MELATONIN INDUCED AUTOPHAGY VIA ACTIVATION OF AMPK AND ERK PATHWAYS AND INHIBITION OF MTOR PATHWAYS IN JURKAT CELLS

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Leukaemia is a haematological malignancy classified by uncontrolled proliferation of haematopoietic cells of bone marrow. To date, a great variety of anti-leukaemia drugs are available, unfortunately, undesirable side effects, cellular resistance and toxicity towards normal cells limit their applications. Melatonin is a hormone mainly produced by pineal gland, it is also known to elicit cytoprotective effects in normal cells while capable to trigger pro-apoptotic signals in cancer cells. Thus, in this study we aim to investigate the molecular mechanism involvement of melatonin induces cytotoxicity in human T-lymphocyte (Jurkat) cells. Jurkat cells were treated with different concentrations of melatonin (0.5, 1.0, 1.5, 2.0, 2.5 mM) and determined the cell viability using MTT assay, autophagy measurement using fluorescence staining, protein expressions were evaluated by western blot. Our results showed that melatonin exerted an anti-proliferative effect through inhibition of cell growth and induced autophagy (red fluorescence) on Jurkat cells in a dose-dependent manner as compared with the control cells. Furthermore, an increased level of autophagy-associated proteins (Beclin-1) expression was detected in melatonin-treated cells when compare with control cells. Additionally, melatonin treated cells had increased AMPK, p-AMPK and p-ERK proteins expression, and decreased mTOR and p-mTOR proteins expression as compared with control. In conclusion, our study shows that melatonin induces autophagy through activation of AMPK and ERK pathways and inhibition of mTOR pathway in Jurkat cells. For future, melatonin may can used as an anti-cancer drug or combine with chemotherapy drugs for cancer treatment.

Keywords: Melatonin, autophagy, Jurkat cells

IN VITRO AND *IN SILICO* STUDIES OF LAWSONE FOR ACUTE SKIN INFLAMMATORY CONDITIONS

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Introduction: The standard treatment for acute skin inflammatory skin condition usually is corticosteroid cream which helps to reduce inflammation remarkably, but it comes with side effects. Thus, researchers nowadays are trying to find alternative treatment, especially those compounds that derived from natural products due to their potential healing properties. *Lawsonia inermis* is a traditional plant that has been used by the older generations to treat wounds, pus and many other skin ailments. Lawsone, one of the main active compounds present in *L. inermis* is predicted to bind to IL-1α, a pro inflammatory cytokine and inhibit its inflammatory activity. Objective: To evaluate the anti-inflammatory effect of lawsone in acute skin inflammatory condition through *in vitro* and *in silico* studies. Methodology: Firstly, the IC_{50} of lawsone on human epidermoid carcinoma cell line (A431) was obtained by using MTT proliferation assay, followed by skin anti-inflammatory assay on ethanol-induced inflammation A431 cells and further analyzed by using AOPI staining. Next, molecular docking was done by utilizing Autodock Tools and Autodock Vina to predict the interactions between lawsone and targeted pro-inflammatory cytokine, IL-1α. The predicted interactions were further analyzed by using PLIP, ProteinsPlus and PyMol. Results: The result showed that lawsone binds to IL-1α with top binding affinity of -5.2 kcal/mol. By utilising PoseView and PLIP, lawsone was found to reside with IL-1α hydrophobic pocket at Ile68 and have hydrogen bonding with Asp65. Lawsone is predicted to act as a potential skin anti-inflammatory agent by significantly increase the number of viable A431 cells *in vitro* (work in progress). Conclusion: Lawsone was found to have relatively good inhibitory activity on IL-1α and expected to show good anti-inflammatory activity in in vitro study.

Keywords*:* Inflammatory skin conditions, lawsone, IL-1α, molecular docking

Syzygium polyanthum ALLEVIATES RENAL DAMAGED IN HYPERTENSIVE RAT MODEL

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Syzygium polyanthum (Wight) Walp. var. *Polyanthum* is a local plant in Malaysia and has been traditionally consumed by Malays as an alternative treatment for hypertension. It was also believed to be an effective remedy for kidney damage secondary to hypertension. However, the scientific evidence on this claim is still insufficient. The previous study only covered the acute and sub-acute study of the antihypertensive effects of *S. polyanthum* leaf extracts (methanolic and aqueous extract) in Spontaneous-Hypertensive Rats (SHR). This study aim is to investigate the effect of aqueous extract of *S. polyanthum* (AESP) leaves on hypertensive-related renal complication. The study focusing in the ability of extract to improve renal structural in hypertensive renal. AESP at 1500 mg/kg was orally administered daily on SHR (n=7), and systolic blood pressure (SBP) was monitored every 2 weeks along the 12 weeks of study period. The other groups; WKY (control normal, n=7), untreated SHR (n=7), and SHR treated with Losartan (20 mg/kg, n=7). AESP significantly reduced the systolic blood pressure after 12 weeks of study ($p<0.0001$). There was significant improvement in renal function. Concurrently, there was also a significant change in renal structure as compare to untreated-SHR. These results suggest that oral administration of *S. polyanthum* able to improve hypertensive-renal damage in SHR rats. In conclusion, this study supported the potential use of *S. polyanthum* leaves as an alternative treatment for hypertension-renal damaged.

Keywords*: Syzygium polyanthum*, hypertension-renal damaged

THE NEUROENHANCEMENT EFFECT OF RAW EXTRACT OF *Centella asiatica* (RECA) ON TRANSDIFFRENTIATION OF FULL TERM HUMAN AMNIOTIC FLUID STEM CELLS (hAFSCs) INTO NEURAL STEM CELLS (NSCs)

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Incidence of neurodegenerative diseases (ND) is now increasing globally. Neuronal dysfunction and progressive neuronal cell death are among the main causes in most ND cases. An increasing number of studies have demonstrated the potential use of neural stem cells (NSCs) to counter the situation. However, due to limited source of brain NSCs, treatment via neuro-transplantation has become very challenging. Thus, finding a new source of NSCs from non-brain sources and inducer of NSCs transdifferentiation is crucial. Recent study showed that highly potent stem cells residing in amniotic fluid can be differentiated into neural lineage in the presence of specific inducer. One potential candidate of the inducer is *Centella asiatica*, which has been consumed traditionally as memory tonic. This study aims to evaluate the neuroenhancement effect of raw extract of *Centella asiatica* (RECA) on transdiffrentiation potential of full term human amniotic fluid stem cells (hAFSCs) into NSCs. hAFSCs are cultured in culture medium (AmnioMAX-II complete medium) prior to MTT assay for dosage determination of RECA. In this study, hAFSCs were treated with RECA at concentration of 10µg/mL and with 5µM of dibutyryl camp (dBcAMP) as positive control, and subjected to undergo transdiffrentiation using monolayer adherent culture technique. The transdiffrentiation of hAFSCs into NSCs were evaluated based on the morphology of NSCs and the expression of NSCs specific markers (Nestin, GFAP and SOX1) through immunocytochemistry. The generation of NSCs was confirmed by the ability of the cells to form neurospheres, the multicellular aggregates of NSCs in low attachment plate through neurosphere assay. Treatment of RECA able to enchance the expression of NSC specific markers and produced high quality neurospheres from hAFSCs as compared to the untreated group. This finding clearly marks RECA as potential NSCs inducer, which is useful for therapeutic application.

Keywords*:* stem cell, amniotic fluid stem cell, human amniotic fluid, full term, neural stem cell, *Centella asiatica*, neurosphere.

MOLECULAR MECHANISM STUDIES OF CURCUMINOID ANALOGUES ON JURKAT T LYMPHOBLASTIC CELLS

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Curcumin is widely used as spice and colouring agent in food, possesses potent anti-oxidant, anti-inflammatory, and anti-tumor promoting activities. However, the poor bioavailability and low water solubility have been an issues for its therapeutic potential as an anti-cancer agent. Therefore, synthetic compound, FLDP-5 and FLDP-8 have been developed to overcome these drawbacks of curcumin. This study is to determine the molecular mechanism of apoptosis induced by FLDP-5 and FLDP-8, curcumin analogues in Jurkat T lymphoblastic leukemia cells. Annexin-V-FITC assay were performed to determine the mode of cell death in FLDP-8 and FLDP-5 treated Jurkat T lymphoblastic leukemia cells. Following treatment of 24 hours, there was a concentration dependent increase of apoptosis in both FLDP-5 and FLDP-8 treated cells. The increase of apoptosis was significant ($p< 0.05$) compared to untreated cells. Furthermore, to determine the role of caspase, cells were pretreated for 1 hour with broad caspase inhibitor and changes in the cell viability were observed. This completely blocked the FLDP-5 and FLDP-8 induced apoptosis. The expression of upstream and downstream caspases in FLDP-5 and FLDP-8 treated cells were determined by western blot analysis. Decrease in pro-caspase 3, 8 and 9 were observed. Taking together, in this study caspase plays a significant role in FLDP-5 and FLDP-8 induced apoptosis with Jurkat cells appeared to be more sensitive to FLDP-5 curcuminoid analogues at an earlier timepoint.

Keywords: curcumin; apoptosis; caspases

Etlingera elatior FLOWER AS ANTI-HYPERGLYCEMIA AGENT: A STUDY ON TYPE 2 DIABETIC RAT MODEL

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Diabetes mellitus is a metabolic chronic disorder that may cause by defective insulin secretion. Un-control diabetes may become worsened and leads for microvascular and macrovascular diabetic complication secondary to hyperglycaemic event. *Etlingera elatior* is a natural based-sources, traditionally used as an alternative options to lower blood sugar level in diabetic sufferer. This study aimed to evaluate the potential anti-diabetic properties in *E. elatior* flower aqueous extract (EEAE) on fasting blood glucose level and its associated complications on streptozotocin-induced Type-2 diabetic rats. In this study, thirty-six (36) rats were used in this subchronic (12 weeks) study. High fat diet (HFD) were given to all rats except the normal group (n=6) for 6 weeks before induction of diabetes. Twelve (12) rats served as normal control (Group A) and obese control (Group B); (non-diabetic). Meanwhile, twenty-four (24) rats were induced to be diabetic with 35 mg/kg of streptozotocin (STZ) and were randomly divided into 4 groups; Group C: Untreated-diabetic rats; Group D: 1000 mg/kg EEAE; Group E: 2000 mg/kg EEAE; and Group F: 250 mg/kg metformin. For 12 weeks, the effects of EEAE treatment on fasting blood glucose, BMI, cholesterol and systolic blood pressure were evaluated. At the end of study, biochemical parameters were evaluated for microalbumin, liver function test and renal function test. Diabetic rats with 1000 mg/kg of EEAE treatment for 12 weeks significantly reduced the fasting blood glucose and improved associated parameters when compared with metformin. Thus, 1000 mg/kg is suggested as the preferable dose to give a profound antidiabetic effects on STZ-induced diabetic rats. Overall, in current finding also shown that *E. elatior* able to minimize the complication associated with diabetes and act as anti-hyperglycemia agent.

Keywords: *Etlingera elatior*, aqueous, streptozotocin, Type-2 diabetes, sub-chronic

THE EFFECTS OF ZERUMBONE ON CELL MIGRATION IN COLON CANCER CELLS

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Colon cancer is the second leading cause of cancer death among male and female. Survival in colorectal cancer patients is poor and greatly affected by its metastasis. Zerumbone (ZER) is the active compound known for its anticancer properties which able to inhibit cancer cell proliferation and apoptosis. This study aims to study the effects of ZER on cell migration in colon cancer cell line. Cytotoxic properties of different concentrations of ZER (6.25 -100 µg/mL) on colon cancer cells (HCT116 cells) and mouse embryonic fibroblast cells (NIH/3T3 cells) were determined by using MTT assay, at different incubation time points (24, 48 and 72 hours). Morphological changes (such as characteristic of apoptosis or necrosis) of HCT116 cells were observed. The effect of ZER on cancer cell migration was determined by using scratch assay. From the MTT results, the IC_{50} values for HCT116 cells treated with ZER were 8.8 ± 0.4 µg/mL (24 hours), 19.7 ± 2.7 µg/mL (48 hours) and 27.0 ± 1.5µg/mL (72 hours). The results showed that the IC_{50} is significantly increased ($p<0.05$) in time-dependent manner. Whereas the IC_{50} values for NIH/3T3 cells treated with ZER were 37.5 ± 1.8 µg/mL (24 hours), 15.0 ± 2.6 µg/mL (48 hours) and 37.5 ± 3.5 µg/mL (72 hours). Three different concentrations (IC_{50}, IC_{25} and IC_{10}) of ZER were used in the morphological study and migration assay. The morphological changes of cells observed under microscope included apoptosis and cell shrinkage. Based on the concentration used, ZER was also shown to inhibit cell migration in HCT116 cells. It is concluded that ZER inhibits cell migration in colon cancer cells.

Keywords*:* Metastasis, migration, Zerumbone, colon cancer

CYTOTOXIC ACTIVITIES OF KELULUT HONEY HARVESTED FROM SELECTED LOCATIONS AGAINST BREAST CANCER CELL LINES

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Kelulut honey (KH) is a natural product-derived food produced by stingless bee from *Trigona* or *Meliponine* species. A study has shown that KH exhibited cytotoxic activity against cancer cells. Previous studies have revealed that geographical origin can affect the cytotoxic activity of honey. However, studies comparing the cytotoxic activities of KH harvested from different locations are currently limited. Therefore, the objective of this study is to compare the cytotoxic activities of KH harvested from different locations against breast cancer cells. In this study, KH was harvested from three selected locations (Sarawak, Selangor and Pahang). Percentages of cell viability of triple negative breast cancer cells (MDA-MB-231) treated with different concentrations (0.5 – 10%) of KH was determined by MTT assay. After incubation for 24, 48 and 72 hours, the concentration which inhibits 50% of the cellular growth (IC_{50}) was plotted. Based on MTT assay, concentration of KH that inhibit the growth of MDA-MB-231 cells (IC_{50}, IC_{25} and IC_{10}) was further selected to evaluate the mode of cell death induce by KH. KH harvested from Selangor significantly ($p<0.05$) exhibited the highest cytotoxic activities against MDA-MB-231 cells, followed by KH harvested from Sarawak and Pahang. The IC_{50} of MDA-MB-231 cells treated with KH harvested from Selangor were as followed: 6.47 ± 0.80 % (24 hours), 3.73 ± 0.47 % (48 hours) and 4.17 ± 0.46 % (72 hours). The IC_{50} is significantly ($p<0.05$) reduced in time-dependent manner against MDA-MB-231 cells. Cells treated with KH showed characteristic of apoptosis such as membrane blebbing, apoptotic body, cell shrinkage and nuclear fragmentation under an inverted light microscope. The results showed that KH harvested from Selangor exhibited the highest cytotoxic activities against breast cancer cells, suggesting that different harvested locations of KH affect its cytotoxic activities.

Keywords*:* Kelulut honey, cytotoxic activities, harvested locations, triple negative breast cancer cells

AN EVALUATION OF NEUROTOXICITY ASSESSMENT IN ZEBRAFISH EMBRYO (*DANIO RERIO*) INDUCED BY KRATOM

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Kratom (*Mitragyna speciosa*) belongs to *Rubiaceae* family. The herb was used as self-medication to treat acute and chronic pain, anxiety and treatment of opioid addiction or dependence. Coping was associated with high kratom consumption (>3 glasses daily) in rural area (McCurdy et al., 2018). Recently, the widely usage of kratom by rural population in Southeast Asia suggested abuse potential and it have been increasingly emerging. Despite of its medicinal properties, the kratom use can lead to dependence as how the major alkaloid, the mitragynine works with unknown effects on addictive profile and behavioral changes of acute and chronic administration. The aim of this study was to investigate kratom effects toward the embryo and explored its potential to alter the embryonic development. The embryo was exposed to five different concentrations (10,50,150,250,300 mg/L) in each well plate (n=10) for each treatment. The embryonic development was observed for every interval of 12 hours (5 days). After short term exposure at 24 hours' post-fertilization, heart rate (bpm), somite formation and tail formation were being observed using inverter microscope. Embryo mortality and hatching rate were tabulated for evaluation of lethality indicator. Findings shown that there were significant changes in alter the embryo development (≥50mg/L). The embryo heart rate in kratom shown gradually lower (40 to 60 beats per minute) than normal embryo (120 to 180 beats per minute). The finding in the embryo tails formation shown defective in kratom after 48hpf. There was significant in embryo delayed hatching rate (≥250mg/L). These data suggest that the short term and long term exposure of kratom consumption significantly alter the embryo development, decline in embryo heart rate and detachment of tail-bud from the yolk sac.

Keywords: Kratom; *Mitragyna speciosa*; Addiction; Embryo development, Neurotoxicity

FABRICATION, DRUG RELEASE AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN LOADED ALGINATE/COCKLE SHELL POWDER NANOBIOCOMPOSITE BONE SCAFFOLD

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Orthopedic implant infection is one of the most challenging issues in bone tissue engineering industry. Hence, local delivery of antibiotics incorporated into the fabricated bone scaffold itself is introduced in order to provide rapid bacteria inhibitory effect to the surrounding. In this study, ciprofloxacin loaded alginate/cockle shell powder nanobiocomposite bone scaffolds are fabricated with 5 wt% and 10 wt% ciprofloxacin respectively. A non-drug loaded nanobiocomposite bone scaffold is fabricated for comparison purpose. Scanning electron microscope (SEM) observation is done to observe the effect of mineralization of the fabricated bone scaffolds using simulated body fluid (SBF) as well as for biofilm formation study of the scaffold using *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial suspensions. In addition, in vitro study of drug encapsulation efficiency of the scaffolds in phosphate buffer saline (PBS), drug release study in SBF and bacteria inhibition study with *Staphylococcus aureus* and *Pseudomonas aeruginosa* are performed. SEM observations showed mineralization of scaffold surface regardless of drug loading indicating the function ability of the scaffold is not reduced with the presence of drugs. SEM observation also noted an inhibition in formation of bacteria biofilm on drug loaded scaffolds compared to the non-drug loaded scaffold (control). However, in vitro studies showed both 5 wt% and 10 wt% ciprofloxacin loaded nanobiocomposite scaffolds have low drug encapsulation efficiency at 4.92% and 3.80% respectively and low level of drug release with no significant difference between the two (p>0.05). The bacteria inhibition studies showed that both compositions of the drug loaded scaffolds and the elution samples of the drug release study can provide some inhibitory effect to the growth of the respective strains of bacteria tested. Therefore, this study shows that ciprofloxacin may not be the most suitable antibiotic to be incorporated into the alginate/cockle shell powder nanobiocomposite bone scaffold in order to provide an optimum antibacterial effect.

Keywords: Bone scaffold; ciprofloxacin; antibacterial activity

EFFECTS OF IRON, ZINC AND VOLUME OF SABOURAUD DEXTROSE BROTH ON FUNGAL GROWTH IN BLOOD SAMPLES

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Systemic fungal infections involve invasion of fungi into bloodstream or deep-seated organs. These infections occur in immunocompromised patients and often lead to mortality if early treatment is not given. However, the currently used blood culture technique is not sensitive enough to detect early stage infection due to the low fungal burden in the bloodstream and fungal inhibiting activity of leukocytes. Fungi need iron and zinc, which are essential trace elements, for growth. Sabouraud dextrose broth (SDB) is a potential growth enhancing medium while sodium polyanethole sulfonate (SPS) inhibits leukocyte activity. Therefore, this study aimed to determine the suitable volume of SDB and incubation period for culturing blood samples and, the effects of addition of SPS, iron and zinc on the growth of fungi that commonly cause systemic infections, *Candida albicans* and *Aspergillus fumigatus*. Ferric chloride and zinc sulfate solutions, both at the concentration of 3 µM and 10 µM were added to whole blood samples spiked with fungal germ tubes and incubated at 37^{o}C, together with 4 mL or 9 mL of SDB, 0.05% SPS and incubated for up to 24 and 48 hours. Blood samples were inoculated onto Sabouraud dextrose agar plates and fungal colony forming units (CFUs) counted. Results showed that 5-fold dilution of blood samples increased fungal growth better than 10-fold dilution, and the growth was enhanced by the addition of SPS. Growth of *A. fumigatus* was observed only after 48 hours incubation. Zinc showed inhibition effect on fungal growth while 3 µM iron was able to increase fungal growth. Therefore, our study demonstrates the fungal growth enhancing effects of 5-fold dilution of blood samples with SDB, addition of SPS and 3 µM iron and incubation of samples for 48 hours. The application of these parameters may increase the sensitivity of blood culture based diagnosis.

Keywords: invasive fungal infections, sensitivity, blood culture

IN SILICO DESIGN OF POTENTIAL CHEMICAL PROBE (DERIVED FROM ZINC NATURAL DERIVATES DATABASE) AS SPECIFIC DETECTOR OF THERMOSTABLE PORCINE PEPTIDE: APPLICATION FOR RAPID HALAL AUTHENTICATION

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The need for rapid Halal authentication procedure has become an international concern due to the increase in meat adulteration cases. To date, halal authentication methods are mostly expensive, time-consuming and need modern equipment and expert personnel to run the test which will limit the methods' reliability to be used as routine authentication procedure. This study is aimed to design and evaluate potential chemical probe(s) specific to detect a thermo-stable porcine peptide (named AQ1) either in raw or thermally processed food products *in* silico. was consistently detected in contaminated-pork meat products and processed pork that was derived from serum albumin protein. Probe design was carried out *in silico*, starting by solving the 3D peptide structure via homology modelling using I-Tasser software. By using LigandScout software, pharmacophore query was generated for the AQ1 peptide template to run virtual screening procedures to retrieve a number of potential chemical probe(s) from Zinc databases. Specific filters (toxicity and Lipinski rule of five filters) from ChemBioserver were applied to minimise the number of hits. Lastly, docking of potential was performed using AutoDock Vina software to predict their binding properties and to selectively choose top 10 potential lead compounds as probe candidate(s)**.** Findings showed 11 possible hits derived from Zinc natural derivatives database that can be retrieved from the webserver. Upon binding, compound no 8680732 presented highest binding affinity to the AQ1 peptide at -5.2 kcal/mol. The compound showed hydrogen bonding with asparagine at residue 5A at 2.14 Armstrong, which is considered as moderately strong hydrogen bonding. Compound no 478771 the second highest binding affinity of -4.8 kcal\mol. No 868072 showed best best binding potential the AQ1 peptide that highlight its potential to be developed as specific chemical probe for halal authentication procedure.

Keywords: AQ1 peptide, chemical probe design, *in silico*

CYTOTOXIC PROPERTIES OF KELULUT HONEY HARVESTED FROM DIFFERENT LOCATION IN LIVER CANCER CELLS

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Kelulut honey (KH) is produced by *Trigona* species, a stingless bee, which has been shown to exert prominent medical properties and could be exploited as a natural nutraceutical agent to treat free radical associated diseases. Recently, KH has shown to possess various pharmacological properties including anti-cancer properties against liver cancer cells. Previous study shown that the pharmacological properties of KH may be influenced by its harvested locations. However, to date, there is no study comparing the cytotoxic properties of KH harvested from different locations *in vitro.* This study aims to compare the cytotoxic properties of KH harvested from different locations against liver cancer cells. Fresh KH was harvested from three different locations (Pahang Selangor, Sarawak). Cytotoxic properties of different concentrations of KH (5-20%) towards liver cancer cells(HepG2) were been determined by using MTT assay, at different time points (24, 48, and 72 hours). Morphological changes (such as characteristic of apoptosis or necrosis) of the liver cancer cells after treated with KH was been observed under light microscope. The IC_{50} value of KH harvested from Selangor on HepG2 cells at three different time points (24, 48, 72 hours) are 1.9±0.31%; 1.2±0.17%; 1.15±0.13% respectively. KH harvested from Pahang shows IC_{50} value of 5.13±0.30% (24 hours); 3.05±0.60% (48 hours); 3.4±0.83%, (72 hours) and IC_{50} value of KH harvested from Sarawak are 2.23±0.27% (24 hours); 2.83±1.11% (48 hours); 1.83±0.17% (72 hours) . The KH harvested from Pahang showed the highest cytotoxic properties (24 hours). And the KH harvested from Selangor showed lowest cytotoxic properties (72 hours). Three different concentration of KH(IC_{50}, IC_{25} and IC_{10}) are used in morphological study. The morphological changes (cell apoptosis and shrinkage) were observed under inverted light microscope. This study showed that KH harvested from Pahang showed the highest cytotoxic properties compared to KH harvested from Sarawak and Selangor towards liver cancer cells. This suggest that different harvested locations of KH may affects its cytotoxic properties.

Keywords: Kelulut honey, cytotoxic properties, harvested locations, liver cancer cells

ANTI-INFLAMMATORY AND ANTI-OXIDANT PROPERTIES OF *ALTERNANTHERA SESSILIS RED*

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Oxidative stress and inflammation are two of the most critical factors implicated in carcinogenesis, degenerative disorders, as well as cardiovascular diseases. Wild type of *Alternanthera sessilis* has been reported to possess numbers of pharmacological activities. However, the cultivar named *Alternanthera sessilis Red (ASR)* is more commonly found in Malaysia with the claimed and believed to be able to reduce the risk of cardiovascular diseases. Current study aimed to evaluate the antioxidant and anti-inflammatory properties of *ASR* in vitro. Anti-oxidant activities were investigated by using ferric reducing ability of plasma (FRAP) assay and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. Anti-inflammatory activities were evaluated by using egg albumin denaturation method. Our result showed that TPC of *ASR* water extract was 14.81 ± 0.08 mg GAE/g dry weight. The TEAC values of the extracts was 12.29 ± 0.26 mg TAE/g dry weight for ABTS. TEAC values determined by ABTS were well correlated with phenolic contents. In protein denaturation method at concentration of 1000 µg/mL extract showed maximum inhibition (96.42%) and standard drug provided (54.84%) inhibition whereas at 250 µg/mL concentration of *ASR* showed minimum inhibition (26.71%). ASR exhibited antioxidant and anti-inflammatory properties and this activities may be associated with its total phenolic content level.

Keywords: Anti-oxidant activities, Anti-inflammatory properties, Phenolic

ANTIMALARIAL ACTIVITY FROM *Canarium odontophyllum* LEAVE EXTRACT AGAINST ERYTHROCYTES INFECTED WITH *Plasmodium falciparum* 3D7

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Malaria belongs in the list of the world as an infectious disease caused many deaths. Therefore, this study was to investigate the activities of malaria in vitro by *Canarium odontophyllum* leaf extract to erythrocytes infected with *Plasmodium falciparum* 3D7. The study began by the initial screening of 2% level of parasitaemia in infected erythrocytes. After desired parasitemia achieved, the infected erythrocytes will given the in vitro treatment with four types of different polarity extract; acetone, methanol, hexane and aqueous which is these four extracts tested for its potential as antimalarial agents. This experiment was conducted to determine the concentration at 50% inhibition of Plasmodium's activity (IC50). IC_{50} were calculated for each extract with different concentration dose from the highest dosage 10mg/mL, then followed by 1 µg/mL, 0.1µg/mL, 0.01 µg/mL, 0.001 µg/mL, 0.0001µg/mL, 0.00001 µg/mL and the lowest dosage was 0.000001 mg/mL using pLDH assay and SYBR Green assay. Thus, the expected results may show that the methanol extract have the lowest value of IC_{50}, then followed by aqueous, acetone and hexane. Hence, the methanol extract then was proceed in the synchronization test where the test purpose was to measured inhibition activity at different morphological stages in *P. falciparum* life cycle such as young trophozoite, mature trophozoite and schizont. Results IC_{50} obtained by synchronization were, young trophozoite show the lowest value followed by mature trophozoite and schizont for the SYBR Green assay and schizont show the lowest value followed by young trophozoite and mature trophozoite. In conclusion, *Canarium odontophyllum* methanol extract has the potential to be used as antimalarial drugs in the future.

Keywords*: Plasmodium falciparum, Canarium odontophyllum*

SYSTEMIC EFFECT OF PTEROSTILBENE IN MOUSE LUNG SQUAMOUS CELL CARCINOMA MODEL

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The aim of the experiment was to study the systemic effect of pterostilbene on other organs than lung in mouse squamous cell carcinoma model. The Balb/C strain were used as they could exhibit an intermediate degree of sensitivity to lung carcinogens and display many similarities to human adenocarcinoma. The model developed lung SCC due to the topically induced of N-nitroso-tris-chloroethylurea (NTCU) and were given pterostilbene (PS) as a chemopreventive agent. The animals were divided into 4 groups which were the control vehicle (received only corn oil), cancer control (received only NTCU diluted in acetone), low dosage of PS treatment (10 mg/kg) and high dosage of PS treatment (50 mg/kg) which were given pterostilbene prior to the application of NTCU. Following sacrifice, the organs such as liver, kidney, skin, spleen and heart were harvested and proceeded to histology observation to determine the morphological and histopathological changes of the organs. The expected results would be the mice the from groups that received low dose of PS will show more carcinogenic characteristics compared to group that received high dose of PS. The oxidative stress level measured was GSH, MDA and SOD to determine the antioxidant activity. The antioxidant level would be high in group that received high dose but not for the group that received low dose of PS. Oxidative stress may lead to cellular damages such as cell membranes, protein and DNA. We suggest that it may possible to detect the effect of carcinogenic compound in changes of DNA structure and it was evaluated with Comet assay with alkaline single cell gel electrophoresis technique. The group that received low dose of PS should display more DNA damages compared to the group that received high dose of PS. In conclusion, pterostilbene would also give systemic effects in lung squamous cell carcinoma mouse model.

Keywords: N-nitroso-tris-chloroethylurea, NTCU, pterostilbene, lung squamous cell carcinoma

ANTI-HYALURONIDASE ACTIVITY IN HONEYS OF MALAYSIA ORIGIN

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Hyaluronidases are involved in many pathological diseases, and their inhibitor showed therapeutic effects on cancer and infectious diseases. This enzyme degrades hyaluronic acid which increase permeability of the host connective tissues. Permeable tissues cause spreading of pathogen, venoms and toxins within human body as well as enhance the cancer progression. Hyaluronidase inhibitor activity was detected in honeys from different origins such as chestnut, oak, heather, pine, buckwheat and mixed blossom where the highest anti-hyaluronidase activity are among chestnut, oak and heather honey. However, such observations are not well described among honey of local origin. In this study, four types of local Malaysia honeys such as Tualang honey, Kelulut, Gelam and commercialized honey were used to determine the anti-hyaluronidase activity. The anti-hyaluronidase activity was measured in the form of half maximal inhibitory concentration (IC_{50}) through hyaluronic acid turbidity reduction assay. All honey showed various degrees of inhibition against hyaluronidase of *Streptococcus pneumoniae* where the honey with the highest anti-hyaluronidase activity is found to be Kelulut honey. In conclusion, local Malaysian honey exhibited good potential as hyaluronidase inhibitor. The findings suggest the potential usage of local natural product as new alternative approach for treatment of cancer and infectious diseases.

Keywords: Hyaluronidase, inhibition, local honeys

ANTILEPTOSPIRAL ACTIVITY OF PHTHALIDE COMPOUND AGAINST PATHOGENIC LEPTOSPIRA

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Leptospirosis is one of the re-emerging zoonotic diseases that the number of cases had been increasing continuously in Malaysia. The disease caused by pathogenic bacteria from genus *Leptospira* by direct contact with the urine of infected animals. Numerous attempts have been made to control the disease especially by chemoprophylaxis but only showed limited success. Therefore, the present study investigates the anti-leptospira potential of phthalide compounds comprise of Px, Px-Br, Py and Py-Br. The compounds were assayed for anti-leptospira activity at different concentration ranged from 1 mg/ml to 1 x 10^{-5} µg/ml using broth microdilution method towards *Leptospira interrogans* (serovar Bataviae) and *Leptospira borgpetersenii* (serovar Javanica). On the other hand, DNA damaging properties of phthalide compounds towards DNA of *Leptospira sp.* was done by incubating the bacteria with the presence or absence of phthalide compounds and analyzed by electrophoresis. Among all these four compounds, anti-leptospira activity of Py-Br compound gave an IC_{50} value of 1 x 10^{-4} µg/ml towards *L. interrogans* serovar Bataviae and Py compound with IC_{50} value of 1 x 10^{-5} µg/ml towards *L. borgpetersenii* serovar Javanica while another three phthalide compounds did not show anti-leptospira activity for this pathogenic species. Since these phthalide compounds showed anti-leptospira activity, the DNA damaging properties of them were tested using their IC_{50} values that were specific to each serovar. The result showed that these Py and Py-Br compounds had DNA damaging properties towards both *Leptospira sp.* as there was fragmentation of DNA band analyzed by electrophoresis. In conclusion, among all phthalide compounds, Py and Py-Br have the potential of anti-leptospira activity by inhibiting the growth and caused DNA damage to the *Leptospira sp.*

Keywords: Leptospira, Phthalide.

COMPARISON OF PHYSICOCHEMICAL, ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF ACACIA, KELULUT AND TUALANG HONEY.

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Honey is a sweet and flavorful natural product derived from nectar of flowers and has been widely use and consumed by human for its health benefits for a long time. Honey composition may vary primarily depends on type of bees and floral sources. However, others factors such as seasonal and environmental also may exhibit different composition and biological effect of honey. These factors also contribute to the difference physicochemical properties and antioxidant activity on different type of honey. The aim of this study was to determine and compare the physicochemical, antioxidant and anti-inflammatory properties of Malaysian Acacia, Kelulut and Tualang honey. Physicochemical properties of honey was determined by pH, moisture content, color intensity, total sugar content, electrical conductivity and total dissolved solid. Total phenolic content (TPC) was measured by using Folin-Ciocalteu's reagent and antioxidant properties was determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and ferric reducing ability of plasma (FRAP) assay. Egg albumin denaturation assay was used to measure the anti-inflammatory level of each honey. pH, moisture content, color intensity, total sugar content, electrical conductivity and total dissolved solid of each honey were varied significantly ($p<0.05$). Tualang honey significantly ($p<0.05$) exhibited the highest TPC value (1.85 ± 0.12 mg GAE/g honey) compared to Kelulut (1.23 ± 0.09 mg GAE/g honey) and Acacia honey (0.65 ± 0.02 mg GAE/g honey). Antioxidant activity by ABTS assay of Acacia honey showed 0.52 ± 0.008 mg TEAC/g honey while Kelulut honey was 1.24 ± 0.02 mg TEAC/g honey and as highest activity obtained from Tualang honey which was 1.82 ± 0.03 mg TEAC/g honey. Acacia honey showed highest anti-inflammatory properties (66.24%) compared to Kelulut (20.88%) and Tualang honey (38.71%). Acacia, Kelulut and Tualang honey exhibited antioxidant and anti-inflammatory properties and these activities may be correlated with its total phenolic content and physicochemical properties.

Keywords*:* Physicochemical, antioxidant, egg albumin assay, Acacia, Kelulut, Tualang

DEVELOPMENT OF AN IN SITU HYBRIDIZATION ASSAY FOR THE DETECTION OF ZIKA VIRUS RNA IN FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUES

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Zika virus (ZIKV) is a positive-sense single-stranded RNA virus belongs to genus *Flavivirus* within *Flaviviridae* family. ZIKV has garnered global attention due to newly ascribed neurological complications such as Guillain Barrésyndrome and fetal microcephaly. The diagnosis of ZIKV infection is challenging due to the similar clinical presentations and antibody cross-reactivity between dengue and ZIKV. Thus, the objectives of this study are: 1) To develop a Digoxigenin-labelled DNA probe for the detection of ZIKV RNA in formalin-fixed and paraffin-embedded tissues, 2) To evaluate the sensitivity of *in situ* hybridization assay for the detection of ZIKV RNA in histopathology specimen. An approximately 300-bp PCR fragment covering ZIKV capsid region was generated from the extracted viral RNA and cloned into pGEM-T-easy vector using standard molecular cloning procedure. The extracted plasmid containing the correct ligated insert was sent for Sanger DNA sequencing before used as a template to produce Digoxigenin-labelled DNA probes using PCR DIG labeling method. Probe specificity was tested on positive controls (ZIKV infected C6/36 cells and human liver blocks) and negative controls (mock-infected C6/36 cells, non-ZIKV flaviviruses infected mouse brain, enterovirus A71 infected mouse skeletal muscles and uninfected human autopsy tissue blocks), respectively. The newly developed probe demonstrated high specificity to ZIKV RNA, without cross-hybridized to other non-Zika flaviviruses RNA (Japanese Encephalitis virus, West Nile virus, Murray Valley Encephalitis virus, Tick-Borne Encephalitis virus, and Dengue virus), mouse and human DNA/RNA. The ISH positive staining was localized only to the cell cytoplasm with no evidence of nucleus staining. In conclusion, this newly developed ISH assay could serve as a sensitive molecular pathology tool for detection and localization of ZIKV infection in formalin-fixed and paraffin-embedded tissues.

Keywords: Zika virus, *In situ* hybridization, formalin-fixed and paraffin-embedded tissue

IN VITRO CYTOTOXIC ACTIVITY OF ROSELLE (*Hibiscus sabdariffa Linn*.) EXTRACT ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC)

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Hibiscus sabdariffa Linn. or Roselle is rich with an organic acid such as ascorbic acid, pectin, and polyphenols such as anthocyanin, phenolic acid, and flavonoid that play a role as an antioxidant agent. Oxidative stress will contribute to the production of highly reactive oxygen species (ROS) that will eventually lead the cascade of atherosclerosis, the main causes of coronary heart disease. The aim of this study was to investigate in vitro cytotoxic activity of two Roselle extracts; polyphenol extract (HPE) and aqueous extract (Aq) in human umbilical vein endothelial cells (HUVEC). Initially, HUVEC were isolated from four donors and being treated with HPE or aqueous extract (Aq) from the Roselle calyx. Both extracts were tested for their possible protective effect and cytotoxicity using the 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on three different dosages (0.01 mg/ml, 0.005mg/ml and 0.001 mg/ml) with two different time points which are 24 hours and 48 hours. As for 24 hours' HPE treatment, the concentration of 0.001 mg/ml showed significant ($p<0.05$) highest cell viability (90.335 ± 0.535) with a constant increase of living cell in 0.01 mg/ml (48.280 ± 0.480) and 0.005 mg/ml (60.345 ± 0.445). However, 0.005 mg/ml of 24 hours' aqueous extract treatment showed a significant ($p<0.05$) higher percentage of cell (92.560 ± 1.923) as compared to other concentration 0.01 mg/ml (90.845 ± 0.488) and 0.001 mg/ml (90.325 ± 0.601). In addition, the results suggested that 24 hours' incubation with both extracts (HPE and Aq) showed a better and have higher cell viability of cell as compared to 48 hours of treatment. We, therefore, conclude that 24 hours of treatment with Roselle extracts; HPE (0.001 mg/ml) and Aq (0.005 mg/ml) would be the best concentration to be used for further investigation on the extracts potential and beneficial in HUVEC studies especially in the context of antioxidant and cardiovascular diseases.

Keywords: Anti-oxidant, Cardiovascular diseases, *Hibiscus sabdariffa Linn*, Human Umbilical Vein Endothelial cells (HUVEC), Reactive oxygen species (ROS)

EXPRESSION OF TOLL-LIKE RECEPTOR (TLR) 2, 4, 8 AND 9 BY MACROPHAGE STIMULATED WITH ASIATIC ACID AND MADECASSOSIDE

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Toll-like receptor (TLR) is a type-1 transmembrane protein on phagocytic cells which involved in the initial recognition of invading pathogens. It plays an important role in the innate immune response against pathogen during phagocytosis. TLR regulates the inflammatory response of infective cells by up-regulation and suppression of genes. Among the TLRs that have been identified, TLR2 and TLR4 are the most important ligands on macrophage that responsible for detecting, engulfing and destroying microorganisms from the surface of the cells, whereas TLR8 and TLR9 act within the endosomal compartment and intrinsically capable to detect foreign nucleic acid that cause by the pathogens. Nowadays, the use of various drugs against microbial infection lead to the emergence of drug-resistances. Thus, this inspired researchers to search for alternative treatment by using natural resources. Therefore, this study was conducted to evaluate the ability of *Centella asiatica* compounds which were asiatic acid and madecassoside in stimulating the expression of TLR2, TLR4, TLR8 and TLR9 in mouse macrophage cell line which were analysed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Overall, the results of the study showed that there were significant differences between the untreated macrophages with the macrophages treated with plant compounds. For TLR4, macrophages treated with 6.25 µg/ml asiatic acid showed the highest expression when compared to untreated macrophages, lipopolysaccharide (LPS)-stimulated macrophages as well as macrophages treated with other *C. asiatica* compounds. However, different result was observed for TLR2. Macrophages treated with either asiatic acid and madecassoside showed a very lower expression of TLR2 in comparison with untreated macrophages and LPS-stimulated macrophages. On the other hand, very low expression bands were observed for TLR8 and TLR9 in all the macrophages. Therefore, these data indicate that asiatic acid and madecassoside have the ability to modulate the innate immune response in mouse macrophage by stimulating TLRs expression on the surface of its transmembrane.

Keywords: *Centella asiatica*, toll like receptor

ELUTION OF VANCOMYCIN FROM FABRICATED ALGINATE/COCKLE SHELL POWDER NANOBIOCOMPOSITE BONE SCAFFOLD FOR POTENTIAL ANTIBACTERIAL AND DRUG RELEASE EVALUATION

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Bacterial infection is a major concern in orthopedic implantation and bone reconstructive surgery. Biofilm formation around an implanted material may lead to serious complication and implant failure. The issue could possibly be addressed through incorporation of local delivery of drugs for a direct and rapid delivery. Therefore, this study aims to investigate the release of drug and its antibacterial activity from alginate/ cockle shell powder nanobiocomposites bone scaffold incorporated with vancomycin. Vancomycin loaded alginate/cockle shell powder nanobiocomposite bone scaffolds are fabricated with 3wt.% and 5wt.% vancomycin respectively for comparison purpose a non drug loaded scaffold is prepared as a control. The mineralizations of the scaffolds using simulated body fluid (SBF) as well as the biofilm formation using *Staphylococcus aureus* immersion were evaluated using Scanning Electron Microscope (SEM). Drug encapsulation study was done by immersing drug loaded and control scaffolds in SBF prior to quantification of eluted drugs at certain time points. Antimicrobial activity of the eluent from each sampling period was tested for growth inhibition of *Staphylococcus aureus* and *Staphylococcus epidermidis* for a period of 21 days. SEM observations showed mineralization of scaffold surface for both drug loaded and control scaffolds. There is significant difference of cumulative amount of vancomycin eluted from scaffolds with higher elution of drugs observed from scaffolds loaded with 5wt.% vancomycin compared to 3wt.% ($P<0.05$). Eluent from both groups showed inhibitory effect against *Staphylococcus aureus* and *Staphylococcus epidermidis* for 21 days. The findings could further be supported with SEM observations showing reduced biofilm formation on surface of 5wt% vancomycin loaded scaffolds compared to control scaffolds. Findings from this study indicates antibacterial properties can be conferred to the fabricated alginate/ cockle shell powder nanobiocomposite bone scaffold with successful incorporation of vancomycin and that the scaffold could be used for local drug delivery application.

Keywords: bone scaffolds, vancomycin, antibacterial

IMAGE ACQUISITION AND QUALITY FOR DIGITILIZED BIOIMAGING OF COLONY FORMING UNIT FOR MOUSE HEMATOPOIETIC STEM / PROGENITOR CELLS

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The hematopoietic colony forming unit (CFU) assay is a valuable stem cell based-assay to assess the multipotency and functionality of hematopoietic stem / progenitor cells (HSPCs). However, the analysis of CFU which is mainly achieved by using manual technique required trained staff and is time-consuming, leading to increase inter- and intra- laboratory variation in colony analysis; of which limits its utilization for therapeutic and research applications. Thus, development of a digitalized bioimaging is needed to overcome these limitations and improving CFU analysis. This study aims to develop digitalized bioimaging for CFU analysis of mouse HSPCs for myeloid, erythroid and pre-B lymphoid lineages. Mouse bone marrow cells were isolated and cultured onto methylcellulose agar to establish CFU for the respective lineages. Then, the CFUs were observed under an inverted micoscope and colonies were captured on day 7 (erythroid and pre-B lymphoid progenitors) and day 14 (myeloid progenitors) using Samsung Galaxy J7 2016 mobile phone with and without microscope lens adaptor, followed by analysis of images using Matlab version 2018. During data acquisition, we noted that images with shadow, incomplete colony morphology and colony located at the edges of the well are considered as poor image quality that cannot be analyzed and used for development of digitalized bioimaging. Meanwhile, the usage of the microscope lens adaptor during image acquisition could shorten the capturing duration and improved image quality as compared to those captured without adaptor. Overall, incorporating digitalized bioimaging platform into CFU assay could lead to a major impact on the analysis of HSPCs functionality which is fundamental to ensure wider applications that require accuracy and reproducibility in CFU results. The invention can prosper the use of CFUs assay in the studies of HSPCs biology, cord blood banking, transplantation lab and research lab.

Keywords: hematopoietic stem / progenitor cells; colony forming unit (CFU); digitalized bioimaging; hematopoietic lineages

ANTI-INFLAMMATORY ACTIVITIES OF CARDAMONIN (2',4'-DIHYDROXY-6'-METHOXYCHALCONE) IN ACUTE AND CHRONIC INFLAMMATORY MURINE MODELS

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Inflammation can potentially cause harm to the body and causing pain, cell swelling and cell damage. The shown symptoms of inflammation are one of the main reasons for most people to seek for medical treatments. Conventional medicines such as Non-steroidal Anti-inflammatory Drugs (NSAIDs) and opioids are widely used in the treatment of pain and inflammation. However, with prolonged consumption of this treatment may cause unwanted multiple side effects such as gastrointestinal disease, renal disorder and adverse cardiovascular event. Therefore, it is necessary to find new sources of anti-inflammatory compounds with potential pharmacological effect in inflammation management to overcome undesirable side effects of modern medicine. Based on the previous finding, cardamonin (2',4'-dihydroxy-6'-methoxychalcone) is believed to have potential to prevent the release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in *in vitro* study. This study aims to investigate the effectiveness of cardamonin on acute and chronic inflammation respectively by using murine models. Anti-inflammatory activities of cardamonin were evaluated using carrageenan-induced paw edema test and cotton pellet-induced granuloma test. The cardamonin (0.3, 1, 3 and 10 mg/kg, i.p.) were given to the mice for all the test as the treatments. Aspirin (ASA) (100 mg/kg, i.p.) and vehicle (ethanol, tween 20, distilled water in 5:5:90 ratio) (10 ml/kg, i.p.) were used as positive and negative control in this experiment, respectively. Male ICR mice (6-8 weeks, 25-35g) were used throughout the study. The result for anti-inflammatory tests showed the injection of cardamonin (i.p.) with four different doses significantly and dose-dependently supressed the development of carrageenan-induced paw edema and granulomatous tissue ($p<0.05$), respectively. This study suggests that cardamonin exerts anti-inflammatory effects in reducing inflammation in acute and chronic inflammatory mice by preventing the release of pro-inflammatory cytokines.

Keywords: anti-inflammatory, cardamonin, in vivo