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Synthesis, Characterization and Biological Properties of Copper(II) and Zinc(II) Complexes against Blood-stage Plasmodium falciparum

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ABSTRACT

Introduction: The spread of multidrug-resistant malaria parasite – Plasmodium sp. to commercially available antimalarial drugs, i.e. artemisinin-based combination therapies (ACTs) and chloroquine (CQ), has become a global treat to eliminate malaria. To limit the impact of antimalarial drug resistance, a new potent and affordable alternative is urgently needed. A number of metal-based compounds (metallodrugs) have been found active against Plasmodium falciparum, the species that causes potentially fatal cerebral malaria, as they are ease in ligand grafting of multi-functional groups. Ferroquine (FQ) is one of the metalloantimalarial drugs that is currently undergoing clinical trials.

Methods: In this study, a series of ternary copper(II) and zinc(II) complexes – Cu(phen)(edda) 1, Zn(phen)(edda) 2, [Cu(phen)(cdmg)] NO3 3 and [Zn(phen)(c-dmg)]NO3 4 were synthesized and characterized by the following tests: Fourier transformed infrared (FTIR), CHN elemental analysis, UV-Vis spectroscopy, molar conductivity and magnetic susceptibility measurements. Results: In vitro hemolytic and antimalarial assays using SYBR Green I dye were done to determine the biological properties of these complexes. Preliminary biological evaluation demonstrated that all the complexes 1, 2, 3 and 4 exhibit toxicity against the sensitive blood-stage Plasmodium falciparum 3D7 with IC50 in μM range. Conclusion: Thus, metal complex is a potentially viable candidate as antimalarial drug to overcome the emergence of drug resistance.

Keywords: metallodrug, Plasmodium falciparum, antimalarial activity
Biofilm Architecture of *Candida rugosa* and Effect of Amphotericin B, Caspofungin, Fluconazole and Voriconazole on its Structure

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ABSTRACT

**Introduction:** One of the most common aetiology of opportunistic fungal infections in humans is *Candida* species. The virulence of *Candida* species is due to repertoire of factors, specifically, the ability to form biofilms. Medical devices such as intravenous catheters, prosthetic heart valves and surgical interventions provide pathogenic microorganisms with a surface to adhere to form biofilm. Fungi present as biofilms are often resistant to antifungal treatment because these biofilms offer a protective barrier that prohibits the drugs to get to the active site of the fungi. The objective of this study is to investigate the biofilm architecture of *Candida rugosa* (*C. rugosa*) at different developmental phases and to identify Sessile Minimum Inhibition Concentrations (SMICs) of amphotericin B, caspofungin, fluconazole, and voriconazole for the biofilm of *C. rugosa*. **Methods:** Confocal scanning laser microscopy (CSLM) and scanning electron microscopy (SEM) were used to visualize *C. rugosa* biofilms at different developmental phases. The antifungal susceptibility test was performed using serial doubling dilution. The growth kinetics of *Candida* biofilms was quantified using XTT reduction assay and crystal violet assay. **Results:** From the antifungal susceptibility test, the biofilms had SMIC of >16μg/mL for amphotericin B, 6μg/mL for caspofungin, >64μg/mL for fluconazole and >16μg/mL for voriconazole. From the SEM micrographs, *C. rugosa* biofilm have a structure composed of an adherent yeast cells and blastopores with hyphal elements. There were significant alterations in the morphology after exposure to antifungal agents. The quantitative measurement of the matrix thickness of embedded yeast cells were obtained from CLSM micrographs. **Conclusion:** In conclusion, the ability of *C. rugosa* to form biofilms may attribute to one of the virulence factors that causes reduced susceptibility to antifungal agents.

**Keywords:** *C. rugosa*, biofilm, antifungal susceptibility, virulence
Usage and completeness of antimicrobial prescribing form within general intensive care unit in a tertiary hospital

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ABSTRACT

Introduction: The incidence of antimicrobial resistance (AMR) has increased worldwide including Malaysia, which may be attributed partly to inappropriate prescribing of antimicrobials. Antimicrobial prescribing form has been introduced to mandate appropriate antimicrobial prescription including documented indication as a key standard of antimicrobial stewardship practice. Hence, this current study aimed to determine the usage and completeness of the designated antimicrobial prescribing form that had been implemented in the General Intensive Care Unit (GICU), Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

Methods: This prospective observational study was carried out in GICU UKMMC from 30 August 2018 to 30 November 2018 by convenience sampling. The information that was recorded in the antimicrobial prescribing form was collected by using the designated data collection form. A total of 68 patients were included and 205 antimicrobial prescribing forms were evaluated.

Results: There were 100% usage of antimicrobial prescribing forms found in this study. However, only 81 ± 8 % of these forms were completely filled. Indication for the antimicrobial prescription was not filled in 47% of the forms. Almost two thirds of the antimicrobial prescriptions were empirically indicated and one percent de-escalation of antimicrobial therapy was filled in the forms. These prescriptions comprised of 91.7% antibiotics, 7.8% antifungals and 0.5% antivirals. The suspected site of infections were primarily from the lungs (27%), gastrointestinal (16%), blood (16%) and central nervous system (14%). Piperacillin/Tazobactam was the most frequent antibiotic prescribed (21%), followed by third and fourth generation cephalosporins (20%). Conclusion: This study provided an overview of the uptake of the antimicrobial prescribing form implementation and highlighted the requirement for supplementary efforts to maximize the compliance of this form.

Keywords: antimicrobial resistant, antimicrobial stewardship, antimicrobial prescribing form, compliance
Assessment of polymyxin B associated nephrotoxicity in ICU patients in a Malaysian tertiary teaching hospital

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ABSTRACT

Introduction: Polymyxins are used as the “last-line therapy” for multi drug resistant (MDR) Gram-negative bacterial infections. However, the development of nephrotoxicity is a major concern. The objectives of this study were to determine the incidence and severity of acute kidney injury (AKI) and to identify risk factors associated with AKI and mortality rate in Malaysian patients on polymyxin B (PMB) for MDR Gram-negative bacterial infections. Methods: A retrospective study was conducted in Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Medical and medication charts were reviewed for all intensive care unit (ICU) patients who received intravenous (IV) PMB from 1st May 2008 to 1st May 2018. Simple and multiple logistic regression were performed to identify risk factors of PMB induced nephrotoxicity. Results: Among the total 572 patients identified, only 31 patients were eligible to be included. The incidence rate of AKI was 45.2% (14 of 31 patients). Univariate analysis showed that age was a significant risk factor of PMB associated nephrotoxicity [OR 1.074; 95% CI 1.002-1.151; P=0.045]. Other four variables (P<0.2) included were female, baseline serum creatinine, baseline estimated glomerular filtration rate (eGFR) and diabetes mellitus. Further analysis by multiple logistics regression showed that age was the variable independently associated with the development of AKI (OR 1.109; 95% CI 1.007-1.221; P=0.035). Furthermore, the development of AKI was not significantly associated with mortality rate. The incidence, severity, risk factor for PMB associated nephrotoxicity and mortality rate of Malaysian ICU patients on PMB were identified. Conclusion: PMB may be used as the salvage therapy of nosocomial infections with extra caution and close monitoring especially in the elderly. Future studies, involving more centers are needed to provide further information regarding PMB induced nephrotoxicity in Malaysian ICU patients.

Keywords: polymyxin B, nephrotoxicity, intensive care unit
Effects of phenylpyruvate feeding with polymyxin B in *Klebsiella pneumoniae*

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**ABSTRACT**

**Introduction:** Polymyxin B (PMB) is one of the remaining antibiotics that is effective against multidrug resistant (MDR) Gram-negative bacteria. However, PMB monotherapy is not able to achieve sustained killing hence, combination with other antibiotics are usually employed. Besides antibiotics, studies are now moving towards non-antibiotic alternatives such as metabolite feeding against MDR pathogens. This study aimed to investigate the susceptibility and bacterial killing of PMB in combination with metabolite phenylpyruvate against *Klebsiella pneumoniae* isolates.

**Methods:** Broth microdilution was used to determine PMB minimum inhibitory concentration (MIC) alone and with phenylpyruvate against two *Klebsiella pneumoniae* isolates. Time kill studies were performed over 24 h (initial inoculum: ~106 CFU/mL), using PMB 2 mg/L and phenylpyruvate 2 mmol/L, alone and in combination, against the PMB-resistant isolate. Microbiological responses were examined using the log-change method.

**Results:** The MIC of PMB was reduced by phenylpyruvate in both isolates. In the time kill studies, during the first hour, PMB monotherapy demonstrated the highest bacterial killing activity even compared to the combination. Phenylpyruvate monotherapy showed negligible activity against *K. pneumoniae*. A significant reduction in bacterial burden was seen at 1 h following PMB monotherapy and combination therapy but an equally rapid regrowth was seen at 4 h. Notably at 24 h, the regrowth following combination therapy was >1-log10 CFU/mL less than PMB monotherapy.

**Conclusion:** Our results suggest that phenylpyruvate increased PMB susceptibility in *K. pneumoniae* and may minimise the emergence of resistance to PMB. Future studies investigating phenylpyruvate at higher concentrations against more isolates are warranted.

**Keywords:** polymyxin, combination therapy, metabolite feeding
Elucidation of antimicrobial activity of non-covalently dispersed carbon nanotubes

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ABSTRACT

Introduction: Microorganisms have begun to develop resistant because of inappropriate and extensive use of antibiotics in a hospital setting. Therefore, it seems to be necessary to find a way to tackle these pathogens by developing a new and effective antimicrobial agents. Carbon nanotubes (CNTs) have attracted growing attention due to their remarkable mechanical strength, electrical properties, and chemical and thermal stability for their potential applications in the field of biomedical as therapeutic and diagnostic nano tools. However, the impact of carbon nanotubes to microbial growth has not been fully investigated. The main aim of this study to investigate the antimicrobial activity of CNT, particularly double-walled and multi-walled nanotubes on representative pathogenic strains such as Gram-positive bacteria Staphylococcus aureus, Gram-negative bacteria Pseudomonas aeruginosa, Klebsiella pneumoniae and fungal strain Candida albicans. The dispersion ability of CNTs (DWCNT and MWCNT) treated with surfactant such as sodium dodecyl-benzenesulfonate (SDBS) and their impact on the microbial growth inhibition were also examined. Methods: A stock concentration of 0.2mg/ml for both double-walled and multi-walled CNT was prepared homogenized by dispersing in surfactant solution by using probe sonication. UV-vis absorbance, Fourier transfer infrared radiation (FTIR) and Scanning electron microscopy (SEM) were used for the characterization of CNTs dispersed in the surfactant solution to study the interaction between molecules of surfactant and CNTs. The antimicrobial activity was determined by analyzing optical density growth curves, viable cell number and zone of inhibition.

Results: This study revealed that microbial growth inhibited by non-covalently dispersed MWCNT was both depend on the concentration and treatment time. In conclusion, the binding of surfactant molecules to the surface of CNT increases its ability to disperse in aqueous solution. Non-covalent method of CNTs dispersion preserved their structure and increased microbial growth inhibition as a result. Conclusion: Multi-walled CNT exhibited higher antimicrobial activity compared to double-walled CNT against selected pathogens.

Keywords: Carbon nanotubes, SDBS, Pathogens, Antimicrobial activity
Electrospun Zein Nanofibers as Carriers of Gallic, Caffeic and p-Coumaric Acid

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ABSTRACT

Introduction: The application of nanofibers in biomedicine has gained increasing interest due to their characteristic large surface area, high porosity and chemical tunability. Electrospinning is one of the most versatile techniques for the production of uniform nanofibers. It has been used for the fabrication of extracellular matrix (ECM)-mimicking fibrous scaffolds for several decades. Electrospun fibrous scaffolds provide nanoscale to microscale fibrous structures with interconnecting pores, resembling natural ECM in tissues, and showing a high potential to facilitate the formation of artificial functional tissues. Furthermore, electrospinning demonstrated potential as a vehicle for the encapsulation of bioactive compounds such as phenolic acids, which was suggested to have significant potential to treat wounds caused by trauma, diabetes, ischemic syndromes and other pathological diseases. This study investigates the application of electrospun nanofibers as bioactive carriers of phenolic acids, where gallic, caffeic and p-coumaric acids were incorporated to zein nanofibers at different concentrations (5%, 10% and 20%).

Methods: The morphology of the produced fibers were examined with scanning electron microscopy (SEM) and exhibited diameters ranging from 396 to 655 nm. Meanwhile, the interaction between the phenolic compounds and zein was examined with attenuated total reflection-Fourier transform infrared (ATR-FTIR). Results: The antioxidant activity of the fibers was determined using 1,10-diphenyl-2-picrylhydrazyl (DPPH) assay and showed that gallic and caffeic acid had retained their active properties after the incorporation to zein electrospun fibers. Conclusion: Overall, the study provides an outline on the potential of electrospinning technique to produce nanofibers that may serve as substrates in skin tissue engineering and carriers for bioactive compounds such as phenolic acids.

Keywords: Nanofibers, electrospinning, bioactive carrier, phenolic acids
Computational comparisons of LipL21 mRNA and amino acids conserved sequences in *Leptospira* strains

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**ABSTRACT**

**Introduction:** Lipoprotein L21 (LipL21) has been used as a molecular marker for leptospirosis as it is highly expressed in pathogenic *Leptospira* species during infection. However, it lacks specificity due to the newly emerging pathogenic serovars. Therefore, interrogation of LipL21 in all serovars needed to understand the pathogenesis of leptospirosis to enable early diagnosis. This study was carried out to determine the suitability of LipL21 as a molecular marker for leptospirosis by identifying the conserved sequences of LipL21 mRNA and amino acids in different *Leptospira* strains.

**Methods:** Location of LipL21 conserved regions in 15 pathogenic and 2 non-pathogenic strains of five *Leptospira* species, were identified using bioinformatics database and tools such as National Center of Biotechnology, Rapid Annotation Subsystem Technology blast search, Muscle program and Jalview software.

**Results:** Multiple sequence alignment analysis revealed that two conserved regions were observed in 10 pathogenic *Leptospira* strains from nucleotide position 29 to 53 and 100 to 137, however conserved amino acid sequences (111-149 and 155-192) were found in all the pathogenic strains. The distinction between gene and amino acid results is due to the degenerate genetic code feature.

**Conclusion:** In conclusion, this study suggests that LipL21 protein has a potential to be used as a diagnostic marker for detection of *Leptospira* pathogens compared to LipL21 mRNA.

**Keywords:** LipL21, Leptospira, Leptospirosis, bioinformatics
OC1

Fear of Negative Appearance Evaluation and Attitude Towards Mammography: Moderating Role of Internal Health Locus of Control, Cancer Worry and Age

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ABSTRACT

Introduction: We aimed to investigate the moderating role of internal health locus of control, breast cancer worry and age in the relationship between fear of negative appearance evaluation and attitude towards mammography in women with no prior mammography screening experience. Methods: A descriptive and cross-sectional, questionnaire-based survey was conducted. Participants included 823 Iranian women aged 30 years and above. We used covariance-based structural equation modeling to test the research hypotheses. Results: The findings provided evidence for the negative association between fear of negative appearance evaluation and women’s attitudes towards mammography. Internal health locus of control, breast cancer worry, and age weakened the detrimental effect of fear of negative appearance evaluation on mammography screening. We found that fear of negative appearance evaluation could more likely act as a barrier towards mammography screening in women who were less likely to have a sense of control over their own health, who were less worried about developing breast cancer, and who were younger. Conclusion: Implications of the study are discussed with recommendations for future research.

Keywords: breast cancer, fear of negative appearance evaluation, attitude towards mammography, internal health locus of control, breast cancer worry, age
Effect of extraction temperature on polyphenolic content and antioxidant potential of *Gynura crepioides* leaves

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**ABSTRACT**

Introduction: *Gynura crepioides* (*G. crepioides*) belongs to the Asteraceae family and native to Southeast Asia, especially Indonesia. *Gynura* genuses are well known for their antioxidant properties. Hence, the current study aimed to study the effect of temperature on polyphenolic content and antioxidant properties on *G. crepioides* leaves extract.

Methods: The extraction of *G. crepioides* leaves was carried out by Ultrasound-assisted extraction (UAE) method for 60 minutes by using ethanol (70 %) at three different temperatures 25°C and 50°C and 75°C. The total polyphenolic content (TPC) was investigated by using Folin-Ciocalteu assay, whereas the antioxidant potential (AOP) was determined by using phosphomolybdenum assay, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results: The results from the study showed that TPC of *G. crepioides* extracted at 25°C was significantly (p < 0.05) lower than 50°C and 75°C with 8.45 ± 0.31 mg GAE/g, 35.83 ± 1.22 mg GAE/g, 35.90 ± 0.78 mg GAE/g respectively. However, the results from AOP has demonstrated lower value of 70.62 ± 0.74 mg AAE/g at 75°C compare to 77.67 ± 0.26 mg AAE/g at 25°C and 81.33 ± 0.68 mg AAE/g at 50°C. From DPPH assay results revealed that extraction temperature at 50°C has EC50 (p < 0.05) lowest value of 92.64 ± 0.56 μg/mL compared to *G. crepioides* extracted at 25°C (98.50 ± 1.18 μg/mL) and 75°C (101.72 ± 9.09 μg/mL). An excellent correlation exhibited between TAC and DPPH radical scavenging assays with *r* = 0.95 and *r*² = 0.89. Present study reveals that UAE method with 70% ethanol, 60 minutes extraction time at 50°C temperature is an optimum to extract highest phenolic content and antioxidants from *G. crepioides* leaves. Conclusion: It was concluded from the study that extraction temperature would affect the extraction of phytochemicals from plants in turn it affects the biological activities.

**Keywords:** *Gynura crepioides*, Folin-Ciocalteu, Phosphomolybdenum assay, DPPH assay
**OC3**

**ZFP36L2 silencing increases sensitivity to Temozolomide via G2M cell cycle arrest and BAX mediated apoptosis**


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**ABSTRACT**

**Introduction:** The mortality rate of glioma patients particularly with glioblastoma multiforme (GBM) remains high even with advancements in the multimodality treatment. This is partly due to chemoresistance of the glioma cells towards drug treatment which finally reduced the survival of GBM patients. In this study, we determined the chemosensitisation and oncogenic characteristics of ZFP36L2 in LN18 GBM cells using RNA interference (RNAi).

**Methods:** Meta-analysis of microarray datasets was used to identify the druggable genes responsive to GBM chemosensitivity. Subsequently, the genes were validated using RNAi screening [pooled small interference RNA (siRNA)]. Temozolomide-resistant LN18 cells were used to evaluate the effects of gene silencing on chemosensitisation to the sub-lethal dose (1/10 of IC50) of temozolomide. Assays such as cell viability, proliferation, migration and invasion and apoptosis assays were carried out. The apoptosis pathway underlying chemosensitisation by ZFP36L2 siRNA was determined using a human apoptosis array kit. Statistical analyses were performed using one-way analysis of variance.

**Results:** ZFP36L2 was identified as a potential marker of GBM based on the meta-analysis and RNAi screening. ZFP36L2 knockdown lead to 1) Apoptosis induction (p < 0.05) 2) Reduced cell migration (p < 0.05) 3) Reduced up to 82% of cell invasion (p < 0.01) and 4) Decreased cellular proliferation in siZFP36L2-treated LN18 cells. Downstream analysis showed that the sub-lethal dose of temozolomide caused major upregulation of BCL2-associated X, apoptosis regulator (BAX).

**Conclusion:** ZFP36L2 is oncogenic and chemosensitive thus may contribute to gliomagenesis through cell proliferation, migration, invasion and apoptosis. RNAi therapy in combination with chemotherapy treatment such as temozolomide may serve as potential therapeutic approach in the future.

**Keywords:** Glioblastoma multiforme, RNAi screening, Temozolomide, Chemosensitisation, ZFP36L2
A study to explore the diagnostic characterisation of soft tissue edema in appendicular bone tumours by Magnetic Resonance Imaging

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ABSTRACT

Introduction: In Malaysia, about 800 children are diagnosed with cancer, a globally a dreadful disease, each year, and osteosarcoma accounts for approximately 3% of such cancer. The early detection of the type and extent of bone cancer is important for effective management through surgery. But, the presence of soft tissue edema around a neoplasm can interfere with accurate local tumor staging and subsequent surgical planning. However very scanty research is done on this; none of the past studies focused specifically on the incidence and quantity of extraosseous edema and its impact. Our interdisciplinary retrospective study with objectives to study the presence of soft tissue edema adjacent to the tumors in the extremities, characterize their pattern and distribution involved 82 patients of wide range of age attending the Hospital Universiti Sains Malaysia with a histologically proven tumor or tumor-like lesion of bone. Methods: This paper emphasizes avoiding misinterpretation of such edema and subsequent over-staging of musculoskeletal tumor. We exclusively used 1T-Magnetic Resonance Imaging due to its excellent resolution. All cases were imaged (T1W, T2W, T1W fat suppressed Gadolinium enhanced, and STIR images) by using 1.5 Tesla MRI unit. STIR images permit study of larger volume of abnormal tissue than spin echo images. Results: Peritumoral edema in 5 cases, Paratumoral edema in 11 patients and mixed type in 45 cases were found including 10 benign tumors. Overall, 5 malignant lesions did not show any soft tissue edema! All the data were analyzed and interpretation and characterisation of the edema was done by an experienced radiologist. The findings were correlated with histopathology examination results. Conclusion: In conclusion, accurate definition of the local extent of a bone tumor and exploration of soft tissue edema is required to maximize the success of diagnostic work-up and staging prior to biopsy and subsequent interventions while minimizing the amount of tissue removed.

Keywords: Muscular edema, Peri and Paratumoral edema, MRI study, Gadolinium enhanced MRI, Bone tumors
Copper Complex Cu(SBCM)2 Induced Cell Cycle Arrest and Apoptosis Towards Oestrogen-Receptor Positive MCF-7 Breast Cancer Cells

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ABSTRACT

Introduction: Copper complexes can be developed as targeted agent for cancer due to increased uptake of copper by cancer cells for angiogenesis. Our previous published data showed that copper complex Cu(SBCM)2 induced apoptosis towards triple-negative MDA-MB-231 breast cancer cells. However, its effect towards other breast cancer subtype remains unknown. Current study was performed to explore the cytotoxicity of Cu(SBCM)2 towards oestrogen-receptor positive MCF-7 breast cancer cells. Methods: MTT assay was employed to study the growth inhibition of Cu(SBCM)2 towards MCF-7 breast cancer cells and human non-cancerous MCF10A breast cells. Morphological changes of Cu(SBCM)2-treated MCF-7 cells was observed under inverted light microscope. Induction of cell cycle arrest and apoptosis were assessed by flow cytometry. The expression of wild-type p53 protein was evaluated by western blot analysis. Intracellular reactive oxygen species of MCF-7 treated with Cu(SBCM)2 was detected using DCFH-DA assay. The cells were then co-treated with Cu(SBCM)2 and antioxidants to evaluate the involvement of ROS in the cytotoxicity of Cu(SBCM)2. Molecular docking study was performed to determine the interaction of Cu(SBCM)2 with DNA, DNA topoisomerase I, and human ribonucleotide reductase. Results: Cu(SBCM)2 induced G2/M phase cell cycle arrest and apoptosis in MCF-7 cells possibly via upregulation of p53 wild-type protein. Cu(SBCM)2 was less toxic towards MCF10A cells. Increased level of intracellular ROS was not detected in MCF-7 cells after treatment with Cu(SBCM)2. However, N-acetylcysteine antioxidant enhance the cytotoxicity of Cu(SBCM)2 in MCF-7 cells. Cu(SBCM)2 showed the greatest affinity for DNA topoisomerase I in comparison to DNA and human ribonucleotide reductase. Conclusions: Cu(SBCM)2 has a potential to be developed as a targeted agent for breast cancer.

Keywords: Breast cancer, Copper complex, p53, Apoptosis, Cell cycle arrest
Development of tissue microarray (TMA) for translational research in colorectal cancer

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ABSTRACT

Introduction: Colorectal cancer is the second most common cause of death in women, and the third most common cause in men. The main treatments available following surgery are chemotherapy and radiotherapy which are not always effective and have side effects. Modern medicine aims to develop targeted cancer therapies that are efficient and cause less side effects to patients. However, this approach requires a thorough understanding of the molecular events that cause cancer cell to grow and divide in order to identify suitable targets. The process of translating the findings into clinical studies can be high cost and technically demanding. However, development of a tissue microarray (TMA), allows immunohistochemical (IHC) staining of multiple cases simultaneously, thereby greatly reducing costs and time. Methods: A TMA was produced from approximately 400 cases of colorectal cancer, along with collection of associated clinical and pathological data. Sections from the TMA were tested for quality by staining with haematoxylin and eosin (H & E), in addition to IHC markers to molecularly classify the colon cancers. Results: The cores from the 384 cases of cancer were successfully transferred to 18 recipient TMA blocks. H & E staining showed good morphological preservation of the cases, reflecting the tumour in the donor blocks. IHC testing was able to successfully classify cases into distinct molecular groupings. Conclusions: The development of a TMA of colorectal cancers provides a valuable tool for the efficient and subsequent molecular classification of colorectal cancer using immunohistochemistry.

Keywords: colorectal cancer, tissue microarray, immunohistochemical staining
Evaluating Antiproliferative Activities of Novel Hybridized Peptides against Hepatocellular Carcinoma Cells (HepG2)

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ABSTRACT

Introduction: Short peptides have acquired increasing interest as promising therapeutics, particularly as anti-cancer alternatives during the recent years. They have been reported to demonstrate incredible anti-cancer potentials through targeting signalling transduction pathways, as well as modulation of cell cycle, tumour suppressor proteins and transcription factors. Peptides are primarily of interest due to its rapid kinetics, high potency and low biocompatibility issue. In search of novel anticancer leads, the main objective of this study is to evaluate the in-vitro antiproliferative properties of hybridized peptides against human hepatocellular carcinoma (HepG2) cells. Methods: Two series of hybridized peptides, ND and DN analogues were designed based on two parent peptides, NDC1 and NDC2, through fragments hybridization approach. Modification of amino acid residues at specific positions of NDC1 and NDC2 was done at the C-terminal. Then, MTT assay was performed to examine the antiproliferative activities of NDC1 and NDC2, through fragments hybridization approach. Modification of amino acid residues at specific positions of NDC1 and NDC2 was done at the C-terminal. Then, MTT assay was performed to examine the antiproliferative activities of NDC1 and NDC2 against HepG2 cells and Vero cells at concentrations ranging from 2-256μg/mL for 24 hours. Results: The parent peptide, NDC1 showed an IC50 value of 87±3.786 μg/mL at 24 hours while NDC2 did not display antiproliferative activity against HepG2 cells. ND1-4 showed higher toxicity against Vero cells compared to HepG2 cells while ND5 did not exhibit antiproliferative activities against both cell lines. In contrary, DN1 and DN4 was found to exhibit antiproliferative activity against HepG2 cells, with IC50 values 170±60.883μg/mL and 170±60.883μg/mL, respectively. Meanwhile, both these hybridized peptides showed minimal toxicity against Vero with IC50 values >256μg/mL. In contrast, DN2, DN3 and DN5 showed minimal antiproliferative activity against HepG2 with IC50 values >256μg/mL. Conclusion: Among the ND and DN hybridized peptides, two hybridized peptides, DN1 and DN5, showed potential anti-proliferative activities against HepG2 with minimal toxicity against Vero. Nevertheless, their activity has been diminished as compared to NDC1 and hence, can be further improved.

Keywords: Hepatocellular carcinoma (HepG2), Hybridized peptides, Anticancer peptide (ACP), Antiproliferative
Chemical Investigation of the Secondary Metabolite Components of Malaysian Endophyte Aspergillus sp. HAB10R12

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ABSTRACT

Introduction: In an on-going research, the endophyte Aspergillus sp. HAB10R12 have been selected for detailed chemical investigation after its crude ethyl acetate extract showed promising anticancer properties with high selectivity. Methods: The former was determined on two cancer cell lines namely, HCT116 and MCF-7 (IC50 = 0.05 and 0.04 μg/mL, respectively) and one non-tumor cell line HeLa (IC50 = 10.5 μg/mL). Results: The result indicates the secondary metabolites produced by the fungus are 200 folds more selective towards cancer cells over normal cells, calling for an immediate detailed investigation of their composition. Preliminary chemical analysis of the crude extract using LC-MS, NMR and UHPLC-UV showed the presence of an uncommon group of diterpene pyrones (NF00659 A1, B1 and A3), previously isolated once with only partial characterization reported. Consequently, large scale isolation of secondary metabolites was carried out and led to the identification of four of the previously isolated diterpene pyrones. Conclusion: The isolation, characterization, relative stereochemistry analysis, and a plausible biosynthesis of the diterpene pyrone compounds is presented herein.

Keywords: Diterpene pyrones, Aspergillus sp., natural products, structure elucidation
Effect of acquisition parameters on the feasibility of computed tomography-based thermometry: An experimental assessment of radiofrequency ablation on phantom study

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ABSTRACT

Introduction: This paper is aimed to evaluate the feasibility of computed tomography (CT) based thermometry for radiofrequency ablation (RFA) through the investigation on the effects of principal CT acquisition parameters to the CT number. Methods: The effects of CT acquisition parameters (tube voltage, tube current, gantry rotation time, and CT reconstructed slice thickness), as well as metal artefacts on CT number were evaluated by using liver tissue equivalent polyacrylamide (PAA) phantom. The correlation between CT number and tissue temperature from 37 \degree C to 80 \degree C was studied with the use of PAA phantom with optimum CT acquisition parameters. Results: The CT numbers show insignificant changes under variation of tube voltages, tube currents, gantry rotation time, and CT reconstructed slice thickness respectively. The CT number difference obtained for each series of the variations are less than 2 HU, which indicates the changes in the CT acquisition parameters has insignificant effects on the CT number shift. On the other hand, the CT reconstructed image is able to improve the metal artefact caused by RF electrode through the increases of CT reconstruction condition. In this paper, A linear regression analysis on the CT number and temperature suggested the CT number is inverse linearly proportional to temperature, with a CT thermal sensitivity of \(-0.521 \pm 0.061\) HU/\degree C (R2 = 0.998). Conclusion: In summary, the results show that assessment of CT thermometry is feasible with the use of current CT technology as it produces high reproducibility and stable CT measurement, which is proved to be independent for CT acquisition parameters.

Keywords: CT-based thermometry, non-invasive thermometry, radiofrequency ablation
Growth Inhibition and G2M Cell Cycle Arrest Induction by Copper (II) Complex on HT-29 Human Colon Cancer Cells

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ABSTRACT

Introduction: Colon cancer is one of the leading cause of cancer death and current treatments often bring about undesired toxicities and resistance. Hence targeted therapeutic regimens for cancer are developed. Anticancer agent incorporated with copper has been synthesized to selectively target cancer cells that are reported to take up more copper compared to normal cells. Cu(SBCM)2 synthesized from the condensation of s-benzyldithiocarbazate and 3-acetylcoumarin was demonstrated to exhibit anti-proliferative effect towards MCF-7 cells and MDA-MB-231 breast cancer cells via cell cycle arrest and apoptosis. However, the mode of cell death of Cu(SBCM)2 on colon cancer cells has not been explored. This study investigated the anti-cancer properties of Cu(SBCM)2 on HT-29, colorectal cancer cell line. Methods: The growth inhibition of the copper complex was determined using MTT assay and xCELLigence real time cell monitoring analysis. Results: Cu(SBCM)2 was shown to inhibit the growth of HT-29 cells significantly in time- and dose-dependent manner with IC50 of 25.23 ± 8.22 uM at 48 hours. Morphological studies using inverted light microscope indicating Cu(SBCM)2-treated HT-29 cells displayed characteristics of apoptosis such as cellular shrinkage and membrane blebbing. Cell cycle analysis was carried out using flowcytometer and Cu(SBCM)2 was found to induce G2M cell cycle arrest at 24 and 48 hours. ROS assay was carried out to determine the involvement of oxidative stress on Cu(SBCM)2 treated HT-29 cells. Nevertheless, results indicated Cu(SBCM)2 significantly suppressed the formation of ROS compared to control. Conclusion: In summary, Cu(SBCM)2 shows promising potential in cancer therapy against colon cancer cells.

Keywords: Colon cancer, copper complex, growth inhibition, cell cycle arrest
Novel Hybridized Peptides, NDs exhibited Antiproliferative Activity against Lung Adenocarcinoma (A549) Cells

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ABSTRACT

Introduction: To date, lung cancer has become increasingly prevalent and remains the leading cause of cancer-related death in both sexes, globally. Despite the advances of cancer treatment, systemic chemotherapy remains as the major treatment option for lung cancer. Nevertheless, the trend of chemotherapy resistance has restricted the efficacy of the chemotherapy treatment, thus leading the urge to develop an alternative chemotherapeutic agent which could give a promising treatment effect. Short peptides have acquired increasing interest as promising therapeutics due to its anticancer potential, rapid kinetics, high potency and low biocompatibility issue. In search of novel anti-cancer leads, the main objective of this study is to evaluate the in vitro antiproliferative properties of hybridized peptide analogues against human lung adenocarcinoma (A549) cell line. Methods: ND and DN analogues were designed based on two parent peptides, NDC1 and NDC2, through fragments hybridization approach. Modification of amino acid residues at specific positions of NDC1 and NDC2 was done at the C-terminal. Then, MTT assay was performed to examine the antiproliferative activities of NDC1, NDC2, NDs and DNs against A549 cells at concentrations ranging from 2-256μg/mL for 24 hours. Results: Findings obtained showed that the parental peptides, NDC1 and NDC2, exhibited IC50 values of 47.5±4.950μg/mL and 239±9.899μg/mL, respectively. All NDs showed excellent antiproliferative activities with IC50 values ranging from 22-71μg/mL. Nevertheless, all DNs did not display antiproliferative activity when tested up to 256μg/mL. We speculated that increased valine and isoleucine with decreased aspartic acid composition in NDs might be associated with their intermediate cytotoxicity strength, comparing with the parent peptides. However, the location of other amino acids in the peptide sequence should still be further investigated as it contributes to the peptide structure, hence leading to its selectivity and potency. Conclusion: As a conclusion, NDs could be further explored to develop a potent anti-cancer therapeutic drug.

Keywords: Anti-cancer peptides; lung cancer; chemotherapy; multidrug resistance; peptide fragments hybridization
Laser-driven hot needle for tissue cauterization and percutaneous hyperthermia cancer therapy

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ABSTRACT

Introduction: Cancer has become a major economic and societal burden. The National Cancer Registry of Malaysia (NCR) estimates that one in four Malaysian (1:4) will develop cancer by the age of 75. This project aims to develop a prototype named “Laser ablation needle” for tissue cauterization and percutaneous hyperthermia cancer therapy. Our ultimate goal is to develop a highly flexible, operator-friendly and cost-effective laser ablation needle for tissue cauterization and hyperthermia cancer therapy, hence to improve the overall cancer survival rate and quality of life among the cancer patient population. Methods: The laser ablation needle is a closed loop opto-electronic control system, consists of a 2 mm Fiber Bragg Grating (FBG) – optical fiber temperature sensor, a laser driven hot needle and a micro-controller. Based on real-time temperature input from the FBG sensor, the micro-controller can perform a dynamic PID control on laser intensity for a safe hyperthermia treatment. In the fabrication, a medical grade optical fiber with a diameter of 800 μm was used for laser delivery. The optical fiber was embedded inside a biocompatible resin-made needle and connected to a 450 nm high power blue laser diode. The FBG temperature sensor was incorporated in the needle for real-time temperature monitoring and control. Focal hyperthermia produced by the laser-driven hot needle was conducted on ex-vivo bovine liver. Results: The rise in temperature was recorded by increasing laser power. The temperature profile was obtained at each depth. Irreversible thermal denaturation during irradiation was captured. Conclusion: These preliminary results suggest that this technique can be applied safely and effectively for cancer treatment. The developed prototype comprised of the diode laser showed that it can deliver its energy via simple optical fiber. This laser is cheaper and much smaller than the conventional high power lasers used in other studies.

Keywords: cancer, hyperthermia, laser ablation
Preparation of Theranostic 153Samarium-labelled Polystyrene Microspheres for Hepatic Radioembolization


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ABSTRACT

Introduction: Hepatic radioembolization is a minimally invasive procedure involving intrarterial administration of radioembolic microspheres for the treatment of liver tumours. In this study, a biocompatible polystyrene (PS) microspheres formulation containing radioactive Samarium-153 (153Sm) was synthesized and tested. The 153Sm emits both diagnostic gamma energy and therapeutic beta radiation, renders the synthesized microspheres an ideal theranostic radioembolic agent for hepatic radioembolization.

Methods: First, the 152SmO3 (20 – 50%, w/v) was encapsulated in PS microspheres using solid-in-oil-in-water solvent evaporation method. The 152Sm-labelled PS microspheres were then activated to 153Sm (Eβmax = 807.6 keV, half-life = 46.3 hours) via 152Sm(n,γ)153Sm reaction in a nuclear reactor with a neutron flux of 2.0 x 1012 n.cm-2.s-1. Physicochemical characterization, gamma spectroscopy and in-vitro radiolabeling studies were carried out to study the properties and stability of the microspheres before and after neutron activation.

Results: The 153Sm-labelled PS microspheres achieved a nominal activity of 4.0 GBq.g-1 after 6 hours of neutron activation. Scanning electron microscope (SEM) and particle size analysis show that the microspheres remained spherical with diameters within 15 – 60 μm after neutron activation. No long half-life radioimpurities were found in the samples as revealed by the gamma spectroscopy results. The 153Sm-labelled PS microspheres achieved radiolabeling efficiency of more than 95% in saline and blood plasma over 480 hours.

Conclusion: A biocompatible 153Sm-radiolabelled PS microspheres formulation has been successfully developed. The formulation achieved desirable properties for theranostic treatment of liver tumours. The formulation is relatively cheaper, easier to be produced and more readily available.

Keywords: Biocompatible, Hepatic Radioembolization, Polystyrene Microspheres, Samarium-153, Theranostic.
PC13

The Role of Hedgehog Signalling Pathway in Human Cancers Development

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ABSTRACT

Introduction: The Hedgehog (Hh) pathway serves as a major regulator in organ development, stem cell maintenance, cell proliferation, and cell differentiation. This pathway is highly regulated and aberrant activation will promote tumorigenesis. Hh pathway notably Sonic Hedgehog pathway was reported to be upregulated and promote tumorigenesis in various human malignancies including colorectal, gastric, lung, prostate, and breast. This review was aimed to discuss the current understanding of Hh pathway activation in different types of human cancers and discuss the development of the therapeutic applications targeting Hh pathway.

Methods: A systematic review was conducted using the electronic research database PubMed Central (PMC) from 2014-2019. The search was limited to studies that are relevant to both Hh signalling pathway and human cancers. A total of 50 articles were selected and their references cited were searched and reviewed.

Results: The results regarding the role of Hh signalling in pancreatic cancer and colorectal cancer are controversial with some reporting tumor promoting activities whereas others tumor suppressive activity. Besides, results from other studies suggesting that Hh signalling pathway plays an oncogenic role by inducing tumor cells proliferation, promoting metastasis and maintaining cancer stem cells in human cancers such as lung, stomach, and breast. To date, Gladegeib (PF-04449913) is the only Hh targeting small molecule inhibitor being studied at FDA Phase 3 clinical trial. Identification of the right tumors and minimization of the side effects remain as the main obstacles in the development of Hh signalling inhibitors.

Conclusion: In conclusion, advancement in our understanding of Hh pathway has provided us opportunity to develop novel therapeutic strategies to fight human cancers with activated Hh pathway but more studies need to be conducted to solve the controversial regarding the role of Hh pathway in certain cancers.

Keywords: Hedgehog pathway, human cancer, Hh signalling inhibitors
Development of Samarium-153 Labelled Radiotracer for Gamma Scintigraphy of Whole Gastric-Intestinal Transit Study

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ABSTRACT

Introduction: Constipation is affecting a quarter of human population at any one time in all age groups. However, a proper gamma scintigraphic study of whole GI transit is rarely performed in Malaysia due to the lack of suitable radiopharmaceutical. Hence, this study was taken to develop a suitable radiotracer formulation for gamma scintigraphy study of whole gastric-intestinal transit.

Methods: The biocompatible polystyrene (PS) incorporated with \(^{152}\text{Sm}_2\text{O}_3\) (5%, w/v) will be used to synthesize the radiotracer. The \(^{152}\text{Sm}\)-labelled PS particles was neutron activated to \(^{153}\text{Sm}\) in a nuclear reactor for 5 minutes. Characterization of the physicochemical properties, gamma spectrometry and in-vitro radiolabeling studies in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were carried out to study the properties and stability of the radiotracer before and after neutron activation.

Results: Scanning electron microscope (SEM) and particle size analysis showed that size, shape and surface morphology of the particles remained after neutron activation. The synthesized \(^{153}\text{Sm}\)-labelled PS radiotracer (100 mg) particles achieved an activity of 3.7 MBq after 46 hrs. As indicated by the gamma spectrometry result, there is no long half-life radioimpurities present in the samples. The \(^{153}\text{Sm}\)-labelled PS particles achieved radiolabeling efficiency of more than 95% in both SGF and SIF over 72 hrs.

Conclusions: A \(^{153}\text{Sm}\)-labelled radiotracer particles formulation has been successfully developed from biocompatible PS. The proposed formulation has the advantage of cheaper, easier to be produced and reduced radiation exposure to staff. Further studies are required to validate the in-vivo performance of \(^{153}\text{Sm}\)-labelled formulation for assessing GI motility and transit in clinical use.

Keywords: GI motility and transit, Samarium-153, Radiotracer, Gamma Scintigraphy
In-vitro Evaluation of Apoptotic Effect of Germacrone on Human Melanoma, Cervical and Gastric Adenocarcinoma

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ABSTRACT

Introduction: Germacrone is a natural product isolated from Rhizoma curcuma with anti-tumor, anti-inflammatory, and anti-bacterial properties. Previous studies have demonstrated that Germacrone exhibits anti-tumor effect in breast and hepatoma cancer cell lines but the studies of its molecular mechanisms and anti-tumor properties in other cancers are not well studied. This study aims to investigate the anti-tumor effect of Germacrone on human skin, cervix, and gastric cancer cell lines and the molecular mechanism underlying the anti-tumor effect of Germacrone.

Methods: A375 (skin malignant melanoma), AGS (gastric adenocarcinoma), and HeLa 229 (cervix adenocarcinoma) cell lines were employed for this research. Treatment of the cell lines with Germacrone has inhibited the cell proliferation in a dose-dependent manner as assessed by MTT assay. The cell lines were incubated with Germacrone for 24 hours followed by detection of the expression of BAX, BAK, p53, BCL2, MCL1, and BCL-XL using Real-time PCR.

Results: Results from Real-time PCR has showed that pro-apoptotic gene BAK was highly expressed in all the human cell lines after the treatment with Germacrone. Furthermore, the expression of pro-apoptotic gene p53 were elevated in both A375 and HeLa 229 cell lines but not in AGS cell lines. The expression level of pro-survival genes BCL2 and MCL1 were found to be decreased in both AGS and A375 cell lines.

Conclusion: In conclusion, Germacrone might be a potent anti-tumor drug candidate for Human Melanoma, Cervix Adenocarcinoma, and Gastric Adenocarcinoma by increasing the expression level of pro-apoptotic proteins BAK. Future studies will focus on studying the cytotoxicity effect of combination of Germacrone with standard chemotherapy drugs on Human Melanoma, Cervix Adenocarcinoma, and Gastric Adenocarcinoma.

Keywords: Germacrone, Rhizoma curcuma, anti-tumour, melanoma, gastric adenocarcinoma, cervical adenocarcinoma
Combination Therapy of a Novel Ruthenium Polypyridyl Complex and Cisplatin for the Treatment of Cancer

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ABSTRACT

Introduction: Combination therapy to treat cancer have been demanded due to the complexity of the disease and to prevent resistance mechanisms commonly found in classic chemotherapeutic methods. Recently, we have reported that [Ru(dppz)2(PIP)]2+ (dppz = dipyridophenazine, PIP = 2-(phenyl)imidazo[4,5-f][1,10]phenanthroline) (RuPIP) immediately stalls replication fork progression in HeLa human cervical cancer cells. Co-incubation with a Chk1 inhibitor achieves synergistic apoptosis in cancer cells. These discoveries indicate that this class of compounds merit further investigation as anticancer drugs, especially within combinational therapy roles. However, information pertaining to the effects of combining ruthenium compounds with existing chemotherapeutic drugs remain scarce. This study aimed to investigate the possible synergistic cytotoxic effects of using RuPIP in combination with cisplatin on different cancer cell lines. Methods: A549, MCF7, Hela and T24 cells were treated with different concentrations of RuPIP or cisplatin alone, as well as different combinations of these two agents at a fixed ratio 1:1 over the course of 72 hr to assess their individual and combination effects. Cell viability was analysed using MTT assay. The combination index (CI) was calculated based on the Chou Talalay Method. Results: Single-agent treatment at 72 hr with RuPIP or cisplatin led to dose-dependent decreases in the viability of the A549, MCF7, Hela and T24 cells at 72 hr. Furthermore, increasing the concentrations of the combinations up to four folds of half maximal inhibitory concentration (IC50) statistically decrease the cell survival rates of A549 and MCF7 cells thus, displayed synergistic effects. Conclusion: Treatment of MCF7 and A549 cells with a combination of RuPIP and cisplatin showed a synergistic effect and thus are promising as a combination therapy for cancer treatment.

Keywords: ruthenium, cisplatin, combination therapy, cancer, Chou Talalay Method
A Novel Combination of Ruthenium Polypyridyl Complex, [Ru(dppz)2PiP]^{2+} and PARP Inhibitor, NU1025 for Cancer Treatment

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ABSTRACT

Introduction: Ruthenium polypyridyl complex (RPC), [Ru(dppz)2PiP]^{2+} or RuPiP, where dppz = dipyridophenazine, and PiP = 2-phenylimidazo[4,5-f][1,10]phenanthroline has been shown to exhibit anticancer activities by stalling the replication fork progression in human cancer cell line, causing DNA double-strand break (DSB) leading to the initiation of DNA damage response (DDR). Poly (ADP-ribose) polymerase (PARP) enzymes are activated in response to DNA damage thus, RuPiP may be advantageously combined with the inhibitors of PARP to improve its efficacy in cancer cell killing. This study was conducted to investigate the cytotoxic effects of RuPiP and selected PARP inhibitor, NU1025, alone or in combination \textit{in vitro} and the possible combinations in order to achieve synergism against three different cancer cell lines. \textbf{Methods:} Cell viability was determined by MTT assay based on established method and the combination index (CI) values were calculated using Chou and Talalay method. \textbf{Results:} Here, we reported that the treatment with RuPiP alone led to dose-dependent decreases in the cell viability meanwhile NU1025 exhibited no toxicity as a single agent. The CI values (<0.9) were interpreted as synergism and our results showed that the combination of RuPiP and NU1025 gave synergistic effects on all three different cancer cell lines. \textbf{Conclusion:} These finding suggest promising benefits of combining RuPiP and NU1025 to optimize cancer treatment in further preclinical and clinical studies.

\textbf{Keywords:} Ruthenium Polypyridyl Complex, Cancer, PARP inhibitor, Synergism, NU1025
Improved In Vitro Toxicity of Ruthenium complex by Mesoporous Silica Nanoparticles Encapsulation

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ABSTRACT

Introduction: Ruthenium compounds are widely studied for its biological activity. However, potent ruthenium drugs often have limited bioavailability due to its poor aqueous solubility. In order to assist the preparation of these hydrophobic compounds, a carrier or drug delivery agent is often introduced. Herein, we encapsulated a hydrophobic ruthenium polypyridyl complex $[\text{Ru}(dppz)\text{PIP}]^{2+}$, ($dppz = \text{dipyrido-}[3,2-a:20,30-c]\text{phenazine, PIP = 2-phenylimidazo}[4,5-f][1,10] \text{phenanthroline}$) in mesoporous silica nanoparticles (MSN). Methods: The MSN was synthesized by using ionic liquid 1-hexadecylphenanthrolinium as a novel template and have 833.99 m$^2$/g in surface area. The cytotoxicity of the synthesized ruthenium complex, MSN and MSN-loaded ruthenium was studied on Hela and A549 cancer cell lines. Results: MSN was non-toxic at lower dosage (<500 μg) while $[\text{Ru}(dppz)\text{PIP}]^{2+}$ displayed moderate toxicity against both cell lines, with IC$_{50}$ of 17.27 μM and 19.92 μM for A549 and Hela, respectively. Remarkably, the encapsulated drug delivered higher toxicity at smaller concentration, with IC$_{50}$ of 2.97 μM and 1.38 μM towards A549 and Hela, respectively. The results signify that $[\text{Ru}(dppz)\text{PIP}]^{2+}$ potential in anti-cancer improved following encapsulation with MSN. Conclusion: This study indicates that mesoporous silica nanoparticles might be a useful drug delivery agent in promoting the bioavailability of hydrophobic ruthenium compound for biological activity.

Keywords: ruthenium, mesoporous silica nanoparticles, anti-cancer
PC20

Development of Peptides Inhibitor Against Human Papillomavirus E7 Protein

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ABSTRACT

Introduction: Human papillomavirus (HPV) is a small, non-enveloped double-stranded circular DNA virus. The high risk types of HPV are claimed to be responsible for over 99% of cervical cancers while the most common oncogenic genotypes 16 and 18, implicated in approximately 70% of all cervical cancers. HPV has six early genes (E1, E2, E4, E5, E6 and E7) that code for regulatory proteins involved in viral DNA replication, transcription control and cellular transformation. The E7 protein is responsible for the escape from cell cycle arrest in HPV infected cells by binding to the retinoblastoma protein (pRB) through its LXCXE binding motif. This inhibits the tumour suppressor protein from regulating the cell cycle progression from G1 to S phase. In this study, the pocket domain of pRB which is targeted by LXCXE is used as a target to design peptide inhibitors using in silico methods. Methods: Biovia Discovery Studio Visualizer and UCSF Chimera softwares were used in designing the peptides. Results: Two crystal structures: 1GUX and 4YOZ were superimposed and studied. The similar amino acid sequences which bind to LXCXE were cut to form the potential peptide inhibitors. Based on this, peptide 1 was selected for further in vitro analysis. The cytotoxicity of the peptide 1 was analysed on three cell lines: CaSki (HPV16+ cervical cancer cell), C33a (cervical cancer cell), and HaCaT (normal keratinocyte). As a result, the IC50 of one of the designed peptide inhibitor – peptide 1, on CaSki cell line was 180 μM. The inhibitory effect of the peptide 1 was also analysed validated using Western Blot using antibodies from the B-Myb and pRB family. The changes in cell cycle before and after treatment of the peptide inhibitor will be further monitored using a cell analyser. Conclusion: Peptide 1 had shown potential as an inhibitor of the HPV E7 protein based on the in silico analysis, but further functional studies are needed to validate its potential.

Keywords: HPV, E7, retinoblastoma protein (pRB)
Effects of amyloid precursor protein on pro-apoptotic pathway in neuronal cells

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ABSTRACT

Introduction: Amyloid plaques, mainly comprising of amyloid-beta peptides derived from its precursor protein, are found deposited in hippocampal and entorhinal cortical regions of patients with Alzheimer’s disease (AD). However, none led to a complete understanding of the molecular mechanisms of the AD in order to generate a new therapy that would eradicate the disease effectively. Activation of pro-apoptotic pathway was found to be associated with the accumulation of amyloid precursor protein (APP). The objective of this study is to examine the effects of APP overexpression on the Bcl-2 family proteins involving in pro-apoptotic pathway in neuronal cells.

Methods: The experiment was first performed with the transfection of HEK 293T cells for generation of lentiviral vector system consisting APP plasmid followed by transduction of SH-SY5Y neuronal cells using lentivirus generated. Subsequently, western blot analysis was conducted to validate the APP overexpression in SH-SY5Y cells. Then, expression levels of Bcl-2 family proteins in the APP overexpressed cells were determined by western blot analysis. The statistical analysis was performed by Microsoft Excel with Student’s t-test.

Results: APP overexpression in SH-SY5Y cells slightly upregulated the pro-apoptotic proteins including Bad, Bid, Bok and Puma but slightly downregulated Bcl-2, Bim and Bax. Conclusion: Our data suggest that APP overexpression regulated the Bcl-2-mediated pathway by a significant downregulation of Bim protein in neuronal cells.

Keywords: Alzheimer’s disease, amyloid precursor protein, Bcl-2 family proteins, proapoptotic Pathway
ABSTRACT

Introduction: Depression is becoming increasingly prevalent as a mental health disorder worldwide. The prevalence of clinical depression is between about five and fifteen percent globally. Clinical depression has also increased in prevalence among the ageing. Some of the etiological factors associated with depression in the ageing include grief and loss, and role transitions. Interpersonal Psychotherapy (IPT), an evidenced based psychotherapy for clinical depression, has been proven to be effective for depression in the ageing. IPT addresses four main problem areas, namely - interpersonal disputes, grief and loss, role transitions and interpersonal sensitivity. The adaptation of IPT for the ageing is IPT for late-life depression or IPT – LLM which was utilized to treat the patient discussed in this case study. Methods: The patient was treated with 12 sessions of psychotherapy which is often the minimum number of sessions required in the treatment of depression with IPT. The initial sessions included the development of an Interpersonal Formulation and Interpersonal Inventory. Her problem areas were grief as her husband had passed away recently, and role transitions. The problem areas were addressed during the 8 middle IPT sessions. The final two sessions were utilized to conclude IPT treatment. Results: The patient was assessed to have a PHQ – 9 (Patient Health Questionnaire--9) score of 17 before treatment with IPT, indicating moderate depression. Her PHQ – 9 score after 12 sessions of IPT was 4 indicating minimal depression. Conclusion: This case study highlights that some of the factors that contribute to depression in the ageing are grief and role transitions, and that IPT is efficacious in the treatment of depression in the ageing.

Keywords: Interpersonal Psychotherapy, Depression, Ageing
The Potential Protective Effect of Curcumin and Piperine on Amyloid-β-42 Induced Cytotoxicity, Fibril Aggregation and Oxidative Damage in SY5Y Cells

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ABSTRACT

Introduction: Amyloid-β (Aβ) peptides play a key role in the pathogenesis of Alzheimer disease and exert various toxic effects on neurons. Dietary phytochemicals are currently used as an adjuvant therapy to accelerate their therapeutic efficacy. Therefore, the present study was designed to investigate the effect of curcumin and its co-administration with piperine against Aβ42 induced cytotoxicity, fibril aggregation and oxidative damage in SH-SY5Y cells.

Methods: The neuroblastoma SH-SY5Y cells were cultured with different treatments of Aβ42, individual curcumin and piperine and combination of curcumin and piperine. Cell viability, Aβ fibril aggregation and oxidative damage such as lipid peroxidation, catalase and glutathione were assessed. The abilities of curcumin and its combination, piperine to scavenge free radicals and to inhibit Aβ aggregation and β-sheeted formation were further assessed.

Results: Curcumin and piperine preserves cell viability, which is decreased by Aβ, indicate that curcumin protects Aβ-induced neuronal damage. Under aggregating conditions in vitro, curcumin and piperine inhibited aggregation as well as disaggregated fibrillar Aβ42, indicating favorable stoichiometry for inhibition. Results also showed that curcumin and piperine as a combination was a better Aβ42 aggregation inhibitor than the individual compounds. Curcumin and piperine depresses Aβ-induced up-regulation of neuronal oxidative stress. The ability of these compounds to scavenge free radicals and inhibit the formation of Aβ aggregation are implicated from the results of this study.

Conclusion: This combination of curcumin and piperine shows a more protective effect on neuronal oxidative damage when they were added into cultured neurons not later than Aβ, especially prior to Aβ. The curcumin and piperine combination prevents neurons from Aβ-induced oxidative stress, indicating a promising therapeutic in preventive medicine for Alzheimer disease.

Keywords: Alzheimer disease, Amyloid beta, Curcumin, Piperine
Acute abdomen with caecal diverticular mass: an elderly issue

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ABSTRACT

Introduction: Acute abdomen in an elderly, especially in the right iliac fossa, is usually the common symptom that needs a precise surgical assessment with acute appendicitis being a common cause. Rarely, other conditions can mimic this presentation such as acute caecal diverticulitis. We describe a rare case of right iliac fossa pain in a 72-year old male which mimicked an acute appendicitis. The ability to recognize such condition is very important as its management is different at various stages. The objective of this case report is to increase awareness of this condition to ensure it is appropriately treated when encountered unexpectedly.

Methods and Results: A 72-year old man was admitted with the complaint of right iliac fossa pain for 5 days. A pre-operative clinical diagnosis of acute appendicitis was made. The patient underwent an emergency laparotomy where an inflammatory mass in the caecum was found. A right hemicolecotomy was performed as there was a suspicion of underlying malignancy. His histopathology report showed a single inflammatory diverticular mass. Acute caecal diverticulitis has a higher incidence in the Asian descent. In Western countries, more than 80% of all diverticula occur in the left sided colon, whereas the incidence of right-sided diverticular disease in Oriental countries can be up to 75%. The pre-operative diagnosis of right caecal diverticulitis is usually challenging to the surgeons, if without radiological imaging. Ultrasonography (USG) and computed tomography (CT) are the important investigations for the diagnosis with their specific findings. The ability to recognize the condition is very important as its management is different at various stages. The morbidity and mortality can be improved by earlier detection and proper management. Conclusion: Acute caecal diverticulitis should be considered in the differential diagnosis in elderly with right iliac fossa pain. The surgical approach can be range from a simple diverticulectomy to a more complex right hemicolecotomy. The decision making should always be tailored to the severity of the condition of the patient.

Keywords: Appendicitis, Diverticulitis, Appendectomy
Effects of amyloid precursor protein overexpression on nuclear factor kappa beta pathway in neuronal cells

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ABSTRACT

Introduction: The aging process is the most significant risk factor for developing Alzheimer’s disease (AD). AD is the most common neurodegenerative disease that causes cognitive and memory impairment in the elderly. Excessive build-up of amyloid protein leads to cell death, brain atrophy, and cognitive and functional decline in AD. The nuclear factor kappa beta (NF-κB) is a family of inducible transcription factors composed of NF-κB1, NF-κB2, RelA, RelB and c-Rel. It is activated by genotoxic agents, as well as oxidative and inflammatory stresses. It regulates expression of genes that control apoptosis, cell cycle progression, cell senescence, and inflammation. NF-κB regulates amyloid precursor protein (APP) processing by activating transcription of β and γ secretases, which promotes amyloid dysregulation in AD. In addition, NF-κB activation is linked with many of the known lifespan regulators including insulin/IGF-1, FOXO, SIRT, and mTOR. Therefore, NF-κB pathway contributes to the pathophysiology of AD. This study aims to evaluate the effects of APP overexpression on NF-κB pathway in neuronal cells. Methods: SH-SY5Y neuronal cells were transduced with APP plasmid. Overexpression of APP in the cells was validated by western blotting. Western blot analysis using antibodies targeting NF-κB signalling pathway was performed using the APP-overexpressed cells. Results: Overexpression of APP in cells caused a significant down-regulation of phospho-NF-κB. Overexpression of APP also slightly up-regulated IkappaB-alpha, IKK alpha, and IKK beta. Conclusion: APP overexpression affected NF-κB pathway by down-regulating NF-κB protein.

Keywords: Alzheimer’s disease, amyloid precursor protein, nuclear factor-kappa B.
Acetylcholinesterase enzyme inhibition potential of natural plant compounds: A therapeutic lead for Alzheimer disease

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ABSTRACT

Introduction: Alzheimer disease (AD) is the most widely recognised neurodegenerative disorder in the ageing population, characterised by progressive neuronal loss. The enhanced level of acetylcholine (ACh) in the human brain is another measure of identifying the progression of the disease. Increased acetylcholinesterase (AChE) level plays a fundamental role in the hydrolysis of ACh which worsens the cognitive function. Though there are several medications or drugs currently used in treating AD, their clinical implications remains debatable due to its adverse effects.

Methods: In this study, the combined nutraceutical effect of natural plant compounds, piperine and curcumin were evaluated for acetylcholinesterase inhibitory assay using Ellman colourimetric method.

Results: Results obtained from the study revealed that combined effects of natural plant compounds showed promising acetylcholinesterase inhibition activity with an IC₅₀ of 104.1 ± 0.08 μg/mL compared to individually treated compounds, i.e., IC₅₀ of curcumin = 134.5 ± 0.05 μg/mL and IC₅₀ of piperine = 62.81 ± 0.00 μg/mL.

Conclusion: The results suggest that the natural plant compounds taken in combination act as natural acetylcholinesterase inhibitors, and could be beneficial in the treatment of AD.

Keywords: Alzheimer disease, In-vitro Acetylcholinesterase, Curcumin, Piperine
The Immunomodulatory Effects of Tualang and Kelulut Honey on Microglial Cell Activities

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ABSTRACT

Introduction: Neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) have become increasingly prevalent in recent years. Microglia, the resident myeloid cells of the Central Nervous System (CNS) are primarily responsible for the production of inflammatory mediators that accumulate and become toxic during a chronic inflammatory response. The accumulation of inflammatory mediators over time inadvertently contributes to the functional impairment of surrounding neurons. Hence, suppressing microglial cell activation can be a solution to control the progress of neurodegenerative disorders. Symptomatic treatments are available, but no curative treatment is currently available, and some are linked to several side effects associated with their use. Honey is a natural product derived from the nectar harvested and modified by honeybees. Its therapeutic effects are widely documented, have been tested and verified extensively in literature. Honey is recognized in modern medicine for its varied pharmacological activities. While the medicinal properties of honeys such as Manuka honey are well established, further investigation is required to elucidate the medicinal properties of locally sourced honeys, namely Tualang (TH) and Kelulut (KH) honeys. In this study, we investigated the immunomodulatory effects of local TH and KH honey on microglial cell activities. Methods: BV2 cells, an immortalized microglial cell line was used in this in vitro study to assess the cell survival when treated with the TH and KH honey. Expression of CD40, CD11b and CD86 were measured using flowcytometry. Results: BV2 cells incubated with TH at concentrations of 0.1% and 0.5%, and KH at concentrations of 0.1% and 0.25% for 24 and 48 hours showed cell survivability above 75%. Both TH and KH decreased ROS production significantly on LPS-induced BV2 cells, but increased ROS production on unstimulated BV2 cells. Additionally, the expression levels of CD40, CD11b and CD86 were also reduced on honey-treated LPS-induced BV2 cells. Conclusion: These results have demonstrated that both TH and KH are capable of suppressing microglial activation. Therefore, we propose the idea of utilizing these honeys as a complementary treatment to suppress the progression of neurodegenerative diseases.

Keywords: Honey, Microglia, Neurodegeneration
PA9

Effect of aerobic exercises on physical fitness and mental well-being in older adults: a scoping review of published systematic reviews

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ABSTRACT

Introduction: There has been growing interest in recent decades in the effect of physical activity in older people as it was believed to play an important role in maintaining functional independence while reducing health care burden at a low cost. Recent reviews showed aerobic exercises were correlated with healthy ageing outcomes, however between-study variance and variation in study population and outcome measurements warrant a more comprehensive assessment of the current evidence. This scoping review of systematic reviews aimed to evaluate and synthesize review evidence on the effect of aerobic exercises on physical and mental well-being in older adults. Methods: Electronic databases, including Cochrane Database of Systematic Reviews, PubMed/MEDLINE, Embase, PsycINFO, CINAHL were searched from inception to 31 December 2018 to identify systematic reviews, with or without meta-analyses that examined the effectiveness of aerobic exercises in older adults. Methodological quality was assessed using Risk of Bias in Systematic reviews (ROBIS) tool. Results: Two hundred and thirteen reviews met the inclusion criteria. In this scoping review, we included 56 reviews that evaluated the effect of aerobic exercises: 24 examined the effect of aerobic exercises on physical outcomes, 26 examined mental outcomes, 5 examined both physical and mental outcomes and 2 examined the effect on quality of life (QoL). Most reviews showed positive effect of aerobic exercises on physical fitness (18 out of 23), mental well-being (15 out of 26) and quality of life (2 out of 2), while others showed inconsistent or negative results. Majority of the reviews reported low to moderate quality with moderate to high risk of bias. Conclusion: Overall, current evidence showed that aerobic exercises appear to be beneficial for physical fitness and QoL in elderly. The effectiveness of aerobic exercises to improve or maintain cognitive function is inconclusive hence would require further evidence prior to recommendation.

Keywords: aerobic, older adults, scoping review
Potential of several types of Malaysian honey in reducing excess weight gain in obese-induced rats

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ABSTRACT

Introduction: Obesity is one of the major health problems in Malaysia. It can lead to other metabolic diseases including diabetes and cardiovascular diseases. Unfortunately, effective treatment to overcome the health problem is yet to be discovered. One of the alternative solutions is by introducing a healthy diet through functional foods. Honey is well-known as one of the functional foods. However, the effectiveness in controlling obesity and then reducing the excess weight gain is still unclear. Several types of local honey were selected to observe their effects on obese-induced rats. Methods: Acacia, Gelam and Pineapple honey were harvested directly from apiaries and forest in Malaysia. Then, the quality of the honey was measured and standardized through physicochemical and antioxidant analyses. Male Sprague Dawley rats were induced to obese by consuming a high-fat diet. Then, the rats were fed with the honey for acute (one single honey consumption for 14 days) and subacute study (honey consumption daily for 16 weeks). Rats fed with orlistat (commercial drug for obesity) and fake honey were used as controls. Physical observation and biochemical analysis were conducted. Results: In the acute study, Gelam and Pineapple honey were significantly reduced the rat’s body weight, glucose, cholesterol and triglycerides level. More profound effects were observed in the subacute study, where all the honey samples were significantly reduced excess weight gain, glucose and the lipid profiles. Meanwhile, orlistat was also demonstrated a reduction in the excess weight gain but with toxicity side effects to the hepatic and renal function. In contrast, fake honey showed significantly increased body weight gain, glucose and the lipid profile in the rats. Conclusion: Based on the results, Malaysian honey samples have the potential to be a part of the daily diet in controlling obesity and reducing excess gain. However, more studies are required to confirm the findings.

Keywords: Malaysian honey, obesity, fake honey, animal model, functional foods
Trends And Awareness Of Lifestyle And Metabolic Diseases
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ABSTRACT

Introduction: The causes of obesity is caloric intake that, over time, is greater than the caloric expenditure. Factors that are associated with this energy imbalance include genetic predisposition, behavioural dynamics, hormonal disturbances, and environmental circumstances. Results: Food Intake: Increased portion sizes, the energy density of today’s commonly eaten foods, and the trend in consuming meals outside the home contribute to the increase in calorie consumption. Satiety helps determine food intake and is partially determined by the volume and weight of the food consumed. Foods that are high in calorie content for a given volume, such as highly processed, low-fiber foods, can lead to excessive calorie intake. Many factors contribute to overeating, including stress, boredom, nutritional insufficiencies, emotional lability, access to food, and the changing of our diet to one that is highly processed. Movement and Physical Activity: In a recent review, Denham et al. (2013) summarized the ability of physical activity to influence epigenetic modifications of histones or DNA in the brain, skeletal muscle, and peripheral blood. Aerobic exercise over many weeks was the primary variable studied that led to activity-induced benefits. Several of the studies found benefits with 30 minutes of daily moderate activity. Yoga is an effective type of movement activity for improving weight and mental well-being. Sleep: There is an association between too little or too much sleep and overweight and obesity. In a survey of more than 54,000 U.S. adults age 45 years or older, sleeping too little (≤6 hours) and sleeping too much (≥10 hours) were significantly associated with obesity. Sleep deprivation is associated with elevated ghrelin, elevated cortisol, elevated insulin, decreased leptin, and increased hunger (Patel and Hu, 2008). Psychosocial Stress: In addition to the negative influences of physiological stress from poor nutrition, insufficient appropriate activity, and inadequate sleep, psychosocial stress can also contribute to excess weight. Psychosocial stress can arise from a wide variety of environmental stressors, such as change in routine, difficult decisions, depression, chronic health issues, lack of access to health care, economic challenges, inadequate social support, abusive relationships, illiteracy, job dissatisfaction, poor adjustment to life-cycle transitions such as retirement, and legal problems. This type of stress is often associated with weight gain, elevated BMI, and poor food choices. Increased cortisol levels can lead to weight gain around the abdominal region (central adiposity). Conclusion: It is important to screen for emotional stress with patients, identify how this stress may be negatively impacting their weight, and help them incorporate stress reduction programs to achieve their weight loss goals.

Keywords: Metabolic diseases, lifestyle factors, food intake, physical activity, sleep deprivation, psychosocial stress
Rodent model of obesity mimicking human metabolic syndrome

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ABSTRACT

Introduction: Polymetabolic syndrome is a malady encompassing centralized accumulation of lipids and subsequent resistance to insulin leading towards diabesity. Currently, this condition is perilous threat to public health and also, creating perplexity for medical scientists. There is an intensive need for the development of obese rodent model having close resemblance with human metabolic syndrome (MetS); so that intricate molecular and/or therapeutic targets can be elucidated. The resultant simulations will be beneficial to explicate not only pathogenesis but also secret conversation of signaling pathways in inducing MetS related complications in other organs. Methods: Currently, there are different methods for the development of rodent models of MetS, for instance, utilizing high lipogenic diet, genetic alterations, induction by chemicals or by combination of high fat diet and few others. In general, combination of cafeteria or western diet and low dose of streptozotocin (STZ) is a fine example of diet induced experimental model. In this model animals are allowed free access to highly palatable, energy dense, unhealthy human food for 12-18 weeks which promotes voluntary hyperphagia resulting in weight gain, increased fat mass and insulin resistance. At the end of feeding period 30mg/kg STZ is given intraperitoneally to mimic human type 2 diabetic condition. Conclusion: Consumption of cafeteria diet with low dose STZ is considered to be the robust model of diabesity offering an exceptional stage to investigate the genomic, molecular, biochemical and cellular mechanisms of obesity and type 2 diabetes.

Keywords: Metabolic syndrome, diabesity, cafeteria diet, streptozotocin
Overweight and obesity among employees at a private university in Malaysia: A cross-sectional study towards a healthy workplace

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ABSTRACT

Introduction: Obesity is a growing public health concern. Poor diet and lifestyle choices are the main contributors to its development. Lifestyle modifications should be aggressively promoted in the community. Recent studies found that worksite obesity prevention and control programs are effective in increasing physical activity and weight reduction among employees. In Malaysia, there is insufficient data on prevalence studies of workplaces. This study investigated the proportion of overweight and obesity among workers in a private university and hope to serve as a baseline for future healthy workplace programs. Methods: This cross-sectional study investigated overweight and obesity and examined its association with occupational stress, sleeping quality, dietary behaviour and physical inactivity among employees in an academic institution. Weight and height were measured, and BMI was calculated and coded as underweight, normal, overweight and obese according to the cut-off points for the Asian population. Results: Out of ninety-five employees, 55 participated in the survey. Among the 55 employees, 4 (7.3%) were underweight, 10 (18.2%) were normal, 25 (45.5%) were overweight, and 16 (29.1%) were obese. The obese and overweight proportion was similar to the national prevalence. Poor sleep quality and high Job stress scores were reported by 40% of the employees, while physical inactivity of more than 6 hours a day was reported by 50%, and the majority (90%) do not consume enough fibre. Among the risk factors investigated, none was associated with obesity. Conclusion: The proportion of overweight and obesity in this community was like the general population. While not significant in this study, the association between obesity and the four risk factors should be further investigated using a larger sample size. Based on the results, community-based health promotion intervention is suggested to reduce obesity among the employees.

Keywords: Obesity; prevalence; risk factors; job stress; sleep deprivation; sedentary; fibre intake
Effects of xanthorrhizol on 3T3-L1 adipocyte hyperplasia and hypertrophy

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ABSTRACT

Introduction: According to the National Health and Morbidity Survey (NHMS) 2015, 47.7% of the Malaysian population are either obese or overweight. The increased obesity prevalence has caused major health problems including cardiovascular diseases and diabetes. Although several anti-obesity drugs have been developed, they are limited due to adverse side effects. Previous studies demonstrated that xanthorrhizol (XNT) reduced the levels of serum free fatty acid and triglyceride in vivo, but the detailed anti-obesity activities and its related mechanisms are yet to be reported. Thus, this study aims to evaluate its abilities to inhibit adipocyte hyperplasia and hypertrophy employing 3T3-L1 adipocytes.

Methods: Statistical significance was established by one-way ANOVA, where p < 0.05 was considered statistically significant. Results: In this study, the IC50 value of XNT (98.3% purity) from Curcuma xanthorrhiza Roxb. in 3T3-L1 adipocytes was 35 ± 0.24 μg/mL. The loss of cell viability was due to 20.01 ± 2.77% of early apoptosis and 24.13 ± 2.03% of late apoptosis. XNT elicited apoptosis via up-regulation of caspase-3 and cleaved PARP-1 protein expression for 4.09-fold and 3.12-fold, respectively. Moreover, XNT decreased adipocyte differentiation for 36.13 ± 3.64% and reduced GPDH activity to 52.26 ± 4.36%. The underlying mechanism was due to impaired expression of PPARγ to 0.36-fold and FAS to 0.38-fold, respectively. On the other hand, XNT increased glycerol release by 45.37 ± 6.08% compared to control. During lipolysis, XNT up-regulated the leptin protein for 2.08-fold but down-regulated the protein level of insulin to 0.36-fold. These results indicated that XNT reduced the volume of adipocytes through modulation of leptin and insulin. Conclusion: To conclude, XNT exerted its anti-obesity mechanisms by suppression of adipocyte hyperplasia through induction of apoptosis and inhibition of adipogenesis whilst reduction of adipocyte hypertrophy through stimulation of lipolysis. Thus, XNT could be developed as a potential anti-obesity agent in the future.

Keywords: Adipocyte, hyperplasia, hypertrophy, obesity, xanthorrhizol
What Can Law Do to Combat Obesity in Malaysia?

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ABSTRACT

Introduction: The obesity rate in Malaysia is increasing exponentially. This is alarming as obesity in a population is likely to lead to health complications and negatively impact national economic productivity. The quality of health of our country is of paramount importance to a sustainable nation. To safeguard the health of our population in this country, there are current laws and potential laws to be implemented to combat obesity. In Malaysia, the government have introduced ‘soft policies’ approach such as Healthy life style programmes and campaigns as means to curb obesity yet its impact is questionable. ‘Hard policies’, such as regulations (e.g. imposing a tax, removal of subsidies, reduction of operational hours of eateries, menu-labelling, curbing of marketing of unhealthy food, implementation of pedestrian and bicycle paths, metabo laws) may be used as legitimate interventions to combat obesity in Malaysia. The aims of this paper is to (i) analyse the legal justifications for implementation of obesity prevention regulations, (ii) to compare and contrast the pros and cons of current and potential obesity prevention regulations to reduce obesity.

Results and Conclusion: The law could be used to facilitate promotion of public health. In light of new scientific advances, gaps in the current regulatory framework, and the increasingly obesogenic environment, this paper proposes potential legal approaches to address obesity in Malaysia. It is important for legal scholars to devise innovative strategies to address obesity from new perspectives. The great potential for the law to rectify the status quo has yet to be fully explored. With reduction of obesity, the fiscal burden of the nation on medical bills could be reduced and productivity could be increased.

Keywords: Law, Obesity, Public Health, Fiscal, Consumer Rights
Purification and Characterization of Charantin from *Momordica Charantia* Linn.

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**ABSTRACT**

**Introduction:** Diabetes mellitus (DM) is one of the top diseases that lead public health concern in Malaysia. It was believed to rise in number up to 4.5 million on cases by year 2020 based on the current figure. *Momordica charantia* Linn (MC), a climber belonging to family Cucurbitaceae, is well known in treating diabetic-related conditions. In earlier studies related to the hypoglycemic properties of MC mainly utilized the crude extract, which contain a mixture of bioactives (charantins, insulin-like peptides and alkaloids). Till now, there is no conclusive result on the major bioactives that play role in the hypoglycemic effect of MC and research regarding the charantin purification was not well established. Hence, the objectives of this study were to purify the charantin from MC and to characterize the purified charantin before further subjected to *in vivo* hypoglycemic study. **Methods:** The crude was first extracted from MC using ethanol as solvent via Soxhlet extraction following by a series of purification steps via washing, centrifugation, and C-18 cartridges. **Results:** The HPLC analysis showed that the charantin of purified extract after passing out from the cartridge exuded at 12.50 min with a concentration of 500 ppm, which is relatively 20 times higher than the crude extract (25 ppm). The structural properties of purified charantin were studied using FTIR and it showed strong peaks of carboxylic acids (2884 nm), alcohols (1023 nm) and diethyl ether (1114 nm) as compared with the standard. The compound was reconfirmed in LC-MS analysis. The result displayed mass spectrum in positive mode indicates the presence of similar compound in the purified extract and standard charantin, as presented by ion m/z = 300. **Conclusion:** The charantin was successfully purified from MC and can act as a potent plant-based hypoglycemic agent for diabetes.

**Keywords:** *Momordica charantia* Linn., charantin, diabetes mellitus, hypoglycemic
PO8

Gliclazide loaded PLGA-HPMC second generation nanocrystals for oral delivery: Design, development and in vitro performance characterization

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ABSTRACT

Introduction: Second generation functionalized nanocrystal is the advancement of nanocrystal technology with great potential to accommodate BCS (Biopharmaceutical Classification System) class II drugs to meet their formulation and drug delivery challenges. Gliclazide is a BCS class II drug used in the treatment of type 2 diabetes, shows poor water solubility and low rate of dissolution, leads to poor and variable oral bioavailability. The second generation poly(D,L-lactide-co-glycolide) (PLGA) Hydroxypropyl methylcellulose (HPMC) based functionalized nanocrystals of gliclazide were prepared by a combination method of emulsion diffusion-high pressure homogenization-solvent evaporation.

Methods: Gliclazide second generation nanocrystals were fabricated with taguchi orthogonal experimental design in combination of step up and top down nanoformulation strategies using drug-polymer (PLGA) ratio at 1:0.5, 1:0.75, 1:1 with HPMC(0.5, 0.75, 1% w/v) as stabilizer. The formulated gliclazide PLGA-HPMC nanocrystals were investigated on particle size, polydispersity index, zeta potential, solubility study, drug entrapment efficiency, in vitro drug release, and surface morphology and compatibility studies. The gliclazide PLGA nanocrystals formulation was prepared with Drug : PLGA at 1: 1 ratio with concentrations 0.75% w/v HPMC at 5 homogenization cycles with 1000bar produce optimized gliclazide nanocrystals. 

Results: The optimized MSGNC8 formulation showed particle size of 239.9 nm, entrapment efficiency 98.62%, and drug release of 43.75%, 82.12% and 98.08% at 3hrs, 24hrs, and 48hrs compared to pure gliclazide % drug release of 28.73%, 67.51% and 78.41% at 3hrs, 24hrs, 48hrs respectively. The solubility study of optimized formulation shows eight folds increased in saturation solubility compared to pure drug. Scanning electron microscopy (SEM) analysis of the gliclazide nanocrystals revealed that gliclazide retained its crystal morphology in polymeric nanocrystals. Further, fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) studies on gliclazide PLGA-HPMC nanocrystals emphasize drug and excipient compatibility in development of gliclazide nanocrystals.

Conclusion: The potential outcomes of research findings emphasize that the developed gliclazide second-generation nanocrystals, which resulted in increase in drug solubility and rate of dissolution with delayed modified release, can be explored in delivery of gliclazide for type 2 diabetes management.

Keywords: Second generation nanocrystals, Emulsion diffusion-High pressure homogenization-solvent evaporation, Gliclazide, D,L-lactide-co-glycolide(PLGA), Hydroxy propyl methylcellulose (HPMC), Type 2 diabetes management
The effect of dietary glycemic index and glycemic load on overweight individuals and type 2 diabetes patients: An overview of the literature


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ABSTRACT

Introduction: Type 2 diabetes is increasing at an alarming rate worldwide. One of the leading factors to this condition is obesity. Low glycemic index (GI) and glycemic load (GL) diets have been proposed as lifestyle changes to address obesity, however, there is a lack of consensus on the optimal approach for weight loss, glycemic control and improving insulin sensitivity. In addition, the outcome of these diets are equivocal, with some studies suggesting beneficial outcomes and others suggesting otherwise. Furthermore, discrepant study designs have led to divergent conclusions. In order to provide a comprehensive overview of the low GI and low GL diets, a systematic review of literature on relevant observational studies and randomised control trials was performed on these databases:- The Cochrane Library, Medline, PubMed, Embase, Cinahl and Web of Science.

Methods: The review was conducted based on the methodological standards for the conduct and reporting of Cochrane intervention reviews, Version 1.07, November 2018. Population, Intervention, Comparison and Outcomes (PICO) tool was used as the organising framework to define key elements of the review question. Results: Pertinent outcome variables include body weight, insulin resistance, HbA1c, fasting serum glucose, BMI, waist-to-hip ratio, triglyceride, HDL and LDL cholesterol. Our current understanding of these diets has been complicated by the reports that were based on different study designs and study populations. This review defines the issues, gaps in the research, study design, and evidence that is needed to inform practice, policy making and future research. There is also a dearth of information on the effect of low GI and GL diets on the Asian populations, specifically on improving insulin resistance. High carbohydrate diets are a mainstay of Asian societies. Conclusion: As cases of obesity and type 2 diabetes surge, there is an urgent need for research on low GI and GL dietary modifications among the Asian populations.

Keywords: Glycemic index, glycemic load, overweight, obesity, type 2 diabetes
PO10

Electrospun deferoxamine nanofiber for diabetic wound healing

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ABSTRACT

Introduction: Diabetic foot ulcer (DFU) is the most distressing complication of diabetes mellitus and often associated with risk of non-traumatic lower extremity amputations. Available formulations and wound dressings for DFU treatment are unfortunately less effective both on controlling and healing DFU. Issues commonly found are associated with providing an optimum environment which facilitates healing process; moist environment, effective oxygen exchange, preventing infection, controlling exudate and also patients compliance. The challenge is therefore to develop a novel drug delivery which address this unmet medical need for better wound treatment of chronic and slow healing DFU. This study aimed to develop a biomaterial based nanofibrous wound dressing formulation containing deferoxamine (DFO), which reported as a potential therapeutic approach to improve wound healing. Deferoxamine regulates the expression and increase stability of hypoxia-inducible factor-1α (HIF-1α), growth factor that crucial in wound repair, and thus increase neovascularization. Preparation and characterization of chosen polymers; chitosan/alginate/polyvinyl alcohol (PVA) for nanofiber formulation will be carried out. Such biodegradable polymer nanofiber is a great benefit for drug delivery owing to its high surface area to volume ratio and high porosity which creates ideal environment to aid in wound healing. Methods: Nanofibers loaded DFO will be fabricated by electrospinning method that utilizes electrostatic force to produce fine fibers from the polymeric solution. Results: Various polymers concentrations and ratios are investigated to obtain the desired fibers characteristics. The selected optimized DFO nanofibers will be studied for its efficacy in wound healing through in-vivo animal studies. Conclusion: The proposed formulation would be an ideal low cost novel wound dressing with improved healing potential for efficient treatment of diabetic foot ulcer.

Keywords: Deferoxamine, electrospinning, diabetic foot ulcer, nanofiber, wound healing
Genetic variants in HBS1L-MYB with Hb subtypes level among Filipino \( \beta^0 \)-deletion carriers coinherited with -\( \alpha^{3.7} \) deletion

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ABSTRACT

Introduction: Filipino \( \beta^0 \)-deletion is predominant among the \( \beta \)-thalassaemia patients in the indigenous population of Sabah, Malaysia particularly among the Kadazandusun. Individuals who co-inherit with \( \alpha \) - and \( \beta \)-thalassaemia will demonstrate milder clinical symptoms with modified complete blood count (CBC) and Hb subtype parameters. \( HBS1L-MYB \) variants act as one of the key regulator of haematopoiesis and erythropoiesis and display strong association with variation of HbF levels. Therefore, this study aims to evaluate the association between genetic variants in \( HBS1L-MYB \) with Hb subtypes level among Filipino \( \beta^0 \)-deletion carriers co-inherited with -\( \alpha^{3.7} \) deletion.

Methods: Filipino \( \beta^0 \)-deletion and -\( \alpha^{3.7} \) deletion were identified using gap-polymerase chain reaction (PCR). A total of 34 subjects found with coinheritance of Filipino \( \beta^0 \)-deletion and -\( \alpha^{3.7} \) deletion were subjected for \( HBS1L-MYB \) intergenic polymorphisms (HMIP) analysis. Hb subtypes level were quantified using BioRad Variant II Hb analyser. Genotyping of \( HBS1L-MYB \) variants rs9399137 and rs11759553 was done using own designed tetra primer ARMS-PCR.

Results: The minor allele frequencies (MAF) of the two HMIP is found more than 0.05 (rs11759553, MAF=0.18 and rs9399137, MAF=0.15), indicating the significance of these variants among the study subjects. Significant difference was found between Hbf level and \( HBS1L-MYB \) variant rs11759553 with p-value less than 0.05 (p=0.001). Subjects with homozygous genotype for rs11759553 (T/T) was found with higher Hbf, followed by heterozygous (A/T) and wild type (A/A). rs11759553 and rs9399137 was found did not influence the level of HbA and HbA2.

Conclusion: This study demonstrates that there are significant associations between certain genetic variants in \( HBS1L-MYB \) with Hb subtypes level among Filipino \( \beta^0 \)-deletion carriers co-inherited with -\( \alpha^{3.7} \) deletion.

Keywords: \( HBS1L-MYB \) variants, Filipino \( \beta^0 \)-deletion, -\( \alpha^{3.7} \) deletion, Hbf level
Association of hematological analysis and α-globin genotypes among eligible blood donors in University Tunku Abdul Rahman (UTAR)

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ABSTRACT

Introduction: Reduction or complete absence of α-globin chain production may result α-thalassemia. Alpha thalassemia carrier may have normal haemoglobin level and thus will be eligible as blood donor. Few complications may happen in which the carrier who donated the blood might be at risk of hypoxia and their blood components might not suitable for transfusion. Thus, it is important to screen for α-thalassemia to prevent any complications happen after donation. The objective of this study is to investigate the interaction of red blood cell indices and α-globin genotypes among eligible blood donors in a private university, Universiti Tunku Abdul Rahman (UTAR), Malaysia.

Methods: A total of 270 eligible blood donors were recruited for this study. Red cell indices were analysed using Horiba hematology analyser and α-globin genotyping was performed for seven alpha deletions, six alpha point mutations and two alpha triplications.

Results: Our study showed high prevalence of α-thalassemia carriers among the eligible blood donors (7.7%, 21/270), with all of them showed normal Hb level (>12 gm/dl). Five genotypes were detected consisting of 249 αα/αα (92.2%), 9 -α3.7/αα (3.3%), 9 --SEA/αα (3.3%), 2 -α4.2/αα (0.7%) and 1 ααCS/αα (0.4%). All α-globin genotypes showed normal Hb level with no significant difference between genotypes (p=0.167). Different α-globin genotypes showed significant difference in RBC, MCV, MCH, MCHC, RDW and Hct/Hb ratio at the p<.05 level due to different extent of α-globin chain reduction.

Conclusion: Our study concluded that by using Hb level alone in screening for the eligibility of blood donors is not sufficient but using full blood count (FBC) screening with borderline MCV and MCH levels might be able to rule out α-thalassemia carriers. FBC and molecular characterisation should be incorporated together to properly rule out α-thalassemia carriers.

Keywords: red blood cell indices, alpha thalassemia carriers, eligible blood donor, multiplex PCR, hemoglobin
Knowledge of Beers Criteria for Potentially Inappropriate Medication Use Among Community Pharmacists in the Klang Valley, Malaysia

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ABSTRACT

Introduction: The geriatric population in Malaysia is expanding rapidly due to increased life expectancies. The vulnerability of this population to the adverse effects of medications due to multiple comorbidities and polypharmacy predisposes them to potentially inappropriate medications (PIMs). The Beers Criteria is a recognized tool for assessing PIMs, but the level of awareness regarding these criteria among community pharmacists is currently unknown. This study aimed to assess the awareness and knowledge of Beers Criteria and its extent of application in practice among community pharmacists in the Klang Valley, Malaysia. Methods: A cross-sectional study was conducted among 218 community pharmacists in the Klang Valley using a validated, self-administered questionnaire. Knowledge on PIMs was assessed using a ten-question clinical vignette based on Beers Criteria. Descriptive and inferential statistics were used to analyze the data. Results: Respondents had a significant proportion of their customer base who were elderly. Only 28% of respondents were aware of Beers Criteria, and of this group only 41% were aware of the latest update. The mean score for the clinical vignette was 5.42 ± 1.98. Awareness of Beers Criteria and years of experience in practice were associated with higher knowledge scores (p < 0.05). Good geriatric practices were reported by respondents with the exception of regular usage of Beers Criteria (16.5% agreement) and regularly asking elderly-looking customers their age (43.6% agreement). Most respondents (74.3%) utilized other clinical resources and were confident in providing care to elderly customers. Conclusion: Awareness of Beers Criteria remains low among community pharmacists. However, pharmacists utilized other resources and demonstrated good geriatric practices. While this shows the adequacy of current practice, efforts to increase awareness of geriatric-specific tools such as Beers Criteria may address specific knowledge gaps and improve the level of care involving the elderly.

Keywords: Beers Criteria, geriatrics, medication safety, community pharmacists
Robust model of systemic inflammation through Lipopolysaccharide

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ABSTRACT

Introduction: Systemic inflammation is the major clinical problem which is constellation of communicable and non-communicable infection equipped with acute to chronic inflammation. It may lead to unfavourable conditions for instance, systemic inflammatory syndrome, burns and sepsis. Systemic inflammation might rotate the steering towards vital clinical maladies including cardiomyopathy, neuroinflammation, hepatitis, liver and kidney diseases and even diabetes. In order to elucidate the molecular insights in these clinical implications, there is an intensive need to design rodent model of systemic inflammation having close association with systemic inflammatory conditions in humans. Methods: Presently, lipopolysaccharide (LPS) induced systemic inflammatory rodent model is widely established, reproducible and acceptable among scientists. In this model animals are treated with intraperitoneal injection of LPS ranging from 1-10 mg/kg which leads to instant release of proinflammatory cytokines to provide robust model of systemic inflammation in order to elucidate pathological conditions and their in-depth mechanism to uncover the new anti-inflammatory therapeutic targets. Conclusion: Robust model would open new window to explore anti-inflammatory activities of phytochemicals, small molecules and drug candidates along with crosstalk of different signaling pathways at molecular level.

Keywords: Lipopolysaccharide (LPS), systemic inflammation, neuroinflammation
What influences the use of local crude herbs among patients with chronic diseases? Preliminary screening of phytochemicals

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ABSTRACT

Introduction: The use of crude herbs for well-being as well as curation and prevention of ailments is evident globally, including Malaysia. To understand the effectiveness and reliability, the presence of phytochemical compounds as the factor influencing the use of crude herbs shall be determined. This study was conducted to screen the presence of phytochemical compounds in the local crude herbs consumed by patients with chronic diseases. This is part of a larger study, where a cross-sectional study was conducted and reported elsewhere. Methods: In total, 15 types of crude herbs were reported by the patients with chronic diseases attending government health clinic at Kampar, Perak. The herbs were extracted using hot and standard cold methods respectively. Results: All the 15 crude herbs’ hot and cold extracts revealed the presence of eight phytochemical compounds, namely, phenols, quinones, tannins, terpenoids, saponins, flavonoids, glycosides, and alkaloids at varying intensity. Saponins, alkaloids, and glycosides were present in all the crude herbs extracts. However, terpenoids, tannins, and phenols were absent in Orthosiphon stamineus, Clinacanthus nutans, and Pandanus amaryllifolius extracts respectively. However, the cold extracts exhibited a higher intensity of phytochemicals compared to hot extracts. The present study confirms the presence of phytochemicals in the local crude herbs consumed by patients with chronic diseases. The screened phytochemicals are bioactive compounds that possess medicinal properties that may trigger the patients to treat their diseases’ underlying conditions. However, the use of prescribed medicine, particularly among aging patients must be taken into account while consuming crude herbs. Conclusion: The findings of this study indicate that structured-evidence based crude herbs use interventions for patients with chronic diseases is warranted.

Keywords: Crude herbs, chronic diseases, phytochemical compounds
PO3

Factors Influences Crude Herbs Use Among Patients with Chronic Diseases Attending a Government Health Clinic: A Cross-sectional Survey


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ABSTRACT

Introduction: Crude herbs can be defined as raw plants materials (e.g. leaves, flowers, roots, etc.) which are not being/minimally processed or dried. Globally, particularly in Malaysia, the use of crude herbs has been increasing. The reasons were as an ailment of diseases underlying conditions and for general wellbeing. In this study, our aim was to investigate factors influences crude herbs use among older patients with chronic diseases. Methods: A cross-sectional survey was conducted using purposive sampling among patients attended government health clinic at Klinik Kesihatan Kampar, Perak. Self-designed questionnaires were used to collect data and data was analysed using SPSS software (ver. 23). Results: A total of 441 participants were enrolled in this study, the response rate was 71.35%. Demographic characteristics of patients who consume crude herbs were; female (57.25%), Malays (45.06%), age between 50-59 years old (31.96%), secondary education level (49.1%), and earned income less than RM3000 (93.27%). Female gender was found associated with the use of crude herbs (p < 0.05). Other socio-demographic characteristics, such as age, race, education level, and salary range found not associated with crude herbs (p > 0.05). The common reasons given by patients to use crude herbs were; family influence, effectiveness in reducing sugar, and accessible and cheaper compared to commercialised herbal drugs. The prevalence of crude herbs use, particularly among ageing patients is alarming. The physicians need to take into account on crude herbs used when prescribing medications. The use of crude herbs can be beneficial but yet can be detrimental if it is consumed while on prescribed medications. Conclusion: The findings of this study indicate that the survey area needs to broaden to other parts of Malaysia, particularly rural is warranted.

Keywords: Chronic diseases, crude herbs, herbs, government health clinics
PO4

Virgin palm kernel oil reduces cardiovascular risk factors
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ABSTRACT

Introduction: Inflammation is one of the major cause of cardiovascular disease, obesity, cancer and stroke. Many dietary compounds containing kernel oil or coconut oil with anti-inflammatory effect can delay the onset of these chronic diseases however the underlying mechanism is unclear. Methods: This study compares the effects of 5% virgin palm kernel oil (VPKO), virgin coconut oil (VCO) and refined, bleached, deodorized olive oil (RBDOO) on selected immune markers in healthy sprague dawley (SD) rats (n=16 per treatment) across 8 weeks. Sera were obtained for four major immunological analyses including cluster of differentiation 4 (CD 4), cluster of differentiation 8 (CD 8), interleukin 6 (IL 6), and c reactive protein (CRP). Results were expressed in mean ± standard error of the mean (mean±SE). Results: Eight weeks fat feeding had no significant difference in weight gain across treatments. Interestingly, we observe significant different on the concentration of CD 4 (p=0.001) with the lowest CD 4 level in rats fed with VPKO 3.87±0.65 ng/ml. The concentration of CD 8 in rats fed with VPKO 8.19±0.25 (p=0.001) ng/ml was comparable to VCO fed rats but was found lower than the control group, RBDOO fed rats. Lower T cell count (CD 4 or CD 8) indicates suppression in inflammation. IL-6 and CRP concentration in rat fed with VPKO 10.89±0.22 pg/ml and 118.39±7.13 ng/ml were slightly higher than that of VCO fed rats but were lower than RBDOO fed rats. Conclusion: We postulate that VPKO could be a potential supplement as an alternative to VCO for relieving inflammation and enhancing body immune system.

Keywords: Virgin palm kernel oil, inflammation, sprague dawley rats
Knowledge and Perception on Blood Safety Issues among Blood Donors at the National Blood Centre: A pilot study

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ABSTRACT

Introduction: Unsafe blood products cause transfusion-transmissible infections among blood receivers. The knowledge and perception of blood donors is important as it is associated with their donation behaviour and hence the safety of blood products. There was no previous study that assessed the knowledge and perception on blood safety issues among blood donors to date. The objective of this study was to assess the knowledge and perception of blood donors on blood safety issues.

Methods: This was a pilot study conducted to pilot test the self-developed questionnaire by the researchers. The questionnaire was available in the Malay language. One-hundred-thirty donors at the National Blood Centre were recruited to complete the self-administered questionnaire. Health sciences professionals, medical students and non-Malaysians were excluded in this study.

Results: A total of 130 donors comprising of 70 males (53.8%) and 60 females (46.2%) responded. The mean age of the respondents is 32.48±8.86 years. Most of the respondents were Malay (55.4%), single (49.2%), working in private sector (46.9%) and regular donor (68.5%). More than half of the respondents did not know that dengue, Zika and mad-cow disease can be contracted through blood transfusion. Ten percent of the respondents answered that bisexual people are eligible to donate blood. 40.7% of the donors agreed to check their HIV status through blood donation. Majority of the donors (60.7%) agreed that the donors’ blood is safe if the screening test is negative. Whereas, 33.9% of the donors disagreed that they shall be responsible if their blood causes infection.

Conclusion: Several knowledge gaps and inappropriate perception among the respondents were identified and these might affect the safety of the blood products. Targeted measures should be taken to rectify donors’ knowledge and perception in order to minimise inappropriate blood donor behaviours and reduce unsafe blood products.

Keywords: knowledge, perception, blood safety, blood donor, national blood centre
The pharmacology and phytochemistry of *Acalypha indica*

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**ABSTRACT**

**Introduction:** *Acalypha indica* is commonly referred to as “pokok kucing galak”. It is an herbaceous species that grow along the earth’s equator line, including the wet, temperate and tropical regions. Domestic cats experience the effect of this plant by reacting very favorably to the root. The first compilation of the ethnopharmacology and phytochemistry of the *Acalypha* plants was published. This genus is the fourth largest genus of the Euphorbiaceae family, with about 500 species. However, the review only represents about one third of the species from the *Acalypha* genus. **Methods:** Hence, this study is performed to obtain updates on the biochemistry of this plant, via literature search. **Results:** From the articles, almost every part of the plant, including the leaves, stems and roots, are used as traditional remedies. Local people consume the plant for therapeutic purposes such as anthelminthic, anti-ulcer, anti-bacteria, anti-microbial and wound healing. In homeopathy practice, it is used for asthma and bronchitis. Nevertheless, there is still a potential risk of using *A. indica*. It was reported that this traditional medicine could induce intravascular haemolysis in patients with a glucose-6-phosphate-dehydrogenase (G6PD) deficiency. Clinical evaluations of *Acalypha* extract could be utilized to justify the ethnomedicinal claims and for the safety of its therapeutic applications. Meanwhile, there is an increase in the phytochemical and chromatographic experiments of *A. indica* that could introduce the extract’s role in pharmaceutical, nutraceutical, zoology and veterinary fields. It contains secondary metabolites, including dihydroactinidiolide; a terpenoid, alkaloids, flavonoids and steroids, for example, brassicasterol. **Conclusion:** The finding of this review concludes that *Acalypha* is a natural source, worth to be further investigated. It is hoped that new biologically active constituents could be discovered, since only few *Acalypha* species were comprehensively studied.

**Keywords:** *Acalypha*, biochemistry, pharmacology, phytochemistry