Abstract

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

PRODUCTION OF TRUNCATED HUMAN BROTHER OF THE REGULATOR OF IMPRINTED SITES (BORIS) IN *ESCHERICHIA COLI* AND IN SILICO ANALYSIS OF BORIS PROTEIN

Maisarah binti Ab. Samad
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Brother of the regulator of the imprinted site (BORIS) is paralog of multifunctional CCCTC-binding transcription factor (CTCF) that exclusively expressed in male germline cells. BORIS protein consists of three domains, N-terminal (N), 11 zinc fingers (ZF) and C-terminal (C) domain. BORIS ZF domain is almost similar with CTCF ZF but the end terminals are divergent. BORIS regulates some crucial germline genes and involves in epigenetic reprogramming. Aberrant expression of BORIS in somatic cells correlates with tumourigenesis, cancer progression and growth retardation. BORIS has narrow genomic landscape and few functional interactions with other proteins.

Objectives: This study aims to produce a set of truncated BORIS proteins using bacterial expression system and to analyse the protein through in silico approaches.

Materials and Methods: In this study, truncated BORIS gene fragments termed as BORIS-N, BORIS-ZF and BORIS-C were amplified via polymerase chain reaction (PCR), then cloned into bacterial expression vector pET16b-SH3(Cys) and chemica lly transformed into *Escherichia coli* (*E. coli*) BL21(DE3)pLysS for protein expressions. The denatured proteins were probed by Western blot using anti-Histidine tag mousemonoclonal antibody.

Results: Western blots showed BORIS-N and BORIS-C proteins aberrantly migrated at 48 and 19 kDa, instead of their theoretical molecular weight of 28.61 and 10.21 kDa, BORIS-ZF protein failed to express and was predicted to be correlated with codon bias usage, protein misfolding and toxicity. The physiochemica l study and in silico analysis were conducted to BORIS protein sequence using Database xxi of Disordered Protein Predictions (D2P2), Predictor of Naturally Disordered Regions (PONDR® FIT and PONDR® VLXT), and DISOPRED3. The intrinsically disordered regions (IDRs) in BORIS were determined, predominantly at N-terminal domain. Computational analysis was performed to predict protein interaction sites at BORIS protein through IDRs functional features. Molecular recognition features (MoRFs) were determined by MoRFpred and DISOPRED, while Eukaryotic Linear Motif (ELM) resource predicted the short linear motifs (SLiMs). Two similar MoRFs were predicted by the two predictors and intersected with LIG_SUMO_SIM_ anti_2 and DOC_MAPK_1, respectively. SUMOylation was predicted to occur in BORIS at SUMO interacting motif. BORIS was also predicted to interact and being regulated by Mitogen-activated protein kinases (MAPK). A hypothesis was constructed to associate the interaction with dysregulation of transforming growth factor beta (TGFB) signalling pathway.

Conclusion: In conclusion, N- and C- termini proteins were successfully expressed except ZF protein. In silico analysis via IDRs was able to drive the discovery of potential interacting partners and functional roles of BORIS.

Supervisor:
Professor Dr Shaharum Shamsuddin

EFFECT OF TUALANG HONEY AGAINST CANDIDA ALBICANS GROWTH AND BIOFILM FORMATION

Norazuroh binti Mohd. Nor
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: *Candida albicans* can form biofilms in vivo and in vitro and is the most common fungal pathogen associated with fungal-biofilm related infections especially in hospital settings.

Objectives: This study was performed to evaluate in vitro inhibitory activity of Tualang honey on pre-formed and established biofilm formation of *C. albicans*.

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 effect of Tualang honey on pre-formed and established biofilm formation of *C. albicans*.

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 formation of pre-formed biofilms and by reducing the size of 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. Tualang honey affects biofilms by slowing the formation of pre-formed

biofilms and by reducing the size of the formed biofilms by disruption of their structure.

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 biofilm formation and disrupt established biofilm.

Supervisor:
Dr Noor Izani Noor Jamil

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

Nur Farah Hani binti Azemi
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

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 *ck*α and *ck*β 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. Computational prediction showed that ckα CpG islands in its promoter are methylated. However, the regulation of *ck*α gene by DNA methylation has neverbeen investigated. DNA methylation level inside the cells can be manipulated by treatments with epigenetic drugs.

Objectives: In this work, preliminary study towards understanding the effect of DNA methylation on ckα expression is carried out. The effects of 5-Azacytidine (demethylating agent) and Budesonide (methylating agent) on HeLa cell growth and DNA methylation level in selected *ck*α promoter CpG island were investigated.

Materials and Methods: MethPrimer and DBCAT programs predicted several CpG islands in the *ck*α promoter that might be the target of DNA methylation. Two epigenetics drugs were used in this study and they were the 5-Azacytidine (5-AzaC) and Budesonide.

Results: MethPrimer and DBCAT programs predicted several CpG islands in the *ck*α 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. Two methods were used to probe the methylation status of a selected *ck*α CpG island. The 216 bp CpG island of interest was selected based on a previous study that showed its importance in the regulation of *ck*α 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 *ck*α CpG island. The second method was restriction enzyme based 5-methylcytosine and 5-hydroxymethylcytosine analysis, which showed that the *ck*α 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 *ck*α 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 *ck*α CpG island.

Supervisor:
Associate Professor Dr See Too Wei Cun

Co-supervisor:
Associate Professor Dr Few Ling Ling

EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THREE TYPES OF MUSA SP. PEEL EXTRACTS

Muhammad Faiz bin Muhammad
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Various of herbal medicines have been screened for their therapeutic potential and their pharmacological properties like the evaluation of antioxidant and antimicrobial effects. One of the plants that has the great potential in promoting these activities is Musa sp. plant, focusing on its peels.

Objectives: Therefore, this study is aimed to evaluate the effect of three types of *Musa sp*. peel extracts by using different solvents.Its antioxidant and antibacterial activities were also studied.

Materials and Methods: *Musa brachycarpa*, *Musa paradisiaca* var. awak and *Musa acuminate* (Cavendish subgroup) species were used in this study. *Musa sp*. peels were bought and collected from central market. The peels were dried and grounded into a fine powder. The extraction was carried out by using successive extraction method. Petroleum ether, methanol and aqueous solvents were used in extraction process to produce *Musa* sp. extractive yields.

Results: In terms of yield percentage, methanol extract has the highest mean of yield percentage (13.09%), followed by petroleum ether (12.04%) and aqueous solution (9.77%). The antioxidant activity of all the three type of *Musa* sp. peel extracts by different solvents was assayed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The aqueous extract of *Musa brachycarpa* showed the highest percentage of inhibition at 91.87% followed by methanol extract at 90.27%. On the other hand, petroleum ether extract has relatively low percentage of inhibition for each of *Musa* sp. peel extracts. The antibacterial

screening was performed by using disc diffusion method. At 20 mg/disc concentration, the methanol extracts have successfully exhibited zone of inhibition to Gram-negative bacteria, *P. mirabilis* and Gram-positive bacteria, *S. aureus* and *S. epidermidis*. MIC and MBC are parts of the various in-vitro microbiological techniques to determine the bactericidal activity of antibacterial agents. The methanol extract of *Musa bracycarpa* and *Musa paradisiaca* var. awak are considered bactericidal against several tested Gram-negative and Gram-positive bacteria with good MIC/MBC ratio ($n < 4$).

Conclusion: Hence, the impact of these antioxidant and antibacterial activities of *Musa sp*. peel extract can be useful in commercializing health products in treating or maintaining some skin issue problems especially aging and acne. However, further research need to be done for validating the efficiency and safety of the peel extracts for people usage.

Supervisor:
Dr Mohd Dasuki bin Sul'ain

Co-supervisor:
Dr Wan Nor Amilah binti Abd Wahab

STUDY OF DENDROPHTHOE PENTANDRA ETHYL ACETATE EXTRACT AS POTENTIAL ANTICANCER CANDIDATES ON SAFETY AND TOXICITY ASPECTS

Lau Shin Yee
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Cancer is often associated with increased risk of death and toxic side effects whereas a lot of cancer patients seek alternative treatments using traditional medicine. *Dendrophthoe pentandra* (DP) is a Malaysian edible plant with traditional claims of medicinal properties such as anticancer.

Objectives: The objective of the research was to investigate the safety and toxicity of DP by evaluating the phytochemicals constituents, heavy metal content, chemical compounds present in DP ethyl acetate extract.

Materials and Methods: The cytotoxicity against brine shrimp and confirmation of toxicity by using selected cell lines (HeLa, L929, Glioma, MD-AMB 231, Hep G2 and MCF-7) were conducted in search of LC50 and IC50 of the plant extract. The crude extracts were studied for qualitative and quantitative analysis of phytochemical compounds. Elemental composition of DP studied using Atomic Absorption Spectrometer, on the other hand, chemical composition of DP was studied by Gas Chromatography-Mass Spectrometry (GC-MS). Plant toxicity were studied using brine shrimp lethality assay using the stock solution of 2000 ppm to prepare working extract solution with 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.2 ppm, 15.6 ppm and 7.8 ppm concentration. Cytotoxicity screening using MTT assay was performed by measuring cell viability on cell lines with treatment of plant extract.

Results: The results showed that DPEA consists of wide range of bioactive compounds indicating the presence of tannins, saponins, flavonoids and alkaloids. The crude extract contained 1.33% of alkaloid, 2.67% of flavonoid, and total phenolic content of 14.87±0.2 µg/g using tannic acid as equivalent. The DPEA extract consist of copper, zinc, manganese which were below the maximum permissible level by World Health Organization guidelines. Using GC-MS, out of 39 compounds detected decanoic acid, palmitic acid, linolenic acid and beta-Sitosterol was suggested to contribute to the medicinal use of DP. The LC50 was undetermined and is predicted to be more than 1000 ppm. DPEA shows antiproliferative activity on MCF-7 of IC50 4.72±0.52 µg/mL and L929 of IC50 18.12±3.46 µg/mL.

Conclusion: In conclusion, the result of the present study revealed that DP plant is relatively safe and can be considered as possible candidates with promising potency to be developed as new chemotherapeutic agent. The wide bioactive compounds in DP perhaps contribute to the traditional usage of DP. Further scientific validation need to be performed to understand more on the pure active compounds and mechanism of DP causes anticancer activity.

Supervisor:
Dr Mohd Dasuki Sul'ain

Co-supervisor:
Dr Wan Ezumi binti Mohd Puad @ Mohd Fuad

SURVEILLANCE OF DENGUE VIRUSES IN AEDES MOSQUITOES IN KOTA BHARU AND BACHOK, KELANTAN

Mohd. Nadzri bin Abu Yazid
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Dengue virus (DENV) infection is a major public health concern with different clinical presentations and often with unpredictable clinical outcomes. The disease is transmitted by the vectors Aedes mosquitoes particularly the *Aedes aegypti* which are commonly found in urban areas and *Aedes albopictus* which are more abundant in rural areas. In year 2014, more than 14,000 dengue cases were reported in Kelantan with 76.6% (10,961 cases) of them occurred in Kota Bharu. A surveillance study on Aedes mosquitoes and dengue virus in these mosquitoes was conducted in two different districts, Kota Bahru and Bachok. In contrast to Bachok, Kota Bharu is an urban area with high cases of dengue annually and was badly affected with a big flood at the end of 2014.

Objectives: This study was initiated to determine the distribution and association of the mosquito vectors in urban and rural areas and to detect dengue viruses in these vectors.

Materials and Methods: Ovitraps were placed at 400 sampling points in selected areas in both districts.

Results: A total of 2,338 *Aedes* mosquitoes were collected from Kota Bharu where 455 (19.5%) were *Ae. aegypti* and 1,883 (80.5%) were *Ae. Albopictus*. From Bachok area,

2,760 Aedes mosquitoes were collected where 121 (4.4%) were *Ae. aegypti* and 2,639 (95.6%) are *Ae. albopictuc*. There is an association between distribution of Aedes mosquitoes and human settlement ($P < 0.05$). *Ae. aegypti* mosquitoes were predominant in the urban area while in contrast, *Ae. albopictus* mosquitoes were more prevalent in the rural area. Ten to 30 Aedes mosquitoes were pooled and virus detections were performed using real time RT-PCR detection, NS1 antigen detection and cell culture.

Conclusion: Albeit the use of various dengue detection methods, no DENV was detectable in any of the tested mosquitoes pools.

Supervisor:
Dr Rafidah Hanim binti Shueb

Co-supervisor:
Dr Nor Fazila binti Che Mat

THE ROLE OF TUMOR-ASSOCIATED MACROPHAGES IN LYMPHOVASCULAR INVASION OF BREAST CARCINOMA

Chong Choi Yen
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: 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 ($X2 = 6.193$, 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:
Dr Sabreena Safuan

DOWNREGULATION OF ONCOGENE PROTEINS IN HELA CELLS TREATED WITH *Oroxylum indicum* LEAVES EXTRACT BY THE ACTIVATION OF TUMOR SUPPRESSOR GENES

Nur Afina binti Mohd. Din
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: The investigation on natural products in treating different types of cancer was widely demonstrated. *Oroxylum indicum* is one of the potential candidates against cervical cancer. It derives from Bignoniaceae family that can be found in most Asian countries and widely used for over thousands of years in Ayurvedic medicine. In Malaysia, it is an edible plant known as "beko". Cervical cancer was reported to be the second most diagnosed cancer in Malaysia. It is caused by Human papillomavirus from the family of *Papillomaviridae* and the development of cervical cancer is highly associated with E6 and E7. The antiproliferative activity of *O. indicum* leaves extract against cervical cancer cell lines, HeLa, was reported in the previous study.

Objectives: In this study, the potential of *O. indicum* in regulating the expression of oncogene proteins (E6 & E7) and tumor suppressor gene (p53 & pRb) on cervical cancer cells is investigated in order to understand its underlying mechanisms.

Materials and Methods: Western blot analysis was used to study the protein expression level and reverse transcriptase polymerase chain reaction was used to study the mRNA expression level.

Results: *O. indicum* was shown to possess high antiproliferative activity on HeLa cells with IC50 of ±6.15 µg/ml. The effect of *O. indicum* on E6 and E7 proteins expression was obtained from Western blot analysis and their protein levels were significantly lower compared to untreated cells. From reverse-transcriptase polymerase chain reaction (RT-PCR), the upregulation of p53 and pRb gene expression was observed in *O. indicum* treated HeLa cells.

Conclusion: In conclusion, *O. indicum* treatment demonstrated the activation of tumor suppressor genes and downregulation of oncogene proteins expression.

Supervisor:
Dr Nor Fazila Che Mat

Co-supervisor:
Dr Noor Izani Noor Jamil

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

Hazwani binti Mohd. Yusof
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

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.

Objectives: The aim 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: 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. The formulation has demonstrated ability to reduce cervical cancer cells viability without any adverse effect observed on the lower reproductive tract in rat model. 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 analysed by DPPH radical scavenging system.

Results: From the result, antiproliferative activity of QIA extract and QI vaginal cream against HeLa cell lines were greater with IC50 value 13.90 ± 2.27, and 20.80 ± 1.94. For expression of HPV E6 and E7, QI vagina cream was able to suppress the expression of both proteins after 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: In conclusion, formulation of QI vaginal cream was able to eliminate cervical cancer cells in vitro without any adverse effect as observed in vivo.

Supervisor:
Associate Professor Dr Hasmah Abdullah

Co-supervisor:
Dr Wan Amir Nizam Wan Ahmad

ISOLATION AND IDENTIFICATION OF MONOCLONAL ANTIBODIES AGAINST *Entamoeba histolytica* ACETYL-CoA SYNTHETASE USING PHAGE DISPLAY ANTIBODY TECHNOLOGY

Foong Tong Ling
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Early diagnosis of amoebic liver abscess (ALA) is crucial as it can potentially lead to fatal outcome. Triad evidences of clinical presentation, radiological imaging and antibody detection tests are routinely used for diagnosis of ALA but they cannot differentiate ALA from other causes of liver abscess. Stool examination and stool antigen detection tests are not useful because most patients with ALA do not have detectable parasites in their stool. Serological methods are normally used to detect antibody or antigen in the serum sample. However, detection of the anti-ameobic antibody cannot distinguish past and current infections as these antibodies can persist for years. Thus, antigen detection by using monoclonal antibody is preferred as it can be used to detect a specific antigen, in which the presence of the antigen in the biological sample is associated with current infection. *E. histolytica* Acetyl-CoA synthetase (EhACS) has recently been reported to be a potential biomarker for detection of acute ALA. EhACS appeared in hamster serum as early as 12-hours post-infection with *E. histolytica*.

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: In this study, phage display technology was deployed to isolate monoclonal antibody by screening a human naïve domain antibody (dAb) library against recombinant EhACS (rEhACS) by bio-panning process. Three rounds of bio-panning from domain antibody phage library against electro-eluted rEhACS were then carried out. Polyclonal phage ELISA of three rounds panning was performed and the results showed increased in signals from the first panning to the third panning. This indicated there was an enrichment of phage particles specific to rEhACS. Monoclonal phage ELISA was then used to screen several potential clones specifically against electro-eluted rEhACS and a panel of non-specific antigens. Sequencing analysis revealed two positive clones, D1 and A5

which were compatible with immunoglobulin variable heavy (VH) chain of the monoclonal antibodies.

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.

Supervisor:
Associate Professor Dr Lim Boon Huat

Co-supervisors:
Professor Dr Armando Acosta Domunguez
Professor Dr Mari Elena Sarmiento Garcia San Miguel

ANTI-PROLIFERATIVE ACTIVITY OF METHANOL EXTRACT OF *Oroxylum indicum* LEAVES ON HELA CELLS VIA INDUCTION OF APOPTOSIS

Nurul Hidayah binti Wahab
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Cervical cancer is the second most common cancer death among women worldwide. Even though current available therapies can increase the survival rate of cancer patients, they still can induce major and long-lasting side effects. Therefore, the utilization of natural products as an anticancer agent provides an alternative. In this regards, leaves of *Oroxylum indicum* (Beko) was chosen as a lot of evidences have shown the anti-proliferative properties of this leaves against a wide range of cancerous cell lines. *O. indicum* have been shown to inhibit the proliferation of cancer cells by acting as apoptosis-inducer, however the mechanism of action was not well explained.

Objectives: This study was done to evaluate the effect of methanol extract of *O. indicum* treatment on human cervical cell lines, HeLa, in term of anti-proliferative properties and the related underlying mechanism.

Materials and Methods: Methylene blue assay (MBA) was used as an anti-proliferative assay.

Results: As a result, the proliferation of HeLa cells was inhibited by methanolic extract of O. indicum at IC50 6.25 ± 2.90 µg/ml. Cisplatin, a widely used anticancer drug, was used as positive control and higher IC50 was obtained from cisplatin treatment (9.37 ± 2.95 µg/ml). As this plant was shown to induce cell death in cancer cells via apoptosis, the expression of several apoptosis signalling molecule were accessed after 24 hours of treatment. Untreated HeLa cells were used as control. From the results obtained, this extract was found to induce apoptosis through activation of Fas-mediated apoptosis pathway by up-regulating the expression of Fas and its ligand, FasL at mRNA level. The up-regulation of both apoptosis molecules eventually promoted the activation of caspase-8, thus, facilitated the execution of apoptosis through caspase-3 activation.

Conclusion: As a conclusion, this present study has proved the potential of methanol extract of *O. indicum* to inhibit the proliferation of HeLa cells and act as apoptosis-inducer by promoting Fas-mediated apoptosis mechanism.

Supervisor:
Dr Nor Fazila Che Mat

Co-supervisor:
Dr Noor Izani Noor Jamil

ISOLATION AND IDENTIFICATION OF A MONOCLONAL ANTIBODY AGAINST *Mycobacterium tuberculosis* ANTIGEN 85 COMPLEX (Ag85) USING ANTIBODY PHAGE DISPLAY

Siti Amalina Bt. Mohd Rozani
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Tuberculosis (TB) has been recognized as a major public health problem and it is present in every part of the world. Prophylaxis, early diagnosis and treatment are the main elements associated with TB control but until now it still remains as a major world health challenge. The development of diagnostic immunologic based tests that can be performed fast, cheap and without the need of laboratory equipment could be suitable for low-income countries where the majority of TB cases are. The possibility to have a specific monoclonal antibody against *Mycobacterium tuberculosis* (Mtb) antigens is an important element for the development of immune based diagnostics. Ag85 complex is the most abundant secreted antigen of Mtb and could be a potential target for diagnostic approach.

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.

Supervisor:
Associate Professor Dr Lim Boon Huat

Co-supervisors:
Professor Dr Armando Acosta Dominguez
Professor Dr Maria Elena Sarmiento Garcia San Miguel

ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF BANANA PULP EXTRACT AGAINST VIBRIO CHOLERAE

Norfaradhiah binti Ruslan
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Diarrheal disease is one of the top leading causes of death in children under five years old. Approximately, more than 700 000 children died due to this disease every year. *V. cholerae* is an important agent of diarrheal disease especially in the developing countries. Oral rehydration therapy and antibiotics are the most common treatments to reduce the severity and lessen the duration of *V. cholerae* infection. However, the emergence of antimicrobial resistance cases in many parts of the world has encouraged the search of new and effective antimicrobial compounds to overcome this issue, especially from natural products such as plants. Banana (*Musa spp.*) has been claimed to have anti-diarrheal activity against several microorganisms, including enteric pathogens. However, the study on the antibacterial and anti-inflammatory activities of banana flesh (pulp) against *V. cholerae* is still unclear.

Objectives: This study was conducted to determine the antibacterial and anti-inflammatory activities of different banana pulp extracts against *V. cholerae*.

Materials and Methods: The antibacterial activity of water and methanol extracts of banana pulps from two different species; Pisang Perak (*Musa acuminata* AA) and Pisang Gala (*Musa balbisiana* BB) against *V. cholerae* was evaluated using agar well and disc diffusion methods, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test. The effects of the extracts on the morphology, behavior and protein profile of the bacteria were also investigated using Gram staining and SDS-PAGE. The anti-inflammatory activity of the extracts was determined by measuring the production of nitric oxide (NO) using ELISA method.

Results: Overall, the results showed that the banana pulp extracts have inhibitory effects against *V. cholerae* in both MIC and MBC tests but not in disc diffusion assay and well diffusion assay. The extracts also influenced the morphology and behavioral pattern of the bacteria, as well as changed the bacterial protein profile. Both water and methanolic extracts of the banana pulp have a modulatory effect on the production of NO by the U-937 monocytic cells infected with *V. cholerae* at different concentrations, indicating the presence of anti- and pro-inflammatory characteristics of the extracts.

Conclusion: In conclusion, the water and methanolic extracts of banana pulps of Pisang Perak and Pisang Gala are potential to be used as indirect antibacterial and anti-inflammatory agents against *V. cholerae* in the future. However, further studies need to be carried out to support this finding.

Supervisor:
Associate Professor Dr Rapeah Suppian

PRELIMINARY TOXICITY EVALUATION OF *Quercus infectoria* GALLS AQUEOUS EXTRACT ON FERTILITY AND EMBRYONIC DEVELOPMENT IN FEMALE SPRAGUE DAWLEY RATS

Nurul Ajilah binti Mohamed Khir
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

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.

Supervisor:
Dr Wan Ezumi Mohd Puad@ Mohd Fuad

Co-supervisor:
Associate Professor Dr Hasmah Abdullah

EVALUATION OF DNA METHYLATION EFFECT ON CpG-ISLAND CONTAINING PROMOTER OF CHOLINE KINASE BETA

Nor Shaheera Mohamad Kamal
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

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 $Mg2+$ 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

Co-supervisor:
Dr Noor Fatmawati Mokhtar

INVESTIGATION OF NEURAL STEM CELL MIGRATORY CAPACITY TOWARDS GLIOMA CELLS (IN VITRO) WITH *Quercus Infectoria* METHANOL EXTRACT

Lohis a/p Balachandran
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Glioma arises from glial cells and it is a type of brain tumour with high prevalence and poor rognosis. Current glioma treatments are combination of surgery, radiotherapy and chemotherapy, however they are able to increase survival rate of glioma patients at a low percentage. Chemotherapy able to kill cancer cells but it also causes damage to healthy cells because it is administered systemically into patient's body. However, targeted anti-cancer therapy aims to destroy cancer cells directly without damaging the neighbouring healthy cells.

Objectives: The aim of this research project was to use neural stem cells as a 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* was extracted using soxhlet technique with 100% and 70% methanol solvent. Optimum half maximal inhibitory concentration (IC50) 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 (IC50:25.27 ± 7.95 µg/mL) and *Q. infectoria* 70% methanol extract (IC50:32.91 ± 2.23 µg/mL) showed anti-proliferative properties against DBTRG-05MG, along with tamoxifen (IC50:19.40 ± 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, we postulated that the neural stem cells (NSCs) 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. Moreover, this study could serve as a platform for targeted anti-cancer therapy to treat glioma using NSCs.

Supervisor:
Dr Tan Suat Cheng

Co-supervisor:
Associate Professor Dr Hasmah Abdullah

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

Nurul Syahida binti Ramli
MSc (Biomedicine)

School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Introduction: Roselle (*Hibiscus sabdariffa* L.) is known to have a rich source of anthocyanin. Anthocyanin 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 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 extract treatment groups; aqueous, aqueous + 1% TFA and ethanol + 1% TFA (administered via oral-gavage for 3 months at dosage of 150 mg/kg). One group was normal rat 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 was also carried out. 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.

Supervisor:
Dr Wan Amir Nizam Wan Ahmad

Co-supervisors:
Dr Sabreena Safuan
Dr Liza Noordin