Abstract

Abstracts of Theses Approved for the PhD/ MSc at the School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

PHARMACOLOGICAL EVALUATION ON THE EFFECT OF SYZYGIUM POLYANTHUM (WIGHT) WALP. LEAVES EXTRACT ON RAT'S BLOOD PRESSURE AND RELATED PARAMETERS

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Introduction: *Syzygium polyanthum* (Wight) Walp. var. Polyanthum (Serai kayu) leaves are traditionally consumed by the Malays as an alternative treatment for hypertension. However, the scientific evidence to support this claim is scarcely reported.

Objectives: The present study investigated the effects of aqueous (AESP) and residual methanolic extracts (met-AESP) of S. polyanthum leaves on rat's blood pressure and related parameters.

Materials and Methods: Both extracts were orally administered on Wistar-Kyoto (WKY) and Spontaneously Hypertensive rats (SHR); where systolic blood pressure (SBP) was monitored within 24 hours. The effects of both extracts were also studied in period of three weeks in SHR. In the subsequent study, both extracts were intravenously administered in anaesthetized WKY and SHR, and the parameters related were recorded. Possible involvement of autonomic receptors and nitric oxide in mediating the blood pressure reduction and vasorelaxation by both extracts was investigated by using respective blockers for on isolated thoracic aorta rings. Phytochemical screening, gas chromatography mass spectrometry, and high performance liquid chromatography were carried out to obtain phytochemical profiles of the extracts.

Results: The orally-administered extracts, either as an acute dose or repeated-dose significantly reduced blood pressure of conscious SHR. The blood pressure reduction by met-AESP was more sustained than AESP in anaesthetized SHR. Both extracts also caused vasorelaxation on aortic rings from WKY and SHR with comparable effects. The reductions in blood pressure and heart rate, were suggested to partly involve α-adrenergic receptors, muscarinic-acetylcholine and β-adrenergic receptors; with suggestion of nitric oxide system involvement. Glycerine, acetic acid, and gallic acid were the potential compounds in AESP; while seselin, linoleic acid, methyl hexadecanoate, oleic acid, and gallic acid were the potential compounds in met-AESP that possibly contributed to the reduction in blood pressure and vasorelaxation by these extracts.

Conclusion: This study supported the traditional use of *S. polyanthum* leaves extracts as an alternative treatment for hypertension.

Supervisor:
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PHOSPHORYLATION AND REGULATION OF HUMAN CHOLINE KINASE BETA BY PROTEIN KINASE A

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Introduction: Choline kinase (CK) is the first enzyme involved in CDP-choline pathway for the biosynthesis of phosphatidylcholine, the major component of membrane phospholipid. CK exists as three isoforms, which are CKα1, CKα2 and CKβ. The regulation of these enzymes is physiologically important. Metabolic alterations of CKα are associated with tumorigenesis, while mutation or deletion of *chk*β gene leads to the development of muscular dystrophy. In anticancer research, inhibition of CK activity has been explored as a potential therapeutic strategy.

Objectives: Post-translational modification is one of the mechanisms to regulate the function of CK. Growing evidences support that yeast and human CKα are regulated by phosphorylation but the phosphorylation of CKβ has never been reported. Hence, the general objective of this work was to study the phosphorylation and regulation of CKβ.

Materials and Methods: In this study, protein kinase A (PKA) was identified as the protein kinase responsible for the phosphorylation of CKβ by in-gel kinase assay. PKA phosphorylation was confirmed with specific PKA inhibitor and Western blotting. *In vitro* assay with commercial PKA further supported CKβ as the substrate for PKA phosphorylation.

Results: The phosphorylation occurred at serine 39 and 40 residues in the N-terminal region of CKβ. Phosphorylation of CKβ was observed in human embryonic kidney cells (HEK293) and liver hepatocellular carcinoma cells (HepG2). Forskolin and 3-isobutyl-1-methylxanthine treatment increased the phosphorylation level of CKβ, while the phosphorylation was inhibited by PKA inhibitor (H-89). The phosphorylation level of CKβ was also increased by epidermal growth factor. The effects of PKA phosphorylation on the biochemical properties of CKβ were subsequently examined. PKA phosphorylation increased the catalytic activities of CKβ with choline, ethanolamine and ATP as substrates. The V_{max}

values for choline, ethanolamine and ATP were increased by 47.1%, 81.8% and 50.8%, respectively. PKA phosphorylation improved the affinity of CKβ for choline and ATP, but decreased the affinity of CKβ for ethanolamine. Consequently, the catalytic efficiencies of CKβ for choline and ATP were increased by 121.0% and 97.5%, respectively. The same effects of PKA phosphorylation on the biochemical properties of CKβ were mimicked by double mutation of the phosphorylated serines to aspartates. PKA phosphorylation also dramatically increased the sensitivity of CKβ to hemicholinium-3 (HC-3), a potent inhibitor of CK. The IC_{50} value for phosphorylated CKβ (50 μM) was 29 times lower than the unphosphorylated enzyme (1.45 mM). In addition, PKA phosphorylation also decreased the stability of CKβ protein against urea denaturation. On the contrary, phosphorylation did not affect the optimum pH, subcellular location and oligomeric state of CKβ.

Conclusion: This study reports the phosphorylation and regulation of CKβ by PKA for the first time. The knowledge provides new insight into the intracellular regulation of CKβ catalytic properties by phosphorylation that might be an important mechanism to modulate lipid metabolism and cell growth.

Supervisor:
Associate Professor Dr Few Ling Ling
Co-supervisor:
Dr Khoo Boon Yin

D2-40 AND CD34 INCREASED THE EXPRESSION OF ICAM-1 IN TRIPLE NEGATIVE BREAST CANCER

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Introduction: A The major cause of mortality from breast cancer is due to dissemination of the primary tumor to the other part of the body through the lymphatic micro vessel invasion (LMVI) and the support of tumor-associated macrophage (TAM).

Objectives: The aims of this study were to investigate the roles of lymphatic and blood vessels, M2 macrophage, and ICAM-1 in dissemination of breast cancer.

Materials and Methods: Haematoxylin and eosin (H&E) and immunohistochemical (IHC) staining on consecutive section of 37 formalin fixed-paraffin embedded (FFPE) breast invasive cancer samples. D2-40, CD34, CD163, and ICAM-1 were used to stain lymphatic vessel, blood vessel, macrophage, and ICAM-1 receptor respectively. ICAM-1 expression on lymphatic and blood vessel was investigated on stimulated MCF-7 and MDA-MB-231 cell lines with D2-40 and CD34 antibodies, followed by flow cytometry reading.

Results: The lymphatic vessel density (LVD) was significantly lower than the blood vessel density (BVD). Increased of total LVD was significantly associated with increased tumor size (X^2=6.193, df=2, P=0.045) and increased of intra-tumoral LVD and lymphatic vessel invasion (LVI) were significantly associated with HER2/neu status (Fisher's Exact test, P=0.022 and P=0.05). Although the BVD was higher than LVD, however the percentage of LVI was higher than BVI 22.24% (145/652) and 5.45% (265/4858) respectively. Generally, LMVI detected in H&E was missed in 50.24% (206/410) compared with LMVI detected in IHC-stained tissues. Expression of ICAM-1 was significantly higher on treated MDA-MB-231 with any endothelial antibodies used in this study compared to MCF-7 (P<0.001).

Conclusion: The specific endothelial antibodies such as D2-40 and CD34 should be considered in histological reporting instead of depending only on H&E to increase the accuracy of individual results. The significant increase of ICAM-1 expression in triple negative samples (TNBC) was proved in both methods used in this study. The finding of lymphatic endothelial antibodies might increase the ICAM-1 level in TNBC provide a foundation for pre-clinical and clinical evaluation. Therefore, ICAM-1 targeted molecule could be the possible alternative therapeutic target for TNBC treatment.

Supervisor:
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PLAQUE INSTABILITY BIOMARKERS: A COMPARISON BETWEEN ACUTE CORONARY SYNDROME AND CHRONIC STABLE ANGINA PATIENTS

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Introduction: Biomarkers play a pivotal role in the diagnosis and management of patients with acute coronary syndrome (ACS). Some biomarkers, such as C-reactive protein (CRP), soluble CD40 ligand (sCD40L), placental growth factor (PlGF) and myeloperoxidase (MPO) have been reported to be involved in plaque destabilization.

Objectives: The levels of these biomarkers were studied in ACS and chronic stable angina (CSA) patients aged ≤ 45 years and aged > 45 years. The relationship between these biomarkers in the coronary circulation and peripheral circulation was also investigated. This study was the first attempt to investigate the expression of peroxisome proliferator-activated receptors (PPARs) in ACS.

Materials and Methods: A total of 79 patients (ACS: n = 39, CSA: n = 40) was recruited. The blood was sampled from the occluded coronary artery (coronary circulation) and also from the median cubital vein antecubital fossa (peripheral circulation). The serum protein levels of CRP, sCD40L and PlGF and plasma levels of MPO were measured using ELISA. The intracellular levels of PPARs were semi-quantified using Western blot. The mRNA levels of the biomarkers were measured by real-time PCR. All ACS patients that underwent six months clinical follow-up was assessed for major adverse cardiac events (MACE) after the acute event.

Results: The peripheral levels of CRP, MPO, sCD40L and PlGF were significantly increased in ACS compared to

CSA patients. Furthermore, the peripheral concentrations of these biomarkers were significantly correlated with the concentrations found in the coronary circulation. The patients aged below 45 years and above 45 years shared similar profiles of biomarkers. The expression of PPAR-γ was significantly increased in the ACS patients and correlated with both sCD40L and MPO. Serum CRP demonstrated the highest area under the curve value of 0.79 ($p < 0.001$) in discriminating ACS, followed by PlGF, MPO and sCD40L. In addition, the biomarkers also showed their promising prognostic abilities in predicting 30-day and six-month MACE in ACS patients.

Conclusion: In conclusion, this study provided additional information on the proteins and gene expression profiles of plaque instability markers in both CSA and ACS patients. The biomarkers contribute to the formation of unstable plaque by triggering vascular inflammation, firous cap thinning and formation of large lipid core in coronary plaque. Their accuracies in discriminating ACS and predicting MACE also showed promising results.

Supervisor:
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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST ENTAMOEBA HISTOLYTICA ACETYL-COA SYNTHETASE USING PHAGE DISPLAY ANTIBODY TECHNOLOGY

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Introduction: Early diagnosis of fatal amoebic liver abscess (ALA) is crucial. Routine antibody detection assays can neither differentiate ALA from other causes of liver abscesses nor distinguish current and past infections as these antibodies persist for long period in infected individuals. Antigen detection assay using monoclonal antibody is preferred as it can detect specific *E. histolytica* antigen circulating in individuals with current infection. *E. histolytica* acetyl-CoA synthetase (EhACS) is recently reported to be a potential biomarker for detection of acute ALA in an animal experiment.

Objectives: This study aimed to produce and characterise a phage monoclonal antibody against recombinant EhACS (rEhACS).

Materials and Methods: A human domain phage antibody library was screened to select a monoclonal antibody which binds specifically to the targeted rEhACS.

Results: Phage display technology was deployed to isolate monoclonal antibody (mAb) by screening a human naïve domain antibody (dAb) library against rEhACS using bio-panning process. Three rounds of bio-panning of dAb antibody phage library against rEhACS were performed, followed by another three rounds of polyclonal phage panning, which showed marked increase in signals from first to third panning. Several potential clones were screened against electro-eluted rEhACS and a panel of non-specific antigens using monoclonal phage ELISA to obtain two positive mAb clones, D1 and A5. Sequencing results showed that they were of variable heavy (VH) chain.

Conclusion: This study has successfully produced and characterized two phage display VH chain monoclonal antibodies against rEhACS. The diagnostic and therapeutic potentials of these two clones would be further investigated in future studies.

EFFECT OF QUERCUS INFECTORIA-BASED VAGINAL CREAM TOWARDS CERVICAL CANCER CELLS

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Introduction: Cervical cancer is the third leading cause of cancer death among females in less developed countries, and almost 70% caused by oncogenic human papillomavirus (HPV) types 16, and 18. To date, most of available therapies usually are associated with side effects to the patients. Therefore, the use of plants as therapeutic agents has been introduced due to its efficiency, safety and economic feasibility. Nowadays, there are numbers of vaginal drug delivery systems have been developed in clinical and research setting. Compared to oral administration of the drug, the vaginal drug delivery has more advantages as an effective route. In this study, *Quercus infectoria* (QI) galls has been chosen as plant of interest due to its anticancer potential as previously reported. Then, QI aqueous (QIA) extracts were selected for formulation of nutraceutical-based vaginal cream namely QI vaginal cream.

Objectives: The aims of this study was to determine the ability of QI extract-based vaginal cream to selectively inhibit proliferation of cervical cancer cells (HeLa).

Materials and Methods: The antiproliferative activity of QIA and QI vaginal cream against HeLa cell lines has been assessed by MTT assay. Then, the expression of HPV E6 and E7 protein in HeLa cell lines treated with QI vaginal cream for 24 hours was determined by Western blot analysis, and the toxicity effect of QI vaginal cream on the lower reproductive tract in female rats model also has been observed by histopathological examination after intravaginal application for 3 weeks. Lastly, antioxidant activity of QIA extract and QI vaginal cream were analyzed by DPPH radical scavenging system.

Result: Antiproliferative activity of QIA extract and QI vaginal cream against HeLa cell lines showed greater IC_{50} value 13.90 ± 2.27, and 20.80 ± 1.94 respectively. The formulated cream showed ability to suppress the expression of HPV E6 and E7, after the treatment. Then, daily application of QI vaginal cream for three weeks did not cause any inflammation to the vaginal mucosa and cervix. QIA extract and QI vaginal cream demonstrated high DPPH radical scavenging activity. The high antioxidant activity might be due to the presence

of gallic acid and tannic acid which are proven to possess antioxidant activity.

Conclusion: The formulation has demonstrated ability to reduce cervical cancer cells viability without any adverse effect observed on the lower reproductive tract in rat model.

Supervisor:
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INVESTIGATION OF NEURAL STEM CELL MIGRATORY CAPACITY TOWARDS GLIOMA CELLS (IN VITRO) WITH QUERCUS INFECTORIA METHANOL EXTRACT

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Introduction: Glioma arises from glial cells and it is a type of brain tumour with high prevalence and poor prognosis. Current glioma treatments are combination of surgery, radiotherapy and chemotherapy. Nonetheless, they are only able to increase survival rate of glioma patients at a low percentage. By its nature, chemotherapy able to kill cancer cells but it also causes damage to healthy cells because it is administered systemically into patient's body. Therefore, targeted anti-cancer therapy which is able to destroy cancer cells specifically without harming the neighbouring healthy cells is necessary.

Objectives: The aim of this research project was to use neural stem cells as a targeted anti-cancer delivery agent to transport anti-proliferative compounds directly to glioma site and kill the cancer cells without damaging the surrounding healthy cells.

Materials and Methods: In this study, crude extract of *Quercus infectoria* gall was extracted using soxhlet technique with 100% and 70% methanol solvent. Optimum half maximal inhibitory concentration (IC_{50}) of human neural stem cell line (H9-hNSC) and human glioblastoma cell line (DBTRG-05MG) were used to determine the optimum concentration for cell migration assay. H9-hNSC was treated with respective optimum concentration of *Q. infectoria* methanol extracts and tamoxifen drug to investigate its migration capacity towards DBTRG-05MG in a modified Boyden chamber.

Results: *Q. infectoria* 100% methanol extract (IC_{50}:25.27 ± 7.95 µg/mL) and *Q. infectoria* 70% methanol extract (IC_{50}:32.91 ± 2.23 µg/mL) showed anti-proliferative properties against DBTRG-05MG, along with tamoxifen (IC_{50}:19.40 ± 3.30 µg/mL). The migration of H9-hNSC with optimum concentrations of *Q. infectoria* methanol extracts and tamoxifen showed migration to DBTRG-05MG and it was able to reduce the number of DBTRG-05MG cells.

Conclusion: In conclusion, neural stem cells could be able to deliver plant extracts and drug towards glioma *in vitro* and reduce the cell number of glioma. However, the mechanisms involved in killing the glioma cells by NSCs are yet to be investigated. In brief, this study could serve as a platform for developing neural stem cell-based targeted anti-cancer therapy to treat glioma.

Supervisor:
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PREVALENS AND FACTORS ASSOCIATED WITH SOIL-TRANSMITTED HELMINTHIASES AMONG LEVEL ONE PRIMARY SCHOOLCHILDREN IN BACHOK DISTRICT OF KELANTAN

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Introduction: *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* are soil-transmitted helminth that infect approximately one-third of the world human population who generally lives in poor communities of developing countries; and the helminthic problems are still unceasing among children living in Bachok district of Kelantan.

Objectives: To determine the incidence of soil-transmitted helminthiases (STH) among level one (year one, two and three) schoolchildren in Bachok district; and the associations between STH and the schoolchildren anthropometric measurements, haemoglobin concentrations, various demographic data, different socio-economic factors as well as their hygienic practices associated with STH.

Materials and Methods: In this study, two sub-districts of Bachok with beaches were randomly selected; in which all primary schools in Tawang and Telong sub-districts were recruited. Since Tawang district has more primary schools (4) than Telong (2), proportionate samplings were performed to obtain 68.9% and 31.1% of the targeted sample size of 270 level one schoolchildren from the two sub-districts, respectively. Formalin-ethyl acetate concentration technique was used to detect helminth ova and/or larvae in the stool samples. A portable haemoglobinometer (Hemocue HB 201+, Sweden) was used to determine capillary haemoglobin concentration from a drop of finger-pricked blood. The anthropometric measurement was taken from all the schoolchildren. Interview was conducted based on a previously validated structured questionnaire to obtain the demography, socio-economic status and hygienic practices of the schoolchildren.

Results: About 22.2% (43/194) of the level one schoolchildren in Bachok district were found to be positive for STH. Fischer's Exact Test chi-square analysis showed that there were no significant difference between STH and body mass index (c^2= 0.461, P<0.05) and capillary haemoglobin concentration (c^2= 0.764, P<0.05) among the level one schoolchildren in Bachok. However, Kendall's tau-b c^2analysis revealed that household with 7 or more family members were found to be significantly associated with STH (c^2= 0.017,

$P<0.05$). In addition not washing hands with soap before meal ($\chi^2= 0.045$, $P<0.05$) was found to be significantly associated with STH. Negative associations were obtained between walking barefooted, drinking unboiled water and eating raw vegetable with STH.

Conclusion: This study revealed that STH is still a health concern among growing-up children in Bachok. In future, it would be interesting to study the effect of reducing the significant unhygienic practices reported in this study to minimize the transmission of STH among level one schoolchildren in Bachok.

Supervisor:
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DRUG SUSCEPTIBILITY TESTING OF MYCOBACTERIUM TUBERCULOSIS USING DIRECT TETRAZOLIUM MICROPLATE ASSAY (TEMA)

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Introduction: A rapid, inexpensive and high-throughput assay for drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) is urgently required especially in developing countries where TB cases are prevalent.

Objectives: The aim of this study was to evaluate the drug susceptibility testing (DST) of MTB to the first-line anti-TB drug using tetrazolium microplate assay (direct TEMA) performed directly on clinical specimens (sputum) by omitting the need for prior isolation of MTB in sputum specimens currently performed by indirect TEMA.

Materials and Methods: A total of 59 acid fast bacilli (AFB) smear positive sputum specimens were directly inoculated into drug-free and serially diluted drug in 7H9-S broth media using tetrazolium dye as growth indicator in the microplate wells. All AFB smear categories with different microscopic bacilli counts (from scanty to 3+) were included in the direct TEMA while the standard inoculum size used in the indirect TEMA was 1.50×10^7 CFU/mL. The minimum inhibitory concentrations (MICs) of isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and streptomycin (SM) were obtained for direct and indirect TEMA with reference to the absolute concentration method (ACM). Receiver Operating Characteristics (ROC) curve was used to determine the cut-off MIC values. The sensitivity, specificity, accuracy and predictive values as well as the mean turnaround time (TAT) for the final sensitivity test results were compared.

Results: The MIC for more than 70% of MTB strains were distributed between 0.0156 to 0.0313 µg/mL for INH; 0.0005 to 0.25 µg/mL for RMP; 0.5 to 2.0 µg/mL for EMB and 0.0625 to 0.25 µg/mL for SM for indirect TEMA whereas 0.0039 to 0.0625 µg/mL for both INH and RMP; 0.25 to 1.0 µg/mL for EMB and 0.0625 to 0.25 µg/mL for SM for direct TEMA. The direct TEMA method performed well by accurately distinguishing between the resistant and susceptible strains of MTB as seen by the area under the ROC curve (AUC) ranged from 0.7569 to 0.9643 against the first-line anti-TB drugs. In indirect TEMA, 80%, 71%, 75% and 100% sensitivities were obtained for INH, RMP, EMB and SM respectively while specificities were 96%, 60%, 38% and 84% for INH, RMP, EMB and SM respectively. In the direct TEMA, 100% sensitivity was obtained for INH, EMB and SM and 71% for RMP. However, the specificities for INH, RMP, EMB and SM were 80%, 71%, 55% and 93% respectively. The overall accuracy and predictive values of direct TEMA were comparable to indirect TEMA. A significant shorter mean TAT of 15 days was observed for direct TEMA followed by indirect TEMA (39 days) and ACM (100 days) ($P < 0.001$).

Conclusion: In conclusion, direct TEMA is a relatively simple, rapid and reliable method for DST screening of MTB in countries with increasing prevalence rates of drug resistance strains.

Supervisor:
Dr Wan Nor Amilah Wan Abdul Wahab
Co-Supervisor:
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EVALUATION OF DNA METHYLATION EFFECT ON CpG-ISLAND CONTAINING PROMOTER OF CHOLINE KINASE BETA

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Introduction: Choline kinase (CK) is the first enzyme in CDP-choline pathway, catalyzes phosphorylation of choline to phosphocholine (PC) in the presence of ATP and Mg^{2+} during *de novo* biosynthesis of phosphatidylcholine, the major eukaryotic membrane phospholipid. Human CK is encoded by two separate genes, *ckα* and *ckβ* which encode three different polypeptides, CKα1, CKα2, and CKβ. Apart from the involvement in PC biosynthesis, loss of *ckβ* gene was also associated with autosomal recessive congenital muscular dystrophy with mitochondrial structural abnormalities in human and murine.

Objectives: Previous studies showed *ckβ* promoter as a TATA-less, GC-rich promoter which led to the assumption that epigenetics regulation at the promoter through DNA methylation might regulate the expression of *ckβ* gene. In this study, DNA methylation status on the second CpG island of *ckβ* promoter was analysed to verify the effect of methylation on *ckβ* promoter.

Materials and Methods: Semi-quantitative measurement of restriction-refractory fragment template amplification with endpoint PCR amplification and quantitative real-time PCR amplification methods were performed to analyze the DNA methylation status on the second CpG island of *ckβ* promoter in HepG2 cell line that

was subjected to a DNA demethylating agent (5-Azacytidine, 5-Aza) and a hypermethylating agent (budesonide).

Results: Restriction enzyme analysis showed that isoschizomer pair methylation sensitive/dependent restriction enzyme (MSRE/MDRE) recognition sites were found at -769 and -899 whereas MSRE *Hha*I recognition site was found at -714 on the second CpG island of *ckβ* promoter. The baseline DNA methylation analysis at -769 and -899 revealed a presence of higher amount of methylcytosine (mC) than unmodified cytosine (C). Both findings shows all the three recognition sites as highly methylated, suggesting the second CpG island of *ckβ* promoter was highly methylated at its normal condition. To study the effect of epigenetic modification on DNA methylation status on the second CpG island of *ckβ* promoter, HepG2 cells were subjected to a DNA demethylating agent (5-Azacytidine, 5-Aza) and a hypermethylating agent (budesonide). Result showed that 5-Aza induce demethylation effect at -714 site as shown by the reduce mC amount and increase amount of C, but hmC (hydroxymethylcytosine) level was not affected. In contrast with its hypermethylating roles, budesonide induced demethylation effect at -714 site as shown by the reduce amount of mC and increase amount of C and resulted in significant increase of hmC level. Analysis at -769 and -899 sites revealed that 5-Aza treatment reduce the amount of mC level, whereas an increase of mC level was seen with budesonide treatment.

Conclusion: In conclusion, this study demonstrated all the three sites on the second CpG island of *ckβ* promoter were methylated, and can be regulated through epigenetic alteration.

Supervisor:
Associate Professor Dr Few Ling Ling
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EFFECT OF TUALANG HONEY AGAINST CANDIDA ALBICANS GROWTH AND BIOFILM FORMATION

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Introduction: *Candida albicans* can form biofilms *in vivo* aa well as *in vitro* and is the most common fungal pathogen associated with fungal-biofilm related infections especially in hospital settings. Previous studies have reported the treatment with antifungal therapy was not effective against *C. albicans* biofilms. Evidence has showed the potential used of *Quercus infectoria* gall extract in the prevention and treatment of candidiasis. However there is lack of evidence on the effect of *Q. infectoria* gall extracts on *Candida albicans* biofilm formation. This study was performed to evaluate the *in vitro* activity of Tualang honey on the pre-formed and established biofilm of *C. albicans*.

Objectives: To determine the biofilm growth kinetic profile. To determine the MIC and MFC of Tualang honey against *C. albicans*. To determine the effect of Tualang honey on the prevention of *C. albicans* biofilms formation. To determine the disruptive effect of Tualang honey on the established biofilms.

Materials and Methods: The minimum inhibitory concentration (MIC) was determined using the two-fold serial dilution technique with Tualang honey concentration ranging from 80% (w/v) to 5% (w/v). The XTT reduction assay and field emission scanning electron microscopy (FESEM) were employed to determine the inhibitory and disruptive effect of Tualang honey on the pre-formed and established biofilms.

Results: The lowest MIC value of Tualang honey was obtained at 80% (w/v) for *C. albicans*. Tualang honey exerted its effect on biofilms by slowing the growth of pre-formed biofilms and by reducing the formed biofilms by disruption of their structure at concentration of 80% (w/v). The FESEM results indicated this honey caused shrinkage to the cell surfaces and decreased biofilm biomass.

Conclusion: The findings from this study concluded that Tualang honey at the concentration of 80% (w/v) is fungistatic and has the ability to reduced biofilms formation and disrupt established biofilms.

Supervisor:
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EFFECT OF EPIGENETIC DRUGS ON CANCER CELL MORPHOLOGY, GROWTH AND LEVEL OF CHOLINE KINASE ALPHA CpG ISLAND DNA METHYLATION

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Introduction: Choline kinase (CK) is the first enzyme in the CDP-choline pathway for the biosynthesis of phosphatidylcholine, a major component of membrane phospholipid. In human, CK is encoded by *ckα* and *ckβ* genes which produce three protein isoforms known as CKα1, CKα2 and CKβ. CKα is involved in tumorigenesis while CKβ is associated with muscular dystrophy. DNA methylation is an important epigenetic mark that regulates gene expression. Aberrant DNA methylation in the form of hypomethylation or hypermethylation is commonly observed in cancers.

Objectives: Computational prediction showed that *ckα* CpG islands in its promoter are methylated. However, the regulation of *ckα* gene by DNA methylation has never been investigated. DNA methylation level inside the cells can be manipulated by treatments with epigenetic drugs. In this work, a preliminary study towards understanding the effect of DNA methylation on *ckα* expression is carried out.

Materials and Methods: The effects of 5-Azacytidine (demethylating agent) and Budesonide (methylating agent) on HeLa cell growth and DNA methylation level in selected *ckα* promoter CpG island were investigated. Two methods were used to probe the methylation status of a selected *ckα* CpG island. The first method used to probe the methylation status was MS-DMSO-PCR and the second method was restriction

enzyme based 5-methylcytosine and 5-hydroxymethylcytosine analysis.

Results: MethPrimer and *DBCAT* programs predicted several CpG islands in the *cka* promoter that might be the target of DNA methylation. HeLa cells treated with 70 µM of Budesonide for 24 hours showed normal cell morphology and 78% cell survival rate compared to control cells as measured by MTT assay. Treatment with 7.5 µM of 5-Azacytidine (5-AzaC) resulted in HeLa cells showing morphological characteristics of apoptosis and 62% of survival rate. The 216 bp CpG island of interest was selected based on a previous study that showed its importance in the regulation of *cka* promoter activity. The first method used to probe the methylation status was MS-DMSO-PCR, which revealed that 24 hours treatment with both 5-AzaC and Budesonide increased the levels of DNA methylation at the *cka* CpG island. The second method was restriction enzyme based 5-methylcytosine and 5-hydroxymethylcytosine analysis, which showed that the *cka* CpG island was methylated and hydroxymethylated after Budesonide treatment.

Conclusion: In conclusion, this study has laid the groundwork for experiments to investigate the effect of DNA methylation on *cka* gene expression by showing the use of epigenetic drugs to manipulate DNA methylation levels without dramatically affecting the cell survival. Most importantly, this study has demonstrated the successful use of MS-DMSO-PCR and restriction enzyme based methods to rapidly assess the levels of methylation and hydroxymethylation in *cka* CpG island.

Supervisor:
Associate Professor Dr See Too Wei Cun

ANTIPROLIFERATIVE EFFECTS OF QUERCUS INFECTORIA GALLS EXTRACT AND ITS MECHANISM OF CELL DEATH ON CERVICAL CANCER (HeLa) CELLS

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Introduction: Cancer is one of the leading causes of death worldwide. To date, most of the available therapies are detrimental and cause side effects to the patients. Therefore, patients turn to alternative treatments by utilizing herbs. Accumulating evidences have shown the wide range of therapeutics properties of *Q. infectoria* (QI) galls including anticancer activity. However, the mechanism of action was not well explained.

Objectives: The aim of this study was to determine the ability of QI galls aqueous (QIA) and ethanol (QIE) extracts to selectively inhibit the proliferation of cervical cancer (HeLa), ovarian cancer (Caov-3) and liver cancer (HepG-2) cell lines without causing harmful effects towards normal cells. The most potent extract then chosen for the determination of mode of cell death (apoptosis), phytochemical screenings and antioxidant activity.

Materials and Methods: The antiproliferative activity of *Q. infectoria* galls aqueous extract (QIA) and ethanol extract (QIE) against cervical cancer (HeLa), ovarian cancer (Caov-3) and liver cancer (HepG-2) cell lines has been accessed by methylene blue assay. The antiproliferative activity towards normal kidney (Vero) and normal fibroblast (L929) cells were also evaluated to determine the toxic effects and selective property of the extracts. In addition, cells treated with DMSO served as negative control and cisplatin as positive control. Dose-response curve were then constructed to determine the IC_{50} values. Then, the mode of cell death in QI-treated cells was determined by Hoechst 33258 staining, FITC-annexin V/propidium iodide double staining and detection of apoptotic proteins. Besides, phytochemicals screening, antioxidant tests and total phenolic content analysis were done to observe the connection of bioactivity.

Results: From the results, as compared to QIE, QIA showed better antiproliferative activity with best growth inhibition towards HeLa cells (IC_{50} value = 13.64 ± 2.39 µg/ml) and exhibit cytoselective property. This current finding also showed that HeLa cells treated with QIA undergone apoptosis, represented by the alteration of nuclear morphology and presence of apoptotic bodies as well increased rate of apoptosis. Moreover, results have shown that QIA induced apoptosis through p53-dependant pathway. Upregulation of p53 has been observed to downregulate Bcl-2 and promoted secretion of cytochrome c, thus facilitated the execution of apoptosis through caspase-3 activation. However, no alteration of Bax expression was detected in this study. Based on the phytochemicals screening, QIA comprises of tannin, flavanoid and alkaloid. Furthermore, the antioxidant profiles showed that QIA exhibited great DPPH radical scavenging and X/XOD superoxide scavenging activities and contains high total phenolic contents. The antiproliferative activity of QIA might be due to the diversity of compounds which act as strong antioxidants.

Conclusion: From this study, it can be concluded that QIA exhibited its selective antiproliferative activity against HeLa cells by induction of apoptotic cell death.

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ETHNOMEDICAL SURVEY, PHYTOCHEMICALS ANALYSIS AND BIOLOGICAL ACTIVITY OF SELECTED ANTIDIABETIC PLANTS USED BY ABORIGINES IN GUA MUSANG, KELANTAN

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Introduction: Diabetes is one of the chronic diseases affecting worldwide population. Presently, there is a growing interest in herbal remedies for diabetic patient. Therefore,

an investigation based on ethnomedicine is required in determined alternative approaches to treat diabetics, such as herbal.

Objectives: The main objective of this study was to document ethnomedical of medicinal plants used by aborigines in Gua Musang, Kelantan. The phytochemical, phytonutrient and biological activities of selected plants were screened.

Materials and Methods: Ethnomedical information was collected by interviewing the aboriginal households (house-to-house interviews) and traditional healers in the village. The antidiabetic potential of aqueous extract of selected medicinal plants was determined using α-glucosidase and α-amylase inhibition assay. Phytochemical and phytonutrient were quantitatively determined using standard procedure and antioxidant activities were determined using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic) asid (ABTS), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and ferric reducing/antioksidan power (FRAP) assays.

Results: The ethnomedicinal data revealed that 46 plant species were used in treating various types of health problems, from common diseases such as muscle aches and fever to chronic diseases such as diabetes, hypertension and malaria. From the species recorded, three plants used to treat diabetes is *Albizia myriophylla*, *Oxalis barrelieri* and *Tacca cristata*. Therefore, those plants were term as antidiabetic plants in this study. All extracts were found to have significant antidiabetic activities. *A. myriophylla* extract showed the highest inhibitory activity against α-amylase in which IC_{50} 15.05 µg/ml, while *O. barrelieri* showed the highest inhibitory activity against α-glucosidase in which IC_{50} 52.40 µg/ml. *O. barrelieri* showed the highest phenolic content (64.30 ± 1.50 mg GAE/g), flavonoid content (19.29 ± 2.90 mg CE/g), tannin (42.59 ± 10.23 mg TAE/g), alkaloid (3.27 ± 0.33%), fat (1.47 ± 0.60%) and protein (10.61 ± 0.72%) while *T. cristata* showed the highest content of saponin (7.17 ± 1.15), ash (10.25 ± 0.15%), carbohydrate (53.51 ± 0.94%) and gross energy (240.93 ± 1.74 kcal/100g). Mineral analysis indicates higher concentrations of magnesium, sodium, calcium, mangan, ferum and zinc in *T. cristata* while higher concentrations of potassium and phosphorus in *O. barrelieri*. *O. barrelieri* extract also had the highest antioxidant acivities in ABTS, DPPH and FRAP assays in which 205.95 µmol Trolox/g, 110.41 µmol Trolox/g and 220.93 µmol Trolox/g were obtained, respectively. Further, the most potent extract which is *O. barrelieri* was subjected to functional beverage development for antidiabetic study in rat model. The results revealed that the *O. barrelieri* juice showed blood glucose lowering effects in STZ-induced diabetic rats.

Conclusion: *A. myriophylla, O. barrelieri* and *T. cristata* were found to possess significant *in vitro* antidiabetic and antioxidant activities. Besides that, *O. barrelieri* juice showed antidiabetic potential in rat animal models.

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TOXICITY EVALUATION OF QUERCUS INFECTORIA GALLS AQUEOUS EXTRACT ON FERTILITY AND EMBRYONIC DEVELOPMENT IN FEMALE SPRAGUE DAWLEY RATS

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Introduction: Water concoction of *Quercus infectoria galls* (QIG) or Manjakani has long been used by the Malay old folks for various purposes. However, there is still scarce of scientific literature pertaining to the safety of QIG particularly during pregnancy.

Objectives: The present study was aimed to evaluate the potential toxicity of QIG aqueous extract on the fertility and embryonic development in female Sprague Dawley rats.

Materials and Methods: Experimental rats were administered with QIG aqueous extract daily via oral gavage at doses of 0 (control), 125, 250, 500 or 1000 mg/kg/day started from pre-mating period, continuously until gestation day 16 and sacrificed on day 20 of pregnancy.

Results: QIG extract did not cause any mortality, adverse health status or abnormal behavioural changes in all rats. Additionally, there were consistent trend on the maternal body weights (MBW), corrected maternal body weight (CMBW) and maternal weigh gain among all groups of animals. The mean length of oestrous cycles was not statistically affected but revealed irregular patterns in some animals upon administration of QIG. The pregnancy parameters including pregnancy index, total number of corpora lutea, number of implantation sites, reproductive organ weights, percentages of pre-implantation loss and post-implantation death revealed no deleterious effects in all groups. All foetuses exhibited normal physical characteristics with the absence of congenital malformation.

Conclusion: Administration of QIG extract of up to 1000 mg/kg/day produced no selective toxicity on the fertility, pregnancy and foetal developmental parameters, except for the moderate changes in oestrous cyclicity data of rats which require further detailed evaluation. Thus, the no observed adverse effect level (NOAEL) detected in this study is 125 mg/kg/day.

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THERAPEUTIC EFFECTS OF ANTHOCYANIN-RICH EXTRACT FROM ROSELLE ON OBESE RAT MODEL

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Introduction: Roselle (*Hibiscus sabdariffa* L.) is known to have a rich source of anthocyanin and being use traditionally as anti-hypertensive supplements. Anthocyanin from other

plants has been reported to have anti-obesity potential among mice model.

Objectives: The main objective of this study was to investigate the therapeutic effects of anthocyanin-rich extract from Roselle calyx in obese rats specifically in reducing obesity and other related complication risks.

Materials and Methods: The study employed 48 male Sprague-Dawleys rats. Obesity was induced by daily administration of self-prepared high fat diet (HFD) for 6 weeks. The obese rats were then further divided into five groups; obese-control, obese-orlistat as well as three obese-Roselle extracts treatment groups; aqueous, aqueous + 1% TFA and ethanol + 1% TFA (administered via oral-gavage for three months at dosage of 150 mg/kg). One group of normal rat served as a control group. Throughout the study period, body weight, Body Mass Index (BMI), percentage of body weight gain and blood pressure were measured. Concurrently, functional vessel study on isolated femoral artery was also carried out to assess vascular reactivity. At the end of 3 months, the animals were sacrificed and the histology of liver and aorta section were also examined.

Results: Feeding HFD for 6 weeks was successful to make the rats obese and concurrently having elevated blood pressure and acute fatty liver. Although no weight loss effects shown in obese rats treated with roselle extract, other complications risks related to obesity such as increased in blood pressure and liver disease were improved.

Conclusion: This study strongly suggests that Roselle possess anti-hypertensive and hepatoprotective action that could be due to the presence of anthocyanin in Roselle calyx.

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MOLECULAR BLOOD GROUP TYPING IN BANJAR, JAWA, MANDAILING AND KELANTAN MALAYS IN PENINSULAR MALAYSIA

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Introduction: Blood group antigens are immunogenic proteins or glycoproteins located on the exofacial surface membrane of red blood cells. These blood groups antigens are encoded by polymorphic genes and their frequencies are highly variable between unrelated populations.

Objectives: The present study was aimed to genotype ABO, Rhesus, Kell, Kidd and Duffy blood group loci in DNA samples collected from four Malay subethnic groups in Peninsular Malaysia. The blood group data collected from these Malay subethnic groups were then subjected to population and health analyses.

Materials and Methods: Blood samples were collected with informed consent from 120 healthy and unrelated individuals. These individuals belong to 4 Malay subethnic groups; Banjar (n=30), Jawa (n=30), Mandailing (n=30) and Kelantan (n=30) Malays. The QIAamp blood DNA Mini Kit (Qiagen®, Hilden, Germany) was used to extract genomic DNA from the blood samples. The extracted DNA samples were typed for ABO, Rhesus, Kell, Kidd and Duffy loci using the BAGene ABO-Type variant, RH-Type and KKD DNA-SSP typing kits (BAG Health Care GmbH, Lich, Germany).

Results: The A, B, O, DCCee, K-k+, Jk(a+b+) and Fy(a+b-) were recorded to be the most frequent blood group phenotypes in the four Malay subethnic groups. A principle coordinate (PCO) plot constructed using ABO and Rhesus phenotype frequencies demonstrated a close genetic relationship between the four Malay sub-ethnic groups and other Southeast Asia (SEA) populations and thus reflects the shared genetic history among these populations. The probability values suggested a lower chance of an exact match even if blood was randomly transfused between a recipient and donor of similar ethnicity. Therefore, blood transfusion centre should provide blood that match at multiple blood group systems and recruit increase number of donors especially those with rare blood group profile.

Conclusion: The present study has successfully genotyped ABO, Rhesus, Kell, Kidd and Duffy blood group loci in Banjar, Jawa, Mandailing and Kelantan Malays using the PCR-SSP technique. Findings from the present study support a common ancestry of Malay subethnic groups and other Austronesian populations. The present study also endorse the application of molecular approaches for blood grouping and highlight the important of supply blood that is match at multiple blood group systems to patients.

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METHOD DEVELOPMENT FOR FORENSIC ANALYSIS OF BODY ODOUR USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction: Body odour of an individual has unique composition due to the influence of specific combination of genetic, diet and environment on the production of the compounds responsible for giving this odour. The characteristic of human odour could be used as a human chemical fingerprint for forensic identification purposes. This research focused on developing an easy, fast and reliable method to collect, prepare and analyse the components of human body odour.

Objectives:

1. To evaluate different sample collection and preparation methods for the analysis of body odour.
2. To evaluate different GC-MS sampling systems such as static headspace GC-MS and liquid injection GC-MS for best suited method for analysis of body odour.
3. To evaluate the effect and also the difference in compounds extracted using different solvents used for analyte extraction from the collection material.
4. To conduct qualitative validation on the developed method.
5. To study intra and inter individual variations based on the compounds detected in the analysis of the body odour using appropriate statistical tools.
6. To determine primary odour components in an individual's body odour and evaluate its use in establishing body odour as a biometric marker.

Materials and Methods: The odour was collected from male subjects using a pre-treated gauze material. The subject was requested to exercise for 30 minutes prior to sampling by using the gauze to wipe over the right arm before sealing the gauze in a 5 mL eppendorf tube. Before sealing dihexyl ketone (internal standard), anhydrous sodium sulphate and also 1 mL methanol were added into the tube and left overnight at room temperature. The gas chromatography-mass spectrometer was used for the detection and identification of the components of human body odour. Two different injection systems; direct static headspace injection without pre-concentration and also the liquid injection system were compared for their suitability for this analysis. Sample preparation techniques for liquid injection by steam distillation followed by liquid-liquid extraction as well as direct extraction method using several different solvents were compared for the effectiveness to extract analyte from the collection material.

Results: This study demonstrated that direct extraction method using methanol as extraction solvent and analysis through liquid injection system via GC-MS were the optimum analysis method for body odour. Qualitative method validation was performed to evaluate the selectivity, specificity, sensitivity, linearity and stability of this method. The evaluated parameters validated the method fitness for its purpose. The analysis of the body odour revealed that it contains majority of organic acids with thirteen carbons and above, alkanes, alkenes, alcohol and others. Analysis of the compounds in the odour of the subjects by applying the developed method reveals that primary odour components can be used to identify an individual. In addition, the compounds present in the sweat of an individual are also useful to identify specific habit of a person, for example; smoking, medications consumed, and drug abuse.

Conclusion: Chemical fingerprint of an individual can be obtained by analysing the skin secretion of an individual. The information obtained from the secretion analysis of the human skin not only contains individualizing characteristics but also about their personal habits and environmental exposures which may be used to aid in the course of crime scene investigation.

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DUAL FORM OF MALNUTRITION IN THE GAZA STRIP–PALESTINE TERRITORIES: PREVALENCE, ASSOCIATED DETERMINANTS AND WOMEN'S KNOWLEDGE AND PERCEPTION OF NUTRITION

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Introduction: In the Gaza Strip, obesity increased among adults and underweight among preschool children were prevalent. The coexistence of these findings led to the hypothesis that obesity and underweight can cluster within a household to emerge dual form of malnutrition in the same household.

Objectives: The main purpose of this study was to understand the profiles of malnutrition among mothers and children aged 2–5 years then in so doing, to address the dual form of malnutrition (overweight mother/underweight child) in the same household in the Gaza Strip. More specifically, to investigate the associated factors with child underweight, maternal overweight pairs, and explore the mothers' nutrition perception and knowledge.

Materials and Methods: This study used quantitative and qualitative mixed methods design. Phase I, cross-sectional study represents a quantitative data set to assess dual form of malnutrition and its associated risk factors among mothers' childbearing aged 18–50 years, and children aged 2–5 years. Some 357 households from three different geographical locations in the Gaza Strip, namely, El Remal urban area, Jabalia refugee camp, and Al Qarara rural area participated in this study. Data were collected using structured questionnaire for face to face interviews. Height and weight for mothers and children were measured, International Physical Activity Questionnaire (IPAQ) was used to measure the physical activity pattern of mothers, 24-Hour recall was used to assess nutrients intake for mother-child pairs, and all 357 children and mothers voluntarily provided blood samples for biochemical tests. In phase II, focus groups discussions were performed to explore mothers' nutrition knowledge and perception. Twenty four of surveyed overweight or obese mothers were recruited from the three locations in the Gaza Strip.

Results: Results from phase I study showed that, about 59.7% of children aged 2–5 years were anemic, 24.4% were underweight Z-score<–1.0, whereas more than half of mothers 64.1% were overweight or obese. The prevalence of dual form of malnutrition was 15.7%. Child birth order had significant association with dual form of malnutrition (OR_{adj}, 1.50, 95% CL (1.22, 1.82); P=<0.001). Household with dual form of malnutrition increased as father's educational decreased (medium or low level) (OR_{adj}, 3.19, 95% CL (1.07, 9.5);

P=0.036), or (OR_{adj}, 3.40, 95% CL (1.12, 10.37); *P*=0.031), respectively. In addition, child with poor appetite was more likely to be underweight and also significantly associated with dual form of malnutrition (OR_{adj}, 6.9, 95% CL (2.35, 20.24); *P*<0.001). Dual form of malnutrition increased among mothers with high nutrition knowledge level (OR_{adj}, 1.23, 95% CL (1.0, 1.52); *P*=0.048). Dual form of malnutrition decreased in households with low monthly income (OR_{adj}, 0.28, 95% CL (0.09, 0.88); *P*=0.030).With regard to nutrient intake, mother's fat intake contributing to obesity was associated with the dual form of malnutrition (OR_{adj}, 1.01, 95% CL (1.0, 1.02); *P*=0.016). In part II, results supported the results of part I, that Palestinian mothers had good nutrition knowledge, but poor nutrition attitude and practice. Knowledge didn't convey to healthy practice, therefore, nutrition knowledge and the negative attitudes of mothers contributed in increasing malnutrition among individuals at household level. Cultural factors and mothers' poor perception have a powerful influence on feeding practices and eating patterns.

Conclusion: This research broadens the understanding of the correlating factors of familial coexistence of underweight children and overweight mothers. In this study, child undernutrition still exists, with increasing levels of maternal obesity as well as the dual burden of malnutrition. Nutrition intervention programs must recognise the coexistence of both extremes of malnutrition at household level, and incorporate this into their targeting strategies in order to manage the dual burden of malnutrition effectively. These new insights suggest programs specifically designed for resource-poor settings to promote healthy eating habits and regular physical activity that prevent both child undernutrition and the adult obesity.

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KNOWLEDGE, PERCEPTION AND EXPERIENCES OF PRIMIPARA MOTHERS ON EARLY BREAST FEEDING INITIATION IN UNIVERSITI KEBANGSAAN MALAYSIA MEDICAL CENTRE

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Introduction: World Health Organization and the United Nations Children's Fund recommend that all infants should be exclusively early breastfed immediately within one hour post-delivery. The decision and practices to early breastfeeding initiation are influenced by many contributing factors. It is well recognized that poor knowledge and practice of early breastfeeding is pronounced among mothers.

Objectives: This research was conducted to assess the predictive factors of knowledge, perceptions, practices and experiences of primipara mothers as well as its related factors towards early breastfeeding initiation (BFI) in Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

Methods: A cross-sectional study was conducted and two hundred and fifteen primiparas (n=215) were recruited via purposive sampling at the postnatal wards, UKMMC. Data was collected using a self-administered questionnaires adapted from the Newborn Feeding Ability and Breastfeeding Initiation Practices. The questionnaires included socio-demographic data, knowledge, perceptions, practices and experiences of mothers towards early BFI.

Results: Half of the respondents (46.5%) had higher knowledge of early initiation of breastfeeding, however the majority of respondents (52.1%) revealed gaining insufficient support from the midwives to assist them in initiating early breastfeeding. There was a significant association between higher education and level of knowledge on early BFI (*P*=0.001). Additionally, there is a significant association between higher income and level of perceptions of early BFI (*P*=0. 015).

Conclusion: It can be concluded that the mothers are still lacking in knowledge of early BFI while intentions regarding breastfeeding their infants is poor. Hence, the BFI training program for both mothers and midwives is needed. This study suggests that systematic assessment of knowledge and practice of ten steps successful breastfeeding among midwives in UKMMC should be established to evaluate their competency in supporting mothers to breastfeed infants.

Supervisor:
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NURSING EDUCATIONAL INTERVENTION TOWARDS THE REDUCTION OF WORK-RELATED LOW BACK PAIN AND ENHANCEMENT OF BODY MECHANICS SKILLS IN THE NURSING PROFESSION

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Introduction: Nurses experience the highest incidence of work-related back injuries, a serious and costly problem that affects their profession. In Malaysia, interventional studies regarding work-related lower back pain (LBP) among nurses are limited. Thus, facts related to this problem are only inferred from regional and international studies.

Objectives: This study attempts to assess the effectiveness of an educational program in reducing work-related LBP, improving nurses' quality of life and nursing skills in using proper and correct body mechanics among Malaysian nurses working at the Hospital Universiti Sains Malaysia (Hospital USM).

Materials and Methods: Pre-test and post-test intervention based on the LBP education and clinical training were selected as method of intervention. Cross-sectional study was selected in the first part of this study using a stratified

random sample of nurses selected from all wards in Hospital USM. The sample size was 300 nurses with 100% response rate. Randomized control trial study was selected for second part with sample of 35 in each control and intervention group.

Results: The study showed that the prevalence of work-related LBP was 51%. Mean score of pain severity in the first part of the study was 2.47. Multiple logistic regression analysis show that three factors that contributed to the occurrence of work-related LBP which are nurses who assume poor body posture at work, who do not have work organization and who perceive poor health status. These groups of nurses increased odds of having work-related LBP at 243.571, 32.058, and 0.066 times respectively. Fifteen of 31 nurses from intervention group did not experience LBP after the educational module, whereas 32 of 35 nurses from the control group experienced LBP (P=0.008). One-way repeated-measures ANOVA showed a significant decreased in LBP severity (P<0.001), time experienced of LBP (P<0.001) in the intervention group after the interventional module. Also, a significant decrease in pain duration was found between control and intervention group after interventional module. A significant improvement in various physical and psychological factors was found between the control and intervention group after the intervention.

Conclusion: This study promoted the effectiveness of educational program module with clinical training into basic nursing education and health care practices in reducing the incidence of work-related LBP among nurses.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODY AGAINST ANTIGEN-85 COMPLEX (AG85) FROM MYCOBACTERIUM TUBERCULOSIS BY ANTIBODY PHAGE DISPLAY

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Introduction: Tuberculosis (TB) is as a major public health problem worldwide. Early diagnosis, prophylaxis and effective treatment regimen are the main strategies used to control TB but the challenge ahead is still daunting, especially in poor developing countries. Hence, development of immunology-based diagnostic tests that are rapid, cheap and equipment-free are important in low-income countries. Antigen detection test requires production of monoclonal antibody against specific Mtb antigen such as Ag85 complex, which is the most abundantly secreted antigen of *Mycobacterium tuberculosis* (Mtb).

Objectives: This study aimed to produce and characterize a phage monoclonal antibody against Mtb Ag85 complex.

Materials and Methods: A human domain phage antibody library was screened to select a monoclonal antibody which binds specifically to the targeted Ag85 complex.

Results: B10 phage display monoclonal antibody clone, which produced reacted against Mtb Ag85 complex and showed negative reaction against the different blocking solutions used during the panning processes. B10 clone was then characterized by DNA sequencing; bioinformatics analysis revealed that the DNA sequence was compatible with a variable (V) domain of a human immunoglobulin (Ig) heavy chain (H).

Conclusion: This study has successfully produced and characterized a phage display monoclonal antibody against Mtb Ag85 complex. The potential of this monoclonal antibody for the development of diagnostic method for TB should be further explored.

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