9TH Malaysian Symposium of Biomedical Science
Antidiabetic Potential of Etlingera elatior Aqueous Extract on Streptozotocin-Induced Diabetic Sprague-Dawley Rats

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Background:
Diabetes mellitus is a chronic metabolic disease affecting people in both developed and developing countries. Poor management of diabetes mellitus leads to serious complications and early death. Modern oral anti-hyperglycaemic drugs are costly and associated with various side effects. This encourages people to seek for safe and cost-effective herb-sources complementary therapies as an alternative treatment of diabetes. Therefore, this study aimed to evaluate the antidiabetic properties of *Etlingera elatior* (Bunga Kantan) flower aqueous extract (BKAE) on streptozotocin (STZ)-induced diabetic rats.

Methods:
Thirty-six (36) rats were used in this sub-chronic (3 months) study. Group 1 was normal rat; Group 2 was untreated-diabetic rat; Group 3 was 250 mg/kg BKAE-treated diabetic rat; Group 4 was 500 mg/kg BKAE-treated diabetic rat; Group 5 was 1000 mg/kg BKAE-treated diabetic rat and Group 6 was 250 mg/kg metformin-treated diabetic rat. The effects of BKAE on blood glucose, body weight, blood pressure and renal function were evaluated. Blood glucose and body weight were measured every two weeks while blood pressure were recorded every month. Histological study of kidney was also carried out using haematoxylin and eosin (H&E) staining.

Results:
Treatments with all three doses of BKAE showed the desired positive effect on the evaluated parameters, comparable to metformin. Twelve-week daily oral treatment with BKAE significantly reduced blood glucose level in diabetic rats. Concurrently, the BKAE-treated rats also showed improved body weight and normalised blood pressure, as compared to untreated-diabetic rats. Besides that, the morphological improvement of kidney for each groups matched their renal function test results. Among three BKAE doses, 1000 mg/kg was found to give the profound antidiabetic effects on STZ-induced diabetic rats.

Conclusion:
Overall, the present finding shown that *E. elatior* has antihyperglycaemic activity and may be therapeutically used in minimize the complications associated with diabetes. Thus, this study support the usage of the plant extracts for traditional treatment of diabetes.

9TH Malaysian Symposium of Biomedical Science
Effect of N-Acetyl cysteine (NAC) Supplementation on Cryopreservation of Hematopoietic Stem/Progenitor Cell: Role on Survival, Oxidative Profile and Repopulation Capacity

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Background:
Transplantation of hematopoietic stem and progenitor cells (HSPCs) has been widely used in clinical application. The technique requires cryopreservation of HSPCs prior to transplantation which could lead to reduced viability and functionality of HSPCs associated with oxidative stress. Thus, supplementation of antioxidant during cryopreservation is recommended. This study aims to investigate the role of an anti-oxidant supplementation, namely N-acetylcysteine (NAC) during cryopreservation on the viability, oxidative profile and repopulation capacity of HSPCs.

Methods:
HSPCs were isolated from mice bone marrow and cultured in growth media (DMEM) for 24 hours. Then, $1x10^6$ of cells were cryopreserved in the presence of 10% DMSO and N-acetylcysteine at 0µM, 0.25µm, 0.5µM and 2.0µM for 48 hours, 2 weeks and 4 weeks at -80°C. Cell viability was determined using tryphan blue exclusion test, while the oxidative profile was measured using glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) assays. The repopulation capacity of HSPCs into hematopoietic lineages comprised of myeloid, erythroid and lymphoid was analysed using colony forming cell assay (CFC) following 4 weeks of cryopreservation.

Results:
Cell viability was significantly reduced ($p<0.05$) following cryopreservation as compared to pre-cryopreservation. The effect was time-dependent as greater viability was noted in long-term cryopreservation (4 weeks) as compared to shorter durations. NAC showed no remarkable evidence except for a significant increase in cell viability ($p<0.05$) following cryopreservation for 48 hours at 0.5µM and 2.0µM. Lowered MDA level and enhanced SOD activity ($p<0.05$) along with unaffected GSH level were noted following cryopreservation as compared to precryopreservation. NAC lowered the MDA level and at 0.25µm, it caused significant increase ($p<0.05$) of GSH level and SOD activity in cryopreserved HSPCs than control. Repopulation capacity of HSPCs to progenitor of respective lineages was significantly reduced following 4 weeks of cryopreservation. NAC shows potential to enhance the clonogenicity of progenitors for myeloid and erythroid while causing reduction for lymphoid.

Conclusion:
Conclusively, cryopreservation impaired the viability and repopulating capacity of HSPCs. However, supplementation of NAC shows potential to minimize these limitations by promoting cells viability, repopulating and antioxidant capacities of HSPCs and the effects are influenced by cryopreservation's time-point and NAC's concentration.

9TH Malaysian Symposium of Biomedical Science

Investigating the effect of Tocotrienol Rich Fraction (TRF) on *Sprague dawley* rats induced- arthritis by complete Freund's adjuvant (CFA)

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Background:
Rheumatoid arthritis (RA) is a chronic inflammatory joint disease leading to disabilities. Current treatment of RA is usually expensive and coupled with many side effects. Thus, exploring other adjuvant from natural source to produce a much safer and less expensive alternative is necessitated. Vitamin E is a nutraceuticals product that becomes progressively popular among Malaysian. Tocotrienol is an isomer of vitamin E which presents at high level in vegetable oil including palm oil. Its properties as cardioprotective, neuroprotective and anti -inflammatory have been evidenced in previous studies. However, its role as an anti-inflammatory in treating RA has not been explored yet. Thus, in this study, TRF was investigated for its anti-arthritic effect in RA animal model.

Method:
Complete Freund's adjuvant (CFA) induced arthritis *Sprague dawley* rat was established and were randomly divided into 6 groups (n=5); consisting of healthy control, arthritis control group, vehicle control group, positive control group, and treatment group . On day 0, all groups except normal group were received subcutaneous injection of 100 µL of CFA at their left hind paw to induce arthritis. Arthritic rats received treatment with two different dose (50 and 100 mg/kg) of TRF, 100% Soy oil (vehicle control), 0.1mg/kg Dexamethasone (positive control) and normal saline (arthritis control) by oral gavage for 21 days, whilst healthy rats only received normal saline through the same route. Arthritis score assessment, body weight and paw diameter measurement were carried out every 3 days interval. After 21 days, animals were sacrificed and blood were collected for hematological and ELISA (COX-2) analyses. Vital organ ie liver and kidney were dissected and weighed for toxicity study

Results:
All doses of TRF showed apparent reduction of arthritic score, paw diameter, and COX-2 level, respectively in CFA- induced rats. However the reduction were not significant when compared to control group. All groups except for Dexamethasone showed increased in body weight indicating the hazardous effect of the drug. In terms of vital organ weight, TRF at all doses were insignificantly difference when compared with normal group, indicating its non-toxic effect. TRF slightly modulates the hematological markers (such as WBC, RBC, PCV, hemoglobin and platelet) when compared with the control group into the normal level.

Conclusion:
In conclusion, this study showed that TRF able to slightly reduce RA in animal model without exhibiting any toxic effects at the dosages used. Therefore, TRF may exhibit significant anti-arthritic effect at higher dosage.

Keywords: Tocotrienol Rich Fraction, Rheumatoid arthritis, Complete Freund's adjuvant, Anti-arthritis

9TH Malaysian Symposium of Biomedical Science

THE EFFECT OF PALM OIL TOCOTRIENOL RICH FRACTION TOWARDS INJURY MARKERS AND LIPID PROFILE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION

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Background:
Many previous studies have reported supplementation of vitamin E is associated to lower the risk of getting cardiovascular disease. Palm oil tocotrienol rich fraction (TRF), a form of vitamin E, has been proven as a cardioprotective agent. A great body of evidence has proven TRF to reduce the occurrence of cardiac cell death, together with decreased release of the biochemistry markers such as AST, ALT, CKMB and LDH and maintaining the serum lipid profile in hypercholesterolemic and cardiovascular disease patients. Hence, this study was aimed to investigate the effect of palm oil TRF towards injury markers and lipid profile in isoproterenol-induced myocardial infarction.

Methods:
A total of 36 male Wistar rats (200-250 g) were randomly divided into 6 groups (n=4~8) namely control, myocardial infarction (MI), TRF (20 mg/kg) (20 TRF), TRF (200 mg/kg) (200 TRF), TRF (20 mg/kg) plus MI (20 TRF+MI), and TRF (200 mg/kg) plus MI (200 TRF+MI). Control and MI rats were administered with stripped oil (1 ml/kg, p.o.), while the remaining rats were divided into two groups to receive TRF (Gold Tri E 70, Sime Darby) at dose of 20 mg/kg and 200 mg/kg (p.o.), respectively. Stripped oil and TRF were given for 84 consecutive days. On the 83rd and 84th day of the treatment, the rats are allocated for MI group were injected with isoproterenol hydroxide (ISO, 85 mg/kg, s.c) with the interval of 24 hours to induce MI condition. After 24 hours of the second ISO injection, all the rats were sacrificed. The blood was drawn and centrifuged for the evaluation of the serum biochemistry and serum lipid profile, while the heart was isolated for histological observation.

Results:
The heart weight and left ventricular weight of three groups; MI, 20 TRF+MI and 200 TRF+MI, showed significant increase when compared with control, 20 TRF, and 200 TRF groups. The biochemical study for plasma injury markers showed significant increase for AST in 20 TRF+MI when compared to control, MI and 20 TRF. However, there was no significance in ALT and CKMB markers in all groups. Supplementation with TRF, did not manage to alter the lipid profile in the MI rats. Histologically, the myocardial-infarcted hearts were presented with necrosis, cell debris and infiltration of inflammatory cells surrounding it. Meanwhile, the TRF-supplemented rats' heart tissues have no apparent lesion.

Conclusion:
In conclusion, TRF supplementation at 20 and 200 mg/kg did not have any effects on both injury markers and lipid profile in isoproterenol-induced myocardial infarction.

9TH Malaysian Symposium of Biomedical Science

UMBELLIFERAE FAMILY EXTRACT AS THE POTENTIAL NEURAL STEM CELL INDUCER FOR RAT AMNIOTIC FLUID STEM CELLS (R3)

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Background:
Increasing number of studies have demonstrated the potential use of stem cell-derived neural stem cells (NSCs) for neurological disorders. However, more efficient method or NSC inducer is essential to increase the NSC conversion from stem cells. Among the inducers, raw extract from a species of family *Umbelliferae*, known as CA, could be the potential candidate. CA has been used in traditional medicine as brain tonic to improve memory and clarity of thinking.

Method:
Here, we aim to evaluate the ability of *Umbelliferae* family extract to exert effect as NSC inducer for rat amniotic fluid stem cells (R3) in serum-contained and serum-free medium using monolayer adherent method. For this study, undifferentiated R3 was first grown in the ESM medium containing fetal bovine serum (FBS) and rat leukemia inhibitory factor (LIF) before being subjected to differentiate into NSCs using monolayer adherent method. R3 was treated with two concentrations of raw extract CA (RECA); 1 and 10 μg/ml while differentiating the cells into NSCs in serum-contained medium (ESM) and serum-free medium (DMEM/F12) for 48 hours.

Results:
After 48 hours of treatment, the cells were checked for the expression of neural stem/progenitor cell protein markers namely Nestin, Sox1, GFAP and Tuj1 qualitatively using immunocytochemistry and quantitatively using flow cytometry. The expression of Nestin, Sox1, Tuj1, and GFAP was observed to be higher in both of the treatment groups as compared to the untreated group (control).

Conclusion:
These results strongly suggest the property of this *Umbelliferae* family extract in enhancing the generation of neural stem cells from these stem cell line under serum and serum-free media. This finding clearly marks CA as the potential neural stem cells inducer, which could be useful to generate NSC from stem cells for future cell-based therapeutic applications.

9TH Malaysian Symposium of Biomedical Science

ANTIOXIDANT ACTIVITIES OF PTEROSTILBENE AS A POTENTIAL UV-PROTECTING AGENT

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Background:
Pterostilbene is reported to have several pharmacological properties including anticancer, anti-inflammatory, antioxidant and antidiabetic. The antioxidant activity of pterostilbene in both in vitro and in vivo models has been proven to possess both preventative and therapeutic benefits in many diseases. Oxidative stress caused by UV radiation is also strongly linked to unfavourable complications for human skin health including photoaging and skin cancer. The interest of antioxidant properties of pterostilbene in reducing oxidative stress caused by UV brought the team to determine the antioxidant effects of pterostilbene in protecting skin from UV radiation.

Methods:
B16 4A5 melanoma cells exposed to UV radiation was used as model in our study to determine the antioxidant properties of pterostilbene. The radical scavenging activity was assessed using DPPH assay. Both enzymatic and non-enzymatic defence systems of oxidative stress were determined using glutathione (GSH) assay, glutathione-s-transferase (GST) assay. The oxidant, malondialdehyde (MDA) level was measured using TBAR assay. The GST protein expression was also identified using the Western blot.

Results:
Our study found that pterostilbene had significant in vitro antioxidant and radical scavenging activities as the level of GSH, GST and DPPH were increased in the cells. GST plays an important role in regulation of the intracellular concentrations of the lipid peroxidation products that were caused by oxidative stress. In addition, the level of oxidant, malondialdehyde (MDA) was able to decrease too.

Conclusion:
A better understanding of antioxidant activities of pterostilbene against oxidative stress caused by UV radiation will eventually lead to the development of potential UV-protecting agent.

9TH Malaysian Symposium of Biomedical Science

SCREENING OF ANTIBACTERIAL POTENCY OF ANTIBACTERIAL PEPTIDE PAM-5 AGAINST SELECTED DRUG RESISTANT PATHOGENIC BACTERIA

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Background:
The alarming threat of global antibiotic resistant bacteria has urged for the development of alternative antibacterial agents. Among these, antibacterial peptides (ABPs) are gaining considerable research attention as potential alternative therapeutic agents against pathogenic bacteria, particularly to antibiotic-resistant bacteria. Previous studies of ABPs were focused on the natural peptides isolated from different sources of living organisms. Even though several studies on synthetic ABPs were documented recently, very few of them were screened for their potency against a wide range of drug-resistant bacteria. In this study, PAM-5, a synthetic peptide which was previously shown to kill several reference strains of pathogenic bacteria, was screened for its potency against several clinically isolated drug-resistant bacteria.

Methods:
Microbroth dilution assay was used to screen for the antibacterial potency of PAM-5 against eight clinically isolated pathogenic bacteria with different antibiotic resistance. These included *Salmonella* Typhi, extended-spectrum β-lactamases producing (ESBL) *Escherichia coli*, Cefazolin (CFZ)-resistant *E. coli* and *Pseudomonas aeruginosa*, Carbapenem-resistant Enterobacteriaceae (CRE) *Klebsiella pneumoniae*, cephalosporin-resistant *Acinetobacter junii*, and Amoxicillin (AMX)- and cephalosporin-resistant *Enterobacter aerogenes* and *Serratia marcescens*. The bacteria were treated with increasing two-fold concentrations of PAM-5 ranging from 2 µg/ml to 256 µg/ml. All the treated bacteria were plated on MH agar to determine the minimal bactericidal concentrations (MBCs) of the peptide against the bacteria. The assays were triplicated to ensure reproducibility.

Results:
PAM-5 consistently demonstrated potent bactericidal effect against *S.* Typhi at the MBC of 32 µg/ml, ESBL-*E. coli* and CFZ-resistant *E. coli* (16 µg/ml), CFZ-resistant *P. aeruginosa* (16 µg/ml), CRE-*K. pneumoniae* (8 µg/ml) and cephalosporin-resistant *Acinetobacter junii* (4 µg/ml). However, PAM-5 is less potent against AMX- and cephalosporin-resistant *E. aerogenes*, as indicated by the high MBC of 128 µg/ml. Moreover, the peptide was not potent against *S. marcescens* at all tested concentrations. These findings suggest that PAM-5 is highly potent against several drug- and multidrug-resistant bacteria, with the exception to *E. aerogenes* and *S. marcescens* as they possess intrinsic resistance towards all peptide compound.

Conclusion:
Conclusively, PAM-5 is worth to be further studied and developed into a potential novel antibacterial agent with moderate spectrum of antibacterial activity.

9TH Malaysian Symposium of Biomedical Science
Effects of *Nigella sativa* Extract on Zebrafish Larval Behaviour

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Background:
Nigella sativa or black cumin seeds have been studied for its potential in protecting various diseases. Previous studies have shown that bioactive compound of *Nigella sativa* is able to alter the behavioural changes and locomotor activity of rodents. However, little information is available on toxicity properties of *Nigella sativa* extract in a zebrafish embryo model as well as effects on locomotor and behavioural changes. This study aimed to identify toxic effect of *Nigella sativa* extract on zebrafish embryo by identifying 50% of lethal concentration (LC50) of the extract and locomotor activity and behaviour at larvae stage.

Methods:
Fish embryo toxicity (FET) was done by determining the LC_{50} of *Nigella sativa* in zebrafish embryo with 6 different concentrations of the extract (100ug/L, 50ug/L, 25ug/L, 12.5ug/L, 6.25ug/L). Lightdark transition method was used to understand the behavioural changes of the larvae and its motility activity at LC10, NOEL, and half NOEL.

Results:
The LC50 of *Nigella sativa* extract is 37.73ug/L. Total distance moved and velocity of zebrafish larval were all significantly different compared to control at second phase of light transition (*p*-value of all treatment were <0.005).

Conclusion:
Nigella sativa extract at different concentrations may change the locomotor behaviour of the zebrafish larvae relating to pharmacological effect on the zebrafish nervous system. The assessment of the effects of natural product on larvae locomotor behaviour may be helpful in discussing possible toxicity of Nigella sativa in comparison with similar natural products.

9TH Malaysian Symposium of Biomedical Science
EFFECT OF *POLYGONUM MINUS* ON RENAL IN CISPLATIN INDUCED RAT

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Background:
Cisplatin (CP) is widely used as chemotherapeutic drug; however it has varies adverse effects. Studies has shown that essential oil of *Polygonum minus* (PM) has the antioxidant and antiinflammatory activity. Thus, this study aimed to investigate the possible protective effect of PM against cisplatin induced nephrotoxicity in rats.

Methods:
Sprague Dawley male rats were randomly divided into 7 groups with 8 rats each. Group 1: normal control, Group 2: Cisplatin group (10mg/kg), Group 3: βCaryophyllene (150mg/kg) + CP, Group 4: Essential oil (100mg/kg) + CP, Group 5: Essential oil (200mg/kg) + CP, Group 6: Essential oil (400mg/kg) + CP, Group 7: Essential oil (400mg/kg). Treatment groups were treated daily for 14 days. On day 15, group 2, 3, 4, 5 and 6 were given CP. Three days after, all seven group rats were anesthetized and sacrificed. Kidneys were harvested for biochemical measurements, oxidant status and histopathology analyses.

Results:
Cisplatin treated group rats resulted an increase in creatinine, blood urea nitrogen (BUN) and Malondialdehyde (MDA), and a reduced of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in renal homogenates. In the groups that is pre-treated with essential of PM showed an improvement: decreased creatinine, BUN and MDA, and increased of GSH, SOD and CAT. In histopathology examination of the pre- treated groups showed reduced damage of structural changes comparing to the CP-induced that showed inflammatory and infiltrated cells.

Conclusion:
This study suggested that *P.minus* showed protective effect on the CPinduced nephrotoxicity through increasing the antioxidant activity and minimizing the oxidative stress.
Additionally, minimizing the functional and structural damages.

9TH Malaysian Symposium of Biomedical Science

ROLE OF N-NITRO-L-ARGININE METHYL ESTER, A NITRIC OXIDE SYNTHASE INHIBITOR IN CHRONIC CONSTRICTION INJURY (CCI)-INDUCED NEUROPATHIC PAIN MICE

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Background:
Neuropathic pain is a chronic condition associated with injuries in both the peripheral and central nervous systems. Nitric oxide (NO) is a diffusible gas that potentially contributes to both physiological and pathological processes in the body including pain and inflammation. Presence of excess NO also contributes to neuropathic pain symptom such as hyperalgesia. N-nitro-L-arginine methyl ester (L-NAME) is L-arginine analogue that is responsible in inhibiting nitric oxide synthase. In present study, we investigated the role of NO synthase inhibitor, L-NAME in the CCI-induced neuropathic pain mice model.

Methodology:
ICR male mice had undergone CCI-surgery of sciatic nerve and the response of pain withdrawal latency was analysed using Hargreaves plantar 14 days after of surgery. The animal was divided into 6 treatments group including sham-operated, vehicle (0.9% NaCl), three dosages of L-NAME (3 mg/kg, 10 mg/kg, and 30 mg/kg), and Amitriptyline (20 mg/kg) and was administered intraperitoneally on day 14 and continued daily for 7 days until day 21.

Result:
Our finding shows that single and repeated dose of intraperitoneally administration of L-NAME at highest dose (30 mg/kg) show significantly inhibited ($p<0.01$; $p<0.001$) thermal hyperalgesia in CCI-induced neuropathic pain using the Hargreaves plantar test. Whereas, administration of 3 mg/kg and 10 mg/kg of L-NAME shows no significant difference when compared to vehicle after single and repeated administration.

Conclusion:
These findings show that L-NAME possess an antihyperalgesic properties in repeated and single administration of CCI-induced neuropathic pain mice.

Keywords: Neuropathic pain, nitric oxide synthase inhibitor, n-nitroarginine methyl ester (L-NAME)

9TH Malaysian Symposium of Biomedical Science

EFFECTS OF *ARTHROSPIRA PLATENSIS* ON SCIATIC NERVE CRUSH INJURY IN ADULT RAT: BEHAVIOURAL ANALYSIS, MORPHOLOGIC AND MORPHOMETRIC STUDIES

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Background:
Sciatica is one of the most common lower back disorders that occurs when the sciatic nerve or parts of it is compressed, irritated or damaged. The neurological presentation may range from minor transient pain to severe sensory disturbance and motor loss with poor recovery. *Arthrospira* (previously *Spirulina*) *platensis* was reported highly nutritious with antioxidant and anti-inflammatory effects. Gamma linolenic acid and phycocyanin are the two main bioactive compounds in *A. platensis* that have the ability to aid nerve regeneration and nerve functional recovery, respectively. This study aims to determine the nerve functional recovery and nerve regenerative effects of oral supplementation with *A. platensis* on rat model of sciatic nerve crush injury.

Method:
A total of thirty five Sprague Dawley rats were randomly divided into four groups: normal (n=5), negative control (n=10) (crush injury with no treatment), positive control (n=10) (crush injury with 500 µg/kg/day methylcobalamin), and experimental (n=10) (crush injury with 180 mg/kg/day A. platensis).

Results:
Oral administration of 180 mg/kg/day *A. platensis* extract showed faster onset of recovery compared to the control groups. Behavioural studies (toe spreading reflex and walking track analysis) demonstrated that rats supplemented with 180 mg/kg/day *A. platensis* presented with a higher degree of functional recovery compared to the ones that were not given any treatment and those given methylcobalamin. Morphological and morphometrical findings indicated an increase in the myelin thickness and myelin sheath layer after administration of *A. platensis*.

Conclusion:
Supplementation with *A. platensis* showed significant increase in functional recovery and nerve regeneration when compared to negative control group while a slight increase in functional recovery and nerve regeneration can be seen when compared to positive control group. These results suggest that *A. platensis* may be beneficial as a therapeutic option for disturbances of nerve interaction.

9TH Malaysian Symposium of Biomedical Science

ALLEVIATION OF LIVER AND RENAL OXIDATIVE STRESS IN PARACETAMOL TOXICITY-INDUCED RATS BY *CASSIA ALATA*

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Paracetamol (PCM) intoxication can result in excessive amount of free radicals and reduce antioxidant defence mechanisms leading to cellular destruction in pathological diseases specifically liver and kidney damage. This study aimed to assess protective and treatment effects of *Cassia alata* leaf aqueous extract against PCM-induced toxicity due to its high alkaloid and flavonoid antioxidant properties. 25 male Sprague Dawley rats were divided into 5 groups; (a) healthy rats, (b) PCM-induced toxicity (3000 mg/kg), (c) Pre-treatment with *C.alata* for 21-days followed by PCM-induced toxicity, (d) PCM-induced toxicity followed by 21-days *C.alata* treatment, and (e) treatment of *C.alata* only for 21 days. Following treatment, rats were sacrificed for measurement of renal and liver Malondialdehyde (MDA) content, catalase enzyme activity (hydrogen peroxide (H_20_2) content) and total antioxidant activity (1-1-diphenyl-2-picryl (DPPH) radical scavenging assay). MDA content were highest in the PCM-induced rats for both renal (0.044±0.005 nmol/mg/protein) and liver (0.037±0.010 nmol/mg/protein), although no significant differences were observed between all groups for liver assessment. In renal, MDA content were significantly higher ($p<0.05$) for PCM-induced group (0.044±0.011 nmol/mg/protein) compared to PCM-induced rats pre-treated with *C.alata* (0.015+0.022 nmol/mg/protein) and the group receiving *C.alata* only (0.012±0.011 nmol/mg/protein). *C.alata* increased catalase enzyme activity as observed by significantly lower H_20_2 content in the liver of pre-treated PCM-induced groups (75.6±1.22 µg/mL) and those treated post toxicity induction (73.0±9.86 µg/ml) compared to PCM-induced group (140.2±14.16 µg/ml). *C.alata* does not appear to affect renal catalase activity in PCM-induced groups, however, *C.alata* supplementation does boost catalase activity in rats supplemented with *C.alata* only where the H_20_2 content (80.0±20.51 µg/ml) is significantly lower than PCM-induced group (145.2±12.65 µg/mL). Total antioxidant activities were similar in all groups except between PCM-induced group (85.37±2.42%) and *C.alata* pre-treated group (76.45±1.13%) in the renal. Our findings show that *C.alata* provides protective and treatment effects in PCM-induced oxidative stress to a certain extent, but does not significantly boost the entire antioxidant network.

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

9TH Malaysian Symposium of Biomedical Science

UV PROTECTING AND ANTI-AGING EFFECTS OF PTEROSTILBENE IN B16 MOUSE MELANOMA CELL LINES

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Background:
Pterostilbene, which is an analogue derivative of resveratrol has been widely researched for its properties as antimicrobial and antioxidant agents. However, its effect on tyrosinase and collagenase activities to act as an anti-aging and UV-protection agent is not yet being assessed.

Methods:
To investigate the anti-melanogenic properties of Pterostilbene, tyrosinase activity inhibitory assay on UV irradiated B164A5 murine cells was carried out. Melanin content from the Pterostilbene treated B164A5 cells was also measured. Collagenase activity of treated B164A5 cells were being determined by using the collagenase activity assay. Western Blotting was being used to obtain supportive data for both tyrosinase and collagenase protein inhibition by Pterostilbene.

Results:
Our study showed that Pterostilbene inhibited tyrosinase activity by dose dependent manner. This was also proven by the reducing melanin content in the treated cells as tyrosinase is the key enzyme for melanin production. Collagenase activity was also shown to be decreasing by the increasing dose of Pterostilbene. Based on the findings from Western Blot, Pterostilbene also reduced the production of tyrosinase and collagenase in B16 cells.

Conclusion:
From the results obtained, we can conclude that Pterostilbene has significant inhibition on tyrosinase and also collagenase activities in B164A5 cell and can be further developed as an alternative UV protection and anti-aging agent.

9TH Malaysian Symposium of Biomedical Science

EFFECTS OF ORALLY ADMINISTERED PTEROSTILBENE ON MELANOGENESIS AND TYROSINASE ACTIVITIES IN UVB-INDUCED MICE

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Background:
Pterostilbene is a stilbenoid and many studies have reported that it possesses many therapeutic properties. Recently, *in vitro* study had been reported that pterostilbene reduced tyrosinase protein expression as well as α-melanocyte-stimulating hormone (MSH). However, these effects have not been studied *in vivo* yet. The aim of our study was to investigate the effects of oral pterostilbene on melanogenesis and tyrosinase activities in UVB-induced mice.

Methods:
Balb/C mice were subjected to 1) corn oil as the control vehicle group without UVB exposure, 2) UVB exposure only, 3) resveratrol given orally to the control positive group with dose 200 mg/kg, 4) 10 mg/kg and 20 mg/kg of oral pterostilbene for the treatment groups. Oral resveratrol and pterostilbene treatments were carried out once a day for 14 consecutive days. UVB radiation with dose 250 mJ/cm^2 was exposed onto mouse skin from vertical position in the mice restrainer on day 9th, 11th and 13th. Thirty mice were used and divided into control and treatment groups. Dorsal skin of mice was also shaved before the UVB exposure.

Results:
We hypthosise that oral pterostilbene can potentially reduce the melanin content in skin of irradiated mice via downregulation of tyrosinase protein. Hyperplasia and acanthosis in epidermal can also be exhibited by UVB-irradiated mice whereas UVB-untreated control mice could not exhibit those features. However, oral pterostilbene and resveratrol could be able to reduce the UVB-induced histological and morphological changes significantly in the UVBirradiated mice. Melanin level in heart is correlated to melanin level in mice skin. However, melanin levels in heart, liver and renal could not affect the physiological of all mice.

Conclusion:
In conclusion, oral pterostilbene can be an antimelanogenesis agent and downregulate tyrosinase protein expression in UVB-induced mice.

9TH Malaysian Symposium of Biomedical Science

ROLE OF CALCIUM CHANNEL BLOCKER, NIFEDIPINE IN NEUROPATHIC PAIN MICE

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Background:
Neuropathic pain is generated when damage or injury occurs at the neurons of peripheral and central nervous system. Calcium channel is responsible for mediating the secretion of neurotransmitters, hormones, synaptic plasticity and gene transcription. When intracellular calcium level increased in neurons, it can lead to alteration in dorsal horn excitability and induced release of glutamate, substance P, and calcitonin gene-related peptide (CGRP) into the synaptic cleft. The consequence of the event lead to increase excitability of dorsal horn projection, excitatory interneurons and reduced excitability of inhibitory interneurons, which can cause neuropathic pain.

Methodology:
Male ICR mice (25-35g) were used in the project. CCI surgical procedures was conducted on day 0 according to the (Bennet and Xie, 1988) procedures with slight modifications. The animals were anesthetized and left sciatic nerve were ligated with silk suture. The skin was closed by nylon suture. In sham-operated group, the sciatic nerve was exposed, but not ligated. Animals (n=6) were divided into treatment groups of Nifedipine, (1, 10 and 20 mg/kg), amitriptyline (10 mg/kg) as the positive control, vehicle (5% DMSO, 95% NaCl, 10ml/kg) and sham group. Daily treatments were given via intraperitoneal route on day 14 and continued daily treatment for 7 days until day 21. Behavioral test such as Hargreaves' test, cold plate test, and rota rod test were conducted on day 0 (baseline) and days 14, 16, 18 and 21.

Result:
Single and repeated dose of intraperitoneally administration of Nifedipine for all doses (1, 10 and 20 mg/kg) significantly inhibited ($p<0.001$) cold allodynia in CCI-induced neuropathic pain mice. Nifedipine at highest dose (20 mg/kg) in single and repeated treatment showed significant difference ($p<0.05$; $p<0.001$) in thermal hyperalgesia when compared to vehicle. Other two doses of Nifedipine (1 and 10 mg/kg) showed significant difference ($p<0.05$; $p<0.001$) for repeated treatment in thermal hyperalgesia.

Conclusion:
These finding showed that Nifedipine was able to attenuate cold allodynia and thermal hyperalgesia in CCI-induced neuropathic pain mice.

Keywords: Nifedipine, Neuropathic pain, CCI, Calcium channel, Allodynia, Hyperalgesia

9TH Malaysian Symposium of Biomedical Science

SYNERGIC EFFECT OF *Nigella sativa* AND *Olea europaea* OILS COMBINED WITH LOCAL MALAYSIAN PLANTS ESSENTIAL OILS AS REPELLENT AGAINST *Aedes aegypti* (DIPTERA: CULICIDAE)

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Background:
Dengue infection is one of the mosquito-borne viral diseases that is transmitted by vector, *Aedes aegypti*. This mosquitoes control is facing a threat due to the increasing mosquito resistance to the insecticides. Mosquito repellents is one of the best way to prevent mosquito bites and prevent the spread of dengue infection. DEET, the repellent with synthetic chemical is the mostly used insect repellent products in which would harm the consumers. As an alternative to DEET, the development of natural-based repellent is being comprehensively conducted. *Nigella sativa* and *Olea europaea* are plants which contains high medicinal properties towards health. Meanwhile, three local Malaysian plants oils used as combination which are *Piper aduncum*, *Piper sarmentosum* and *Litsea elliptica.*

Methods:
This study aims to investigate the synergic effect of cream formulation containing *N. sativa* and *O.europaea* oils when combined with local Malaysian plants oils as repellents under laboratory conditions, using human volunteers. *N. sativa* and *O.europaea* are purchased from the local pharmacy. *P. aduncum*, *P. sarmentosum* and *L. elliptica* were extracted using Clevenger extraction method.

Results:
The value of ED_{50} and ED_{95} of each oil were determined by using Buescher et al. 1982 method. The value of ED_{50} and ED_{95} of *N. sativa* are 3.01% (0.11 mg/cm^2) and 16.46% (0.62 mg/cm^2) respectively. *O. europaea* shows the value of ED_{50} of 2.25% (0.08 mg/cm^2) and value of ED_{95} is 11.60% (0.43 mg/cm^2). Each oil was formulated into cream and tested following SIRIM standard method 2007. The protection time of *N. sativa* for the first 4 hours was 100% at the value of ED_{95} and decrease by 81.89% at the 8 hours. Meanwhile, for the first 4 hours, *O. europaea* showed 96.00% protection time at the value of ED_{95} and drop by 66.67% at 8 hours. The combination of both *N. sativa* and *O. europaea* showed 88.50% protection time for 8 hours. Combination of *N. sativa* and *O. europaea* with three local Malaysian oils showed 100% protection for the whole 6 hours.

Conclusion:
There is synergic effect shown when *N. sativa* and *O. europaea* oils when combined with three local Malaysian plants as repellent against *Ae. aegypti*.