks (261 units/l ALT, 118 units/l ALP, and 12 µmol/l bilirubin). Fluconazole was then discontinued. Reduction in ALT level to 118 units/l was seen 10 days after fluconazole was withheld. We report a case with hepatotoxicity associated with fluconazole, whereby mild improvement was seen upon drug discontinuation. The drug adverse effect relationship could be classified as "possible" based on Naranjo's scale. Thus, like other azoles, fluconazole is associated with liver toxicity. Liver function tests should be monitored especially if the dose is high.

TRADITIONAL CHINESE MEDICINE ASSOCIATED WITH SUBARACHNOID HEAMORRHAGE AND DEATH: A CASE REPORT

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There are a lot of problems arising from the use of traditional medicine due to adulteration of synthetic drugs. The objective is to present a case of traditional chinese medicine (TCM) associated with subarachnoid haemorrhage (SAH) and death. A 57 year old Chinese male was admitted after found lying unconscious. He was later diagnosed as having SAH. He was presented with Cushing's syndrome, uncontrolled hypertension and electrolyte abnormalities. The past medical history showed he had been taking TCM (in a form of capsule) for his knee pain for duration of six years. In the ward, he was treated with IV Tramadol, IV Metoclopramide, potassium supplement and IV hydrocortisone. He had two episodes of fit and failed to be resuscitated. The cause of death was stated as SAH. The Naranjo's scale showed that this effect falls under probable.

The TCM used was suspected to contain steroid based on patient's symptoms. Furthermore, many studies have shown adulteration of TCM with prescription drugs and the most common adulterant reported is steroid. As a conclusion, adulterated TCM may cause severe adverse effect or even death especially if used for long term.

ERYTHROMYCIN-INDUCED HEPATOTOXICITY: A CASE REPORT

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Hepatotoxicity of macrolides is mostly associated with erythromycin. The objective is to discuss a case of erythromycin-induced hepatotoxicity in intensive care unit (ICU), Penang General Hospital. A 69 year old Chinese man was admitted to the ICU due to hypoxemia and worsening of Glasgow Comma Scale, presented with symptoms of pneumonia, IV ceftriaxone 2 gm BD and IV erythromycin 500 mg QID were initiated on the first day. IV Acyclovir 500 mg TDS was also started on day 1. Five days after the administration of erythromycin and ceftriaxone, his liver enzymes showed a rapid elevation. Due to this incident, IV erythromycin was discontinued. Two days after discontinuing erythromycin, the liver enzymes eventually improved and were showing a decreasing trend. Liver injury in this patient was possibly caused by erythromycin because after discontinuing erythromycin, the liver enzymes eventually improved. There have also been a few reported cases of liver injury induced by erythromycin. The likelihood that this incident was drug-related could be classified as "possible" based on the Naranjo's causal relationship algorithm. Immuno-compromised patients with hypoalbuminemia have high risk of erythromycin-induced hepatotoxicity. If the use of erythromycin could not be avoided, the patient with risk factors should be closely monitored with regular liver function tests.

PATTERN OF ANTIMICROBIAL RESISTANCE AT THE GENERAL INTENSIVE CARE UNIT, PENANG GENERAL HOSPITAL IN YEARS 2003 AND 2004

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Distribution of antimicrobial resistant pathogen changes with time, and varies among different locations in the hospital. An area specific antimicrobial

susceptibility data is important to develop rational prescribing practice of antimicrobials. The objectives are to identify the common microbial isolates in the ICU of Hospital Pulau Pinang and their resistance patterns in 2003 and 2004 as well as to recommend appropriate empirical therapy according to the resistance pattern. The WHONET 5.2 software was used to collect the data. Data was taken for the first 10 months of 2003 and 2004. SPSS 10.0 was used to analyse the data. Most common isolates found in ICU in both 2003 and 2004 were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus coagulase negative*, *Klebsiella pneumoniae* and *Acinetobacter species*. The frequencies of resistant strains are MRSA 63.6% (2003) to 76.7% (2004); MRSE 61.3% to 72%; ESBL-producing *Klebsiella pneumoniae* 54.4% to 61.9%; ceftazidime resistant *Pseudomonas aeruginosa* 15.7% to 46.2% and imipenem resistant *Acinetobacter sp.* 79.2% to 61.3%. Majority of blood isolates were Gram positive and respiratory tract isolates were Gram negative. Periodic area-specific antimicrobial surveillance is vital to identify bacterial resistance.

A STUDY OF CORONARY ARTERY DISEASE RISK FACTOR PROFILES IN HYPERTENSIVE PATIENTS

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Several studies have shown that risk profiles such as high serum cholesterol, low HDL cholesterol, smoking and diabetes mellitus are coronary risk factors in hypertensive patients. Ninety-eight Hypertensive patients (50 females and 48 males) were interviewed and investigated to assess risk factors for coronary artery disease. Blood sugar, total cholesterol, serum HDL cholesterol levels and serum lipid profiles were estimated. The results of our study showed high clustering of various risk factors for CAD among our hypertensive population. 76% had high cholesterol, 43% had HDL < 45, high systolic pressure was seen in 57%, all had high diastolic pressure, 41% were diabetics and 66% were > 50 years. Because of the high clustering of risk factors high prevalence of CAD of 27% was seen in our samples. Percentage of 10 year CAD risk was calculated using Coronary Disease Risk Prediction score of Framingham study (2). CAD risk score > 20% was seen in majority of the patients (74.08%) and > 40% in 20.4% of the patients. 26.53% had clinical /ECG evidence of CAD. Aggressive reduction of modifiable risk factors is needed in hypertensive subjects for CAD risk reduction. It was observed that increasing age, decreases in serum HDL, increasing serum total cholesterol, smoking and diabetes mellitus were responsible for increased risk for coronary artery disease in hypertensive patients.

CYTOCHROME P450 3A5 GENETIC POLYMORPHISM: INFLUENCE ON IMMUNOSUPPRESSIVE THERAPY

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Immunosuppressants cyclosporine and tacrolimus are metabolised by cytochrome P450 3A enzymes which are coded by a group of CYP3A genes, including CYP3A5 gene. Substitution of A nucleotide (CYP3A5*1) by G nucleotide at loci 6986 (CYP3A5*3) in intron 3 of CYP3A5 gene, is associated with lower expression of CYP3A5 enzyme which lead to higher plasma drug concentration. We investigated the influences of CYP3A5 polymorphism on the concentration-adjusted dose requirement (mg/kg/day) and the C_0 (ng/l)/Dose(mg/kg/day) ratio of cyclosporine and tacrolimus at month sixth post-transplantation, whereby C_0 is trough level and Dose is daily dosage of cyclosporine or tacrolimus, in renal transplant patients. Forty-seven cyclosporine-dependent and fourteen tacrolimus-dependent renal transplant patients were recruited for the study. Among cyclosporine-dependent renal transplant patients, cyclosporine dose requirement ranged 1.0–7.0 mg/kg/day. This confirms the large interindividual variations in cyclosporine pharmacokinetics. CYP3A5*1/*1 (54±125 mg/kg/day) cyclosporine-dependent patients were found to have significantly higher dose requirement compared to those with cyclosporine pharmacokinetics. *1/*3 (3.6 ± 1.04 mg/kg/day, p = 0.02) or *3/*3 (3.6 ± 1.31 mg/kg/day, p = 0.03). However, there is no significant difference between group *1/*3 and *3/*3 in dose requirement of cyclosporine. For tacrolimus-dependent patients, *1/*3 and *3/*3 patients are significantly different in dose requirement and C_0 /Dose ratio: 0.24(*1/*3) vs 0.09(*3/*3) mg/kg/day, p = 0.011 and 45.7(*1/*3) vs 125.0(*3/*3), p = 0.011, respectively. Our results showed that CYP3A5 genotype could be the major factor that contributes to inter-individual variation in the pharmacokinetics of cyclosporine and tacrolimus.

NEGATIVE CORRELATION BETWEEN BODY FAT AND PHYSICAL FITNESS: A MESSAGE TO THE YOUNG OVERWEIGHT/OBESE SUBJECTS

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The human body is primarily composed of fat and fat free mass. An accurate estimation of body composition is important in planning a comprehensive program for total physical fitness. Cardiovascular abnormalities have been

reported in obese subjects. Similarly, metabolic adaptations and altered autonomic functions are recognised in the undernourished. It is expected that the physical work performance is reduced in both groups. One study has reported that the work performance was better in the undernourished than the normal group. Since the physical fitness efficiency is rarely quantified in relation to the body fat, the present study was undertaken for further investigations. The objectives of this study were: 1) to measure the body fat in the overweight (OW) obese, underweight (UW) and normal subjects. 2) To evaluate the relationship between the body fat and physical fitness. Six healthy male subjects for each group (OW/Obese, UW/UN, Normal) were recruited. Measurements of body weight, height, body mass index. Body fat, lean body was assessed by-aAnthropometry (body girth measurements), b) Skin-fold calipers (fat-o-meter). Physical fitness efficiency by Harvard step test. Results indicated that the overweight subjects had significantly reduced physical fitness scores while the undernourished had good scores as compared to the normal group. The study highlights a negative correlation between body fat and physical efficiency. The overweight need to shed their excess weight to achieve good grades of physical efficiency.

BUPRENORPHINE: ITS EFFECTIVENESS AND DRAWBACKS IN HEROIN DEPENDENCE TREATMENT

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Buprenorphine is a partial opioid agonist, newly introduced to treat opioid dependence in Indonesia. In this study we would like to investigate the effectiveness and drawbacks of buprenorphine on Indonesian opioid dependents treated in Sembada Hospital, Medan Indonesia. This study was a descriptive study, conducted in Sembada Hospital Medan. Study participants were opioid dependent who met DSM-IV criteria for drug-dependence who had been on buprenorphine treatment for at least one month. They were interviewed when they came to get their buprenorphine supplies, based on the already prepared questionnaires. There were 32 participants. Buprenorphine was effective to keep them away from opioid because there was no withdrawal to opioid (96.88%). The euphoria produced by buprenorphine was weaker than heroin, but they obtained calmness and feeling good (100%) and self confidence (96.88%). Economically the cost was much less. Buprenorphine produced no tolerance and some of them could use it in a non-daily dose. All of them felt more secure legally. Some of them (34.38%) reported improvement in their sex life. Side-effects were mild, mostly constipation (46.88%) or hardened stool (62.5%). They did worry about possibility of being dependent to buprenorphine. The availability of the drug is very important for drug compliance. In conclusion, buprenorphine is effective as a substitution therapy in heroin dependence. It produced no withdrawal to heroin. Euphoria produced by buprenorphine was weaker than heroin, but they obtained calmness, feeling good and self-confident. The cost was less and legally

they felt more secure. The side-effects were mild. The availability of the drug is important for drug compliance.

MELATONIN AND AFFECTIVE DISORDERS: A REVIEW STUDY OF RECENT ASPECTS

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There has been a continuing search for “biological markers” for affective disorders during the past 50 years. The occurrence of disturbed diurnal rhythms, delayed sleep onset, early morning awakening, body temperature variations and hormonal rhythms have all suggested the possible disturbances in circadian time keeping system as one of the major contributing factor that underlies affective disorders, like endogenous depression, bipolar affective disorders and seasonal affective disorder. Both pineal gland and its hormone melatonin have been shown to be essential for synchronization of internal bodily rhythms with those of external light-dark cycles. Studies of melatonin levels in depressives have shown that its amplitude as well as phase position are altered during illness. In bipolar affective disorder patients, changes in the phase of melatonin are very well-documented. Treatment of patients with affective disorders has been shown to correct both the phase position and amplitude of melatonin levels. Melatonin has been suggested both as a “state marker” and “trait marker” in affective disorders. In addition to this, bipolar patients exhibit greater suppression of nocturnal plasma melatonin levels to bright light application (500 lux) when compared to healthy controls. This super sensitivity to light for melatonin suppression is being used even to identify the people among normal subjects who are prone to develop bipolar affective disorders. A change in onset, duration and offset of melatonin secretion is seen in patients with seasonal affective disorder. These patients generate “a biological signal” in accordance with change in seasons. From all these it is evident that melatonin has a definite role to play in the etiology of affective disorders like endogenous depression, bipolar affective disorders and seasonal affective disorder.

NUFERA™: ANTI-INFLAMMATORY ACTIVITY OF VIRGIN COCONUT OIL ON SUPERFICIAL WOUND

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Coconut has been acknowledged as a multifunctional plant which has been used as food and traditional medicine for years. With this belief, Nufera™ a biotechnologically processed virgin coconut oil was developed. This study was carried out to show the effectiveness of Nufera™ as anti-inflammatory agent on

an open wound. A test was conducted on a patient with newly formed wound with swelling in a period of 14 days by topical application direct on the wound. After an hour of application, the wound started to dry with noticeable redness seen around the wound. After 24 h, scab was formed with lesser redness. Patient signifies that no pain felt at this point. Nufera™ was applied twice daily until the end of this study. On the fifth day of treatment, slighter redness was observed and patient reported that the scab can easily be removed. The skin appeared with subsided redness on the seventh day with the presence of scab, and swelling was further diminished. Complete wound healing with formation of normal skin composition was observed on the fourteenth day of treatment. The patient stated that this product helps reduced the pain and dried the wound quicker. These characteristics indicate anti-inflammatory activity of Nufera™ which expedites the healing process as well.

RAPID INFUSION OF VANCOMYCIN-INDUCED RED MAN SYNDROME

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To report a case of vancomycin-induced red man syndrome (RMS) in patient with end stage renal failure when given in rapid infusion rate. A 31 year old Malay woman developed rash over face and upper torso while receiving the first dose infusion of vancomycin 1 g (10 mg/ml over 1 h) to treat her catheter related sepsis. From history the patient was not allergic to vancomycin, and the drug serum concentration was within therapeutic level. When the second dose of vancomycin was administered four days later in slower infusion rate (5 mg/ml over 2 h) she tolerated the dose and no reaction occurred. Vancomycin-induced "red man syndrome" is mediated partly by histamine release, and its severity is correlated with the area under the plasma histamine concentration-time curve. The actual mechanism is not clearly understood because the reaction does not usually occur at slow infusion rate. It is not a true allergic reaction and it is related to the release of histamine. In conclusion, slow infusion rate (at least 2 h) is recommended in patients receiving vancomycin to avoid RMS. If the symptom still occurs, pre-treatment with antihistamine is recommended in future.

DOCUMENTATION OF SELF-CARE ACTIONS TAKEN BY KELANTANESE WOMEN DURING MENOPAUSE

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Menopause is associated with numerous symptoms and women often resort to a number of self-care actions, which include the use of both modern and traditional remedies. This study documents some of the traditional and alternative remedies used by Kelantanese women to manage their postmenopausal symptoms. A semi-structured questionnaire in the Malay language was administered to 326 naturally menopausal healthy women (mean age of 57.1 ± 6.58 (SD) years) residing in Kelantan. Mean age at menopause was 49.4 ± 3.4 (SD) years and 75% of these women were within the first 10 years of menopause. The mode for the number of symptoms complained by each woman was 8 (range 0-16). The commonest symptoms were tiredness (79.1%), reduced level of concentration (77.5%), musculo-skeletal aches and pains (70.6%), backache (67.7%) and night sweat (53%). Apart from hormonal replacement therapy, other self-care actions included traditional medicine; *akar kayu*, *kacip fatimah*, *jamu*, *ginseng*, or/and alternative medicine; Evening Primrose Oil, Royal Jelly, Omega and KY jelly. Prescribed medication was used to relieve aches and pains, prevent osteoporosis or coronary heart disease, and reduce urogenital symptoms. The percentage of women taking self-care actions depended upon the symptom. It ranged from 47.8% for the reduced level of concentration to 100% for crying spells and anxiety. Their choice of self-care actions might be influenced by their cultural, religious, educational and socio-economic factors. In conclusion, it appears that in addition to modern medicines the use of traditional and alternative remedies formed a significant component of the self-care actions taken by the majority of the Kelantanese women to help see them through the menopause transition.

ALLOPURINOL-INDUCED STEVENS-JOHNSON SYNDROME IN PATIENT WITH RENAL IMPAIRMENT

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Stevens-Johnson syndrome (SJS), also known as erythema multiforme major, is a systemic disease mostly involving the skin and mucous membranes. SJS is believed to be the result of a cell-mediated hypersensitivity reaction to a number of immunologic stimuli including drugs and infectious agents. We report a case of SJS syndrome induced by allopurinol in a patient with chronic renal failure. A 76 year old Chinese male was referred from a private institution for further management of erythema multiforme. He was advised by a friend to take

allopurinol to help with the joint pain. Past medical history includes hypertension with chronic renal failure. Overall patient's skin and mucous membrane condition improved once allopurinol was discontinued. Allopurinol may cause a severe, and sometimes fatal, hypersensitivity reaction in patients with pre-existing renal disease. The average time of onset of the syndrome after starting allopurinol is two to six weeks but can be considerably longer. If it is given in the correct dosage, modified with respect to renal function, then severe toxicity reactions are seldom seen. The incidence of hypersensitivity reactions would be further reduced if allopurinol is prescribed only where there is clear evidence of therapeutic benefit.

EVIDENCE BASED MANAGEMENT OF CARDIAC COMPLICATIONS SECONDARY TO GRAVES' THYROTOXICOSIS

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The objective of this case presentation is to discuss the evidence based management of cardiac complications of Graves' thyrotoxicosis. Thyrocardiac complications such as atrial fibrillation and congestive heart failure are common problem especially in the elderly. A 61 year old Malay man is a known case of hyperthyroidism who defaulted treatment and developed atrial fibrillation secondary to the disease. Patient also diagnosed with congestive heart failure on admission, classified under NYHA class II. Standard regimen of heart failure was prescribed. His heart rate remained uncontrolled until the addition of beta blocker. Correction of the underlying hyperthyroidism is the primary consideration of management of the cardiac complications. Based on American College of Cardiology and Malaysian Consensus guidelines, the addition of beta blocker to digoxin would achieve adequate rate control in thyrotoxic atrial fibrillation. This is attributed to increased renal clearance of digoxin and increased sensitivity to catecholamines due to elevated number of beta adrenoceptors in hyperthyroidism. In conclusion, evidenced based therapeutic intervention in management of thyrotoxic atrial fibrillation may improve the patient outcome.

HEALING EFFECT OF TOPICALLY APPLIED CHITOSAN GEL ON DIABETIC FOOT ULCERS: A PRELIMINARY CLINICAL STUDY

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Non-healing foot ulcers in patient with diabetes are the leading causes of complications such as infection and amputation. Ulceration is the most common

single precursor to amputation and has been identified as a causative factor in 85% of lower extremity amputations. The objective was study the healing effect of chitosan gel on diabetic ulcers. A total of six patients were enrolled in the study. Patients received chitosan gel dressing. Dressings were changed when clinically required. The study end point was the complete closure of the wound. Overall results of this study showed topical chitosan gel is effective in wound healing. In conclusion, chitosan gel appears to be useful as a topical agent in the healing effect of diabetic foot ulcers.

PYRIDOXINE-DEPENDENT SEIZURE IN A CHILD: A CASE STUDY

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Normally seizure can be controlled by anti-epileptic drugs (AED). The objective is to discuss a case of pyridoxine-dependent seizure in a child. A 26 months old Indian female girl had been admitted several times due to recurrent seizures. She developed multiple seizure episodes daily and admitted for uncontrolled seizure. Seizure episodes were presented as complex partial seizure with symptoms of up-rolling eye ball, drooling of saliva, stiffening of upper limbs, lip smacking, head nodding and post-ictal drowsiness. She had been treated with AED, carbamazepine 125 mg BD and clonazepam 0.375 mg BD. Recurrent seizure in this patient was suspected due to medication non-compliance. However, her seizures still occurred despite compliance with AED. Fortunately after receiving pyridoxine 100 mg BD treatment she had no more seizure episode. This child is suspected having inborn pyridoxine-dependent-seizure (IPDS). Furthermore, few studies have shown these IPDSs occurred when pyridoxine supplements are interrupted and seizures controlled after re-administration of pyridoxine. In conclusion, pyridoxine-dependent seizure is a rare disorder which is readily treatable. Newborn or children who do not respond to AED, administration of pyridoxine therapy should be considered.

STANDARD HEPARINISATION AND HEPARIN LOCK INDUCED BLEEDING IN HEAMODIALYSIS PATIENT: A CASE REPORT

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Little is known about the systemic effect of heparin used as an anticoagulant during hemodialysis to induce bleeding. The objective is to report a case of standard heparinisation and heparin lock induced bleeding in hemodialysis

patients. A 24 year old Malay female was admitted due to sudden fit during haemodialysis. She is a known case of systemic lupus erythematosus (SLE) with end stage renal failure (ESRF). These two problems may contribute to higher risk of bleeding. On the second day of admission, she developed massive bleeding characterised by bleeding from the palate and haematemesis with fresh blood. Her INR was 7.5, PT was 97.8 s and APTT was >180 s at that time. She was put on a heparin free dialysis but still had maleana suspected to be due to the heparin lock. The standard dose of heparin lock given was 5,000 U per catheter initially and no bleeding was observed after the dose was reduced to 2,500 U. An objective causality assessment using Naranjo algorithm revealed that these effects are possible. As a conclusion, special precaution should be taken when using heparin in patient with high risk of bleeding and this include the need to modify the dose given.

CEFOPERAZONE/SULBACTAM ASSOCIATED WITH COAGULOPATHY IN A CRITICALLY ILL PATIENT: A CASE REPORT

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To discuss the coagulopathy associated with cefoperazone/sulbactam (cper/sulb) administration in a critically ill patient. A 61 year old Indian female admitted to Hospital Penang with problems of arrhythmia and acute pulmonary oedema. This patient also has underlying hypertension, ischaemic heart disease, type 2 diabetes and renal impairment. After 59 days of ICU admission, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated from the culture of tracheal aspiration, which were treated with IV cper/sulb. Patient has an episode of massive maleana after 13 days of IV cper/sulb administration. The coagulopathy profile was deranged on day 16 of IV cper/sulb administration with prolonged PT and INR of 19.2 and 1.6 s, respectively, which was treated with four units of fresh frozen plasma (FFP). Cefoperazone, a third generation cephalosporin with methylthiotetrazole (MTT) side chain, has been reported infrequently to cause hypofibrinemia and haemorrhage. The risk factors of cefoperazone-induced bleeding were mainly malnutrition, renal insufficiency, or hepatic insufficiency. Prophylaxis or treatment with vitamin K and FFP may be needed in patients at high risk or when bleeding occurred. Discontinuation of cper/sulb may be necessary if coagulopathy do not resolved after the interventions. The patients with high risk of bleeding need to be identified and the use of cper/sulb should be monitored closely.

**CORRELATION OF PLASMA B-TYPE NATRIURETIC PEPTIDE
LEVEL WITH EJECTION FRACTION IN
PRIMARY HYPERTENSIVE PATIENTS**

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Currently we are in the midst of a chronic disease epidemic of heart failure, one of the worst complications for primary hypertensive patients. Ejection fraction is an important measure for left ventricular function, as well as an indicator for the presence of heart failure. Earlier data suggests that B-type natriuretic peptide level partially reflects the ventricular pressure. The aim of this work is to investigate whether plasma concentration of BNP reflects the heart's capability with the ejection fraction as the indicator. This study was conducted on 47 hypertensive patients referred for echocardiography to evaluate the ventricular function. Patients with diabetes mellitus, previous history of myocardial infarction were excluded from the study. The cardiologist making the assessment of left ventricular function was blinded to BNP levels. The BNP levels were assessed using the Triage Meter from Biosite Diagnostics. Results showed that BNP levels display a negative correlation with ejection fraction (Spearman correlation test) and it is clearly shown in the BNP versus ejection fraction scatter plot graph. The significant result (paired t-test, $p < 0.05$) proves that both predictors are very important and relates to each other. The study which is the first to be conducted in Malaysia may be helpful in ruling out the diagnosis of heart failure or as the best screening method.

**THERAPEUTIC CHALLENGES IN A SYSTEMIC LUPUS
ERYTHEMATOSUS PATIENT WITH STEROID INDUCED
COMPLICATIONS: A CASE REPORT**

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Corticosteroid therapy is frequently used for the treatment of systemic lupus erythematosus (SLE). Although it prolongs the remission of the disease, it may cause number of side effects. The objective is to discuss a case report of 17 years old male, with history of SLE and lupus nephritis, presented with cushing syndrome secondary to prolong use of steroid. Initially he has been treated with high doses of steroids, but because of the steroid's side effects it has been planned for the patient to treat with low doses of steroids along with IV cyclophosphamide (12 cycles). However his disease became active again after he has defaulted his treatment. In the ward he was again treated with high doses of steroids despite of already having severe steroid induced complications. Steroids along with chemotherapy is the only choice to treat SLE, but serious complications are associated with prolong use, such as cushing syndrome,

osteoporosis, hyperlipidaemia etc. In conclusion although not much proper guidelines and clinical trials are available, the treatment of this disease is still a challenge especially in treating the disease and in preventing the steroid induced side effects.

**PROBIOTIC SUPPLEMENTATION IMPROVES TOLERANCE TO
HELICOBACTER PYLORI ERADICATION THERAPY - A PLACEBO-
CONTROLLED, DOUBLE-BLIND RANDOMISED STUDY**

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Helicobacter pylori (*Hp*) is the major cause of chronic gastritis, and a risk factor for peptic ulcer and gastric cancer. In this study we investigated the effect of probiotic supplementation on the tolerance and efficacy of *Hp* eradication treatment in a randomised, double-blind, placebo-controlled trial. A total of 338 volunteers were screened for *Hp* infection. The eligibility criteria were met by 47 subjects whose *Hp* infection was verified at the outset and re-evaluated after the treatment by the ¹³C-urea breath test and by EIA serology. The subjects were randomised to receive probiotic therapy (*Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* LC750, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS) or a placebo during *Hp* eradication and for three weeks following the treatment, and recorded their daily symptoms in a standardised diary. When the frequencies of new or aggravated symptoms were evaluated, no significant differences were found for individual symptoms. However, the probiotic group showed less treatment related symptoms as measured by the total symptom score change ($p = 0.038$) throughout the *Hp* eradication therapy in contrast to the placebo group. The *Hp* eradication rate was higher in the group receiving probiotic therapy (91% vs. 79%, $p = 0.42$). In this group the recovery of probiotic bacteria in the faeces increased significantly ($p < 0.001$). The data suggest an improved tolerance to the eradication treatment when total symptom severity was taken into account. Furthermore, the results show that probiotic bacteria are able to survive in the gastrointestinal tract despite the intensive antimicrobial therapy.

A PROBIOTIC MIXTURE ALLEVIATES SYMPTOMS IN IBS PATIENTS – A CONTROLLED SIX-MONTH INTERVENTION

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Irritable bowel syndrome (IBS) is a widespread functional gastrointestinal disorder of unknown aetiology. The effect of probiotics in IBS remains unclear. The aim of this study was to investigate whether a probiotic mixture containing *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* JS might be effective in alleviating IBS symptoms. One hundred-three patients fulfilling the Rome criteria I or II took part in this six-month double-blind placebo-controlled trial. The patients received daily a probiotic capsule or a placebo capsule. Gastrointestinal symptoms and bowel habits were recorded in a symptom diary. At the end of the intervention the total symptom score (abdominalpain+distension+flatulence+borborygmi) was 7.7 (95% CI -13.9 to -1.6) points lower in the probiotic group ($p = 0.02$). This represents a median reduction of 42% in the symptom score in the probiotic group compared to a median reduction of 6% in the placebo group. In individual symptoms, borborygmi was milder in the probiotic group ($p = 0.008$), and for the rest of the symptoms there was a non-significant trend towards milder symptoms in the probiotic group. There were no significant differences between the groups regarding bowel habits. The results show that the probiotic mixture may be effective in alleviating IBS symptoms. Considering the high prevalence of IBS and the lack of effective therapies, even a slight reduction in symptoms could have public health consequences.

MISCELLANEOUS

DURIAN (*DURIO ZIBETHINUS*) RIND: AN UNCONVENTIONAL SOURCE OF NUTRACEUTICALS

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Crude durian rind pectin (CDRP) powder was produced from the dried durian (*Durio zibethinus*) rind powder (DRP) by an acid extraction and ethanol precipitation methods. The physical characteristics of CDRP were compared with those of commercial medium rapid set citrus pectin (MRS). The CDRP powder was darker-yellowish in color with good solubility in cold water. When dissolved, the CDRP produced a higher viscosity solution as compared to those

of MRS pectin. The viscosity of both type of pectin solutions however increased with increasing pectin concentration. At pH 3, CDRP formed gels when dissolved with 65% sugar, but gel was not formed in the presence of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ at pH 5.4. This suggests that the CDRP consisted mainly of high methoxyl pectin. The CDRP acid-sugar gels were darker in colour and less transparent in appearance than that of MRS pectin gels. The CDRP gels were higher in hardness and springiness but lower in cohesiveness as compared to that of MRS pectin gels. No appreciable difference in the fracturability, adhesiveness and gumminess attributes were noted between the two gels. Both type of gels however showed an increase in hardness, adhesiveness, fracturability and gumminess but a decrease in cohesiveness with increasing pectin concentration. Results from this study indicate that durian rind can be a potential source of nutraceuticals.

BIOSYNTHESIS OF 2, 3-DIHYDROXYBENZOIC ACID IN TRANSGENIC *CATHARANTHUS ROSEUS* CELL CULTURES OVEREXPRESSING ISOCHORISMATE SYNTHASE

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[1-¹³C] pyruvate feeding has been used to examine the biosynthesis pathway of 2,3-dihydroxy benzoic acid (2,3-DHBA) in *Catharanthus roseus* (*C. roseus*) cell cultures. Preliminary experiments with feeding of labeled pyruvate or phenylalanine to an elicited wild-type *C. roseus* cell cultures did not result in incorporation of any label in 2,3-DHBA. However, the involvement of isochorismate *synthase* (ICS, E.C. 5.4.99.6) in the biosynthesis of 2,3-DHBA was tested using a transgenic *C. roseus* cell line which constitutively overexpressed the *C. roseus* ics-gene. Higher level of 2,3-DHBA was detected after elicitation in the transgenic *C. roseus* cell line if compared with untransformed cell line. Moreover, this cell did show the corporation of labeled [1-¹³C] pyruvate. The label was mainly found in the position C-7 (carboxyl group) of 2,3-DHBA, as well as in the C-3 and C-4 positions. This pattern is consistent with the previous incorporation study using [1-¹³C] glucose in a wild-type *C. roseus* cell line. Isochorismate as an intermediate in the biosynthesis of 2,3-DHBA and confirms the involvement of ICS in this pathway.

DETECTION OF JUVENILE HORMONE III (JH III) IN CELL SUSPENSION CULTURE OF *CYPERUS AROMATICUS*

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Cyperus aromaticus (*C. aromatics*) from Cyperaceae family is a kind of rhizomatous sedge with a triangular stem. It is commonly known as 'rumpit ganda' in Malaysia. Insect juvenile hormone III (JH III), methyl-10R, 11-epoxy-3, 7, 11-trimethy 2E, 6E-dodecadienoate were detected in *Cyperus iria* L. and *C. aromaticus*. In insect, juvenile hormones (JHs) are regulators for the metamorphosis and physiological processes. They are important in insect species for several reproductive functions such as the maintaining of the larval characters at the moulting stage without differentiating into the adult stage. Two weeks old callus tissues were used to establish the cell suspension culture of *C. aromaticus* by inoculating 0.5 g cells into 30 ml liquid MS medium supplemented with 4.5 mg/l 2-4D and 5.5 mg/l NAA. The cultures were maintained in culture room regulated with a temperature of 25° C ± 2°C and continuous lighting with intensity of 2000 ± 500 lux. The highest biomass, 3.21 g (dry weight 0.42 g) was obtained after 18 days of culture from the initial weight of 0.5 g cell. The crude extracts of *C. aromaticus* cells analysed by High-Pressure (High-Performance) Liquid Chromatography (HPLC) detected the presence of JH III.

PRODUCTION OF EMBRYOGENIC CALLI OF *HYOSCYAMUS NIGER* L.

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Black henbane (*Hyoscyamus niger* Linn.) (*H. niger*) is one of the few Solanaceous plants which has been used as medicine for more than 4000 years. Currently, black henbane is cultivated as a source of tropane alkaloids for the pharmaceutical industry. Drugs based on tropane alkaloids are applied in modern medicine as painkillers and antispasmodics. The production of embryogenic calli of *H. niger* was carried out as an initial step towards establishing somatic embryogenesis system that could enhance the breeding via somaclonal variation and genetic transformation. Whitish and friable embryogenic calli were induced from the roots of *in vitro* plantlet, the petiole and the leaf segments of *H. niger*. Embryogenic calli were best produced on Murashige and Skoog (1962) medium (MS) supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 10 mg/l parachlorophenoxy-acetic acid (picloram) for the root explants, MS + 4 mg/l picloram for the petiole explants and MS + 6 mg/l picloram for leaf explants under continuous light intensity of 2000 ± 500 lux. Initially globular structures were observed after 21 days of culture and histological studies confirmed the formation of embryogenic calli.

However, the tissue lost its embryogenic potential upon repeated subculture cycles.

MICROPROPAGATION AND *IN VITRO* FLOWERING OF *PHYLLANTHUS NIRURI* (EUPHORBIACEAE)

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An efficient protocol was developed for a rapid and large-scale production of the valuable medicinal plant *Phyllanthus niruri* (Euphorbiaceae) via axillary bud proliferation using nodal segments of the mature plants. The nodal segments were cultured on MS medium (Murashige and Skoog, 1962) supplemented with various growth regulators of benzyladenine (BA), indole-3-butyric acid (IBA) and kinetin (KN). Maximum shoot multiplication with the formation of 6.6 shoots per explant was achieved on MS medium supplemented with 1.0 mg/l BA. *In vitro* flowering and fruiting occurred in 97% of the microshoots on MS medium without growth regulators. With the same medium, all the shoots produced roots. The first *in vitro* flowering was observed seven days after initial proliferation of nodal segments while fruiting occurred 15 days after culture. More shoots were produced (11.8 shoots per explant) after the separated multiple shoots were transferred to MS medium supplemented with 1.0 mg/l BA. The highest frequency of flowering and fruiting (90%–100%) were obtained when the plantlets were transferred to a growth regulator-free MS medium after the third subculture cycle. The continuous subculture of the nodal segments enabled the production of healthy shoots. The established micropropagation protocol could be used for raising a stock of genetically homogenous plant material.

SELECTION OF ELITE CLONES OF MISAI KUCING (*ORTHOSIPHON STAMINEUS* BENTH.) FOR THE PRODUCTION OF ROSMARINIC ACID USING CELL SUSPENSION CULTURES

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Orthosiphon stamineus [*O. aristatus* (Bl.) Miq.], belongs to the Lamiaceae family, is a medicinal herb of South-East Asia. The leaves are used as tea in Java for the treatment of bladder and kidney ailments. Traditionally, the tea is used for removing uric acid stones from kidney. Rosmarinic acid (RA), a natural antioxidant is commonly found in this plant. *In vitro* plantlets of *O. stamineus* could be established on MS medium (Murashige and Skoog, 1962) supplemented with 30g/l of sucrose. The plantlets were separated into three category (fast growing, intermediate growing and slow growing) based on the plant height during every subculture cycle. Leaves from the plantlets were used as explants for callus induction in MS medium supplemented with 1.0mg/l of 24-D and

1.0mg/l of NAA (callus induction medium). The friable calli that were formed from the cutting edge of the leaves were transferred into liquid callus induction medium for the establishment of the cell suspension culture of *O. stamineus*. HPLC analysis showed that RA was present in all the selected cell lines. The cell lines from fast growing groups harvested after 12 days of culture contained the highest amount of RA.

DETERMINATION OF TICLOPIDINE IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD WITH ULTRAVIOLET DETECTION

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A simple high performance liquid chromatography method for determination of ticlopidine in human plasma using ultraviolet detection was developed and validated. Plasma sample of 0.2 ml was added with imipramine HCl as internal standard and 4 M of sodium hydroxide before extracted with n-heptane and isoamyl alcohol (99:1, v/v). The extraction solvent was transferred into reactivial and evaporated to dryness. The residue was reconstituted with 100 μ l of mobile phase and 20 μ l was injected onto the column. A Synergi 4 μ Fusion-RP 80 Phenomenex column (250 x 4.6 mm ID, 4 μ m) was used for chromatographic separation. The mobile phase was comprised of acetonitrile, methanol and 0.05 M KH₂PO₄ (20:25:55, v/v) added with 0.2% of triethylamine before adjusted to pH 4.0 with o-phosphoric acid. The flow rate was 1.0 ml/min and the detection wavelength was 235 nm. Ticlopidine and imipramine were well-separated and free from interference by endogenous components in the plasma. The standard calibration curve was linear between 50 to 1000 ng/ml with a correlation coefficient value of 0.9991. The extraction recovery values of ticlopidine and imipramine were 86% and 90%, respectively. The average intra-day and inter-day coefficient of variation values were less than 5.7%. The limit of quantification was 50 ng/ml and the limit of detection was 25 ng/ml at signal to noise ratio of 3:1. This method could be employed to quantify ticlopidine in human plasma samples obtained from bioavailability studies.

DETERMINATION OF INSULIN IN HUMAN PLASMA USING SIMPLE AND SENSITIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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A simple, sensitive, accurate and precise high performance liquid chromatography with ultraviolet detection method for the determination of insulin in human plasma was developed and validated. An aliquot of 10 μ l of human plasma was extracted with 1 ml of dichloromethane. The organic phase

was transferred into reacti-vial and evaporated until approximately 200 μl about 200 μl of 0.05 M hydrochloric acid was added for back extraction. The balance of 200 μl of dichloromethane was then evaporated to dryness before 20 μl of aqueous phase was injected into the column. A reversed phase Luna 5 μm C18 (2) Phenomenex column (150 x 4.6 mm ID, 5 μm) was used for chromatographic separation. The mobile phase consisted of 0.2 M of sodium sulphate anhydrous adjusted to pH 2.3 with orthophosphoric acid and acetonitrile (74:26, v/v). The flow rate was set at 1.2 ml/min and the detection wavelength was 214 nm. There was no interference from the endogenous compounds. This method produced linear calibration curve over insulin concentration range of 1.2-7.6 $\mu\text{g/ml}$, with correlation coefficient value greater than 0.99. The average intra-day and inter-day coefficient of variation values were less than 6.4 %. The limit of detection and quantification of the method were 0.6 and 1.2 $\mu\text{g/ml}$, respectively. The extraction recovery for insulin was above 75%. This method could be employed for the determination of insulin in human plasma samples obtained from bioavailability study.

EVALUATION OF EUDRAGIT RS-30D AND KOLLICOAT SR-30D IN THE DEVELOPMENT OF CONTROLLED RELEASE PSEUDOEPHEDRINE HCl PELLETS

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The objective of this study was to evaluate the drug sustaining capability of two polymeric aqueous dispersions, Eudragit RS30D (Copolymers of acrylate and methacrylates with quarternary ammonium group) and Kollicoat SR-30D (Polyvinyl acetate stabilized with povidone and sodium lauryl sulfate) in the development of controlled release pseudoephedrine HCl pellets. The pellets containing 30% w/w drug loading were prepared using extrusion-spheronization technique followed by coating with polymeric aqueous dispersions using fluidised bed coater. Pellets of size range between 0.8 mm and 1.25 mm were selected for the *in vitro* release studies. Three different dissolution media, 0.1 M HCl, pH 4 and distilled water were used for drug release studies. The amount of drug released was quantified using UV spectrophotometry. The results showed that increasing the polymer coating level from 10% w/w to 30% w/w reduced drug release rate. At a coating level of 20% and above, pellets coated with Kollicoat SR-30D could be sustained more than 12 h. In contrast, pellets coated with Eudragit RS30D could not be sustained more than 2 h even at a coating level of 30%. Hence, at the same coating level, Kollicoat SR-30D delayed the drug release to a significantly greater extent than Eudragit RS30D. The drug release was closely similar in the three dissolution media. In conclusion, controlled release pellets containing pseudoephedrine HCl were successfully developed and the drug release could be modified in a predictable manner by varying the types and content of polymer used.

EVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SULPIRIDE IN HUMAN PLASMA

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A simple and sensitive high-performance liquid chromatography method with fluorescence detection for the determination of sulpiride in human plasma was developed and validated. A Luna 5u C18 (2) (5 μ m, 150 x 4.6 mm ID) Phenomenex column was used for chromatographic separation. The fluorescence detector was operated at excitation and emission wavelengths of 300 nm and 345 nm, respectively. Sulpiride and metoclopramide hydrochloride internal standard were extracted from 0.5 ml of plasma sample with ethylacetate and dichloromethane (3:2, v/v) after alkaline treatment. The mobile phase was comprised of 0.01 M phosphoric acid, acetonitrile and methanol (75:15:10, v/v) with 0.15% of triethylamine before adjusted to pH 6.0 with glacial acetic acid. The flow-rate was set at 1.0 ml/min. The standard calibration curve was linear over a concentration range between 15.6 ng/ml and 4000.0 ng/ml, with a correlation coefficient of 0.9996. The mean recovery values for sulpiride and metoclopramide hydrochloride were 94% and 92%, respectively. For both within-day and between-day assays, the accuracy values were not more than 12.0%, while for the precision all the coefficient of variation values were not more than 10.7% at the concentrations determined. The limit of quantification was 15.6 ng/ml and the limit of detection of 10.0 ng/ml was obtained at a signal-to-noise ratio of 3:1. This method was used to quantify the plasma levels of sulpiride in 16 healthy adult male volunteers in a single-dose, two-period, two-sequence, two-treatment cross-over bioequivalence study.

IMPROVED ORAL BIOAVAILABILITY OF CEFOTAXIME USING pH-DEPENDENT EUDRAGIT L100 NANOPARTICLES

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Nanoparticles have attracted considerable interest as oral delivery systems for peptidomimetic drugs due to their ability to protect the compounds from degradation in the gastrointestinal tract, as well as facilitating their absorption into the systemic circulation. The objective of present study was to develop a pH-dependent Eudragit L100 nanoparticle delivery system that could improve the oral bioavailability of a hydrophilic peptidomimetic model drug, cefotaxime. Cefotaxime-loaded nanoparticles were prepared using a pH-dependent controlled nanoprecipitation method. The particle size of the nanoparticles produced by this method were less than 150 nm, and of low polydispersity index when measured using Photon Correlation Spectroscopy (zetasizer 1000HS). However, the amount of cefotaxime loaded in the nanoparticles using the

nanoprecipitation method was relatively low, being approximately 4.0% w/w an *in vivo* study using Sprague Dawley rats showed that the oral bioavailability of cefotaxime was increased approximately four folds when administered using the nanoparticles compared to an aqueous solution of the drug. Hence, in conclusion, the Eudragit L100 nanoparticles were capable of increasing the oral bioavailability of the model peptidomimetic drug.

EFFECTS OF MOLECULAR WEIGHT AND SOLUBILITY OF DRUG ON MICROENCAPSULATION

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The physicochemical properties of drug molecules play an important role in the success of the final microencapsulated dosage design. The objective of this study was to study the feasibility of encapsulating six different drug molecules, ketoconazole, ketoprofen, magnesium stearate, pseudoephedrine hydrochloride, diclofenac sodium, and paracetamol, using a coacervation-phase separation method. The effects of molecular weight and solubility of drugs on microencapsulation were also investigated. Drug loaded microparticles were prepared by adding an ethanolic drug solution to a gelatin solution. In the case of magnesium stearate, ethanol was added to a dispersion of magnesium stearate in gelatin solution. Formaldehyde solution was used to rigidise the gelatin coat before the microparticles were washed and freeze-dried. The morphology and particle size values of the encapsulated microparticles were examined using light microscopy and Mastersizer, respectively. The mean particle size of encapsulated ketoconazole, ketoprofen, and magnesium stearate microparticles were approximately 178, 98, and 388 μm , respectively, and correlated directly to their individual molecular weight. In comparison, the morphology of encapsulated ketoconazole microparticles was more spherical than those of ketoprofen or magnesium stearate microparticles. Drugs, such as pseudoephedrine hydrochloride, diclofenac sodium, and paracetamol, which are soluble in both water and ethanol, failed to form microparticles. In conclusion, ketoconazole, ketoprofen, and magnesium stearate were successfully formulated as microparticles using coacervation phase separation method. The solubility of drug affected the process of microencapsulation, while the increase in molecular weight of drug increased the particle size of the final microparticles.

**ELUCIDATION AND HPTLC QUALITATIVE ANALYSIS OF
OLEANOLIC ACID ISOLATED FROM THE ANTI-
INFLAMMATORY SUB-FRACTION OF *ORTHOSIPHON*
STAMINEUS BENTH. EXTRACTS**

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Oleanolic acid is a triterpenoid compound that exists widely in natural plants in the form of free acid or aglycones for triterpenoid saponins. Saponins can be chemically categorised as comprising an aglycone linked to one or more sugar chains. There are two groups of saponins, one contains a steroidal aglycone and the other contains a triterpenoid aglycone. Oleanolic acid, the aglycone for many triterpenoid saponins in medicinal plants, has been isolated from more than 120 plant species are shown to be an active ingredients in producing biological effects. The objectives of this research are to identify and elucidate oleanolic acid (isolated) present in *Orthosiphon stamineus* and to perform qualitative high performance thin layer chromatography (HPTLC) analysis. The isolated compound from the anti-inflammatory active sub-fraction was identified and elucidated by spectroscopic methods like ¹H-NMR, ¹³C-NMR, GC-MS, and FT-IR. Qualitative analysis was carried out using CAMAG LINOMAT 5 auto sampler where the isolated compound and active sub-fraction were applied to a 20 cm x 10 cm precoated thin layer chromatography (TLC) plate. The plate was developed in chloroform: ethanol (85:15) solvent system placed in a glass chamber. Then it was sprayed with anisaldehyde-sulphuric acid reagent and heated for about 5 min at 100°C. Evaluation of the developed plate was performed using CAMAG TLC SCANNER 3 under UV light at 254 nm and 365 nm. Data acquisition and processing were done using the winCATS software. The compound was obtained as a white solid powder with the melting point of 198°C-200°C; FTIR (KBr) : 3419 cm⁻¹ (OH), 2921 cm⁻¹ (CH), 1699 cm⁻¹ (C=O), 1627 cm⁻¹ (C=C), 1463 cm⁻¹ (-CH₂-), 1375 cm⁻¹ (-CH₃), 1052 cm⁻¹ (C-OH); molecular formula C₃₀H₄₈O₃, m/z 456. Based on the data obtained from IR, ¹H-NMR, ¹³C-NMR, GC-MS and by comparison with the literature values, the compound isolated was identified as 3β-hydroxy-olea-12-en-28-oic acid (oleanolic acid). Oleanolic acid gave a blue fluorescent colour at R_f 0.95 under UV 365 nm after spraying with anisaldehyde-sulphuric acid reagent. HPTLC chromatogram of the active sub-fraction showed six main peaks when observed under UV light at 254 nm and 365 nm respectively. One of the peaks at R_f 0.95 was found to be oleanolic acid based on the comparison of the R_f value of pure isolated oleanolic acid.

INFLUENCE OF AGING ON CYCLOSPORIN A RELEASED FROM GELUCIRE MATRICES

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A study was conducted to evaluate the influence of aging on the *in vitro* release of cyclosporin A from Gelucire matrices. Cyclosporin A was first incorporated into Gelucire[®] 44/14 using heat-fusion method at a ratio of 1:10 (cyclosporin A: Gelucire[®] 44/14, w/w). The molten mixture was then filled into hard gelatin capsules and stored at room temperature (approximately 25°C). The *in vitro* release profiles of the freshly prepared samples and aged samples were determined using an *in vitro* dissolution test while the thermal behaviour was characterised using differential scanning calorimetry (DSC). The *in vitro* dissolution test was carried out using the rotating basket method set at 100 rpm with 1000 ml 0.1 N HCl maintained at 37°C whereas the DSC instrument was run from 2°C to 60°C at a rate of 2°C/min. A significant aging effect was observed whereby the *in vitro* release of cyclosporin A from the matrices decreased upon aging although the melting point of the Gelucire matrices was maintained at approximately 41°C. Various additives were evaluated to overcome the aging effect on Gelucire matrices. The additives evaluated included a solubiliser (diethylene glycol monoethyl ether), emulsifiers (sorbitan monooleate, polyoxyethylene sorbitan monooleate) and lecithin (phospholipids). Addition of emulsifiers up to 10% did not improve the stability of the formulation whilst addition of the solubiliser was capable of retarding the aging effect of the formulation. Further investigation is needed to stabilise the self-emulsifying formulation.

IN VITRO RELEASE EVALUATION OF SUSTAINED RELEASE GLICLAZIDE MATRIX TABLETS PREPARED USING EUDRAGIT RSPO AND KOLLIDON-SR

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Eudragit RSPO (copolymer synthesised from acrylic and methacrylic acid ester) and Kollidon SR (copolymer of polyvinyl acetate and povidone) are widely recommended to be used in the preparation of sustained release delivery systems. Sustained release gliclazide matrix tablets were prepared using Eudragit RSPO and Kollidon SR as retarding agents. The concentrations of the two polymers employed were 15%, 20%, 25% and 30% of the tablet weight. The drug release was investigated in phosphate buffer solution at pH 7.2 using USP dissolution apparatus II (paddle method). Increase in polymer concentration decreased the drug release for both polymers. At 30% polymer concentration, the drug release could be sustained for more than 12 h. The drug sustaining

properties of both polymers at same concentrations appeared to be closely similar. When the release data were fitted into the release kinetic equations of zero order, first order and Higuchi square root of time, the drug release profiles of formulations containing Eudragit RSPO were better described by first order release kinetic. In the case of Kollidon SR, the formulations were better fitted with zero order kinetic release.

OPTIMISATION OF ENZYMATIC METHOD IN ENHANCEMENT OF CHITOSAN SOLUBILITY IN WATER

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Chitosan has received much attention as a functional biopolymer with applications in various fields such as pharmaceuticals, food, cosmetics and medicines. The application of chitosan has limitation because it is only soluble in dilute aqueous acidic solution. The aim of this study was to optimise a simple enzymatic method in improving the solubility of chitosan in water using cheap and commercially available hemicellulase enzyme, which could increase the ease of handling and reduce production cost. Chitosan was dissolved in 2% v/v acetic acid before enzymatic breakdown with hemicellulase. The enzyme was denatured and separated from the reaction mixture by boiling. The filtrate was concentrated using a rotary evaporator and then freeze-dried. Solubility measurement was carried out to determine the optimum conditions required to produce chitosan with highest solubility. Parameters that included enzyme concentration, reaction temperature, reaction time and pH, were investigated. It was found that by increasing the enzyme concentration, reaction time and reaction temperature, the solubility of chitosan increased. The optimum conditions were achieved at 20% enzyme concentration, 6 h reaction time, and 40°C reaction temperature. Above the optimum conditions, there was no statistically significant difference in the solubility of chitosan. In contrast, increasing the pH value decreased the solubility of chitosan. The presence of HCl at pH 2.5 and 3.5 could have hydrolysed chitosan to HCl-glucosamine. As such, pH 4.5 was taken as the optimum condition since HCl was not added at this pH.

EFFECT OF DIFFERENT DRUG-POLYMER PREPARATION METHODS ON PHYSICAL AND DRUG RELEASE PROPERTIES OF SUPPOSITORY

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The effect of different preparation methods of drug-polymer (diclofenac sodium [DcNa]- Carbopol 934P [CP]) mixture, namely, physical mixing (P1), co-grinding (P2) and wet granulation (P3), on physical and drug release properties of

suppositories containing palm oil base (ChocExa), was evaluated. No consistent trend in base hardness was observed in suppository incorporated with DcNa-CP mixture prepared with different methods, although P1 method recorded the highest hardness values in the presence of 1% and 2% w/w CP. Furthermore, among the three different preparation methods, DcNa-CP preparations with 2% w/w CP have significantly higher hardness values than 1% w/w CP. On the other hand, incorporation of DcNa-CP prolonged the softening time of all suppositories in the order of blank base < blank incorporated with drug < base incorporated with DcNa-CP mixture of P2 method < base incorporated with DcNa-CP mixture of P1 method < base incorporated with DcNa-CP mixture of P3 method. Incorporation of DcNa-CP mixture prepared using P1 and P3 methods prolonged softening time significantly compared to blank base and base incorporated with drug. Moreover, in the presence of CP in all DcNa-CP preparations, drug release was fastest in base incorporated with drug only, followed by base incorporated with DcNa-CP prepared using P3 method, P2 method and lastly P1 method. Nevertheless, an increase in CP content reduced the rate of drug release. In conclusion, the base hardness and softening time of suppositories incorporated with DcNa-CP prepared using P2 and P3 methods were comparable to that of P1, but with faster drug release.

**APPLICATION OF RESPONSE SURFACE METHODOLOGY IN
INVESTIGATION OF RHEOLOGICAL PROPERTIES AND
TEXTURE PROFILES OF CARPOBOL® 934P-NF (CP) AND
PLASDONE® K-90 BIOADHESIVE GEL**

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Carpobol® 934P-NF (CP) and Plasdone® K-90 (PD, polyvinylpyrrolidone) were evaluated as suitable base in the preparation of bioadhesive gel. The effect of the compositions of CP and PD on the rheological and texture properties was investigated. A response surface methodology, Central Composite Design, was employed for optimisation in the experimental design. The rheological properties of the gel preparations, namely, flow pattern, zero-rate viscosity, and thixotropy, were examined using continuous shear rheometry. On the other hand, the texture properties, namely, compressibility, hardness, adhesiveness, cohesiveness, gumminess, and springiness, were determined using texture analyser. The gel preparations demonstrated plastic flow with negligible hysteresis. For pure CP gels, increasing CP concentration increased the compressibility, hardness, adhesiveness, gumminess, and zero-rate viscosity significantly. Incorporation of PD into CP gel greatly diminished the texture properties of the gels. There was interaction between the two input parameters, CP and PD, as shown by the quadratic equations. The increase in CP and PD proportions in the gels increased the viscosity, adhesiveness, springiness, and gumminess but reduced the cohesiveness. In conclusion, application of response surface methodology enabled prediction of the parameters of rheological and texture properties except compressibility and hardness in the development of CP-PD gels.

**FREE RADICAL SCAVENGING CAPACITY AND XANTHINE
OXIDASE ACTIVITY OF *PHYLLANTHUS RETICULATUS*'S LEAVES
BY ASSAY-GUIDED COMPARISON**

**ROZAINA MAHMOD, TENGKU SIFZIZUL TENGKU MUHAMMAD AND
SHAIDA FARIZA SULAIMAN**

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The study goals are to identify the xanthine oxidase inhibition and the free radical scavenging activity of the extracts from the leaves of *Phyllanthus reticulatus* (*P. reticulatus*). Xanthine oxidase catalyses the oxidation process of hypoxanthine to xanthine and uric acid as end products. This process possessed a crucial role in gout. The free radical scavenging activities were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH radical has an absorption band at 515 nm which disappears due to reduction by *P. reticulatus* free radical scavenging activity. Among the five tested extracts, 80% methanol was found to give the best results for free radical scavenging activity and xanthine oxidase inhibitory activity, i.e 2.124 µg/ml and 78.119 µg/ml, respectively thus the extract was fractionised using paper chromatography and resulted in 15 fractions. Fmp 3 showed the best EC₅₀ value of 1.066 µg/ml (free radical scavenging activity) and IC₅₀ value of 0.010 µg/ml (xanthine oxidase inhibitory activity).

**A COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITIES
OF EXTRACTS FROM THE HEALTHY AND INFECTED LEAVES OF
*TRIPHASIA TRIFOLIA***

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Triphasia trifolia (lime berry) is a spiny shrub of the family Rutaceae which has been used in traditional medicine to treat various microbial diseases. The objective of this study is to compare *in vitro* the antibacterial efficacy of the extracts from the healthy leaves and infected leaves of *Triphasia trifolia*. These extracts were subjected to antibacterial screening against 24 species of Gram-positive and Gram-negative bacteria. Hexane, chloroform and methanol extracts from leaves of the plant were tested for their antibacterial activity using disk diffusion method, with 20 µl load extract volume per disc. Results from the antibacterial tests demonstrated that the hexane and chloroform extracts from infected leaves exhibited activity against various species of bacteria. Chloroform extract from infected leaves showed greater antibacterial activity against *Salmonella paratyphi* than that of hexane and methanol extracts. These results suggest that chloroform extract of *Triphasia trifolia* infected leaves is more potent in bacterial inhibition and might contain post infectious compounds such as phytoalexin.

COMPARATIVE STUDY OF CONFORMATIONAL ANALYSIS TECHNIQUES FOR PHARMACOPHORE GENERATION IN CATALYST

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Pharmacophore is the molecular framework that carries the essential features responsible for a drug's biological activity. A pharmacophore model describes distance between important atoms or group of atoms in a ligand that are required for interaction with a receptor. In order to generate a pharmacophore model, various conformers of a compound must be generated to produce a good representation of a compound's conformational space. However, we have observed that seemingly trivial differences in conformer generation can profoundly affect the models produced. The aim of this study is to analyse a literature data set in order to determine which is the best conformational tool for producing good Catalyst hypotheses. Two conformational analysis techniques of Catalyst/Best and Catalyst/Fast were selected. All computational experiments were conducted on a Silicon Graphics Octane, running under the IRIX 6.5 operating system. Pharmacophore model generation was made using Accelrys's Catalyst/Hypogen (version 4.7) modeling environment. The Catalyst/Best conformational technique produces a comparable pharmacophore model when compared to the Catalyst/Fast but at the expense of a longer calculation time. Fast conformation generation tools perform as well as best conformer generating tools.

PROTEOMIC STUDY OF COLON CANCER TISSUES

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Colorectal cancer continues to be the second leading cause of death for both men and women, despite majority advances in basic research over the last two decades, response rate to therapy remain poor. Prognosis uncertainty of colon cancer can be overcome by defining the tumor-associated changes that occur at either the gene or protein levels. In our present study, we are applying the proteomics approach to study the possible changes in cancer tissues as compared to normal tissues. Proteins are the functional components in cells, whereby they serve as the building blocks and the active components that regulate cells activities. Therefore, understanding of the differential protein expression in cancer tissue compared to normal tissue will serve as the foundation for future development of drug-target therapy in cancer research. The current study reveals the use of optimised protein extraction buffers to extract proteins from both colon normal tissues and colon cancerous tissues. The protein profiles of four different colon cancer patients were analysed using SDS-PAGE. The results show that besides the commoner proteins between the normal and cancerous tissues, there are variations in protein band between the two tissues, indicating the presence of

cancer-related proteins. The differentially expressed protein bands were excised from the gel and in-gel digestion of the proteins was carried out prior to LC/MS/MS analysis. MS/MS spectra were searched against the protein database using Mascot software. Furthermore, the functions of the proteins were identified using SWISS-PROT program.

IN VITRO PRODUCTION OF TROPANE ALKALOIDS FROM *HYOSCYAMUS NIGER* L.

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In vitro plantlets of *Hyoscyamus niger* L. (*H. niger*) could be produced by inducing multiple shoots formation from the apical shoots using Murashige and Skoog (1962) (MS) medium supplemented with 8.0 mg/l benzyladenine (BA) and 0.5 mg/l 3-indole butyric acid (IBA). The separated shoots produced roots on basic MS medium to form complete plantlets. The leaf explants produced compact callus on MS medium supplemented with 2.0 mg/l picloram. The compact callus showed the presence of both hyoscyamine and scopolamine alkaloids. The *in vitro* roots of *H. niger* could be mass produced in aerated tubes using liquid MS medium supplemented with 0.5 mg/l IBA. GC-MS analysis revealed the presence of both hyoscyamine and scopolamine in all the *H. niger* roots from various sources which included roots of the *in vitro* plantlets, roots induced on solid MS medium supplemented with 2.0 mg/l IBA, roots produced on shaken flasks and aerated tubes using liquid MS medium supplemented with 0.5 mg/l IBA. Both hyoscyamine and scopolamine were not detected in the aerial part of the *in vitro* plantlets as well as the liquid culture medium from the shake flasks and the aerated tubes.

MODELLING DRUG ABSORPTION USING MULTIVARIATE ANALYSIS

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Physico-chemical properties play a key role in drug metabolism and pharmacokinetics. These properties can be measured as well as can be calculated. It has been highlighted that a number of 3D descriptors such as 3D molecular surface property are strongly correlated to the more traditional physico-chemical properties. The calculation of other 1D, 2D and 3D descriptors is possible using a range of software packages. The aim of this study is to evaluate various descriptors for inclusion in drug absorption model. Validation of the model will be carried out using known orally delivered drugs and "drug-like" molecules. A data set related to intestinal absorption and oral availability was selected. Various physico-chemical parameters as well as structural and geometrical

descriptors were evaluated to build a statistical pattern recognition model of passive intestinal absorption. Multivariate data analysis was calculated using The Unscrambler 9.0. A pattern recognition model is appropriate given the available quantity and quality of data. Use of standard multivariate methods permits classifying a new molecule to some class of molecules of interest. Computational ADME models such as the passive intestinal absorption described herein is useful if they can predict with reasonable accuracy the ADME property of the compounds of interest.

CHARACTERISATION OF *IN VITRO* MODULATORY EFFECTS OF FLAVONOIDS ON HUMAN CYTOCHROME P450 (CYP) 2C8 ISOENZYME

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Flavonoids exhibit a wide range of biological activities arising mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors. Among the enzymes interacting with flavonoids are the cytochromes P450 (CYPs), the monooxygenases metabolising xenobiotics and endogenous substrates. CYP2C8 is an important human liver isoform involved in metabolism of paclitaxel, amodiaquine and rosiglitazone. While modulatory effects of flavonoids on many CYP isoforms have been characterised, CYP2C8-flavonoid interactions have not been well-characterised. The work described in the present project aimed to express CYP2C8 in bacterial expression system and to use the expressed protein to characterise potency and mechanism of naturally occurring flavonoids on CYP2C8 activity. CYP2C8 protein was expressed in DH5 α bacterial cells according to established protocols. Expressed CYP2C8 activity and its susceptibility to flavonoid effect were examined using the substrate probe tolbutamide in an established high-pressure liquid chromatography (HPLC) assay. CYP2C8 was expressed at high level as judged from the Western blotting analyses as well as detectable protein spectral activity in spectrophotometric analyses. The expressed CYP2C8 catalysed tolbutamide hydroxylation actively in the HPLC assay. Hydroxytolbutamide formation fitted best the single Michaelis-Menten kinetic, with apparent K_m and V_{max} values of 1157.6 μ M and 12.1 pmol/min/mg protein, respectively. Screening of five flavonoid compounds on tolbutamide hydroxylase assay indicated that the flavonoids inhibited CYP2C8 with different potency but all of them exhibited mechanism based inhibition. Structural factors important for CYP2C8 inhibition were the molecular

shape (volume to surface ratio), the number of hydroxyl groups as well as glycosylation of the hydroxyl group. Quercetin and luteolin were the most potent inhibitors among the flavonoids examined in this study. In conclusion, naturally occurring flavonoids have differential effects on CYP2C8 and certain structural factors may be important to exert inhibitory effect to the isoform.

FURTHER EVALUATION OF SOYBEAN OIL-IN-WATER MICROEMULSION AS DOSAGE FORM FOR DELIVERY OF CYCLOSPORIN A ORALLY

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Our earlier study (Elwalied et al. 2003) had showed that, among the vegetable oils tested, soybean oil was found to be the best oil phase for producing cyclosporin A oil in water microemulsion. This conclusion was based on the solubility of the drug in various oils and the triangle phase-diagrams of the microemulsions produced. Our later study (Elwalied *et al.* 2004) which had been concentrated on soybean oil microemulsions, had showed that the size of microemulsion droplets did not change significantly after storage of 90 days at 4°C and 27°C. Extrapolation of the results indicated that the emulsions can be stored for more than three years without any significant change in droplets size. The present study is conducted with the aim of justifying further the suitability of soybean oil microemulsion as oral dosage form for cyclosporin A. In this study various amounts of cyclosporin A were incorporated into soy bean microemulsions. The size of microemulsions droplets were measured using Malvern Zeta Sizer. The effect of different pH on the droplets size for duration of 4 h was also investigated. To compliment the study further, the effects of surfactant concentrations on the solubility of cyclosporin A was also investigated. The amounts of Cyclosporin A needed to produce saturated solutions in soybean oil containing various amounts of Span 80 were measured by HPLC method. The results of the present study showed that the presence of cyclosporin A in the microemulsions significantly increased the size of microemulsions droplets. The higher the concentration of the drug in the microemulsion, the bigger the droplets size would be. Microemulsion droplets appeared to increase in size after dilution with buffer solutions at pH 1, 2, 3, 4, 5 and 6 at 37°C and monitored for 4 h, but the change in droplets size was not significant statistically. The solubility of cyclosporin A in soybean oil increased from 22.07 mg/ml in the absent of surfactant to 144.5 mg/ml and 664.6 mg/ml in present of 16% and 33% Span 80, respectively. This result indicate that higher amount of cyclosporin A can be loaded into the soybean microemulsion, thus it is possible to give the emulsion orally at the required dose.

PHARMACISTS AND DRUG MARKETING

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Recently, the number of pharmaceutical companies is rising all over the world especially in Pakistan. Despite of the fact that there have been big multinational pharmaceutical company mergers in the recent past and a lot more expected in future, there is a mushroom growth of the local pharmaceutical industry. Several factors are involved in this rapid growth; patent expiry, availability of better and easily accessible healthcare facilities and a lot of novel molecules being developed, are just a few to be mentioned. In this perspective, the dissemination of information about the usage of these drugs by a medical practitioner is also getting immense importance. Marketing of a complex product like pharmaceuticals is by no means straight forward. The fact that marketing a pharmaceutical is based on the interpretation and presentation of its efficacy as observed in clinical trials is the core theme. So, questions might arise as what should be the ethical way of presenting and interpreting this scientific data and by whom? Who is the most trustworthy person in a medical practitioner's point of view to be consulted for the prescribing information of a drug? The representative of a pharmaceutical company, the hospital pharmacist or the pharmacist working as a pharmaceutical company representative? This presentation will encompass these important questions in an attempt to develop and suggest guidelines for providing better healthcare facilities and will meet the need of monitoring drug marketing.

THE EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE EXTRACT OF ANGSANA LEAVES (*PTEROCARPUS INDICUS* WILLD.)

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A study has been conducted on the *in vitro* antibacterial activity of the *Pterocarpus indicus* Willd. leaves extract against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The leaves extracts were prepared by percolation using ethanol as solvent and the ethanol extract was then fractionated with chloroform and n-hexane. The *in vitro* antibacterial activity was determined by measuring the diameter of inhibition zones in Mueller-Hinton agar medium by Kirby Bauer method. The ethanol extract of Angsana leaves exhibited a strong inhibition against the growth of *Staphylococcus aureus*, but only weak inhibition against *Streptococcus pyogenes*. The chloroform and n-hexane extracts of Angsana

leaves did not inhibit the growth of all bacteria used in the study. Ethanol extract of *Angsana* leaves is useful as an antibacterial agent against *Staphylococcus aureus*, but not against *Streptococcus pyogenes*.

SCREENING OF *GYNURA PROCUMBENS* FOR ANTITUBERCULAR ACTIVITY

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Tuberculosis (TB) remains a serious health problem in many regions of the world, especially in developing nations. It is a contagious disease and is becoming epidemic in some parts of the world. It is estimated that 30%–60% of adults in developing countries are infected with *Mycobacterium tuberculosis*. Therefore, it is essential to have new antituberculosis agents, preferably those that can be readily and simply produced from some local source. In this study, we investigate the antitubercular activity of *Gynura procumbens* or locally known as 'sambung nyawa'. Different extracts of *Gynura procumbens* (methanol, pet. ether, ethyl acetate, chloroform and butanol) were investigated for its antitubercular activities using two conventional methods, 1% proportion method and disk inhibition zone. Results from both methods indicated that only the methanol, pet. ether and ethyl acetate extracts showed promising results with a minimum inhibitory concentration (MIC) of 15 µg/ml. The chloroform and butanol extracts did not exert any inhibition action on *Mycobacterium tuberculosis*. Thus these results, though preliminary, may suggest the possible role of *Gynura procumbens* as an antitubercular agent.

MORPHOLOGICAL ALTERATIONS OF BACTERIA DUE TO THE ANTIMICROBIAL PROPERTY OF NUFERA EXTRACT

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Local food such as coconut has contributed to traditional folk medicine for decades in Asia. With the reemerging of pathogens, coconut products raw and processed have great potential for the development of new drugs especially against resistant organisms. The main objective of this work was to determine the presence of anti-bacterial activity of the coconut extract via observing morphological alteration of the bacteria after being exposed to the oil extract. The sensitivity plate method was employed using methicillin resistant strain of

Staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa*. Observation using scanning electron microscopy reveals morphological changes on bacteria when exposed to the essential oil extract. The outer membrane were distorted and some had holes or pore formation on its surface. The micrograph also showed the formation of "finger-like" projection on bacterial surface, which was commonly seen in other membrane active agents. Such disrupted membrane picture reveals the mode of antibacterial action of the extract on the selected microorganisms.

ANTIMICROBIAL ACTIVITY OF THE NUFERA™ OIL EXTRACT ON GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Cocos nucifera, traditionally consumed as food is recognised as having specific pharmacological properties. The multifunctional food not only provides energy but claimed to possess antimicrobial fatty acids in its raw state. The aim of this study was to evaluate the antimicrobial activity of the oil extract of Nufera, a commercially available food product of *Cocos nucifera* on different groups of microorganisms. The extract was subjected to different solvent composition and concentrated on a filter paper disc. The air-dried extract disc was placed on to the agar plate of lawn bacterial colonies to examine inhibition of growth of the test microorganisms. The inhibitory effect of the extract was most effective for *Streptococcus pneumoniae*, *Candida albicans*, and *Staphylococcus aureus* including the methicillin resistant strain while for the Gram negative group of bacteria *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Vibrio cholera* also demonstrated clear zone of inhibition. However, the strains of *Streptococcus viridans*, *Bacillus sp.*, and *Klebsiella sp* showed resistance against *in vitro* treatment of the extract. The investigation presently confirms that there is a relatively high degree of limited antibacterial activities in the processed extract of Nufera. Further identification of the active fraction would enhance the validation of the potentiality of the extract as the future antibacterial agent.

ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF THE EXTRACTS FROM *PHYLLANTHUS RETICULATUS*'S LEAVES**ROZAINA MAHMOD, TENGKU SIFZIZUL TENGKUL MUHAMMAD AND SHAIDA FARIZA SULAIMAN**

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Acetylcholinesterase is one of the major forms of brain cholinesterases besides butyrylcholinesterase. It functions as a tetrameric protein that catalyses the hydrolysis of acetylcholine to prevent progressive loss of cholinergic neurons in Alzheimer's disease. The study goal is to determine acetylcholinesterase inhibition from the leaves of *Phyllanthus reticulatus*. Five crude extracts were tested using eight different concentrations and 80% methanol extract showed the lowest IC₅₀ value, i.e. 6.609 µg/ml. The assay was also tested against fractions of 80% methanol extract. Out of 50 fractions, Fmp 3 exhibited the lowest IC₅₀ value, i.e. 0.001 µg/ml. Further purification and isolation of bioactive compounds from Fmp 3 will be carried out to elucidate the structure.

STORAGE, THERMAL STABILITIES AND ANTIOXIDATIVE ACTIVITY OF *CARISSA CARANDAS* ANTHOCYANINS: EFFECTS OF CO-PIGMENTATION**SITI RAFIDAH YUSOF AND SHAIDA FARIZA SULAIMAN**

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Co-pigments were added in *Carissa carandas* syrup to determine the effect of co-pigmentation on storage, thermal stabilities and antioxidative activity of Pelargonidin 3-*O*-glucoside. Ten co-pigments selected for the study are: quercetin, naringin, rutin, (+)-catechin, L-tartaric acid, caffeic acid, aluminium chloride, iron(II) sulphate, iron(II) chloride and gum arabic. Spectrophotometric analysis was carried out and experimental results indicated that Pelargonidin 3-*O*-glucoside-copigment complexes showed good stability up to 60 days of storage at room temperature (in the absence of light). No microbial growth occurred in the stored syrup. Thermal stability of Pelargonidin 3-*O*-glucoside was also improved after the addition of copigments. Pelargonidin 3-*O*-glucoside-caffeic acid and Pelargonidin 3-*O*-glucoside-rutin complexes exhibited the highest stability for both storage and thermal treatments. The antioxidative activity was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Syrup with caffeic acid and (+)-catechin as copigment with the concentration of 0.2 mg/ml showed the best antioxidative activity with 40% and 37% of radical scavenging activity respectively.

SCAVENGING EFFECTS OF *TRIPHASIA TRIFOLIA* EXTRACTS ON 2,2-DIPHENYL-1-PICRYLHYDRAZYL RADICAL

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The aim of this study was to evaluate the scavenging effects of *Triphasia trifolia* extracts. Thirteen different extracts from leaves, stems and fruits of *Triphasia trifolia* have been tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method. The results indicated that some extracts possess high radical scavenging activity from other extracts after 30 min of incubation period at 37°C. Methanol (EC₅₀ = 0.299 mg/ml) extract from infected leaves of *Triphasia trifolia* indicated the highest percentage of DPPH scavenging activity as compared to other extracts, thus this extract may be considered as a potential source of antioxidants from plant.

THE CYTOTOXICITY EFFECT AND CELL DEATH MECHANISM OF HEXANE EXTRACT OF *MURDANNIA JAPONICA* ON HUMAN HEPATOCELLULAR CARCINOMA CELL LINE, HEPG2

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Traditional medicines are widely used throughout the world. In this study, cytotoxicity of hexane extract of *Murdannia japonica* (MJ) on human liver cancer cell, HepG2, and the mechanism of cell death were determined. Cytotoxicity test was carried out using Methylene Blue Assay (MBA) and reverse-transcription polymerase chains reactions (RT-PCR) was used to identify the apoptotic gene expression of HepG2 cell line treated with the extract. The results obtained indicate that the EC₅₀ of the extract was 37.17 µg/ml. EC₅₀ concentration was found to induce the expression of caspase-3 gene at 4, 8, 12, 16 and 24 h of incubation. The greatest expression of caspase-3 was observed at the 4th h of incubation. In conclusion, the hexane extract of MJ has the ability of inducing the expression of apoptotic gene which caused the programmed cell death.

BRINE SHRIMP LETHALITY ASSAY OF SELECTED LOCAL PLANTS

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The brine shrimp lethality examination (BSLT) is a general test for preliminary assessment of cytotoxicity activity in plant extracts. In this study the cytotoxicity

activity of ethanol and aqueous extract of three local plants namely *Cleome gynandra*, *Citrus aurantifolia* and *Mikania cordata* were evaluated using brine shrimp (*Artemia salina leach*) lethality assay. The brine shrimp lethality test was carried out using the 96-well microplate method. About 100 µl of the plants extract (3000 µg/ml) was dispensed (in triplicate) into the first and second well of the microplate row. Two fold serial dilutions with 100 µl sea salt solution were made across the plates starting from well number 2 to 8 (inclusive) to give a final concentration of 24.44 µg/ml. The serial dilutions were performed in triplicates. A suspension containing 7–15 mature *Artemia salina nauplii* were then added to each well and the plates were left at 30°C for 24 h. Control wells contained all of the above except the plants extract, being replaced with sea salt solution. The plates were then examined under a binocular microscope (x12.5) and the numbers of dead *nauplii* were counted. About 100 µl of methanol was then added to each well to kill the remaining *nauplii*. The total number of *nauplii* was counted and the average percentage of death and survival at each dose was recorded. The aqueous extract of all three plants evaluated were found to be non-toxic in BSLT with LC₅₀ value of > 2500 µg/ml, 2138 µg/ml and > 3000 µg/ml, respectively. In ethanol extract, *Citrus aurantifolia* exhibited low cytotoxicity activity with LC₅₀ value of 243.2 µg/ml whereas *Cleome gynandra* and *Mikania cordata* did not demonstrate cytotoxicity activity with LC₅₀ value of 2000 µg/ml and 1986 µg/ml respectively. From the results, we suggest that the aqueous and ethanol extract of *Cleome gynandra*, *Citrus aurantifolia* and *Mikania cordata* were inactive in a brine shrimp lethality assay.

METABOLITE PROFILE FINGERPRINT FOR MONITORING BATCH CONSISTENCY OF *ORTHOSIPHON STAMINEUS* EXTRACT

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Monitoring of herbal extract uniformity and consistency is a routine task in quality control of phytopharmaceutical industry. Metabolic fingerprinting via FTIR enables detecting changes in the overall metabolic profiles of the plant extracts prior to targeted metabolite analyses using the standard chromatographic methods (e.g. GC/MS and HPLC). The complex bio (chemical) fingerprint analyzed using chemometric method. To develop a rapid and non-destructive method based on FTIR fingerprinting couple with the chemometric data analysis via Principal Component Analysis (PCA) for monitoring consistency of the extract formulation obtained from different supplier. The FTIR spectra were recorded using Thermo Nicolet FTIR Nexus spectrometer coupled with DTGS (deuterated tri-glycine sulphate) detector (4000–400 cm⁻¹ at resolution 4 cm⁻¹ with 16 scans). Air background spectrum was recorded before each sample and run in triplicates. Spectral preprocessing i.e. baseline correction and normalisation were performed prior to spectral analysis via PCA using *The Unscrambler 9.0* (CAMO, Trondheim, Norway). From the 3D matrix plot, the

presence of the sharp absorption peak at 1600–1760 cm^{-1} assigned to the carbonyl group (C=O), characterised the present of high content of terpenoids and flavanoids the in complex mixture of *Orthosiphon stamineus* extract. Inter-batch variations are observed from the 2D score plot obtained from PCA and thus indicate variability in the (bio) chemical constituent. Sample identity (i.e., supplier, mode of extraction and batch-to-batch consistency) could be monitored easily with the aid of chemometric treatment of the overall metabolite fingerprint. The developed method could be used as a rapid quality control tool for standardisation of phytopharmaceutical product.

ANALYSIS OF *EURYCOMA LONGIFOLIA* JACK (TONGKAT ALI) EXTRACTS USING TASTE SENSOR

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The roots of *Eurycoma longifolia* (*E. longifolia*) Jack have been used as an essential ingredient in herbal medicine. In this study, samples of *E. longifolia* extracts from different locality, parts of plant and mode of extraction were analysed using taste sensor. The methodology for evaluating herbal medicines is based on the bio-sensing principle that mimics human gustatory system through the incorporation of biological lipid material as sensing element. The potentiometric data collected show fingerprints profile of the whole chemical components in the extracts where good reproducibility and repeatability of the results were observed. The collected data were further investigated utilising chemometric algorithm namely Principal Component Analysis. Overall, results generated from the sensing system showed good separation and classification of *E. longifolia* based on the plant's botanical identity i.e. parts, source and also the mode of extraction and drying method.

ASSESSMENT OF HERBAL CRUDE AND EXTRACT OF *LABISIA PUMILA* BY CHEMOMETRICS - ASSISTED INTERPRETATION OF FTIR

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Pharmacognosical analysis of medical herbs remains as a challenging issue for analytical chemists, as herbs are a complicated system of mixtures. The application of infrared (IR) spectroscopy in herbal analysis is still very limited compared to its application in other areas (food and beverage industry, microbiology, pharmaceutical etc.) This article attempts to expand the use of FTIR spectroscopy and at the same time creating interest among prospective researcher in herbal analysis especially in *Labisia pumila*. A case study was conducted by incorporating appropriate chemometric methods (Cluster Analysis;

CA, Principal Component Analysis; PCA and Soft Independent Modelling of Class Analogy; SIMCA) as tools for extracting relevant chemical information from obtained infrared data. The developed method can be used as a quality tool for rapid authentication from wide variety of herbal samples.

A SIMPLE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF CYCLOSPORIN A

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A simple reversed-phase high performance liquid chromatography (HPLC) method with ultraviolet detection was developed for the determination of cyclosporin A in Gelucire matrices and dissolution samples (0.1 N HCl). Cyclosporin A in Gelucire matrices was initially dissolved in tetrahydrofuran before dilution using mobile phase while the dissolution samples were injected directly into HPLC system. Separation was performed using a GENESIS CN column (250 × 4.6 mm I.D.; 4 µm) maintained at 55°C. The mobile phase consisted of acetonitrile-0.04 M potassium dihydrogen phosphate (57:43, v/v) adjusted to pH 3.5 with 85% phosphoric acid. Analysis was run at a flow rate of 1.0 ml/min with the detector operating at a wavelength of 210 nm and a sensitivity range of 0.005 a.u.f.s. The retention time for cyclosporin A was 6.4 min. The linearity of the assay method was in the range of 0.31 µg/ml to 20.00 µg/ml with a correlation coefficient greater than 0.9999. Limit of detection was 0.05 µg/ml at the signal-to-noise ratio of 3:1 whereas limit of quantification was 0.31 µg/ml. The within-day and between-day coefficient of variation, CV% (denoting precision) values were all less than 2.5% while the accuracy did not deviate by more than 5% from the actual concentration values. In conclusion, the method is simple, precise and accurate for the determination of cyclosporin A in Gelucire matrices and dissolution samples.

SOME PROPERTIES OF PEGAGA, MENGGUDU AND PEGAGA-PINEAPPLE AGGLOMERATES AND COMPRESSED TABLETS

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Pegaga, mengkudu, pegaga-pineapple extracts were dried in a fluidised bed granulator to produce the agglomerates. The spraying process was carried out pneumatically by a 1.0 mm diameter nozzle. One kilogram of ground sugar was used as a carrier in the fluidised bed dryer. The inlet temperature used was 80°C and the process temperature was 50°C in the drying chamber. The fluidising airflow rate used was 20 m³/h. The atomisation pressure at the spray nozzle was 3.0 bar. The flow rate of the peristaltic pump used was 6 g/min. The drying

duration was 2 h. The colour of the agglomerates showed that *pegaga* agglomerate had light green colour, *mengkudu* agglomerate had light beige colour and *pegaga*-pineapple agglomerate had greenish-yellow hue. The moisture content of the agglomerates ranged from 0.43%–0.55%. The bulk density of about 0.76 g/ml of the agglomerates showed that the agglomerates were relatively porous which was desirable for tableting. Tableting was carried out by using a 10-station tableting machine consisting of round-shaped punches and dies. The mean weight of the tablets was less than 1.3 g and the mean thickness was 6.4 mm which were relatively heavier and thicker compared to the pharmaceutical tablets. The mean friability of *mengkudu* tablet at 0.01% was less friable than *pegaga*-pineapple and *pegaga* tablet at 0.31% and 0.42%, respectively.

DETECTION OF BIO-INSECTICIDAL COMPOUNDS FROM MICROPROPAGATED PLANTLETS OF *SPILANTHES ACMELLA* L.

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Micropropagated plantlets of *Spilanthus acmella* L. (*S. acmella*) could be established via shoot proliferation using the axillary buds and nodal segments as the explants. Multiple shoots were successfully induced from the axillary buds using Murashige and Skoog (1962) medium (MS) supplemented with 0.5 mg/l N₆-Benzyladenine (BA). More multiple shoots were produced from vertically placed explants especially in large culture vessels. A higher number of multiple shoots were produced from the nodal segments using the same medium compared to the axillary buds. The best temperature for the growth of the *in vitro* plantlets of *S. acmella* was 24°C–26°C and this range of temperature tended to produce healthy shoots without any morphological abnormality. The acclimatisation protocol which consisted of three weeks incubation in the growth chamber followed by one week under shaded area had resulted in a 100% survival of plantlets when transferred to the field. GC-MS analysis detected the presence of the naturally occurring insecticide, N-isobutyl-2E,6Z,8E-decatrienamide (spilanthol) in the mother plants, the flower heads and the micropropagated plantlets of *S. acmella* with similar retention time (43.18–43.21 min). However, N-isobutyl-2E,4Z,10E-dodecatetraenamide (an isomer of N-isobutyl-2E,4E,8E,10Z-dodecatetraenamide – a potent mosquito larvicide) was only detected in the micropropagated plantlets of *S. acmella*.

**THE ADVANTAGES OF HEAT-TREATMENT OF SERUM IN
SAMPLES AND STANDARD SOLUTION IN LOCALLY
FORMULATED ENZYME IMMUNOASSAY FOR 17OHP**

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Previously we have reported the formulation of a locally made enzyme-based immunoassay system for the measurement of serum and plasma 17 α -hydroxyprogesterone (17OHP) to be used for screening of congenital adrenal hyperplasia (CAH) in newborn. This assay measures total 17OHP in serum or plasma samples without the need of extraction by solvents or buffers. Thus to ensure equivalence of conditions in samples and standard curve, the working solution for setting up the standard curve was prepared from human pooled sera that had been heat-treated and subsequently charcoal-treated to remove endogenous steroids. In this report we would like to provide experimental data on the advantages of using heat-treated serum for the measurement of 17OHP. Heat-treatment extends the sensitivity of the standard curve and thus that of the assay. Heat-treatment of the samples in alkaline buffer denatures the protein and frees up the bound steroid thus allowing the assay system to measure the total free steroid without the need for further extraction. Limited measurements of the baseline levels of 17OHP in Malaysians have been made and results are compatible with those reported in the literature.