# THE EFFECTS OF Clinacanthus nutans AQUEOUS EXTRACT ON RAT MODEL OF POLYCYSTIC OVARIAN SYNDROME (PCOS)

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#### **Background:**

Polycystic ovarian syndrome (PCOS) is the most common endocrine abnormality in reproductive aged women affecting approximately 5 to 10% of this population. One of the diagnostic criteria is hyperandrogenism that affect the ovulation process and oocyte development, hence, increasing the rate of infertility. *Clinacanthus nutans* (CN) has been found to possess a wide range of bioactive compounds. The presence of isoflavones was believed to possess an anti-oestrogenic property that may help in the treatment of this syndrome. This study aimed to determine the effects of CN extract on rat model of PCOS.

#### **Methods:**

A total of 15 virgins, adult female Sprague Dawley (SD) rats were divided into 5 groups (n = 3) namely control group, vehicle only group, dehydroepiandrosterone (DHEA) + vehicle group, PCOS-metformin group, and PCOS-CN group. An optimisation for PCOS induction in 21-day duration was conducted in these three groups of rats: (a) control group, (b) vehicle only group, and (c) DHEA + vehicle group. PCOS-metformin and PCOS-CN groups were classified as the treatment groups have been given the respective treatments for 15-day post-induction.

#### **Results:**

In the induction phase, the body weight of the rats between control group and vehicle only group (p = 0.032), as well as control group and DHEA + vehicle group (p = 0.007), were significantly increased on Day 21 (final body weight). Besides that, PCOS induction by using DHEA via intraperitoneal administration significantly increased the number of follicular cyst (p = 0.0145) and significantly decreased the number of corpus luteum (p = 0.020) between control group and DHEA + vehicle group. However, CN aqueous extract did not reduce any of the DHEA-induced PCOS characteristics in the rat model.

#### **Conclusion:**

DHEA had successfully established PCOS characteristics in this rat model, yet CN aqueous extract failed to improve PCOS. However, this study warrants further investigation and replication before conclusive final conclusion can be made.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science COMPARISON ON PHYTOCHEMICALS SCREENING, ANTIOXIDANTS AND TOXICOLOGICAL EVALUATION OF AQUEOUS EXTRACTS OF *Dioscorea* hispida AND Plectranthus rotundifolius TUBERS.

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#### **Background:**

In Malaysia, there are many plants that have been used in the traditional medicine of Malaysian such as tubers of *Dioscorea hispida* (DH) and *Plectranthus rotundifolius* (PR) Both have been claimed effective in treating the ailments such as skin infection, microbial infection and wound healing. PR is not poisonous while DH is poisonous. However, both tubers are edible if prepared properly. In this study, the phytochemical constituents, antioxidant and toxicity of aqueous extract of both tubers were investigated with respects to their medicinal value in traditional health care system.

#### **Methods:**

The aqueous extracts of DH (DHAE) and PR (PRAE) were used to screen the phytochemical constituents such as tannins, saponins, alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids and phenols. The antioxidant activity of the aqueous tuber extracts was determined in the terms of their total phenolic (TPC) and flavonoid content (TFC). In evaluation of their toxicity, the brine shrimp lethality test (BSLT) was carried out and the  $LC_{50}$  value of DH and PR tuber extracts was determined.

#### **Results:**

Both DHAE and PRAE showed the presence of tannins, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols, and the absence of cardiac glycosides. Both DHAE and PRAE were found to have a high total phenolic content (TPC) and total flavonoid content (TFC). It is showed that the TPC and TFC values of DHAE were  $21.34\times10^3\pm0.10$  mg GAE/g and  $7.90\times10^3\pm0.01$  mg QE/g respectively. The TPC and TFC values of PRAE were  $32.41\times10^3\pm0.15$  mg GAE/g and  $6.31\times10^3\pm0.01$  mg QE/g respectively. In comparison, DHAE exhibited lower TPC and higher TFC values than PRAE. In BSLT, DHAE demonstrated lower LC<sub>50</sub> value compared to PRAE, with LC<sub>50</sub> value of  $1.13\times10^3\,\mu\text{g/mL}$  and  $1.23\times10^3\,\mu\text{g/mL}$ . However, both DHAE and PRAE were found to be non-toxic since LC<sub>50</sub> values of both extracts were more than  $1.0\times10^3\,\mu\text{g/mL}$ .

#### **Conclusion:**

The study revealed that both DHAE and PRAE possessed the similar group of phytochemical constituents and contain high antioxidants, and both are safe.

# THE EFFECT OF CO-ADMINISTRATION OF MINOCYCLINE AND IFENPRODIL ON TACTILE ALLODYNIA AND C-FOS PROTEIN EXPRESSION IN SPINAL CORD OF STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHY RAT

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#### **Background:**

Neuropathy pain is intense and damaging noxious stimuli that aroused from nerve damage in the diabetic patient. Non-neuronal cells such as microglial cells and N-Methyl-D-Aspartate receptor had been proved to influence this neuropathy pain processing. The objective of this study is to compare the effect of minocycline and ifenprodil treatment as given alone and also by combination on tactile allodynia and c-Fos protein expression in spinal cord of streptozotocin-induced diabetic rat.

#### **Methods:**

This study was involved 5 groups of male Sprague-Dawley rats weighing 200-250g each rat. The group were consisted of Saline group (C) (n=4), Streptozotocin group (STZ) (n=4), Streptozotocin + minocycline (50 mg/kg intraperitoneally/1hour before tactile allodynia on Day 10) (STZ + M) (n=4), Streptozotocin + ifenprodil (1 mg/kg intraperitoneally/30 minutes before tactile allodynia on Day 10) (STZ + I) (n=4) and Streptozotocin + minocycline + ifenprodil (intraperitoneally/1 hour and 30 minutes before tactile allodynia on Day 10) (STZ + M+I) (n=4). All rats except for rats in C group were induced for diabetes by injection of 60mg/kg STZ intraperitoneally on day 0, followed by the assessment of diabetes status by measuring tail vein blood glucose level on day 3. The rats then were tested for tactile allodynia measurement using automatic Von Frey instrument at day 0, 3 and 10 after diabetes induction. Minocycline and/or ifenprodil treatment were given on day 10 before performed the tactile allodynia test. Then, the rats were kept 1 day before sacrificed for immunohistochemistry analysis to detect Fos-like immunoreactivity (FLI) in the rat's spinal cord.

#### **Results:**

The pain threshold for diabetic group (STZ) was significantly decreased on days 3 when compared to day 0 (p<0.05). For diabetic treated groups (STZ+M, STZ+I and STZ+M+I groups), there were significant decrease on pain threshold on the days 3 but increased back during days 10 compared to day 0 (p<0.05). The pain threshold showed significant difference when compared between control to the STZ+ M and STZ+ I groups at days 10 (p<0.05) where the STZ+M group was showed stronger effect. The expression of FLI at both ipsilateral and contralateral sides of the spinal cord was found to be increase in STZ group compared to other groups (p<0.05). The FLI expression in the STZ+ M, STZ+ I and STZ+M+I groups were found significant reduced compared to the STZ (p<0.05) where the STZ+M group was showed better effect.

#### **Conclusion:**

As conclusion, combination treatment of minocycline and ifenprodil treatment (STZ+M+I) did not produce better results in attenuated the tactile allodynia and FLI expression if compared to minocycline and ifenprodil that had given as alone treatment.

# Data Mining for Remedial Potential from The Manuscript of Tama, the Royal Physician of Pontianak

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#### **Background:**

The existence of manuscripts in Malay Archipelago is a symbol of the Malay intellectual tradition and identity in the previous centuries. However, it will not be of much value if it is not preserved and be made evident by means of research. Thus, it is vital that these scripts are identified, read and analyzed. With only 11 publications on the *Nusantara*'s ancient medical literature in 2016, this study adds to the effort of reviving the value of Malay intellectual heritage and deducing its potential as grounds for further medical research. The main interest in this particular study is to understand and analyze Kitab Obat-Obat dan Azimat (MSS B15), a Malay medical manuscript from the early 19<sup>th</sup> century. It documents the medicinal exercise of Tama, the Physician to the royal household of His Majesty of Pontianak (currently the capital city of West Kalimantan, Indonesia). Mainly, the text contains the prescriptions used at the time. Hence, a retrospective study for potential therapeutic agent is proposed. This study, specifically, seeks to identify the *materia medica* employed and to collect scientific evidence from literature search for the aforementioned.

#### **Methods:**

Kitab Obat-Obat dan Azimat (MSS B15) was selected from a list of digitized Malay manuscripts available on British Library website. The manuscript was roughly assessed for its credibility and legibility by reading a few random pages. Following selection of text, all 42 written pages were transliterated from *Jawi* to Romanized Malay (*Rumi*). From the transliterated text, contents were extracted and organized in appropriate categories namely ingredients used and usage. Findings on the relative ingredients and its use from modern literature are also included.

#### **Results:** (expected results)

The result of this study includes the numerical and descriptive presentation of single compound and compounded materials, the different body systems that the remedies are applied for, use of local and imported materials, prescriptions proposed and methods of healing from MSS B15. The comparative analysis with other Malay medical texts with contemporary scientific literatures would be the next step in data mining exercise of this text.

#### **Conclusion:**

The systematized analysis of the *materia medica* in MSS B15 presented benefits to illuminate further medical research in line with modern science.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Gene Expression of Metastasis Biomarkers (MMP-9 and E-Cadherin) in Breast Cancer Cell MCF7 Treated with Neolamarckia cadamba

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#### **Background:**

Metastasis is the spread of tumor from its original site to distant parts of the body. Cancer metastasis are responsible for majority of cancer-related death. In breast cancer, metastasis affects the bone and the lung, and less frequently the liver, brain, and adrenal medulla. MMP-9 and ECadherin are among important biomarkers in metastasis. *Neolamarckia cadamba* (NC) is a tropical tree species that has been shown to have anticancer properties in vitro. In this study, we sought to better understand metastasis process and to identify a new drug target.

#### **Methods:**

NC extracts was prepared. After required cell was seeded, the IC<sub>50</sub> concentration of extracts was determined using Trypan Blue Exclusion Method (TBE). Total RNA was extract and reverse transcribed to cDNA. Quantitative polymerase chain reaction (qPCR) primers were designed and optimized. qPCR plate was designed with each treated cDNA template was run together with untreated cDNA template, non-template control, and housekeeping genes GAPDH using Real-Time PCR Detection System. Expression analysis was performed using CFX manager software. Statistical analysis was performed using Independent t-test.

## **Results:** (expected result)

The NM extract able to exhibit effect on gene expression of metastasis biomarker (MMP-9 and ECadherin).

**Conclusion:** (to be determined)

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Teratogenicity of *Christia vespertilionis* in a zebrafish model.

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#### **Background:**

Chistria vespertilionis is an herbaceous plant that is traditionally used as medicine to treat the illness since many years ago. This plant can be found in some regions of South-East Asia. People usually consumed it by drinking tea from the leaves. Christia vespertilionis has potential as anti-cancer and antimalaria agents. Zebrafish is one of the suitable animal models for toxicity testing as it may give results in a short period of time and the transparency of their embryos allow the direct observation of organ morphology during treatment.

#### Method:

The identity of the *Chistria vespertilionis* leaves was certified by vouchering process and solvent extraction procedures were conducted to produce crude extract. *Christia vespertilionis* extract stock solution was prepared by dissolving 0.1g of it into 1 mL 0.1% Dimethyl sulfoxide (DMSO). The solution then stored in -20°c chiller until used. For the preparation of test solution, a serial of dilution were performed to produce eight different concentrations. In order to investigate its toxic dose, toxicity assessment was conducted on zebrafish model *in vivo*. Zebrafish embryos were exposed with eight different concentrations of the plant extract and the toxicity effects were evaluated. The concentrations used are 25  $\mu$ g/mL, 50  $\mu$ g/mL, 100  $\mu$ g/mL, 200  $\mu$ g/mL, 400  $\mu$ g/mL, 800  $\mu$ g/mL, 1600  $\mu$ g/mL and 3200  $\mu$ g/mL. The LC<sub>50</sub> value was determined by conducting probit analysis. Three selected concentrations then used to examine the developmental effects of *Christia vespertilionis*. At the end of the 72 and 96 hour post-fertilization (hpf), the morphological and cardiac activity effects were observed and compared to controls.

#### **Result (expexted):**

Lethal effect of *Christia vespertilionis* on zebrafish embryo is dose dependent and the  $LC_{50}$  value of *Christia vespertilionis* is approximately 2371  $\mu$ g/L. Abnormal developmental features were observed at 72hpf and 96hpf such as heart edema, pericardia edema, reduce heartbeat, declined blood flow rate, delay hatching period and smaller eye size.

#### **Conclusion:**

These findings indicated the potential developmental toxicity of *Christia vespertilionis* on zebrafish in early development.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Cytotoxicity Testing Of Doxycycline-*Nigella sativa*- Eugenol Emulsion (DNE) Fusion On Human Periodontal Ligament Fibroblast (HPdLF) Cell Lines

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#### **Background:**

Periodontal diseases can associated with other type of disease which is atherosclerosis. Researchers hypothesized that the higher the severity of periodontitis, the higher the percentage of having atherosclerosis because they found that 70% of patients they focused, had the atheroma plaque together with the severe periodontitis. Thus, some new findings about the treatments to cure the periodontal diseases are needed. In this research, the fusion medicine of doxycycline, *Nigella sativa* and eugenol medicine will be used to test its cytotoxicity to the human periodontal ligament cells as none of the researches before had used these fusion to test for the treatment of periodontitis.

#### **Methods:**

The in-vitro testing method used in this experiment to see the growth of the cell culture of human periodontal ligaments in a suitable and favors medium to the cells. The use of in-vitro testing method was to get the original cells from the cultured cells that maintained the characteristics and identity of the primary or secondary cells bought from ATCC suppliers. The cells that had been cultured were tested with different concentration of DNE fusion in 24-well plates and their morphologies were observed. After that, testing the cells with the MTT agent were carried out and observed their behavior. The formazan crystal formation was observed by reading their absorbance by using ELISA microtiter plate reader at 50nm wavelength with reference 690nm.

#### **Results:** (expected result)

The results demonstrated that the Doxycycline-*Nigella sativa*-Eugenol (DNE) fusion was non-cytotoxic at certain concentration on the human periodontal ligament fibroblast cell lines.

#### **Conclusion:**

This Doxycycline-*Nigella sativa*-Eugenol (DNE) fusion exhibit increased effectiveness in terms of invitro tissue healing and tissue regeneration. The synergistic effect between them can be established for the periodontitis treatment.

# IN VITRO ACTIVITY OF Eleusine indica (Linn.) Geartn LEAVES POLAR EXTRACTS IN WOUND HEALING PROCESSES AND ANTIOXIDANT ACTIVITY IN HUMAN DERMAL FIBROBLAST ADULT (HDF-A CELL LINE)

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#### **Background:**

Each year millions of people around the world experience both acute and chronic skin injuries. *Eleusine indica (Linn.) Geartn (E.indica)* is a potent plant having antimicrobial, anti-oxidant and anti-inflammatory properties and their use will help in healing of wounds in a natural and cost-effective way. Thus, aim of this research is to evaluate the in vitro activity of *E.indica* leaves polar extracts in wound healing processes and antioxidant activity in human dermal fibroblast adult (HDF-a cell line).

#### **Methods:**

HDF-a cell was treated with methanol, ethanol and aqueous extracts of *E.indica* leaves with concentration 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/ml for 24 hours for cytotoxicity assay. Cell's proliferations with the chosen concentration from cytotoxicity assay were measured after 24, 48 and 72 hour's treatment by MTT assay. Concentrations of each extract with the high cell's proliferation were next used to monitored wound closure by scratch assay with 24, 48 and 72 hour's treatment and antioxidant activity cells-based. Antioxidant activity was performed by treated the HDF-a cell with hydrogen peroxide and followed by extracts and vice versa and concomitantly.

#### **Results:**

Ethanol extracts showed the highest cell's viability as compared to methanol and aqueous extracts. There is significant different (p<0.05) of cell's viability for ethanol extracts at 1000  $\mu$ g/ml with 157.52  $\pm$  8.31% as compared to untreated cells, 100  $\pm$  0%. Methanol and aqueous extracts showed the cell's viability lower as compared to untreated cells but insignificant (p>0.05). However, all the extract did not show IC<sub>50</sub> value.

#### **Conclusion:**

Improvement in the cell's proliferation, cell's migration on the treated cells and the ability of the extracts in protecting the cells from the oxidative stress shows that *E.indica* extracts can be used as a wound healing agent.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science THE EFFECT OF *POLYGONUM MINUS* ON THE TESTES OF CISPLATIN-TREATED RATS

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#### **Background:**

Cisplatin has been used extensively in chemotherapy treatment for various types of cancer such as bladder cancer, head and neck cancer, esophageal cancer and others. However, cisplatin also comes with side effects that can affect normal cells including normal reproductive cells. While *Polygonum minus* or better known as kesum is one of the easily available plants in our country, often used in traditional medicine to treat various disorders. Therefore this study was aimed to identify the effect of *Polygonum minus* on testis of the cisplatin-treated male rats.

#### **Methods:**

A total of 56 male Sprague-Dawley rats weighing between 180-220 grams were randomly divided into seven group n (8). Control group; Group 1 (normal control), Group 2 (cisplatin 10mg/kg), Group 3 (βCaryophyllene 150mg/kg). Treatment group consists of Group 4 (essential oil 100mg/kg, cisplatin10mg/kg), Group 5 (essential oil 200mg/kg, cisplatin 10mg/kg), Group 6 (essential oil 400mg/kg, cisplatin 10mg/kg), and Group 7 (essential oil 400mg/kg). β-Caryophyllene and essential oil was administered every day before the cisplatin is administered at day 15<sup>th</sup>. 3 days after cisplatin was administered, the rats were sacrificed and the testis were isolated and prepared for homogenate.

#### **Results:**

The malondialdehyde (MDA) level is higher on the group 2 compare to other groups. Superoxide dismutase (SOD) level shows lowest reading in group 5 and 6. Other than that, glutathione (GSH) level decreases with higher dose of essential oil.

#### **Conclusion:**

This finding shows that the essential oil of *Polygonum minus* can give protective effect in testis of the cisplatin-treated male rats by reducing the oxidative stress.

# IN VITRO EVALUATION OF WOUND HEALING EFFECT OF HEXANE, DICHLOROMETHANE AND ETHYL ACETATE EXTRACT OF *ELEUSINE INDICA* (LINN.) GAERTN LEAVES AGAINST HUMAN DERMAL FIBROBLAST CELL

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#### **Background:**

Eleusine indica (Linn.) Gaertn widely accessible in tropical and subtropical countries had been used by local population as traditional medicine to treat various discomfort and diseases. Eleusine indica has been reported to have antioxidant, anti-inflammatory and antibacterial properties, which are the prerequisite characteristics of a good wound healing agent. Non-polar and semi-polar extracts of Eleusine indica has been proven to contain phytochemical compounds that promote wound healing in V-79 cell lines. To the best of our knowledge, its potential contribution against open wound healing using human dermal fibroblast has not been assessed. This study was designed to evaluate the wound healing effect of hexane, dichloromethane and ethyl acetate extracts of Eleusine indica against human dermal fibroblast.

#### **Methods:**

The plants were sampled from General Hospital Kuala Lumpur, Malaysia and the leaves are subjected to maceration extraction method. Seven concentrations were prepared: 31.25, 62.5, 125, 250, 500, 1000, 2000  $\mu$ g/mL. Non-polar (hexane, dichloromethane) and semi-polar (ethyl acetate) extracts of *Eleusine indica* leaves were tested their 24-hour effect against the viability of human dermal fibroblast (HDF) using MTT assay.

#### **Results:**

Hexane extract showed IC50 value in human dermal fibroblast cell viability at 1000  $\mu$ g/mL while dichloromethane and ethyl acetate extracts show no IC50 value up to 2000  $\mu$ g/mL. Hexane extract showed significant cytotoxic effect to human dermal fibroblast at 1000 and 2000  $\mu$ g/mL, dichloromethane extract at 2000  $\mu$ g/mL and ethyl acetate extract at 125 to 2000  $\mu$ g/mL.

#### **Conclusion:**

Human dermal fibroblast cell proliferation at treatment incubation time more than 24 hours should be assessed as *Eleusine indica* hold promising potentials as an alternative to modern topical wound healing agent.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Molecular Investigation of Palmitic Acid on Endometrial Cancer Proliferation

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#### **Background:**

Endometrial cancer (EC) is the fourth most common cancer among women in Malaysia. Obesity, which is manifested by elevated plasma free fatty acids in patients, is an established risk factor for EC. Evidently, cancer cells will alter their fatty acid metabolism to sustain cell growth and proliferation, fulfil energy requirements and provide metabolites for anabolic processes. However, how obesity affects EC metabolism and function to promote EC progression is still poorly understood. The objective of this study is to investigate how the condition mimicking obesity can alter endometrial cancer metabolism and subsequently affects its biology.

#### **Methods:**

Palmitic acid, the most abundant form of endogenous fatty acid, is used to mimic the condition of obesity in this study. The proliferation of ECC-1 cells treated with different concentration of palmitic acid were determined by using methyl thiazolyl tetrazolium (MTT) assay. ECC-1 cells were also treated with bovine serum albumin (BSA) vehicle control, 2 and 8  $\mu$ M palmitic acid. The metabolic phenotype of EC cells exposed to this condition was profiled by measuring the key metabolic genes involved in fatty acid metabolism using quantitative real time polymerase chain reaction (RT-qPCR).

#### **Results:**

ECC-1 cell proliferation increased the highest when treated with palmitic acid in the range of 2-8  $\mu M$ . Most tumour cells will alter their lipid metabolism through de novo fatty acid synthesis. However, it is likely that under the condition of elevated free fatty acids, the tumour cells take this advantage to promote their proliferation. Indeed, EC cells treated with 8  $\mu M$  showed increased uptake of free fatty acids through upregulation of CD36, a fatty acid transporter by two fold and subsequently affect the metabolism of EC cells. Gene expression of acyl-CoA synthetase long chain family member 1 (ACSL1) is also upregulated to activate and direct fatty acids into either catabolic or anabolic metabolism. The fatty acids are potentially directed to anabolic metabolism, through upregulation of diacylglycerol acyltransferase (DGAT1), key enzyme which promotes triglyceride and phospholipid synthesis.

#### **Conclusion:**

In conclusion, under conditions mimicking obesity, ECC-1 cells have altered metabolism associated with increased anabolic metabolism, which potentially promotes EC cell proliferation.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science ANTIDEPRESSANT & ANXIOLYTIC EFFECTS OF ZERUMBONE IN CHRONIC CONSTRICTION INJURY (CCI)-INDUCED NEUROPATHIC PAIN MICE

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#### **Background:**

Neuropathic pain is a chronic condition that is difficult to be treated. Currently available therapies are either ineffective or non-specific and produce several of adverse effects, thus requiring newer treatment approaches to be developed. Zerumbone (ZER) is a sesquiterpene isolated from the edible plant *Zingiber zerumbet*. Zer is found to exert various medicinal benefits such as anti-nociceptive, anti-neuropathic pain and anti-inflammatory activities. Previous study reported that neuropathic pain causing depression through alteration of monoamine neurotransmitter which are noradrenaline (NA) serotonin (5-HT) and dopamine that affects multiple areas of the brain including the prefrontal cortex, hippocampus, amygdala, and thalamus. Besides that, previous study reported that, depression induces anxiety-like behaviour was influences by the corticotrophin releasing hormone (CRH) secretion which eventually lead to HPA axis activation.

#### Methodology:

Male ICR mice (23-25g, n=6) will be used in this experiment. The CCI will be performed on the sciatic nerve. The animals will be anesthetized and the left sciatic nerve will be ligated with a silk suture. In naïve mice group will not undergo any surgery procedure. Zer will be dissolved in Tween 20, dimethyl sulfoxide (DMSO) and physiological saline 0.9% at a ratio of 5:5:90 and will be prepared freshly before the experiment. Treatment groups consist of vehicle, Zer (1, 5 & 10 mg/kg) and amitriptyline (20 mg/kg) and will be administered intraperitoneally at an injectable volume of 10 mL/kg, on day 7 and continued daily for 14 days until day 21. The antidepressant and anxiolytic effects of Zer will be evaluated by performing forced swimming test and plus maze test on day 0, 14 and 21. The results will be analysed using two-way ANOVA followed by Bonferroni's post-hoc test with significant level at p<0.05.

#### **Results:**

ZER at 1mg/kg, 5 mg/kg and 10 mg/kg (I.P.) significantly reduced the depression-like behaviour and anxiety - like behaviour in CCI-induced neuropathic pain mice.

#### **Dicussion & conclusion:**

Zer exhibits antidepressant and anxiolytic properties possibly by regulating serotogenic, noradrenergic, dopamine pathway or GABAergic pathway and possessing anti-inflammatory properties.

Keywords: Zerumbone, depression, neuropathic pain, anxiolytic

PROTECTIVE AND TREATMENT EFFECTS OF CASSIA ALATA ON LIVER AND

RENAL MORPHOLOGY OF PARACETAMOL TOXICITY-INDUCED RATS

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**Introduction:** 

Paracetamol (PCM) functions as a pain reliever (non-narcotic analgesics) and widely used as

antipyretics to reduce fever. Numerous cases have shown that excessive PCM intake may lead to

hepatotoxicity and nephrotoxicity and this is the basis of PCM abuse used as a way to commit

suicide.

**Objective:** 

To investigate the protective and treatment effects of Cassia alata leaf aqueous extract against PCM

toxicity.

Methodology:

25 male Sprague Dawley rats were divided into 5 groups consisting of 5 rats in each group; (a)

Negative control (standard diet) for 7 days, (b) Positive control (induced with single dose PCM

toxicity at 3000 mg/kg); (c) Pretreatment with Cassia alata followed by PCM-induced toxicity, (d)

Induced with PCM toxicity followed by 21-days Cassia alata treatment and (e) Supplementation

of Cassia alata extract only for 21 days. Following treatment, rats were sacrificed, kidney and liver

harvested, then stained with Hematoxylin and Eosin (H&E).

**Results:** 

There are no differences of liver morphology in all groups observed. In the renal however, all

groups treated with Cassia alata showed improvement in renal morphology indicated by the

histological features (damaged glomeruli, dilated tubules and endothelial rupture in capsule), the

size of glomerulus and ratio of normal versus damaged glomerulus compared to PCM-induced rats.

**Conclusion:** 

Findings of this study show minimal protective effects of Cassia alata which can be assured in the

kidney of our experimental rat model but not in the liver.

**Keywords**: Paracetamol toxicity, cassia alata, hepatotoxicity, nephrotoxicity

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Synergistic Effect of Combine *Zingiber officinale* Methanol Extract and Ciprofloxacin against Foodborne Bacteria

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The outbreaks of foodborne illness with increasing morbidity worldwide reveal the severity of this condition and the need to develop treatment to combat it. The emergence of the antibiotic resistance bacteria due to excessive antibiotic prescription has led to the need in development of new antibiotic concoctions to increase the potency of these antimicrobial drugs. Thus, this study was designed to determine the synergism of combination between *Zingiber officinale* methanol extract and ciprofloxacin against foodborne bacteria. The antimicrobial activity of the plant extract was assessed using disk diffusion method and the zone of inhibitions was measured in millimetres. The minimum inhibitory concentration (MIC) of the plant extract and antibiotics against the two foodborne bacteria were evaluated using broth microdilution method. The synergistic effect between plant extract and antibiotic were then determined using checkerboard assay method. The result show that the antimicrobial activity of extract against *Shigella dysenteriae* ishigher than *Salmonella typhi*. However, there is no synergism between extract and antibiotic for both bacteria except antagonism on *Salmonella typhi*.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF KELULUT HONEY FROM DIFFERENT LOCATION IN MALAYSIA

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#### **Background:**

Previous study revealed that the antioxidant levels in Tualang honey may vary based on their locations of harvest. Therefore, we hypothesized that Kelulut honey (KH) harvested from different locations will vary in pharmacological properties.

#### **Methods:**

KH was harvested from three different locations in Malaysia (Sarawak, Pahang, and Selangor). The total phenolic content (TPC) for each sample was determined using Folin-Ciocalteu reagents. The antioxidant capacity of each sample was determined by its ability to scavenge DPPH, ABTS and FRAP radical. Gallic acid and Trolox was used as a standard for calibration curve construction respectively. Percentages of cell viability of murine macrophage cells (RAW264.7) treated with different concentration of KH were measured using MTT assay. The highest concentration of KH that not cause toxicity effect in RAW264.7 cells were chose for anti-inflammatory assay. LPS-induced RAW264.7 cells have been used to determine the anti-inflammatory activities. Dexamethasone was used as a positive control. The inhibitory effect of KH on NO production was measured by Griess assay. Statistical analysis were performed using the Statistical Package for Sosial Science (SPSS). Results were analysed by One-way analysis of variance (ANOVA), followed by post hoc test. A pvalue less than 0.05 were considered significant.

#### **Result:**

A comparative analysis of the total phenolic content and antioxidant levels of KH harvested from three different locations were performed. The TPC varies from  $368.4 \pm 23.5$  to  $2247.5 \pm 258.5$  µg GAE/g KH in the descending order: Selangor > Sarawak > Pahang. The antioxidant activities (DPPH, ABTS, FRAP) varies from  $249.9 \pm 13.5$  to  $839.8 \pm 97.4$  µg TEAC/g KH in a similar descending pattern with TPC. Based on MTT results, two concentrations (1% and 0.5%) that showed no cytotoxic activities towards RAW 264.7 cells after 24 hours of incubation were further selected for antiinflammatory assay. Antiinflammatory assay results showed that KH harvested from different locations significantly inhibited NO production in LPS-induced RAW 264.7 cells with KH harvested from Selangor showed the highest inhibitory effect followed by Sarawak and Pahang.

#### Conclusion

These results suggest that harvested locations of KH were found to affect its antioxidant levels and antiinflammatory activities.

Keywords: Kelulut honey, total phenolic content, antioxidant capacity, anti-inflammatory activities

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science In Silico Study of Lawsone For Skin Inflammatory Conditions

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#### **Background:**

Lawsonie is an active phytoconstituent of *Lawsonia inermis*, a plant that is popularly known as henna, inai and mehndi. *Lawsonia inermis* had been long used for both cosmeceutical and medicinal purposes. Scientifically, the plant has been reported to possess various pharmacological properties such as antibacterial, anti-fungal, anti-pyretic, analgesic, hypoglycaemic, antiinflammatory, immuno-stimulant and anti-oxidant properties. Traditional folks believed that applying grounded leaves of *L. inermis* on skin helps in treating skin inflammation and it is still practiced up to these days. This shows the potential of *Lawsonia inermis* as an alternative treatment to the current skin anti-inflammatory drugs which are mainly steroid-based that causes unwanted side effects. In this study, lawsone is predicted to bind to TNF- $\alpha$ , which is a pro-inflammatory cytokine, and inhibit its inflammatory activity, therefore, reducing the skin inflammation.

#### Methodology:

Structures of lawsone and TNF- $\alpha$  were obtained from the database and prepared using AutoDock Tools software prior to molecular docking. Molecular docking studies are done utilising Autodock Vina software, which predicts the interaction binding sites and binding affinity. The interaction between lawsone and TNF- $\alpha$  were further analysed by utilising Protein-Ligand Interaction Profiler and PyMol.

#### **Results:**

The result showed that lawsone has good binding affinity with protein TNF- $\alpha$ , with highest binding energy of -6.4. Lawsone were docked to the hydrophobic binding pocket of TNF- $\alpha$  and interacted with the protein's binding residues at GLY<sup>121</sup> from chain A and TYR<sup>59</sup> and TYR<sup>151</sup> from chain B. The predicted interaction of lawsone and TNF- $\alpha$  would be investigated via *in vitro* methods in future studies.

#### **Conclusions:**

The study highlights the potential of lawsone as an effective healing modality for skinrelated inflammatory conditions via good inhibition of TNF- $\alpha$  in silico.

**Keywords**: Lawsone, anti-inflammatory, molecular docking, TNF-α

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Profiling of King Cobra (*Ophiophagus hannah*) venoms from four different geographical regions

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#### **Background:**

Snakebite envenomation can be fatal and is a serious public health concern in tropics. King cobra (*Ophiophagus hannah*) is a medically important snake in Asia. The species is widely distributed and its venom composition may have significant geographical variation that influences its toxicity and neutralization by anti-venom. This study aimed to investigate and compare the protein profiles of king cobra venoms from 4 different regions i.e. Indonesia, Malaysia, Thailand and China.

#### Methods:

Profiling of venom toxin was investigated using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) followed by the venom proteome Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to profile the protein fractions.

#### **Results and Discussion:**

Reverse phase-HPLC of *O. hannah* from Indonesia (OH-I), Malaysia (OH-M), Thailand (OH-T) and China (OH-C) yielded 23 (OH-I), 27 (OH-M), 27 (OH-T) and 32 (OH-C) fractions respectively. Based on chromatograms, OH-M and OH-T venoms shared similar pattern of protein elution revealing similar retention times for the fractions eluted. The abundant low molecular weight proteins were likely the three-finger toxins (3-FTX which were 6-8 kDa) in the *O. hannah* venom. Previous study had shown that *O. hannah* from Malaysia and Thailand contained lethal postsynaptic neurotoxins (NTXs) called α-NTX which is composed of approximately 70 amino acids residues. In this study, the chromatographic and SDS PAGE profiles for the Indonesian and Chinese OH venoms appeared different from the Thai and Malaysian OH venoms. In particular, peak 1 from profile of *O. hannah* venom from China was average 10 times lower than profiles for *O. hannah* from Indonesia, Malaysia and Thailand. In *O. hannah* venom from Indonesia, peak 1-6 showed higher areas under the curve compared with the venom profiles for *O. hannah* from Malaysia and Thailand. The details of variation are analysed using LC-MC/MS for the eluted fractions of the four OH venom samples.

#### **Conclusion:**

The profiles revealed that the Malaysian and Thai king cobra (OH) venoms were similar in their protein profiles; however the Indonesia and China venom profiles vary from one and another among the four samples. This may have implication on the venom composition, biological activities and immunoreactivities of anti-venoms.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Comparative Protein Profiling and Immunoreactivity of the venoms of two Indonesian Snakes: White-lipped Pit Viper (*Trimeresurus albolabris*) and Island Pit Viper (*Trimeresurus insularis*)

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#### **Background:**

Rural populations in Indonesia are heavily affected by snakebite envenomation. The green tree pit vipers of *Trimeresurus* sp. are common biting snakes in the country. The white-lipped pit viper (*Trimeresurus albolabris*) is distributed in the western part of Indonesia while the island pit viper (*Trimeresurus insularis*) is found in the eastern part of Java and the Lesser Sunda Islands. Both the species are highly similar in morphology, however, the compositions of their venoms have not been comprehensively profiled for comparison. The immunoreactivities of different commercial antivenoms to the venoms also remain unknown.

#### **Methods:**

The venoms of the two Indonesian *Trimeresurus* sp. were fractionated by reverse-phase high performance liquid chromatography (RP-HPLC). The venom fractions were subjected to gel electrophoresis using sodium dodecyl sulphate—polyacrylamide gel electrophoresis (SDS-PAGE) and protein identification with liquid-chromatography tandem mass spectrometry (LC-MS/MS). The immunoreactivities of Thai Green Pit Viper Antivenom (GPVAV) and Taiwanese Hemato Bivalent Antivenom (HBAV) to the two venoms were elucidated using enzyme-linked immunosorbent assay (ELISA).

#### **Result and Discussion:**

The chromatographic elution and gel distribution patterns varied between the two Indonesian *T. albolabris* and *T. insularis* venom, indicating that the venoms of these two closely related species were different. Despite that, GPVAV and HBAV exhibited immunoreactivity to both the Indonesian *Trimeresurus* venoms. In comparison, GPVAV showed a stronger binding activity than HBAV for both the *Trimeresurus* sp. venoms. The finding implied that the Indonesian *T. albolabris and T. insularis* venoms possessed protein antigenicity that is more similar to the venom of Thai *T. albolabris* (used in raising GPVAV) than to the Taiwanese *Protobothrops mucrosquamatus* and *Trimeresurus stejnegeri* (used in raising HBAV).

#### **Conclusion:**

The current study demonstrated the variations of the Indonesian *T. albolabris* and *T. insularis* venoms using RP-HPLC and SDS-PAGE. Proteomic analysis of the two venoms is in progress to elucidate the composition details of the two venoms in comparison. The immunoreactivities of GPVAV and HBAV to the two venoms supported the need for further study in animal model to evaluate and validate the *in vivo* efficacy of the antivenoms.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Evaluation of Antioxidant Activity of Solvent Leaf Extracts of Citrus hystrix and Syzygium polyanthum

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### **Background:**

Antioxidants can be defined as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Living organism carry out various biochemical processes during cellular metabolism, and this may produce reactive oxygen species (ROS) as a by-product. ROS is a subset of free radicals that eventually cause oxidation of biomolecules, causing deleterious effect to the human health. Phytochemicals from plants have been suggested to be useful in preventing free radicals induced cellular oxidation, and such an activity may help prevent development of chronic disease such as cancer. In this study, the antioxidant potential of leaf extracts of *Syzygium polyanthum* (SP) and *Citrus hystrix* (CH) were assessed. SP is a plant species in the Myrtaceae family with papery leaf texture while CH from Rutaceae family, present with large winged petiole leaves.

#### **Methods:**

The phytochemicals from the dried leaves were sequentially extracted using solvents of varying polarity (Hexane, Ethyl Acetate, Methanol, Water). The extracts were subjected to *in-vitro* antioxidant activity assays namely Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH assay, ABTS assay and O<sub>2</sub><sup>-</sup> anion assay. The protective effect of the SP-MeOH and SP-W was analysed at the cellular level using ATCC colon cancer cell line (HCT116).

#### **Results & Discussion:**

The total yield for the extraction was 27.16 % for CH, compared to 20.10 % for SP. For both the plants, the yield of non-polar solvent is lower than the polar solvents indicating the predominance of polar phytochemicals. SP-MeOH displayed highest TPC value of  $155.53 \pm 4.72$  mg GAE/g DE, followed by SP-W, SP-EA and SP-HX extracts. Unlike TPC assay, the flavonoid content was significantly higher in SP-EA compared to other extracts. The *in vitro* antioxidant activity of the extracts was studied using DPPH, ABTS and  $O_2$  radical scavenging assay. As compared to CH, the SP extracts scavenge the radicals more effectively. The cellular antioxidant assay (CAA) results showed that the protective effect of SP-W and SP-MeOH in HCT116 cell line was comparable to the positive control, quercetin.

#### **Conclusion:**

SP-MeOH and SP-W presented the most notable antioxidant activity and this highlight the potential use of SP for nutraceutical industry.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science DERIVATIZATION OF QUERCETIN-LYSINE CONJUGATE: OPTIMIZATION AND EVALUATION OF PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES

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#### **Background:**

Quercetin is a flavonoid that possesses health benefit such as antioxidant property. However, quercetin is known to be unstable under certain environmental influences and there is a need to improve its stability. Study shows that flavonoids can interact with lysine to form thermodynamically stable derivatives. Hence, the present study was designed to optimize conditions required to form quercetinlysine (Q-L) conjugate and to evaluate its physicochemical and antioxidant properties.

#### **Methods:**

Derivatization of Q-L was performed by mixing equimolar concentrations (0.6mM) of quercetin and lysine in 0.1M Tris-HCl buffer, pH9 at 4°C for 24h. Physicochemical property of the samples (quercetin and Q-L) was compared using UV/Vis absorbance spectroscopy scan and analytical high performance liquid chromatography (HPLC). Antioxidant property was analyzed using ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assays. Separately, stability of quercetin dissolved in 0.1M Tris-HCl buffer pH9, dimethyl sulfoxide (DMSO) and ethanol for 24h was tested at room temperature and 4°C. Samples were evaluated for physicochemical and antioxidant properties at various time points.

#### **Results:**

Q-L showed significant change in solubility. It dissolved well in water unlike quercetin in ethanol. A single peak that appeared at 6min for quercetin was disappeared for Q-L in analytical HPLC analysis. However, there was a remarkable change in absorption spectrum and antioxidant activity of Q-L compared to quercetin. The conjugate yielded a single band with maximum peak at 284nm whereas quercetin showed two distinctive bands with maximums falling at 304nm and 375nm. Q-L was inefficient in scavenging DPPH radicals and showed lower FRAP value compared to quercetin. Quercetin dissolved in 0.1M Tris-HCl buffer pH9 showed significant change in absorption profile and decrease in antioxidant property which correlated with results obtained for Q-L. This indicated that quercetin has become unstable during the conjugation process.

#### **Conclusion:**

The change in solubility and analytical HPLC profile of Q-L indicated possible formation of the conjugate. However, it was clear that buffer solution at pH 9 is not a suitable model to perform the conjugation as it affects the stability of quercetin. Alkaline buffer at different pH, ethanol or DMSO may serve as better medium to perform the conjugation.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science KNOWLEDGE AND PRACTICE OF NUTRITIONAL CANCER THERAPY BASED ON POLYAMINE DEFICIENT DIET AMAONG CANCER PATIENTS IN KUANTAN, PAHANG

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Keywords: Cancer, Nutrition, Polyamines, Food polyamines, Dietary polyamines

#### **Background:**

Nutritional cancer therapy is a complimentary strategy in treating cancer in which would minimise unwanted side effects. Polyamine deficient diet (PDD) is one of nutritional cancer therapy that can be applied to prevent the progression of cancer cells in the body. It is a strategy to reduce polyamines (PAs) intake by cancer patient. Many researches have been done on PDD in United Stated, United Kingdom, Japan and Sweden on food database on polyamine content. However, no polyamine database available in Malaysia. Therefore, it is doubtful on the knowledge and practice of diet intake of cancer patients in Malaysia. To begin with, this cross-sectional study aims to measure the level of knowledge and practice on nutritional cancer therapy of among cancer patients in Kuantan, Pahang. This study also aims to determine the factor that associate with patient's awareness and practices on therapy.

#### Methodology:

A total of 100 cancer patients in Kuantan, Pahang participated in this study. Convenience sampling is been used and consent was obtained before distributing the questionnaires. Self-administered questionnaire was distributed among respondents through hard copy and google form. The level of knowledge and practice of respondent was analyzed by descriptive statistical analysis. The association between factors that might influence and the level of knowledge and practice was analysed by independent T-test and one-way ANOVA.

#### **Results:**

The analysis showed that the level of knowledge and practice on polyamine deficient diet among cancer patients in Kuantan, Pahang is significantly low. However, most of the respondents believed that nutritional cancer therapy can helps in prevention of cancer.

#### **Conclusion:**

It is hoped that the outcome of this study will provide information to related agencies in implementing of nutritional cancer therapy as a complementary strategy in cancer therapy and planning any healthrelated programs in future to increase the knowledge of cancer patients.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science DETERMINATION ON BONE-ASSOCIATED BIOLOGICAL MARKER LEVELS IN BREAST CANCER PATIENTS

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#### **Background:**

Breast cancer is the most common cancer among women and is the second highest cause of death after lung cancer. The risk of breast cancer incidence increases by age and reproductive status. Many studies have assessed the relationship of breast cancer risk and estrogen exposure. In addition, estrogen and calcium also have an association with bone mineral density. Cancers enhance calcium release from bone causing an increase in calcium in serum, leading to bone fragility. Therefore, this study aims to determination the bone-associated biological marker levels in untreated breast cancer patients.

#### **Methods:**

A total of 20 breast cancer patients from Hospital Kuala Lumpur were involved in this study. Breast cancer patients above 40 years old postmenopausal women were selected as respondents. Patients were required to undergo dual energy x-ray absorptiometry (DEXA) scanning to determine the bone mineral density (BMD) and also to undergo breast biopsy.

#### **Results:**

Breast biopsy analysis revealed that 16 women had 'Invasive carcinoma' (80%), 2 women had 'Features favouring fibroadenoma' (10%), 1 woman had 'Complex proliferating breast lesion' (5%) and 1 woman had 'fibrocystic change' (5%). For BMD analysis, the hip score revealed that the patients had either 'Osteopenia', 'Osteoporosis' or 'Normal' with 25%, 10% and 65% respectively. Statistical analysis showed that there was a correlation between breast biopsy findings and BMD of hip (r = 0.517; p < 0.05). All the respondents have low level of estrogen where 5% at 'low level', 35% at 'moderate low' and 60% at 'very low' level, whereas calcium level were high in the blood serum. Statistical analysis also showed that there was no correlation between estrogen and calcium levels on BMD result.

#### **Conclusion:**

From the analysis, it can be concluded that there is no relationship between breast cancer patients with estrogen and calcium levels.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science Effects of Low Frequency Microwave Radiation Exposure on Erythrocytes and Leukocytes in Sprague Dawley Rats.

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Exposures to microwave (MW) radiation cause a variety of changes in biological systems, including the hematopoietic and immune systems. This study was carried out to evaluate the effects of low-frequency MW radiation on erythrocytes and leukocytes in Sprague Dawley rats. Twelve males Sprague Dawley rats with an average body mass of 250 g were divided into control group (n=6) and exposed group (n=6). The exposed group was irradiated to 2.45 GHz MW radiation for eight weeks (5 days a week, 8 hours a day). After eight weeks of exposure, blood samples were collected for hematological analysis and the morphology of leukocytes were observed under microscope. Results showed that MW radiation exposure caused significant increase in the total erythrocyte count, total leukocyte count and total differential count of lymphocyte, neutrophil, monocyte and eosinophil. The basophil count was significantly decreased in comparison to control group. However, the mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were not significantly affected by MW radiation exposure. For morphology, neutrophil showed alteration in cell by degradation of cytoplasm occurred; while lymphocyte, monocyte, basophil and eosinophil were normal after exposure. In summary, these results demonstrate that exposures to MW radiation may affect some hematological parameters and morphological changes in rats.

# 9<sup>TH</sup> Malaysian Symposium of Biomedical Science THE EFFECTS OF *Lignosus rhinocerus* EXTRACT ON MUSCARINIC RECEPTOR FUNCTION IN ISOLATED RAT'S TRACHEA

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#### **Background:**

Lignosus rhinocerus (Tiger milk mushroom) has been traditionally used as an anti-asthmatic agent.—The immunomodulatory properties and phytochemical compositions of *L. rhinocerus* might be the factors that contribute to the anti-asthmatic effects. Despite controlling inflammation of the airway, the receptors on the trachea also are important to help in lung function by controlling smooth muscle contraction during an asthma attack. Thus, the aim of this study was to determine the effects of *L. rhinocerus* extract on the function of muscarinic receptor found in the trachea isolated from Sprague Dawley rat.

#### **Methods:**

*L. rhinocerus* extract was prepared using the hot water extraction method by Soxhlet extractor and was freeze-dried and then milled into powder. The rat's trachea was isolated and used in the functional myograph study (*in-vitro*) to measure the contractility and relaxation of the trachea muscle.

#### **Result:**

The extract was shown to be able to partially relax the carbachol-induced contraction of the isolated trachea at the highest concentration (1gm/ml). In contrast to the control drug used in the study, atropine was able to fully relaxed the isolated trachea at a concentration of 10nM.

#### Conclusion:

In conclusion, we demonstrated that the L. rhinocerus extract has vasorelaxant effect on pre-contractile trachea with the involvement of muscarinic receptor. However, the highest concentration of the extract which is 1g/ml only be able to produce partial effect of relaxation on isolated tracheal ring.; thus further study is needed to explore the possible effects of the L. rhinocerus extract on other airway smooth muscle receptors.