Malaysian Journal of Pharmaceutical Sciences
Vol. 9, No. 1, (2011)

IN VIVO ORAL TOXICOLOGICAL STUDIES OF NUFERA® VIRGIN COCONUT OIL

AHMAD H. IBRAHIM[1], KHAIRUL ANNUAR ZIT[2], SAWSAN S. AL-RAWI[3], MOHAMED B KHADEER AHAMED[1] AND AMIN MALIK SHAH ABDUL MAJID[1]

[1]Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

[2]Genomelife Sdn. Bhd. (709881-A), E-2 Blok E, Excella Business Park, Jalan Ampang Putra, Taman Ampang Hilir, 55100 Kuala Lumpur, Malaysia

[3]Department of Environmental Technology, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Justification: Nufera is a trade mark for commercially available edible coconut oil supplied by Genomelife Sdn. Bhd., which is being utilised by a large population of people in Malaysia. It is extracted through fermentation process. Primarily, it is extracted from the kernel of matured coconut. Coconut oil has various applications in food, medicine, and industry.

Objectives: The present study aims to determine the safety of Nufera® virgin coconut oil by determining its possible harmful or noxious effects after acute and subchronic administration in Sprague-Dawley rats.

Materials and Methods: Acute and subchronic tests were the principle experiment conducted at EMAN test laboratory, USM, following the OECD guidelines. In acute toxicity test, up and down method (limit dose) was conducted. A single dose of 5000 mg/kg of the Nufera® virgin coconut oil (VCO) was given orally to 5 healthy female adult rats. The rats were observed for mortality and clinical signs for 3 h and then periodically for 14 days. While in the subchronic toxicity study, a daily dose (175, 550 and 2000 mg/kg) of Nufera® VCO was administered orally to 3 different groups of female and male rats, respectively for 28 days. At the end of experiment, all animals were euthanised, followed by gross necropsy, histopathology of vital organs and hematology.

Results: The results showed that, in acute toxicity study, Nufera® VCO at a dose of 5000 mg/kg caused neither visible signs of toxicity nor mortality. All five rats were healthy throughout the experiment and survived until the end of observation period. In subchronic toxicity, administration of the Nufera® VCO at 175, 550 and 2000 mg/kg for 28 days did not produce any mortality and there were no significant differences in the general condition, growth, organ weights, hematological parameters, clinical chemistry values, or gross and microscopic appearance of the organs from the treatment groups as compared to the control group. The LD_{50} for the Nufera® VCO is found to be more than 5000 mg/kg body weight whereas, the observed adverse effect level (NOAEL) was found to be 2000 mg/kg per day for 28 days.

Implication: It can be concluded that, Nufera® VCO did not cause any mortality nor did it cause abnormalities in necropsy and histopathology findings. There were no acute or subchronic toxicity observed and the Nufera® VCO is devoid of any adverse risk. Hematological and histological observations reveal some considerable enhancement of general health of the animals with respect to their growth and food intake. Nufera® VCO possessed bioenhancement effect that requires further in depth study to investigate the action of its mechanism.

PHARMACOLOGICAL EVALUATION OF *NARDOSTACHYS JATAMANSI* FOR ITS SPASMOLYTIC, BRONCHODILATOR, VASODILATOR AND PLATELET AGGREGATION INHIBITORY ACTIVITIES

ASHFAQ AHMAD[1], KHALID HUSSAIN JANBAZ[2], ANWARUL HASSAN GILANIA[3], MALIK HASSAN MEHMOOD[3], MUNNAVVAR ZUBAID ABDUL SATTAR[1] AND HASSAAN A. RATHORE[1]

[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

[2]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

[3]Natural Product Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi 74800, Pakistan

Introduction: The dried roots and rhizomes of *Nardostachys jatamansi* DC, commonly known as maskroot, have been found to have many traditional uses. The infusion of the roots has been used to help treat mental disorders, insomnia, and disorder of the blood and circulatory systems. In the native systems, the medicinal plant has been mentioned as having hepatotonic, cardiotonic, diuretic and analgesic properties. Scientific investigations on *N. jatamansi* revealed its antibacterial and antifungal activities. It is also found to be effective in the prevention of cognitive impairment and neuro-degeneration. Plants roots were reported as possessing antioxidant, analgesic and antimalarial activities. Although not supported by scientific data, this plant is traditionally believed to be effective in treating gastrointestinal tract ailments, asthma, blood pressure and platelet aggregation inhibition.

Objectives: The aim of this study was to evaluate the traditional use of *N. jatamansi* for its spasmolytic, bronchodilator, vasodilator and platelet aggregation inhibition activities.

Materials and Methods: The spasmolytic activity of *N. jatamansi* was investigated on isolated tissues of rabbit jejunum and guinea pig ileum. Spasmolytic activities were confirmed both by in vitro and in vivo methods. Bronchodilator activity was validated by using isolated tissue rings of the rabbit trachea. Vasorelaxant effect was confirmed by using aorta of rats and rabbits while platelet aggregation inhibition was confirmed by using the human blood sample.

Malay J Pharm Sci, Vol. 9, No. 1 (2011): 27–37

Results: The phytochemical investigations on methanolic extract of dried rhizomes of *N. jatamansi* exhibited saponins, flavonoides, terpines, sterols, tannins and anthraqinones, among the detected constituents of plant. The investigations on isolated tissue preparations revealed that crude methanolic extract of *N. jatamansi* exerted non-specific relaxant effect against high K^+ (rabbit jejunum, rabbit aorta, rat aorta and rabbit trachea); phenylephrine (rabbit aorta and rat aorta); and carbachol (rabbit trachea)-induced contractions possibly mediated through Ca^{+2}-channel blocking mechanisms. The crude methanolic extract of the plant was also found to exert gastrointestinal muscle relaxant activity since it was shown to have demonstrated antidiarrhoeal effect against castor oil-induced diarrhoea in mice. These findings validate the vernacular use of the rhizomes of *N. jatamansi* DC in gastrointestinal, cardiovascular and respiratory ailments channel blocking mechanism. ADP induced platelet aggregation was inhibited by Nj. Cr.

Conclusion: The present investigation can be concluded that *N. jatamansi* exhibited relaxant effect on gastrointestinal and bronchiolar smooth muscles possibly mediated through multiple modes of action but mainly blockade of Ca^{+2} channels is presumably involved. The relaxant activity on gastrointestinal muscle was reflected as crude plant extract caused inhibition of castor oil-induced diarrhoea in mice. The crude plant extract caused inhibition of ADP-induced platelets aggregation.

SYNERGISTIC HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITY OF *VERNONIA AMYGDALINA* AND *AZADIRACHTA INDICA*

ITEM J. ATANGWHO[1, 2], PATRICK E. EBONG[1], MOHD ZAINI ASMAWI[2] AND MARIAM AHMAD[2]

[1]Department of Biochemistry, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria

[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

The synergistic hypoglycemic and antihyperglycemic activity of *Vernonia amygdalina* (VA) and *Azadirachta indica* (AI) was evaluated in this study, with a view to scientifically validate the practice of combining these herbs as a management for diabetes in traditional medicine. We therefore, monitored daily changes in blood glucose and body weight of streptozotocin-induced diabetic (SDR) and non diabetic (NDR) rats treated with the combined extracts (100 mg/kg b.w. each of VA and AI, p.o) and insulin (Humulin, 5 units/kg b.w., s.c) for 28 days. Glucose levels in whole blood, serum and liver-whole-homogenate (LWH) were assayed, along with serum α-amylase activity in SDR and NDR at the end of the 28 days. Results showed significant decrease ($p<0.05$) in blood glucose of both SDR and NDR. However, both decreases were prompt with the combined extracts and insulin compared to extracts of VA and AI alone, which only began 7 days after commencement of administration. Moreover, the time-course variation in blood glucose of rats treated with combined extracts was similar and almost paralleled that of insulin-treated rats, but more steady with extracts treatment than insulin. This trend in blood

glucose regulation was also replicated in body weight regulation. Of the four treatments, VA significantly increased (p<0.05) LWH glucose of NDR, whereas AI significantly decreased (p<0.05) LWH glucose in SDR. However, these opposing effects were modulated in the group administered with a combination of the two extracts. Although α-amylase activity was non significantly decreased (p>0.05) in NDR by the respective extracts and their combination, it was significantly decreased (p<0.05) by insulin treatment. In the SDR, the activity of the enzyme which was decreased in the diabetic control relative to the NDR control, became increased significantly (p<0.05) upon treatment with individual extracts and their combination; but not with insulin treatment. Combined extracts of VA and AI have exhibited positive synergistic action on glucose control in both diabetic and non diabetic rats compared to the single extracts, which may justify their combination in traditional settings.

IN VIVO STUDY OF ANTITUMOUR ACTIVITY OF THE STANDARDIZED 50% ETHANOLIC EXTRACT OF *ORTHOSIPHON STAMINEUS* AGAINST COLORECTAL CARCINOMA INDUCED ORTHOTOPICALLY IN NUDE MICE

FOUAD SALEIH RESQ AL-SUEDE[1], MOHAMMED KHADEER AHAMED[1], MOHD. OMAR B. ABD. KADIR[2], ZHARI ISMAIL[1] AND AMIN MALIK SHAH ABDUL MAJID[1]

[1]School of Pharmaceutical Sciences,
[2]School of Industrial Technology, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Objectives: Orthosiphon stamineus Benth. (Lamiaceae), a traditional medicinal herb, has been used as a diuretic and remedy for angiogenesis-related disorders. Phytochemical studies revealed that it is enriched with flavonoid, terpenoid, caffeic acid derivatives, chromene and phenolics. The orthotopic transplantation model of human tumour has been demonstrated to be more "patient-like" animal tumour model. The purpose of this study is to establish the in vivo antitumour activity of the standardised 50% ethanolic extract of *O. stamineus* against colorectal carcinoma induced orthotopically in nu/nu nude mice.

Materials and Methods: Explants tissue of colon tumours grown subcutaneously in nude mice was transplanted into cecum wall of the other group nude mice. After the complete healing of the incision wound, the treatment with the extract (200 mg/kg) was initiated for 6 weeks. During this period, the body weight was recorded every week. At the end of the experiment, the animals were euthanised and the tumours were dissected out and sizes were measured by digital callipers.

Results: All mice accepted the tumour implantation procedure well and resumed normal activities within 10–20 min after cessation of anaesthesia. There were no surgical complications, infections or deaths. The tumour uptake percentage was 80%. There are no significant changes in the weight of both groups before and after the treatment (p>0.05). The extract inhibited the tumour growth significantly by 82.8 ± 6 (p< 0.05).

Conclusion: An orthotopic transplantation model for human colon in nude mice has been established. *O. stamineus* may be having a potential source for chemotherapeutics to treat neoplasia.

TOXICITY EFFECT OF WATER EXTRACTS OF *HOLOTHURIA ATRA* IN MICE

NURUL ALIA AZIZAN[1] AND RIDZWAN HASHIM[2]
[1]Faculty of Medicine and Health Science, Universiti Sultan Zainal Abidin (UniSZA), Kampus Kota, 20400 Kuala Terengganu, Terengganu
[2]Office of the Campus Director, International Islamic University Malaysia (IIUM), Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan, Pahang

Holothuria atra is a well known marine animal among the local traditional practitioners. It is believed to have medicinal values and is also consumed as food by the locals mainly based on local culture and beliefs. The aim was to determine the lethal median dose (LD_{50}) and histopathological toxicity of water extract of *H. atra* after intra peritoneum administration in mice. The behavioral changes and mortality were assessed in mice after administrations of water extract of *H. atra* for up to 14 days. Then, after day 14, histopathological examination of the liver was done. Seven doses of *H. atra* solution; 10 mg/kg, 20 mg/kg, 30 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg were administered and compared to a control group which was administered normal saline. In the acute study in mice, the water extracts of *H. atra* caused dose-dependent general behavior adverse effects and mortality. The main behavioral sign of toxicity observed was hypoactivity, noticed immediately after administration of the extract which was more obvious at the higher doses and persisted until death. Mortality increased with increasing doses: the calculated LD_{50} was 41 mg/kg in mice. The liver toxicity was confirmed by histopathological examination, which indicated the presence of abnormal hepatocytes with a distorted shape and undefined cell lining as well as enlarged nuclei in low doses groups. High doses groups indicated a more prominent distortion of the polyhedral hepatocytes with undefined cell lining, massive cytoplasm, pyknotic, karyorhexis and karyolytic nuclei (necrosis of hepatocytes). Control group showed polyhedral hepatocytes with defined cell lining arranged in cords and normal round nuclei, with granular cytoplasm. In conclusion, because of the relatively low LD_{50} value in the acute study in mice, it may be concluded that the *H. atra* extract is toxic.

ACTIVITY GUIDED ISOLATION OF ANTIINFLAMMATORY CONSTITUENTS FROM *KAEMPFERIA GALANGA* (ZINGIBERACEAE) L. EXTRACTS

MUHAMMAD IHTISHAM UMAR[1], MOHAMMAD ZAINI ASMAWI[1], AMIRIN SADIKUN[2] AND RABIA ALTAF[1]

[1]Department of Pharmacology,
[2]Department of Chemistry, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Ethnopharmacological Relevance: Kaempferia galanga (KG) is a valuable medicinal herb of Zingiberaceae that exhibits a number of pharmacological activities. Extracts of KG have potent larvicidal, mosquito repellent, analgesic, vasorelaxant, nematicidal, antineoplastic, sedative, antimicrobial, antiallergic, antioxident and wound healing properties. A considerable amount of work has already been done in the isolation and identification of phytoconstituents with reference to the above mentioned pharmacological effects of the herb. Most of these pharmacological effects of KG extracts are due to ethyl trans para methoxycinnamate (EPMC) and ethyl cinnamate. The herb is also reported for its antiinflammatory effect; however the constituents in KG extracts responsible for this antiinflammatory effect are not yet identified.

Objectives: The aim of this study was to isolate the antiinflammatory constituents from the rhizomes of KG.

Materials and Methods: Rhizomes of KG were collected from Sungai Putani (Penang, Malaysia). The rhizomes were dried, powdered and then serially extracted in petroleum ether, chloroform, methanol and water. After freeze-drying, the extracts were tested for their antiinflammatory potential in rats by using carrageenan induced paw oedema. The most effective extract was further dissolved in n-hexane to get fraction 1. The residue was dissolved in a mixture of chloroform and n-hexane (50% chloroform: 50% n-hexane) to get fraction 2. The residue after the collection of fraction 2 was taken as fraction 3. All the three fractions were further evaluated for antiinflammatory potential by using carrageenan induced paw oedema in rats. The most active fraction out of the three was further dissolved in a mixture of chloroform and n-hexane (75% chloroform: 25% n-hexane) to get sub-fraction 1 whereas the residue was taken as sub-fraction 2. Both sub-fractions were further investigated for the antiinflammatory effect in rats using the above mentioned model. The most effective sub-fraction was subjected to gas chromatography-mass spectroscopy (GC-MS) for the identification of active constituents.

Results: Constituents in chloroform extract with more trends towards polarity were found to be responsible for the antiinflammatory effect of KG. Although most of the pharmacological effects of KG are due to EPMC, the antiinflammatory effect of KG is not due to the same.

Conclusion: The study concludes that the extracts of KG have potent antiinflammatory effect that is even comparable with indomethacin. With already evident potent analgesic effect, these results appreciate the use of KG extracts for the management and treatment of

inflammatory complications instead of conventional non steroidal antiinflammatory drugs that have major gastric side effects.

STUDY OF CARDIOVASCULAR EFFECTS OF *GYNURA PROCUMBENS* LEAF EXTRACTS ON ISOLATED RAT THORACIC AORTA AND ATRIUMS

NAVNEET KAUR, AMMAR EHSAN, RABYAH B. ALI, MOHD. ZAINI ASMAWI AND AMIRIN SADIKUN

School of Pharmaceutical Sciences, Universiti Sains Malaysia,
11800 USM Pulau Pinang, Malaysia

Introduction: Gynura procumbens (Lour.) Merr. (Compositae), or locally known as *sambung nyawa* is cultivated in most of the Southeast Asian countries and have been traditionally used for various ailments including antihypertensive.

Objectives: The preliminary study aimed to investigate the cardiovascular effects of the different ethanolic aqueous (95%, 75%, 50% and 25% v/v) and water extract of *G. procumbens* leaves on isolated rat thoracic aorta and, right and left atriums.

Material and Methods: Five different extracts and then butanol and aqueous fractions of active extract of *G. procumbens* leaves were studied for vasodilation effects on endothelium intact, precontracted with phenylepherine, rat thoracic aorta. Latter the cardiac effects (chronotropic and ionotropic effects) of these extracts were studied.

Results: Significant vasodilation and –ve chronotropic and –ve ionotropic effect were observed with the water extract as compared to the ethanolic extracts of *G. procumbens*. On fractionation of active water extract, the butanol and aqueous fraction didn't show the vasodilation effect. The cardiac effects were similar as GPWE.

Conclusion: The finding suggests that water extract of *G. procumbens* has promising cardiovascular effects and needs further investigations.

IN VIVO ANTIHYPERTENSIVE POTENTIAL OF *ORTHOSIPHON STAMINEUS* IN SPONTANEOUS HYPERTENSIVE RATS (SHR): A PRELIMINARY STUDY

NURUL ALIA AZIZAN[1], RASHIDI AHMAD[2], KHALEED MOHAMED MOHAMED KORIEM[3], MOHD ZIKRI AHMAD[2] AND ZAINI ASMAWI[4]

[1]Faculty of Medicine and Health Science, Universiti Sultan Zainal Abidin, Kampus Kota, 20400 Kuala Terengganu, Terengganu, Malaysia

[2]Emergency Department, School of Medical Sciences, Health Campus USM, 16150 Kubang Kerian, Kelantan, Malaysia

[3]Advanced Medical and Dental Institute, Universiti Sains Malaysia, No 1-8 (Lot 8), Persiaran Seksyen 4/1, Bandar Putra Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

[4]School of Pharmaceuticals Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Orthosiphon stamineus Benth. (*OS*) is widely used in Southeast Asia as a traditional folk medicinal herb and claimed to treat many diseases such as fever, high blood pressure, gout and diabetes mellitus. This study was conducted to investigate the antihypertensive effect of the standardised methanol extracts of *OS* in spontaneous hypertensive rats (SHR). The antihypertensive effect of standardised methanol extracts was examined by treating different groups of SHR with a single oral administration (10 mL/kg) for 14 days. The rats were randomly divided into five different groups of three rats each; Group 1 received Irbesartan (IR) drug (20 mg/kg) that served as positive control; Group 2 received distilled water and served as negative control; and treatment groups (Group 3, 4 and 5) were treated with *OS* extracts at three different doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. Systolic blood pressure (SBP) was measured by using tail cuff method on day 0 (before initiating treatment) and at the end of observation period (day 14th). Daily oral administration of the methanol *OS* extract at three different doses for two weeks exhibited a significant decrease in SBP (p<0.05) in SHR. The SBP was reduced significantly at the end of treatment (day 14th) compared to the pre treatment day (day 0) at these dosage: 250 mg/kg (p-value: 0.012); 500 mg/kg (p-value: 0.006); 1000 mg/kg (p-value: 0.044). In conclusion, these results provide evidence of the effectiveness of standardised methanol extracts of *OS* by reducing systolic blood pressure in SHR.

Malay J Pharm Sci, Vol. 9, No. 1 (2011): 27–37

ANTIDIABETIC ACTIVITY OF *PHALERIA MACROCARPA* F RUITS EXTRACTS IN STZ-INDUCED DIABETIC RATS

RABYAH B. ALI, NAVNEET KAUR, ALI JIMALE MOHAMED, MOHD ZAINI ASMAWI AND ROZAIHANIM MAHMUD
School of Pharmaceutical Sciences, Universiti Sains Malaysia,
11800 USM Pulau Pinang, Malaysia

Introduction: Phaleria macrocarpa is a popular herbal medicinal plant in Indonesia with the local name *mohkota dewa*. This medicinal plant is popular because its leaves and fruits are considered to be able to cure various diseases such as cancer, impotency, diabetes mellitus, allergies, dysentery, rheumatism, high blood pressure, stroke, migraine and cholesterol related ailments.

Objectives: This study was carried out to investigate the hypoglycemic effect of *P. macrocarpa* fruit extract on normal rats and diabetes induced rats for 12 days.

Materials and Methods: The powder of *P. macrocarpa* fruits was extracted with petroleum ether and methanol using soxhlet apparatus and then extracted with water by maceration. Glucose levels in the rats with hypoglycemia were induced by streptozotocine (65 mg/kg body weight) with metformine 500 mg/kg as positive control. An Intraperitoneal Glucose Tolerance Test (IPGTT) was also performed with 1 g/kg glucose loading on normal rats using glibenclimde (10 mg/kg) as standard.

Results: For the normal rats, for both hypoglycemic and IGPTT, methanol extract of *P. macrocarpa* reduced blood glucose level but was not significant. However methanol extract of *P. macrocarpa* fruits was found to have significant antihyperglycemic effect on STZ-induced diabetic rats compared to the control group.

Conclusion: It is concluded that the fruits of *P. macrocarpa* significantly lowered the blood glucose level in the diabetic rats as alleged by its traditional use.

PHARMACOKINETICS STUDY OF KETOPROFEN IN HEALTHY SHEEP UNDER LOCAL CONDITIONS OF PAKISTAN

S. AFZAL, A. ALI, M. ASHRAF, M. USMAN AND S. AMIN
Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Ketoprofen belongs to the family of non steroid antiinflammatory drugs (NSAIDS). It acts as an antiinflammatory, analgesic and antipyretic drug. The purpose of this study was to evaluate the pharmacokinetic of ketoprofen in healthy sheep under local conditions of Pakistan. Pharmacokinetics parameters of ketoprofen were studied in sheep after a single intravenous dose of 3 mg/kg body mass. Blood samples were collected before and within

24 hours after drug administration at predetermined time intervals. Plasma was separated immediately and concentration of ketoprofen at different time intervals were determined by validated HPLC method using phosphate buffer (pH 7.0) and acetonitrile (75:25) as mobile phase at wavelength of 254 nm. Pharmacokinetic parameters were determined from the plasma concentration-time curves by using two-compartment model. On an average with ketoprofen, the area under curve (AUC) was 5.47±2.72h.mg/L, distribution half life (t½α) was 0.12±0.10 h, elimination half life (t½β) was 1.91±0.95 h, total body clearance (Cl) was 0.65±0.25l/kg/h, steady state volume of distribution (Vdss) was 0.82±0.46 L/kg, maximum plasma concentration (Cmax) was 6.68±2.28 mg/L and time to reach maximum concentration (Tmax) was 0.12±0.02 h. Pharmacokinetic parameters observed in Pakistani sheep showed that dosage adjustment is required and dose should be higher than the recommended dose as prescribed in literature.

THE SOD MIMETIC TEMPOL RESTORES RENAL VASCULAR VASODILATATION IN CYCLOSPORINE A INDUCED RENAL INSUFFICIENCY IN SPRAGUE-DAWLEY RATS

TAN YONG CHIA[1], MUNAVVAR A. SATTAR[1], MOHAMMED H. ABDULLAH[1], FAIZ UD DIN AHMAD[1], ZAID O. IBRAHEEM[1], KHOO JOO LI[1], YEN PEI PEI[1], HASSAAN A. RATHORE[1] AND GURJEET KAUR CHATAR SINGH[2]

[1]School of Pharmaceutical Sciences,

[2]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Introduction: An increasing body of evidence suggests that oxidative stress is involved in the pathogenesis of a wide range of cardiovascular diseases, including hypertension and renal failure. Besides that, oxidative stress may contribute to the progression of renal disease indirectly by promoting hypertension or directly by inducing glomerular damage and renal ischemia. Much interest centers on the concept that dramatic increase in mortality in patients with chronic renal failure (CRF) may be secondary to the deleterious cardiovascular effects of oxidative stress. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a superoxide dismutase (SOD) mimetic is a water soluble stable and paramagnetic nitroxyl radical or nitroxide, and hence it is very efficient in scavenging free radical. SOD catalyzes the conversion of O_2^- to H_2O_2, which is then turned into water by catalase reaction.

Objectives: To interest of this study was to investigate the altered haemodynamics and the vascular dysfunction in Cyclosporine A (CsA) induced renal insufficiency model and whether it could be ameliorated by Tempol.

Materials and Methods: Sixteen male Sprague Dawley (SD) rats with a body weight range of 180–230 g were assigned into two groups (n=8) as SD rats treated with CsA (CA) and treated with Tempol + CsA (TCA) respectively. CA rats received CsA for 21 days at 25 mg/kg/day p.o; TCA rats received Tempol for 21 days at 30 mg/kg/day p.o. The vascular and renal haemodynamics were measured at the end of the treatment period. Mean arterial blood pressure (MAP), renal excretory function and metabolic study were

also determined at day 0 and day 21 respectively. Renal histology was also performed to investigate the morphological changes during pathological condition. Data were presented as mean±S.E.M and were analysed using ANOVA with significance at $p<0.05$.

Results: The metabolic and renal functional parameters showed significant ($p<0.05$) improvement after 21 days of treatment of Tempol in CA rats; with body weight (CA: 215±9 g; TCA: 239±3 g), fluid intakes (CA: 24±6 mL; TCA: 28±3 mL), creatinine clearance (CA: 3.4±0.2 mL/min/kg; TCA: 4.7±0.4 mL/min/kg), glomerular filtration rate (CA: 0.5±0.1 mL/min/kg; TCA: 6.5±1.7 mL/min/kg), urinary protein excretion (CA: 49.3±1.3 mg/mL/kg; TCA: 12.80±1.2 mg/mL/kg). Mean arterial blood pressure was reduced significantly ($p<0.05$) (CA: 119±3 mmHg; 102±7 mmHg). The overall mean response to renal vasoconstriction due to bolus injection of exogenous agonists were also improved ($p<0.05$) significantly with noradrenaline (CA: 35.52±1.4%; TCA: 49.49±2.7%), phenylephrine (CA: 44.51±4.3%; TCA: 51.82±3.5%), methoxamine (CA: 28.36±7.6%; TCA: 37.48±6.2%) and aqngiotensin II (CA: 24.78±1.6%; TCA: 31.22±3.4%) respectively. Kidney of CA rats showed severe disruption in the glomerulus and tubular structure mainly in subcapsular area with abscess neutrophils inflammation surrounded by foamy macrophages. However, SD rats treated with Tempol did not show any abnormality in the kidney.

Conclusion: The SOD mimetic Tempol restores vasodilation in CsA treated SD rats. Furthermore, 3 weeks administration of Tempol also ameliorates the adverse of CsA on renal functional and structural abnormalities. In summary, Tempol may be acting as a powerful antioxidant that stimulates the endothelium dependent vasodilation factor by lowering or scavenging the oxidative free radicals by which free radical always suggested to be the culprit of many chronic diseases such as renal failure and hypertension.