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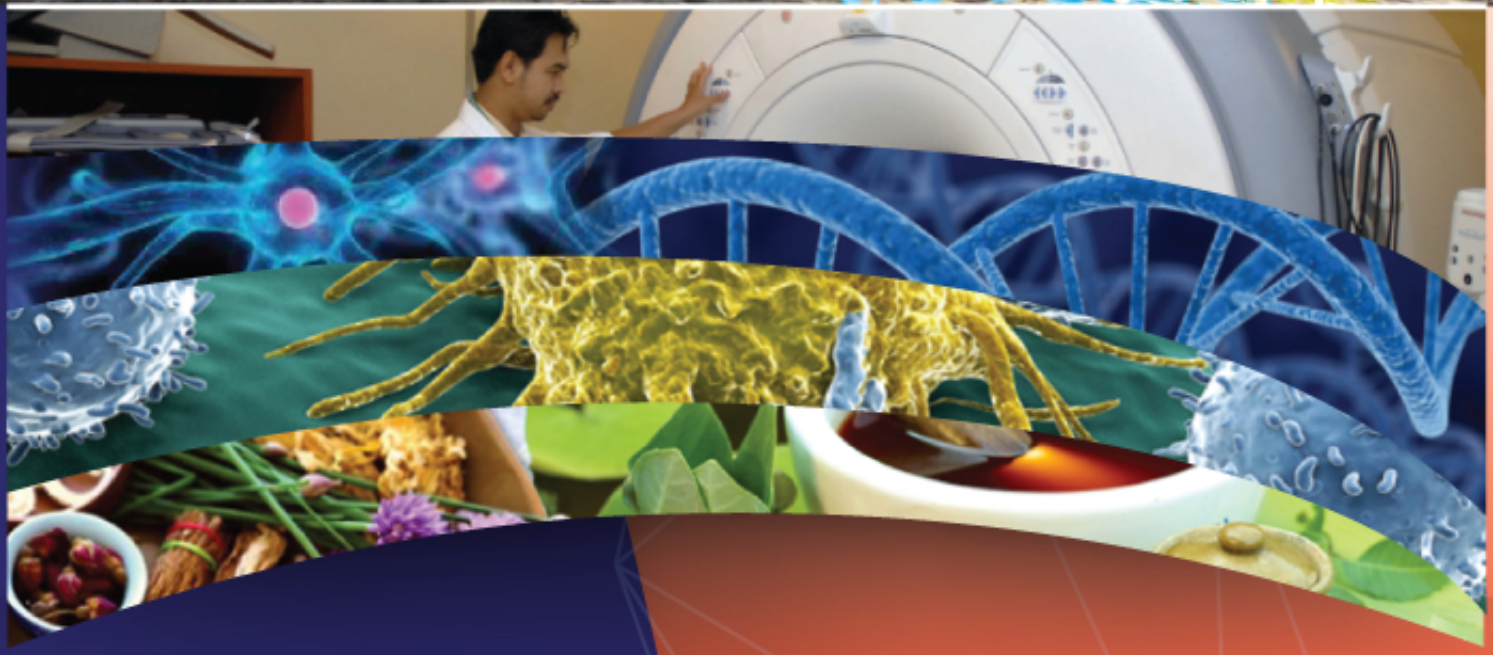

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Water-Soluble Chitosan Nanoparticles from Acylation of Short Chain Hydrophobic Alkyl Group

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Purpose: Chitosan has attained intense interest in drug delivery due to its biocompatibility, biodegradability and low toxicity nature of the chitosan. However, the poor solubility of the chitosan in water has restricted its application in physiological environment. Herein we modified the chitosan into water-soluble chitosan nanoparticles via depolymerization and acylation with short chain of alkyl group. The modified chitosan was evaluated for its structural and thermal analysis. **Methods**: The commercially obtained chitosan was first chemically depolymerized into 25 kDa molecular weight and subsequently acylated with different mole ratio of butyric anhydride (C4) to amino group of chitosan with the presence of methanol. The modification of the chitosan was characterized by fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR). Thermal properties of the chitosan were studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Transmission electron microscopy (TEM) and field emission scanning electron microscopy (FESEM) were used to observe the morphology of the particles. **Result**: The acylation was confirmed by increase of degree of acylation in FT-IR spectrum and existence of new peak of alkyl group in NMR spectrum. The endothermic peak of the acylated chitosan was shifted to lower temperature with the decrease in heat enthalpy in DSC curve as more butyric anhydride is added. Similarly, the acylated chitosan revealed significant weight loss of 11% compared to water-soluble chitosan in TGA analysis. Imaging from TEM and FESEM showed the formation of spherical chitosan nanoparticles. **Conclusion**: Water-soluble chitosan nanoparticles have been prepared successfully through depolymerization and acylation and the size was confirmed with TEM.

Keywords: **Chitosan nanoparticle, water-soluble, acylation**

Breast Cancer Patients Taking Anastrozole for More Than One Year Exhibit Higher Risk of Developing Anastrozole-Related Mood Disturbance

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Purpose: Although anastrozole (Anas) plays a key role in the management of endocrine sensitive post-menopausal (PM) breast cancer (BC), there is variability in adverse reactions such as mood disturbance and dizziness associated with its use. Theses adverse reactions although less frequent can affect the quality of life of BC patients. The aim of this study was to determine the clinical and demographic factors associated with these adverse events. **Methods**: This is a cross-sectional study of estrogen receptor (ER) positive PM women (n = 97) with stages I to III BC receiving Anas. Multivariate analyses were performed to investigate the factors associated with Anas-induced mood disturbance and dizziness. **Results**: Approximately, 20.6% and 13.4% of the subjects experienced mood disturbance and dizziness respectively. Patients with more than three years of Anas treatment had higher odds of having mood disturbance (adjusted odds ratio 20.31, confidence interval 1.75 to 235.31, p = 0.016). No significant association was established between serum estrogen levels and development of mood disturbance and dizziness. **Conclusions**: The study confirmed that mood disturbances and dizziness are not the most commonly reported adverse reactions among ER+ PM BC women receiving Anas and duration of Anas treatment may be an important predictor of mood disturbance in these patients.

Keywords: **Anastrozole, mood disturbance, dizziness**

Preliminary Study on The Effects of EphA2 Inhibition in Angiogenesis Signaling Pathways of Human Malignant Glioma Cells

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Purpose: Brain tumor "glioblastoma multiforme (GBM)" is assigned under fourth pathologic grades (grade IV) of astrocytic tumor. Current GBM treatments commonly aim to target the bulk of the tumor, and yet still less targeting on angiogenesis pathway which is crucial for tumor growth. EphA2 plays important role in regulating angiogenesis and known to be over expressed in GBM tumors. This study aims to investigate the roles of EphA2 in angiogenesis signaling pathway by discovering its relation to angiogenesis related markers; VEGF and VEGFR-2. **Methods**: Small interfering RNA (siRNA) targeting EphA2 with the appropriate transfection reagent was used to transfect U-87 glioma cells for gene silencing. Quantitative real time PCR was used to evaluate the expression difference before and after the inhibition. The similar method also been applied to observe the relation of EphA2 gene silencing to tumor angiogenesis related markers; VEGF and VEGF-R2. **Results**: Relative gene expression level of EpHA2 in siRNA-EphA2 treated group compared to untreated group showed successful knockdown of EphA2 gene expression by -0.357 of fold change, similar to VEGF that was found down regulated by -1.41 of fold change. In contrast, VEGFR-2 was up-regulated by +2.38 of fold change after EphA2 knockdown. **Conclusions**: Our preliminary results have shown that siRNA targeting EphA2 successfully inhibited its gene expression and subsequently down regulated VEGF gene expression. The findings demonstrated that EphA2 acts in concert with VEGF signaling pathway in promoting angiogenesis. Contrarily, VEGFR-2 expression was found up-regulated following EphA2 gene knockdown which could suggest that VEGFR2 and EphA signaling pathways play non-redundant roles in GBM angiogenesis.

Keywords: **EphA2, angiogenesis, glioblastoma multiforme (GBM), siRNA**

Complete Depletion of CpG Motifs from pDNA Affects Transcription

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Purpose: CpG-free plasmid (pZCpG) exhibited extended transgene expression in mouse lungs, with minimal inflammatory response. Recently, promising results were reported in cystic fibrosis gene therapy clinical trial using pZCpG. When extending pZCpG towards *ex vivo* approach, we observed limited transgene expression in cell lines. Our objective is to determine the basis for the pZCpG-depleted expression *in vitro*. **Methods**: Two novel plasmids (pDNA) of similar pZCpG backbone with variable CpG content of GFP transgene were constructed. These pDNAs were transfected into human cell lines (HEK-293FT, MCF7, H1299 & SH-SY5Y) using lipid- and polymer-based gene transfer agent separately and protein expression was analyzed up to 14 days. Evaluation of transfection efficiency and pDNA toxicity was conducted at Day 1 post-transfection. To determine if the difference in protein expression was at mRNA level, RNA distribution analysis was performed by evaluating nuclear and cytoplasmic mRNA at Day 1 post-transfection. **Result**: The pZGFP (0 CpG) exhibited lower expression than pRGFP (60 CpG) at all time points. At single cell level, the pZGFP showed lower mean fluorescence intensity than pRGFP. These differences were also observed despite using polymer-based agent. The low pZGFP expression was not due to low pDNA copy number, toxicity or mRNA transport rate as no significant differences were observed. However, GFP mRNA of pRGFP was higher than pZGFP. **Conclusion**: Complete depletion of CpG motifs from pDNA resulted in the low expression in vitro and this may be due to the discrepancies at the transcriptional level. The influence of DNA methylation is currently being assessed.

Keywords: CpG motif, gene therapy, epigenetics

SKP2: An Emerging Proto-Oncogene in Acute Myeloid Leukaemia t (8;21)

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Purpose: S-phase protein kinase 2 (SKP2) is a well-known proto-oncogene in a myriad of cancers and leukaemia. This includes lymphoma, melanoma, nasopharyngeal cancer, prostate cancer and breast cancer. Nevertheless, its role as a proto-oncogene in acute myeloid leukaemia is currently limited. Therefore, this study was carried out to investigate the role of SKP2 as an oncogene in AML t(8;21). **Method:** In this study, an electroporation based siRNA mediated gene knockdown approach was used to down regulate *SKP2* in a AML t(8;21) positive cell line, Kasumi-1. Electroporation was carried out every four days. **Results:** Prolonged gene knockdown experiments on Kasumi-1 cells with si*SKP2* (siRNA directed against *SKP2*) at a concentration of 100nm showed a 66% reduction in *SKP2* transcript levels ten days after the knockdown. SKP2 protein levels exhibited a 70% reduction as well. SKP2 suppression had dire consequences on the propagation of AML t(8;21) cells. This included a reduction of cell clonogenicity by 40% and an increase of senescent cells by more than two fold as compared to the control. Furthermore, the expression of telomerase reverse transcriptase (TERT), an important component in the self-renewal machinery of the cells was severely impeded. Transcript levels were reduced by 47% while protein levels decreased by 69%. **Conclusion:** Suppressed levels of SKP2 significantly affected the proliferation and self-renewal of AML t(8;21) cells. These observations show that SKP2 is likely a proto-oncogene in AML t(8;21) and thus a potential therapeutic target.

Keywords: **S-phase protein kinase 2 (SKP2), acute myeloid leukaemia (AML), small interfering RNA (siRNA)**

Transgene Expression from Lentiviral Vector Driven by Different Constitutive Promoters in Mouse Pluripotent Stem Cells

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Purpose: Scientists have been exploring the possibility of correcting disease via gene therapy on induced Pluripotent Stem (iPS) cells. However, transient transgene expression following lentiviral (LV) gene delivery in mammalian cells has been a major setback for somatic cells gene therapy. Here, we assessed the duration of Green Fluorescence Protein (GFP) expression mediated by lentivirus driven by either human elongation factor 1α (EF1α) or cytomegalovirus (CMV) promoter in mouse iPS cells and mouse embryonic stem (ES) cells. **Methods**: iPS and ES cells were transduced with LV carrying GFP driven by either EFIα or CMV with the optimal multiplicity of infection. The resultant GFP expressing cells were sorted by FACSAria. GFP expression time point study for 30 days was performed. Percentage of GFP positive cells and mean fluorescent intensity (MFI) were determined by FACSCanto. **Results**: LV/EF1α transduced iPS cells exhibited significant GFP expression (80% expressing cells) with persistent level of MFI for 30 days. However, iPS cells differentiation was observed following LV/CMV gene delivery at day 10, but not in LV/EF1α transduced iPS cells. Both LV/EF1α and LV/CMV presented significant GFP expression (> 50%) in mouse ES cells for up to 30 days but surprisingly, LV/EF1α showed significantly higher MFI when compared to LV/CMV. Pluripotency analysis indicated that the cells retained their pluripotency potential throughout the study period. **Conclusion**: The research findings could provide an insight for the application of pluripotent stem cells for persistent correction of genetic disorders using gene therapy technology.

Keywords: **Gene expression, iPS cells, embryonic stem cells, gene therapy**

Generation of Induced Pluripotent Stem Cells (iPSC) from a Haemophilia A B6;129S4-F8$^{tm1Kaz/J}$ Mouse Tail Fibroblast

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Purpose: The ability to reprogram somatic cells to pluripotent stem cells may circumvent any ethical issues surrounding the usage of embryonic stem cells in regenerative medicine. Therefore, the goal of this study is to generate induced pluripotent stem (iPS) cells from fibroblasts of a haemophilia A mouse model. The generated haemophilia A iPS cells can either be used for a gene therapy proof-of-principle study or to give a better understanding of the molecular control of the disease. **Methods**: Primary mouse-tail fibroblasts were successfully isolated using an explant culture technique. Polycistronic lentiviral vector carrying Oct4, Sox2, Klf4 and c-Myc (OSKM) factors was generated from HEK293FT cells. FACS analysis titration method was performed to determine the viral titer. MOI analysis was performed to determine the lowest amount of virus needed to yield the highest expression of Oct4. MOI of 10 and 25 were used to transduce the primary mouse-tail fibroblasts for iPS cells generation. **Results**: LV/OSKM with a titer of 9.2 x 10^8 TU/mL was produced. Primary tail-tip fibroblasts were maintained, passaged and cryopreserved in a standard culture condition. The primary mouse-tail fibroblasts showed morphological changes after 10 days of transduction with LV/OSKM at MOI of 10 and 25. Strangely, the cells exhibited neuronal-like morphology instead of the expected iPS cells like colonies. **Conclusion**: Generation of iPS cells from primary mouse fibroblast using lentiviral vector carrying OSKM factors was unsuccessful. Instead, the primary mouse-tail fibroblasts exhibited neuronal-like cells morphology. This phenomenon is currently being elucidated.

Keywords: **iPSC, lentivirus, reprogramming**

Mitochondrial DNA Quantity Changes in Leucocytes of Cancer Patients on Cisplatin and 5-fluorouracil Regimen

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Purpose: Cisplatin and 5-Fluorouracil are the critical components of therapeutic regimen in a broad range of malignancies. Their therapeutic efficacy is limited due to the development of toxic side effects and drug resistance by the cancer cells. Cisplatin toxic side effects are usually associated with mitochondrial injury in vivo and in vitro. In vitro evidence indicates that cisplatin decreases mitochondrial DNA (mtDNA) copy number. There is limited information on the effect of cisplatin on human mtDNA quantity. In order to clarify the effects of cisplatin and 5-FU on mtDNA in human, this study focused on the changes of mtDNA quantitation in cancer patients who were treated with cisplatin-based regimens. **Methods**: 24 blood samples from head and neck cancer patients were collected before chemotherapy and after second cycle of chemotherapy. Total DNA was extracted from peripheral blood leukocytes by salting out method. Validation of primer pairs were done by conventional PCR and checked by gel electrophoresis. The relative changes of mtDNA quantity were determined by quantitative PCR targeting *Cyt-B* genes normalizing with the nuclear gene *PARL*. **Result**: Comparing 24 samples, there was an increased mtDNA level after second cycle of chemotherapy, though it did not reach the statistically significant level (P=0.31, Wilcoxon signed ranked test). However, 58% of the total patients had relatively increased mtDNA amount while 42% of the total patients showed decreased mtDNA amount after second cycle of chemotherapy (P<0.05, paired t-test). **Conclusion**: Overall, Cisplatin-based chemotherapy can affect mtDNA quantity changes in leucocytes of head and neck cancer patients.

Keywords: **Mitochondrial DNA, cisplatin, 5-fluorouracil**

Synthesis and Characterization of Polyurethane (PU) Blended with Polyethersulfone (PES) Hollow Fiber Membrane for Blood Purification

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Purpose: Biocompatibility of a blood contact materials has been crucial for any extracorporeal blood aphereses and purification procedure. Polyurethane (PU) has been widely used as a biomaterial in various medical devices. Thus, in present work, PU with three different functional groups, carboxyl, hydroxyl and sulphonyl group on its molecular structure, were synthesized. The synthesized PU were blended with polyethersulfone (PES) to improve the performance, permeability and biocompatibility of the traditional PES hollow fiber membrane for blood purification application. **Methods**: PU as a biocompatibility enhancer for PES was synthesized by using diphenylmethane diisocyanate (MDI) and dimethylolpropionic (DMPA). PES-PU hollow-fiber membranes with various loading of PU (0-5 wt %) were fabricated via the dry-wet phase inversion spinning technique. The morphology, physical and chemical properties of the hollow fiber membranes were investigated. The effects of blended PES-PU hollow fibers membrane on the performance of the membranes were evaluated by pure water flux and BSA rejection. **Results**: The fabricated PES/PU hollow fiber membrane possessed an asymmetric structure, with long finger-like voids at the outer surface and a thin selective structure on the inner surface. The selective layer helps in removing small uremic toxin and in retaining larger protein. The experiments showed a reduction in protein adsorption by incorporation of PU in the PES membrane. The study also showed that there is an increase in the hydrophilicity with the increase of PU loading, which led to the increase in permeability of the membrane. **Conclusion**: These results suggested that the addition of PU into PES has successfully enhanced the permeability and biocompatibility of the membrane and thus, allow practical application in the field of blood purification.

Keywords: **Polyurethane, hollow fiber membrane, blood purification**

Characterization and Performance Evaluation of Hydrophilic PES/MWCNTs Hemodialysis Membrane

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Purpose: Membrane hydrophilicity is an important factor to achieve high flux and excellent protein rejection during hemodialysis treatment for end stage renal failure patient. In this work, multi-walled carbon nanotubes (MWCNTs) are incorporated to enhance the hydrophilicity of polyethersulfone (PES) hemodialysis membrane. **Methods**: Prior to the mixing, surface functionalization of MWCNTs using poly (citric acid) was carried out to introduce oxygen-rich species such as carboxyl groups and hydroxyl for better dispersion. Poly (citric acid)-grafted-MWCNTs (PCA-g-MWCNTs) were prepared by grafting citric acid monohydrate onto the surface of purified MWCNTs. 0.05% of PCA-g-MWCNTs were then dispersed in PES and polyvinylpyrrolidone blend. The neat PES membrane and PES/MWCNTs MMM were fabricated via dry-wet spinning technique, characterized in terms of morphology and hydrophilic properties before tested for pure water flux (PWF) and protein rejection using bovine serum albumin (BSA). **Results**: The decrease in contact angle value from 77.56° to 56.06° for PES/MWCNTs membrane indicated the increase in surface hydrophilicity, which rendered positive impacts on the PWF and BSA rejection of the membrane. The PWF increased from 15.8 $Lm^{-2}h^{-1}$ to 95.36 $Lm^{-2}h^{-1}$ as PCA-g-MWCNTs were incorporated due to the attachment of abundant hydrophilic groups that present on the MWCNTs, which improved the affinity of membrane towards water molecules. Same trend was observed for protein rejection, in which the PES/MWCNTs membrane rejected 95.2% of BSA compared to 90.2% protein rejection obtained from neat PES membrane. **Conclusions**: The incorporation of PCA-g-MWCNTs enhanced the features and performance of the PES membrane for hemodialysis application.

Keywords: **Poly (citric acid), multi-walled carbon nanotubes, polyethersulfone, hemodialysis membrane**

Relation between Decision to Refuse Treatment and Educational Background in Breast Cancer Patients

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Introduction: Data from Malaysian Cancer Statistic 2006 has shown breast cancer was the most common cancer among female and also the most important cancer among population regardless of sex in Peninsular Malaysia. It is the most common cancer in Malaysian women followed by cervix uteri and colon cancers. Despite massive campaigns to increase awareness by government and NGOs, there are still diagnosed patients refusing treatment, which generally due to strong beliefs in traditional treatments and loss of autonomy in making decision. **Purpose**: To study percentage of breast cancer patients in Hospital Sultan Abdul Halim (HSAH), Sungai Petani who refused treatment from 2013 till 2015. Secondly, to analyze relation between incidence of patients declining recommended treatment and educational background during the period from 2013-2015. **Method**: Retrospective data has been collected from total patients who were diagnosed with breast cancers in Hospital Sultan Abdul Halim since 2013 and list of defaulters was isolated. Their educational background has been studied from patient's medical files and via phone calls. **Conclusion**: There is a relationship between educational background of breast cancer patients and their decision to refuse medical and/or surgical treatment.

GCMS Analysis of Chemical Compounds of *Clinacanthus nutans*

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Purpose: This study was carried out to detect the chemical compounds present in the leaves of *Clinacanthus nutans* by GC-MS. **Methods**: The powdered leaves of *Clinacanthus nutans* (*C. nutans*) were extracted separately with hexane, chloroform and methanol successively in the increasing order of their polarity. The chemical compounds present in the extracts of *C. nutans* were analyzed using Agilent Technologies Gas Chromatography-Mass Spectra (7890A/5975C GCMSD). The mass spectra of the compounds detected in the extracts were matched with the National Institute of Standards of Technology (NIST) library and WILEY library. **Result**(s): Some of the chemical compounds identified were mainly triterpene, fatty acids, plasticizers, hydrocarbon and phenolic components. In the hexane extract, 1, 2, 4-trimethylbenzene (0.26%), hexadecanoic acid (2.47%), trans-phytol (2.52%), neophytadiene (10.58%) and squalene (31.01%) were present in the leaves of *C. nutans*. Meanwhile, for chloroform extracts, p-cumylphenol (19.10%), bisphenol A (69.26%) and 1, 2-benzenedicarboxylic acid, diisooctyl ester (11.57%) were found to be in abundance whereas betaine (0.79%), butanoic acid,4-(dimethylamino)-, methyl ester (0.71%), hexadecanoic acid (1.96%), bisphenol A (49.39%), alpha-tocopherol (13.28%), lanosterol (7.48%) and trans-9-octadecanoic acid (2.21%) were identified in the methanol extract. **Conclusion**: The results indicate that the leaf extracts of *C.nutans* are made up of various chemical compounds that could be further explored for its bioactivity potential such as anti-oxidant, antimicrobial and anti-inflammatory properties.

***Keywords: Clinacanthus nutans*, GCMS, chemical compounds**

Morphology and Performance Evaluation of PSf/PVP Hemodialysis Membrane

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Purpose: Membrane morphology plays important roles in achieving high flux and excellent uremic toxin removal for efficient hemodialysis therapy. Hemodialysis membrane morphology correlates to spinning parameters applied during fabrication of membrane. In this work, the effect of air-gap on the morphology and liquid separation performance of the polysulfone (PSf) hemodialysis membrane is investigated. **Methods**: PSf hollow fiber membranes were prepared via dry-wet spinning process from dope solutions comprises of 18 weight percent (wt%) PSf and 4.8 wt% polyvinylpyrrolidone in N-methyl-2-pyrrolidone. The membrane morphology was characterized using scanning electron microscope (SEM), before tested with ultrafiltration system to measure pure water flux (PWF) and protein rejection using bovine serum albumin (BSA). **Results**: SEM analysis revealed that the air gap does change the structure of the membranes due to elongational stress because of the gravitational pull on the PSf hollow fibers. At low air gap (3 cm), the lower average pore size on the outer surface reduced the PWF while at high air-gap (50 cm), larger average pore size of membranes permitted water molecules to pass through easier and faster. It was observed that the PWF of the membrane increased significantly with air gap due to the increasing pore size. Membrane fabricated at 50 cm air gap obtained better PWF (28.45 Lm-2h-1) and protein rejection (94.47%) compared to the membranes fabricated at 3 and 30 cm air gap. **Conclusion**: The effect of air gap enhanced the morphology and performance of PSf membrane for hemodialysis application.

Keywords: **Hemodialysis, polysulfone, air-gap, membrane, polyvinylpyrrolidone**

Characterization and Protein Absorption of Poly (1,8-octanediol citrates) (POC) Blend Polyethersulfone (PES) Hemodialysis Membrane

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Purpose: Protein absorption is one of the key factor contributing to blood compatibility of hemodialysis membranes. Absorption of protein on the membrane surface trigger the thrombogenic process. In order to reduce the amount of protein absorption on the membrane surface, a citric acid-based elastomers (CABEs), poly (1,8-octanediol citrate) (POC) was used to modify polyethersulfone (PES) membrane. The effect of POC concentration loading in PES membranes on the protein absorption was tested. **Method**: Four PES-POC flat-sheet membrane samples (M-0, M-1, M-3 and M-3) were prepared by blending with different POC concentration loading (0%, 1%, 2%, 3%) respectively through phase-inversion method. The prepared membranes were then characterized using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), contact angle and surface charge using Zeta-potential. Membrane samples were incubated with 1mg/ml of protein solution (bovine serum albumin (BSA) or fibrinogen (FBG)) in phosphate buffer at 37^{o}C for 2 hours. After thorough wash, the membranes were incubated with 2% SDS solution at 37^{o}C for 1 hour. The protein concentration in the SDS solution was measured using a Micro BCA™ Protein Assay Reagent Kit. **Result**: Zeta-potential analysis revealed that the four membrane samples exhibit negative charge properties. Contact angle showed the hydrophilicity of the membranes sample are ranged between 61^{o} and 67^{o}. Our result showed a decrease amount of BSA and FBG absorbed as POC concentration loading was increased. **Conclusion**: These results suggest that the PES membranes modified with POC could be a potential membrane for the hemodialysis application.

Keywords: **Poly (1,8-octanediol citrate) (POC), polyethersulfone (PES), protein**

Measurement of Attenuation Values Using Single-Energy and Dual-Energy Computed Tomography

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Purpose: The aim of this study was to compare the attenuation values of a fabricated thyroid phantom material using both Single-Energy and Dual-Energy CT and to show that the mass attenuation coefficients are able to be determined using the Dual-Energy CT as a new method to validate the phantom material. **Methods**: A paediatric thyroid phantom was designed to mimic a real shape and size of a paediatric age of 9 years old. High density polyethylene was used to fabricate the thyroid phantom material. The fabricated phantom was scanned using Single-Energy and Dual-Energy Computed Tomography, which the current and the slice width were settled automatically by the machines. The CT numbers were evaluated and the mass attenuation coefficients (μ/ρ) of the phantom material were obtained at each applied energy for both modes. The experiment was repeated at fixed current of 300 mAs and slice width of 5mm for both scanning modes. The results were compared with the National Institute of Standards and Technology's (NIST's) tables. **Results**: The obtained μ/ρ of the phantom material from both modes, showed very similar match with the NIST's values. However, the fused image of the Dual-Energy CT showed a perfect match with the values listed in NIST's tables. No significant effect on the results at fixed current and slice width had shown for both modes. **Conclusion**: The Single-Energy and the Dual-Energy CT can be used to determine the mass attenuation coefficients of the phantom material, as they showed perfect match with the NIST's values especially the perfusion image from the Dual-Energy CT and it can be used as a new method to obtain the mass attenuation coefficients of the phantom material.

Keywords: **Attenuation value, dual energy, single energy, CT, thyroid, pediatric, phantom**

A Comparative Transcriptomic Analysis Reveals Conserved Features of Non-Small Cell Lung Cancer Stems Cells

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Purpose: The aim of this study was to identify the transcriptomic composition of putative lung cancer stem cells (CSCs) as compared to the normal lung stem cells. **Methods**: To understand the molecular characteristics of lung CSCs, microarray analysis was performed using Affymetrix 1.0 ST array on putative lung CSCs isolated from non-small cell lung cancer cell line (A549 and NCI-H2170) and normal putative lung stem cells isolated from primary human bronchial/tracheal epithelial cells (PHBEC). The cells were isolated based on double positive expression of stem cell surface markers CD166/CD44 and CD166/EpCAM using the FACSAria III cell sorter. **Results**: The microarray data were analyzed by comparing the putative lung CSCs with normal putative lung stem cells. Among all genes identified, 379 genes were found to be conserved in all three different putative lung CSCs phenotype in which 38 (10.0%) genes were up-regulated and 341 (90.0%) genes were down regulated. Bioinformatics analysis of the genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) revealed the genes involved in biological process and pathways associated with cancer and stem cells including the ectoderm development and lung development process, ECM-receptor interaction pathway, pathways in cancer, small cell lung cancer and apoptosis pathway. **Conclusions**: This study provides new insights into molecular regulatory mechanisms of lung CSCs development. We anticipate that these results will provide foundations for further studies in providing a better treatment for lung cancer.

Keywords: **Lung cancer, cancer stem cells, bioinformatics**

Genetic Variants Associated with Opioid Addiction among Malay Population

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Purpose: Opioid dependence is a novelty seeking personality trait and have significant consequences on personal, family and society. In this study, we have used candidate gene association to identify variants that associated with vulnerability to develop heroin addiction in the same gender and ethnicity. The goal of this study was to determine the frequency of SNPs rs1800955, rs737866 and rs10494334 among Malaysians and study their association with the phenotype of opioid dependent. **Methods**: A total of 469 opioid dependents and 543 Malay male volunteer subjects were included in this study. SNPs were genotyped using Taqman SNP genotyping assay. The results obtained were analyzed for allele and genotype frequencies. An association between genotypes and the opioid dependence phenotype was also performed using Haploview 4.2. **Results**: All the three markers genotyped for both cases and randomly ascertained controls were in Hardy-Weinberg equilibrium. The -521 C/T SNP (rs1800955) of the DRD4 gene showed nominal association with a possible protective effect of the C allele (p= 0.0571) odds ratio1.1965; 95% CI (0.9946- 1.4393) compared to allele T. However, there were no significant association between opiate dependent and volunteers for SNP rs737866 and rs10494334. **Conclusions**: This study suggests that variant C allele of rs1800955 may contribute to vulnerability to opiate dependence among Malaysian population and this finding may guide future studies to identify genetic risk factors for opioid dependence.

Keywords: **Opioid, vulnerability, SNPs**

Gene Expression Differences between Leukaemia Cell Lines and Leukaemia Tissue Cells

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Purpose: Aim of this study was to determine the capacity of Kasumi-1 and SKNO-1 cell lines as model systems for leukaemia investigation- by identifying significant alterations in gene expression pattern in cell lines in comparison with clinical tumour material and normal cells. **Methods**: Whole genome expression in t(8;21) positive myeloblasts from bone marrow aspirates of two AML patients, and Kasumi-1 and SKNO-1 cell lines were array analysed using HumanHT-12 v3 expression BeadChip (Illumina, San-Diego, USA) targeting 48,803 transcripts. For comparison, CD34+ cells from bone marrow aspirates of a normal individual were also array analysed in parallel. **Results**: Of the 48,803 transcripts studied, 34,073 transcripts were shown to be differentially expressed. The hierarchical cluster analysis of these differentially expressed genes (DEG) revealed that model cell lines (Kasumi-1 and SKNO-1) formed an independent cluster branching away from patients' tumour cells. However, when the comparisons were made using the overlapping 6,092 DEG in patient samples and corresponding cell lines, the resulting dendrogram showed that the clinical material and the cell lines were clustered together indicating that the patterns of gene expressions were homogeneous to each other. Spearman correlation coefficient for the former comparison was 0.451 ($p<0.0001$) and the latter was 0.822 ($p<0.0001$). **Conclusion**: Kasumi-1 and SKNO-1 cell cultures are excellent model systems of choice to get a first approximation of *in-vivo* activity in cancer biology. Our results indicate that stringent fold change threshold is important when these cells are used as model systems for meaningful downstream investigations including dysregulated biological pathways towards leukaemogenesis.

Keywords: **Leukaemia, cell lines, microarray**

Tracheal Epithelium Regeneration in Rabbit by Airway Progenitor Epithelial Cells and Adipose-Derived Mesenchymal Stem Cells

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Purpose: This study was aimed to evaluate the potential of airway progenitor epithelial cells (AEC) and adipose-derived mesenchymal stem cells (AD-MSC) in tissue regeneration and repair of denuded trachea derived from rabbit. **Methods**: Rabbit trachea was sectioned into 1cm rings and the epithelium was removed with brushing-induced technique, which exposed the tracheal basement membrane and maintained the cartilage structure. The segmented trachea was placed in a microcentrifuge tube filled with growth medium. AEC and AD-MSC were labelled with BrdU and seeded into the trachea. Incubation was performed in a MACSmix™ tube rotator for 1d and 5d. Haematoxylin and eosin and immunofluorescence staining were performed as to observe the cellular engraftment and regeneration of epithelium layer. **Results**: There were no epithelial cells detected in the mucosa region of the control group. In contrast, a new lining of epithelium layer was formed in the denuded trachea basement membrane of the treated group. Immunofluorescence staining showed that the thin lining of the engraftment was accumulated with BrdU-labelled *cells* which was found attached to the *basement membrane.* Engraftment was remained visible and formed into multiple lining of epithelium up to 5 days compared to the control group. **Conclusions**: Our results demonstrate a promising data of the potential use of AEC and AD-MSC as sources of cells for regenerating tracheal epithelium for tissue engineering applications. However, further study to prolong the incubation time of the denuded trachea with progenitor and stem cells is required to assess the pseudostratified epithelium formation.

Keywords: **Trachea regeneration, airway epithelial cells, mesenchymal stem cell**

Breast Cancer Defaulters, Why?

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Purpose: Breast cancer is the most common cancer among women in Malaysia. There were 3292 cases of newly diagnosed breast cancer in Malaysia in 2007. The estimated mortality rate for breast cancer was 11.4 in 100000 population which contributed by factors such as presentation at advanced stage of disease, poor compliance and high refusal rate for treatment and opted for alternative medication. To study causes that leading to refusal of conventional treatment amongst defaulters in patients who has been diagnosed as breast cancer. To identify the reasons for choosing alternative medicine amongst defaulters. **Methods**: Data collected from pool of patients who has been diagnosed as breast cancer in HSAH, Sg. Petani from year 2013 until 2015. Collected data further channelised into those who refusing conventional treatment and those who opted for alternative medication. **Results**: This study proves that there are multiple factors leading to refusal of standard conventional treatment amongst defaulters. Data shows there is strong correlation between defaulted breast cancer patients with alternative medicine. **Conclusions**: Breast cancer awareness and psychological counselling are recommended for all patients with breast symptoms to increase knowledge and understanding regarding nature of breast cancer and its standard treatment. The lack of autonomy in decision-making by the women was the major reason for refusal of conventional treatment of breast cancer and opted for alternative medication and this was mostly due to strong cultural beliefs and practices around family and women's right.

Inhibitory Effect of Curcumin on CD166+/EpCAM+ of Cancer Stem Cells (CSCs) in A549 (Adenocarcinoma) and H2170 (Squamous) Cell Lines

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Purpose: The present study was aimed to observe the effect of curcumin by targeting the CD166+/EpCAM+ of CSCs derived from A549 (adenocarcinoma) and H2170 (squamous) cell lines. **Methods**: The lung CSCs with phenotype CD166+/EpCAM+ from A549 and H2170 cells was cultured and were then treated with curcumin and cisplatin to determine the inhibitory concentration (IC50). The effectiveness of curcumin was evaluated using MTS assay and cells apoptosis was analyzed using flow cytometry. Several assays were conducted to evaluate the effect of curcumin including the migration ability and colonies formation. **Results**: The IC50 obtained from curcumin and cisplatin on A549 was 40μM and 4μM while H2170 was 30μM and 4μM respectively. The study showed that curcumin enhanced cisplatin effects through combined treatment even at the lower doses of cisplatin. This has resulted in high number of cells undergone apoptosis by 18% and 90% in A549 and H2170 respectively. Furthermore, the combined treatment was also found to be reduced the migration of both A549 and H2170 cells to only 9% and 21% respectively. Besides, the effect of combined treatment was also evaluated on the capability of the cell to form colonies and the study found that the formation of colonies on both cells were suppressed indicating the inhibitory effects of curcumin on CSCs stemness. **Conclusion**: Taken together, the study was successfully showed the inhibitory effect of curcumin on CD166+/EpCAM+ of CSCs both on A549 and H2170 cells thus may provide a new strategy in targeting CSC especially in NSCLC.

Keywords: **A549 (adenocarcinoma), H2170 (squamous), cancer stem cells (CSCs), non-small cell lung cancer (NSCLC), curcumin, cisplatin**

Ursodeoxycholic Acid Protects Cardiomyocytes Against Hypoxia by Regulating Caspase -3/-9 and Survival Signaling Proteins

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Purpose: Ursodeoxycholic acid (UDCA) is the most hydrophilic bile acid and commonly used in the treatment on liver diseases. Recently, UDCA has been shown in protecting heart against arrhythmia. In heart, loss of cardiomyocytes due to hypoxia could leads to heart diseases. Mechanism involves in UDCA cardioprotection are not well understood. Therefore, this study aimed to investigate the mechanism involve in UDCA cardioprotection against hypoxia by using *in vitro* model. **Methods**: The primary neonatal rat cardiomyocytes isolated from 0-2 days old neonates. Cells were treated with UDCA and followed by chemical induction of hypoxia; 100 µm cobalt chloride ($CoCl_2$) for 24 hours. Cells were then lysed and subjected for ELISA assay and western blotting to measure expression of caspase-3, caspase-9, Erk and Akt. All data were analyzed by pair t-test for beating assessment and one-way ANOVA for MTS assay, ELISA assay and western blot ($p < 0.05$). **Results**: $CoCl_2$ (Mean ± SEM; 72 ± 2.08 bpm) reduced cardiomyocytes beating rates significantly compared to untreated (117 ± 2.31 bpm). UDCA-$CoCl_2$ (95 ± 3.48 bpm) improves beating rate compared to hypoxia only. Hypoxia induces up-regulation of caspase-3/-9 activation compare to untreated. UDCA treated cells followed by $CoCl_2$ treatment shown to downregulate the caspase-3/-9 activation compare to $CoCl_2$ only. Up-regulation of survival signaling protein expression Erk (42/44 kDa) and Akt (60 kDa) in UDCA-$CoCl_2$ compare to hypoxia only. **Conclusion**: In conclusion, UDCA protects cardiomyocytes against hypoxia by inhibiting caspase -3/-9 and upregulating of survival signaling protein (Erk and Akt).

Keywords: **Ursodeoxycholic acid, apoptosis, caspases, cardioprotection**

The Regenerative Effect of Aerosolised-Honey in Animal Model of Asthma

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Purpose: The present study was aimed to evaluate the potential use of *Tualang* honey in remodeling the airway tissue and its effect on inflammatory cell response following ovalbumin-induced airway hyperresponsive in rabbits using aerosolisation method of treatment. **Methods**: Upon injury, the *Tualang* honey was given in two different doses; 25% (v/v) and 50% (v/v) using ultrasonic nebulizer (Mabis Mist^{TM}). The effects of aerosolised-honey treatment were evaluated based on its role as either to prevent the occurrence of asthma or as rescuing agent in relieving the asthma related symptoms. Research findings were interpreted based on histopathological analyses which were included morphometric analyses, goblet cell counting and scoring of inflammatory cells. **Results**: Inhalation of honey (regardless of the doses) was proven to significantly restore the airway structure in the regions of epithelial, mucosal and submucosal. This study also demonstrated the inhibitory effect of honey on goblet cell hyperplasia which led to the reduction of mucus secretion. These effects contribute in relieving the airway obstruction due to airway thickening and mucus accumulation in the airway lumen. The beneficial effect of honey was further supported by reduction in the number of eosinophils infiltrated in the airways. Eosinophilic inflammation is the pathological hallmark of asthma. **Conclusion**: The present study provides the evidence that honey aerosolisation was effective in inhibiting ovalbumin-induced airway inflammation by alleviating the asthma-related features and also prevents the occurrence of asthma. The aerosolisation technique used in this study suggests its potential application as an alternative strategy for treating patients with asthma.

Keywords: **Honey aerosolisation, asthma, airway inflammation**

Comparison Study with Other International Protocols for Myocardial Perfusion Scan in Nuclear Medicine at Different Hospitals in Jeddah

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Purpose: The main purpose of this project is to assist nuclear medicine practitioners in recommending, performing, interpreting, and reporting the results of myocardial perfusion imaging studies related to other procedures such as European Association of Nuclear Medicine (EANM), ICRP, and other association then compare that procedures with some procedures done by some local hospitals in Jeddah. Additionally, comparing the salient features of the available protocols will be studied, where the advantages and disadvantages of each protocol should be examined. **Methods**: This study was carried out for five hospitals to demonstrate that all of those hospitals used the same protocols and regulations of Myocardial Perfusion Scan upon to other regulation of associations based on: the Radiopharmaceutical, Dose, Post injection time, Collimator, Patient protection, Quality control and Quality assurance regarding. Current study conducted by using a questionnaire based on the standard criteria that were determined by International Commission of Radiation Protection (ICRP) and European Association of Nuclear Medicine (EANM). These criteria were answered by a Nuclear Medicine Technician/Technologist for each hospital in this study. **Results**: It was found that all the hospitals using the same type of Radiopharmaceutical that is ^{99m}TC - CESTAMIBI beside of ^{99m}TC – TETROFOSMIN while the recommended radiopharmaceutical is Thallium-201 and two technetium-99m labeled radiopharmaceuticals (MIBI and TETROFOSMIN). Regarding ICRP and EANM the administrated dose should be 120 MBq (3.24mCi) for rest and 80 MBq (2.16mCi) for stress procedures so it was found that just one hospital followed the roles. In fact, there were just two hospitals applied the similar post injection scanning time that is 30 – 60 min as ICRP and EANM. **Conclusion**: close attention to the details of acquisition protocols, processing techniques, and image interpretation is needed to ensure high diagnostic quality in myocardial perfusion studies.

Keywords: **Myocardial perfusion, nuclear medicine, protocol, ICRP, EANM**

Allergenic Potential of Airborne Algae Isolated from Malaysia

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Purpose: The aim of this study was to investigate the allergenic potential of the isolated airborne green and blue-green algae, namely *Scenedesmus* sp., *Cylindrospermum* sp. and *Hapalosiphon* sp. **Methods**: The suspension of freeze dried airborne algae (1 and 0.01 mg/mL) were adminstered into balb-c mice model through intra-nasal route to determine their allergenic potential. **Results**: Results showed that *Scenedesmus* sp. (1 mg/mL) increased systemic Ig E levels in mice by 3-8 fold compared to pre-treatment. On the other hand, *Cylindrospermum* sp. and *Hapalosiphon* sp. at similar concentration caused systmic Ig E to increase by 2-4 fold. The potential of airborne algae causing Ig E mediated type 1 hypersensitivity was elucidated using other immunological markers such as cytokine interleukin (IL)- 4, 5, 6 and interferon-ɣ. When the amount of interleukins in mouse serum between day 0 and day 53 (day of sacrifice) were compared, mice exposed to *Hapalosiphon* sp. (1mg/mL) expressed IL4 and 6 by 8 fold while the *Cylindrospermum* sp. (1mg/mL) increased the expression of IL4 and IFɣ by 8 and 2 fold respectively. **Conclusion**: In conclusion, repeated exposure to the three selected airborne algae may stimulate immune response and generate Ig E in a mouse model.

Fabrication of Melt Derived 45S5 Bioactive Glass

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Purpose: To fabricate melt-derived bioactive glass (BG) and assess its biocompatibility towards stem cell from human exfoliated deciduous tooth (SHED). **Methods:** Melt-derived 45S5 BG ($SiO2$-CaO-Na_2O-P_2O_5) was fabricated and subjected to characterization using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Stem cells from human exfoliated deciduous tooth (SHED) were used to evaluate the biocompatibility of 45S5 by exposing the cells to various concentration of BG-conditioned medium (1-10 mg/ml) using alamarBlue assay. **Results:** XRD result indicated that BG produced have an amorphous structure. FTIR analysis showed that Si-O-Si bending, Si-O stretching and Si-O-Si stretching (asymmetric) bands are present within the BG structure indicating the characteristic of silicate network. SHED exposed to BG-conditioned medium showed increasing proliferative activity at lower concentration of BG powder to liquid ratio from Days 1-7. Higher BG concentration such as 8-10 mg/ml reduced SHED activity and is not suitable for cell growth. **Conclusion:** 45S5 BG with lower concentration of powder to liquid ratio (1-2 mg/ml) promoted SHED proliferation and may be used for future work.

Keywords: **Bioactive glass, SHED, proliferation**

Stimuli-Responsive Linear Disulphide Polymers for Potential Colon-Targeted Drug Delivery System

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Purpose: To synthesise and characterise cystamine- and cysteamine-based linear disulphide polymers for potential colon targeted drug delivery. **Method**: The synthesised cysteamine-based protected dithiol monomer was eluted with column chromatography; mobile phase: diethyl ether : dichlomethane = 6 : 4. Removal of trityl protection group was conducted using trifluoroacetic acid in the presence of scavenger, triethylsilane. The dithiol monomer was self-polymerised or with ethylene diethanethiol at different ratios to produce cysteamine-based polymers. Meanwhile, cystamine-based polymer was synthesised *via* reflux polymerisation. All synthesised compounds including dithiol monomers and polymers were characterised using spectroscopic methods. **Results**: Successful formation of cysteamine-based deprotected dithiol monomer was monitored by the disappearance of aromatics signals at 1774-1956 cm^{-1} in IR and further confirmed with the presence of new IR peak at 2545 cm^{-1} (S-H). Polymerisation was successful with the absence of S-H peak in the IR spectrum for cysteamine-based polymers. Meanwhile, the formation of cystamine-based polymer was corroborated by the presence of a secondary amide at 3300 cm^{-1} and carbonyl peaks at 1640 cm^{-1} in the IR spectrum. Porous morphology was observed in SEM and the presence of carbon, oxygen and sulphur elements in the cystamine-based polymer was confirmed by EDX. **Conclusion**: Both cystamine- and cysteamine-based disulphide polymers were successfully synthesised and characterised through spectroscopic techniques.

Keywords: **Cystamine, cysteamine, disulphide, polymers**

Antiproliferative Activity of *Clinacanthus nutans* Water Extract Through Human Lung Cancer Cell (A549)

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Introduction: *Clinachantus nutans* is widely grown in tropical Asia and locally known 'belalai gajah' or Sabah snake grass. It has been used as a natural product to treat skin rashes, snake bites, lesion caused by herpes, diabetic, fever and cancer. This plant content several potent bioactive compounds which mostly located in its leaves. The major bioactive constituent is flavones that may possess potential phytochemical properties for cancer treatment. Therefore, the objectives of this research are to determine the optimum concentration of flavones in water extract using Peleg's model and to evaluate potential antiproliferative activity on human lung cancer cell (A549). **Purpose**: *Clinachantus nutans* is widely grown in tropical Asia and locally known 'belalai gajah' or Sabah snake grass. It has been used as a natural product to treat skin rashes, snake bites, lesion caused by herpes, diabetic, fever and cancer. This plant content several potent bioactive compounds which mostly located in its leaves. The major bioactive constituent is flavones that may possess potential phytochemical properties for cancer treatment. Therefore, the objectives of this research are to determine the optimum concentration of flavones in water extract using Peleg's model and to evaluate potential antiproliferative activity on human lung cancer cell (A549). **Methods**: The extraction process was carried out on fresh and dried leaves at 28 to 30 ˚C with liquid-to-solid ratio of 10 ml/g (1:10, w/v) (n = 3) for 72 hrs. The extracts were collected intermittently (1 ml/2 hrs) prior to flavones content (mg) and its concentration (mg/ml) analysis using RP-HPLC. The extract which contained the highest amount of flavones was used to evaluate the inhibitory concentration (IC_{50}) via 2-D cell culture of human lung cancer cell for 72 hrs through MTS assay. **Results**: Based on the results obtained, the optimum flavones concentration (2800 ppm) and yield (700 mg) were observed at 14 hrs of extraction ($t_{exhaustive}$). The optimized aqueous extract exhibited antiproliferative effect on A549 cell line with IC_{50} of 24.6 µg/ml for 3 days of incubation. **Conclusion**: In conclusion, flavones in *Clinachantus nutans* water extract possess potential antiproliferative properties against human lung cancer cell (A549), suggesting an alternative approach for cancer treatment.

Nanoparticles-Hydrogel Composite as a Novel Intra-Pocket Drug Delivery System for the Treatment of Periodontitis

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Purpose: The aim of this article is to acquaint the reader with the current and future nanotechnological approaches that are being investigated for the treatment of periodontitis, with a particular attention to the Nanoparticles-Hydrogel composite, owing to its unique potentials of achieving multi-drug therapy and providing beneficial characteristics of nanoparticles (NPs) and that of hydrogel system. **Methods**: This article was developed through comprehensive review of the literatures which involved searches in ISI Web of Knowledge, PubMed, Google, Google Scholar, SpringerLink, Scopus, ScienceDirect, ProQuest, EBSCOHost and SAGE Premier, using the keywords "Nanocomposite for periodontitis," "Hydrogel for periodontitis," "Nanotechnological approaches for periodontitis" and "Periodontal intra-pocket drug delivery system." The articles obtained were screened, selected, reviewed and analyzed. **Result**: The recent innovations in nanotechnological drug carrier systems seems promising in the treatment of periodontitis. Several polymeric nanoparticles-hydrogel co-formulations have been investigated in recent years, using both natural and synthetic polymers. Some of the excellent results and rewards achieved from these novel approaches are the use of bioadhesive polymers to achieve prolong drug release, the increment of intra-pocket drug penetration, the enhancement of mechanical properties using chemical crosslinkers and the possibility of loading multiple drugs in a unit delivery system. **Conclusion**: Recent advances in nanotechnology have shown that NPs possesses great potentials as drug carriers in the treatment of periodontal infection. The future utilization of these advantages will significantly improve dental care. The co-formulation of Nanoparticles-hydrogel composite showed additional advantages compared to simple NPs or hydrogels as local intra-pocket drug delivery systems.

Keywords: **Nanocomposite, hydrogel, nanoparticles, periodontal intra-pocket drug delivery system, nanotechnological approaches**

Inhibition to Functional Expression of ABC Transporters: A Acreening Option for Inhibitors of Anticancer Drug Resistance from Herbal Medicine

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Purpose: Inhibition to ATP-binding cassette (ABC) efflux transporter such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP), can be postulated to reverse drug resistance of cancer pharmacotherapy. We investigated *in vitro* effects of commercialized inhibitors to P-gp, MRPs and BCRP using dual dye retention assay in HK-2 (human proximal tubule) cell line[1]. We propose the use of transporter-mediated dye efflux assay to study functional expression of ABC transporters as a screening method to inhibitors of anticancer drug resistance from herbal medicine. **Methods**: In this study, mRNA expression of the transporters was assessed by quantitative polymerase chain reaction (qPCR). Dual dye assay was developed for the evaluation of functional expression of the ABC transporters; Hoechst 33342 (H33342) dye assay was used to assess P-gp or BCRP function, whilst MRP-specific fluorescent dye (glutathione methylfluorescein, GSMF) assay was used to assess activity of the MRPs. Intracellular dye retention was used to indirectly assess activity of the indicated efflux transporters. **Results**: In the HK-2 cells, qPCR confirmed mRNA expression of several members of the MRPs, P-gp, but not BCRP. The H33342 retention was observed in the presence of cyclosporin A (P-gp inhibitor) but not with KO143 (BCRP inhibitor), suggesting the functional expression of P-gp only. Similarly, the GSMF retention was observed in the presence of MK571 (MRP inhibitor) indicating MRP function in the cells. **Conclusions**: Dual dye assay developed by this study can be suggested for simultaneous screening to inhibitors of P-gp, BCRP and MRP transporters from herbal medicine.

Keywords: **ABC transporters, dye efflux assay, anticancer drug resistance**

Repellent Activity of Essential Oils from Jasmine and Chamomile Flowers Against *Aedes aegypti*

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Purpose: *Aedes aegypti* is an important vector responsible for the transmission of dengue and yellow fever which cause major human morbidity and mortality. Vaccines are not available for many insect-borne diseases and therefore personal protection plays a crucial role to reduce the risk of infection. Natural repellents such as essential oils may provide means of protection that are safe, pleasant to use and environmentally sustainable. **Methods**: Essential oils of jasmine (*Jasmine officinale*) and chamomile (*Anthemis nobilis*) were studied for their repellent activities against *Aedes aegypti* using rat model. Each essential oil was tested individually at the concentrations of 5%, 10% and 20% (v/v). The combination of both oils at different ratios was further assessed. Briefly, the test material was applied onto the depilated abdomen of the Sprague Dawley rats. The rat confined in a wire mesh cage was placed on top of the mosquito cage containing blood-starved female mosquitoes. The number of mosquito bite(s) at the treated area for 5 min exposure of every half-hour interval was recorded for the duration of 2 h. **Results**: Essential oils of jasmine and chamomile at 20% showed higher repellent activity against *Aedes aegypti* compared to 5% and 10%. The combination of oils at 20% in 1:1 ratio exhibited 100% repellency for 60 min, reduced to 90.2% and 88.2% after 90 min and 120 min, respectively. **Conclusions**: In this study, combination of jasmine and chamomile essential oils showed promising repellent activity against *Aedes aegypti*. Thus, the chemical composition of these oils should be further studied to explore their activity.

Keywords: E*ssential oil, repellent, *Aedes aegypti

Ovarian Scanner: Automated Ovary Tumor Segmentation and Classification in Ultrasonic Images by Hybrid Classifier Approach for Analysis and Decision Making for Gynecologist

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Purpose: Ovarian cancer is leading cause of cancer in woman after breast cancer in Malaysia. Accurate diagnosis of ovarian cancer from acquired images is dependent on the expertise and experience of ultra-sonographers or physicians, and is therefore, associated with inter observer variability's. The main challenge in the research is to find accuracy of detecting the tumor from ultrasonic images for radiologist and gynecologist. The objective of this work is to develop a computer-aided detection system to facilitate the identification of ovarian cancer from the confocal microendoscope system which can be acquired in real time. **Methods**: To achieve the goal, we propose a framework to enhance, detect and classify tumor in the ultrasonic ovarian images. Intelligent segmentation of tumor is first pre-processed by median filtering and segmented by region growing method and features are extracted for classifying the tumor. Selection of the features was performed using traditional feature selection techniques including linear discriminant analysis and principal component analysis that combines the results of these methods. The selected features are used for classification. The proposed classification is based on hybrid classifier which combines the Artificial Neural Network and Decision Tree classifier. **Conclusion**(s): The expected results will show that it is possible to automatically identify patients with ovarian cancer based on texture features extracted from confocal microendoscope images and that the machine performance is superior to that of the human observer. The proposed CAD based ovarian scanner prototype will improve the classification accuracy, sensitivity, specificity, and positive predictive value in detecting ovarian tumor.

Keywords: **Ovary tumor, segmentation, classification**

An Anti-Senescence Role of a Cell Cycle Protein, Cyclin D1 in Breast Cancer Cells

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Purpose: To demonstrate a novel function of cyclin D1 in suppressing senescence formation in breast cancer cells. **Methods**: The stable cyclin D1 and/or retinoblastoma protein (pRb)-knockdown cells were created by express short hairpin RNA (shRNA) in a luminal breast cancer cell line (MCF7). Senescent cells were identified by the senescence-associated β-galactosidase (SA β-gal) assay, expression of senescence markers, including *p16*, *p21* and *lamin B1* using real-time PCR, and immunofluorescent staining for heterochromatin trimethylation at Lys9 of histone3 (H3K9me3). Reactive oxygen species (ROS) were measured using 2',7'-dichlorofluorescein diacetate (DCFDA) and detected by flow cytometry. **Results**: Cyclin D1 depletion resulted in cellular senescence in a breast cancer, which associated with decreased cell proliferation, changed cell morphology; their volume increased and they lose their original shape by acquiring a flattened cytoplasm. This was confirmed by SA β-gal assay and expression of senescence markers including increased *p16*, *p21* and decreased *lamin B1* levels, and induction of heterochromatin H3K9me3 foci. We found accumulated level of endogenous ROS, and attenuated response to exogenous ROS in the cyclin D1-depeted breast cancer cells. Pretreatment of cyclin D1-depleted cancer cells with an antioxidant N-acetylcysteine (NAC) reduced the number SA β-gal positive cells significantly. Moreover, suppression of ROS by cyclin D1 is independent of the pRb inactivating function of CDK4-cyclin D1. **Conclusion**: Cyclin D1 maybe required to maintain low non-toxic level of ROS in the breast cancer cells, and to prevent the cancer senescence.

Keywords: **Cyclin D1, oxidative stress, senescence, breast cancer**

Rates of Depression and Anxiety in Recently Diagnosed Patients with Head and Neck Cancer in Malaysia: A 6 Months Follow-Up Study

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Introduction: Depression and anxiety are common psychological sequelae of head and neck cancer. These psychological issues are often disregard but deserves further study. **Objectives***:* To determine the rates of major depression and generalized anxiety disorder over time and their relationships with socio-demographic factors. **Methods***:* At the baseline, 60 recently diagnosed head and neck cancer patients were recruited in a tertiary referral centre and teaching hospital in Malaysia. Mini International Neuropsychiatric Interview 6.0.0 (MINI) was administered to diagnose Major Depressive Disorder and Generalized Anxiety Disorder. Six months after baseline assessment, only 50 patients completed the study where MINI assessment was repeated. **Results***:* The rates of Major Depressive Disorder reduced non-significantly from baseline to follow up assessment (Baseline: n= 8, 16%; Follow up: n= 6, 12%, p= 0.727) while Generalized Anxiety Disorder increased significantly from baseline to follow up (Baseline: n= 3, 6%; Follow up: n= 11, 22%, p= 0.008). Logistic regression analyses demonstrated socio-demographic factors did not contribute to Major Depressive Disorder. However, low monthly income increased risk of Generalized Anxiety Disorder by 16.49 times (p= 0.026) but other socio-demographic factors did not contribute to Generalized Anxiety Disorder. **Discussion***:* Increased rates of Generalized Anxiety Disorder were observed across a brief time period at follow up assessment. Low monthly income significantly increased risk of the disorder, which is an interesting juxtaposition of psychosocial factors in a medical illness.

A Case of Psychotic Disorder Due to Dengue Fever

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Introduction: Neuropsychiatric presentation in dengue is considered atypical presentation. Literature which reported Psychotic Disorder due to dengue fever is rare and the case we reported here clearly presented with psychotic symptoms during dengue fever. **Clinical presentation**: We are reporting a case of a 57 years old Malay gentleman was referred to Psychiatric Outpatient Clinic with complaint of visual and auditory hallucination, persecutory delusion, interrupted sleep accompanied by slight agitation for 4 days. He also gave a history of high grade fever, myalgia, nausea and vomiting and flu like symptoms for past 5 days prior to referral; in which onset was 1 day prior to onset of psychotic symptoms. There was no history of alcohol and drug intake before, no past and family history of mental illnesses. Nevertheless, he was a known case of Diabetes Mellitus with hyperlipidemia. Serological testing revealed highly elevated titres of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) against Dengue Virus. There were no neurological deficits noted, no altered sensorium and cognitive impairment during the episode. There was no evidence of encephalitis and metabolic disturbances. The patient was diagnosed with Psychotic Disorder due to Dengue Fever, with hallucinations and was rehydrated with intravenous fluid and treated with Oral Quetiapine. Patient's psychotic symptoms resolved over the next 3 days and his platelet count and blood investigations normalised within 1 week of admission. **Conclusion**: This case report demonstrated patients who presented with acute onset of psychosis accompanied by symptoms of viral fever should be screened for dengue fever.

Computational Investigation of β-Catenin-TCF4 Inhibition with Zerumbone

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Purpose: Aberration in the Wnt-beta catenin signaling pathway is the major cause proliferation of several cancer cells. Zerumbone, a sesquiterpene with known anticancer activity, has not been investigated in this pathway. Because of importance of the pathway, specifically β-catenin-TCF4 transcription complex, and the compound in cancer, the research can contribute significantly to our understanding of action of zerumbone at molecular level by correlating with available experimental data. **Methods**: We investigated its activity against the β-catenin-TCF4 transcription complex using molecular docking and MMPB(GB)SA binding energy calculations. Discovery Studio 2.5.5 (Accelrys Inc.) and AMBER12 were used for the experiment on a GPU accelerated machine. **Results**: The docked complex of drug and the β-catenin-TCF4 transcription complex showed a binding energy value of -80 kcal/mol, while the MMPB(GB)SA calculation indicate PBTOTAL and GBTOTAL values of -11.19kcal/mol and -11.31kcal/mol, respectively. **Conclusion**: β-catenin-TCF4 transcription complex is important as it positions the TCF4 for binding to the DNA promotor site for protein transcription. Inhibition of the transcription complex would ensure the instability of the TCF4-DNA complex. The free binding energy of the complex indicated that zerumbone could potentially inhibit the transcription complex and hence inhibit cancer cell proliferation.

Keywords: **Wnt-beta catenin signaling pathway, zerumbone, beta-catenin-TCF4 transcription complex, molecular dynamics, free binding energy**

Rapid Detection of Human Papillomavirus Genotype 16 by Loop-Mediated Isothermal Amplification

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Purpose: Human papillomavirus 16 (HPV16) has strong association with oral cancer. The usage of loop mediated isothermal amplification (LAMP) for detection of HPV 16 showed promising results with high specificity and sensitivity. This study was aimed to develop the loop-mediated isothermal amplification (LAMP) reaction for rapid detection of HPV16 in HPV-related oral squamous cell carcinoma (OSCC). **Methods**: A set of six primers were designed to specifically distinguish E6 oncoprotein in HPV 16. The specificity was checked using HPV types 18, 35, 43 and 56. The sensitivity was determined by 10-fold serial dilution in 25 µl of Loopamp DNA amplification kit. Different reactions time (20, 30, 40, 60, 80,100 minutes) and temperature (50, 55, 60, 65, 70^{o}C) were optimized for the detection of HPV16. Amplification results were inspected by addition of SYBR Green I for naked-eye evaluation and re-confirmed with gel electrophoresis. **Results**: The LAMP reactions were positive at reaction temperature of 50^{o}C, 55^{o}C, 60^{o}C, and 65^{o}C but negative in 70^{o}C. All reactions were positive regardless the time incubation and the detection limit of the assay was up to 10^{6} pg/mL. No cross-reactivity with other HPV genotypes was observed. **Conclusion**: This colorimetric LAMP assay has potential usefulness for the rapid screening of HPV genotype 16 especially in resource-limited setting. Further studies using clinical specimens to evaluate assay performance are in progress.

Keywords: **Loop-mediated isothermal amplification (LAMP), human papillomavirus 16 (HPV 16), oral squamous cell carcinoma (OSCC)**

Cytotoxic Effect of *Phaleria macrocarpa (scheff.) boerl,* on Human Colon Cancer Cell Line (HT29)

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Purpose: Natural or dietary compounds are believed to have potent anticancer activity, low toxicity and cause very few adverse side effects *Phaleria macrocarpa (scheff.) boerl,* (PM) or commonly known as Mahkota Dewa are believed to have phytochemical compounds such as phenolic compound and antioxidant activity that can retard various cancers activity, thus it has potential to kill and inhibit proliferation of the human colon cancer cells HT29 cells. The aim of the experiment was to study Inhibitory Concentration dose (IC50) of *Phaleria macrocarpa (scheff.) boerl,* crude fruit (WF) and seed (WS) water extracts on HT29 cell line and to observe the morphological changes. **Methods**: The Inhibitory Concentration dose (IC50) of *Phaleria macrocarpa (scheff.) boer*l, (PM), WF and WS extract were assessed by Tryphan Blue Viability Assay (TBVA) and morphological changes were observed under microscope. **Results**: WF and WS were shown to inhibit cell growth in a dose dependent manner with IC50 of 100µg/ml and 160 µg/ml respectively. Brief morphological observations under light microscope at different magnification demonstrated the presence of apoptosis mechanism. The morphologic feature of apoptosis can be easily seen especially the presence of apoptotic bodies and detachment of HT29 cells from its surrounding clusters. Other hallmarks of apoptosis observed are shrinkage of the cytoplasm and convolution of outlines. **Conclusion**: *Phaleria macrocarpa (scheff.) boer*l is a potential alternative to be further studied for its anti-cancer properties.

***Keywords: Phaleria macrocarpa (scheff.) boerl*, cell viability, anti-cancer, apoptosis**

Image Driven Pharmacokinetics Using Molecular Imaging for Cancer Nanotherapeutics

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Purpose: The physical properties of nanocarriers such as size, charge, shape and surface chemistry plays an important role in its *in vivo* distribution. To facilitate biodistribution analyses, it is advantageous if the circulation time and organ accumulation of nanocarriers could be visualized non-invasively *in vivo* in real time. A molecular imaging technique that is both sensitive and quantitative will allow the biodistribution of the constructs to be tracked for up to a few days allowing for real-time biodistribution data in an intact living system to be obtained and quantified. **Methods**: A series of control protein polymers and fusion proteins of low and high molecular weight was expressed. Using a bifunctional chelating agent and after subsequent complexation with Cu-64, the biodistribution and microPET imaging studies was performed in mice with palpable MDA-MB-231 tumors at multiple time points. **Results**: Small micelles and linear polypeptides exhibited significant liver and renal accumulation respectively. Passive accumulation in tumors via the EPR effect or through specific association of fusion proteins with the integrin receptors upregulated on the tumor was observed. **Conclusion**: The outcome of this study reinforces the importance of size in determining the blood half-life of nanocarriers used. In addition inclusion of a targeting moiety confers specificity and selectivity to the construct towards tumors. Thus, through the use of molecular imaging it is thus possible to non-invasively optimize carrier characteristics for the delivery of therapeutic drugs and proteins.

Keywords: **Biodistribution, molecular imaging, pharmacokinetics, protein polymer, positron emission tomography**

Evaluation of a Multiplex PCR Assay for the Detection of Porcine DNA in Meat-Based Food Products

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Purpose: Pork adulteration continues to be a major concern to the consumers due to health and ethical issues. A gel-based multiplex PCR assay was successfully developed to detect porcine DNA in meat-based food products. The assay detection limit is 1 pg DNA per reaction and 0.5% of adulterated meat. In this study, the assay was further evaluated with processed meat products available in the local market around Northern region of peninsular Malaysia. **Methods**: 0.1g of meat samples were homogenized overnight in 1 mL DNA extraction buffer and further subjected to 200-fold dilution prior to DNA amplification. The PCR amplify two targets 1) long interspersed repetitive element from *Sus scrofa* and 2) a mitochondrial gene conserved in all vertebrates. The resulting PCR amplicons were separated in 2% agarose gel and visualized under 302nm UV illumination. **Result**s: A total of 45 samples of processed meat products were analyzed. Results showed 100% sensitivity and specificity. The processed meats containing pork showed positive results whereby two amplification bands were observed. The remaining 41 samples gave only a single amplification band indicating the PCR reactions were valid, suggesting no adulteration occurred in the tested commercial product samples. **Conclusion**: Despite the presence of PCR inhibitor in processed food, the in-house DNA extraction method included in the assay allowed accurate porcine DNA detection. The simple and sensitive multiplex PCR has proven to be a reliable tool for detection of pork contamination in meat-based food products particularly in inspection program to enforce labeling regulation in food industry.

Keywords: **DNA extraction, food adulteration, multiplex PCR, porcine DNA**

β-Carboline: A New Potential Anticancer Agent for Leukemia

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Purpose: β-carboline has been widely investigated for various health indications, one of which is the recently discussed anticancer. Current antileukemic therapeutics are still insufficient due to low efficacy, low selectivity and severe side effects. Hence this study investigated potential anticancer activity of β-carboline derivatives *in vitro*. **Methods**: β-carboline derivatives were synthesized using various substitutions and tested using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for anticancer activity and selectivity index against four cancer (human leukemia, colon, cervical and liver) and two non-cancerous (human foreskin and mouse embryo fibroblasts) cell lines. Mode of death was evaluated using MTT, acridine orange and ethidium bromide (AO/EB) fluorescence staining and caspases 3/7 assay, followed by cell cycle analysis using flow cytometry. Mutagenicity was further tested through Ames test using *Salmonella typhimurium* strains. **Results**: Based on the IC_{50} values of the primary and secondary screenings, derivative CDR007 was selected for its potent and highly selective inhibitory activity against K562 human leukemia cell line. Evaluation of the mode of cell death has found that CDR007 acted as a cytostatic agent through caspases-independent apoptosis. Flow cytometric analysis of cell cycle progression induced by CDR007 demonstrated G_0/G_1 phase arrest. Furthermore, mutagenicity test indicated that CDR007 is non-mutagenic in the presence and absence of metabolic activation system. **Conclusions**: This study supported that CDR007 has promising outcome in leukemia treatment, thus should be further explored. More studies on the mechanism of action of CDR007 and its genotoxicity effect should be conducted for a more comprehensive understanding of its potential.

Keywords: **β-Carboline, leukemia, apoptosis**

Modulatory Effects of *Clidemia hirta* Against Carbon Tetrachloride (CCl_4) - Induced Fulminant Hepatic Failure and Necrosis in Mice

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Purpose: Abundance of local herbal plant with varies of phytochemicals was known due to their beneficial effect in conventional medicinal practice. This study assesses the phytochemical constituent (Screening, Total Phenolic & Total Flavanoid) and antioxidant activity of the *Clidemia hirta* and its effect against carbon tetrachloride (CCl_4) - induced liver injury in mice. **Methods**: The total phenolic content was determined according to the Folin–Ciocalteau method. Total flavonoids content was determined by using the Aluminium chloride colorimetric method. The determination of antioxidant activity was done according to the method of free radical scavenging of DPPH (2,2-diphenyl-2-picrylhydrazyl). The standard methods will be adopted for the preparation of tissue fractionations for biochemical estimation. **Results**: The presence of saponin, flavanoid, steroid, tannins and cardiac glycosides were positively found in all respective qualitative test conducted. Total phenolics content was 62.184 ± 0.211 milligrams of gallic acid equivalent (GAE). The total flavonoid content was 91.889 ± 2.940 milligrams of catechin equivalents (CAE).The inhibition concentration (IC_{50}) for *Clidemia hirta* was 45.481µg/ml. The biochemical results will be discussed. **Conclusion**: *Clidemia hirta* showed significantly high antioxidant activity together with high phenolic and flavanoid content. It exhibits potent antioxidant ability that can be observed from the high free radical scavenging efficiency.

Keywords: **Antioxidant, carbon tetrachloride, hepatic injury, chemoprevention**

Association between Mammographic Breast Density and Age in the Multi-Ethnic Population Malay, Chinese and Indian in Malaysia

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Purpose: Dense breast tissue increases the risk of breast cancer. Young women and premenopausal status are generally associated with dense breast tissue. It is assumed that breast density gradually decreases after menopause [1]. Genetic and environmental factors may be the reasons of the differences in breast density among races [2]. This study was aimed to assess the association between the mammographic breast density and age in the three main ethnics in Malaysia namely Malay, Chinese and Indian. **Methods**: Subjects were selected from women with a range of age between 35 to 73 years who underwent diagnostic or screening mammography at National Cancer Society Malaysia. Mammograms were obtained with dedicated Hologic 3D Selenia Dimensions mammographic unit. This study focuses on dense breast subjects, hence only patients of BI-RADS category 2, 3 and 4 were selected and categorized by the radiologists depending on the radiographic appearance of the breast on mammography. **Results**: Breast density appears to be greater in women between ages 40 to 49 years (46.30% and 58.82% for BI-RADS 3 and 4 respectively). Women between 50 and 59 years old show a lower density (43.75% for BI-RADS 2). There is an inverse relationship between patient age and mammographic breast density. For ethnic or racial comparison, Chinese women appear to be mostly in BI-RADS 4, while Indian women are mostly in BI-RADS 2. **Conclusion**: In the evaluation of potential benefit of extended imaging for breast cancer screening, breast density should be considered especially for women at increased risk for the disease.

Keywords: **Mammography, breast density, BI-RADS, age**

Chemopreventive Effect of Hydrazone Derivative on Cervical Cancer Cells

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Purpose: Cervical cancer is one of the common cancer of female reproductive organs. Synthetic chemical derivatives such as hydrazones are widely been used in cancer research. This study is to investigate the chemopreventive effects of the synthetic hydrazone derivative on cervical cancer cells. **Methods**: IC50 value had been determined and cell proliferation assay was done with Hydrazone derivative on cervical cancer cells. Cytotoxicity test was performed on normal mouse skin fibroblast cells. Apoptotic and cell cycle activities were analyzed with flow cytometry. Western blot analysis been done in few selected proteins. **Results**: The results showed an inhibitory concentration (IC50) of the compound at 0.03mg/ml in a dose and time-dependent manner on the cervical cancer cells. The hydrazone derivative inhibits the proliferation of cervical cancer cells in comparison to the untreated control. In addition, the hydrazone derivative causes S and G2/M phase cell cycle arrest without induction of apoptosis.The western blot analysis showed the treatment had up-regulated the expression of p-NF-kB and cyclin E2 proteins and down-regulated the $p27^{kip1}$ and PCNA proteins expressions. **Conclusion**: In conclusion, the findings in this study suggested that the hydrazone derivative arrest the cell cycle and act as chemopreventive in cervical cancer cells.

Keywords: **Chemoprevention, hydrazone, cervical cancer cells**

Loss of *Noxa -/-* Delays Incidence and Growth of Melanoma in a Mouse Melanoma Model

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Purpose: To evaluate the contribution of the intrinsic apoptosis pathway to melanoma biology in a genetically modified mouse melanoma model. **Methods**: We utilized a mouse melanoma model; *Cdkn2a-/-, Tyr-HRAS^{G12V}*; ("control cohort") which was crossed to either a *Noxa* or a *Puma*-knockout line resulting in *Cdkn2a -/-, Tyr-HRAS^{G12V}, Noxa -/-* ("*Noxa -/-* cohort") or *Cdkn2a -/-, Tyr-HRAS^{G12V}, Puma -/-* ("*Puma -/-* cohort"). Each mouse was monitored for well-being and melanoma growth for 240 days. **Results**: We hypothesized that *Noxa -/-* or *Puma -/-* will lead to faster appearance and invasive growth of melanoma. Mice from all three cohorts developed melanomas in the ears, trunk, tail, foot and anus. Melanoma penetrance was high in the control (92%) and *Puma -/-* (100%) cohorts but is low in the *Noxa -/-* (89%) cohort. Furthermore, melanoma onset in the *Noxa -/-* cohort was significantly delayed as compared to the *Puma -/-* ($p < 0.05$****) and control ($p < 0.05$***) cohorts. Growth of melanomas was slower and the number of melanomas per mouse is also decreased in the *Noxa -/-* cohort. Interestingly, a number of mice in the *Puma -/-* cohort developed intracranial tumours. **Conclusions**: Contrary to our hypothesis, *Noxa -/-* cohort exhibited decreased melanoma penetrance, delayed melanomagenesis and decreased number of melanomas. Melanomas also grew slower. We hypothesize three possibilities for the delay in melanomagenesis in the *Noxa -/-* cohort (1) overcompensation by more potent BH3-only proteins; (2) down-regulation of the anti-apoptotic proteins and (3) a concerted effect between two BH3-only proteins.

Keywords: **Melanomagenesis, oncology, dermatology**

In Vitro the Cytotoxic Activity of *Orthosiphon stamineus* (Misai Kucing) Standardized Ethanolic Extract in Combination with Chemodrug on Pancreatic Cancer Cell Lines

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Purpose: Gemcitabine is used as the first line of treatment for pancreatic cancer. Unfortunately, most patients with advanced pancreatic cancer develop resistant towards gemcitabine hence the need to find new complementary therapies. *Orthosiphon stamineus* (*O.s*) is a medicinal plant which is widely used as traditional medicine in South-East Asia for rheumatism, tumorous, diabetic blindness and psoriasis. However, not much is known about the use of *O.s* as complementary medicine in cancer treatment. The present study aims to investigate the complementary effect of *O.s* with gemcitabine in pancreatic cancer cell lines. **Methods**: The cytotoxic activity of *O.s* (standardized ethanol extract) was assessed through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay at 72 hrs and cell colony formation assay over a period of 10 days, either as stand alone or in combination with gemcitabine in Panc-1 and MiaPaCa2 pancreatic cancer cell lines. **Results**: *O.s* did not exhibit cytotoxic effect in Panc-1 and MiaPaCa-2 pancreatic cancer cell lines. Nevertheless, *O*.s sensitised pancreatic cell lines to gemcitabine treatment by reducing cell viability and cell survival. Combination treatment using *O.s* and gemcitabine was synergistic in yielding greater growth inhibition in Panc-1 and MiaPaCa-2. In addition, *O.s* and gemcitabine combination significantly reduced pancreatic cancer cell colony survival. **Conclusion**: *O.s* sensitised pancreatic cancer cells to gemcitabine treatment. More work is being carried out to study the mechanism of action of O.s and gemcitabine in pancreatic cancer *in vitro* and *in vivo*. This could pave way for novel strategies in complementary treatment of pancreatic cancer.

Keywords: **Gemcitabine, misai kucing, pancreatic cancer**

Retroperitoneal Sarcoma-Rare But Relevant

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Retroperitoneal sarcoma is a rare tumor which only account for 1-2 % of all solid malignancies. Generally it carries the poor prognosis because it present late. Patient usually presented with abdominal pain, abdomen distension, loss of weight and loss of appetite. The CT imaging remained the gold standard investigation in the management of retroperitoneal sarcoma. The ultrasound guided or CT guided biopsy remain controversial. Some surgeon suggest that imaging guided biopsy should only performed in the selective cases that biopsy result will make the different for the entire management plan, example retroperitoneal lymphoma, metastatic lymph node from the testicular malignancy. The main treatment for the retroperitoneal sarcoma is complete surgical en- bloc resection (R0). However, most of the time it is difficult to achieve as the tumor invades into adjacent structure likes inferior vena cava, abdominal aorta, pancreas, colon and others. Multidisciplinary discussion is useful when retroperitoneal sarcoma resectability in question. Adjuvant chemotherapy and radiotherapy not show the encouraging results in the recent clinical trials. The novel study such as targeted therapy will be the future direction. Phase 1 trials reported to date with 2 targeted therapies show favorable toxicity profiles, making this strategy a feasible and promising issue to be explored. Future genetic study, identification of new pathways and their correspondent inhibitor and the arise of innovative agents such as oncolytic viruses, make the final endpoint of cure sarcomas a goal not so far to be reached.

***Keywords*: Retroperitoneal sarcoma, radiotherapy, targeted therapy**

Biocomputational Genome-Wide Analysis of sRNA In *Leptospira interrogans serovar Lai*

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Purpose: Leptospirosis is an endemic disease in Malaysia. *Leptospira interrogans* serovar Lai, is a pathogenic spirochetes bacteria known to be the causative agent of leptospirosis. Most of the *discovered coding genes related to adhesion, invasion and the haematological changes that characterize leptospirosis were reported in the complete genome deposited in NCBI*. However, to date, only a few candidates of non-protein coding RNAs (sRNA) has been reported in *Leptospira interrogans* serovar Lai. sRNAs in bacteria are important as they involved in various cellular regulating roles including environmental adaptation and regulation of virulence and pathogenicity. These sRNA could shed light into the pathophysiological function of the infection of *Leptospira Interrogans* serovar Lai. Biocomputational tools were selected for the discoveries of sRNA in *Leptospira Interrogans* serovar Lai. **Methods**: By integrating few sRNA prediction programs into a pipeline, whole-genome sequences were compared among Leptospira spp. to identify orthologs sRNA candidates. RNAz predict structurally conserved and thermodynamically stable RNA secondary structures in whole-genome sequence alignments as structural conserved RNA. nocoRNAc remove structural conserved RNA that overlaps with coding sequences and detect transcriptional features such as rho-independent termination signals (Transterm) and promoter region (SIDD). Potential sRNA candidate will bear a conserved RNA structured with transcription features. **Results**: A total of 215 sRNA (122 antisense to annotated genes; 93 overlap intergenic region candidates) were detected biocomputationaly in *Leptospira interrogans* serovar Lai. **Conclusion**: The computational pipeline has successfully predicted 215 candidate sRNA from pathogenic *Leptospira interrogans* serovar Lai. Expression profiling of these sRNA will be further experimentally validated.

Keywords*: sRNA, nocoRNAc, RNAz, *Leptospira interrogans serovar Lai

In Vitro Studies of Surface Modified PLA Microspheres Treated with NaOH

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Purpose: Poly(lactic) acid (PLA) is well known as therapeutic drug vehicle because of its properties including biocompatibility, biodegradability and has good mechanical properties. But, its hydrophobicity is not preferable for drug delivery systems (DDS). Thus, surface modifications through Sodium Hydroxide (NaOH) hydrolysis at different concentrations had been made to make it hydrophilic by introducing –OH functional groups. All surface modified microspheres had been examined for morphological changes in terms of surface roughness and wall thickness prior to in vitro studies through cells attachment, cells migration and cells differentiation. **Methods**: Prior to the completion of PLA microspheres surface modifications, cross sections had been done through microtome to observe the difference in PLA morphology for each concentration of NaOH used. For in vitro studies, three different assays had been performed; cells attachment, cells migration and cells differentiation. All these studies were conducted to observe the interaction of the cells towards surface modified PLA microspheres treatments. DAPI fluorescent staining was used for cells attachment study, colcemid treatment for cells migration and osteogenesis differentiation kit for osteogenic differentiation of mesenchymal stem cells (MSCs) in tissue-culture vessels. **Result**(s): As the concentration of NaOH used to modify PLA microspheres increases, structure roughness and porosity increases. Changes from hydrophobic to hydrophilic property also enhances in cells studies as increase in cells attachment, cells migration and cells differentiation were observed. **Conclusion**(s): Surface modified PLA microspheres with NaOH enhances the cells attachment, cells migration and cells differentiation and non-toxic for biological environments.

Keywords*: Poly(lactic acid), surface modification, hydrophilicity, *in vitro

Solid State Sintering Approach on Producing Porous Tricalcium Phosphate Bioceramics

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Purpose: Porous Tricalcium Phosphate (TCP) is recognized in biomaterials research due to excellent biocompatibility, biodegrability and bioresorbability. However, the processes on producing porous β-TCP are complicated and costly. Therefore, a new method of solid state sintering was conducted to overcome the problems. **Method**: TCP bioceramic was prepared via solid state reaction and pressed into pellet form at 20 MPa. The sintered pellets (1100°C to 1400°C) were characterized by X-ray diffraction (XRD), density and porosity measurement, diametral tensile strength test (DTS) and scanning electron microscopy (SEM) evaluation. **Results**: β-TCP (β-$Ca_3(PO_4)_2$) phase was maintained at 1100°C and 1200°C whilst α-TCP (α- $Ca_3(PO_4)_2$) phase had formed as the second phase at 1300°C and 1400°C. The highest apparent porosity is measured at 1100°C which is 60.06% and with density of 1.19 g/cm^3. Besides, the DTS values are in the range of 0.77 to 2.38 MPa which lower at the low sintering temperature. Interconnected pores were observed at the fracture surfaces of the sintered pellets, and strut is thick which would provide higher DTS value. **Conclusion**: TCP bioceramics with interconnected pores was produce via solid state reaction.

***Keywords*: Porous; tricalcium phosphate; bioceramics; interconnected pores**

The Effect of Murine Norovirus-1 (MNV-1) Infection on the Foam Cell Formation

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Purpose: To investigate the effect of Murine norovirus (MNV-1) infection to the foam cell formation. **Methods:** Genetically defined MNV-1 strain CW1 was produced using an established reverse genetics technique. Mouse primary macrophage cells and mouse leukemic macrophage (RAW264.7) cell lines were infected with MNV-1 (0.01 MOI) and treated with oxLDL (80 μg/ml) for 6, 12, and 24 hours. The treated cells were then stained using oil red O to assess foam cell formation. Total RNA were also isolated from treated cells and were reverse-transcribed into cDNA and further subjected to PCR to detect the presence of MNV-1. **Results:** OxLDL treatment on mouse primary macrophages were accelerated the formation of foam cell formation over time but not in RAW264.7 cells. After 12 hours post-infection, MNV-1 were eliminated almost 70% of both type of cells. The similar findings were also observed on both type of cells that have been treated with combination of MNV-1 and oxLDL. **Conclusion:** OxLDL stimulated foam cell formation in primary macrophages but not in RAW264.7 cells. The elimination of both type of cells after 12 hours of treatment of MNV-1 alone and combination of MNV-1 and oxLDL need further investigation as the role of MNV-1 infection on the foam cell formation is still unknown.

***Keywords*: MNV-1, RAW 264.7, primary macrophages, foam cells, oxLDL, oil red O**

Cell Evaluation of HA/TCP as Bone Substitution

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Purpose: In this study, the application of HA/TCP as 3D scaffolds was evaluated *in vitro* in order to examine for any adverse effect of using the material in the grafting. **Methods**: The 3D scaffold of HA/TCP with ratio of 20:80 and 70.30 was produced by using a cylindrical mould which gave into 1.6cm in diameter pellets. The toxic properties of the scaffold were evaluated by testing the scaffolds on IMR-90, normal human fibroblast cell. The cell viability assay was carried out at 24h, 48h and 72h time point using Presto Blue Cell Viability Reagent™. The morphological changes of the cell were also observed, which was focusing on the any formation of the vacuolization of the cytoplasm, cell membrane integrity using inverted microscope. The capability of the cell to attach on the pellets' surface also was observed after 21d incubation period by using SEM imaging. Fixation with 10% glutaraldehyde was done for SEM imaging. **Results**: Both ratio of HA/TCP pellets were non-toxic to the IMR-90, which reflected by more than 50 percent of cell viability percentage. Besides no toxic properties, the cell viability graph showed an increment pattern in cell growth. This data was reflected with normal cell morphology observation. In SEM imaging, there were some cell attachment to the pellets' surface was observed. **Conclusion**: This study suggesting that the HA/TCP might have osteoconduction properties, thus enhance the cell-attachment at the surface of the scaffold.

***Keywords*: Bioceramic, HA/TCP, 3D scaffold, bone substitution, cell evaluation, cell toxicity**

The Relationship between Anti-Adipogenic and Anti-Carcinogenic Effects of Fisetin via Activation of CCN2/TGF-β in 3T3-L1 and SK-MEL-28 Cells

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Purpose: Fisetin can be found mainly in trees and shrubs of Fabaceae and Anacardiaceae family. Although, several biological activities have been reported, precise role and molecular mechanisms of fisetin in anti-adipogenic and anti-carcinogenic effects are still unclear. Here, our study was focused that the evaluation of fisetin in 3T3-L1 adipogenesis and melanosis of SK-MEL-28 and elucidation of molecular relationship between anti-adipogenic and anti-carcinogenic effects of fisetin. **Methods**: The cytotoxicity was evaluated by MTT assay and the effect of fisetin on the 3T3-L1 adipogenesis was examined by Oil Red O staining. The mRNA expression levels of transcriptional factors were evaluated by RT-PCR analysis. The proteins levels were examined by western blotting. **Results**: The concentrations of 10 to 100 μM fisetin treatments for 24 h did not affect to the viability. Thus, up to 100 μM of fisetin was chosen for further studies. The treatment of 1 to 100 μM fisetin dose dependently inhibited 3T3-L1 adipogenesis by 1.31%, 19.04%, 62.31%, 75.44% and 101.02%, respectively when compared to mature adipocytes. The down-regulated mRNA expressions of transcriptional factors were observed in fisetin-treated 3T3-L1 cells. We also observed that the fisetin treatment significantly inhibited melanin synthesis in malignant SK-MEL-28 melanoma. In addition, we elucidated that the suppression of both adipogenesis and melanosis by fisetin treatment was mediated by activation of CCN2/TGF- β signaling pathway. **Conclusion**: These results suggest that the fisetin has the potential as natural functional foods and drug in prevention for obesity and cancer.

***Keywords*: CCN2, TGF- β, fisetin, adipogenesis, melanosis**

Radiation Dose Assessment to the Adult Patients Undergo Repeat PET/CT Examination

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Purpose: Patient undergoing Positron Emission Tomography Computed Tomography (PET/CT) has a possibility to repeat PET/CT examination on the same day of appointment. With a single combined PET/CT examination, patient exposed to CT and PET radiation dose. While for repeat PET/CT scan cases on the same day, patient received extra CT radiation dose. Radiation dose assessment needs to be done. **Methods**: Number of 85 patients was collected within 3 month period. Criteria patient for this study include patient age of 17 years above and diagnose with suspected metastasis cases. Effective dose from PET scan was calculated using dose coefficient reported in International Commission on Radiological Protection (ICRP) Publication 106 for F18 FDG while effective dose from CT scan was determined using k coefficient reported in ICRP publication 102 and Dose Length Product (DLP) value. **Results**: The mean effective dose result in single scan from PET and CT were found to be 6.99±1.53mSv and 9.50±4.81mSv respectively. Repeat PET/CT scan which contribute average effective dose from CT were found to be 6.75±3.81mSv. The mean whole-body effective dose received by patient undergoing the combined PET/CT examination was 23.24mSv. These results shows that radiation dose to the patient increased with almost 29% from a single combined PET/CT examination which 16.25mSv. **Conclusions**: The increase of radiation dose to the patient need to be considered by the physician and physicist. This result could be as reference for dosimetry of patient undergoing PET/CT examination in Malaysia.

***Keywords*: Radiation dose assessment, F18 FDG, effective dose, repeat PET/CT scan, adult**

Estimation of Radiation Dose Exposure and Remaining of ^{18}F-FDG Radioactivity in Patient Body Before Discharged

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Purpose: Administration of Radiopharmaceutical Fluorine-18 Fluoro-deoxyglucose (^{18}F-FDG) is considered as outpatient cases based on diagnostic activity less than 1100MBq. Although the activity of ^{18}F-FDG given to the patient less than 1100MBq, radiation dose to the public shall be less than 0.5 uSvhr^{-1} and comply with as low as reasonably achievable (ALARA) principle. The aim for this study is to estimate radiation dose to the patient based on patients excretion activity after received intravenous injection of ^{18}F-FDG and remaining ^{18}F-FDG left in the body before discharged from the PET/CT centre. **Methods**: Number of 55 patients for this study. The measurement was done on patient's abdomen body surface and at 1 meter set distance from anterior patient body. Calibrated portable survey meter was used to measure dose rate exposure from the patients. This procedure was done before patients leave the centre. **Results**: The mean administered of ^{18}F-FDG was 378.37MBq±80.78. The dose rate measurement at 1 meter distance and abdomen body surface was 7.96uSvhr^{-1}±2.93 uSvhr^{-1} and 127.43uSvhr^{-1}±47.68uSvhr^{-1} respectively. Duration spending time at this PET/CT centre after received the F18-FDG intravenous injection was around 189.85min±40.35min. Estimation of patient excretion was 31.47MBq±19.89MBq. It was found that the increasing of ^{18}F-FDG administered by interval of 50MBq and these will increase patients' excretion of 3.7MBq. Within 7.31hr after received the injection, at 1 meter distance and abdomen body surface, the radiation dose rate estimate to be reduced from 7.96uSvhr^{-1} to 0.5uSvhr^{-1} and 14.61hr to 0.5uSvhr^{-1} respectively. Patient recommended staying away from children after 12 hours from receiving the injection. **Conclusions**: The radiation exposure to the public is lower and safe.

***Keywords*: Radiation dose assessment, F18 FDG, effective dose, PET/CT**

Determination of Cellular Uptake Using Biodegradable Nanoparticles

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Purpose: The efficiency of drug delivery depends on the physicochemistry of the carrier and the interaction between carrier and tissues in the gastro intestinal tract. *Phyllanthus watsonii*, which is locally known as dukung anak, are found in tropical forests in Malaysia. The major constituents of the extracts are geraniin. Extracts from the plants have been associated with anti-hypertensive, anti-viral and anti-diabetes properties. However, the bioavailability of the phytochemicals was often hampered by the challenges during the delivery and cellular uptake. This research aims to study endocytosis based on the nanocarrier properties and their relations to tissue organisation. **Methods**: Polymeric nanocarriers (PEG-PLGA) were synthesised using double emulsion method. CRL1790, normal colon human epithelial cells were used as the cell model. Other experiments had done include controlled released, cell viability and cellular uptake activities on biochemically micropatterned surfaces. **Results**: The mean size of nanoparticles (NP) produced were 132 nm with zeta potential value of -39.8mV. The PDI value of this NPs is 0.237. Control release study shows the extracts in NPs were released within 2.5 hours. The presence of 1 to 5ug/ml NPs containing the extract shows no effect on cell viability. Images of SEM showed cells can interact with the nanoparticles. **Conclusions**: The PLGA-PEG NPs have been synthesised with optimal size and low polydispersity. The NPs with and without the extract also provide an acceptable control release and cell viability.

***Keywords*: Polymeric nanoparticles, cell viability, cellular uptake**

Role of UV Protection Agent against Skin Cancer

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Purpose: Skin cancer is the most common form of cancer and greater than 90% of skin cancer cases are due to excessive skin exposure to ultraviolet radiation from the sun (Sheehan *et al*, 2002). Reducing exposure towards ultraviolet (UV) A and B radiation in range between 290nm to 400nm via the application of sunscreen appears to be effective methods in preventing skin cancer. **Methods**: In this study, three commercial sunscreens with the same value of SPF (sun protection factor) 50 are chosen. These sunscreen samples were defined as sample I, sample II and sample III. Each sunscreen samples was applied homogenously on a glass slides using spin coating technique before tested their UV protection ability. The UV transmission of each samples were measured through UV-VIS spectrophotometer. **Results**: In this experiment, two values of transmission were observed at wavelength 330 nm (UVA wavelength) and 300 nm (UVB wavelength). For the transmission at wavelength 330 nm, sample II shows the best UV protection ability with the value of transmission of only 0.09%, while sample I and III with transmission of 4.37% and 0.23% respectively. For the transmission at wavelength 300 nm, again sample II shows the best UV protection ability with the value of transmission of 0.03, while sample I and III with transmission of 1.49% and 0.16% respectively. **Conclusions**: The reported data supports the function of sunscreen as a UV protection agent and thus, has a high ability in reducing the risk of skin cancer due to solar UV radiation.

***Keywords*: Radiation, sunscreens, transmission, UV protection**

DNA Biosensor : Regulatory T Cells Intracellular Staining

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Purpose: Regulatory T cells (Tregs) play a vital role in maintaining immune homeostasis and peripheral tolerance. However, the role of Tregs in cancer has increasingly been recognized as a key component in suppression of effector cells, which leads to development of cancer and is now a major challenge for tumour immunotherapy. Forkhead box P3 (FOXP3) has been identified as the master regulator of Tregs function but it is located intracellularly. This renders in depth studies on Tregs and its plasticity near impossible as isolation of Tregs would require permeabilizing these cells in order to stain for FOXP3. In order to allow intracellular staining that would allow for the isolation of live cells, we aim to develop a nanocluster probe specifically for detection of FOXP3 for identification of Tregs. **Methods**: Here we proposed an optimized method for the detection of single-stranded and double-stranded synthetic oligonucleotides of FOXP3 target by hybridization of the silver nanocluster probe to its target. The hybridised samples were viewed under UV illuminator and fluorescence intensity was measured by fluorescence spectroscopy. **Result**: The system forms a three-way junction by successful hybridization of AgNC, G-rich strand (G-rich) to the target FOXP3, which generated a shift in fluorescence spectra with a marked increase in fluorescence intensity. **Conclusion**: This silver nanocluster system were able to detect FOXP3 target sequence successfully with a shift in excitation and emission wavelengths of AgNC.

***Keywords*: Silver nanocluster (AgNC), DNA detection, fluorescence shift**

Aerosol-Based Mesenchymal Stem Cell Delivery Reduces Airway Inflammation and Histopathological Changes in Animal Model of Chronic Lung Injury

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Purpose: This study was aimed to determine the efficacy of aerosol-based mesenchymal stem cell (MSC) delivery in the setting of chroninc lung injury. **Methods**: For chronic lung injury model, the rabbits were sensitized with both i.p and inhalation of ovalbumin. The MSC was aerosolized into rabbit lungs using the MicroSprayer® Aerosolizer Model IA-1B 48 hours after insult. Post mortem was performed 3 days following cell delivery. Histopathological assessments of the lung tissues and cellular engraftments were quantitatively scored following treatments. **Results**: Our results revealed that aerosolized MSC treatment significantly improved the remodelling of the airways as structural changes of the basement membrane, epithelium, mucosa, and submucosal regions of the airway that caused by the induction with OVA was significantly reduced in the MSC treated group ($p<0.001$). The airway inflammation score of MSC treated group indicated a significant reduction of the inflammation and granulocytes infiltration at the peribronchiale and perivascular regions ($p<0.05$). The MSC treatment also alleviated the number of airway inflammatory cells in the BALF and the goblet cell hyperplasia. The aerosol-based MSC cell delivery method was also resulted in the engraftment of MSC in the recipient lungs 3 days following transplantation. **Conclusions**: We have established an aerosol-based cell delivery as a feasible tool for cell therapy of chronic lung injury using the Microsprayer® Aerosolizer device. Our findings suggest that MSC cell delivery via aerosolization method promotes lung regeneration and repair, therefore can be a valuable tool for future therapy to treat chronic lung injury.

***Keywords*: Aerosol-based cell therapy, mesenchymal stem cell, chronic lung injury**

Synthesis, Characterisation and Evaluation of Silver Nanoparticles from *Pleurotus sajor-caju* on *Candida albicans*

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Purpose: Silver nanoparticle (AgNPs) is widely studied due to its antifungal and antimicrobial activities against broad range of microorganisms. The *C. albicans* is one of the pathogenic yeast that have been reported to be inhibited by AgNPs. Green method used to synthesise AgNPs also has been intensively studied due to its environmentally friendly and the used of non-toxic reducing agent. Therefore in this study, *Pleurotus sajor caju* mushroom or grey oyster mushroom extract was used to synthesis AgNPs. **Methods**: Characterisation of synthesised AgNPs have been carried out by UV-Vis spectrophotometer, dynamic light scattering analysis, Transmission Electron Microscope (TEM), Fourier Transform Infrared (FTIR) spectrometer and X-ray diffraction (XRD) analysis. Meanwhile, the antifungal activity of AgNPs on *C. albicans* was determined through broth microdilution technique. Then the study on inhibition of AgNPs on *C. albicans* has been extended by determining the impact of AgNPs on *C. albicans* isocitrate lyase gene (Ca*ICL1*) which encoded for isocitrate lyase enzyme which was important in glyoxylate cycle. **Results**: The formation of AgNPs has been confirmed by the color changed from pale yellow to reddish brown after 72 hours of incubation with 1mM $AgNO_3$. The mean particle size of AgNPs obtained was 11.68 nm with the zeta potential value is -8.54 mV. The FTIR spectra also suggested that the protein and polysaccharide in *P. sajor caju* extract was responsible in reducing silver ion to AgNPs. Meanwhile, XRD spectra also showed that the nanoparticle is the face-centred cubic (fcc) structure of silver. Then, the minimum inhibitory concentration (MIC) of AgNPs was 250 mg/L while the minimum fungicidal concentration (MFC) was 500 mg/L. The impacts of AgNPs on the expression of Ca*ICL1* showed that the the gene was downregulated at 2 and 4 hour incubation and then upregulated at 6 hours incubation. **Conclusion**: The AgNPs is useful as antifungal agent to inhibit *C. albicans* growth. Thus, the mechanism of inhibition of AgNPs through genomic study may provide information for future development in AgNPs as potential antifungal drug.

***Keywords*: Silver nanoparticles, *Pleurotus sajor caju*, MIC, rt-qpcr**

Selection of Stable Carotenoid Hyper-producing *Xanthophyllomyces dendrorhous* Mutants through Chemical Mutagenesis

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Purpose: Recent studies of astaxanthin on its involvement in cancer prevention, enhancement of immune response and strong antioxidant properties, other than its traditional role as a pigmentation source in aquaculture, have attracted attention of researchers on its potential benefits to human being. The yeast *Xanthophyllomyces dendrorhous* is one of the most promising natural sources of astaxanthin. It produces astaxanthin through isoprenoid pathway. However, the astaxanthin content in wild type *Xanthopyllomyces dendrorhous* is very low. The objective of the study is to obtain a hyperproducer of astaxanthin from wild type *Xanthopyllomyces dendrorhous* through random mutagenesis by chemical mutagens. **Methods**: *Xanthophyllomyces dendrorhous* wild type strain DSMZ 5626 was used for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and ethyl methanosulfonate (EMS). The hyperproducing mutants were screened using β-ionone and selected mutants were used for carotenoid extraction and stability test. **Results**: Mutant colonies of yellow, pink and red colours were obtained from the originally orange wild type. MNNG gave better results as a mutagen as the mutants exhibited increase of astaxanthin content up to 100%, while EMS-mutated mutants did not show promising yield of astaxanthin and the rate of reverse mutation was very high. Stability study was carried out for 10 generations and two stable mutant strains (M34 and M39) were selected for further study. **Conclusion**: The hyperproducing mutants will not only satisfy the increasing demand of consumers for natural astaxanthin, it will also contribute to the competitiveness of natural astaxanthin versus synthetic astaxanthin in term of production cost.

***Keywords*: *Xanthopyllomyces dendrorhous*, astaxanthin, hyperproducing mutant**

Porcupine bezoar exhibit anti-cancer activities on Human Breast Adenocarcinoma cells (MCF-7)

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Purpose: Currently there were many treatments available to treat cancer patients, for instance chemotherapy, radiation therapy, immune therapy, and many more. Unfortunately, advances in cancer therapy have resulted in increasing numbers of survivors which left to deal with side effects of their treatments. Therefore, discovery of alternative treatment with minimal side effect is crucial. Thus, study on bezoar take place as there were no scientific studies about porcupine bezoar to this date in Malaysia. This lead to the premise that porcupine bezoar could have greater potential for the chemoprevention activities. **Methods**: MCF-7 cells were treated with bezoar for cell proliferation, cell cycle, apoptosis and qPCR analysis. Cell viability of treated and untreated MCF-7 was examined by Trypan Blue Exclusion Assay. The cellular morphology of MCF-7 cells was observed by phase contrast microscopy. Flow cytometry analyzed cell apoptosis with annexin V/ propidium iodide (PI) and cell cycle with PI. qPCR run in detecting gene expression involved in apoptosis and cell cycle arrest. **Result**(s): Based on the conducted study and experiments, it is evident that the bezoar treatment in human gingival fibroblast (HGF-1) cells didn't show cytotoxicity effect. Upon treated with optimized concentration of bezoar for 24, 48 and 72 hours, it shows inhibition of proliferation by 45.5, 31.25 and 37.1% respectively, significantly apoptosis and cell cycle arrest occurs. Bezoar treatment also induced apoptotic-liked in morphological changes. Upon qPCR analysis, its indicated MCF-7 undergo intrinsic pathway of apoptosis and arrest at G1/G0. **Conclusion**(s): Porcupine bezoar can be alternative treatment for cancer with minimal side effects. Yet furthermore insights need to be studied.

***Keywords*: Bezoar, Apoptosis, Cell cycle, Anticancer, MCF-7**

Cloning, Expression and Purification of Recombinant Human G6PD

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Purpose: This study aimed at cloning, expressing and purifying recombinant human G6PD and G6PD (Viangchan) using heterologous system. The expression and enzyme activity of both proteins were determined and compared. **Methods:** G6PD gene was PCR amplified using pOTB7 cloning vector (Dharmacon-). For G6PD Viangchan gene, a mutation at location 871 (G>A) was introduced by using QuickChange Site-Directed Mutagenesis Kit (Agilent-). Both purified amplicons were ligated to the expression vector pET26b (+), transformed into *E. coli* DH5α competent cells and the sequences of both plasmids were confirmed by full DNA sequencing. The plasmids were re-transformed into BL21 (DE3) cells for protein expression. The cells were induced with different IPTG concentration and incubated for several time points. SDS-PAGE and Western blot analysis were carried out to confirm the size and the presence of the proteins, respectively. The proteins were purified and quantified, and the enzyme activity assay was performed. **Results:** Full length G6PD (normal) and G6PD (Viangchan) genes were successfully cloned into pET26b (+). Heterologous expression of the proteins was done by induction with 1 mM IPTG for 18 hours at 25 °C. From 100 ml of culture, 28.94 µg of WT G6PD and 15.62 µg G6PD Viangchan proteins were obtained, respectively. 22mU/mL G6PD activity was detected in WT G6PD whilst 2.38 mU/mL in n G6PD Viangchan correspondingly. **Conclusion:** Recombinant human WT and Viangchan G6PD proteins were successfully produced and purified using heterologous expression system. As expected, the activity of recombinant WT G6PD was higher than the mutant G6PD (Viangchan).

***Keywords*: G6PD, cloning, protein purification, enzyme activity**

The Establishment of Callus and Cell Suspension Culture of Sabah Snake Grass (*Clinacanthus nutans*) for Cancer Studies

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Purpose: *Clinacanthus nutans* is a well-known medicinal plant where recently, the consumption of fresh leaves was reported to possess the ability to suppress cancer advancement at critical stages. This investigation aims to determine a suitable media for the production of friable callus in establishing cell suspension culture with the purpose to harness anti-cancer secondary metabolites. **Methods**: Friable callus was induced from *ex vitro* young leaves on solidified MS medium with 0.25mg/L 2,4-D and 0.75mg/L Kinetin after 8 weeks of culture. Three parameters (light intensities, orientation of explants and types of cytokinin) were studied to optimize the culture conditions for callus induction. **Results**: It was observed that full light and 16H photoperiod significantly increased cell biomass in comparison to the explants incubated in total darkness. Higher callus biomass was induced on the adaxial surface of leaf explants in contact with the culture media. Furthermore, 0.75mg/L TDZ was found as an effective cytokinin being used alongside 0.25mg/L 2,4-D for callus induction. Results indicated that explants cultured on MS medium with 0.25mg/L 2,4-D and 0.75 TDZ produced the highest cell biomass in comparison to Kinetin, Zeatin and BAP with the average of 0.592g. **Conclusions**: A suitable callus proliferation media is needed to allow rapid proliferation of callus. It was found that MS medium supplemented with 0.50mg/L 2,4-D and 1.00mg/L Kinetin achieved the highest callus growth index indicating this formulation as a suitable callus proliferation media. Further studies will involve the formulation of proliferation medium followed by establishment of cell suspension culture and tests on selected cancer cell lines.

***Keywords*: Callus culture, *Clinacanthus nutans*, plant cell suspension culture**

Anti-Proliferative Effect of Myo-Inositol (MI) on Androgen-Independent Prostate Cancer DU 145 Cells

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Purpose: Although Myo-inositol (MI) is known as an antitumor agent but the actual mechanism is still remain unclear. Therefore, in this study we try to elucidate the effect of MI on androgen receptor by using androgen-independent prostate cancer cell, DU145. In this first phase of the study, we aimed to identify the inhibitory effect of MI (synthetic compound) to DU145 and its anti-proliferative activity. **Methods**: DU 145 cancer cell line was treated with MI to determine half maximal inhibitory concentration (IC50). Cell concentration and cell viability of cancer cells treated with MI was analysed by Trypan Blue Exclusion Assay (TBEA). IC50 of this finding was used in the cell proliferation assay. **Results**: IC50 of MI on DU 145 was 2.5 mM/ml. The cell concentration for control is 32.18 $x10^4$ cells and plummeted to 17.04 $x10^4$ cells when treated with IC50 dose for 72 hours. Cell viability of cancer cells for cell proliferation assay was decreased to 28% after 8 days of treatment. **Conclusions**: These findings indicate that MI may inhibit proliferation of DU 145 via androgen independent pathway but further analysis will be done in elucidate the actual mechanism.

***Keywords*: Myo-inositol, DU 145, anti-proliferative**

Generation of RNA Aptamers against *Mycobacterium tuberculosis* Secretory Protein ESAT-6: A Preliminary Study

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Introduction: ESAT-6 is a secretory protein produced by Mycobacterium tuberculosis and is released in early stages of infection. It forms a heterodimer complex with another protein called CFP-10 and has been implicated with Mycobacterium sp. pathogenicity. Aptamers are chemical ligands made up of short nucleotides sequences that are able to bind to target proteins with high affinity and specificity. They are developed using a process called Systemic Evolution of Ligands via Exponential Enrichment (SELEX). Due to their chemical stability and high specificity against the target, aptamers have the potential to become very useful biological tools. **Objective**: The objective of this study is to develop RNA aptamers that bind specifically to ESAT-6 protein. **Methodology**: Eleven SELEX cycles were carried out using the N40-randomised RNA pool. Stringency of the binding reaction in each SELEX cycles was increased gradually by varying the amounts of protein, RNA pool and the competitor. The resulting RNA pool from the 11th cycle of SELEX was subjected to filter binding assay to assess its binding against ESAT-6 protein. **Results**: RNA pool was successfully derived from SELEX cycle 11. Filter Binding assay against the target protein at 800 and 1600 nM confirmed that binding enrichment of the RNA pool has occurred. **Conclusion**: Filter Binding assay suggested the presence of potential binders in the RNA pool. Further SELEX cycles will be carried out to improve the binding enrichment of the RNA pool and for sequence deconvolution. Sequencing will be carried out to identify the putative aptamer.

***Keywords*: Aptamer; *M. tuberculosis*; ESAT-6**

In Vitro Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine on Normal Prostate Cell Line

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Purpose: 2-Amino- l -methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is one of the most abundant mutagenic heterocyclic amines found in cooked meat. It is formed through heat-dependent condensation of creatinine, phenylalanine and sugar at levels ranging from non-detectable level up to 865 ng/day with human exposure level estimated to be less than 0.1 ppm. The objective of this study is to evaluate the carcinogenic effects of PhIP in different vehicle concentrations on normal prostate cell line, RWPE, at human exposure level. **Methods**: The cells were treated with the highest concentration of PhIP exposed in human, 10^{-7}M, which was diluted in three different concentrations of DMSO, namely 0.003%, 0.025% or 0.25%. The experiment was carried out with or without pooled human liver microsomes as bioactivation system. Cytotoxicity and cell proliferation effects of PhIP on normal prostate cell line, RWPE-1 were determined using MTS assay. Cell transformation assay were carried out to assess its carcinogenic transformation. **Results**: In MTS assay, no significant different is observed in cell responses in all DMSO concentrations tested. However, in cell transformation assay, all cells treated with 10^{-7} M in all DMSO concentrations with activation system showed anchorage-independent growth and colony formation. **Conclusion**: At human exposure level, PhIP can induce carcinogenic transformation in normal human prostate cell line, RWPE-1 albeit no effect on the cell proliferation.

***Keywords*: PhIP, RWPE-1, *in vitro* carcinogenicity**

Antiangiogenic Effects of Sophorolipids Extracted from Malaysian Palm Oil

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Purpose: In the present study, an attempt was made to screen the antiangiogenic activity of Sophorolipids (SLs) extracted from Malaysian palm oil via fermentation against human vascular endothelial cell line (EA.hy 926). **Method**: Palm oil is used to produce Sophorolipids in the presence of non-pathogenic yeast, *Candida bombicola* via fermentation. MTT Assay was performed to study the antiproliferative effects against EA.hy 926 cell line. Rat aorta ring assay and *in vitro* scratch wound healing assays were performed to study the antiangiogenic effect. **Results**: An IC_{50} of 114.27 ± 10.60 µg/mL were obtained in MTT Assay. Preliminary study also found that the extract inhibited growth of new blood vessels and inhibited the migration of EA.hy 926 cells in scratch assay pointing towards potential antiangiogenic effect.
Conclusion: The Sophorolipids showed moderate antiangiogenic activity and one of the possible mechanisms of action is by inhibiting the formation of new blood vessels.

***Keywords*: Sophorolipids, EA.hy 926, MTT Assay, rat aorta, scratch assay**

Dosimetric Study of Three Dimensional Conformal Radiotherapy (3D CRT) of Localized Prostate Cancer using a Custom Made Male Pelvic Phantom

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Purpose: Verification of dose delivery is a basic necessity of quality assurance in radiotherapy treatment. To verify the delivery of radiotherapy doses and dose distributions it is necessary to replicate the entire treatment process as accurately as possible in a way that allows the direct measurement of doses. A male pelvic phantom was constructed to investigate the treatment planning system; three-dimensional conformal radiotherapy (3D CRT) technique for localized prostate cancer treatment as well as the organ at risks (OARs); bladder, rectum and both femoral head using 6 MV photon energy. **Methods**: A CT dataset of a male pelvis was used to define the geometric boundaries of principle tissues – prostate, bladder, rectum and both femoral head. Dose measurements to the target and OARs were obtained from the thermos-luminescent dosimeters (TLD) and Gafchromic external beam therapy (EBT) 2 film. Points of interest were located throughout the dataset to identify appropriate placement of TLD chips. The center of the prostate was identified as the location for point-measurement with seventeen (17) holes to place the TLDs while another eighty three (83) holes were distributed among the organ at risks. A piece of EBT 2 film was located at the center of the 31 pieces of Perspex where the target was located. Teflon was used to construct both femoral head. **Results**: The pelvic phantom has been constructed with different density materials match well with typical tissues. All calibrated TLD and EBT 2 film displayed a linear response (R^2=0.983) with respect to the measured doses at d_{max} from 25 cGy to 250 cGy. **Conclusion**: The good agreement between planned and measured dose shows that the phantom is a useful and efficient tool for 3D CRT technique dosimetric verification.

***Keywords*: 3D CRT, dosimetric study, radiotherapy**

Health Promotion and Disease Prevention Study to Identify the Life Style Issues

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A research study was conducted to identify the life style issues in population in Chitral and Gilgit Baltistan in Pakistan and suggest Behavior Change through Communication (BCC). The life style issues identified included excessive intake of salty tea, a housewife and manhood syndrome. The life style of salty intake was a precursor of many diseases in the population including high blood pressure, gastritis and renal diseases, while housewife and manhood syndrome were identified as precursors of depression and suicide. Intervention methods suggested was behavioral change through communication. Community mobilization, initiation of Health Promotion and Disease prevention programs were suggested. Training of community mobilizers and health workers was undertaken to apply BCC interventions. At the same time, screening programs for high blood pressure were enhanced and mental health screening programs and e-health consultation programs were initiated in the community for the first time. The program is currently in the intervention phase and intended to bring about life style changes in increasing the community to reduce salty tea and enhance mental health to overcome depression and suicide.

***Keywords*: Life style, behavior change through communication (BCC), excessive intake of salty tea, a housewife and manhood syndrome**

Pets and Mental Wellbeing: Is There an Association?

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Purpose: To assess the association of pet keeping with mental well being among a subset of Karachi population. **Methods:** A cross-sectional study was conducted in 2015. A sample of n=550 were taken from general population accompanying the patients at the outpatient departments of public and private hospitals situated in Southern town of Karachi. Convenience sampling technique was used for selection of the participants. Self administered questionnaire was given to the participants. Questionnaire was based on validated PHQ 9. Data was analyzed using SPSS version 20, and associations were made using chi-square test. **Results:** Out of the n=550 participants, 48% were males. Mean age of the sample was 41+/- 12. Overall 23% of the sample was found to have scores less than 14 and greater than 5 on PHQ 9 validated tool signifying mild depression. Significant difference (p value 0.001) was observed in the mean scores of pet owners versus non pet owners. Higher mean scores 14+/- 3 was observed in people who had no pets. **Conclusion:** Participants who were pet owners were found to be more mentally stable as depression was found to be more common in non pet owners.

Transcriptome Analysis of *Proteus mirabilis* during Oxidative Stress Adaptation

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Purpose: Bacteria are well known for their fast adaptation to the stress condition. Bacteria undergos some changes in the gene expression which enable us to understand their adaptation to the stress condition. A transcriptome study is conducted to compare the differential gene expression between normal and oxidative stress condition of *P. mirabilis*. This study enables us to understand the adaptation of *P. mirabilis* during stress. **Methods**: *P. mirabilis* were cultured in Luria broth and the total RNA was extracted during exponential and oxidative stress which was induce using hydrogen peroxide. These extracted RNA was sequenced via Illumina HiSeq 2000 Platform. The obtained fastQ format transcriptome sequence was aligned with reference genome of *P. mirabilis* using Bowtie2. Differential analysis was conducted using DESeq and KEGG software was used to understand the metabolic pathways of selected genes. **Results**: From the DESeq analysis showed that 1693 genes are up-regulated and 1688 genes are down-regulated during oxidative stress condition. 79 genes are having equal expression at both conditions. Most of the phage protein and dimethyl sulfoxide reductase genes are up-regulated. Respiratory nitrate reductase, tetrathionate reductase, flagellar related proteins, tryptophan synthase and alkyl hydroperoxide reductase were shown to be down-regulated during oxidative stress. **Conclusion**: Interestingly, alkyl hydroperoxide reductase is down-regulated where these gene suppose to be up-regulated and most of phage protein is up regulated during oxidative stress. This is preliminary data obtained from bioinformatics analysis. Further research need to be done to deeply understand the adaptation of *P.mirabilis* in oxidative stress.

***Keywords*: *P. mirabilis*, differential expression, oxidative stress**

Over-Expression and Purification of an RNA Chaperone, Hfq Protein of *Proteus mirabilis*

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Purpose: Hfq is a RNA chaperone protein present in many bacterial species including *P. mirabilis*. It plays a vital role in virulence of various bacteria. Hfq exhibits its function by binding with npcRNA which will be needed for the *trans*-acting npcRNA, mRNA interaction. To gain further insights on the target npcRNAs that interact with this RNA chaperone, Hfq with high purity is needed. **Methods**: *Hfq* gene from *P. mirabilis* was amplified and cloned in to pET-28b+ vector. Ligated mixture was then transformed into TOP-10 cells. The transformed bacterial colony with recombinant plasmid was screened by antibacterial selection and confirmed by sequencing. The recombinant plasmid was transformed into *E.coli* BL21and induced expression with IPTG. The over expressed hfq protein was purified using Ni-NTA affinity chromatography. The best elution of the Hfq protein was actualized using 1 M of imidazole. **Results**: *Hfq* gene had been successfully cloned in to pET-28b+ vector. Recombinant plasmid was successfully transformed into TOP10 and BL-21. A highly purified Hfq protein was obtained and confirmed by SDS PAGE. **Conclusion**: In this study we successfully purified hfq protein from *P. mirabilis* for further RNA binding study. The recomninant protein will be applied in immune coprecipitation study, using total RNA from *P. mirabilis,* to fish our npcRNA that's co-function with hfq.

***Keywords*: *P. mirabilis*, Hfq, recombinant protein**

Construction and Cloning of Reporter-Tagged Replicon for I*n vitro* Replication Study of Murine Norovirus-1 (MNV-1)

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Purpose: This research aimed for construction and cloning of Nanoluciferase-tagged replicon system to be used *in vitro* replication study of murine norovirus (MNV-1). **Methods:** The Nanoluciferase reporter protein was engineered to be expressed as a fusion protein to MNV-1 minor capsid protein, VP2. Foot and mouth disease virus 2A (FMDV2A) sequence was inserted between the 5'end of Nanoluciferase gene and the VP2 start sequence for the co-translational 'cleavage' of the fusion proteins during transcript expression in cells. Nanoluciferase gene with FMDV2A amplification was performed through series of standard and overlapping polymerase chain reactions using templates pNL1.3 and pT7:MNV 3'Rz plasmids. The resulting amplicon was then cloned into three readily available backbones plasmids of MNV-1 cDNA clone; wild-type, NS7 frame-shift and NS7 active site mutants utilising the unique restriction sites SacII and Nhe1. Transformation was done using *E. coli* DH5alpha competent cells and the correct clones were screened using restriction enzymes analysis and confirmed by DNA sequencing. **Result:** Nanoluciferase-tagged MNV-1 cDNA clone for all backbones was successfully produced; pT7:MNV-NL2A-VP2-3'Rz-wt, pT7:MNV-NL2A-VP2-3'Rz-F/S and pT7:MNV-NL2A-VP2-3'Rz-ΔNS7. Restriction enzyme digestion analysis indicated the Nanoluciferase gene was successfully inserted into the specific site on the parental cDNA clone (complete MNV-1 genome) and further confirmed for correct sequence of the Nanoluciferase gene and FMDV2A via DNA sequencing (data not shown). **Conclusion:** This study has successfully produced Nanoluciferase-tagged MNV-1 cDNA clones which could potentially provide a robust experimental assay in assessing the viral RNA replication *in vitro* and further characterisation could be performed to assess its translational effectiveness.

Keywords: **Construction, cloning, murine norovirus–1, replicon system, nanoluciferase gene**

Ultrasound-Assisted Surfactant Enhanced Emulsification Microextraction Method Coupled with Gas Chromatography-Mass Spectrometry for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Sugarcane Samples

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Purpose: To develop a simple, efficient, fast and environmental friendly ultrasound assisted surfactant enhanced emulsification microextraction (UASEME) combined with gas chromatography-mass spectrometry (GC-MS) for detection of selected polycyclic aromatic hydrocarbon in sugarcane samples. **Methods**: 30µL toluene was injected into 10mL volumetric flask. Then followed by the addition of 15µL of Tween 20. Then, the sample solution was added into the volumetric flask. The upper part of the volumetric flask was covered with cap and turned up-side down. The sample was ultrasonicated for 2 min and the solution turned into cloudy mixture. After extraction process, two immiscible phases were formed and 20 µL of the upper layer was taken out using microsyringe. Finally the clean extract was diluted with 80 µL of methanol prior to GC-MS analysis. **Result**: There was no contamination of PHE and FLU in tap water samples, however PHE was detected in all nine sugarcane juice samples at concentration lower than LOQ value. **Conclusion**: The UASEME method was successfully applied for the extraction of PHE and FLU from sugarcane juice samples coupled with GC-MS detection. UASEME provides many advantages such as faster extraction time, good linearity, and low consumption of organic solvent. It offers good LODs and acceptable range of precision and recovery. This is the first work reports on the extraction of PHE and FLU using UASEME method from sugarcane juice samples in Malaysia. Thus, the proposed method can be contemplated as simple, efficient, and environmentally friendly method for analysis of polycyclic aromatic hydrocarbons (PAHs) compounds.

***Keywords*: Microextraction, polycyclic aromatic hydrocarbons (PAHs), sugarcane**

The Value of FDG PET-CT in the Assessment of Primary Extra Gastrointestinal Stromal Tumor of the Peritoneum and Omentum

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Purpose: Primary extragastrointestinal stromal tumor (EGIST) of the peritoneum and omentum is extremely rare. Being normally highly FDG avid, FDG PET-CT has the capability to screen for the possible underlying occult primary malignancy in the normally occurring sites along the gastrointestinal tract as well as the detection of distant metastasis to assist further in the management of this disease. **Method**: A case report to enlighten the natural history of EGIST and to illustrate the diagnostic potential of FDG PET-CT in evaluating EGIST. **Case Presentation**: A sixty six year old lady presented with lower abdominal mass and pain since May 2015. Early colonoscopic examination revealed multiple colonic polyps. HPE of the polyps revealed tubular adenoma; moderate dysplasia. CT scan in June 2015 noted multiple heterogenously enhancing peritoneal and omental masses with calcifications suggestive of metastatic deposits. USG guided percutaneous biopsy of the peritoneal mass performed on 18.8.15 resulted as epithelioid GIST, however inconclusive of primary extra GIST or a metastatic GIST. PET-CT was requested to determine the possible underlying occult primary malignancy as well as to look for evidence of distant metastasis before initiation of therapy. The result of PET-CT is consistent with primary malignant extragastrointestinal stromal tumor of the peritoneum and omentum with no evidence of distant metastasis. **Conclusion**: FDG PET-CT has a potential role in assisting the diagnosis of EGIST of the peritoneum and omentum and can be utilised further in the management of this disease as described in this case report.

***Keywords*: Primary extragastrointestinal stromal tumor, gastrointestinal stromal tumor, FDG PET-CT**

Selection of CD36-Peptide Phage Using Phage Display Technique

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Purpose: This study aimed at selecting specific CD36 peptide phage. CD36, a scavenger receptor that express heavily on macrophages, is able to internalise different classes of ligands. Thus, exploiting this receptor can be a promising strategy for non-viral gene delivery to treat athero-inflammatory disorders. **Methods**: Ph.D-12 Phage Display Peptide Library (NEB) was first used in double negative selections against a plastic well and BSA. Positive selection was then carried out against rhCD36 protein. After several TBST washings, the bound phage were eluted with 0.2 M Glycine-HCl (pH 2.2) and neutralised with 1 M Tris-HCl (pH 9.1). A total of 50 clones (after the third and fourth pannings) were selected. PCR was performed to confirm clones with randomised peptides prior to DNA sequencing. Possible CD36 motif was identified by sequence analysis. CD36-binding ability of the clones was assessed by ELISA using HRP-conjugated anti-M13 phage antibody. BSA and streptavidin phage were used as irrelevant protein and phage controls, respectively. The colorimetric measurement was done at 450 nm wavelength. **Results**: PCR results revealed 44 out of 50 clones selected contained the randomised peptides. In total, 10 different clones with unique DNA sequences have been identified. 3 clones bearing N_L motif were further characterised by ELISA, which showed an increase in the phage binding as more phage were added into CD36-coated wells. Unfortunately, the same binding trend can be seen when BSA was used. **Conclusion**: The selected phage clones are able to bind non-specifically to rhCD36 protein. Hence, the remaining clones will be subjected to ELISA to identify the best CD36 binders.

***Keywords*: Phage display, CD36, gene delivery**

Image Guided Radiotherapy (IGRT) for Head and Neck Cancer (HNC) in AMDI USM: A Prelimenary Study of Patients' Setup Reproducibility

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Purpose: Advanced radiotherapy techniques use sophisticated linear accelerator (linac) to shape the high energy x-ray beam delivery more accurately to the tumour while sparing the normal tissues. The beam is delivered to the target daily, fraction-by-fraction, over several weeks. Irradiation is guided with imaging modalities incorporated as part of the linac known as image guided radiotherapy (IGRT). This retrospective study investigates the reproducibility of HNC patient setup at a new centre using IGRT performed before the treatment. **Methods**: Prior to treatment, the patient position was immobalised with a head mask on a CT scanner during radiotherapy simulation. The structure of the tumour was delineated as volumetric target margins on the CT images acquired, with an extension of 3 mm margin in all direction. Cone beam CT images were acquired before radiotherapy treatment and were compared with the intended treatment setup acquired during simulation. The position differences were corrected if exceeded 3 mm. The data were recorded for five patients for every imaging performed over their course of treatment. **Results**: The maximum position differences measured for every patient range between 2 mm to 5 mm. Four of the patients exceeded the 3 mm margin of uncertainty added to the volumetric treatment margin. **Conclusions**: A 3 mm margin is sufficient provided that daily imaging and setup correction are performed. A 5 mm margin extension appears to be safe in the setting where IGRT is not available. Successful implementation of IGRT in radiotherapy requires coordinated effort of the medical physicists, the radiation oncologists and the radiation therapists.

***Keywords*: Advanced radiotherapy, IGRT, IMRT**

UV-Vis Spectrophotometric-Based Metabolite Profiling of *Clinacanthus nutans* Leaves Possessing Antioxidant Activity

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Purpose: To identify potential active metabolites responsible for antioxidant activity in *Clinacanthus nutans* (*C. nutans*) leaves extracts using UV-Vis spectrophotometric-based metabolomics approach. **Methods**: *C. nutans* leaves were ultrasonicated using different solvents and resulting extracts were subjected to 2,2-diphenylpicrylhydrazyl (DPPH) assay to evaluate for their free radicals scavenging potential. SIMCA-P software was used to conduct principal component analysis (PCA), partial least squares to latent structures (PLS) and orthogonal PLS discriminant analysis (OPLS-DA) on the UV spectrophotometry data to assess for similarities and differences among samples as well as the correlation between X and Y variables. CV-ANOVA, Variable Importance in Projections (VIP) and permutation and were performed to validate OPLS-DA and PLS models. **Results**: OPLS-DA well discriminated the extracts into four distinct clusters (p-value < 0.0001). Components at wavelengths 266, 267, 265 nm in PLS and 332, 333, 331 nm in OPLS-DA were important variables that best differentiated the extracts. Ethanol and aqueous extracts showed positive correlation with DPPH activity, with ethanolic extract achieving the highest DPPH activity among all. Orientin, homoorientin, schaftoside, vitexin, isovitexin, and caffeic acid which detected at wavelengths 303 to 365 nm, were potential metabolites that responsible for DPPH antioxidant activity. **Conclusion**: This study demonstrated that the correlation of metabolomic approach with bioassay data can be an excellent strategy for identification of bioactive metabolites from crude plant extract.

***Keywords*: *Clinacanthus nutans* (Burm.f) Lindau, UV-Vis spectrophotometry, DPPH activity, multivariate data analysis, metabolite profiling**

Tissue Characterisation in Liver Using Dual-Energy Computed Tomography

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The aim of this study was to review the techniques to improve the images in detecting the liver lesions using the dual-energy computed tomography (DECT). DECT has improved the tissue characterisation during the past few years, which provided extra information compared with the single-energy computed tomography. The use of low and high voltages in DECT provides a fused image that improved the detection of the liver tumor due to the higher contrast-to-noise ratio (CNR) of the tumor compared to the liver. The utilisation of the contrast agents in CT scanning improved the image quality by enhancing the contrast-to–noise (CNR) and signal–to–noise ratio (SNR) while reducing beam-hardening artifacts. Many studies show that using different agents with suitable protocols such as iodine mapping and morphine co-medicine in potential donors, can enhance the CT imaging visualisation and increase the ability of liver tumor detection. Besides, scanning protocols such as tube current (mAs), collimation, pitch, gantry rotation times also very important when underwent CT examination. This also increases the image quality and gives better lesion visibility with lower dose. Furthermore, using the current modulation software provided by the manufacturers and low voltage technique in detecting hypervascular liver lesions, help to reduce the dose to the patient. The dose in DECT is lower comparing to the CT perfusion and conventional SECT, and maintains the image quality in the same time. DECT had shown an encouraging future in improving the characterisation and detection of the liver lesions with comparing with the conventional CT.

***Keywords*: Dual-energy, contrast, CT, tissue characterisation, liver, lesions**

Intestinal Parasitism among Rural and Urban School Children in the State of Selangor, Malaysia

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Purpose: This study was conducted to determine and compare the prevalence of intestinal parasitic infections among rural and urban school children in Selangor. The stool samples and demographic data were collected from 751 students of ten primary schools, five from urban schools (SK Bukit Lanjan, SK Bukit Bangkong, SK Batu 9, SJKT Ampang and SJKC Kg Baru Ampang) and another 5 from rural schools (SK Sungai Melut, SK Bukit Tampoi, SK Dengkil, SJKT Dengkil and SJKC Dengkil). **Methods**: Intestinal parasites were then screened by using several techniques such as direct smear, formalin ether sedimentation, acid fast (Ziehl-Neelsen) staining and cultivation in complete Jone's medium. Demographic variables were analysed using SPSS software. **Results**: Prevalence of Intestinal helmints infections such as *Ascaris* (24.1% and 21.3%), *Trichuris* (22.1% and 16.1%) and hookworms (12.4% and 3.5%) were respectively identified for both rural and urban school children. For intestinal protozoa, *Blastocystis* appears to be 15.5% and 13.9% in rural and urban school children, respectively. **Conclusion**: Although infections were found to be appearing in both rural and urban community, the prevalence clearly show that rural community is more prone to infection.

Cytotoxic Activity of *Crinum asiaticum* Leaf Methanol Extract

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Purpose: The objective of this study is to investigate the cytotoxic (potential anti-cancer) activity of *Crinum asiaticum* and to justify scientifically, its use in the traditional medicine, as an anti-tumour agent. Also, it is of significance to exploit novel anti-cancer drugs from medicinal plants and study the possible mechanism of action (e.g. anti-angiogenesis) of the *Crinum asiaticum* leaf methanol extract (CALM). **Methods**: The plant was first separated into four different parts, i.e. root, bulb, stem and leaf. The methanol (crude) extracts of these parts were further fractionated using various solvents, i.e. hexane, ether, butanol and distilled water. The cytotoxic activity (cytotoxicity) was then screened (using MTT assay) on following cell lines: Vero, Hep-2, HCT, MCF and EAHY. The anti-angiogenic effect was studied using rat aorta ring assay (inhibition of new blood vessel growth). **Results**: All the extracts (root, bulb, stem and leaf) showed cytotoxicity microscopically and quantitatively. However, only the leaf methanol extract showed significant cytotoxicity (about 70%, $P < 0.05$) against Vero cells, whilst the other methanol extracts did not show significant effects. Thus, only CALM extract was used for further tests, on other cell lines. The IC_{50} values ranged from 1.5 µg/ml (for MCF cells) to 50 µg/ml (for Vero cells). It was also found that the ether fraction showed the highest toxicity on Vero cells, followed by hexane, and finally butanol fraction. Tests on Hep-2 cells showed that the hexane fraction showed the highest toxicity, followed by butanol, and finally ether fraction. Preliminary study also found that the extract inhibited new blood vessel growth, thus, exhibiting anti-angiogenic effect. **Conclusions**: The CALM extract showed moderate cytotoxicity (IC_{50} values between 30 to 100 µg/ml) on all the selected cell lines (Vero, Hep-2, HCT and EAHY) except for MCF (actively cytotoxic), as indicated by the IC_{50} value of below 30 µg/ml, and one of the possible mechanisms of action is anti-angiogenesis.

***Keywords*: *Crinum asiaticum,* cytotoxic, anti-angiogenic**

In Silico Study on Gene of MSX1 and PAX9 Mutations with Tooth Formation

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Purpose: To determine the relationship between gene MSX1 and PAX9 mutations with tooth formation. **Methods**: This is a retrospective study comprises two approaches, bioinformatics and oral embryology. For the former, we investigated the protein sequences and their 3D protein structure templates of MSX1 and PAX9 genes were retrieved from NCBI database (http://www.ncbi.nlm.nih.gov), BLAST and Protein Data Bank (http://www.rcsb.org). The 3D protein structure models of MSX1 and PAX9 genes were constructed by SWISSMODEL (http://swissmodel.expasy.org) and further visualized by DeepViewer (http://spdbv.vital-it.ch/index.html, ver 4.1.0). For the later, the process of tooth formation in normal and mutated tooth was comprehensively studied by reviewing the current scientific articles. **Results**: The selected normal and mutated MSX1 gene IDs are NP_002439.2 and AAA58665.1, respectively. Meanwhile, for PAX9 gene IDs are XP_006720218.1 (normal) and NP_006185.1 (mutated) proteins. The structure templates for protein modelling are given as [MSX1: normal (1FF9.pdb) and mutated (3f4n.1.A.pdb); PAX9: normal (3NR8.pdb) and mutated (2GAG.pdb)]. Protein sequence comparison revealed a few mutations as follows, MSX1: Ala45Thr, Gly97Ala, Val98Ser and Pro99Arg and PAX9: Ser340Ala, Leu339Ser, Ser338Ala, and Leu336Ser. The structural comparison between normal and mutated proteins each, MSX1 and PAX9 were 12.68Å and 123.90Å of the RMSD values, respectively. Mutation of MSX 1 and PAX9 (which were required in the mesenchyme for transition from bud to cap stage) will arrest tooth development at bud stage and responsible for a specific pattern of tooth agenesis. **Conclusion**: MSX1 and PAX9 are the most important genes that related with tooth agenesis.

***Keywords*: *In silico*, MSX1, PAX9, mutations, tooth agenesis**

In Silico Study on E6 and E7 Human Papillomavirus (HPV) Oncoproteins

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Purpose: This *in silico* study compares the HR and LR HPVs based on their E6 and E7 oncoprotein structures. **Methods**: Available HPV protein sequences (E6: 1283; E7: 918) from National Center for Biotechnology Information (NCBI) are aligned, compared using Basic Local Alignment Search Tool (BLAST) and subsequent homology modelling of protein structure in SWISS-MODEL workspace. A final template for each oncoprotein of selected HPVs is viewed under RasMol and Root-Mean-Square Deviation (RMSD) values are calculated with Deepviewer. **Results**: HPV-6 and -11 have closely related E6 and E7 oncoprotein structures (RMSD: 0.02-0.05Å) while HPV-16 and -18 show moderately distinct structures (RMSD: 0.95-1.17Å). When comparing HR HPV with LR HPV, HPV18 E6 is structurally closer to LR HPV E6 (RMSD: 0.08Å). HPV16 has a relatively closer E7 structure to LR HPV (RMSD: 0.07Å) compared to HPV18 (RMSD: 1.06Å). The structural differences contribute to the functional differences between the HPV types. **Conclusion**: Our *in silico* study demonstrates the unique and specific protein and oncoprotein structures among HR and LR HPVs and serves as the platform for further exploration in designing genome-based drugs and vaccine.

***Keywords*: *In silico*, E6, E7, HPV**

A Comparative Study of Two Different Normoxic Polymer Gel Dosimeters on Dose Response and Dose Sensitivity

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Purpose: Polymer gel dosimeters are fabricated from radiation sensitive chemicals which, upon irradiation, polymerize as a function of the absorbed dose. In general, the accuracy of a polymer gel dosimeter is determined by several dosimetric characteristics including the gel composition. This study concentrated on the radiation properties and the specific effects of the cross-linker on normoxic gel dosimeters evaluated in terms of the R2–dose response and R2–dose sensitivity. **Methods**: Two different types of normoxic polymer gel dosimeter were selected with one type consisting of cross-linkers (PAGAT) and the other type being free of cross-linkers (MAGAT). The gel dosimeter was irradiated using linear accelerator (LINAC) at a dose range of 1 Gy to 20 Gy, 24-h post-manufacturing. The imaging of gel dosimeter by Magnetic Resonance Imaging (MRI) was done a day post-irradiation using an optimized protocol. **Results**: The R2–dose response between PAGAT and MAGAT gel dosimeters showed a significant difference. When cross-linker concentration increase, the R2-dose sensitivity of PAGAT gel dosimeter increased while R2–dose sensitivity of MAGAT gel dosimeter decreased. **Conclusion**: The MAGAT gel dosimeter showed better dose response and sensitivity compared to the PAGAT gel dosimeter

***Keywords*: Polymer gel dosimeter, R2-dose response, R2- dose sensitivity, MRI**

Mismatch Rejection of SIFT Keypoints in Temporal Breast MRI

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Purpose: Registering the temporal pairs of breast MRI provides an aid for better detection and treatment of breast cancer. However, mismatch between the salient points has caused misregistration. Therefore, this study proposed a technique of mismatch rejection in Scale Invariant Feature Transform (SIFT) using the slope line. **Methods**: The proposed technique is implemented on temporal pairs of breast MRI acquired from six patients. We select the T2 images for each pair and calculate their SIFT keypoints. Then, the corresponding descriptor which contains 128 features for each keypoint are determined and matched using Euclidean distance. Although keypoints between images have similar descriptor, however their visual appearance are different. To reject the mismatched, we propose a new rejection factor by estimating the slope line between the matched keypoints and compared it with a threshold. The slope line between the corrected matched is expected to be small. **Results**: The proposed technique exceeded an accuracy of 90% in rejecting mismatch keypoints. The threshold of 0.024 is selected as it gives the best results for all images tested. **Conclusions**: The proposed technique using the slope line has attained a high accuracy for mismatch rejection of SIFT keypoints in temporal breast MRI. This will increase the accuracy of registration that will improve the detection and treatment of breast cancer. However, this technique is limited to keypoints matching with a very small rotation. As the view of MRI images are rarely rotated, the proposed technique will be sufficient for mismatch rejection of SIFT keypoints.

***Keywords*: Mismatch rejection, temporal breast MRI, SIFT**

Thyroid Hormone Stimulation of Autophagy is Essential for Mitochondrial Biogenesis and Activity in Skeletal Muscle

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Thyroid hormone (TH) and autophagy share similar functions in regulating skeletal muscle growth, regeneration and differentiation. Although TH recently has been shown to increase autophagy in liver, the regulation and role of autophagy by this hormone in skeletal muscle is not known. Here, using both in vitro and in vivo models, we demonstrated that TH induces autophagy in a dose- and time-dependent manner in skeletal muscle. TH induction of autophagy involved ROS stimulation of AMPK-mTOR-Ulk1 signaling. TH also increased mRNA and protein expression of key autophagy genes, LC3, p62, and Ulk1, as well as genes that modulated autophagy and FOXO1/3a. TH increased mitochondrial protein synthesis and number as well as basal mitochondrial O2 consumption, ATP turnover, and maximal respiratory capacity. Surprisingly, mitochondrial activity and biogenesis were blunted when autophagy was blocked in muscle cells by Atg5 shRNA. Induction of ROS and AMPK by TH played a significant role in the upregulation of PPARGC1A, the key regulator of mitochondrial synthesis. In summary, our findings showed that TH-mediated autophagy was essential for stimulation of mitochondrial biogenesis and activity in skeletal muscle. Moreover, autophagy and mitochondrial biogenesis were coupled in skeletal muscle via TH induction of mitochondrial activity and ROS generation.

***Keywords*: Thyroid hormone, autophagy, muscles, mitochondrial biogenesis, oxidative phosphorylatio**