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MULTIPLE SITES OF ACTION OF POLYCHLORINATED BIPHENYLS (PCB) IN MAMMALIAN CELLS

KOIBUCHI, N.
Department of Integrative Physiology, Gunma University Graduate School of Medicine, Gunma, Japan

Endocrine disrupting chemicals (EDCs) disrupt development and homeostasis of many organs including brain, liver and reproductive organs. The possible molecular targets of such EDCs have been considered to be nuclear hormone receptors (NRs), where the EDCs act as agonists or antagonists. The NRs such as thyroid and steroid hormone receptors are ligand-dependent transcription factors. Until recently, more attention has been given, particularly among such NRs, to the effects of EDCs on oestrogen receptors (ERs). Recent evidences have shown, however, that EDCs may also disrupt our endocrine system through a non-ER-mediated pathway. Among such EDCs, we have been studying the effect of polychlorinated biphenyls (PCBs) on thyroid hormone (TH) receptor (TR)- and other NR-mediated transcription. TH plays an important role in brain development. Hypothyroidism during pre- and post-natal period results in abnormal brain development known as cretinism in human. Since perinatal exposure to PCB induces a mild decrease in IQ, it has been hypothesised that the effect of PCB may be exerted, at least in part, through the TH system. However, the molecular mechanism of PCB action has not been clarified. Recently, we have confirmed that PCB suppressed TR-mediated transcription not by antagonising TH binding, but by dissociating TR from TH response element at DNA. Such suppressive effect was greater with polyortho type of PCB than coplanar type PCB and dioxins. Furthermore, we have also shown that PCB not only suppressed TR action but it also activated the action of steroid and xenobiotic receptors (SXR) that regulate cytochrome P450 (CYP) mono-oxygenase 3A4 gene expression. Thus, PCB may also affect metabolism of various drugs such as tamoxifen. PCBs also have a direct action on neuronal membrane. By using cultured brain stem neurons, we have shown that PCB induced the increase in the resting membrane potential. On the other hand, it suppressed the depolarisation induced by extracellular stimulus such as low pH. Such changes were accompanied by an increase in intracellular calcium, which then induced an increase in immediate-early gene expression such as c-Jun. In conclusion, PCB may affect multiple pathways to alter the development and homeostasis of many organs such as the brain and liver.

ENDOCRINE DISRUPTOR ISSUES IN JAPAN AND OECD: CURRENT STRATEGIES AND OUR OWN RESEARCH FROM DAPHNIA TO MOUSE

IGUCHI, T.
Center for Integrative Bioscience, Okazaki National Research Institutes, Okazaki and CREST, JST, Japan

Monitoring of environmental chemicals in Japan has revealed that several endocrine active chemicals are found in river water, sediments and wildlife as well as in the human umbilical cord. In 2001–2002, risk assessments of tributyltin, nonylphenol and octylphenol have been conducted by the Ministry of Environment, Japan. Risk assessments of di(2-ethylhexyl)phthalate and di-isononyl phthalate have also been performed by the Ministry of Health, Labor and Welfare using a toxicological point of view in 2001. Currently, monitoring of chemicals in top predators has been conducted. In OECD, several validation management groups are working on establishing testing methods for mammals, using rats; amphibians, using *Xenopus laevis*; birds, using Japanese quail; fish, using medaka, zebrafish and fathead minnow; and invertebrates, and for the standardisation of screening methods, using receptor binding, reporter gene assay, gene expression and 3-dimensional computational methods. In our laboratory, we identified several steroid hormone receptors in fish and alligators. We also found that juvenile hormone agonists affect reproduction of Daphnids. Microarray application is promising in identifying hormone responsive genes and understanding molecular mechanisms of endocrine disrupting chemicals on animal species including humans. An overview of the recent progress in endocrine disruptor research will be provided.

NOVEL REAL-TIME AND SUPERSENSITIVE TECHNIQUES TO ASSESS THE REPRODUCTIVE TOXICITY OF ENVIRONMENTAL POLLUTANTS: *IN VITRO* FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) TECHNIQUE AND *IN VIVO* FUNCTIONAL NUCLEAR MAGNETIC RESONANCE (NMR) IMAGING TECHNIQUE

MANABE, N., SUGIMOTO, M., NISHIZAWA, H. AND IMANISHI, S.
Unit of Anatomy and Cell Biology, Department of Animal Sciences,
Kyoto University, Kyoto, Japan

Most people consume a large amount of food and tap water every day and ingest many types of pollutants present in the food and water. Recently, chemical environmental pollution, including that by endocrine disruptors (EDs), has been a great social problem. However, the toxicological properties of these pollutants have not been fully elucidated. Generally, the levels of pollutants in food and tap water are very low, and the intake period is extremely long. Therefore, supersensitive and real-time assessment of the toxicity of the pollutants is needed. We have developed novel highly sensitive and real-time techniques for the assessment of the reproductive toxicity of environmental pollutants. *In vitro* fluorescence resonance energy transfer (FRET) is a novel technique for visualising the intracellular signal-transducing pathway. We made two constructs. Cyan fluorescent protein (CFP) and oestradiol receptor alpha (ER) were bound with a four-

peptide bridge, and yellow fluorescent protein (YFP) and Heat shock protein 90 (Hsp90) were bound with a four-peptide bridge. These constructs were integrated into expression vectors, and then the vectors were double-infected into hepatoma HepG2 cells. FRET was shown in such transfected cells. When oestradiol and ED-compounds were added, the FRET disappeared. Thus, the real-time signal-transducing process was observed. *In vivo* nuclear magnetic resonance (NMR) imaging is also a novel technique for visualising the 3-dimensional (3D) structure and physiological function of adenosine triphosphoric acid (ATP) metabolism in living mouse embryos. NMR imaging with enhanced spatial resolution due to the use of a strong static magnetic field and highly magnetic field gradients is useful for non-invasive and continuous investigation of objects. Conventional proton magnetic resonance imaging (1H-MRI) is widely used in the clinical field, especially for detection of disorders in soft tissue that cannot be detected by X-ray computed tomography. In our NMR imaging, the spatial resolution is enhanced to sub-millimeter orders, while that in clinical MRI is of the order of 1 mm. Using our NMR imaging technique, non-invasive and 3D observation of small specimens (mouse embryos) can be performed at a level similar to low-power light microscopy. Moreover, we developed *in vivo* 31P-magnetic resonance imaging (31P-MRI) to evaluate physiological properties. Briefly, *in vivo* 31P-NMR spectra were acquired on a FT-NMR spectrometer (JNM-□□□□□-400, JEOL, Tokyo, Japan), which was equipped with a vertical 9.20-Tesla, 89-mm bore (inside gradient) superconducting magnet. To obtain *in vivo* 31P-NMR spectra of mouse embryos, the mother mouse was mounted on a surface coil probe (two-turns, 20 mm in diameter), which was tuned to 31P at 161.7 MHz. The relative ATP levels were obtained by taking the ratio of the beta-phosphate peak of ATP. Human and porcine ovarian tissues transplanted to the renal capsule of severe combined immunodeficient (SCID) mice are useful for evaluating the *in vivo* toxicity of compounds on the functions of the ovary, oocyte, follicle and luteal body. Small tissue blocks prepared from porcine ovaries were placed into the capsule space of the kidneys of SCID mice. After 14 days of xenotransplantation, antral follicles were seen. Xenoplanted SCID mice were orally administered ED compounds. The effects of the ED compounds on the follicle growth and development were estimated. Somatic clone mouse embryos at preimplantation stages, early embryos, are supersensitive against compounds which have developmental toxicity. Somatic cell clone embryos are useful for assessing the toxicity of compounds. Somatic clone mice were made using F1 mice (male JF1 mouse x female 129 mouse). Donor cells were prepared from cumulus cells (ovarian follicular cells). Two-cell, four-cell, morula (segmentation sphere) and early blastocyst embryos of somatic clone mice were incubated in the culture medium containing extremely low levels of ED compounds, and their developmental process was estimated.

LOW-DOSE EXPOSURE TO DIOXINS, THE MECHANISM OF TOXICITIESAND HEALTH RISK ASSESSMENT

TOHYAMA, C.
Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan

Dioxin and related compounds (described as dioxins below) released into the environment mainly via combustion by a large number of incinerators have aroused a serious social concern in Japan for the last several years. The general public feared the possible threat to human health by dioxins. After the re-evaluation of health risk of dioxins in a consultation at the World Health Organisation, the Government of Japan formulated expert committees to re-evaluate the health risk of dioxins and to set up the tolerable daily intake (TDI), a safety standard for dioxin and related compounds in 1999. In the re-evaluation process, laboratory animal data on various toxicity end-points in reproduction, brain, and behavioural and immunological functions were adopted, and the TDI value was recommended to be 4 pgTEQ/kg/day. Since some ambiguity for the extrapolation of animal data to man as well as the toxicity mechanism by dioxins remains to be solved, we have investigated how low level perinatal exposure to dioxins affected various parameters in reproduction, brain, and behavioural and immunological functions, focusing upon the most sensitive period of life, from fertilisation to delivery. In this lecture, I will summarise and discuss our novel findings about toxicities of dioxins from the following four aspects; end-point by low-dose dioxins, critical windows, arylhydrocarbon receptor (AhR) dependency/independency, and species/strain differences in susceptibility. When pregnant Holtzman rats were administered 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on gestation day (GD) 15, androgen receptor mRNA and significant shortening of anogenital distance were suppressed at 50 ng TCDD/kg bw. Another experiment showed that GD15 turned out to be a critical window. In contrast, using a cross-fostering protocol, we found that exposure to dioxins via lactation and not via placenta, is a prerequisite for the disruption of thyroid hormone and retinoid metabolism. The immuno-suppression was found in mice exposed to TCDD in adulthood, but female mice born to dams exposed to TCDD *in utero* had a higher IgE in serum after antigen treatment, suggesting the possibility of the development of enhanced allergic reaction, depending upon the time of exposure. The use of AhR-null mice showed that most of the above-mentioned effects so far examined were mediated via AhR. On the other hand, *in utero* and lactational exposure to polychlorinated biphenyl (PCB)77 and PCB153 resulted in the disruption of thyroid hormone homeostasis in an AhR-independent manner. This independency was shown by experiments in which rats exposed to these PCB isomers had suppressed level of serum thyroid hormones but without the elevation of UGT1A6 mRNA as well as CYP1A1. To evaluate the sensitivity of the response in humans to exposure to dioxins, we compared TCDD-induced teratogenicity, such as cleft palate and hydronephrosis, among three mice strains, C57Bl/6, DBA/2 and humanised AhR knock-in mice. Interestingly, humanised AhR knock-in mice responded least to TCDD, strongly suggesting that humans are relatively less sensitive than other animal species. At the same time, a very similar incidence of hydronephrosis among the three strains suggests that factors besides AhR play a significant role in the aetiology of hydronephrosis. We also found that despite the identical base sequence of AhR in Holtzman and Sprague-Dawley rats, the placenta from

Holtzman rats was found to be more vulnerable to low doses of TCDD, suggesting the possible involvement of modifying factors for the placental-foetal toxicities.

ENDOCRINE DISRUPTING CHEMICALS (EDCs): A MALAYSIAN CONTRIBUTION

MUSTAFA, A.M.
Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Endocrine disrupting chemicals (EDCs) are a group of chemicals that may cause disruptions in the functions of the endocrine system. These chemicals include industrial chemicals, pollutants, drugs, agricultural chemicals such as pesticides, chemicals used for domestic and medical purposes, and chemicals found in vegetables and fruits. This paper will focus on several EDC groups of chemicals, the phyto-oestrogens, bisphenol A and some pesticides. These include isoflavonoids, which are plant products that are essentially confined to legumes. They were classified as "phyto-oestrogens" because of their oestrogenic properties. Studies have suggested that phyto-oestrogens possess a similar activity as the natural hormone, oestrogen. Phyto-oestrogens are weaker than natural oestrogens. Bisphenol A also possesses oestrogenic effects and is distributed in the environment through leaching from plastic bottles, cans and many other domestic products. People who consume soy products or phyto-oestrogen pills as a natural therapy may be exposing themselves to some health risks. Studies on Malaysian vegetarian diet showed the presence of phyto-oestrogens at an average level of 4–6 mg of diadzein and genistein/gm dry weight of the fresh product. Consumption of high amounts of these compounds daily with soya milk and other vegetables may result in high plasma levels of genistein and diadzein. In individuals taking 300 gm of *tofu fa* and 325 ml of soya milk, the plasma levels of diadzein and genistein can reach a level of 10–20 ng/ml six hours after ingestion.

SCREENING/TESTING SCHEME FOR ENDOCRINE DISRUPTING CHEMICALS

KANNO, J.
Cellular and Molecular Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan

It has been found that the hormonally active chemicals (HACs) in our environment are often monitored to be oestrogenic or anti-androgenic. Therefore, their primary functions have been considered as binding to oestrogen receptors (ERs) and/or androgen receptors to induce a sequence of receptor-mediated biological events. On the other hand, the endocrine disrupting chemicals (EDCs) can be defined as those HACs that induce adverse effects in intact organisms or chemicals that induce “receptor-mediated toxicity”. It is easy to develop a screening system for hormonal activities. However, it would be rather difficult to develop the “definitive testing(s)” that can assess the adverse effects of HACs. Provisionally, the large-scale bioassays such as traditional multigeneration studies and its

variants are considered as the definitive testing. These types of testing are costly and time-consuming, so that the number of chemicals to be tested is very much limited. Other possible test protocols that would be able to monitor receptor-mediated toxicity are also considered to become as large-scale as the traditional multigeneration tests. Under these circumstances, the MHLW took a strategy to compile the Screening/Testing Scheme which comprises two components, that is, a screening system to generate the prioritised chemical list from tens of thousands of chemicals surrounding us, and a definitive testing system to assess adverse effects of chemicals that are given high priority in the prioritised chemical list. To effectively make a prioritised list of chemicals, the screening system was composed of three elements. These are, the *in silico* 3D-QSAR for virtual screening of receptor binding capability, *in vitro* reporter gene assay using HeLa cell based stable transfectants for ER alpha and ER beta, and as the *in vivo* screening, the uterotrophic assay (for oestrogenic chemicals) and the Hershberger assay (for androgenic chemicals). The order of chemicals listed in the prioritised chemical is constantly being revised as new data are received. The "definitive testing" is under development. Recognising the limitation of the traditional multigeneration studies, we are proposing a "rodent full life cycle test (one life span test)" which is aimed at monitoring neurological, immunological and endocrinological end-points from conception to senescence. In addition to the reproductive end-points, this test is expected to cover development, maturation, maintenance and senescence of the Neuro-Immuno-Endocrine network, including social behaviours and immune status.

HEPATOPROTECTIVE ACTIVITY OF THREE LOCAL *PHYLLANTHUS* SPECIES: *PHYLLANTHUS NIRURI, PHYLLANTHUS URINARIA* AND *PHYLLANTHUS DEBILIS*

ABDUL HYE KHAN[1], MUNAVVAR ZUBAID ABDUL SATTAR[1] AND PHANG, N.L. [2]

[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2]Nova Laboratories Sdn. Bhd., Sepang, Selangor, Malaysia

Healing properties of traditional plants are being investigated in light of recent scientific developments throughout the world for their potent pharmacological activities, low toxicity, and economic viability. Liver disease is one of the major public health problems in the present time. The conventional therapeutic approaches are sometimes inadequate and limited due to serious side-effects, and this urges the need for alternative approaches. In line with our efforts on developing novel hepatoprotective drugs of plant origin, we investigated three homegrown *Phyllanthus* species for putative hepatoprotective effect. In several prominent traditional health care systems, the plants of this genus are used for numerous ailments, including liver diseases. In the present study, we used carbon tetrachloride intoxicated animal model using male ICR mice (Charles River strain) to evaluate hepatoprotective activity. Carbon tetrachloride produces a toxic insult on the target organ largely through its metabolite, the trichloromethyl radical ($CCl^{3\bullet}$). A possible role of superoxide radicals is also suggested in such toxic insult. In our study, a substantial liver protective activity was observed by the crude methanolic extracts of all the three plants as evident from the significant ($P < 0.05$) dose-dependent (60, 120 and 180 mg/kg/day) reduction in the marker enzymes (SGPT and SGOT), as compared to the

control. Moreover, we found *Phyllanthus niruri* offered better protection over carbon tetrachloride-induced liver toxicity than the other two species. From our preliminary findings, we speculate that a possible antioxidant effect of these plants might be responsible for the observed liver protective activity.

SCREENING OF LOCAL PLANTS FOR ANGIOTENSIN-CONVERTING ENZYME INHIBITION

WONG, W.J.[1], SAM, C.K.[1] AND CHENG, H.M.[2]
[1] Institute of Postgraduate Studies, University of Malaya, Kuala Lumpur, Malaysia
[2] Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Our laboratory has been testing the antioxidant activity of botanical samples. We have now extended the analysis to include antihypertensive properties, such as angiotensin-converting enzyme inhibition (ACEI) activity, since the renin-angiotensin system (RAS) pathway has recently been shown to also modulate the oxidative balance. A sensitive, fixed-time, spectrophotometric assay for angiotensin-converting enzyme (ACE) measures the rate of hippuric acid generation from hippuryl-L-histidyl-L-leucine (HHL). The ACE activity from rabbit lung acetone powder extract is based on the colorimetric determination of hippurate using cyanuric chloride (2,4,6-trichloro-s-triazine)/dioxan reagent measured at the absorbance of 382 nm. Using an ACE inhibitor (Zestril®, AstraZeneca, USA) as a standard (25 mg/ml), 20 plants were investigated for their ACEI activity. For some of the samples, the leaves, stems and roots were separately tested. Aqueous extracts (extraction ratio: 0.1 g/ml) were prepared. For lemon (*Citrus hystrix*), inhibition of ACE activities relative to Zestril® were 25% (leaves) and 9.9% (stems). For mint (*Mentha arvensis* L), inhibition of ACE activities were 33% (leaves) and 30% (stems). The leaves of most plants showed the greatest level of ACEI activity. For parsley (*Coriandrum sativum* L), the relative ACEI of the plant parts were different; 0% for leaves, 65% for stems, and 91% for roots. For Chinese boxthorn (*Lycium chinense* Mill), inhibition of ACE activities were 15% (leaves) and 52% (stems). No ACEI was found for the leaves or the stems of mustard (*Brassica juncea* L), guava (*Psidium guajava* L), sweet potato (*Ipomoea batatas*), cogon grass (*Imperata cylindrical* L Beauv) and carrot (*Daucus carota* var *sativa*).

EFFECTS OF FLAVONOIDS ON ENDOTHELIAL DYSFUNCTION IN AORTA FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

AJAY, M.[1], MUSTAFA, A.M.[1], ACHIKE, F.I.[2] AND MUSTAFA, M.R.[1]
[1] Department of Pharmacology, Faculty of Medicine, University of Malaya
[2] International Medical University, Kuala Lumpur, Malaysia

Diabetes mellitus (DM) is a known risk factor for the development of cardiovascular disease. Diabetes mellitus has been shown to be associated with impaired endothelial function, as demonstrated by decreased endothelium-dependent relaxations (EDR), which is postulated to be the result of increased free radical (ROS) production. ROS inactivates endothelium-derived nitric oxide (EDNO) to peroxynitrate, and hence leads to oxidative

stress. Flavonoids have been shown to have cardiovascular beneficial effects. This study investigated the effects of quercetin, a bioflavonoid, on the reactivity of arteries from streptozotocin (STZ)-induced diabetic rats. The contractile responses to the α-adrenoceptor agonist, phenylephrine (PE), and the EDR to acetylcholine (ACh) were markedly increased and decreased, respectively, in STZ-diabetic aortas compared with age-matched euglycaemic controls. The maximal vasodilation responses to endothelium-independent vasodilator sodium nitroprusside (SNP) were slightly reduced in diabetic aortas compared to controls, whilst sensitivity of diabetic aortas to SNP remained unaltered. In addition, in the presence of ω-nitro-L-arginine methyl ester (L-NAME), the PE-induced contractions remains comparable in quercetin-treated diabetic aortas compared with untreated diabetic aortas. From these findings, it is concluded that quercetin modulates endothelial dysfunctions in STZ-induced diabetic rat aortas by preventing NO inactivation and this may explain, at least in part, the reported beneficial actions of flavonoids on the vascular complications associated with diabetes.

POSSIBLE TOXIC EFFECTS OF *TINOSPORA CRISPA* ON LIVER AND KIDNEY OF SPRAGUE-DAWLEY RATS

TAN, P.T., CHAN KIT LAM AND ABAS HAJI HUSSIN
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

Tinospora crispa (*T. crispa*) Miers, locally known as 'akar seruntum', has been used as an herbal remedy for diabetes mellitus, hyperglycaemic and metabolic disorders. This study investigated the possible toxic effects of *T. crispa* on the kidney and liver of Sprague-Dawley (SD) rats. Doses of 10, 100, and 500 mg/kg of the chloroform extract derived from methanolic-soluble residue of *T. crispa* were administrated orally to female and male SD rats (n=6) for 14 days. The control groups were treated with the respective vehicles. The blood samples were collected by cardiac puncture. Serums alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), urea and creatinine were determined using COBAS INTEGRA® model 700. The results showed that only the adult male rats fed with 500 mg/kg of *T. crispa* had a significant increase in the ALT level ($P < 0.05$), as compared to their respective control groups. However, the other biochemistry parameters were not significantly affected. The toxic effects observed were found to be sex and age dependent. In conclusion, caution should be observed for chronic intake of 500 mg/kg or more of *T. crispa* because of possible toxicity to the liver.

THE HYPOGLYCAEMIC ACTION OF AQUEOUS EXTRACT OF *GYNURA PROCUMBENS* IN DIABETIC RATS

ZURINA HASSAN, MARIAM AHMAD AND AHMAD PAUZI MD YUSOF
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

The hypoglycaemic effect of aqueous extract of the leaves of *Gynura procumbens* was examined in normal and streptozotocin (STZ)-induced diabetic rats. The extract at a dose of 1 g/kg body weight reduced the mean blood glucose level of STZ-induced diabetic rats ($p < 0.05$) but not in normal rats. In diabetic rats, the extract reduced the glucose level at hour 2, 5, 6 and 7 when administered by gastric intubation. In glucose tolerance test where the rats were loaded with glucose (500 mg/kg body weight) intraperitoneally to induce hyperglycaemia, the extract did not reduce the glucose levels ($p > 0.05$) to as low as the values produced by glibenclamide (standard drug) in both normal and STZ-induced diabetic rats. The plasma insulin of STZ-induced diabetic rats was also measured using rat insulin ELISA kits. The result obtained showed that the aqueous extract did not significantly increase plasma insulin in these rats. In another set of experiments, the extract did not produce stimulation of insulin secretion from the RIN-5F cell line, a clonal pancreatic β-cell. Taken together, these findings indicate that the hypoglycaemic activity of the aqueous extract of *Gynura procumbens* leaves involves an extra-pancreatic action and is not due to an insulinotropic activity.

THE HYPOGLYCAEMIC PROPERTIES OF *OXALIS BARRELIERI* AND ITS EFFECT ON WOUND HEALING

KUMAR, P.E.[1], TAUFIK, M.[1], AHMAD, Z.[1], HAKIM, N.[1], SULAIMAN, R.[1] AND ITHNIN, H. [2]
[1] Departments of Biomedical Sciences and [2] Clinical Sciences,
Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Oxalis barrelieri, also known as 'belimbing tanah' in Malaysia, is believed to be an herbal remedy for diabetes mellitus. Diabetes mellitus gives rise to many complications, including delayed wound healing. The objective of this study is to determine the hypoglycaemic properties of *Oxalis barrelieri* and its effect on wound healing. Sprague Dawley (SD) rats used in the experiment were divided into four groups: normal control (N), diabetic control (DC), diabetic rats treated with extract of *Oxalis barrelieri* (TX) or Diabetmin® (DMIN), a commercial anti-diabetic drug. *Oxalis barrelieri* extraction was prepared in 95% ethanol. Diabetes mellitus was induced in three groups (DC, TX and DMIN) by intraperitoneal injection of streptozotocin (60 mg/kg body weight) in citrate buffer. Group DC received only the vehicle, which is olive oil. Group TX received the ethanol extract of *Oxalis barrelieri* (300 mg/kg). Group DMIN received Diabetmin® in distilled water. All treatments were given orally on alternate days for four weeks. Overnight fasting blood glucose level was determined at the end of each week by using Optium® glucose strips. Data was analysed using a one-way ANOVA. On week 1, all rats

showed normal blood glucose level. On the second week, after the injection of streptozotocin, all the three groups (DC, TX and DMIN) showed elevated glucose levels of approximately 400 mg/dl. Significant differences ($P < 0.05$) were observed in week 2, 3, 4, 5 and 6 for all the treatment groups compared to those in group N. The DMIN group showed significant differences ($P < 0.05$) on weeks 3, 4, 5 and 6 when compared with the DC group. The TX group showed a significant decrease ($P < 0.05$) of blood glucose level on the 4th week when compared with group DC. Group TX exhibited lower blood glucose levels when compared with group DC. The trend of blood glucose level of TX group and DMIN group was the same and this indicated the potential hypoglycaemic property of the plant. This finding shows that the extract of *Oxalis barrelieri* has hypoglycaemic effects on diabetic-induced rats. Wound healing studies were carried out on another 24 SD rats divided into four groups. Division of groups, treatment and condition were the same as in the previous study. Treatments were given daily. A two-centimetre long, full-skin thickness surgical wound was created by a midline incision on the dorsal surface and allowed to heal. On the 6th and the 9th day, three rats were randomly selected per group. The wounds together with the surrounding area were excised and processed for haematoxylin and eosin staining. Qualitative assessments showed that the *Oxalis barrelieri* extract treated group promoted angiogenesis. Epithelisation and collagen formation were also enhanced significantly in rats from group TX. Congestion, necrosis and infiltration of lymphocytes were reduced in group TX. As a conclusion, this study confirms the potential hypoglycaemic and wound healing properties of the *Oxalis barrelieri* ethanolic extract on diabetic rats. Thus, this study has revealed the scientific basis for the traditional use of this plant.

THE EFFECTS OF ANGIOTENSIN II, ADRENALINE AND VALSARTAN ON ISOLATED BOVINE CORONARY ARTERY

KU ZAIFAH, N. AND RAZAK, T.A.
Department of Basic Medical Sciences, Kuliyyah of Medicine,
International Islamic University Malaysia, Kuantan, Pahang, Malaysia

Coronary artery disease is the main cause of mortality and morbidity in many countries. Angina pectoris is one of its clinical presentations. The basic pathologic change of angina is myocardial ischaemia. Neuroendocrine hormones play an important role in the development of the disease. Angiotensin II and adrenaline are two important neuroendocrine factors in coronary or ischaemic heart disease. Their interaction at the tissue level was the main focus of this study. This is an *in vitro* study that looked at the effects of angiotensin II, adrenaline, and angiotensin II receptor blocker on the isolated bovine coronary artery. The experiment consisted of two parts. The first part was the determination of effective potassium chloride concentration that causes 70% contraction (KCl-EC70) of the bovine artery. The second part of the study was that of the arterial response to various drugs, alone or in combination after precontraction at KCl-EC70. Drugs used (adrenaline, angiotensin II, and valsartan [angiotensin II receptor blocker]) were at five times the normal plasma human value, in order to mimic the plasma neurohormonal levels during myocardial ischaemia in human. The Wilcoxon-signed rank test was used to analyse the data obtained. The concentration for KCl-EC70 was 0.3 g/ml. There was a significant reduction in the contraction of the bovine coronary artery in the

presence of adrenaline alone and in the presence of both valsartan and angiotensin II. Angiotensin II on its own did not cause a significant relaxation or contraction of the artery. No other drug or drug combination caused any significant change. The presence of increased levels of adrenaline induced relaxation of the isolated bovine coronary artery. This relaxation most likely occurred via activation of the beta-adrenoceptors. Angiotensin II probably facilitated and helped maintain the contraction induced by potassium chloride. This effect was abolished by valsartan. It was unlikely for valsartan to act through any other mechanisms as it has a predilection towards the AT_1 receptor. Adrenaline and valsartan at five times the normal plasma human concentration cause bovine coronary artery relaxation. Valsartan may have a promising role in the treatment of myocardial ischaemia due to its indirect vasodilatory effect.

EFFECTS OF DES-ASP-ANGIOTENSIN I ON THE ACTION OF ANGIOTENSIN II IN ISOLATED MESENTERIC VASCULATURE OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

DHARMANI, M.[1], MUSTAFA, M.R.[1], ACHIKE, F.I.[2], AND SIM, M.K.[3]
[1] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur
[2] International Medical University, Kuala Lumpur, Malaysia
[3] Department of Pharmacology, National University of Singapore, Singapore

In recent years, researches have revealed other angiotensin peptides besides angiotensin II that may be an essential component of the renin-angiotensin system. Des-Asp-angiotensin I (DAA-I), a nonapeptide, have been shown to be physiologically involved in the central regulation of blood pressure. This nonapeptide has been shown to attenuate the vascular contractile response of angiotensin III but not that of angiotensin II in rat aortic rings. The present study was conducted to investigate the modulatory effects of DAA-I on angiotensin II-induced vasoconstrictions in the isolated perfused mesenteric arterial bed of normoglycaemic Wistar-Kyoto (WKY) rats and streptozotocin (STZ)-induced diabetic rats. Male rats, aged 12 weeks were injected with STZ (75 mg/kg, ip) to induce diabetes. The control group was given equal volume of the vehicle. After eight weeks, the superior mesenteric arterial bed was excised from phenobarbitone-anaesthetised rats and perfused with oxygenated Krebs at a rate of 5 ml/min. Changes in perfusion pressure to bolus injections of angiotensin II (10^{-10} M–10^{-6} M) were observed before, and 30 min after pre-treatment with DAA-I (10^{-9} M–10^{-18} M). There were no significant differences in the angiotensin II-induced vasoconstrictions between the diabetic and the normoglycaemic animals. Pre-treatment with DAA-I (10^{-9} M–10^{-18} M) attenuated the angiotensin II (10^{-10} M–10^{-7} M) responses in the isolated perfused mesentery of the normoglycaemic rats. However, DAA-I failed to reduce the angiotensin II responses in the diabetic rat. PD123319, an AT_2 receptor antagonist, did not affect the attenuation of angiotensin II in the presence of DAA-I, suggesting that the nonapeptide does not act through AT_2 receptor. Indomethacin blocked the attenuation of angiotensin II-induced vasoconstrictions by DAA-I, suggesting that the nonapeptide may be acting through indomethacin-sensitive angiotensin receptor. The present results suggest that DAA-I possess vasomodulatory actions against angiotensin II, and this protective action of the nonapeptide may be compromised in diabetes.

THE ROLE OF PERIPHERAL SYMPATHECTOMY ON RENAL HAEMODYNAMICS IN HYPERTENSION

WONG, K.Y.[1], MUNAVVAR ZUBAID ABDUL SATTAR[1], NOR AZIZAN ABDULLAH[2] AND EDWARD J. JOHNS[3]

[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[3] Department of Physiology, University College Cork, College Road Cork, Ireland

The sympathetic nervous system (SNS) is an important regulator of the activities of heart and peripheral vasculature. This study examined the role of the peripheral SNS on the control of renal haemodynamics in hypertension. For this purpose, Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were utilised. Chemical sympathectomy was carried out by the administration of 6-OHDA intraperitoneally to animals at a dose of 50 mg/kg on day 1, 100 mg/kg on day 2 and 50 mg/kg on days 5 and 8. In haemodynamic study, the animals were anaesthetised (60 mg/kg ip, sodium pentobarbitone), followed by cannulation of carotid artery and jugular vein and isolation of renal artery. Renal blood flow (RBF) was measured using an electromagnetic flow probe. Arterial blood pressure was measured using a pressure transducer. All data were recorded in computerised data acquisition system and expressed as mean ± S.E.M and compared by two-way ANOVA followed by Bonferroni test with a significance level of 5%. A substantial change was observed in the sympathectomised rats in term of haemodynamics. There was a marked reduction of blood pressure in sympathectomised hypertensive rats. Noradrenaline and phenylephrine were found to exert a significant difference ($p < 0.05$) in the peripheral and renal haemodynamics of both of the normo- and hypertensive rats when administered peripherally or intrarenally. However, a significant change ($p < 0.05$) was observed in the blood pressure when methoxamine was administered peripherally. Moreover, when administered intrarenally it caused a significant reduction in RBF only, in both the normo- and hypertensive rats. Angiotensin II, administered peripherally or intrarenally, caused a significant change ($p < 0.05$) in the peripheral and renal haemodynamics in normotensive rats only. These results further support previous findings that α-adrenoceptors are involved in mediating the pressor responses in both the peripheral and renal resistance vessels, thus indicating an important role of the sympathetic nervous system in hypertension.

THE EFFECT OF CLONIDINE ANALOGUE (AL12) ON BLOOD PRESSURE RESPONSE AND RENAL FUNCTION IN DIABETIC WISTAR-KYOTO RATS

MIA LAZHARI[1], MUNAVVAR ZUBAID ABDUL SATTAR[1], NOR AZIZAN ABDULLAH[2] AND EDWARD J. JOHNS[3]

[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[3] Department of Physiology, University College Cork, College Road Cork, Ireland

The action of clonidine to reduce blood pressure is mediated within the central nervous system by its action at α_2-adrenoceptors to decrease the sympathetic outflow, including that to the kidney. A reduction of blood pressure to the same extent following administration of a clonidine-like compound, AL12, had no apparent effect on renal function. High blood pressure presents a major threat in patients with either type 1 or type 2 diabetes. It greatly increases the risks for complications, such as end-stage renal disease, coronary artery disease, stroke, peripheral vascular and diabetic retinopathy. The purpose of the present study was to compare the effect of AL12 on blood pressure responses and renal function in male diabetic Wistar-Kyoto rats. Male, 250–350 g Wistar-Kyoto rats were used for the induction of diabetes. After one week of acclimatisation in the animal holding facility, the animals were fasted overnight and then injected with a single dose of streptozotocin (STZ), 55 mg/kg, ip. Blood samples for glucose level measurement were taken from the tail vein 48 h after STZ injection and the animals were considered diabetes when blood glucose level reached 16.7 mmol/l. Animals were given orally AL12 10 mg/kg daily, for six days. The animals were kept individually in metabolic cages for 24 h on days 1, 3 and 5 and water intake and urine output were recorded. The urine samples were stored at –20^{0}C for later analysis of urinary sodium using flame photometry. On day 7, the animals were anaesthetised with pentobarbitone sodium (60 mg/kg, ip). After tracheotomy, the left jugular vein and carotid artery were cannulated to allow continuous infusion of saline at 6 ml/kg/h containing pentobarbitone sodium (12.5 mg/kg/h) and to measure the arterial blood pressure, respectively. Methoxamine (2, 4 and 8 μg), noradrenaline (200, 400 and 800 ng), angiotensin II (5, 10 and 20 ng) and phenylephrine (2, 4 and 8 μg) were infused through the jugular vein and the blood pressure responses were recorded. Data (mean ± s.e.m) was compared using the two-way ANOVA and followed by the Duncan test with a significance level of 5%. The results obtained indicated that the group of rats that were given AL12 showed significantly higher blood pressure response to all the agonists. The results in metabolic study demonstrated that the water intake and urine output were increased by 15.9% and 16.1%, respectively ($p < 0.05$), whereas urine excretion of sodium were decreased by 3.6% ($p < 0.05$) when compared with the respective control values. These findings support the hypothesis that AL12 may exhibit actions similar to those of clonidine, but the level of peripheral sympathetic suppression induced by the compound may be greater as reflected by a larger response to the adrenergic agonists.

STUDY OF RENAL HAEMODYNAMICS IN WKY AND SHR BY ADRENERGICALLY AND ANGIOTENSIN II -INDUCED VASOCONSTRICTION WITH OR WITHOUT SELECTIVE ADRENERGIC AND CALCIUM CHANNEL BLOCKERS

AIDIAHMAD DEWA[1], MUNAVVAR ZUBAID ABDUL SATTAR[1], NOR AZIZAN ABDULLAH[2] AND EDWARD J. JOHNS[3]

[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2] Department of Pharmacology, Faculty of Medicine, University of Malaya Kuala Lumpur, Malaysia
[3] Department of Physiology, University College Cork, College Road Cork, Ireland

Our earlier results suggested the co-existence of α- and angiotensin II receptors at the level of the renal resistance vessels in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. This study was designed to study the probable vasoconstrictor effects mediated by α-$_{1D}$ receptors and calcium channels. The animals were anaesthetised with pentobarbitone sodium (60 mg/kg, ip) and a tracheotomy was carried out to facilitate artificial respiration, if necessary. The left jugular vein and the right carotid artery were cannulated for continuous infusion of anaesthesia and measurement of the arterial blood pressure respectively. After a midline incision, the left kidney was exposed and an electromagnetic flow probe was placed on the renal artery to determine the renal blood flow (RBF). The left iliac artery was cannulated such that the beveled tip of cannula faced the renal artery. The renal nerves were placed on bipolar electrodes for electrical stimulations. A mixture of saline and pentobarbitone sodium (12.5 mg/kg/h) was infused (6 ml/h) close-renal arterially. Upon completion of the surgery, 2 ml of saline were injected intravenously as a primer and the animal was allowed to stabilise for an hour. The reductions in RBF to electrical stimulation (1, 2, 4, 6, 8 and 10 Hz at 15 V, 2 ms for 15 s), bolus doses of phenylephrine (0.25, 0.50, 1.0 and 2.0 μg), methoxamine (1, 2, 3 and 4 μg) and angiotensin II (2.5, 5.0, 10 and 20 ng) were determined before and after bolus doses of BMY 7378 (100 and 200 μg/kg plus 25 and 50 μg/kg/h, respectively) and amlodipine (200 and 400 μg/kg plus 50 and 100 μg/kg/h, respectively). Data (mean $\pm$ s.e.m) were compared using the two-way ANOVA and followed by Bonferroni test with a significance level of 5%. The results showed that renal vasoconstrictor effects were attenuated by BMY 7378 in both the WKY and SHR except for the responses to methoxamine in the WKY, and to angiotensin II in both the WKY and SHR. Administration of amlodipine resulted in a significant reduction in renal vasoconstrictor responses in the WKY and SHR. These data collectively suggest that the renal haemodynamics regulation at the level of the renal resistance vessels in WKY and SHR by the sympathetic and local renin-angiotensin systems is also mediated through calcium channels, but not through the α-$_{1D}$ except in the SHR.

EFFECTS OF CENTRAL ADMINISTRATION OF ANGIOTENSIN PEPTIDES AND ANALOGUE ON WATER INTAKE AND BLOOD PRESSURE IN RATS

MOK, J.S.L[1], TAN, S.K.[2] AND ABDUL GHANI, S.R.[2]
[1] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2] Elective Phase Two Medical Students, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

It is well-established that central injections of angiotensin (A) II and its precursor, AI, effectively increase the arterial blood pressure and also stimulate drinking in several species of animals. In contrast, the heptapeptide A-(1-7), which is a biologically active product of AI, was found not to possess any dipsogenic or vasoconstrictor activity. Angiotensin III (AIII), however, was found to produce similar or reduced pressor and dipsogenic responses when administered into the brain. So far, the central effects of [Val^5]-AII, a synthetic AT_1 receptor agonist, have not been well-documented. In this study, we evaluated the central pressor and dipsogenic activities of these angiotensin peptides and the analogue. Adult female Wistar-Kyoto rats weighing 220–280 g were anaesthetised with methohexital sodium (Brevital® sodium, Lilly; 40 mg/kg, ip). Intracerebroventricular (icv) cannulae were stereotaxically implanted. At least three days after the operation, the left femoral artery was cannulated using a Week's catheter under the same anaesthesia for the continuous recording of pulsatile and mean arterial blood pressure (MABP) on a MacLab recording system. The animals were allowed to recover overnight before testing. Throughout the experiment, the animals were unrestrained and had free access to water. Dipsogenic and MABP responses to icv AI, AII, AIII, A-(1-7) and [Val^5]-AII, at doses of 0.3, 0.6, and 1.2 μg/kg, were measured. In other separate experiments, the responses to 0.6 μg/kg AI (icv), before and after 15 min pre-treatment with 100 or 300 μg/kg of synthetic angiotensin-converting enzyme inhibitor (ACEI, pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, Sigma), were also measured. Water intake in the 15 min period was estimated by weighing the bottle before and after each treatment. Drinking latency was recorded. Statistical comparisons were made by two-way and one-way ANOVA followed by Bonferroni test. AI and AII produced a similar, dose-related polydipsia, which was significantly different ($p < 0.05$) at 1.2 μg/kg dose compared to that of the lowest dose tested. On the other hand, AIII or [Val^5]-AII also stimulated drinking, but these effects were found not to be dose-related. AIII, however, appeared to produce a dose-related decrease in the amount of water consumed. At the 300 ng/kg dose, AIII produced only 49.4% of the dipsogenic effects of AII. For each drug, drinking latencies were not significantly different at all the dose levels tested. All the peptides, except for A-(1-7), produced similar, dose-related increases in MABP. In contrast, A-(1-7) failed to stimulate drinking or alter the blood pressure at all the doses tested. Pre-treatment with 100 or 300 μg/kg angiotensin-converting enzyme inhibition (ACEI) significantly attenuated the dipsogenic responses to 600 ng/kg AI in a dose-related manner ($p < 0.05$ in both cases). Drinking latency to 600 ng/kg AI was significantly ($p < 0.01$) increased following pre-treatment with 300 μg/kg of ACEI. The pressor response to icv injections of AI was significantly ($p < 0.05$) attenuated only by 300 μg/kg of ACEI. Our data suggests that the central pressor effects are mediated mainly through AT_1 receptors, whereas, the dipsogenic effects are mediated, only in part, via these receptors.

SYNTHETIC PEPTIDES OF MITE TROPOMYOSIN ALLERGEN: TOWARDS FINDING A DIAGNOSTIC USE

SOON, S.C., CHENG, H.M. AND SAM, C.K.
Lab C503, Institute of Postgraduate Studies, University of Malaya,
Kuala Lumpur, Malaysia

When the mapping of both B-cell and T-cell epitope(s) on antigens became a prime activity in immunology, many allergens too, were scrutinised for the exact fragment(s) or peptide(s) (in the case of protein allergens) which can be recognised by specific antibodies leading on to the whole allergic reactions. The correct identification of antigenic epitopes will greatly aid the diagnosis and prognosis of a disease, allergy included. Ultimately, the identification of epitope(s) on allergens as recognised by antibodies particularly IgE, is useful in pinpointing events underlying the genetics and development of allergy, all in the hope of contributing to the advancement of immunotherapy or in the production of vaccines for allergy prevention in the ultimate quest of eradicating or desensitising allergy. Various allergenic components of the house dust mites have been identified as major triggers of allergy. Among these, the tropomyosin component of *Dermatophagoides farinae* was selected, considering its high homology with other invertebrate tropomyosins, frequently present in seafood. Mite tropomyosin was also reported to react with high frequency (80.6%) with specific IgE in the sera. Therefore, both linear and conformational peptides of mite tropomyosin, (designated Der f10) were synthesised as non-cleavable peptides on pins using the Multipin Peptide Synthesis technique. These linear mite tropomyosin peptides proved to be reactive when tested in a modified ELISA pepscan, showing IgE reactivity at four prominent regions representing the location of B-cell epitopes on Der f10. Finer mapping using smaller 5-mer linear peptides confirmed the location of four immunodominant epitopes: EVRAL, LQKEV, VDRLE and EDELV showing over 75% IgE-binding reactivity. To mimic the coiled-coil structure of the tropomyosin, conformational peptides were synthesized. When tested in pepscan, peptides KEARMMAEDADRKYDE, ITDEERMDGLE-NQLKE, EDADRKYDEVARKLAM, EVARKLAMVEADLER, ERAEERAETG-ESKIVE and ETGESKIVELEEELRV further suggested immunodominant sites on the mite tropomyosin. Subsequently, an online programme predicting antigenicity of mite tropomyosin showed good correlation between these mapped epitopes and the predicted ones. These reactive mite tropomyosin peptides can be assembled in a mixture and thus serve as a tool for mite hypersensitivity detection in allergic patients.

ANTIOXIDANT ACTIVITY OF MICROALGAE SAMPLES IN ABTS RADICAL CATION DECOLOURISATION ASSAY

CHUAN, J.[1], CHU, W.L.[2], PHANG, S.M.[1] AND CHENG, H.M.[3]
[1] Institute of Postgraduate Studies, University of Malaya, Kuala Lumpur, Malaysia
[2] International Medical University, Kuala Lumpur, Malaysia
[3] Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Microalgae have been widely used as food materials as well as medicinal ingredients for their therapeutic effects in oriental countries such as Japan, Korea and China. In this study, the antioxidant activity of five species of microalgae, *Chlorella vulgaris* 001 (*C. vulgaris*), *Oocystis* sp. 074, *Spirulina platensis* 161 (*S. platensis*), *Chlamydomonas* UMACC 229 and *Navicula* UMACC 231, were investigated using Trolox equivalent antioxidant activity (TEAC). Both aqueous and ethanolic microalgae fractions were tested. The antioxidant profile in the various growth phases of the microalgae was studied. Aqueous extracts from *S. platensis*, *Oocystis* sp., and *Chlamydomonas* sp. were higher in TEAC activity than ethanol extracts. In all the species, there was a general pattern of increasing antioxidant activity in the aqueous extracts sampled every 5 days for 30 days. For the ethanolic extracts, the peak increase in antioxidant activity appears to occur earlier, about 18 days. The Antarctic microalgae species, *Navicula* sp. was unique in that there was a progressive decreasing antioxidant activity from the highest TEAC activity at day 2 (2.8 mM) to 1.5 mM at day 18. Aqueous extracts of *S. platensis*, *Oocystis* sp., and *C. vulgaris* showed the highest TEAC values, at around 3.0 mM. The other two microalgae species gave hydrophilic TEAC values of around 2 mM. Thus, in general, TEAC values for both aqueous and ethanol extracts gradually increased from lag phase to exponential phase, after which the values decreased slightly during the linear growth phase. The observations indicate that growth properties and growth conditions of microalgae are accompanied by changes in their free radical scavenging activity.

EVALUATION OF MULTIPLE CARDIAC PUNCTURE SAMPLING ON THE BLOOD PROFILE OF RATS AND ITS USE IN HYPERURICAEMIC STUDIES

VIKNESWARAN, M. AND CHAN, K.L.
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

The methods frequently used for multiple blood sampling in rats are jugular vein cannulation, tail-cut, cardiac puncture and retro-orbital sinus. This study was undertaken to investigate the effects of multiple blood sampling by cardiac puncture on the blood composition of rats and to apply the method in antihyperuricaemic studies. The experiments were performed in groups (n = 6) of adult male Sprague-Dawley rats. In the first experiment, three intraanimal cardiac puncture blood samples were taken from rats at intersample intervals of 1, 3, 5 and 7 days (referred as Group 1, 3, 5 and 7, respectively) and their blood composition was each determined. In the second experiment, hyperuricaemia was induced chemically using potassium oxonate (200 mg/kg) and uric acid (1 and 2 g/kg). Blood samples were obtained by cardiac puncture and their uric acid

concentrations were determined. Rats tolerated this simple blood collection technique as evidenced by the absence of overt morbidity or abnormal behaviour. In Group 1, the multiple sampling caused a significant ($p < 0.01$) reduction in red blood cells count, haematocrit and haemoglobin concentration, whereas in Groups 3 and 5, there was a significant ($p < 0.01$) reduction in haematocrit and haemoglobin concentrations. In Group 7, there was a significant ($p < 0.01$) reduction only in the mean corpuscle haemoglobin concentration. However, there were no changes observed in the mean corpuscle volume, mean corpuscle haemoglobin, platelet count, mean platelet volume and white blood cells count due to multiple sampling in any of the groups. Based on the results obtained, three-day sampling intervals between consecutive blood samples were used for the antihyperuricaemia studies. The use of cardiac puncture sampling in antihyperuricaemia studies showed a significant ($p < 0.05$) increase in the plasma uric acid level in the hyperuricaemic group as compared to the control group.

EFFECT OF DIABETES ON *MORINDA CITRIFOLIA* L ('MENGKUDU') - AMINOPYRINE METABOLISM IN RAT LIVER

AL-MOSALI, M., NORHAYATI ISMAIL AND ABAS HJ HUSSIN
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

The objective of this study is to investigate the *in vitro* effect of fresh aqueous extract of the 'noni' or 'mengkudu' fruit (*Morinda citrifolia* L.; family: Rubiaceae) and two liquid commercial products of 'noni' or 'mengkudu' (Tahitiian and Hawaiian) on the metabolism of a model drug (aminopyrine) in streptozotocin (60 mg/kg body weight; iv)-induced diabetic rat hepatocytes. Our *in vitro* results showed a significant increase in aminopyrine N-demethylase activity in the presence of 100 µg/ml ($P < 0.01$) aqueous extract in the hepatocytes of adult female rats. Aminopyrine N-demethylase activity increased significantly in both adult and young male rat hepatocytes in the presence of 100 µg/ml, but decreased significantly in young female rat hepatocytes in the presence of 0.1, 1.0 ng/ml ($P < 0.05$) and 10 ng/ml ($P < 0.01$) Tahitian 'noni' preparation. Aminopyrine N-demethylase activity was increased significantly in the adult female hepatocytes in the presence of 50 and 100 µg/ml ($P < 0.01$) of Hawaiian 'noni' preparations. The results obtained suggest that the stimulated enzyme activity is dependent on the type of the juice of 'noni' fruit tested and is also dependent on age and gender.

IN VITRO STUDY ON THE INFLUENCE OF *ORTHOSIPHON STAMINEUS* ON LIVER DRUG METABOLISING ENZYMES IN STREPTOZOTOCIN-INDUCED DIABETIC RAT

CHIN, J.H.[1], SABARIAH ISMAIL[2] AND ABAS HJ HUSSIN[1]
[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia,
[2] Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia

Orthosiphon stamineus (*O. stamineus*; 'Misai Kucing') is an herbaceous plant that is widely used in Malaysia to treat kidney problems, gout and diabetes mellitus. The aim of this study is to investigate whether the methanol extract of *O. stamineus* has any influence on

the phase I and phase II liver metabolising enzymes in streptozotocin (STZ)-induced diabetic rat liver. Adult male Sprague-Dawley (SD) rats (180–200 g) were induced diabetese by 50 mg/kg of STZ via intravenous administration, and the liver was used as a source of hepatocytes, microsomes and cytosolic liver fraction. Six concentrations of the methanol extract, ranging from 0.00001 mg/ml to 1 mg/ml, were used in this *in vitro* study. A phase I liver metabolising enzyme known as aminopyrine N-demethylase and two phase II liver metabolising enzymes known as glutathione-S-transferase (GST) and UDP-glucuronosyltransferase (UGT) were studied. The results were analysed by the Dunnett test. From the data obtained, aminopyrine N-demethylase activity in diabetic rats was not affected by the methanol extract of *O. stamineus*. UGT activity in diabetic rats was increased significantly ($P < 0.05$) in the presence of 0.0001 mg/ml and 0.001 mg/ml of the methanol extract of *O. stamineus*. However, in the presence of 1 mg/ml of methanol extract of *O. stamineus*, a significant decrease in UGT activity was observed as compared to the respective control group. For GST assay, GST activity was decreased significantly in the presence of 1 mg/ml of methanol extract of *O. stamineus*. Other concentrations of *O. stamineus* did not affect the GST activity in diabetic rats. It is suggested that methanol extract of *O. stamineus* could affect the activities of phase II liver metabolising enzymes, UGT and GST, but has no influence on the activity of phase I liver metabolising enzyme, aminopyrine N-demethylase, in diabetic rats.

VASORELAXANT EFFECT OF *TRIMERESURUS PURPUREOMACULATUS* VENOM ON RAT AORTIC RINGS

SIM, S.M.[1], ZHANG, W.B.[2] AND KWAN, C.Y.[2]

[1] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

[2] Department of Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Canada

Previous work from this laboratory has shown that the crude *Trimeresurus purpureomaculatus* (*T. purpureomaculatus*; shore pit viper) venom possesses hypotensive and vasorelaxant effects, and that this vascular relaxation effect appeared to consist of endothelium-dependent and endothelium-independent components. Thus, this study aimed to investigate further the possible pharmacological mechanism(s) that could account for the relaxant effect of the crude venom on the vascular system. Rat aortic rings (3–4 mm length) were suspended in 4-ml organ baths containing Krebs-Henseleit solution aerated with 5% CO_2 in O_2 and kept at 37°C. The contractions of the aortic rings were measured isometrically, and the inhibitory effects of the crude venom on the contractile response induced by various stimulants were investigated. Our results indicated that the crude *T. purpureomaculatus* venom relaxed aortic rings pre-contracted with phenylephrine (PE, 1 μM) in a dose-dependent manner (50–200 μg/ml), but had no effect on the contraction induced by 60 mM KCl. Removal of the endothelium and pre-incubation with L-NAME (300 μM) partially inhibited but did not abolish the relaxant response of the crude venom on PE-induced contraction. Furthermore, this relaxant effect of the crude venom (100 μg/ml) on PE-induced contraction was not significantly inhibited by atropine (1 μM), propranolol (10 μM), or indomethacin (10 μM). While barium chloride (30 μM) partially attenuated the vasodilatory effect of the crude venom on PE-pre-contracted aortic

rings, 4-aminopyridine (3 mM) and glibenclamide (10 μM) did not seem to have an affect; but tetraethylammonium (5 mM) totally abolished the vasorelaxant effect of the crude venom. Our findings confirm that the crude *T. purpureomaculatus* venom produces vasorelaxant effect via mechanisms that involve both endothelium-dependent and endothelium-independent components, but it does not appear to act on the L-type Ca^{2+} channel, neither on muscarinic, β_2-adrenergic nor prostaglandin receptors. Our results also suggest that nitric oxide, endothelium-derived hyperpolarising factor, and certain K^+ channels may be involved in mediating the vasorelaxant effect of the crude venom. As the crude venom may contain two or more vasoactive substances, each with its own different mechanism of vasorelaxant action, further investigation using purified fractions of *T. purpureomaculatus* venom may help to elucidate the contribution of each pharmacologically active component of the venom to its overall vascular effect.

SYSTEMIC AND INTRAPERITONEAL EXPRESSION OF IL-6 AND IL-8: THEIR ROLES IN ENDOMETRIOSIS-INDUCED EMBRYOTOXICITY

NOORDIN, L.[1], TAN, G.J.S.[2] AND OTHMAN, M.S.[3]
[1] Department of Physiology, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Malaysia
[2] School of Biomedical Sciences, the University of Notre Dame Australia, Fremantle, Australia
[3] Department of Obstetrics and Gynaecology, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Malaysia

The aetiology of endometriosis-associated infertility remains poorly understood. Peritoneal fluid and serum have long been the focus of investigation as possible mediators of infertility in endometriosis through their toxic effect on early embryo growth. The adverse effect of these biological fluids on early embryo growth may be associated with cytokines, since endometriosis is a local pelvic inflammatory disease. We have shown previously that in women with endometriosis, the peritoneal fluid was embryotoxic. The present study was thus undertaken to determine whether interleukin (IL)-6 and IL-8 that are present in peritoneal fluid or in serum might mediate the embryotoxic effect of endometriosis. Peritoneal fluid and serum were obtained from 21 infertile women with endometriosis of varying severity (7: minimal or mild; 7: moderate; 7: severe) and 7 infertile women without endometriosis. The levels of IL-6 and IL-8 in the peritoneal fluid and serum from both groups were measured using the ELISA method. Two-cell mouse embryos were cultured in 1 ml modified Whitten's medium as previously described, in the presence or absence of IL-6 and IL-8 (100 and 1000 pg/ml, respectively). The embryos were cultured and observed for three days. The levels of IL-6 were significantly higher in the peritoneal fluid with endometriosis as compared to those without endometriosis ($p<0.05$, Mann-Whitney U-test), and were correlated with the severity of endometriosis ($p<0.05$, Kruskal-Wallis test), but not of IL-8. No significant difference was noted in the levels of serum IL-6 and IL-8 between the two groups. IL-6 was found to inhibit the growth of early mouse embryos at each stage of development ($p < 0.001$, Fisher's exact test). IL-8, however, inhibited the development of the morula to blastocyst ($p < 0.001$, Fisher's exact test), but not the early development of the two-cell mouse embryos. The increased levels of IL-6 in the peritoneal fluid from women with endometriosis together

with the embryotoxicity of both interleukins, suggest that interleukins (specifically IL-6) may be involved in the mechanism of embryotoxicity in endometriosis. The elevated levels of peritoneal fluid IL-6 do not correlate with the serum levels suggesting that the changes in the cytokines resulting from endometriosis occur locally. The embryotoxic effect in endometriosis could come directly from the peritoneal fluid.

PATHOPHYSIOLOGICAL DETERMINANTS OF EXERCISE CAPACITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

RAJA AHMAD, R.E.A.[1], JOHGALINGAM, V.T.[1], AMUDHA, K.[2], CHIA, A.[3], SUPPIAH, S.[3], AHMAD, Y.[3], ASMAWI, R.[3], HUSSAIN, N.A.[4], CHOY, A.M.[4], WAN AHMAD, W.A.[4], TAN, K.H.[4], HUSAIN, R.[1] AND LANG, C.C.[4]

Departments of [1] Physiology and [2] Clinical Pharmacology,
Faculty of Medicine, University Malaya
[3] Echocardiography Unit, University Malaya Medical Center
[4] Cardiology Unit, Department of Medicine, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Patients with type 2 diabetes mellitus (DM) have previously been shown to have a decreased level of aerobic fitness, as indicated by lower value of maximal oxygen consumption (VO_2max) during graded exercise test. Limitation in exercise capacity among type 2 diabetic patients is multi-factorial and may be related to the impairment in exercising cardiac output, endothelial dysfunction and skeletal muscle deconditioning. This study aims to define the cardiovascular determinants of exercise capacity in patients with uncomplicated type 2 DM. We hypothesise a relationship between VO_2max and cardiac diastolic function and wall stress, as well as vascular endothelial function. In this on-going study, we have investigated 34 diabetic subjects without overt cardiac complications, and compared them with 24 age- and gender-matched healthy controls. Exercise capacity was reflected by the value of VO_2max, measured during symptom-limited graded treadmill cardiopulmonary exercise testing. Cardiac function was assessed by resting echocardiography. The cardiac neurohormonal level, N-terminal pro-brain natriuretic peptide (NT-ProBNP), was measured at rest, during sub-maximal exercise, and at maximal exercise, to assess intramyocardial wall stress. Flow-mediated flow index (FMD) was established through brachial artery Doppler ultrasound for assessment of vascular endothelial function. Our preliminary data revealed significant reduction of VO_2max in the DM group (25.11 ± 8.20 ml/kg/min; $p < 0.01$), compared to controls (30.96 ± 6.11 ml/kg/min). The NT-ProBNP levels were not significantly elevated during exercise in both groups. There were significant differences between the ratio of early (E) to late (A) peak transmitral blood flow (E/A ratio) and FMD indices between diabetic and non-diabetics patients (1.08 ± 0.36 vs. 1.28 ± 0.33; 2.83 ± 4.53 vs. 6.03 ± 6.44%, both $p < 0.05$). In the DM group, there was a significant inverse correlation between VO_2max and the percentage of glycated haemoglobin$_{A1C}$ (%Hb_{A1C}) and early peak transmitral filling ($r = -0.40$; $p < 0.05$). The VO_2max was also positively correlated with creatinine levels ($r = 0.42$; $p < 0.05$) and percentage of heart rate increment during exercise ($r = 0.58$; $p < 0.001$). Our preliminary data suggest that there is a relative reduction in the exercise capacity, myocardial diastolic function and vascular endothelial function in patients with uncomplicated type 2 DM, compared to their normal counterparts. The limitation of

exercise capacity in the former may be related to the extent of chronic glycaemia, impairment in cardiac diastolic function, as well as an attenuated heart rate response that occur during exercise.

ENDOCRINE-MODULATING EFFECTS OF PHYTO-OESTROGENS ON SELECTED OESTROGEN-DEPENDENT TISSUES OF MICE

JEGATHAMBIGAI, R.[1], KASSIM, N.M.[2], MUSTAFA, A.M.[3] AND OTHMAN, I.[1]
Departments of [1] Molecular Medicine, [2] Anatomy and [3] Pharmacology,
Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Concerns have been raised regarding the role of environmental and dietary oestrogens as possible contributors to the increased incidence of various abnormalities in oestrogen target tissues of both sexes. These abnormalities include breast cancer, endometriosis, fibroids and uterine adenocarcinoma in females as well as alterations in sex differentiation, decreased sperm concentration, benign prostatic hyperplasia, prostatic cancer, testicular cancer, reproductive problems and decreased thyroid hormone levels in males. The study was to determine the effects of phyto-oestrogens on selected morphological and hormonal parameters of adult male and neonatal female mice. Morphological changes in testis, epididymis and thyroid of adult males, and ovaries of neonatal females were determined. Hormonal parameters such as serum and testicular testosterone and serum total triiodothyronine (T_3) were also determined in males. Adult males were treated orally with either genistein or diadzein at low or high doses for 14 days, whereas neonatal female mice were treated with subcutaneous injections of either genistein or diadzein for 7 days at low or high doses. In both cases, control animals received the vehicle only. All the animals were sacrificed 24 h after the last treatment. Reproductive organs and thyroid were dissected, weighed and processed for light microscopy. Serum and tissue samples were analysed for testosterone and total T_3 levels in males. The phyto-oestrogen-treated males showed a decreasing trend in the body-weight and testicular weights. Histologically, the testis showed degenerative changes, multinucleated giant-like cells, immature germ cells and reduction in seminiferous tubule diameters. The epididymis, exhibited a reduction in the number of sperms in most tubular lumens of both groups. There was also a reduction in the epididymal epithelial cell height. There were no significant morphological changes in the thyroids of the treated groups. In the female-treated groups, the ovaries exhibited poly-oocyte follicles with increased incidence in the high-dose groups. The serum and testicular testosterone levels and total T_3 levels were also significantly reduced in both dose groups with greater reduction in the high-dose groups. Hence, exposure of phyto-oestrogens causes significant morphological and hormonal changes in mice. Such exposures may affect the developing foetus and the male reproductive system of mice.

ROLE OF ANDROGEN ON GROWTH AND REGRESSION OF GUBERNA-CULUM DURING DESCENT OF TESTIS

MD YON, H. AND KASSIM, N.M.
Department of Anatomy, Faculty of Medicine, University of Malaya,
Kuala Lumpur, Malaysia

Androgen has been recognised to play a role in the process of testicular descent. Gubernaculum, which is a structure connecting the caudal end of testis to the inguinal region, is the structure believed to pull the testis down towards the internal inguinal ring prior to descent of testis into the scrotum. The present study was conducted to determine the development of muscular component in gubernaculum during testicular descent in rats. Prenatal and neonatal rats were obtained from time-mated pregnant mothers. Animals were divided into control and experimental groups treated with vehicle and an anti-androgen agent, flutamide, respectively. Animals were sacrificed at various stages of development, from prenatal day 18 (E18) to birth. Tissue specimens were processed for light microscopy and immunohistochemical (IHC) staining. The gubernaculum at E18 to E20 of both the control and treated animals were divided into gubernacular cone (Gn) and gubernacular cord (Gc). Gn is a structure consisting of mesenchymal tissue with myoblasts located peripherally while Gc connects the testis to the apex of Gn. The findings from IHC staining of Gn for myosin in muscle fibres of control animals showed positive staining while those of the treated Gn were hardly stained. The muscle fibres in Gn were observed to become more prominent as the animals progressively developed. By E21 and at birth, Gn of control animals had begun to evaginate into the inguinal canal towards the developing scrotal sac. The muscle fibres of Gn in treated animals only began to show positive myosin staining at E21 and at birth; there were no signs of evagination. Hence, androgen is found to be essential for normal growth and regression of gubernaculum, particularly for the development of the muscular component.

EFFECTS OF BISPHENOL A ON THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF JUVENILE MALE SPRAGUE-DAWLEY RATS

KAUR, G.[1], MUSTAFA, A.M.[1], KASSIM, N.M.[1] AND ISMAIL, R.[2]
Departments of [1] Pharmacology and [2] Physiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Bisphenol A (BPA), which is a component of plastic materials, has been classified as an endocrine disrupting chemical (EDC) that causes biological effects. The objective of this study is to determine the effects of BPA on the reproductive system of juvenile male Sprague-Dawley (SD) rats at various doses. Juvenile male SD rats were administered orally with BPA at low, medium and high doses for 42 days. The control group was administered with Tween 80 in distilled water only. The animals were subsequently sacrificed and the testis, epididymis and seminal vesicles were obtained. These tissues were weighed and prepared for histological assessment. BPA at all doses did not affect the weight of the testis, epididymis and seminal vesicle. However, BPA was observed to cause testicular damage in the treated groups when compared to the control. The severity of the

damage increased with increasing doses of BPA. Formation of giant-like cells, sloughing and disruption of intercellular junctions between germ cells were observed even at the lowest dose of BPA used. The seminiferous tubules of the animals exposed to the high dose contained sperms with no tails in the lumen, but instead were filled with cellular debris and immature germ cells. There were also very few maturing spermatids observed in the tubular lining. In addition, the epididymis of all the treated groups was observed to have either empty lumen or be filled with rounded immature germ cells and cellular debris. In contrast, the epididymis of the control group was filled with spermatozoa. These observed histological changes indicated that the treated rats have altered reproductive functions. The present findings show that BPA, even at low doses, may disrupt the male reproductive system.

EFFECT OF ALPRAZOLAM ON PUPS OF PREGNANT ALBINO RATS

DHRUBA, C.
Department of Human Biology, International Medical University,
Kuala Lumpur, Malaysia

Alprazolam is one of the most widely prescribed benzodiazepines, and is generally considered a safe and effective drug for the treatment of anxiety. Alprazolam administration changes EEG data and affects the corticotropin-releasing factor (CRF). However, the safety of the drug in pregnancy and on the newborn has not been well established. It was decided to administer 1 mg/kg body weight of alprazolam orally for the first seven days of pregnancy to the experimental group, to look for the effects of alprazolam on pups and the liver of pups. The same amount of saline was administered to the normal group. On the 20th day of gestation all the animals were sacrificed by cervical dislocation. Thereafter, pups were taken out by laparotomy and their number, length, weight and gross anomalies were noted. All the pups were examined immediately and then fixed in Bouin's fluid. The liver of each pup was taken out and after processing, 10 μm thick serial sections were cut and stained with H and E. It was observed that all the indices were lower in the experimental group. Histological changes in the liver of the experimental pups were noted. The liver of the experimental group revealed fatty degenerative changes and derangements of the hepatic lobular architecture. It can be concluded that administration of alprazolam during the first seven days of pregnancy interferes with normal embryogenesis and may also interfere with placental circulation, since two pups were dead *in utero* in one of the experimental groups.

EFFECTS OF ANGIOTENSIN II ON ISOLATED PERFUSED STREPTOZOTOCIN-INDUCED DIABETIC RAT HEART

GOPAL, S.[1], ACHIKE, F.I.[2] AND MUSTAFA, M.R.[1]
[1] Department of Pharmacology, Faculty of Medicine, University of Malaya
[2] International Medical University, Kuala Lumpur, Malaysia

Angiotensin II (ang II) is the physiologically active product of the renin-angiotensin system and plays an important role in the pathogenesis of arterial hypertension, cardiac

hypertrophy, chronic heart failure, myocardial infarction and fibrosis. Contrasting results have been published on the inotropic effect of ang II in different models and animal species. Using the Langendorff preparation we investigated the effect of ang II on myocardial contractility, coronary perfusion pressure and heart rate in streptozotocin (STZ)-induced diabetic Wistar-Kyoto (WKY) rats and the normal WKY (control) rats. The myocardial contractility was slightly increased in normal rats. In diabetic rats, the myocardial contractility was either not affected by the lower doses of ang II (10^{-13}–10^{-8} M) or was significantly lowered by higher doses (10^{-7}–10^{-6} M). The coronary perfusion pressure was increased in a dose-dependent manner (in both normal and diabetic rat hearts) and was enhanced in diabetic rats. The heart rate was not affected by ang II (10^{-13}–10^{-6} M) in both normal and diabetic hearts. Our results suggest that either AT_1-receptor was down-regulated or AT_2-receptor was up-regulated in diabetic rat myocardium. Coronary smooth muscles were more sensitive in diabetics compared to normal rats, suggesting that AT_1-receptors were up-regulated in coronary smooth muscles in diabetic rats. Our results also suggest that ang II has no effect on the sino-atrial conduction. Further investigations are required.

ANTICARDIAC HYPERTROPHIC ACTION OF LOSARTAN AND ITS EFFECT ON CARDIAC ANGIOTENSIN RECEPTORS

CHEN, W.S. AND SIM, M.K.
Department of Pharmacology, National University of Singapore, Singapore

The effects of losartan on angiotensin receptors in hypertrophic rat hearts were studied. Losartan is the prototype of the new class of drugs known as the angiotensin receptor blockers or ARBs, which act by blocking the angiotensin AT_1 receptor. Losartan is approved for the treatment of hypertension and has recently (September 2002) been approved for nephropathy associated with type 2 diabetes and hypertension. Male Sprague Dawley rats (220 to 250 g) whose abdominal aorta was coarcted were intraperitoneally administered with 0.5 ml saline or various doses of losartan (0.15 to 10 mg/kg/day) for four days. Following this, the ventricles were salvaged and weighed. The hypertrophy index (weight of ventricles in mg/body weight in g) of each rat was determined. Total protein extract was prepared for Western Blot analysis and ventricle membrane was prepared for saturation binding using [^{125}I]-Sar^1-Ile^8-angiotenisn II. Losartan dose-dependently attenuated cardiac hypertrophy in the coarcted rats. Significant effect was observed with a dose of 0.625 mg/kg/day for four days. Higher doses did not produce further increases in anticardiac hypertrophic effect. Cardiac hypertrophy was accompanied by an increase in [^{125}I]-Sar^1-Ile^8-angiotensin II binding, which was mainly due to the up-regulation of AT_2 receptors. Treatment with losartan resulted in dose-dependent suppression of AT_1 and AT_2 receptor protein levels and reduction of [^{125}I]-Sar^1-Ile^8-angiotensin II binding sites. It is suggested that the anticardiac hypertrophic action of losartan is a result of its ability to suppress the expression of enhanced cardiac angiotensin receptors.

THE INVOLVEMENT OF RHO-KINASE IN THE POSITIVE INOTROPIC EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ IN RAT ATRIUM

LING, R.H.L., MARZUKI, A. AND YEW, S.F.
Department of Pharmacy, Universiti Kebangsaan Malaysia,
Kuala Lumpur, Malaysia

Over the past decade, much interest has been generated on the myocardial Ca^{2+} sensitisation. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been reported to produce a positive inotropic effect (an increase in cardiac contractility) by increasing myofibril Ca^{2+} sensitivity through activation of the FP receptor, phosphoinositide breakdown and possibly through activation of the Na^{+}-H^{+} exchanger via protein kinase C. Recently, the FP receptor has been found to activate GTPase Rho, which also has been implicated in enhancing myofibril Ca^{2+} sensitivity. Therefore, the objective of this study was to investigate the involvement of Rho-kinase, a target of GTPase Rho, in the positive inotropic effect of $PGF_{2\alpha}$ in the rat atrium. Left atria isolated from adult male Wistar rats were attached to platinum electrodes mounted in tissue baths containing Krebs-Henseleit buffer (37°C), pulled to a basal tension of 0.5 g and stimulated to contract (40 V, 0.2 Hz, 0.4 ms pulse duration). Developed tension was monitored with an isometric tension transducer and recorded using a Grass 7D Polygraph. $PGF_{2\alpha}$ was added cumulatively (0.0001 to 1 μM) into the tissue bath (first concentration-response curve), and after washout, the Rho-kinase inhibitor Y-27632 (10 μM or 100 μM) was added 30 min prior to the cumulative addition of $PGF_{2\alpha}$ again into the same atrial preparation (second concentration-response curve). Time-matched controls without the addition of inhibitor were also performed in a similar manner. In separate studies, the cytoplasmic fractions of the control or $PGF_{2\alpha}$-stimulated (1 μM, 10 min) left atria were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated in anti-ROCK-II/ROK-β or anti-ROCK-I/ROK-α antibody, and then in anti-mouse horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands, detected by 4-chloronaphtol/diaminebenzidine, were then analysed using a densitometer. $PGF_{2\alpha}$ increased atrial contractility in a concentration-dependent manner by as much as 56.8 ± 2.8% (mean ± SEM, n = 6, basal developed tension = 0.61 g) at 1 μM. The inhibitor, Y-27632, at 100μM depressed the $PGF_{2\alpha}$ positive inotropic effect of the same group of atria so that the maximum increase was only 24.0 ± 4.0% (n = 6, $p < 0.05$, paired t-test). Time-matched studies showed no significant difference between the two concentration-response curves of $PGF_{2\alpha}$ in the same atrium. The density of the immunoreactive bands for ROCK-II/ROK-β, but not ROCK-I/ROK-α, $PGF_{2\alpha}$-stimulated atria was in significantly increased compared to control atria. To conclude Rho-kinase, specifically ROCK-II/ROK-β, is involved in the positive inotropic effect of $PGF_{2\alpha}$ in rat atrium.

MODULATION OF ANGIOTENSIN II-INDUCED CONTRACTION BY REACTIVE OXYGEN SPECIES IN STREPTOZOTOCIN-INDUCED DIABETIC RAT MESENTERIC ARTERIES

CHIN, L.C.[1], MUSTAFA, M.R.[1] AND ACHIKE, F.I.[2]
[1] Department of Pharmacology, University of Malaya, Kuala Lumpur
[2] International Medical University, Kuala Lumpur, Malaysia

Angiotensin II (Ang II) is known to play a fundamental role in controlling the functional and structural integrity of the arterial wall and may be important in pathological mechanisms underlying vascular complications in diabetes. Increased release of endothelium-derived reactive oxygen species (ROS) may influence Ang II-induced contraction of diabetic animal vasculature. This study, therefore, examined the role of ROS in modulating the Ang II contraction in mesenteric arteries from streptozotocin (STZ)-induced diabetic rats and their age-matched controls. Changes in isometric tension of mesenteric arteries in response to graded concentrations (10^{-11}, 10^{-9} and 10^{-7} M) of Ang II were measured using the Mulvany wire myograph. To assess the involvement of ROS the tissues were incubated with ROS inhibitors for 30 min prior to and during Ang II-stimulated contraction. All preparations were endothelium-intact. Ang II-induced contraction was observed to be significantly lower in the diabetic animal mesenteric arteries compared to the normal. Pre-treatment with the hydrogen peroxide (H_2O_2) inhibitor catalase (CAT; 800 U/ml), resulted in significant increases in the Ang II-induced contraction in diabetic but not in normal arteries. In the presence of the superoxide anions scavenger, superoxide dismutase (SOD, 150 U/ml), Ang II-induced contraction was significantly attenuated in both normal and diabetic arteries. These results suggest that the presence of H_2O_2 may explain the reduced contractile effect of Ang II in diabetic tissues, while superoxide anions in both normal and pathological (diabetic) states may enhance the contractile effect of Ang II.

SCANNING ELECTRON MICROSCOPIC STUDIES CONFIRM THE NICOTINE-INDUCED DAMAGE OF OOCYTES

RAJIKIN, M.H.[1], ELDA, S.L.[2], ZAITON, Z.[2], ROZZANA, M.S.[2], GAPOR, A.[3], MEGAT RADZI, M.A.R.[4] AND ABDULLAH, R.B.[5]
[1] Faculty of Medicine, Universiti Teknologi Mara, Petaling Jaya,
[2] Physiology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur
[3] Malaysian Palm Oil Board, Bangi
[4] Electron Microscopy Unit, Pathology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur
[5] Institute of Advanced Studies, University of Malaya, Kuala Lumpur, Malaysia

It has been established that nicotine retards the *in vitro* development of pre-implantation of embryos, reduces the number of litters and causes low birth weight. Since these could be due to the pro-oxidant activity of nicotine, oral antioxidant supplementation may be beneficial in alleviating these detrimental effects. The present study was undertaken to evaluate the effects of nicotine and subsequent supplementation of tocotrienol (T3) on the

development of the oocytes. Three groups of five mice each were treated daily for 30 days as follows: (1) normal saline, (2) nicotine (5 mg/kg body weight [bw]), and (3) nicotine plus tocotrienol (60 mg/kg bw). After 30 days, plasma malondealdehyde (MDA) levels were measured and the scanning electron microscopic studies (SEM) of the oocytes were done. The results showed that the level of plasma MDA in the nicotine-treated group was higher (1.66 ± 0.25 μmol/mg protein) than the control group (0.85 ± 0.14 μmol/mg protein) ($P < 0.05$). However, the plasma MDA level in the nicotine-plus-T3 group was significantly reduced (0.27 ± 0.08 μmol/mg protein) compared to that of the nicotine group ($P < 0.05$). The plasma MDA level in the nicotine-treated group was in agreement with the image obtained from the scanning electron micrograph which showed each of the oocyte being non-spherical with severely torn zona pellucida (zp). Supplementation of T3 produced a more spherical oocyte and an intact zp. Although the surface of the oocyte was rough, the effect was less severe than that of the nicotine group. In conclusion, tocotrienol appears to reduce the detrimental effects on oocytes treated with nicotine.

BRAND'S ESSENCE OF CHICKEN PREVENTS ENDOTHELIAL DYSFUNCTION IN AORTIC RINGS OF SPONTANEOUSLY HYPERTENSIVE RATS

AJAY, M. AND MUSTAFA, M.R.
Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Brand's Essence of Chicken (BEC), a popular chicken extract used as a traditional remedy, has been reported to exhibit anti-hypertensive, anti-cardiohypertrophic and anti-arteriosclerotic actions. Impairment in endothelial functions has been associated with or contributes to the development of vascular diseases. In the present study, the effects of chronic administration of BEC (0.8 ml/kg body weight, corresponding to approximately a human consumption of one bottle per day) on endothelial function in spontaneously hypertensive rats (SHR) and their age-matched normotensive Wistar-Kyoto rats (WKY) were investigated. Male rats (4–5 weeks old) were divided into three groups ($n = 8$) and fed orally for eight weeks with normal saline, gelatin or BEC. At the end of the treatment, the non-invasive systolic blood pressure (SBP) and the aortic endothelial function, in terms of responses to acetylcholine (ACh) and sodium nitroprusside (SNP), were measured. The results showed a reduction of 6 mmHg in SBP in BEC-treated group (186.05 ± 3.27 mmHg) of SHR animals compared to the saline-treated group (192.56 ± 3.60 mmHg). As compared with saline-treated animals (71.31 ± 4.34%), the endothelium-dependent relaxation responses to ACh (10 μM) were markedly improved in the aortic rings isolated from BEC-fed SHR animals (83.24 ± 4.60%), whilst the relaxation to SNP remains comparable in all treatment groups. In normotensive WKY rats, chronic administration of BEC was found to have no significant effect on SBP and on the responses to ACh and SNP. The results are in agreement with previous studies and suggest that the cardiovascular protective actions of BEC in hypertensive subjects are likely to be due to its modulator effect on endothelial function.

ANTINOCICEPTIVE EFFECTS OF *CHANNA STRIATUS* ('HARUAN'), *CHANNA MICROPELTES* ('TOMAN') AND *CHANNA LUCIUS* ('BUJUK') EXTRACTS

SOLIHAH, M.H.[1], SOMCHIT, M.N.[1], ISRAF, D.A.[1,2], AHMAD, Z.[1], ARIFAH, A.K.[3], MAT JAIS, A.M.[4] AND ZAKARIA, M.S.[5]

[1] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia

[2] Laboratory of Natural Product, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia

[3] Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia

[4] College University of Technology and Management Malaysia

[5] Laboratory of Marine Sciences, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia

Channa striatus (*C. striatus*; 'haruan'), *Channa micropeltes* (*C. micropeltes*; 'toman') and *Channa lucius* (*C. lucius*; 'bujuk') are snakehead fish belonging to the *Channidae* family. *C. striatus* has been reported to possess wound healing, anti-nociceptive and anti-eczema properties. However, no previous study has been done on the pharmacological benefits of the other two closely related snakehead fish. Therefore, in the present study, three species of *Channa* were used to evaluate and compare their antinociceptive properties. The aqueous extract was prepared by using distilled water. The antinociceptive activity of three local *Channa* spp. was studied in mice using the writhing test, a visceral pain model. Doses of 15 and 30 mg/kg of aqueous extracts were administered intraperitoneally 30 min before 0.6% acetic acid injection. Aqueous extracts of *C. striatus*, *C. micropeltes* and *C. lucius* at a dose of 15 mg/kg showed significant antinociceptive effects as indicated by 57.63%, 47.45% and 30.84% reduction of writhes, respectively compared to the control. At a dose of 30 mg/kg, aqueous extracts of *C. striatus* showed significant inhibition of 59.28% compared to *C. micropeltes* (27.40%) and *C. lucius* (22.75%). Administration of morphine (0.4 mg/kg) caused 99.25% inhibition. These results showed that aqueous extracts of the three local *Channa* spp. have antinociceptive effects which were expressed in a dose-dependent manner. *C. striatus* had the most potent activity when compared to the other two species.

REDUCTION OF CARDIAC INFARCT SIZE AND VASCULAR NEOINTIMA GROWTH BY CHICKEN MEAT EXTRACT IN RATS

XU, X.G. AND SIM, M.K.

Department of Pharmacology, Faculty of Medicine, National University of Singapore, Singapore

The cardiovascular actions of a commercial chicken-meat extract known as Brand's Essence of Chicken (BEC; Cerebos Pacific Ltd, Singapore) were investigated in balloon catheter-injured rat carotid arteries, and in ischemic re-perfused rat hearts. Injury of the

rat right carotid artery with a balloon catheter led to the development of neointima in the injured section of the artery. The development reached a maximum stage at 14 days post-injury. Daily oral administration of BEC for 14 days in carotid artery-injured rats, dose-dependently attenuated the development of neointima. Effective attenuation was observed with 1.6 ml of BEC. Occlusion of the left main coronary artery of the rat heart for 45 min caused sizable infarct scarring of the left ventricular wall in the heart at 14 days post-reperfusion. Daily oral administration of BEC for 14 days, dose-dependently attenuated the infarct size and transmurality at 14 days post-reperfusion. Effective attenuation was observed with 0.8 ml of BEC. The effective doses of BEC, extrapolated to the commercial package (a bottle of BEC contains 70 ml of the extract) were 1.6 and 0.8 bottle per 70 kg man, respectively. The present findings are the first demonstration of the two specific cardioprotective actions of BEC. An earlier study using normo- and hypertensive rats showed that BEC attenuated the development of hypertension-related cardiac hypertrophy and arteriosclerosis, and the age-related elevation of blood pressure in normotensive rats. These findings suggest the presence of cardioprotective compounds, possibly peptides, in BEC, and warrant further study of the product.

BILATERAL SYMPATHETIC ASYMMETRY IN HAND AS INDICATED BY ELECTRICAL CONDUCTANCE OF THE SKIN

MOHAN, S.M
Perak College of Medicine, Ipoh, Malaysia

Bilateral asymmetry in the level of sympathetic activity was determined by recording the volar skin conductance in the right and left hands of the 58 young (19–23 years) university students (male = 30, female = 28). Volar skin conductance is the measure of electrical conductance between fingers of a hand with microsiemens (μS) as the unit. All except seven subjects were Chinese. Hand dominance was determined by a hand dominance inventory. A note on familial sinistrality (presence of left hander among parents or siblings) of each subject was kept. Volar skin conductance of the two hands was recorded on PowerLab equipment. Data was analysed using Student t-test (paired samples, two tailed). When all subjects were grouped together, the volar skin conductance on the left side (22.98 ± 8.56 μS, mean ± SD) was significantly ($p < 0.01$) higher than on the right side (21.65 ± 7.57 μS). Similarly, in the pooled group of right-handers (n = 47), the skin conductance on the left side (23.40 ± 9.23 μS) was significantly ($p < 0.01$) higher than on right side (21.77 ± 7.96 μS). Among the right-handers, the right-handers without familial sinistrality (n = 34), the skin conductance on left side (24.76 ± 10.0 μS) was significantly ($p < 0.05$) higher than on right side (22.19 ± 8.85 μS). However right-handers with familial sinistrality (n = 13) and pooled group of left-handers (n = 11), left-handers with (n = 5) or without (n = 6) familial sinistrality did not demonstrate significant difference in skin conductance between left and right hands. It is postulated that the lower sympathetic activity in terms of skin conductance on the right side may, especially in the right-handers without familial sinistrality, indicates a similar situation regarding the sympathetic activity to the arm blood vessels. Relatively lower sympathetic activity to right arm is likely to result in more vasodilatation and better blood flow in the right arm than in the left arm. This may have a role in the establishment of right handedness in the right-handers without familial sinistrality.

PHARMACOGENETICS: ON THE WAY TOWARD INDIRECTLY TAILORED DRUG THERAPY

ENG, H.S., TAN, S.Y.[1], LANG, C.C.[1], PHIPPS, M.E.[2], MUSTAFA, A.M.[1] AND MOHAMED, Z.[3]
Departments of [1]Pharmacology, [2]Medicine and [3]Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

P-glycoprotein (P-gp), a product of the multidrug-resistance MDR-1 gene, functions as an energy-dependent exporter of substances from the inside of cells and from membranes to the outside. Therefore, the degree of expression and the functionality of the MDR-1 gene can directly affect the therapeutic effectiveness of drugs. The variation in the activities of P-gp may be caused by genetic polymorphisms in MDR-1 gene. Genetic polymorphism is a condition in which a genetic character occurs in more than one form, resulting in the coexistence of more then one phenotype for drug metabolism in the same population. By screening for genetic variants, every individual can be classified as a poor (PM), or an extensive (EM) metaboliser, based on the number of functional genes present. Previous studies showed that the Exon 21 G2677T is associated with tacrolimus blood level and it may act as positive predictor of tacrolimus-induced neurotoxicity. This polymorphism is believed to have linkage with C1236T in Exon 12. We have set out to determine the allele frequencies of MDR-1 (Exon 12 C1236T and Exon 21 G2677T/A) gene in Malaysian population. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to genotype at location 2677, Exon 21 and location 1236, Exon 12 in MDR-1 gene. Out of a total of 72 DNA samples, 57 samples were successfully genotyped for Exon 21 G2677T/A. The allele frequencies for G, T and A nucleotide are 0.63, 0.36 and 0.01, respectively. For Exon 12 C1236T, only 55 samples were genotyped. The allele frequency for T nucleotide at this locus is 0.7, whereas C nucleotide is 0.3. In conclusion, we have genotyped 55 samples for both allelic polymorphisms at the loci 1236 Exon 12 and 2677 Exon 21. However, we propose to recruit more subjects in order to give a better indication of the allele frequency in the Malaysian population.

SHORT-TERM EFFECTS OF WEIGHT TRAINING ON BONE DENSITY AND STRENGTH IN HEALTHY YOUNG UNTRAINED MALE MALAYSIAN ADULTS

SELVARAJAH, V.S.[1], TEOH, H.T.[1] AND HUSAIN, R.[2]
[1] Sports Centre, University of Malaya, Kuala Lumpur, Malaysia
[2] Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Long-term weight training of 6 to 12 months is said to be effective in improving bone mineral density (BMD). The purpose of this study was to determine the effectiveness of a short-term, 6-week weight training-program on BMD and strength in healthy young untrained male Malaysian adults. Forty five subjects with the mean age of 22.9 ± 1.2 years volunteered for this study and were randomly divided into three groups. The three groups consisted of high intensity, 70% of 1RM (HI) training group, low intensity, 50% of 1RM (LI) training group and the control group (CG). The training groups went through a

series of 10 exercises utilising Nautilus weight machines with 10 repetitions per set for three sets. Measurements of BMD (g/cm^2) utilising the Lunar DPX IQ 4566 dual energy x-ray absorptiometry (DEXA) were obtained for the lumbar spine (L1–L4) and the left femoral neck. Strength tests were conducted on Nautilus weight machines for the bench press and leg press using the indirect 1RM measurement technique. Training at HI resulted in a significant increase ($p < 0.05$) in BMD of the lumbar spine (1.3%) and significant increases ($p < 0.01$) in bench press (16.4%) and leg press (53%). Training at LI resulted in significant increases ($p <= 0.01$) in BMD of the lumbar spine (1.9%), bench press (13.7%) and leg press (36.2%). There was no significant increase in BMD of the femoral neck in both training groups. These data indicate that short-term weight training was successful in increasing BMD of the lumbar spine in both training groups in healthy young untrained male Malaysian adults. It is also interesting to note that LI training produced a higher BMD increase of the spine compared to the HI training group. Thus, it appears that training at LI for six weeks is sufficient to get an increase in BMD at the spine. However, strength gains were superior in the HI training group than in the LI training group.

THE EFFECTS OF RAMADAN FASTING ON SOME ANTHROPOMETRIC PARAMETERS

ISMAIL, R., SUBRAMANIAN, R. AND HUSAIN, R.
Department of Physiology, Faculty of Medicine, Universiti Malaya,
Kuala Lumpur, Malaysia

Various studies have reported changes in the anthropometric parameters of Muslims observing the fast during the month of Ramadan. However, the findings differed from one report to another which could be attributed to the season during which the fasting was observed, diet, as well as cultural differences. The aim of the present study was to investigate the effects of Ramadan fasting on some anthropometric parameters of young Malaysian adults and to compare the changes, if any, between male and female subjects. Forty female and 13 male healthy young adults, aged between 18 to 24 years, volunteered for the study, which was carried out in the Hijra year of 1422. Anthropometric parameters were determined four times; one week prior to Ramadan (Pre-Ram), at the end of the first week of Ramadan (Ram1), beginning of the fourth week of Ramadan (Ram2) and one-month post-Ramadan (post-Ram). The height, weight and the circumference of waist and hip measurements were carried out using standard procedures. The body mass index (BMI) of subjects was calculated using the formula; weight (kg)/height (m)^2. The percentage body fat of subjects was determined by a body composition analyser (Biodynamics, model 310). Ramadan fasting significantly reduced the weight ($p < 0.001$), BMI ($p < 0.0.001$) and percentage body fat ($p < 0.05$) of the female subjects as early as one week after fasting (Ram1), and the effects continued through Ram2 and post-Ram. These reductions showed a tendency to return to pre-Ram values but remained significant ($p < 0.05$) one month after Ramadan (post-Ram). In the male subjects, however, a significant reduction in the weight ($p < 0.01$), BMI ($p < 0.01$) and the percentage body fat ($p < 0.01$) was only observed after three weeks of fasting (Ram2). The weight and BMI of the male subjects returned to pre-Ram values during post-Ram, while the percentage body fat, although showing a trend to return to the pre-Ram value, remained significantly lower

($p < 0.05$). Ramadan fasting did not significantly reduce the waist measurement of female subjects but reduced the measurement for males only during Ram2. In contrast, fasting significantly reduced the hip measurement obtained during Ram1 and Ram2 in the males and females, $p < 0.01$ and $p < 0.001$, respectively. However, the calculated waist:hip ratio of both the male and female subjects was not significantly altered by Ramadan fasting. Ramadan fasting was found to lower the weight, BMI, the percentage body fat and the hip measurement of both male and female subjects. These effects, however, appeared to be more pronounced in female subjects compared to males during the early days of fasting. The females were also observed to be slower in reverting to pre-Ram conditions compared to male subjects. Generally, the effects of Ramadan fasting were only transient and thus, we could conclude that this ritual was not detrimental to health.

THE EFFECTS OF TOCOPHEROL AND TOCOTRIENOLS ON BONE HISTO-MORPHOMETRY PARAMETERS IN RATS EXPOSED TO FERRIC NITRILOTRI-ACETATE

AHMAD, N.S.[1], LUKE, D.A.[2], KHALID, B.A.K[1], AND IMA NIRWANA, S.[1]
[1] Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[2] Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

The ferric component in ferric nitrilotriacetate (FeNTA) is able to generate oxygen-derived free radicals, and be deposited in bone. These effects can cause stimulation of bone resorption by osteoclasts, and impairment of bone formation and mineralisation by osteoblasts. The aim of this study was to examine the effects of FeNTA on structural bone histomorphometric parameters and to determine whether supplementation of alpha-tocopherol or palm tocotrienols would protect the bone from FeNTA toxicity. FeNTA (2 mg/kg of rat body weight) was injected intraperitoneally with or without the supplementation of 100 mg/kg of alpha-tocopherol or palm tocotrienols for eight weeks. FeNTA injection reduced trabecular bone volume (BV/TV) and trabecular thickness (TbTh) but had no effect on trabecular number (TbN) or trabecular separation (TbS). These changes were prevented with the supplementation of palm tocotrienols but not with a similar dose of alpha-tocopherol. These data suggest that FeNTA toxicity may be caused by the stimulation of bone resorption and impairment of bone formation by iron deposition which then resulted in thinning and reduction of trabecular bone volume. Only palm tocotrienols supplementation was able to protect the bone from the toxic effects of FeNTA. Therefore, vitamin E may be potentially useful in the treatment of bone diseases, especially those that are associated with iron or free-radicals overload.

DIURETIC PROPERTIES OF SEVERAL LOCAL MEDICINAL PLANTS IN RATS

ADAM, Y.[1], SOMCHIT, M.N.[1], SULAIMAN, M.R.[1], NASARUDDIN, A.A.[1, 2] AND ZURAINI, A.[1]

[1] Department of Biomedical Science, Faculty of Allied Health Science, Universiti Putra Malaysia, Malaysia

[2] Anatomy Unit, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

Natural plants are commonly used by the locals in the treatment of many diseases. In the treatment of dysuria, many types of medicinal plants are used, such as *Carica papaya* (*C. papaya*; papaya), *Ananas comusus* (*A. comusus*; pineapple), *Orthosiphon stamineus* (*O. stamineus*; 'Misai Kucing') and *Imperata cylindrica* (*I. cylindrica*; lalang). The aim of this study was to evaluate the diuretic properties of these local plants. Spraque-Dawley rats were divided into 13 groups of 6 and placed in individual metabolic cages. Negative control group (Group 1) was given distilled water orally. The positive control groups (Group 2 and 3) were given frusemide and hydrochlorothiazide at 10 mg/kg in distilled water, respectively. Treatment groups (Groups 4–13) were given plant extracts at a dose of 5 and 10 mg/kg for each rat. Urine samples were collected and the volume measured every hour for 4 h. The electrolyte content of the urine was determined by using ion selective electrode (ISE) analyser. The blood of each rat was taken to determine glucose, blood urea nitrogen (BUN) and creatinine levels. *C. papaya*, *A. comosus* and *O. stamineus* extracts exhibited diuretic activities. The *C. papaya* extract increased the 4-h urine volume when administered at both 5 and 10 mg/kg. The *A. comosus* extract increased the urine volume only at 10 mg/kg. However, *O. stamineus* extract increased the urine volume even at 5 mg/kg. No increase was recorded for the 4-h urinary excretion of Na^+ and Cl^- for all the plant extracts. However, there were significant increases in the 4-h urinary excretion of K^+ for *C. papaya*, *A. comosus* and *O. stamineus* extracts. All the extracts, except for *I. cylindrical*, significantly increased the BUN and creatinine levels of the blood. However, only *O. stamineus* extract increased the level of blood glucose. In conclusion, three of the plants investigated had diuretic activity, which was similar to that of frusemide and hydrochlorothiazide.

THE SHORT- AND LONG-TERM EFFECTS OF *GARCINIA ATROVIRIDIS* (ASAM GELUGOR) ON TESTICULAR 11ß-HYDROXYSTEROID DEHYDROGENASE OXIDATIVE ACTIVITY AND PLASMA TESTOSTERONE LEVELS IN RATS

NWE, K.H.H.[1], NORHAZLINA, A.W.[1], ZARA, O.M.[2], MORAT, P.B.[2] AND HAMID, A.[3]

[1]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[2]Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[3]Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Leydig cells of matured rat testis contain a high level of 11ß-hydroxysteroid dehydrogenase (11ß-HSD) enzyme that oxidatively inactivates glucocorticoid, and thereby, protecting the testis from the inhibitory effects of corticosterone (B) on testosterone (T) production. Previous studies showed that in normal rats the plasma T levels changed with testicular 11ß-HSD oxidative activities in most of the treatments. On the other hand, high levels of T could reduce testicular 11ß-HSD oxidative activity. The objective of the present study was to determine the short- (1 week) and long- (14 weeks) term effects of *Garcinia atroviridis* (GA; asam gelugor) on testicular 11ß-HSD oxidative activity and plasma T levels in rats. GA was extracted using ethanol (GAE) or ethyl acetate (GAEA). B was administered only in the short-term treatments. The percentage oxidative activities of 11ß-HSD and plasma total T levels (by Coat-A-Count diagnostic products) were determined. The results showed that both in the short- and long-term treatments with the above extracts, GA caused significant reduction in the testicular 11ß-HSD oxidative activity except for the GA with ethanol extract (slight increase but not significant). Regarding plasma T levels, all the short-term treatments of GA with ethanol or ethyl acetate showed a slight increase, but it was not significant, compared to the normal control group. However, the groups of rats given GAE together with B showed a significant increase in plasma T level compared to the rats given GAE or B alone. On the other hand, the long-term treatment of GAEA extract showed a significant rise in the plasma T levels compared to the normal control. It seems that GA and B have inhibitory effects on one another on both the parameters. An increase in plasma T levels probably reduces the testicular 11ß-HSD oxidative activities as a negative feed back mechanism. In conclusion, GA might be useful in male reproductive health especially if the condition is associated with high glucocorticoid and low plasma T levels.

THE EFFECTS OF HIGH DOSE OF *EURYCOMA LONGIFOLIA* JACK ON TESTICULAR 11β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY, TESTOSTERONE AND CORTICOSTERONE PLASMA LEVELS IN ADRENALECTOMISED RATS

NORHAZLINA, A.W.[1], KHH, N.W.E[1], ARTINI, A.R.[2] AND MORAT, P.B.[2]
[1] Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[2] Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Previous studies have shown that a moderately high dose of corticosterone (B) reduced testicular 11β-hydroxysteroid dehydrogenase (11β-HSD) oxidative activity and plasma testosterone (T) levels in normal rats, but in adrenalectomised (ADX) rats, the parameters studied remained unchanged. On the other hand, a high dose of B was reported to cause Leydig cell apoptosis. Recently, a low dose of water extract of *Eurycoma longifolia* Jack roots (EL) has been shown to increase testicular 11β-HSD activity and T levels in ADX rats and shown to counteract the effects of B on both parameters. Therefore, the present study was aimed to determine if a high dose of EL could antagonise the effects of a high dose of B on testicular 11β-HSD oxidative activity, and T and B levels in the ADX rats. In this study, bilateral adrenalectomy was performed on Wistar male rats (200–250 g body weight). After 24 h, the rats were given either EL (orally), B (intramuscularly) or combination of EL and B for seven consecutive days. On the 7th day of treatment, the rats were sacrificed 60 min after the last treatment. Results obtained showed that testicular 11β-HSD activity was significantly increased in all treatment groups compared to ADX control. Meanwhile, plasma B levels in ADX rats given a combination of EL and B (ADX+B+EL) were significantly lower than ADX rats given B (ADX+B). However, T levels did not show any significant change in all groups compared to ADX control. In conclusion, a high dose of EL could reduce plasma B levels but it was unable to increase testicular 11β-HSD activity and T levels in ADX rats given a high dose of B.

SOME PHARMACOLOGICAL PROPERTIES OF THE SUDANESE MEDICINAL PLANT *CLERODENDRON CAPITATUM*

ABDELWAHAB, S.I.[1], MOHAMED, O.Y.[2], ABDELWAHAB, H.M.[3] AND SOMCHIT, M.N.[1]
[1]Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Malaysia
[2]Faculty of Pharmacy, University of Khartoum, Sudan
[3]Institute of Medicinal Plants, Khartoum, Sudan

The medicinal plant *Clerodendron capitatum* (Verbenaceae) is used traditionally in Sudan in the treatment of male sexual impotence. The methanolic extract of the roots of the plant (percentage yield 9.67%) was studied to investigate some pharmacological properties. The extract was tested in isolated tissue preparations of guinea pig atria, rat uterus, rabbit jejunum and aortic strips, and toad rectus abdominis muscle. In a dose-dependent manner, the extract showed inhibitory effects on the contractility and the rate of guinea

pig atria ($p < 0.05$), these effects were found to be refractory prior to the addition of glibenclamide, atropine and simultaneous administration of calcium chloride. The extract revealed no relaxant, contracting or blocking effects on the tissue preparations of rabbit aortic strips and toad rectus abdominis muscle. The drugs which were used to test the blocking effects in these two preparations were adrenaline and acetylcholine, respectively. In rat uterus and rabbit jejunum preparations the extract showed a serotonin-like activity in a dose-dependent manner ($p < 0.05$), and this effect was found to be reversed by prior addition of a non-selective 5-hydroxytryptamine antagonist, cyproheptadine. The results from different isolated tissues preparations showed that methanolic extracts of the plant roots possess non-cholinergic and non-adrenergic properties. The effect may be due to changes in the intracellular contents.

A STUDY ON THE VASORELAXANT ACTIVITIES OF DERIVATIVES OF ETHYL CINNAMATE

ABD RANI, F. [1], MOHAMAD, K.[1], AWANG, K.[2], MUSTAFA, M.R.[3] AND OTHMAN, R.[1]

[1] Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

[2] Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

[3] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Ethyl cinnamate is a compound extracted from the rhizome of *Kaempferia galanga*. Recent studies showed that ethyl cinnamate is the active component which contributes to the vasorelaxant property of the plant. The vasorelaxant activity of ethyl cinnamate on rat aorta involves inhibition of Ca^{2+} influx through the voltage-dependant and the receptor-operated Ca^{2+} channels. In this study, a total of 11 derivatives of ethyl cinnamate were studied for their vasorelaxant activity against pre-contracted thoracic aorta from male Sprague-Dawley rats. The vasorelaxant activity of the compounds was tested against high K^+ and phenylephrine- (PE) induced contractions. The results showed that there is no significant difference in the vasorelaxant activity of the compounds against high K^+ or PE-induced contractions, except for cinnamoyl chloride, which showed a higher vasorelaxant activity against PE-induced contractions. The compounds found to have higher vasorelaxant activity compared to ethyl cinnamate are ethyl-2,4,6-trimethylphenylacetate, coumarin, cinnamoyl chloride and ethyl α-cyanocinnamate. The findings also suggest that various changes in the chemical structure of the compounds lead to varying degrees of vasorelaxant activity.

ANTIMALARIAL ACTIVITIES OF SOME SELECTED TRADITIONAL HERBAL PLANTS AGAINST *PLASMODIUM BERGHEI IN VIVO*

BASIR, R.[1], CHAN, K.L.[1], VIKNESWARAN, M.[1] AND ISMAIL, S.[2]
[1] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2] Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia

Malaria remains as one the most important parasitic diseases in the tropical and subtropical areas in the world. There were 300–500 million death cases due to malaria reported annually. Treatment against malaria has becoming extremely difficult due to the widespread resistance of the parasites towards the available antimalarial drugs. This prompted the search for new antimalarial agents in order to curb the disease. In the present study, we investigated the antimalarial activity of five species of traditional herbal plants, which included *Eurycoma longifolia* (*E. longifolia*), *Andrographis paniculata* (*A. paniculata*), *Alyxia lucida*, *Araisia* and *Orthosiphon stamineus* (*O. stamineus*). *Plasmodium berghei* infection in ICR mice was used as an *in-vivo* model of the disease. Mice infected with the parasites were treated with the methanol extracts of the plant species and a four-day suppressive test against the parasites was carried out. The course of the infection was monitored throughout the treatment. Three of the plants extract (*E. longifolia*, *A. paniculata* and *Araisia*) showed considerable antimalarial activity. *A. Lucida* extract only showed weak activity against parasites inhibition whereas *O. stamineus* did not show any antimalarial activity at all. This preliminary result suggests that *E. longifolia*, *A. paniculata* and *Araisia* extracts have the potential to be developed as antimalarial agents.

EFFECTS OF AN ANTIGOUT TRADITIONAL MEDICINE ON TESTIS MORPHOLOGY

ZAINI, F.[1], MUSTAFA, A.M.[1] AND KASSIM, N.M.[2]
Departments of [1]Pharmacology and [2] Anatomy, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

The use of herbal medicine as an alternative to modern medicine has been widely accepted since the advancement of knowledge in this area. There are various forms of herbal medicine available in the market; some of which have not been clinically proven to be effective and some may not be safe enough for use. The present study was designed to examine the effects of selected anti-gout traditional medicine on the male reproductive system. The effects of one of these traditional medicines screened are presented here; however, its name could not be revealed due to restrictions. Male albino ICR mice (six weeks old) were divided into three groups (n = 7). Group 1 served as control while groups 2 and 3 received the anti-gout medicine at 0.1 g/kg (low dose) and 1 g/kg (high dose), respectively. The animals were monitored for 14 days and subsequently sacrificed. The testes were dissected, weighed and prepared for histological examinations. No significant changes were observed in the body weights of treated animals compared to those in the control group. However, there was a reduction in the testes weight and size in the treated mice. The treated mice were observed to have mass disruption of the seminiferous tubules as well as a reduction in the tubular diameter. In addition, the germ cells lining the tubules appeared sloughed and no mature spermatids were observed in

the lumen. Hence, it can be concluded that the anti-gout herbal medicine studied may cause disruptions in testicular morphology of male albino ICR mice. Analysis by GCMS on the crude drug revealed the presence of very high concentration of benzoic acid which may have been used as a preservative for this product and could be partly responsible for the morphological changes seen in the testes of these mice.

A DRUG METABOLISM STUDY IN RAT LIVER MICROSOMES SUPPLEMENTED WITH VITAMIN E; TOCOTRIENOL-RICH FRACTION AND α-TOCOPHEROL

GHAZALI, R.A.[1], WAN SALLEH, W.K.H. [1], KHAZA'AI, H.[2], MUTALIB, M.S.A.[2] AND SHARIF, R.[1]

[1] Department of Biomedical Sciences, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur

[2] Malaysian Palm Oil Board, Bangi, Malaysia

The effects of tocotrienol-rich (TRF) and α-tocopherol (TCF) fractions on drug metabolising enzyme activities in rat liver microsomes were investigated. A total of 25 Wistar male rats were used where the TRF and TCF were administered through forced-feeding, and the study was conducted throughout a period of 30 days. In this research, the effects of TRF and TCF at doses of 250 mg/kg and 1000 mg/kg on 7-ethoxycoumarin O-deethylation activities and the estimation of glutathione levels were investigated. The results showed that the 7-ethoxycoumarin O-deethylation activities on all groups, TRF250, TRF1000, TCF250 and TCF1000, were significantly higher ($p < 0.05$) than that in the untreated group. This study also showed that the glutathione levels in the three groups, TRF250, TRF1000 and TCF250, were significantly higher ($p < 0.05$) than that in the untreated group, while the glutathione level of TCF1000 was higher, but not significant ($p > 0.05$), when compared to that in the untreated group. These observations suggest a possible interaction between Vitamin E and the detoxification pathways of the body.

LACK OF HYPOGLYCAEMIC EFFECT OF *GYNURA PROCUMBENS* ON STREPTOZOTOCIN-INDUCED DIABETIC SPRAGUE-DAWLEY RATS

AMIRUDDIN, F.K. AND ISMAIL, R.

Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Gynura procumbens (*G. procumbens*) is a herbal plant which is extensively used in South East Asia for its medicinal properties. The extract from the plant has been shown to lower blood pressure of rats and recently has also been reported to have a hypoglycaemic effect on streptozotocin (STZ)-induced diabetic rats. STZ on its own has also been reported to have a blood pressure-lowering effect. The aim of the present study was to investigate the effects of two extracts from *G. procumbens* on the weight, blood pressure and plasma glucose concentration of STZ-induced diabetic Sprague-Dawley (SD) rats. Leaves of *G. procumbens* were oven-dried, blended and subsequently extracted using hexane and ethanol. Diabetes mellitus was induced in SD rats by injecting a single dose of STZ at

60 mg/kg of body weight intraperitoneally. Rats having a plasma glucose concentration of more than 16 mmol/l were considered diabetic and subsequently used for the study. These rats were treated by oral administration once a day for three weeks with various doses of either ethanolic or hexanic extract of *G. procumbens*. Another group of diabetic rats was treated with the vehicle to act as the control group. The weight, blood pressure and blood glucose concentration were monitored weekly. The weights of the rats were observed to be progressively decreasing from week 0 to week 3. However, there was no significant difference between the weights of the control group compared to those of the treated animals. Similarly, there was no significant difference in the blood pressure of the three groups of animals. Both the ethanolic and hexanic extracts of *G. procumbens* were found not to significantly reduce the blood glucose concentrations when compared to the control group. In contrast to previous reports, this study did not show significant hypoglycaemic effect of either ethanolic or hexanic extracts of *G. procumbens* on STZ-induced diabetic rats.

HYPOTENSIVE ACTIVITY OF *GYNURA PROCUMBENS*: POSSIBLE MECHANISM OF ACTION

HOE, S.Z. AND LAM, S.K.
Department of Physiology, Faculty of Medicine, University of Malaya,
Kuala Lumpur, Malaysia

The leaves of the plant, *Gynura procumbens* Merr (Compositae), have been used by various ethnic groups in Malaysia as a remedy for hypertension. Previous studies have shown that the final aqueous fraction (FAq) obtained from the crude ethanolic extract of the leaves produced a significant fall in the systolic (SBP), diastolic (DBP) and thus, the mean arterial blood pressure (MAP) of rats in a dose-dependent manner without any significant decrease in the heart rate (HR). However, the mechanism of action of these compounds that results in decreasing blood pressure (BP) has not been studied. In the present study, therefore, the hypotensive effect of the FAq was further elucidated by using various pharmacological antagonists. Anaesthetised Sprague-Dawley (SD) rats were pre-treated with FAq before given bolus injections of various antagonists that have effects on the cardiovascular system. Changes in the BP of the rats were monitored directly from the cannulated carotid artery connected to a pressure transducer using the Macintosh MacLab setup. Administrations of hexamethonium (10.0 mg/kg) and atropine (2.0 mg/kg) significantly ($p < 0.05$) prevented the fall in the blood pressure due to the FAq, while phentolamine (2.0 mg/kg) and propranolol (2.0 mg/kg) did not show any significant effect. These findings suggest that the hypotensive action of the FAq appears to be brought about by activation of the ganglionic and muscarinic cholinergic receptors of the autonomic nervous system, and seems not to be mediated through effects on α- or β-adrenoceptors.

PHARMACOLOGICAL CHARACTERISATION OF *BUNGARUS CANDIDUS* (MALAYAN KRAIT) VENOM AND ONE OF ITS PHOSPHOLIPASES A_2

CHONG, Y.J.[1], GEH, S.L.[2], SIM, S.M.[2], TAN, N.H.[1]
Departments of [1] Molecular Medicine and [2] Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Bungarus candidus (Malayan krait) is a medically important poisonous land snake in South Asia. Several cases of severe Malayan krait venom poisoning have been reported. Two major phospholipases A_2 (named PLA-1 and PLA-2) were isolated from the crude venom using high performance gel-filtration chromatography Superdex Peptide HR 10/30 and Lichrospher WP 300 RP-18 reverse phase chromatography. Both phospholipases A_2 were lethal to mice, their LD_{50} being 0.18 µg/g, IV. The molecular weight of PLA-1 and PLA-2 was approximately 10 KDa each, as determined by SDS-PAGE. PLA-1 has a higher phospholipase A_2 activity and was chosen for pharmacological characterisation. The crude *Bungarus* venom and PLA-1 were tested in anaesthetised rats (250–350 g) to observe their effects on the systemic blood pressure, respiratory movements and skeletal muscle contractions, and all these three parameters being monitored simultaneously on a MacLab data-acquisition system. The blood pressure was monitored from a cannulated common carotid artery and the heart rate was estimated from the blood pressure recording. The respiratory movements were monitored by tying a string onto the skin above the diaphragm; this string was then attached to a force displacement transducer connected to the MacLab. The contractions of the gastrocnemius muscle were elicited through stimulation of the sciatic nerve at 0.1 Hz, 0.1 ms pulse width and at supramaximal voltage of 6–8 V. Our results showed that in anaesthetised rats injected with the crude venom, both the low (200 µg/kg, IV, n = 4) and high (1200 µg/kg, IV, n = 4) doses progressively depressed the respiratory movements and subsequently, the systemic blood pressure. The gastrocnemius muscle exhibited slow-onset contracture and its twitch tension progressively decreased until completely blocked. When the test animals were artificially ventilated at the time when the mean blood pressure was 38.4%–66.3% (n = 8) of the control (n = 4), there was a prompt recovery of the pressure, after which the animals survived for several hours. It was observed that the crude venom at low and high doses predominantly caused blockade of neuromuscular transmission, and that the depression of the cardiovascular system was secondary to the inhibitory effect on the respiration. Additionally, rats injected with PLA-1, at 15 µg/kg, IV (n = 2) and 200 µg/kg, IV (n = 3), were seen to exhibit a gradual decrease in gastrocnemius muscle contractions with no significant depression of their respiration. Interestingly, PLA-1 produced noticeable contracture of the gastrocnemius muscle whilst inhibiting limb muscle contractility. Our preliminary study suggests that the *Bungarus* crude venom has a predominant neuromuscular blocking action and that PLA-1 found in the crude venom could contribute to this neuromuscular blocking effect by depressing skeletal muscle function.

EFFECT OF ESSENTIAL OIL OF *CANANGA ODORATA* (YLANG-YLANG) ON BLOOD PRESSURE AND HEART RATE OF RATS

RAUP, B., SUBRAMANIAN, R. AND ISMAIL, R.
Department of Physiology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia

Essential oils (EO) have been used in aromatherapy to treat various ailments since ancient times. Essential oils have been claimed to have a relaxing or a stimulating effect, and are also thought to influence the cardiovascular system. The EO of ylang-ylang (*Cananga odorata*) has been claimed by aroma therapists to be effective in treating hypertension. However, scientific evidence supporting these claims is still lacking. The aim of this study was to investigate the effects of EO of ylang-ylang on the blood pressure and heart rate of normotensive Sprague-Dawley rats. The rats were anaesthetised, and the carotid artery was cannulated and connected to a pressure transducer for direct blood pressure recording (MacLab system). ECG leads were attached to record the heart rate. The baseline blood pressure and heart rate were recorded. The EO was then applied on to a filter paper placed in a glass funnel and allowed to vaporise under a lamp. The funnel was arranged to enclose the rat's snout and the rat was allowed to breathe in the vapour for a period of 15 min. During this period, the blood pressure and heart rate were recorded continuously. The same procedure was repeated with various doses of the EO. The EO of ylang-ylang was found to reduce both the systolic and diastolic blood pressures, and consequently, the mean arterial blood pressure at most of the doses used (5, 30, 50, 80 and 100 µl). The reduction in blood pressure was found to be statistically significant at certain doses. On the other hand, the EO was found to significantly increase the systolic and mean arterial blood pressures at 10 µl. However, the effect of EO of ylang-ylang on heart rate was inconsistent. These conflicting effects of the oil at different doses may be due to the various alkaloids found in the oil, some of which may possess hypertensive while others may have hypotensive properties, along with positive and negative chronotropic effects. This could be one of the reasons why aroma therapists prefer to use a mixture of oils for treating various ailments, rather than a single oil. In conclusion, the findings of this study demonstrated that the use of essential oil of ylang-ylang in aromatherapy might have some scientific basis. However, further studies are required to ascertain the mode of action of the oil and the optimal amount to be used in treatment.

ANTI-ULCER EFFECT OF *PIPER BETEL*, *SOLANUM NIGRUM* AND *ZINGIBER CASSUMUNAR* ON ULCERATION INDUCED BY VARIOUS ULCEROGENS IN RATS

SHAMIMA, A.R., SOMCHIT, M.N., SULAIMAN, M.R. AND NASARUDDIN, A.A.
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Piper betel, *Solanum nigrum* and *Zingiber cassumunar* have been reported to possess several herbal medicinal properties. In this study, we have evaluated their anti-ulcer properties in traditional use. The effects of ulcerogens, aspirin, ethanol, indomethacin and acetic acid, on the stomach of rats treated with ethanol extracts of *Piper betel*, *Solanum nigrum* and

Zingiber cassumunar were studied. Cimetidine was given in the positive control group, whereas, normal saline was given as a negative control. Each group (n = 6) was given the extracts for seven days and on the 8th day, the ulcerogens were given orally after 24 h fasting. On the 9th day, all the rats were euthanised with ether and the stomachs were examined macroscopically, followed by fixation in 10% formalin for histopathology procedure. All treated groups given the extracts showed a decrease in the ulceration score based on macroscopic examination. The results were also confirmed by histological evaluation of the severity of ulceration and the degree of associated inflammation. These results showed that all the extracts have anti-ulcer properties.

THE EFFECT OF AQUEOUS-METHANOLIC EXTRACT OF *ORTHOSIPHON STAMINEUS* ON HYPERURICAEMIC RATS

ARAFAT, O., THAM, S.Y., SADIKUN, A., ISMAIL, Z. AND ASMAWI, M.Z.
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

Orthosiphon stamineus (OS) is used in Malay traditional medicine for treating many diseases, including nephrolithiasis and gout, the two disorders associated with hyperuricaemia. The effect of oral treatment of OS extract was examined on hyperuricaemic rats to justify its use in traditional medicine. Hyperuricaemia was induced in groups of rats (n = 6) by injecting the uricase inhibitor, potassium oxonate (250 mg/kg; ip). An hour later, the rats were treated orally with either 50% aqueous-methanolic extract of OS (0.25, 0.5, 1 and 2 g/kg) or allopurinol 50 mg/kg or saline. Blood samples were collected from the tail veins at 0, 2, 4, 6 and 8 h after oral treatment. The blood was allowed to clot for an hour before being centrifuged to separate the serum. The uric acid concentration in serum was then analysed using HPLC. The concentration analysed showed that potassium oxonate significantly increased the uric acid level in saline-treated rats at 3, 5 and 7 h after hyperuricaemia induction. Oral administration of 0.5, 1 and 2 g/kg OS extract significantly reduced the uric acid level ($p < 0.01$) to a level lower than that of the saline-treated rats at 2, 4 and 6 h after treatment. Allopurinol administration significantly decreased the uric acid level ($p < 0.01$) starting from two hours after treatment until the end of the experiment. Results suggest that treatment with 50% aqueous-methanolic extract of OS reduces the formation of uric acid and alleviates hyperuricaemia. The reduction in the uric acid level would reduce the possibility of the deposition and formation of uric acid crystals which may lead to several disorders such as gout and kidney stone formation (nephrolithiasis). These results provide supporting scientific evidence for the use of OS in the treatment of nephrolithiasis and gout in traditional medicine.

CLINICAL STUDIES

EFFECTS OF IRBESARTAN ON PLASMA 'HOMOCYSTEINE-INSULIN RELATIONSHIP': A CAUSE OR AN EFFECT?

AYOB, A.[1] AND RAZAK, T.A.[2]
[1]Pharmacology Unit, Department of Basic Medical Sciences, Kulliyyah of Medicine,
[2]Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Malaysia

Several studies have demonstrated a correlation between plasma total homocysteine (tHcy) concentrations and insulin levels. Plasma tHcy levels are lowered by insulin and can be elevated in insulin-resistant states. However, it is uncertain whether plasma tHcy and insulin are related to each other, and whether in hypertensive patients, the relationship can be influenced by irbesartan, an angiotensin II receptor antagonist. The determination of tHcy concentrations and insulin levels were carried out to find any possible association. A non-randomised, run-in placebo and a three-month follow up clinical study was carried out in 34 patients (12 women and 22 men) with uncomplicated mild-to-moderate essential hypertension. The age of the patients ranges from 31 to 67 years (52.1 ± 1.47 years; mean ± SEM). The patients were kept on placebo for four weeks, and the first blood sampling was performed, followed by commencement of irbesartan 150 mg daily for 12 weeks period. A second blood sampling was performed at the final visit (week 12). At week 4 and 8, irbesartan was titrated to 300 mg daily should the patients' blood pressures were not controlled. All patients fasted overnight for at least 10 h before the blood samples were collected. The tHcy and insulin concentrations were measured by an enzyme immunoassay technique. There was a significant difference ($P < 0.001$) between the placebo and irbesartan in lowering both the systolic and diastolic blood pressures. The plasma insulin levels were significantly increased (7.04 ± 0.58 µIU/ml and 11.12 ± 1.15 µIU/ml; mean ± SEM; $P < 0.001$), and the tHcy concentrations were significantly decreased (11.44 ± 0.45 µmol/l and 9.33 ± 0.38 µmol/ml; $P < 0.0001$) after therapy. A significant negative correlation was found between tHcy and insulin levels at baseline ($P < 0.05$, $r = -0.472$). However, there was no significant correlation found after therapy ($P > 0.05$, $r = -0.046$). The present findings show that there is a significant relationship between tHcy and plasma insulin in hypertensive patients, particularly at baseline. It may indicate that the tHcy metabolism is substantially affected by insulin action. Apart from lowering blood pressure, irbesartan has also influenced the 'tHcy-insulin relationship'. This suggests that there is a potential causal or effectual relationship between irbesartan with tHcy and/or insulin. An established mechanism is yet to be identified.

ACUTE EFFECTS OF ATORVASTATIN ON ENDOTHELIAL FUNCTION IN HEALTHY SUBJECTS AT RISK FOR TYPE 2 DIABETES MELLITUS

AMUDHA, K.[1], CHAN, S.P.[2], AZMAN, W.[2], IMRAN, Z.A.[2], MUSTAFA, M.R.[1] AND LANG, C.C.[2]
Departments of [1] Pharmacology and [2]Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Endothelial dysfunction (ED), an important determinant of altered vascular reactivity, plays a major role in the genesis of vascular complications in diabetes mellitus (DM). The aim of our study was to assess whether ED is present in the first-degree relatives (FDRs) of patients with type 2 DM and if so, whether it is reversible with HMG-CoA reductase inhibitor therapy that has an established action on vascular function in DM. Sixty normotensive and normoglycaemic subjects (mean age 27 ± 4 years; BMI < 25) with ≥ 1 parent with type 2 DM were compared with 20 age-, sex- and BMI-matched controls (negative family history of diabetes). At baseline, we evaluated the endothelial function, insulin sensitivity, sICAM-1, IL-6 and hsCRP levels in both groups. FDRs were then randomised to receive atorvastatin (80 mg) or placebo in a double-blind, placebo-controlled, parallel group design for four weeks. Endothelial function was assessed by high-resolution vascular ultrasound, whilst insulin resistance and sensitivity, by HOMA and QUICKI, IL-6 and sICAM-1 by ELISA, and hsCRP by immunoturbidimetric assay. At baseline, endothelium-dependent dilatation was significantly reduced in FDRs when compared to controls (3.8 ± 0.8% vs 12.6 ± 0.9%; $p < 0.001$). FDRs were significantly more insulin resistant ($p < 0.01$), and less insulin sensitive ($p < 0.01$), and had significantly high levels of sICAM-1 ($p < 0.001$), IL-6 (6.6 ± 0.1 vs 3.3±1.3 ng/ml; $p < 0.001$), and hsCRP (2.3 ± 3.2 vs 0.92 ± 1.42mg/l; $p > 0.05$) with no differences in endothelium-independent dilatation and lipid profile between groups. After four weeks of atorvastatin therapy, endothelial function was significantly improved (8.9 ± 3.5% vs 4.9 ± 4.6%; $p < 0.05$) and hsCRP levels were somewhat, but not significantly, reduced ($p > 0.05$). Serum triglyceride, total cholesterol and LDL levels were significantly reduced ($p < 0.001$) in the atorvastatin group. We have shown for the first time that significant impairment of vascular reactivity can be detected early in the FDRs of subjects with type 2 DM. This was associated with altered insulin sensitivity and elevated levels of IL-6 and hsCRP. This ED was significantly reversed by acute atorvastatin therapy - an effect, which may be related to its hypolipidaemic and anti–inflammatory actions.

METHOD OPTIMISATION AND VALIDATION STUDY ON THE USE OF LASER DOPPLER FLOWMETRY TO ASSESS HUMAN MICROVASCULAR HEALTH

TEE, G.B.[1], RASOOL, A.H.G.[2], HALIM, A.S.[3] AND RAHMAN, A.R.A.[4]
Departments of [1] Pharmacology and [2]Reconstructive Sciences, School of Medical Science, Universiti Sains Malaysia, Kubang Kerian, Malaysia
[3] Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kubang Kerian, Malaysia

Human postocclusive forearm skin reactive hyperaemia is a potential means of identifying early signs of cardiovascular diseases. In this study, we investigated the effect of varying occlusion times in assessing postocclusive forearm skin reactive hyperaemia using laser Doppler fluximetry (LDF). We also determined the intraday and interday reproducibility of this method. Twenty healthy male volunteers were studied on three separate days (at least 24 h apart) via a randomised design. The volunteers were studied in a supine position while fasted. Laser Doppler probes were placed on the volar surface of the antebrachium. Baseline readings were taken before upper arm blood flow was occluded using a blood pressure cuff at a pressure of 200 mmHg. Occlusion duration was randomised to 1, 2 or 3 min for each day. Skin blood flux was measured before, during and after occlusion using LDF. The primary outcome calculated was maximal change in skin blood flux before and after occlusion, expressed in arbitrary unit (AU). To address intraday and interday reproducibility, skin reactive hyperaemia was performed twice within each study day for two days. Intraday assessments were separated by 60–90 min; while the interday assessments were 24–72 h apart. Skin blood flux changes (mean ± SEM) after 1, 2 and 3 min occlusion period were 15.39 ± 1.27 AU, 24.84 ± 1.62 AU and 32.14 ± 1.73 AU, respectively. Using repeated measures analysis of variance, significant difference ($p < 0.05$) in skin blood flux changes were revealed between these three occlusion durations, where 3 min occlusion produced significantly greater change in skin blood flux compared to 1 and 2 min. The intraday and interday coefficient of variation of skin blood flux measurements were 4.77% and 6.50%, respectively. It is recommended that studies using postocclusive forearm skin reactive hyperaemia should occlude the forearm for at least 3 min. This procedure is suitable to assess or screen microvascular health as it is a simple, non-invasive, well-tolerated and reproducible method.

PALM TOCOTRIENOLS: TRACING THEIR METABOLISM AND BIOKINETICS

SYED FAIRUS[1], MD NOR, R.[1], CHENG, H.M.[2] AND SUNDRAM, K.[1]
[1] Food Technology and Nutrition Unit, Malaysian Palm Oil Board
[2] Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Detection of tocotrienols in human plasma has proven difficult even after long periods of supplementation. The rapid disappearance of tocotrienols has raised questions about their physiological effects in humans. In order to better understand the metabolic fate of ingested tocotrienols and their related biokinetics in humans, a post-prandial intervention

was undertaken. Seven healthy volunteers (four males and three females) were pre-conditioned on a tocotrienol-free diet for seven days. On the 8th day, all volunteers were administered a single dose of vitamin E supplement (either 1010.5 mg palm tocotrienols or 1098 mg α-tocopherol) in the form of capsule. Blood was sampled at baseline (fasted), 2, 4, 5, 6, 8 and 24 h after supplementation. The tocopherol and tocotrienol concentrations in total plasma, triglyceride-rich particles (TRP), low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions for each bleeding interval were determined. Following the intervention with palm tocotrienols treatment, tocotrienols were detected in total plasma and TRP, LDL and HDL. However, the concentrations of the tocotrienols detected were minimal, while α-tocopherol remained the major circulating plasma vitamin E isomer. Tocotrienols concentration in total plasma, TRP and LDL peaked between 4 to 6 h and at 8 h in HDL after supplementation. Alpha-tocopherol was the major vitamin E isomer detected in plasma in both palm tocotrienols and α-tocopherol treatments. The rapid disappearance of tocotrienols in plasma and all lipoprotein fractions suggests that tocotrienols have a very short duration of absorption and distribution in circulating blood.

BILATERAL SYMPATHETIC ASYMMETRY IN THE EYES AS INDICATED BY INTRAOCULAR PRESSURE

MOHAN, S.M.
Perak College of Medicine, Ipoh, Malaysia

Bilateral sympathetic asymmetry between the right and left sides of the body of an individual is a less known phenomenon. The nasal cycle and nostril dominance are clear examples of the manifestation of bilateral sympathetic asymmetry. Sympathetic activity as indicated by volar galvanic skin resistance is lesser on the right arm than on the left arm. Eye dominance is an example of bilateral asymmetry in the body, as is handedness. As sympathetic stimulation causes a fall in the intraocular pressure (IOP), it may be stated that the variations in the IOP between the two sides indicate bilateral sympathetic asymmetry in the eyes. The present study examined the asymmetry in the sympathetic activity in the eyes in terms of IOP. Intraocular pressure in 150 newborns (one day old), 80 young adults (21.78 ± 1.43 years; mean ± SD) and 159 old people (53.58 ± 10.23 years) was measured with Tono-Pen under topical anaesthesia. The comparison of IOP in the right and left eyes was done using a paired t-test (one tail). The IOP was significantly ($p < 0.05$) higher in the right eye (16.16 ± 2.93 mmHg) than in the left eye (15.79 ± 3.19 mmHg) of the new born babies. Similarly, the IOP was significantly higher ($p < 0.05$) in the right eye (15.04 ± 2.84 mmHg) than in the left eye (14.71 ± 2.82 mmHg) of the young adults. There was no significant difference in the IOP of the right eye (15.16 ± 2.82 mmHg) and the left eye (15.03 ± 3.31 mmHg) of the old people. As higher IOP indicates lower sympathetic activity, it can be assumed that sympathetic activity is lower in the right eye than in the left eye. As the right eye is usually the dominant eye, it may be said that the sympathetic activity in terms of IOP is lower in the dominant eye. Appropriate level of IOP is essential to maintain the shape of the eye ball and the optical alignment. Hence the lower level of sympathetic activity to the right eye supports relatively higher level of IOP for better visual function of the dominant right eye. The lack of asymmetry in the old people is similar to the diminution of the cerebral hemispherical dominance in the geriatric

population. We intend to hypothesise that the sympathetic asymmetry in bilaterally placed organs helps to establish the dominant pattern of the body.

LEVELS OF PLASMA OESTROGEN AND TESTOSTERONE IN HEALTHY VEGETARIANS AND NON-VEGETARIANS

NORAZIT, A.[1], HUSAIN, R.[2], ISMAIL, R.[2], RAJA AHMAD, R.E.A.[2], SEELAN, S.[1] AND MUSTAFA, A.M.[1]

[1]SUCXeS Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

[2]Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

This study was conducted to examine the difference in hormonal levels of volunteers with a high intake compared to that with a low intake of a phyto-oestrogen-rich diet. The sample consisted of 20 non-vegetarian and 10 vegetarian healthy Malaysian males. The subjects (n = 30) were between 20–30 years of age and consisted of all three major races, i.e., Malays, Chinese, and Indians. Oestradiol and testosterone levels were measured using radioimmunoassay kits (DSL-4300 ACTIVE® Oestradiol Coated Tube Radioimmunoassay kit and DSL-4000 ACTIVE® Testosterone Coated Tube Radioimmunoassay kit). The phyto-oestrogens measured in this study using Liquid Chromatography-Mass Spectrometry (LC-MS) consisted of diadzein, genistein, diadzin, genistin and coumesterol. The results showed that the vegetarians (290.5 pg/ml) had a statistically higher ($P < 0.05$) mean level of oestradiol compared to the non-vegetarians (190 pg/ml). The mean testosterone level in the vegetarian sample (4.38 ng/ml) was also statistically lower ($P < 0.05$) compared to the non-vegetarian sample (6.23 ng/ml). The accumulative mean level of phyto-oestrogens in the vegetarian sample (22.66 ug/ml) was also statistically higher ($P < 0.05$) compared to the non-vegetarian sample (11.19 ug/ml). This study also determined the correlation between individual phyto-oestrogens, oestradiol and testosterone. Diadzin had a positive correlation (Pearson Correlation = 0.453) with oestradiol, but a negative correlation (Pearson Correlation = –0.434) with testosterone. Genistin only had a negative correlation (Pearson Correlation = –0.377) with testosterone. All other phyto-oestrogens correlation was not significant. All results analysed were statistically significant at $P < 0.05$.

LEVEL OF OESTRADIOL IN HEALTHY MALE MALAYSIAN VOLUNTEERS

NORAZIT, A.[1], HUSAIN, R.[2], ISMAIL, R.[2], RAJA AHMAD, R.E.A.[2], PANG, H.C[1], SUHAIMI, J.[1] AND MUSTAFA, A.M.[1]
[1]SUCXeS Laboratory, Department of Pharmacology, University of Malaya, Kuala Lumpur, Malaysia
[2]Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

The oestradiol level of 120 male volunteers was measured to examine the normal range of circulating levels of oestradiol in Malaysian males and also to examine the influence of diet on these levels. The volunteers comprised vegetarians (n = 21) and non-vegetarians (n = 99) aged between 20 and 30 years. The volunteers comprised all the major races in Malaysia, ie, Malays (n = 48), Chinese (n = 43), Indians (n = 26), and others (n = 3). Oestradiol levels were measured using a radioimmunoassay kit (DSL-4300 ACTIVE® Oestradiol Coated Tube radioimmunoassay kit). The values obtained ranged from 70 pg/ml up to 800 pg/ml. The mean level of oestradiol was 236.05 pg/ml. The reference level measured by the manufacturer was greater than 74 pg/ml. It was observed that vegetarians have a significantly higher ($P < 0.05$) mean level of circulating oestradiol (329.3 pg/ml) compared to the non-vegetarians (216.3 pg/ml). The results were then analysed by race. The only significant difference ($P < 0.01$) in the mean levels of oestradiol was between the Malays (197.23 pg/ml) and the Indians (313.85 pg/ml). The difference between the Indians and the Chinese (235.79 pg/ml) was not significant. All results analysed were statistically significant at $P < 0.05$.

LEVELS OF NITRIC OXIDE AND NITRIC OXIDE SYNTHASE ACTIVITY IN FOETOPLACENTAL TISSUES FROM WOMEN WITH PRE-ECLAMPSIA

WAN ALI, W.M. AND SINGH, H.J.
Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia

Nitric oxide (NO), a potent vasodilator has been postulated to have a role in the aetiology of pre-eclampsia. Reduced production of NO in the placenta has been claimed to be the possible cause for the impaired vasodilatation and therefore placental insufficiency seen in pre-eclampsia. The aim of our study was therefore to measure the levels of NO and nitric oxide synthase (NOS) activity between foetoplacental tissues from normotensive pregnant women (NTPW) and women with pre-eclampsia (PE). Amnion, chorion and placental cotyledon were collected immediately after delivery from 12 NTPW and 12 PE women, washed thoroughly with 0.5 M phosphate buffer, pH 7.5 at room temperature, weighed 2 g and individually homogenised in 2 ml of the same buffer for 3 min. This is followed by centrifugation at 4000g, 4°C for 15 min. The homogenisation and centrifugation were repeated three times and the supernatant collected were pooled individually according to the specific tissue and kept at –70°C until assayed. Levels of NO and activity of NOS were measured using the Griess reaction technique. There was no significant difference in the levels of NO or in the activity of NOS between the amnion, chorion and placental

cotyledon from NTPW or PE or between corresponding tissues from both the groups. The absence of any significant difference in NO or NOS activity between tissues from both the groups might suggest to us to exclude any defect in nitric oxide production by foetoplacetal tissues in pre-eclampsia.

MISCELLANEOUS

IMPOSEX IN *THAIS* SPP. AS INDICATOR FOR TRIBUTYLTIN CONTAMINATION IN THE COASTAL WATERS OF WEST COAST OF PENINSULAR MALAYSIA

ISMAIL, A., FERDAUS, M.Y., SYAIZWAN, Z.Z. AND ISMAIL, A.R.
Department of Biology, Faculty of Science and Environmental Studies,
Universiti Putra Malaysia, Serdang, Malaysia

Imposex is a phenomenon whereby male sex characteristics (penis and/or vas deferens sequences [VDS]) are induced in female gastropods in addition to the female system, and may cause a reduction in population reproduction. This malformation was first described for the dogwhelk *Nucella lapillus* L. (*N. lapillus*), an estuarine snail. Imposex occurrences in Thais gastropods have been reported in several species such as *Thais clavigera* and *Thais bronni* in Japan, and *Thais bitubercullaris* and *Thais jubilae* in Singapore. The abnormality was linked to the presence of exposure to organotins, particularly tributyltin (TBT) in coastal waters. TBT, with a concentration as low as 10 ng/l in water, promotes imposex in rock shells *Thais clavigera.* Therefore, imposex in neogastropods have been used worldwide as an indication for TBT contamination. Imposex is an example of the effects of endocrine disruptor chemicals (EDCs). TBT-induced imposex in *N. lapillus* is a result of an increase of testosterone level in treated female snails. TBT inhibited cytochrome-P450-dependent aromatase normally responsible for the conversion of testosterone to 17β-oestradiol in female snails. In the present study, four species of rockshells (*Thais gradata*, *Thais bitubercularis*, *Thais tuberosa* and *Thais hippocastanum*), collected from 27 sites along the west coast of Peninsular Malaysia during 2001–2004 period, showed varying degrees of imposex. The observed imposex development in this study included a pseudopenis and/or a VDS and could be classified as stage 1, 2, 3, 4, 5 and 6 according to the VDS classification scheme and range of penis length (RPL). One hundred percent of the 950 female snails of *Thais* spp. showed imposex characters. Most of the imposex cases recorded in this study occurred at stage 2 (71.59%), followed by stage 1 (14.26%) then stage 4 (8.34%), while the rest are at stages 3, 5 and 6. Both the incidence of imposex and the degree of imposex development in these snails were greater in samples collected from marinas, ports and areas with high boating or shipping activities. Lesser imposex levels were found in areas with lower boating activities. The number of imposex cases in this study also showed similar patterns of change with TBT levels in the sediment, water column and in *Perna viridis* tissue collected from the Straits of Malacca. These findings confirmed that imposex in *Thais* spp. can be used as an indicator to assess TBT contamination along the west coast of Peninsular Malaysia.

DEVELOPMENT OF MEDIUM THROUGHPUT MUSCARINIC RECEPTOR BINDING ASSAY

YAP, K.F.[1], CHUNG, L.Y.[1] AND MUSTAFA, M.R.[2]
Departments of [1] Pharmacy and [2] Pharmacology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

A filtration-based competitive radioligand muscarinic receptor binding assay suitable for medium throughput screening has been developed. In this assay, 96-well GF/C filter plate was adapted and integrated with FilterMate cell harvester and TopCount NXT Microplate Scintillation and Luminescence Counter. Using rat brain as the source of muscarinic receptor, a linear relationship of protein concentration and radioligand binding was established up to a protein concentration of 133 µg protein/well. The parameters investigated include radioligand concentration, pH, incubation time, temperature and washings. In general, the optimum protocol contained 36 µg protein/well, 0.5 nM [^{3}H] N-methylscopolamine ([^{3}H] NMS) and 0.05 M Tris HCl buffer pH 7.4. The receptor-radioligand equilibration was reached after 90 min incubation at 21°C. Saturation analysis of [^{3}H] NMS gave B_{max} of 293 fmol/mg protein and K_D of 0.0589 nM. The B_{max} value shows the muscarinic receptor density is high whilst K_D value suggested [^{3}H] NMS has high affinity towards the receptor and is a suitable radioligand source for filtration assay. The linear Rosenthal plot suggests single site binding in this receptor-radioligand interaction. K_i obtained from competition experiments with known muscarinic receptor ligands were: atropine sulfate salt (0.299 nM), scopolamine methyl bromide (0.0515 nM) and dicyclomine hydrochloride (1.59 nM). Low intra-plate variability (CV, 5.64%) and Z factor of 0.81 clearly show that this assay protocol is robust and reliable as a medium throughput screening assay.

A VALIDATED GAS CHROMATOGRAPH-MASS SPECTROMETER METHOD FOR THE DETERMINATION OF BISPHENOL A IN HUMAN URINE SAMPLE

THANNIMALAY, L.[1], MUSTAFA, A.M.[1] AND CHEN, S.S.[2]
[1] Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur
[2] Environment and Bioprocess Technology Centre, SIRIM Berhad, Shah Alam, Malaysia

A rapid, sensitive and specific gas chromatograph-mass spectrometer (GCMS) method for the determination of bisphenol A (BPA) in human urine was developed and validated. BPA was extracted from urine samples using solid-phase extraction (SPE) with a C18 cartridge. ^{14}D-BPA analogue was used as the surrogate standard to increase method accuracy and precision. ^{14}D-BPA was added to the urine sample and enzymatic deconjugation was performed prior to SPE to determine the total amount of free-BPA and BPA-glucuronide. BPA was analysed after derivatisation with bis(trimethylsilyl)-tri-fluoroacetamide (BSTFA) using GCMS with a quadrapole detector in the selected ion monitoring (SIM). The average recoveries for 0.1, 0.5 and 0.9 ng/ml were 92%, 98% and 94%, respectively. The coefficient of variations (CV) for the recoveries was below 15%. The average interday precision for each 0.1, 0.5 and 0.9 ng/ml were 14.8%, 4.6% and 9.5%, respectively. The average intraday precision for each 0.1, 0.5 and 0.9 ng/ml

were 10.1%, 9.9 and 10.4%, respectively. The calibration curve for the extracted BPA in urine was linear over the range of 0.1–0.9 ng/ml concentration (r^2 = 0.996). The limit of quantification (LOQ) for this method is 0.1 ng/ml of BPA, which is also the lowest validated concentration. This method can be used for the determination of BPA in human urine sample.

METHOD DEVELOPMENT FOR DETERMINATION OF CHLORAMPHENICOL IN WASTEWATER

MALINTAN, N.T. AND MUSTAFA, A.M.
Department of Pharmacology, Faculty of Medicine, University of Malaya,
Kuala Lumpur, Malaysia.

The need to monitor antibiotic residues in the content of effluent and food is increasing along with the escalating incidence of antibiotic resistance in human and the widespread use of antibiotics in animal husbandry. This study is to develop a method to investigate the presence of chloramphenicol in wastewater. This method was developed using HPLC (Shimadzu LC-10 AT) with the spectrophotometric detector (SPD-10A VP) set at 278 nm. The injection volume was 200 µl. Acetonitrile:water 70:30 (pH 4.3) was used as the mobile phase and the flow rate was 1.2 ml/min. Sample preparation was done using liquid-liquid extraction method. Blank or test samples (500 ml) were extracted with ethyl acetate and evaporated to dryness in the water bath (40°C) under nitrogen stream. A C18 HPLC column (SUPELCOSIL 25 cm x 4.6 mm) was used in the analysis and the retention time for chloramphenicol was 7 min. The analysis was done using an external standard calibration method. The recovery of extracted chloramphenicol was between 73% and 85% for concentration ranging from 5 to 100 ng/ml. The limit of detection and quantification were 5 ng/ml and 7.5 ng/ml, respectively.

A STUDY OF SOME VARIABLES IN A TETRAZOLIUM DYE (MTT) BASED ASSAY FOR CYTOTOXIC TESTING IN HUMAN CANCER CELLS

TAN, G.K., CHEAH, S.H. AND KIM, K.H.
Department of Physiology, Faculty of Medicine, University of Malaya,
Kuala Lumpur, Malaysia

The tetrazolium dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, first described by Mosmann, is a simple and important method for large-scale screening programmes of anticancer compounds. It is based on the ability of dehydrogenase enzymes in the living cells to metabolise yellow MTT salt to purple formazan and the number of viable cells is spectrophotometrically measurable as the amount of the MTT reaction products present. We have studied various factors involved in the optimal use of this assay for the measurement of the rate of cell growth and for cytotoxic testing of various compounds in human normal cell (Chang cells) and cancer cells (MCF-7, PC-3 and CaSki cells). These factors include the relationship between the number of cells per well and the optical density (OD) of the formazan produced, optimization of the time course for the formazan development after the addition of MTT

solution and incubation time after the addition of dimethyl sulphoxide (DMSO). Cells for experiments were taken from exponential phase cultures growing in 75 cm^2 tissue culture flasks. The OD measurements were carried out using microplate reader with wavelength 554 nm and reference wavelength 690 nm. The amount of formazan produced by a given number of cells varied between different cell lines. Nevertheless, a linear relationship was seen between the number of the cells plated (1–8×10^3 cells/well) and the resulting absorbance in the assay at day 3. The OD of PC-3 and MCF, with initial plating number 8×10^3 cells/well, reached the maximum detectable limit of the microplate reader used on day 3. Therefore, we propose that the plated cell number should not exceed 8×10^3 cells/well for the assay designed to be incubated for three days. In addition, the resulting OD of the formazan product was also dependent upon the incubation time (up to 3 h) after the addition of 50 µl of MTT solution (2 mg/ml) to 200 µl medium. Hence, we have adopted 3 h as the optimum incubation time after adding MTT solution. Besides that, our own experience supported the preference of Alley *et al*. and Twentyman *et al.* for DMSO as the solvent of choice to dissolve the formazan crystals. DMSO was able to dissolve the formazan crystals extremely rapidly and the OD remained stable throughout the observation period (15, 30, 45 and 60 min). We cannot deny that there are several disadvantages of this assay, including the safety hazards to personnel due to exposure to large quantities of DMSO, and the inefficient metabolism of MTT by some human cell lines. However, as long as handling is done with caution, this assay is rapid and easy to conduct and hence is suitable for the determination of growth and cytotoxic sensitivity of the cell lines.

MEASUREMENT OF TOTAL 17α-HYDROXYPROGESTERONE IN NORMAL HUMAN SERUM

CHONG, H., CHEAH, S.H., RAGAVAN, M. AND JOHGALINGAM, V.T.
Monoclonal Laboratory, Department of Physiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

The determination of 17α-hydroxyprogesterone (17OHP) in plasma or serum is clinically useful for the diagnosis and management of congenital adrenal hyperplasia (CAH) and is conventionally done by radioimmunoassay using isotopic ligands. In this study, we assessed the feasibility of measuring 17OHP in normal human serum/plasma using our in-house formulated enzyme immunoassay (EIA) system. This assay system was set up using monoclonal antibody (Mab) generated in our laboratory. The assay was conducted by the direct measurement of 17OHP in heat-treated human serum. A 96-well EIA microplate was pre-coated with 17α-hydroxyprogesterone-3-(o-carboxymethyl)oxime-bovine serum albumin (17OHP-BSA) overnight in carbonate/ bicarbonate buffer, pH 9.5. The coated plate was then incubated with standard 17OHP prepared in steroid-stripped serum/plasma and patients' samples, together with an appropriate dilution of Mab. The 17OHP in the standards or samples competed with the immobilized 17OHP-BSA for binding with the Mab, and the amount of Mab bound to the plate after washing was inversely proportionate to the quantity of the 17OHP in the standards or serum/plasma samples. The bound antibody was visualized with a secondary antibody (anti-mouse IgG conjugated to peroxidase). Colour formation was done with ABTS as substrate. The absorbance was measured in an ELISA plate reader at 405 nm. The detection limit

(ranging between 0.05–1.0 ng/ml) depended on the Mab used and its dilution factor, the turn-around time for this system could be as low as 3 to 4 h. The procedure was simple and well-suited for routine analysis of a larger number of samples. This method has been applied to measure 17OHP in human serum/plasma samples obtained from University Malaya Medical Centre (UMMC) blood bank, student volunteers and from the paediatric clinic. A total of 80 adult sera, 48 children (aged between 1–12 y) sera and 60 adult plasma were assayed using this method. The average total 17OHP were found to be 16 ± 7 (Mean ± SE) for adult serum, 25 ± 11 for children and 30 ± 11 for adult plasma, respectively.

ON-LINE PRE-TREATMENT METHOD FOR HPLC AND LCMS USING COLUMN-SWITCHING TECHNIQUE

HAMADA, N.[1], ZHAN, Z.[1] AND IIDA, J.[2]
[1] Customer Support Centre, Shimadzu (Asia Pacific) Pte Ltd, Singapore
[2] Analytical and Measuring Instruments Division, Shimadzu Corporation, Kyoto, Japan

In the analysis of drugs in biological samples by High Performance Liquid Chromatography (HPLC) and/or Liquid Chromatography Mass Spectrometer (LCMS), preparation of samples to remove proteins is essential. Manual preparation of samples is time-consuming work. Automated sample preparation using pre-column extraction and column switching was therefore examined. The system used consisted of two flow-lines, which were connected to each other by a flow change-over valve. A pump, a high-pressure flow change-over valve, a low-pressure switching valve, and HPLC and/or LCMS were used. The instruments used were all from Shimadzu Corporation, Japan. The drug of interest was trapped by hydrophobic interaction on pre-column Shim-pack MAYI-ODS (methylcellulose-immobilized reversed phase pre-treatment column). Proteins and non-volatile salts in the sample were removed in this stage. After that, the high-pressure flow change-over valve was turned and the drug on the pre-column was introduced onto the analytical column for HPLC and/or LCMS. In this study, the basic performance of this system such as the elution behaviour of proteins on the pre-column and the reproducibility of the total analysis were examined. This system made the direct injection of serum and a stable automated analysis possible. In addition, we will show that large-volume sample injection using bypass-line technique for food analysis can give higher sensitivity.

DEVELOPMENT OF A RADIOIMMUNOASSAY FOR ALPHA-FOETOPROTEIN USING LOCALLY PRODUCED ANTISERA

CHEAH, S.H., LIM, S.L., RAGAVAN, M. AND JOHGALINGAM, V.T.
Department of Physiology, Faculty of Medicine, University of Malaya,
Kuala Lumpur, Malaysia

Alpha-foetoprotein (AFP), a protein normally found in the foetus, is also produced by adults under certain pathological conditions. It is a useful tumour marker for certain types of cancers such as hepatocellular carcinoma and germ cell carcinoma. A locally produced

antibody would therefore be useful in producing relatively inexpensive immunoassay methods for in-house diagnosis and also for research purposes. Rabbit polyclonal antisera was produced by immunising five rabbits with highly purified AFP in Freund's complete adjuvant followed by booster shots of AFP in Freund's incomplete adjuvant. Blood was collected via the ear vein about 7–10 days after booster shots. The antiserum was characterised using a double antibody precipitation method with ^{125}I-labelled AFP (^{125}I-AFP). The ^{125}I-AFP was prepared in the laboratory using the chloramine-T method. The antisera from the various rabbits and blood collections that showed high antibody titres and similar binding characteristics were pooled and used to develop a competitive radioimmunoassay (RIA). The final protocol of the RIA that was set up used the double antibody precipitation method done over two days. On day 1 the following mixture was added to assay tubes at room temperature: 100 µl standard AFP solutions or serum sample, 100 µl anti-AFP rabbit serum at appropriate dilution, 100 µl ^{125}I-AFP. The tubes were incubated overnight at 4°C. On the following day, 100 µl normal rabbit serum, 100 µl donkey anti-rabbit serum, 500 µl of 8% polyethylene glycol (PEG) were added. The mixture was incubated for a further 30 min at room temperature and the bound radioactivity was separated from the unbound fraction by centrifugation. The supernatant was decanted and the radioactivity of the precipitate was counted in a gamma-counter. The quantity of radioactivity in the precipitate (antibody-bound ^{125}I-AFP) varied inversely with the amount of AFP in the standard solutions. Thus a standard curve could be generated and the quantity of AFP in the tubes containing the serum samples could be determined from the quantum of radioactivity that was precipitated. The assay was quite specific and did not compete with a number of other proteins tested. The useful range of standard curve was from 0 to 350 ng/ml. The intra-assay and inter-assay CV done by measuring repeatedly two positive patient samples were between 3%–4% and 4.5%–6%, respectively. Measurement of eight patient serum samples sent for testing at the Clinical Diagnostics Laboratory (CDL) of University of Malaya Medical Centre (UMMC) showed good agreement between the results obtained by this assay and the commercial kits used by CDL. Therefore the RIA procedure using the locally raised antiserum against AFP may be used as a diagnostic tool and for research purposes at relatively low cost.

MOLECULAR BASIS OF ETHNIC DIFFERENCES IN DRUG RESPONSES AND DISPOSITIONS: EVIDENCE FROM META-ANALYSES OF ALLELIC, GENOTYPIC AND PHENOTYPIC DISTRIBUTION OF CYP2C SUBFAMILY

WONG, L.P.[1], CHAN, E.S.Y.[2], ATIYA, A.S.[1] AND LANG, C.C.[1]
[1] Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2] NMRC Clinical Trials and Epidemiology Research Unit, Singapore

Molecular mechanisms responsible for interethnic differences in drug responses and disposition have been extensively reported and reviewed in Caucasians and Blacks of African descent. This study reviewed, reanalysed and further assessed the most recent data on population-based genetic variations and molecular basis for ethnic differences in the drug metabolising enzyme CYP2C subfamily. Meta-analytical technique was applied to obtain a more precise pooled estimate of global population polymorphism distribution of CYP2C9 (*2 and *3) allelic variants, and CYP2C19PM phenotype and/or genotypes

across various ethnic groups. More importantly, this review incorporated the most current data from studies done in Malaysia, as well as studies from other East Asian and South East Asian population groups. This study revealed evidence of drug metabolising enzyme polymorphisms, and provided the most comprehensive summaries and comparisons of global phenotype, genotype and allelic polymorphic distribution of the CYP2C subfamily across various ethnic populations using meta-analytical technique. A better understanding of the molecular basis underlying ethnic differences in drug responses and disposition will contribute to improved individualised drug therapy. Most important of all, such population-specific differences may help explain adverse effects and drug reactions in patients of different population background.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF GALLS OF *QUERCUS INFECTORIA*

BASRI, D.F., FAN, S.H. AND ZIN, N.M.
Department of Biomedical Science, Faculty of Allied Health Sciences,
Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

The galls of *Quercus infectoria* (*Q. infectoria*) or 'biji manjakani' are widely employed as postpartum Malay traditional medication to restore the elasticity of the uterus as well as to treat infections associated with episiotomy tear. Known as 'majuphal' in India, it is popularly used as dental powders and also as remedy for toothache and gingivitis. In this paper, the aqueous and acetone extracts from the galls of *Q. infectoria* were screened against three Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Bacillus subtilis* (*B. subtilis*), and three Gram-negative bacteria, *Escherichia coli* O157:H7 (*E. coli*), *Salmonella typhimurium* NCTC 74 (*S. typhimurium*) and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) using disc diffusion method. Out of six bacterial species tested, *S. aureus* was the most susceptible microorganism with an intermediate degree of sensitivity towards the extracts displaying inhibition zones of 14.34 ± 0.73 mm (aqueous extract) and 13.87 ± 0.36 mm (acetone extract), compared to 18.20 mm (gentamicin 10 μg/disc). On the other hand, the extracts showed weak inhibitory effect against *S. epidermidis*, *B. subtilis*, *S. typhimurium* and *P. aeruginosa*, while there was no inhibition zone observed for *E coli*. The minimum inhibition concentration (MIC) values of the extracts ranged from 0.08 to 1.25 mg/ml whereas the maximum bactericidal concentration (MBC) values ranged from 0.31 to 2.50 mg/ml. The MBC values of the aqueous extracts against *S. aureus* and *S. typhimurium* were higher than their MIC values. The MBC value of the acetone extract against *S. aureus* was also higher than its MIC value. Interestingly, however, the MIC and MBC values of the acetone extract against *S. typhimurium* were the same (1.25 mg/ml). Our findings suggest that both the aqueous and acetone extracts of the galls of *Q. infectoria* are bacteriostatic against *S. aureus*, whereas the acetone extracts were bactericidal against *S. typhimurium*. As such, the galls of *Q. infectoria* are potentially good sources of antibacterial agents. The antimicrobial property of the extracts of the galls of *Q. infectoria* in this study could possibly be due to the presence of tannin.

ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL COMPOUNDS OF *CALLICARPA FARINOSA*

CHUNG, P.Y. [1], CHUNG, L.Y. [1], NGEOW, Y.F. [2] AND GOH, S.H.[3]
Departments of [1]Pharmacy, and [2] Medical Microbiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia
[3] Forest Research Institute Malaysia, Kuala Lumpur, Malaysia

Antibiotic resistance has become an alarmingly prevalent problem worldwide, increasing the mortality and morbidity associated with infectious diseases and the cost of treatment. Therefore, the development of novel antimicrobials is an important strategy for combating antimicrobial resistance. Plant-based resources have enormous but largely untapped, therapeutic potential for treating infectious diseases. Antimicrobial activities against reference Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and *Candida albicans* were tested on 209 plant extracts obtained from more than 30 families of plants found in the state of Sabah, Malaysia. The plant extracts were tested by a disc-diffusion technique in which antimicrobial activity was evaluated based on the ability of the plant extracts to diffuse through agar to affect the target organisms. *Callicarpa farinosa* (Verbenaceae) which exhibited high antimicrobial activities against reference and clinical strains of *Staphylococcus aureus* was selected for further separation and isolation. The chloroform extract of the bark of *Callicarpa farinosa* afforded five compounds found to be pentacyclic triterpenoids. The antimicrobial activities of all these purified compounds were examined against reference and clinical strains of *Staphylococcus aureus* (methicillin-sensitive and methicillin-resistant). All five compounds showed activity with minimum inhibition concentration (MIC) ranging from 4 to 31 µg/ml. However, the MIC for the test organisms could be lowered to a range of 3 to 5 µg/ml with a combination of two different compounds. The range obtained was comparable to vancomycin which has a MIC of 4 µg/ml. These compounds could be further developed to overcome the problems of resistance by MRSA as vancomycin is fast losing its effectiveness as an option for treatment. Other plant extracts worthy of further investigation are the extracts of *Callicarpa erioclona* (Verbenaceae), *Siphonodesma friflora* (Verbenaceae) and *Homalium panayanum* (Flacourticeae).

ANTIMICROBIAL SCREENING FOR SOME MALAYSIAN FLORA

SOMCHIT, M.N. AND ABDELWAHAB, S.I.
Department of Biomedical Sciences, Faculty of Medicine and Health Science,
Universiti Putra Malaysia, Malaysia

The flora in South East Asia is very rich and Malaysia possesses a variant and large number of plants that are used traditionally in the treatment of a variety of diseases. In this study we aim to investigate the antimicrobial activity of some Malaysian flora from different families, namely, *Curcuma phaeocalis*, *C. aeruginosa*, *C. xanthorrhiza*, *C. domestica*, *Zingiber minor*, *Azadarichta indica*, *Solanum nigrum*, *Piper betel* and *Averrhoa bilimbi*. Different concentrations from the plant ethanolic extracts were loaded onto Whatman No. 1 filter

paper discs (∅, 6 mm) and the discs were placed in the microbial cultures of Gram-positive bacteria (*Staphylcoccus aureus*, *Bacillus sibtilis*, *Micrococcus lotus* and *Enterococci faecalis*); Gram-negative bacteria (*Pseudomonas aeuroginosa*, *Escherichia coli*, *Salmonella infantis* and *S. enteritidis*); yeast-like fungi (*Candida albicans*, *C. tropicalis* and *Cryptococcus neoformis*); and filamentous fungi (*Aspergillus fumigatous*, *A. ochraceus* and *Microsporum canis*). After incubation periods, the clear zones around the discs were noted. None of the above mentioned species was found to exhibit any antimcrobial activity up to 150 μg/disc.

ADVANCED GCMS TECHNIQUES FOR TRACE ANALYSIS

HUI, L.L.C.[1], NAKAGAWA, K.[2], TANAKA, K.[2] AND MIYAGAWA, H.[2]
[1] Shimadzu (Asia Pacific) Pte Ltd, Singapore Science Park, Singapore
[2] Shimadzu Corporation Japan, Analytical Instruments Division 1, Kyoto, Japan

Achieving a high sensitivity and selectivity is always the most challenging task in trace analysis of complex matrix samples. This paper reports two techniques to improve the performances of GCMS methods in this application. High-pressure injection method described in this report involves the use of an increasing pressure of the carrier-gas when a sample is injected. This high pressure during sample injection compresses the vaporised gas samples. As a result, a larger amount of the sample, than is normally possible with a conventional method, can be introduced, and thus the sensitivity of the method is increased. One cannot rely on the relative retention times to confirm target analytes in a sample if co-elution occurs in GC analysis. Even using an MS with a conventional EI source in GCMS analysis, the co-elution problem may not be solved. This is because the ratios of the mass peak intensities of the indicative ions to the target ion will not match the criteria (within an acceptable tolerance) of identification if some fragment ions of the same m/z values from the co-elutes are present. It is found that NCI is much more selective than EI, and it can identify more effectively the analytes with co-elution occurring. This is particularly useful in the detection and identification of electron-affinitive-type in complex matrices.