

CONSTRUCTION AND EVALUATION OF MULTIGENE MUTANTS OF VIBRIO CHOLERAЕ 0139 AS VACCINE CANDIDATES

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Introduction: Cholera is a major health issue, affecting millions of lives annually. In light of the recurrent outbreaks of cholera, there is a pressing need for the development of vaccines that allow rapid mass vaccination.

Objectives: In this study, genetically modified vaccine candidates, VCUSM21P and VCUSM22P, were designed. VCUSM21P is a prototrophic vaccine which encodes non-toxic cholera toxin A (ctxA) subunit immunogen and has accessory cholera enterotoxin (ace), zonula occludens toxin (zot), and repeats-in-toxin CIA (rtxC/A) mutations. On the other hand, VCUSM22P is ace, zot, ctxA, rtxCIA, and hemagglutinin/protease (hap) mutant. Both mutants were found not to disassemble the actin of HEP2 cells.

Methods: Mouse colonisation assay was used to determine VCUSM21P and VCUSM22P colonization ability in-vivo. Rabbit ileal loop assay was performed to evaluate the reactogenicity caused by them. The immune responses provoked by the 2 vaccine candidates and their protective function against cholera were evaluated in a rabbit model. The mutants were found to be good colonizer of the mouse intestine. In the ileal loop assay using non-immunised rabbits, fluid accumulation was found in loops injected with 1×10^6 and 1×10^8 colony forming unit (CFU) of wild Type (WT) V. cholerae. Unlike the WT V. cholerae challenge, 1×10^6 and 1×10^8 CFU of the mutants did not cause any reactogenicity in non immunised rabbits. Immunisation using 1×10^{10} CFU of the mutants induced both IgA and IgG antibodies production against cholera toxin (CT) and 139 lipopolysaccharides (LPS), as well as elevated vibriocidal antibody.

Result: The reactogenicity caused by the WT V. cholerae in rabbits immunised with 1×10^{10} CFU of the mutants was found to be reduced as evidenced by the absence of fluid in loops administered with 1×10^2 - 1×10^7 CFU of WT V. cholerae. In the Removable Intestinal Tie Adult Rabbit Diarrhoea (RITARD) experiment, the non-immunised rabbits were found unprotected against a lethal challenge with 1×10^9 CFU WT V. cholerae. However, 100% of rabbits immunised with the mutants survived the WT V. cholerae challenge.

Conclusion: Immunohistochemical, histopathological, and ultrastructural examination of non-immunised rabbit's ileum challenged with WT V. cholerae revealed severe ileal damages. But less severe damages were noted following the WT V. cholerae challenge in the ileum of rabbits immunized with VCUSM21P and VCUSM22P. The multigene mutants could be used for vaccination against potentially fatal V. cholerae 0139.

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THE RELATIONSHIP BETWEEN PROTEIN AND MRNA EXPRESSION OF TRANSCRIPTION REPRESSOR DREAM, C-FOS AND PRODYNORPHIN IN MODULATING PAIN RESPONSES INDUCED BY FORMALIN IN THE RAT SPINAL CORD

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Introduction: Downstream Regulatory Element Antagonist Modulator (DREAM) protein acts as a transcription repressor for c-Fos and prodynorphin gene which is involved in modulating pain processes.

Objectives: This study was conducted to investigate the relationship between DREAM, c-Fos, prodynorphin protein and mRNA expression through opioid and non-opioid receptors in the modulation of pain responses induced by formalin in the rat spinal cord.

Methods: Male Sprague Dawley rats weighing between 250–300 g were divided into 5 major groups. Group 1 consisted of rats treated with pre-emptive administration of ketamine (anaesthetic drug) (5 mg/kg body weight) intraperitoneally (i.p) and given formalin injection (K + F group) ($n = 36$) after 30 minutes. Group 2 consisted of rats treated with preemptive administration of norbinaltorphimine dihydrochloride (norBNI) (kappa opioid receptor antagonist) (2 mg/kg body weight) (i.p) and given formalin injection (N + F group) ($n = 36$) after 24 hours. Rats in group 3 were treated with preemptive administration of both ketamine and norBNI (i.p) and given formalin injection (NK + F group) ($n = 36$). Rats in group 4 were only treated with normal saline and given formalin injection (F group) ($n = 36$) while rats in

group 5 were not given any treatment and considered as a control group (C group) ($n = 18$). Each experimental group except the control group was further divided into subgroups ($n = 6$) consists of rats that were sacrificed at 2 and 4 hours after formalin injection. 50 μ l of formalin 5% (1.85% formaldehyde solution), was subcutaneously injected into the plantar surface of the left hind paw of the rat. The pain behaviour responses were recorded for 1 hour for rats in the C, F, K + F, N + F, and NK+F groups. The rats were then sacrificed 2 and 4 hours after formalin injection and the lumbar L4/L5 segments of spinal cords were removed for immunohistochemistry, Western blot, and real-time PCR analysis. Noxious stimuli (formalin injection) in this study increased the total number of FLI and PLI neurons, consistent with an increase in relative c-Fos and prodynorphin mRNA levels at 2 hours after formalin injection on the ipsilateral side. The mean relative DREAM protein in the nuclear extract and relative DREAM mRNA levels were also increased at the same time. These effects probably contributed to increase in pain behaviour responses during the tonic phase of the formalin test. However, pre-emptive administration of ketamine prevented the increase of total number of FLI and PLI neurons and relative c-Fos and prodynorphin mRNA level at 2 hours after formalin injection on the ipsilateral side. At the same time, this study found that the relative DREAM mRNA level was decreased but the mean relative DREAM protein level in the nuclear extract was increased at this time. These effects may reduce pain behavior responses during the tonic phase of the formalin test in this group. Pre-emptive administration of norBNI (kappa opioid receptor antagonist) increased the total number of FLI and PLI neurons, consistent with the relative c-Fos and prodynorphin mRNA level at 2 hours after formalin injection on the ipsilateral side. These resulting in decreased mean relative DREAM protein level in the nuclear extract and relative DREAM mRNA level at this time. Furthermore, pre-emptive administration of norBNI enhanced pain behaviour responses during the tonic phase of the formalin test. Pre-emptive administration of both ketamine and norBNI eliminated some of the effects of pre-emptive administration of ketamine and norBNI. These findings suggest that both NMDA and kappa opioid receptors are involved in modulating acute pain responses in this study. However, the changes in c-Fos and DREAM mRNA and protein expression at 2 hours are not prolonged and reverse to the basal state at 4 hours except for the changes in prodynorphin mRNA and protein expression.

Result: These findings suggest that prodynorphin mRNA and protein expression are important in persistent pain mechanisms. In addition, the mean relative DREAM protein in the cytoplasm extract was unaffected in each of the experimental groups in this study after 2 and 4 hours following formalin injection.

Conclusion: In conclusion, this study shows that DREAM protein acts as a transcription repressor for c-Fos and prodynorphin gene, and can be modulated by NMDA and kappa opioid receptor action during acute pain. The

modulation is achieved through changes in the localization of DREAM protein in the nucleus or cytoplasm of neurons. This is an important mechanism which permits the upregulation and downregulation of c-Fos and prodynorphin gene and its protein expression in the rat spinal cord during acute pain processes.

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PREVALENCE AND MICROSCOPIC STUDY OF THE HERBS USED IN PREGNANCY

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Introduction: The use of herbal medicine is common in Malaysia including its use during pregnancy. However, there have been very few formal studies of herbal consumption in pregnancy and even fewer looking at ultra-structural features and trace elements of herbal medicine.

Objectives: This study was to determine the prevalence of use and to identify possible chemical properties and morphological structure of herbal medicine found to be popularly used among the Kelantanese Malay women during the pregnancy period.

Methods: A study was conducted among 460 Kelantanese Malay women at antenatal and postnatal ward Hospital USM from September to December 2007 using structured close-ended questionnaires. The surface morphology and the microstructure of *Anastatica Hierochuntica L.* were captured by Supra 50 VPSE-SEM LEO and Olympus SZ40 stereo microscope with image analyzer. Elemental analysis was done by using Energy Dispersive X-Ray (EDX). Of these 460 women, 55.7% were housewives, 61% had attended secondary education, 85% were aged between 21–40 years and 57.2% were para 2 to 5. Herbal medicine used during pregnancy was 34.3% while 73% utilized herbal in labor because in the belief that it can shorten labor and makes labor easier. The most commonly used herbal medicine in pregnancy was *Anastatica Hierochuntica L.* (Sanggul Fatimah) (60.1%), followed by coconut oil (35.4%), and herbs prepared by traditional midwives (6.3%). The majority of women (89.2%) used only 1 type of herbal medicine and took it when necessary (53.2%) with 1 capsule/glass (38%) per day. Herbal medicines used by pregnant women were commonly unsupervised (81%), with most women getting information from their parents (60.7%) and buying the products directly from traditional midwives (32.2%) and 77% agreed upon its efficacy and safety. *Anastatica Hierochuntica L.* structures

were viewed under VPSEM and were discussed.

Result: Micro diffraction analysis of the herb revealed inert significant presence of useful mineral such as magnesium, aluminium, potassium, zinc, iron, and calcium as well as elements like carbon, oxygen, and silica.

Conclusion: Herbal medicine is still being commonly used among the Malay Kelantanese women communities. A detailed study is therefore needed to establish among others, the efficacy and safety of these herbs where the well-being of mother and fetus are of paramount.

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SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF ADIPONECTIN GENE AND ITS ASSOCIATION WITH SERUM ADIPONECTIN CONCENTRATION AND METABOLIC SYNDROME RISK FACTORS AMONG MALAY ADULTS

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Introduction: Metabolic syndrome is a cluster of risk factors that include central obesity, hypertriglyceridaemia, reduced HDL cholesterol, hypertension, and hyperglycemia. Accumulating evidences support the hypothesis that hypoadiponectinemia, a type of adipokine, confer increased risk for metabolic diseases. Of interest, some of the common polymorphisms in the promoter region, exon and intron 2 of the human adiponectin gene are associated with risk factors of metabolic syndrome.

Objectives: The present study aims to investigate the association of several single nucleotide polymorphisms in the adiponectin gene with serum adiponectin concentration and metabolic syndrome risk factors among Malay adults.

Methods: A total of 298 Malay adults were recruited in this study. Measurements for waist circumference and blood pressure were taken-before drawing an overnight fasting blood. Biochemical tests for triglycerides, HDL cholesterol, and glucose were carried out by using commercially available kits. Plasma adiponectin concentration was measured using Human Adiponectin ELISA kit. A total of 5 sites of single nucleotide polymorphisms in adiponectin gene (SNPs -11426, -11391, and -11377 at proximal promoter and SNPs +276 and +45 at exon 2 regions) were screened using mini sequencing method.

Result: Findings from this study showed that the adiponectin concentration in the subjects with MS was significantly lower than those without MS ($P < 0.05$). The

adiponectin concentration was also significantly associated with only hypertriglyceridemia ($P < 0.001$) and reduced HDL cholesterol ($P < 0.001$). None of the studied SNPs or haplotypes showed any significant association with the adiponectin concentration. Moreover, only SNP -11426 was significantly associated with MS ($P < 0.05$), while SNP +276 was associated with hypertriglyceridemia ($P < 0.05$), and haplotype -11426/-11377 was associated with hyperglycemia ($P < 0.05$). Overall, there was no statistically significant interaction between the status of MS and SNPs or haplotypes with respect to the adiponectin concentration. However, there was a significant association between the adiponectin concentration and the status of reduced HDL cholesterol with SNP-11426 ($P < 0.05$). Besides, a significant association was also observed in the adiponectin concentration and hypertriglyceridemia with haplotype 11426/+45 ($P < 0.05$).

Conclusion: In conclusion, hypoadiponectinemia and SNPs and haplotypes of adiponectin gene may contribute to the development of metabolic syndrome and its risk factors, via unknown mechanisms in Malay population.

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'ULAM': CONSUMPTION AMONGST KELANTANESE MALAY FROM SELECTED DISTRICTS AND THEIR ANTIOXIDANT PROPERTIES

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Introduction: Eating styles have been shown to exert a major impact on the quality of life and this can be shown in the health status of the individual. So the saying "We are what we eat".

Objectives: The present study was carried out to evaluate the consumption of 'ulam' amongst Kelantanese Malay who are synonyms with the famous Kelantanese cuisine of nasi kerabu and nasi ulam.

Methods: 168 participants, 67 male and 101 female had agreed to participate in this survey. They were residents from the districts of Batu Mengkebang, Salor and Tawang, aged 18 years old and above. The result showed 32.8% of male and 32.7% of female participants took 'ulam' daily. Meanwhile, participants who aged 40–59 years old contributed the major percentage in practicing daily intake of 'ulam'. The study shows the relationship between income and 'ulam' intake in each district. The result also revealed that 83.6% of male and 74.3% of female participants combined various type of 'ulams' at one sitting. Most of them took 2 type of 'ulams' during

meal time. Majority of participants agreed that 'ulam' can increase appetite and are safe to consume. Topography of the area of study and availability of 'ulam' in the vicinity possibly influenced the type of 'ulam' consumed in each district. In conclusion, there was similarity between the genders in practice of 'ulam' intake. It is hoped that this study will provide relevant information pertaining to the consumption and properties of various 'ulam' that are being consumed in society, especially those 'ulam' that are edible wild plants available in Kelantan. Methanolic extract of 10 'ulam' were investigated for their antioxidants properties. Antioxidant activity of the methanolic extracts of *Luffa acutangula* (Petola segi), *Oroxylum indicum* (Buah beko), *Jenerih* (no scientific name available as yet), *Leucaena leucocephala* (Petai belalang), *Emilia Sonchifolia* (Bayam peraksi), *Acrostichum aureum* (Piai), *Garcinia xanthochymus* (Asam kandis), *Syzygium inophylla* (Gelam tikus), *Curcuma longa* (Bunga kunyit) and *Moringa Oleifera* (Merungai) were screened by using DPPH radical Scavenging (0.5 mg mL⁻¹) and Ferric thiocyanate assay (1 mg mL⁻¹).

Result: Out of the 10 'ulam', *Syzygium inophylla*, *Curcuma longa*, *Emilia Sonchifolia*, *Moringa Oleifera* and *Oroxylum indicum* showed significantly higher in antioxidant activity (> 90%) detected by both assay.

Conclusion: In conclusion, the outcome of the study may help in contribution towards the development of dietary control or production of nutraceutical for chronic diseases. This study assisted in increasing the available information regarding 'ulam' that used among Malaysian and it can also help in identification of potent sources of natural antioxidant.

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DETERMINATION OF MACRONUTRIENTS IN SELECTED FOODS FOUND IN KELANTAN

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Objectives: The present study was conducted to determine the nutrient composition of selected prepared foods popularly consumed among the people of Kelantan state of Malaysia. The outcome of this study is vital in establishing reliable data on the nutrient composition of foods consumed by the community.

Methods: 100 types of foods that are prepared and served in Kelantan had been chosen in this study. Food samples for analyse were purchased from 3 places. Macronutrients such as calorie, carbohydrate, moisture, protein, fat, and ash were analyzed using the standard methods of AOAC (1995). Range of carbohydrate foods analyzed was between 4.71–96.49 g per

100g, protein, between 0.11–31.99 g per 100 g and fat between 0.23–26.81 g per 100 g.

Result: The results show that the foods analysed generally high in carbohydrate content. Foods that were high in carbohydrate, which is more than 70 g per 100 g is 'dodol', 'pengat pisang' dan 'gelembung buaya'. Even though these foods are high in carbohydrate, the Kelantanese communities still able to consume them by controlling the amount of food intake.

Conclusion: These results will be used in the estimation of nutrient intake related to the study of diet and health relationship for Kelantan.

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THE ROLE OF DREAM IN THE REGULATION OF FORMALIN INDUCED PAIN IN RAPID EYE MOVEMENT (REM) SLEEP DEPRIVED RATS

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Introduction: Rapid eye movement (REM) sleep deprivation has been shown to decrease pain threshold after various pain stimulus. Down regulatory antagonist modulator (DREAM) is a transcriptional repressor of prodynorphin gene.

Objectives: This study evaluates the effect on DREAM in relation to REM sleep deprivation, formalin induced pain, or combination of both; and its relationship to the formalin induced pain behavioural responses.

Methods: Male Sprague Dawley rats (250–300 g) were divided into 4 major treatments; free moving control ($n = 36$), REM sleep deprivation ($n = 36$), tank control ($n = 36$), and sleep recovery ($n = 36$). REM sleep deprivation was elicited for 72 hours using the inverted flower pot technique. Each group was further divided into 2 groups which consisted of rats that were either injected with 2.5% formalin or not. Food consumption and body weight gain were measured before and after the treatment. The formalin induced pain behavioural responses were recorded for 1 hour for rats that subjected to formalin injection. The ventrobasal thalamic complex of brain (VB) were removed from each group for immunohistochemistry ($n = 6$), Western blot ($n = 6$) and real-time PCR analysis ($n = 6$) separately. The 'inverted flower' pot technique was confirmed to induce REM sleep deprivation in the REM sleep deprived, and sleep recovered rats by the classic pattern of hyperphagia with converse loss of body weight. There is a marked hypoalgesia demonstrated in the second phase of formalin induced pain in the REM sleep deprived

rats REM sleep deprivation per se did induce morphological change and reduced the number of DREAM positive neurons (DPN) bilaterally. There was an increase in nuclear DREAM extraction bilaterally. After 72 hours sleep recovery, the morphological changes still persisted with reduction of the DREAM mRNA bilaterally. Formalin induced pain reduced the number of DPN bilaterally and increased the nuclear DREAM extraction contralateral to formalin injected site. Interestingly, REM sleep deprivation with formalin test increased the number of DPN, cytoplasmic, and nuclear DREAM extraction bilaterally which was more on the contralateral side except for nuclear extraction.

Result: There was a significant decrease of DREAM mRNA ipsilaterally. However the changes seem to be reversible as no change is seen in DPN, DREAM extractions, and mRNA in sleep recovery group with formalin induced pain.

Conclusion: In conclusion, REM sleep deprivation and formalin induced pain per se generated their own distinct effects on DREAM. Nevertheless, the combination of both treatments resulted in dynamic intracellular changes which reflected the survival ability of neuronal cells, at least by preserving its basic functions. As DREAM is a transcriptional regulator of prodynorphin, the functional survivability of the neuronal cells was reflected behaviourally by the significant hypoaesthesia after both REM sleep deprivation and formalin induced pain.

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FRACTURE PHENOMENA IN SODA LIME SILICA GLASS CAUSED BY BULLET IMPACTS

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Objectives: A series of studies was performed under controlled experimental conditions to investigate the fracture patterns produced in static loading (ball dropping experiments) and also the impacts of bullets of different calibres and nose shapes, fired from different weapons, onto soda lime silica glass of different dimensions and thicknesses.

Methods: The results obtained in static loading experiments confirmed the earlier findings reported in the literature. In the bullet induced glass fracture experiments, all the bullets were fully metal jacketed except 0.38 Special ones that had exposed lead. Nose types varied from round nose and flat nose to hollow point. The velocity of the bullets varied from 220–1020 m/s. 2 chronographs, 1 placed in front of the glass target and the other immediately behind it, measured the striking and remaining velocities of the bullets.

The bullets after penetration of the glass were recovered using a bullet catch. The resulting crack patterns on glass were studied for their characteristics and surface markings. Some of them were also analysed quantitatively using the concept of fractal dimension that measured the complexity of irregular patterns. Observations revealed substantial differences in the behavior of the bullets to produce fracture patterns in the glass. These patterns were much different from those produced during static loading tests. Each bullet of a specific calibre and type produced a unique pattern by which it can be identified. Further, the same calibre bullets (9 mm round nose and flat nose and 5.56 mm rifle calibre) discharged from 2 different weapons (pistol and sub machine gun for 9 mm calibre/ and rifle and Carbine for 5.56 mm calibre) produced distinguishable patterns according to each weapon. The bullets deformed by mushrooming and shearing of its tip confirming the ductile nature of the projectiles at high velocities. The percentage of bullet deformation showed linear relationship to the complexity of the resulting fracture pattern: the greater the percentage the more complicated the patterns that were formed. The velocity loss for a specific calibre for a given thickness of glass was almost same irrespective of the striking velocity.

Result: Significantly, the fractal dimensions of the patterns varied linearly with the kinetic energy lost to glass during the penetration of the bullet. It was found that the dimension of the glass target had an influence in the fracture patterns caused. The larger dimensional glass had less cracking patterns and the characteristics were confined mostly to the regions close to the hole and the crater. The smaller dimensional glass had the patterns spread throughout the glass. This can be understood qualitatively. The waves that were setup in the glass by the impact of the bullet, responsible for the fracture pattern, were influenced by the boundary conditions obtained at the glass frame. These boundary conditions obviously affected the propagation of the waves created more when the boundary is near to the point of bullet impact than when it is far away.

Conclusion: A quantitative study of the influence of glass dimensions on the fracture patterns should be worthwhile. The data and analysis presented in the thesis demonstrated that they can be used in real crime scene reconstructions involving shooting incidents including those in which bullets have passed through intermediate glass targets. Soda lime silica glass finds extensive use in the windows of buildings and it is also an important glass component in laminated and bullet proof glass. The current study might also help the material scientists to understand better the behaviour of this type of glass subjected to high velocity bullet impacts so that better bullet proof glass constructions could be conceived.

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TOTAL SULFATED GLYCOSAMINOGLYCAN (GAG) OF MALAYSIAN SEA CUCUMBERS *STICHOPUS HERMANNI* AND *STICHOPUS VASTUS* AND ITS EFFECTS ON WOUND HEALING IN RATS

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Introduction: Sea cucumbers have long been exploited as a source of medicinal compounds due to the presence of sulfated glycosaminoglycans (GAGs).

Objectives: The aim of this study was to investigate the occurrence of total sulfated GAG from the integument body wall, the visceral internal organs, and the coelomic fluid of Malaysian sea cucumbers; *Stichopus hermanni* and *Stichopus vastus* and evaluate the effect of total sulfated GAG on wound healing in rats using macroscopic and microscopic evaluations.

Methods: In both species, the integument body wall was the highest source of total, O- and N-sulfated GAGs followed by the visceral internal organs and the coelomic fluid. There was more O-sulfated GAGs compared to N-sulfated GAGs for percentage (%) division in both species. In the full-thickness excisional wound model using 47 female Sprague-dawley rats, 20 µl of 1 µg/ml concentration of total sulfated GAG from each anatomical part of each sea cucumber species were applied to the wound area (6 mm diameter) from Day 0 to Day 12, while phosphate buffered saline (PBS) was applied to control group. The progress of healing was assessed through macroscopic examination and analysis of epithelization, inflammatory cells, fibroblasts proliferation, new vessels formation and collagen fibers organisation using light microscope (LM), transmission electron microscope (TEM), and scanning electron microscope (SEM).

Result: Macroscopic examination revealed significantly ($P < 0.0167$) wound contraction percentage (%) on each observation occurred in sulfated GAGs treated group from *S.vastus coelomic fluid* (day 1 [8.33, IqR 9.38], day 6 [33.33, IqR 6.25], and day 12 [75.00, IqR 2.08]) as compared to control group (day 1 [0.00, IqR 0.00], day 6 [8.33, IqR 9.38], and day 12 [54.17, IqR 18.75]). The epithelisation progress of *S. vastus* integument body wall and coelomic fluid sulfated GAGs treated groups was significantly ($P < 0.0167$) greater compared to control group. LM and SEM evaluations showed that all treatment groups have fully bridged the excised wound on the 12th day of observations. LM and TEM evaluations showed enhanced fibroblasts proliferation with significant ($P < 0.0167$) finding occurred in sulfated GAGs treated group from *S. vastus* coelomic fluid compared to control group. For new vessels formation, LM and TEM showed a significant ($P < 0.05$) increase in the sulfated GAGs treated group from *S. vastus* anatomical parts compared to control group. LM,

TEM, and SEM evaluations showed that sulfated GAGs from *S. vastus* anatomical parts stimulate dense organisation of collagen fibers on the 12th day of observation, significantly ($P < 0.05$) compared to control group.

Conclusion: This study strongly indicate that sulfated GAGs in particularly from *S.vastus* coelomic fluid, seems to hasten the wound healing event through positive effect on acceleration of wound contraction percentage (%), enhance epithelization migration, fibroblast proliferation, angiogenesis process, and collagen organization.

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ASSOCIATION OF MITOCHONDRIAL DNA 10398 POLYMORPHISM WITH BREAST CANCER AND APOPTOSIS IN MALAY POPULATION

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Introduction: The mitochondrial DNA 10398 polymorphism has been observed to associate with breast cancer in several populations.

Objectives: In this study, mitochondrial DNA 10398 polymorphism was screened in 101 Malay female patients with invasive breast cancer and 90 age-matched healthy female controls using minisequencing method.

Methods: The results showed a statistically significant difference with P -value of $P = 0.007$ (OR, 2.29; 95% CI, 1.252–4.200) with the proportion of G variant was higher (73%) than A variant in patients (27%) as well as in controls (G = 54%, A = 46%). The breast cancer tissues were then analyzed using immunohistochemistry method to investigate the relation of this polymorphism in affecting apoptotic level in breast cancer.

Result: No significant difference was observed between the expression of pro (Bax) and anti-apoptotic (Bcl-2) proteins among patients carrying A variant ($P = 0.48$). However, significant difference was observed in patients with G variant ($P = 0.016$).

Conclusion: These results indicate that mtDNA 10398 polymorphism may be useful as a breast cancer risk marker in this population.

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DEVELOPMENT OF FLUORESCING METIDCILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AS A TOOL FOR SCREENING OF ANTIBACTERIAL PROPERTIES OF MIMUSOPS ELENGI LINN

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Introduction: *Staphylococcus aureus* is a gram-positive bacterium that can cause abscesses, various pyogenic infections (e.g., endocarditis and osteomyelitis), food poisoning, and toxic-shock syndrome. It is also one of the most common causes of nosocomial infection (pneumoniae, septicaemia, and surgical-wound infections). The Methicillin Resistant *Staphylococcus aureus* (MRSA) is a strain that is resistant to β -lactam antibiotic by virtue of changes in the penicillin-binding protein within their cell membrane. Consequently, all antibiotics that has β -lactam ring like penicillin group and cephalosporin group are unable to inhibit the growth of this organism.

Green fluorescent protein (GFP) is a protein of unknown function found in the jellyfish, *Aequorea victoria*. This GFP-fusion protein shows a punctuate pattern when localises in the cytoplasm.

Objectives: The present study focused on the development of a fluorescing MRSA by the construction of MRSA vector carrying GFP gene with the intention of determining whether the fluorescing MRSA strain can be used as a tool for the rapid screening of antibacterial properties of natural product.

Methods: The natural products that had been used were aqueous and several organic solvents extracts from the bark of plant *Mimusops elengi* Linn, known locally as 'Bunga Tanjung' plant. This plant has been reported to contain a potent antibacterial component. In this study, phytochemical investigation of aqueous, ethanolic and ethyl acetate extracts of *M elengi* Linn. Studies revealed the presence of alkaloids, flavonoids, and tannin compounds. Whereas, the diethyl ether and petroleum ether extracts revealed the presence of alkaloids only but absence of flavonoids and tannin compounds. These secondary metabolites are known to be synthesized in response to microbial infection.

Result: The present study is, therefore, designed to assess the antibacterial potency of different solvent extracts (aqueous, diethyl ether, ethyl acetate, ethanolic, and petroleum ether) of the bark of *M elengi* Linn. on MRSA. The antibacterial activity of each extract was tested for their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) which can inhibit the growth of at least 99.9 % of the bacterial colonies.

Conclusion: The aqueous, diethyl ether, and ethyl acetate extracts had recorded the same results of MIC (16 μ g/ml) and MBC (32 μ g/ml) against MRSA. Whereas, the ethanolic and petroleum ether extracts had recorded higher results of MIC and MBC (i.e., 32 μ g/ml and 64 μ g/ml) against MRSA. These results showed that all aqueous and organic solvents extracts had antibacterial activities against MRSA.

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