INTERNATIONAL CONFERENCE ON DRUG DISCOVERY AND TRANSLATIONAL MEDICINE 2021 (ICDDTM'21)

Advancing Precision Medicine with Emerging Technologies: Bridging Gap Between Academia and Industry

7th December 2021 (Virtual Pre-conference Workshops)
8th – 9th December 2021 (Virtual Conference)

Organisers:
Universiti Putra Malaysia, Universiti Teknologi MARA, Universiti Sains Malaysia, UCSI University, MAHSA University, Taylor's University, University Malaya, Monash University Malaysia, Eman Biodiscoveries Sdn. Bhd., Malaysian Association for Cancer Research and GHHS Healthcare

Editorial Information

Acknowledgement:
The scientific committee thanks Dr Geraldine Law-Lee for reviewing an abstract submitted for presentation at the ICDDTM'21

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PLENARY TALK 1

Harnessing Digital Medicine To Optimise Drug Development And N-Of-1 Healthcare

Prof. Dr. Dean Ho[1,2,3,4]

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ABSTRACT

Drug discovery and development are different segments of the therapy roadmap that need to be seamlessly integrated. Discovering promising candidates represents the first of many steps needed to optimally harness a drug's potential, particularly due to the fact that monotherapies may yield improved but sub-optimal clinical outcomes compared to standard care. As such, these candidates often need to be delivered in combination with other therapies, and the methodologies used to design these combinations vary widely. Importantly, traditional and truly optimised drug development can be the difference between no efficacy and life-saving outcomes. In the quest for truly optimised drug development - whether novel or repurposed - multiple challenges need to be overcome. The right drugs and corresponding doses need to be identified, which will have a profound impact on the drugs that ultimately comprise that combination. Using traditional approaches, this can be an insurmountable barrier given the very large drug and dose parameter space that is created. In addition, a one-size-fits-all approach serves as a barrier to truly individualised, or N-of-1 treatment, as even effective drugs given at incorrect dosages can result in little to no efficacy. Furthermore, these doses may need to be modulated dynamically during the course treatment, since the patient response to treatment can also be dynamic. This talk will address our recent clinical development studies at WisDM and N.1 to dynamically tailor patient-specific treatment outcomes, reduce healthcare costs, and increase accessibility to practice-changing and optimised medicine.

PLENARY TALK 2

SARS Cov 2 Vaccines: Current State Of The Art

Prof. Dr. Richard M. Novak

University of Illinois Hospital, USA

ABSTRACT

The sudden and rapid arrival and spread of COVID 19 led to an unprecedented vaccine development effort worldwide. The successful trials and approval of multiple vaccines has not been equaled by their acceptance, production and distribution. The predominant vaccines will be discussed and compared. Emergence of variant viruses, waning immunity and the introduction of booster doses will be discussed. The application of vaccine in pregnancy will also be addressed.

PLENARY TALK 3

Regenerating Tissues … Cell By Cell!

Prof. Dr. John EJ Rasko, AO[1,2,3]

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[2] Professor, Sydney Medical School, University of Sydney; Head, Gene and Stem Cell Therapy Program, Centenary Institute
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ABSTRACT

Advanced therapeutic medicinal products based on Gene and Stem Cell Therapy are increasingly being approved throughout the world. Immunotherapies including checkpoint inhibitors and CAR-T cells have captured the attention of many scientists, physicians and cancer sufferers. The convergence of substantial incremental technical advances towards combined cell and gene therapy has led to improved clinical outcomes in immune deficiencies, haemoglobinopathies, blindness, immunotherapies and other inherited diseases. In the regenerative medicine field, there is a pressing need to standardize cell manufacturing protocols for widespread clinical testing. Strict compliance with government regulation and oversight is essential to maintain the safety of all therapeutic products. We recently completed the first trial of iPSC-derived Mesenchymal stromal cells in Steroid-Resistant Acute GvHD. MSCs have been widely investigated as a treatment for graft versus host disease (GvHD), but with mixed results. Factors such as MSC donor variability and the effects of prolonged culture expansion may contribute to inconsistent or disappointing outcomes. The novel Cymerus™ manufacturing process facilitates virtually limitless production of well-defined and consistent MSCs from a single human iPSC bank, using clonogenic progenitor-based technology. This avoids both inter-donor variability, batch-to-batch variation and the need for prolonged in vitro expansion of MSCs. We have conducted a multi-centre, open label study of Cymerus MSCs (CYP-001) in adults with steroid-resistant acute GvHD. The primary objective was assessment of safety and tolerability, while the secondary objective was efficacy, based on best responses by Day 28/Day 100 and overall survival. This is the first completed study worldwide with any iPSC-derived product. It has yielded encouraging safety and efficacy data, which support further clinical development of Cymerus iPSC-derived MSCs for GvHD and other indications.

PLENARY TALK 4

Advances In Drug Delivery

Prof. Dr. Edward W. Boyer

Harvard Medical School, Ohio State University, United States

ABSTRACT

Delivery of medications, especially depot medications, is changing, as are the triggers for release of medications. This lecture will review new reservoirs of medications, new sensors to stimulate drug release, and methods for powering these interesting devices.

PLENARY TALK 5

Therapeutic Applications From The Human Gastrointestinal Microbiome

Dr. Samuel Foster

Hudson Institute of Medical Research, Australia

ABSTRACT

The human microbiome represents an important and largely unexplored source and target for novel therapeutic interventions. While faecal transplantation has demonstrated the potential of these approaches, future microbiome-based medicines will likely consist of defined combinations of live bacteria and rationally selected prebiotics. This next generation of microbiome-based medicines has substantial potential in both the treatment of diseases from inflammatory bowel disease to cancers and targeted health improvements through interventions such as optimised immune development in infants. Until recently, technological constraints have limited these advances; however, developments in sampling methods, bacterial culturing and metagenomic sequencing and analysis coupled with experimental validation have advanced this field. We have now cultured over 10,000 bacterial isolates from patient specific samples and applied cutting-edge metagenomic analysis methods to identify key clinically relevant disease candidates. Combining advanced microbiological and cell culture-based assay systems with detailed bacterial genomics and in vitro assessment of host-microbe interactions has provided the capacity for functional understanding of both the microbiome and the host factors driving disease phenotypes observed within patient cohorts. These advances provide the capacity to identify and validate key bacterial strains and the host responses they induce within the patients. This research will represent the basis for future therapeutic development.

INVITED TALK 1

Targeting KRAS With Small-Molecule Non-Covalent Inhibitors

Prof. Dr. Alemayehu Gorfe

University of Texas, USA

ABSTRACT

KRAS4B (KRAS) is arguably the most sought-after anti-cancer drug target that is found mutated in many forms of cancer, including in 98% of pancreatic and 45% of colorectal cancers. The FDA has recently approved a covalent inhibitor specific to the G12C variant of KRAS that is common in small cell lung cancer. However, no comparable progress has been made on G12D, G12V, G13D or Q61H mutants which together represent over 78% of KRAS-driven cancers. My presentation will focus on our development and application of a dynamics-guided discovery workflow to target these mutants with small-molecule non-covalent inhibitors.

INVITED TALK 2

Mapping The Spatiotemporal Complexity Of Glioblastoma Tumors

Assoc. Prof. Dr. Carol Tang

Senior Principal Investigator, Neuro-Oncology Research Program, National Neuroscience Institute (NNI), Singapore

ABSTRACT

Glioblastoma tumors are among the most untreatable CNS malignancies where patients survive no more than fifteen months after the initial diagnosis. International efforts have now demonstrated that cellular and molecular heterogeneity of tumor tissue confounds treatment efficacy. This has significant implications in the design of therapeutic approaches as histologically identical GBM tumors can now no longer be treated similarly. This talk will describe NNI's brain tumor resource, Glioportal, as an empowering tool for preclinical validation prior to advancement of clinical trial. The resource comprises molecularly annotated patient tumors, established cell lines and matching orthotopic xenograft tumors. We will also describe a "use and application" study of Glioportal that evaluates novel stratification methods. We propose that molecular stratification identifies patient cohorts most likely to respond to targeted therapies. This in turn spares non-responders of chemotherapeutic side effects and financial costs. With the recent induction into GBM AGILE, our stratification methodologies proffer an opportunity to integrate directly into the existing WP1066 adaptive trial. The NNI neuro-oncology program seeks to translate basic science discoveries to advance precision oncology-driven therapeutic goals.

INVITED TALK 3

Botanical Drug Development In The Malaysian Herbal Industry: Issues, Challenges And Breakthroughs From The Perspective Of A Biotechnology Company

Prof. Dr. Aman Shah Abdul Majid[1], Assoc. Prof. Dr. Amin Malik Shah Abdul Majid, Farizah Ahmad

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[2] Medical Director, NatureCeuticals

ABSTRACT

The herbal industry is a fast-growing industry worldwide with an estimated global annual market worth of more than US$60 billion (RM 251.1 billion). The growing trend in herbal industry is led by the increased demand in herbal supplements, health related functional food, herbs-based sports energy drinks and cosmeceuticals among others. The raw material demand by the industry for natural products, plant-based phytochemicals in crude or processed form are also on the rise and often driven by consumers perception of a safer and natural alternative to conventional products. High incidence of adverse drug interactions and long-term safety concerns with allophatic medicines use are also contributing factors to the shift in the demand for alternative medication, from traditional medication systems such as Traditional Chinese Medicine (TCM), Ayurveda, Kampo, Jamu to other folk medication systems. Malaysia's strategic location with its vast biodiversity, multi-ethnic cultures, a strong governance and established research institutions of international standing, offers a unique combination for the development of a robust herbal industry as well as providing a good source of raw materials. Southeast Asians in general welcome alternative medications of TCM, Ayurveda, Jamu and others. Traditional medicine based on local herbs have long been widely practised in Malaysian households and regionally. This folk medication system can be sourced for potential lead ingredients as botanical drugs or phytomedicine medicines for therapeutic use for the prevention and management of specific illnesses through robust scientific research and development (R&D). Realising the blend of huge economic potential and local heritage in herbal knowledge, Malaysia has initiated the high value herbal products (botanicals) initiatives under the Economic Transformation Program (ETP) new key economic areas (NKEA) EPP1 which has been put under the governance of the Ministry of Agriculture and Agro-based Industries Malaysia. This program consolidates the researchers from local universities and the local research institutes, the herbal industrial players as well as the regulatory authorities to plan and execute the program. Under the EPP1 project, the main aim is to raise the scientific content of the herbal products of the local NKEA companies which can be considered as a path-breaker for the industry and the country. They are tasked to produce standardised high value herbal products through the R&D of the preclinical and clinical studies to generate the safety, quality and efficacy data in order to gain approval for health claims of the products. This paper draws on the status of the herbal industry and the EPP1 initiatives undertaken by NatureCeuticals Sdn Bhd (NC) as a case study. NC is one of the leading Biotechnology companies that the government had engaged to develop botanicals with high claims from pre-clinical to clinical studies. Highlights on NC'S several major issues and challenges confronted in implementing the project are discussed.

INVITED TALK 4

Precision In Biological Regenerative Medicine: Targeted Organ Specific Precursor/Progenitor Stem Cells And Peptides

Prof. Dato' Sri Dr. Mike K.S. Chan[1,2,3]

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[2] Associate Professor, American Academy of Stem Cell Physicians (AASCP)
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ABSTRACT

Regenerative medicine is the field of medicine that focuses on developing and applying therapies to regrow, repair or replace damaged or diseased cells, tissues and organs. Regenerative medicine includes the use of stem cells, peptides, extracts as well as tissue engineering and regeneration. European therapeutic paradigm works on principles that the human body is inherently capable of self-healing and regeneration to a certain extent. These abilities depend on existing individual variability conditions with anatomical, physiological, mental and spiritual balance where conventional medicine has limitation to. As known to all, stem cells in our body deteriorates as we age over time. The human body comprises of about 37.2 trillion of live cells and we have about 1 stem cell, the super cell so called the Parent, Mother or Father cells out of 10,000 live cells from birth. We all age at an extremely fast pace and by the time we are 60, we are probably left with only 5% stem cells in our body, explaining well in a way our immunity drops acutely down over time disproportionately in which risk of mortality sets in. Bearing in mind that all our 220 tissues and organs in the body have a life span as well as with a different timeline and regenerational turnover time. There are several different types of stem cells from totipotent to pluripotent (embryonic stem cells) to multipotent (tissue stem cells) and unipotent (progenitor/precursor stem cells). Renal diseases and neurodegenerative diseases like Dementia, Alzheimer's diseases, Parkinson diseases, Autism, Down Syndrome, Cerebral Palsy and Global Delay Development are among the chronic diseases which are unable to resolve on their own where there exists no amicable "cure" in conventional medicine treatments. Regenerative medicine complements conventional medicine to seek and unearth therapies that support the body to repair, regenerate and restore itself and may be a solution to reach a state of fine fettle. Peptides are known as short chains of amino acids linked by peptide bonds which transfer information BETWEEN the cells and INSIDE the cells. In other words, peptides are big molecules which the cells use to build new cells. Peptides help stem cells to create new cells. Being administered to human body or any other living entity, long chain peptides break down to short chain peptides and amino acids, serving as a building material for the cells and performing the role of bioregulators for cells' functioning the so-called biopeptides. There are two types of peptides, synthetic peptides which are chemically synthesized small polymers of amino acids and organic peptides which are naturally occurring amino acids. When we take Precision into Biological Regenerative Medicine, we can probably consider the phrase of "law of similars" and "similia similibus curantur" (Like Treats Like), the organon of the Art of Healing first moulded by Swiss physician Paracelsus in the 15th century and Samuel Hahnemann in the 18th century. If you can throw all darts in in the dart board and hit bull's eye, it's Precision (full 50 points per dart thrown than to have 10 darts hitting all over the board with 0, 1, 2 ,3, 5, 7, 8, 10, 20 and 25 points). Precision and specificity are key here with body ability to treat by itself in biological regenerative medicine. An 'A' for 'A' disease, 'B' for 'B' disease, 'C' for 'C' disease and 'Z' for 'Z' disease rather than an A for A to Z diseases (all different disorders). With the organisation of the 12 systems in the human body to organs and tissues, the repair and regeneration must be at the cellular level to precisely regenerate the right type of cells and stem cells of the organ. Recollect that there are a varying number of cells in each organ, e.g., there are 67 different types of cells in the brain, 75 different types of cells in the liver, 13 different types of cells in the pancreas and so forth. Targeted organ specific stem cells, (e.g., cells and peptide extracts of substantial migra, frontal lobe, corpus callosum, temporal lobe of the brain) apart from Mesenchymal stem cells can be envisaged as part of the 'Precision' protocols. The same applies for renal and epithelial cells for kidney disfunction and hepatocytes and for liver, as well as cardiomyocytes for cardio problems and the list goes on. The European century old therapeutic paradigm here with stem cells and peptides of human, xeno cells transplantation and peptides (of Specific Pathogen Free (SPF)/Virus Antigen Free (VAF)) sources and vegetable based phytosomes organ specific enhancing therapies as a complementary option probably opens up more options and alternatives into treatment of concentrated diseases where conventional methods are exhausted as long as The Hippocratic Oath "Primum Non Nocere" (First, do no harm) is maintained.

INVITED TALK 5

An Overall Perspective On Stem Cell Applications In Musculoskeletal Sciences

Prof. Dr. Tunku Kamarul Zaman bin Tunku Zainol Abidin[1,2]

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ABSTRACT

The rapid growth of the stem cell market is accredited to the rising number of clinical trials and the rise of stem cell therapies being employed globally, more so in the field of musculoskeletal sciences or Orthopaedic sciences. In this field, mesenchymal stem cells (MSC) are widely accepted, but not generally accepted as standard therapy. In general, the use of MSC is more common and of interest. To put things into perspective, as of 2021, the number of publications relating to MSC for the past 30 years have reached close to 80,000 in total versus that of less than 500 observational/clinical studies for ESC. There have been approximately 1,138 registered clinical trials involving MSC, with 61% of these involving Phase 2 clinical trials. Most of the clinical trials involved the field of traumatology, neurology, cardiology and immunology, whilst the most frequent pathologies targeted for treatment includes knee osteoarthritis, ischemic heart disease and dilated cardiomyopathy. Of these studies, only 18 clinical trials published their end results. Based on a recent publication, of the 903 selected clinical trials reviewed, it was found that Malaysia is one of the countries listed currently conducting 8 clinical trials; a single phase 1 trial with the remaining in phase 2 development. The main source of MSC continue to come from bone marrow, followed by adipose tissue. Whilst the majority of studies have used autologous sources, there is an increasing trend of using allogenic sources owing to their ease of supply and that these cells being readily available as an "off-the-shelf" product. There is also a growing trend of using more advanced cell culture and harvesting systems in the up-scaling process, and the use of more natural sources of growth factors as supplements. Whilst not in clinical trials, it is of interest to note that in several pre-clinical studies, newer technologies such as gene-editing, transfection models, pre-programmed phenotypic differentiation and also clonal selection of MSC as proof of concepts, and may find their way into clinical trials at a later stage. The worry with many such advances is that there will be several parties that will lay claim to the potential of these cells in treating diseases, with many attempting to blatantly market untested/unproven products in an attempt to make a quick profit from selling the "stem cell" label to the public; while others proceed to intertwine poorly designed studies with components of pseudoscience like approaches to quickly exploit the unwearied healthcare market. All of these may prove to be detrimental to both patients and, of the reputation and development of this field of study. In the present lecture, we will highlight the progress, development and trends in MSC in research and applications in musculoskeletal sciences as a general view.

INVITED TALK 6

Understanding The Role Of GBA In Parkinson's Disease

Prof. Dr. Martin A Kennedy[1], Oscar E. E. Graham[1], Allison L. Miller[1], Toni. Pitcher[2], George D. Mellick[3], Antony Cooper[4], Justin O'Sullivan[5], Tim Anderson[2]

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ABSTRACT

GBA is a gene that encodes an enzyme called β-glucocerebrosidase, and mutations in the gene are known to be the most common genetic contributor to Parkinson's disease. Parkinson's disease patients with GBA coding mutations generally have more severe symptoms, faster disease progression, and reduced survival compared with noncarriers. Different GBA variants appear to impact variably on clinical phenotype. In addition, the GBA enzyme and its associated lysosomal pathway activities are targets for drugs under development or testing in Parkinson's disease. We implemented a nanopore DNA sequencing method for the GBA gene, which identifies coding and non-coding variants. Because this platform enables long-read single molecule sequencing, it also allows accurate identification of haplotypes of GBA which include non-coding variants as well as mutations in the coding sequence. We applied the GBA nanopore sequencing method to individuals from two well-characterised Parkinson's disease cohorts (including >400 cases), and discovered more than 50 individual haplotypes, which could be clustered into two main haplotype groups. We found a significant association between these GBA haplotypes and symptom onset age and diagnosis age, even once all individuals with coding variants had been removed. Our data supports a role for non-coding variation, and particular GBA haplotypes, as modifiers of disease onset and diagnose age in Parkinson's disease. We outline possible reasons for this and directions for future research.

INVITED TALK 7

Opportunities And Challenges Of Using Real-World Evidence In Kratom Drug Development

Prof. Dr. Vicknasingam Balasingam Kasinather

Centre for Drug Research, Malaysia

ABSTRACT

Kratom or ketum have been used traditionally by societies in Malaysia and Thailand for about a century. It has been used for various social and therapeutic purposes. In recent years, researchers have conducted field studies among kratom users to document these social and therapeutic claims. Fundamental researchers using animal models have used the field study data to conduct animal studies to substantiate the findings from the field. The use of real-world evidence does not typically follow the traditional drug development model and are similar to how medical marijuana research has been conducted. The presentation discusses how real-world data can be used as an opportunity for kratom drug development and at the same time poses challenges of using real-world data in meeting the scientific rigour of the traditional path of drug development. While safety data is paramount in drug development, a progressive and innovative approach taking into consideration real-world evidence need to be incorporated to advance kratom drug development.

INVITED TALK 8

A Novel Animal Model Of Alzheimer's Disease

Prof. Dr. George J. Augustine

Lee Kong Chian School of Medicine, NTU, Singapore

ABSTRACT

We have developed a novel mouse model of Alzheimer's Disease (AD) based on two of the main risk factors for AD: ApoE4 lipoprotein (ApoE4) and amyloid precursor protein (APP). By mating mice harbouring AD-associated mutations in their APP gene with mice with a single allele of ApoE4, we created double-mutant AD mice. These mice developed APP-derived plaques in their brains starting at age 3 months and an increase in microglia, particularly microglia associated with plaques. Behaviourally, the double-mutant mice are largely similar to their single-mutant counterparts. However, long-term synaptic potentiation in the hippocampus decays much faster in the double-mutant mice than in the single-mutant mice. Hippocampal transcriptome analysis indicates an up-regulation of several genes associated with vascular development or function. We are currently analyzing the structure and function of brain vasculature in vivo, using 2-photon imaging. In summary, our novel mouse model points toward the importance of vasculature in AD.

INVITED TALK 9

Translating Research Into Clinical Practice In Chronic Colitis To Colorectal Cancer Model

Prof. Dr. Raja Affendi Raja Ali

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ABSTRACT

Early diagnosis of inflammatory bowel disease (IBD) has high tendency to develop cancer. Currently, cancer surveillance is done by colonoscopy at an earlier age. To avoid frequent colonoscopy, a biomarker is required to predict the course of the disease. Our Gut Research group has discovered the transcriptomic changes similar to colorectal cancer in those suffered IBD for more than 20 years. One of the important pathways in driving chronic inflammation to cancer is phosphatidylinositol 3-kinases (PI3K) signaling pathway. To understand further on the pathway in IBD, we studied the regulation of *SGK2,* a gene related to the pathway in *in vivo* and *in vitro* models. CRISP-Cas 9 on *SGK2* has resulted in reduction in the expression of inactivated form of GSK3β and active β-catenin. In-vitro knockdown of *SGK2* in SW480 cell lines showed inhibition of cell proliferation, migration, and invasion by mediating the GSK3β/β-catenin pathway, thus acting as a potential biomarker for the development of UC into colitis-associated cancer (CAC). Targeted sequencing was performed on thirteen PI3K-related genes using Agilent SureSelect Human All Exome V6 in human samples of CAC and sporadic colorectal cancer. Median age of all patients was 64 (IQR:9) years old. A total of 64 mutations found in 13 genes in all samples and 26 synonymous, missense and indel mutations in CAC. *IL12RB1, IL31, TYK2* and *OSMR* variants were found exclusively in CAC patients. Tyrosine kinase 2 (*TYK2*) variant; rs280523 has a significant role in mediating signalling and functional response of key cytokines in IBD pathogenesis. Downstream genes related to TYK2 such as *IL12, IL23* and *IFN* are the potential new targets for IBD therapy. Our findings contribute knowledge in understanding the transformation of chronic IBD to cancer through cytokine-induced PI3K. Clinical assessment is required to combine with molecular findings to achieve precision medicine in IBD.

INVITED TALK 10

The Gut Microbiome: Faecal Microbiota Transplants And Beyond

Adj Assoc. Prof Dr. David Ong Eng Hui[1,2]

[1] Co-founder, Asian Microbiome Library, Singapore
[2] Department of Medicine, National University of Singapore

ABSTRACT

What is the hype about the gut microbiome? What is the gut microbiome and what are its links with the rest of the body? What are the applications of the gut microbiome in maintaining health? Faecal Microbiome Transplant (FMT) has been used to treat *Clostridium difficile* Colitis and is now being used to treat a host of other diseases. With the use of FMT for the mainstream treatment of refractory *C. difficile*, scientific interest in FMT for other diseases has also increased and the area of probiotics and microbiome-based therapies has also taken off. I will be discussing some of the developments in this exciting area.

PDGFB Paracrine Signalling Fuels Growth Promoting-mTORC1 Hyperactivation In Clear Cell Renal Cell Carcinoma (ccRCC)

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ABSTRACT

Introduction: ccRCC is the most common subtype of kidney cancer. Advanced stage ccRCC is highly mortal and there is still a lack of efficient ccRCC diagnostic and therapeutic strategies to date. We have discovered a super enhancer-driven KLF6 transcriptional network that functionally linked ccRCC hyper-vascularization and mTORC1 hyperactivation, which are the important ccRCC clinical targets. KLF6 transactivated the expression of pro-angiogenic PDGFB that would activate the mTORC1 activity in ccRCC via PI3K-AKT signalling. Nonetheless, the mode of this activation remains unexplored, whether ccRCC cells could secrete PDGFB extracellularly and stimulate the neighbouring cells mTORC1 activity in a paracrine manner. **Methods:** To test this, we performed PDGFB Western blotting and ELISA on several ccRCC cell lines' serum-free conditioned media. We also assessed the level of secreted PDGFB in 786-M1A cells upon PDGFB and KLF6 targeting using CRISPR-Cas9, and the overexpression of exogenous PDGFB. P-S6 ribosomal Western blotting was performed to assess the KLF6-targeted cells mTORC1 activity upon culturing with conditioned media of parental, PDGFB-overexpressed and PDGFB-targeted cells. **Results:** ccRCC cells did secrete significant amount of PDGFB into the extracellular milieu whereby the amount of secreted PDGFB positively correlated with intracellular PDGFB expression. PDGFB/KLF6 targeting and PDGFB overexpression significantly reduced and increased the level of secreted PDGFB, respectively. We previously demonstrated that KLF6 inhibition impaired the 786-M1A cells mTORC1 activity due to reduced PDGFB expression. Herein, we observed mTORC1 activity restoration in these KLF6-targeted cells upon culturing with conditioned media of parental and PDGFB-overexpressed cells. **Conclusion:** ccRCC cells secrete PDGFB, not only important to sustain their own mTORC1 activity, but also in the neighbouring cells to support the tumorigenesis. Our present findings further unravelled the molecular network that modulates the previously unaddressed frequent mTORC1 hyperactivation in ccRCC, which would potentially pave the way for the development of better ccRCC clinical strategies.

Keywords: Kidney Cancer, mTOR, PDGFB, Paracrine Signalling, CRISPR

Dentatin Is Superior To 5-Fluorouracil In Induction Of Intracellular Reactive Oxygen Species In Colorectal Carcinoma Cells

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ABSTRACT

Introduction: The first-line anti-colorectal cancer chemotherapeutic drug, 5- Fluorouracil (5-FU) is associated with fatal complications. Dentatin (DTN) isolated from *Clausena excavata* has demonstrated anti-tumour capacity in a few cancers cell lines and was found to be relatively non-toxic to normal cells. In this investigation, we compared the anti-tumour effects of DTN and 5-FU on HCT-116 colorectal carcinoma cells. **Methods:** 48 hour-DTN- and 5-FU-treated HCT-116 cells were subjected to determination of cytotoxicity and cell migration, via MTT and wound healing assays, respectively. Intracellular reactive oxygen species (ROS) was determined using ELISA. Meanwhile, morphological changes were observed under the inverted microscope. Apoptotic cells were acquired via flow cytometry and further validated using fluorometric assay to determine caspase-3 activity. **Results:** Both compounds were cytotoxic in a dose dependent manner. DTN exerted lower IC50 value compared to 5-FU. Meanwhile, at their respective IC50, wound healing assay revealed that both compounds inhibited cell migration. Strikingly, DTN induced excessive intracellular ROS which is 2-fold higher than 5-FU. In addition, cell morphology observation showed high presentation of cell blebbing and apoptotic bodies. Apoptotic assay further confirmed both compounds induce apoptosis and activation of apoptotic mediator caspase 3. **Conclusion:** DTN is superior to 5-FU in induction of intracellular ROS, which leads to apoptotic cell death via caspase 3 activation in colorectal carcinoma cells. This study provides a basis to DTN as an essential lead compound in combating colorectal carcinoma, that warrants further investigations.

Keywords: Dentatin, *Clausena excavate*, Reactive Oxygen Species, Natural Compounds, Apoptosis

Simultaneous Inhibition Of Proteasome And Autophagy Synergistically Enhance Cytotoxicity Of Doxorubicin In Breast Cancer Cells

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ABSTRACT

Introduction: Two protein degradation pathways exist to degrade intracellular proteins, namely ubiquitin–proteasome system (UPS) and autophagy. Studies have elucidated the involvement of UPS and autophagy in the development of doxorubicin resistance in breast cancer cells. Following anticancer treatments, autophagy acts either as cytoprotective mechanism to endure therapy-induced stresses or augment cell death induced by anticancer agents. This study aimed to investigate the role of autophagy in breast cancer cells co-treated with doxorubicin and proteasome inhibitor. **Methods:** The expression of autophagy protein (LC3A/B and Beclin-1) and UPS protein (ubiquitin) in MDA-MB-231 and MCF-7 cells following 6 hours treatment with doxorubicin, ixazomib and/or hydroxychloroquine were determined by western blot analysis. The combinatorial effects of doxorubicin, ixazomib and hydroxychloroquine in MDA-MB-231 and MCF-7 cells were determined by MTT assay. The combination index (CI) of the MTT assay was calculated using the CompuSyn Software. **Results:** Doxorubicin and ixazomib co-treatment increased the expression of Beclin-1 (3.8-fold and 3.5-fold) and LC3-II proteins (13.5-fold and 1.9-fold) in MDA- MB-231 and MCF-7 cells, respectively. The triple-combination of doxorubicin and ixazomib with lysosomal inhibitor, hydroxychloroquine further increased the expression of LC3-II proteins by 45.0-fold and 16.5-fold in MDA-MB-231 and MCF-7 cells, respectively. These findings confirmed that doxorubicin and ixazomib co-treatment induced autophagy in MDA-MB-231 and MCF-7 cells. MTT assay showed that the triple-combination synergistically inhibited breast cancer cell growth, achieving CI as low as 0.575 and 0.126 in MDA-MB-231 and MCF-7 cells, respectively. The triple-combination also induced greater ubiquitinated proteins accumulation (2.5-fold and 3.0-fold) as compared to (1.7-fold and 1.9-fold) induced by doxorubicin and ixazomib co-treatment in MDA-MB-231 and MCF-7 cells, respectively. **Conclusion:** Autophagy induced by doxorubicin and ixazomib co-treatment serves a cytoprotective role in breast cancer cells. Simultaneous inhibition of UPS and autophagy with ixazomib and hydroxychloroquine synergistically enhanced doxorubicin-mediated breast cancer cells killing.

Keywords: Breast Cancer, Autophagy, Ubiquitin-Proteasome System, Doxorubicin

Hybrid Anticancer Peptides DN1 And DN4 Exert Selective Cytotoxicity Against Hepatocellular Carcinoma Cells By Inducing Both Intrinsic And Extrinsic Apoptotic Pathways

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ABSTRACT

Introduction: Hepatocellular carcinoma (HCC) remains to be the dominant histological subtype of primary liver cancer with least effectiveness to most treatments due to drug resistance and wide range of side effects. Meanwhile, therapeutic peptides have emerged as promising therapeutic alternatives in pharmaceutical industry, especially to fight cancer. Here, we aim to investigate the in-vitro anti-proliferative activities and cell death mechanism of two series of hybrid peptides (ND and DN series) designed based on IsCT peptide (from *Opithacantus madagascariensis*) and A4 peptide (from Escherichia coli bacterial membrane anchor and aurein 1.2) against HCC cells. **Methods:** Cytotoxicity and cytostaticity of hybrid peptides on HepG2, Vero and THLE-3 cells were first assessed. Upon selecting hybrid peptides with higher selectivity against HepG2 cells, mode of cell death and signaling pathways activated were determined. **Results:** Our results indicated that ND series (IC50 ranging from 22 – 72 µg/mL) displayed stronger cytotoxicity on all cell lines tested as compared to the DN series (IC50 ≥105 µg/mL). Among these, DN1 and DN4 showed observable selectivity against HepG2 cells in a dose-dependent manner in the absence of cytostatic activity. Through sequence-activity relationship study, we discovered that modulation of cationicity and hydrophobicity in DN1 and D4 were found to correlate with their selectivity and cytotoxicity against HepG2 cells. Subsequent findings delineated that both peptides caused apoptotic cell death in HepG2 cells via minimum up-regulation of p53, accompanied by concurrent activation of both intrinsic and extrinsic apoptosis regulatory proteins that were cross-linked by Bid protein. **Conclusion:** The current findings denote evidence of DN1 and DN4 as potential candidate to be further explored as an alternative in HCC and other primary cancers' treatment. Further modulation of physicochemical properties of DN1 and DN4 can be done in search of novel ACP variants with enhanced potency as anticancer therapeutics.

Keywords: Anticancer Peptides; Cytotoxic Peptides; Hepatocellular Carcinoma; Hybrid Peptides; HepG2

Rapid Changes In Mitochondrial DNA May Participate In The Development Of Cisplatin-Resistant Oral Squamous Cell Carcinomas

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ABSTRACT

Introduction: The genomes of cancer cells are constantly evolving to adapt to environmental changes. Cancer cells accrue a multitude of mutations during their transformation into malignant tumours, making it difficult to pinpoint the true tumorigenic mutations. In this study, we aimed to ascertain changes in mitochondrial DNA (mtDNA) that drove the development of cisplatin-resistant oral squamous cell carcinoma (OSCC). **Methods:** We derived drug-resistant cells from two human OSCC cell lines, namely H103 and SAS, by repeated treatments with cisplatin. Before and after cisplatin treatments, we extracted mtDNA and sequenced it using a nanopore sequencer, MinION, to obtain the mutational and methylation profiles of the cells. Comparisons of pre- and post-treatment mtDNA sequences allowed us to eliminate functionally irrelevant mtDNA changes. We also determined cisplatin-induced changes in mtDNA content (using quantitative PCR) and mitochondrial function i.e., mitochondrial oxygen consumption rates, mitochondrial membrane potentials, and production of reactive oxygen species (ROS). **Results:** We found that the cisplatin treatments resulted in positive selection for a m.3910G>C mutation in the MT-ND1 gene of the SAS cells and a reduction in their mtDNA content. However, we found no differences in the mtDNA content and sequences between the cisplatin-resistant and parental H103 cells, bar several mutations whose allele fractions altered significantly post-treatment. Both cisplatin-resistant SAS and H103 cells demonstrated changes in their CpG methylation patterns. In keeping with the mtDNA changes, the cisplatin-resistant SAS and H103 cells became less sensitive to ROS-induced cytotoxicity caused by cisplatin, probably because of mitochondrial dysfunction. **Conclusion:** Our findings suggest that a constellation of mtDNA changes, namely reductions in mtDNA content, point mutations, enrichment of low-frequency, survival-enhancing mutations, and epigenetic changes, could quickly emerge during the development of drug-resistant cancer cells. These changes are functionally important and may participate in the cellular adaptation in response to environmental changes.

Keywords: Mitochondrial DNA, Induced Cisplatin-Resistant Cells, Nanopore Sequencing, Oral Squamous Cell Carcinoma

Anti-Proliferative Activity Of Novel Indole Schiff Base β-Diiminato Ligand Through Apoptosis Against Triple Negative Breast Cancer Cells

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ABSTRACT

Introduction: Breast cancer still stands first in terms of incidence and mortality rates among women worldwide. Triple-negative breast cancer (TNBC) characterized by lack of expression of estrogen receptor (ER-), progesterone receptor (PR-) and human epidermal growth factor receptor 2 (HER2-) is the most lethal and aggressive breast cancer subtype. Due to the lack of effective therapeutic strategies, there is still crucial need to develop more effective anti-cancer agents. Schiff bases have attracted attention as promising agents in cancer drug discovery related to their azomethine functional group. This study was conducted to evaluate the inhibitory effect and cytotoxic mechanism of a novel indole Schiff base β-diiminato ligand against MDA-MB-231 cells, a TNBC model. **Methods:** The cell viability was investigated through MTT colorimetric assay and cytotoxicity using trypan blue and lactate dehydrogenase release (LDH) assays. Apoptosis was analyzed through microscopic observation. All the experiments were conducted following 24h treatment with compound. **Results:** The complex significantly inhibited cell proliferation in MDA-MB-231 breast cancer cell line with the IC50 value of 2.41±0.29 µg/mL. In addition, inverted phase contrast microscope observation revealed cell shrinkage, rounding of the cell shape and membrane blebbing formation, and Hoechst 33342/PI dual-staining assay showed nuclear shrinkage, chromatin condensation, and fragmentation, representing induction of apoptosis in cancer cells upon treatment in a dose-dependent manner (0, 2.5 and 5 µg/mL concentrations). **Conclusion:** The results of this study suggest that this β-diiminato ligand is able to inhibit cell proliferation and induce apoptosis in TNBC breast cancer cells. The complex is a potential candidate for future cancer studies.

Keywords: Indole Schiff Base, Breast Cancer, Anti-Cancer Activity, Apoptosis, Drug Discovery

Cytotoxicity-Guided Solvent Fractionation Of Methanolic Crude Extract Of *Synclisia scabrida* Miers Ex Oliv. Root

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ABSTRACT

Introduction: Cancer is a life-threatening disease, posing therapeutic challenges in the quest for total cure. Researchers have been looking for potential therapeutic agents from natural sources. *Synclisia scarbida*, a Nigerian plant which has recently been reported to have anticancer efficacy by local traditional practitioners, was chosen for further research in our study. **Methods:** Methanol extract (ME) of *S. scabrida* root was chosen for its high yield and potency. Preliminary phytochemical studies on ME were carried out using standard methods. The methanol extract (9.0 g) was fractionated into nine fractions using column chromatography (MeOH:DCM = 1.4:8.6). The fraction4–8 (1.6 g) was combined and partitioned to obtain 24 subfractions. Based on TLC, subfractions 1–7 (2.3 g) and 8–21 (712.1 mg) were further purified by PTLC (IPA:DCM = 6:4) to yield five sub-subfractions for each case. The 3rd and 4th fractions from subfractions 1–7 were combined again. The third band (204.1 mg) of PTLC subfractions 8–21 was fractionated further to yield three sub-subfractions. MTT assay was employed to evaluate anticancer potentials of the fractions/subfractions of *S. scabrida* against HCT-116 (colon), MCF-7 (breast) and PANC-1 (pancreas) cancer cells. **Results:** The presence of alkaloids, tannins, and steroids was confirmed in an initial phytochemical screening of ME. The combined fractions 4–8 had an IC50 value of 23±14 µg/mL against PANC-1. Subfractions 1–7 had IC50 values of 78±20, 47±8 and 33±6 µg/mL in PANC-1, HCT-116, and MCF-7, respectively. However, the combined sub-subfraction 3- 4 yielded from subfractions 1–7 was only active against HCT-116 (58±3 µg/mL) and MCF-7 (43±6 µg/mL). **Conclusion:** PANC-1 was initially reported to be susceptible to fractions 4-8; however, the efficacy of the subfractions decreased for the PANC-1 cells, which could be due to the loss (degradation) of potent phytoconstituents during the purification process. From the preliminary study, it was confirmed that MCF-7 is the most sensitive to *S. scabrida* sub-fractions. Thus, more research is needed for the careful isolation of pure phytochemicals and to confirm the anticancer potential of *S. scabrida*.

Keywords: *Synclisia scabrida*, Phytochemicals, Anticancer Potential, Cell Viability Assay

Anti-angiogenic *Lactobacillus plantarum* LAB12-derived Cell Free Supernatant Downregulated VEGF And Upregulated TSP-1 In HCT116

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ABSTRACT

Introduction: The limitations of conventional chemotherapy and targeted therapy has called for alternative strategies in management of colorectal cancer (CRC). The fact that sporadic CRC are associated with diet raises the possiblity of disease prevention through consumption of probiotics. There is growing evidence that indicates the strain-dependent usefulness of probiotic-derived bioactive metabolites against CRC. As such, the present study examined the *in vitro* anti-angiogenic potential of cell free supernatant (CFS) fermented by unique probiotic lactic acid bacteria (LAB) isolated from locally fermented food. **Methods:** This study first examined the 24-hour differential cytotoxicity of LAB-derived CFS against HCT116 (human colon carcinoma cell line) and HUVEC (human umbilical vein endothelial cells) by using the Sulforhodamine B Assay. The LAB-derived CFS was assessed for its anti-angiogenic potential at the highest subtoxic dose using immunocytochemistry (for HCT116) and the tube formation assay (for HUVEC). **Results:** Out of the 12 LAB-derived CFS, LAB1 and LAB12 as well as LAB3 and LAB4 were the two most potent *Lactobacillus* spp. and *Pediococcus* spp., respectively against HCT116. Subsequent screening against HUVEC found the LAB-derived CFS to elicit differential cytotoxicity between HCT116 and HUVEC, with greater selectivity towards HCT116. Immunocytostaining of HCT116 treated with LAB12-derived CFS, in particular, showed downregulation of the pro-angiogenic vascular endothelial growth factor (VEGF) and upregulation of the anti-angiogenic thrombospondin (TSP-1). HUVEC exposed to LAB12-derived CFS formed significantly lesser tube-like structures ($p<0.05$) even in the presence of VEGF. **Conclusion:** The present findings strongly implied the anti-angiogenic effect of LAB12 against HCT116 and the potential use of probiotics as for prevention against CRC.

Keywords: Angiogenesis, Colorectal Cancer, Probiotics, Vascular Endothelial Growth Factor, Thrombospondin

Supercritical Fluid Extraction Of Black Pepper Essential Oil And Oleoresin With Potential Anticancer Property

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ABSTRACT

Introduction: One of the limitations of current anticancer drugs is the side effect of toxic chemical residues during the synthesis process. Therefore, discovery of natural sources of anticancer drug using green and environmentally friendly extraction technology is the new direction in biomedical research. Black pepper (Piper nigrum) or the "King of Spices" has been shown to exert anti-inflammatory, cardioprotective and anticancer effects. Most of the reported black pepper extracts were extracted using ethanol, dichloromethane, chloroform, and petroleum ether, but these organic solvents can be toxic to human. In contrast, supercritical fluid extraction (SFE) technology utilizes non-toxic supercritical CO2 (scCO2) as the "solvent" that leaves no chemical residue in the product. Therefore, this study aimed to extract essential oil and oleoresin of Sarawak black pepper using SFE and investigate their anticancer properties. **Methods:** The essential oil and oleoresin of black pepper were simultaneously extracted and separated using the pilot-scale SFE. Ultraviolet-visible (UV-Vis) spectrophotometer and gas chromatography-mass spectrometry (GCMS) were used to identify the chemical compounds in the extracts. The potential anticancer property of the essential oil and oleoresin towards human breast cancer cells (4T1 and MDA-MB-231) were evaluated using MTT assays. **Results:** The percentage yield of essential oil and oleoresin were 1.26% and 5.28%, respectively. GCMS and UV-Vis results revealed the presence of caryophyllene, α-pinene, and α-copaene in the essential oil while piperine existed in the oleoresin. The MTT results showed that the oleoresin exhibited at least 2-fold cytotoxicity (IC50 = 0.6 mg/mL) towards 4T1 and MDA-MB-231 compared to the essential oil, but less cytotoxic than the studied crude extracts (IC50 = 38 µg/mL) obtained using organic solvents. **Conclusion:** The black pepper oleoresin has potential anti-breast cancer property. Its lower cytotoxicity compared to the organic solvent-extracted extracts is probably due to the presence of toxic solvent residue and warrants further investigation.

Keywords: Supercritical Fluid Extraction, Black Pepper, Oleoresin, Essential Oil, Anticancer

Assessment Of The Antioxidant And Anticancer Potential Of Crude Methanolic Extract Of Malaysian Marine *Chlorella* sp. *In Vitro*

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ABSTRACT

Introduction: Microorganisms and terrestrial plants are the main source of natural anticancer and antioxidant agents for decades. Though marine organisms, especially marine microalgae are often considered as a mother lode of potential antitumor compounds, this source is yet to be thoroughly investigated. Therefore, the project aims to investigate the anticancer activity of crude methanolic extract of Malaysian marine *Chlorella* sp. against a human breast cancer cell line (MCF-7) and evaluate its antioxidant potential and phytochemical contents. **Methods:** Freeze-dried *Chlorella* sp. biomass was extracted with methanol solvent through sonication for 20 minutes and maceration for one hour. The microalgal extract (1 mg/ml) was used to determine antioxidant activity by using DPPH free- radical scavenging and ferric reducing antioxidant power (FRAP) assays with gallic acid and ascorbic acid as standards respectively. The algal extract was evaluated for their cytotoxic effect at a concentration of 100 µg/mL against MCF-7 cell line using MTT assay. The total phenolic and flavonoid contents were also determined. **Results:** The methanolic extract (1 mg/mL) of *Chlorella* sp. showed moderate radical inhibition activity (52%), when compared to gallic acid (98%), and also exhibited good ferric reducing power (25.53 ± 0.02 mg ACE/ g extract) in FRAP assay. The extract (100 µg/mL) inhibited MCF-7 cells by reducing the cell viability up to 24% after 48 hours of incubation. The methanolic extract showed 38.8 ± 0.01 mg GAE/ g extract and 278.89 ± 0.09 mg QE/ g extract for total phenolic and flavonoid content, respectively. **Conclusion:** The methanolic extract of Malaysian marine *Chlorella* sp. was found to inhibit the MCF-7 breast cancer cell line and seems to be a good source of antioxidants.

Keywords: Antioxidant, Breast Cancer, Crude Extract, Microalgae, Phytochemical

SRJ23 Induces Autophagy Regulatory Proteins *In Vitro* And Suppresses Growth Of Gemcitabine-Resistant Pancreatic Cancer Xenografts

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ABSTRACT

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is currently the seventh leading cause of cancer deaths globally. Mutation of Kirsten-Ras (K-Ras) oncogene is prevalent in pancreatic cancer patients. PDACs are aggressive and lethal as patients developed remarkable resistance to available conventional chemotherapy, gemcitabine, in a short treatment period. SRJ23 (3,19-(3-chloro-4-fluorobenzylidene andrographolide) was found to inhibit K-Ras oncogenic signalling *in vitro*. In this investigation, the anti-tumour effect of SRJ23 against gemcitabine resistant Capan-2 (CP2GR-5μM) and gemcitabine resistant Panc-1 (PIGR-3.5μM) pancreatic cancer xenografts were evaluated. **Methods:** Immunoblotting protocol was carried out on CP2GR-5μM cells treated with 10 μM SRJ23 to detect autophagy and apoptosis regulatory proteins [(Beclin-1 and ATG12) and (Bax, Bcl-2, Caspase-3), respectively]. CP2GR-5μM and PIGR-3.5μM tumour xenografts were established in 6-8 weeks female nude mice subcutaneously. Mice bearing tumour of 100-300 mm3 were randomised into 4 treatment groups. Each mouse was treated with a single intraperitoneal dose of 100 mg/kg SRJ23. Tumour size and mice body weight were measured for 14 days. The mice were euthanised 14 days post-treatment. Tumour, liver, kidneys and spleen were harvested and weighed. **Results:** Immunoblotting results revealed 10 μM SRJ23 to suppress expression of Beclin-1, ATG12 and Caspase-3 and enhance expression of Bax and Bcl-2. *In vivo* results indicated significant ($p<0.05$) tumour growth suppression by a single treatment of 100 mg/kg SRJ23. The significant effect was noted in CP2GR-5μM and PIGR-3.5μM tumour xenografts beginning day 7 and day 5, respectively. Moreover, mice from the SRJ23 treatment group did not exhibit adverse toxicity during treatment period. **Conclusion:** Immunoblotting results imply that SRJ23 is able to halt the formation of phagophore as opposed to gemcitabine that promotes the formation of autophagosomes. In parallel, SRJ23 initiates the formation of apoptosome *in vitro*. It is highly plausible that SRJ23 inhibited autophagy and induced apoptosis in tumour xenografts to cause tumour shrinkage.

Keywords: Autophagy, Apoptosis, SRJ23, Gemcitabine-Resistant Pancreatic Cancer Xenograft, PDAC Resistance

Interplay Between Autophagy And Apoptosis In A Pancreatic Cancer Cell Line Treated With SRJ23, Gemcitabine Or Combination Of Both Agents

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ABSTRACT

Introduction: Pancreatic cancer is an aggressive disease. Although its occurrence rate remains low in Malaysia, it is the third leading cause of cancer-related death in the USA because of limited treatment options. Gemcitabine is a standard chemotherapy for pancreatic cancer. Most pancreatic cancers harbour K-Ras mutations that lead to constitutive activation of mitogen-activated protein kinase (MAPK) signalling pathway. The aim of this study was to explore the potential of Ras inhibitor, SRJ23 in modulating autophagy and apoptosis in a pancreatic cancer cell line, Capan-2 alone or in combination with gemcitabine. **Methods:** 3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used in assessing the growth inhibition of SRJ23 alone or in combination with gemcitabine at a concentration of 0.1-100 μM for 96 hr against Capan-2 cells. Compusyn software was used to determine the combination index of SRJ23 and gemcitabine drug combination treatment. Immunoblotting assay was used to detect the expression of autophagy markers (LC3, Beclin-1, ATG5) and apoptosis markers (Bax, Bcl-2, caspase-3 and cleaved caspase-3). Additionally, MDC staining was used to detect the change of autophagy at morphological level. **Results:** Combination of 10 μM SRJ23 with 10μM gemcitabine showed synergistic growth inhibitory effect with combination index of 0.87. Furthermore, SRJ23 or gemcitabine treatment alone induced autophagy by increasing the protein expression of autophagy marker, LC3B-II and it further elevated the autophagy protein level when treated in combination. Simultaneously, SRJ23 and gemcitabine combination treatment significantly increased the protein expression of apoptotic marker, cleaved caspase-3 compared to its single treatment. MDC staining showed that the fluorescent density was higher and the number of MDC-labelled particles in Capan-2 cells was greater in the SRJ23 and gemcitabine combination treatment compared to the single treatments. **Conclusion:** The findings suggested that combination treatment of SRJ23 and gemcitabine was indeed beneficial in regulating autophagy to promote apoptosis of Capan-2 cells.

Keywords: Pancreatic Cancer, SRJ23, Gemcitabine, Apoptosis, Autophagy

Zoanthids-Associated Marine Bacteria As A Source Of Active Metabolites For Their Application In Human And Animal Health

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ABSTRACT

Introduction: Ecuador is considered a megadiverse country in its marine habitats. Thus, the Reserva Marina el Pelado (REMAPE) shows a great variety of marine organisms, which can be a source of bioactive compounds. Recent studies have shown that the secondary metabolites found in invertebrates are produced by the symbiotic microorganisms. In this context, it is intended to isolate metabolites produced by bacteria associated with zoanthids as a source of active molecules that can solve human and animal health problems. **Methods:** Sample Collection, Processing, and Identification. Bacterial DNA extraction and 16S rDNA gene analysis. Preparation of bacterial extracts. In vitro evaluation of biotechnological potential in aquaculture. Determination of the anti-Vibrio activity of enriched inoculums. Determination of the anti-Vibrio activity of bacterial extracts. Effects on Carcinoma Cell Lines. **Results:** An abundant and high diversity of bacteria associated with zoanthids is evidenced. A total of 12 bacterial strains were isolated and characterised, which showed varied results in the biochemical profile. All of them showed positive results for the use of citrate except Z10. Molecular identification by means of 16s rRNA gene sequencing resulted in the identification of 9 bacterial species. All bacteria showed> 99% similarity when analysed in the NCBI GenBank. Of the nine bacterial species isolated, eight did not show antimicrobial activities, except for one, which showed antimicrobial activities against all the pathogens evaluated. The present exclusion zone showed antimicrobial activity, which indicates bacteria associated with zoanthids producing bioactive compounds. **Conclusion:** We performed an assessment on the potential of zoanthids-associated bacteria isolates, to produce bioactive compounds and the biological activity of their extracts. One molecule isolated from zoanthids presented activity against some carcinoma cell lines, indicating the metabolic potential of the isolates. Zoanthids-associated bacteria strains also presented activity against all shrimp pathogens evaluated.

Keywords: Secondary Metabolites, Natural Products, Bacteria, Marine Invertebrates, Drug Discovery

Phytochemical Content And *In Vitro* Antibacterial Activity Of Malaysian *Calotropis procera* (Ait.) Twigs Extracts

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ABSTRACT

Introduction: *Calotropis procera* (Ait.) (family Apocynaceae) is an ancient medicinal plant that is used in Ayurvedic, Arabic, Sudanese and Unani traditional medicinal systems for treatment against several diseases like dysentery, leprosy, skin and wounds infections, and fever. *Calotropis procera* contains different phytoconstituents such as glycosides, flavonoids, triterpenes, steroids, proteins, and enzymes. The present study aimed to undertake a preliminary phytochemical screening and assess the in vitro antibacterial effects of different solvent extracts of *C. procera* twigs against a panel of Gram-negative and Gram-positive bacteria. **Methods:** Briefly, dried and powdered *C. procera* twigs were extracted using three solvents of increasing polarity (i.e., n-hexane, dichloromethane and methanol) through the cold maceration method for 72 hours. Standard phytochemical tests were carried out to detect the presence of phytochemicals in the extracts. The antibacterial activity of the extracts (dissolved in DMSO) was assessed at dose of 3 mg/well against six Gram-negative and six Gram-positive bacteria using the agar well diffusion method. Ampicillin and DMSO were used as positive and negative controls, respectively. **Results:** The methanolic extract was presented with abundant flavonoids and phenolics, while the n-hexane and DCM extracts were rich in sterols. The methanolic twig extract was found to be active (ZOI; 9-12.5 mm) against the tested bacteria. Escherichia coli and Proteus vulgaris were the two most sensitive bacteria while MRSA was the least sensitive bacterium (ZOI; 9 mm) among all tested bacteria. **Conclusion:** The antibacterial potential of *C. procera* twigs could be due to phenolic and flavonoids polar constituents present in methanolic extract. The present findings warrant further in-depth phytochemical and antimicrobial investigations of antibacterial lead(s) for antibiotic drug discovery.

Keywords: Antibacterial Activity, *Calotropis procera*, Phytochemicals, Methanolic Extract

Molecular Docking Of *Piper betle* Bioactive Compounds On Inducing Wound Healing By Increasing The Proliferation Rate Of Fibroblast Cells

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ABSTRACT

Introduction: Wound healing is a process which goes through a complex regulatory system of various molecules that ultimately resulted in a new formation of healthy cells. Chronic wound such as diabetic ulcer, is a significant issue that threatens the quality of life of many patients in Malaysia. Many of these patients had to go through limbs amputation which at the same time would cost them financially. *Piper betle* is a wild plant that grows across Sabah which were found to increase the rate of wound healing. Herbal medicines have been studied for decades due to their potential medical effects, particularly on wound healing. **Methods:** In this study, we perform computational molecular docking of 10 bioactive compounds found in *Piper betle* using AutoDock Vina to evaluate their structure and predict the favoured orientation between the ligands to 3 different receptors responsible in regulating the rate of fibroblast cell proliferation. **Results:** We were able to observe the three-dimensional (3D) structure of the bonded molecules using Pymol software and found that most of the bioactive compounds were able to form hydrogen-bonding with the receptors at various binding affinity. **Conclusion:** Application of these compounds may be possible to regulate the level of the receptors with regards to increasing rate of fibroblast cell proliferation.

Keywords: Fibroblast Cell Proliferation, Bioactive Compound, Molecular Docking, Chronic Wound, Wound Healing

Assessment Of The Anti-Bacterial Activity Of A Simple Nanoformulation, Gallic Acid Loaded Graphene Oxide (GAGO) Against Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains

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ABSTRACT

Introduction: Reckless use of antibiotics has caused a dramatic increase in the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA). MRSA infections have invaded the community, producing a new strain known as the community-acquired MRSA (CA-MRSA) with the presence of Panton-valentine leucocidin (pvl) genes, a pore-forming toxin. The present study aims to support the hypothesis that a simple, safe and cost-efficient gallic acid loaded graphene oxide (GAGO) nanoformulation would be able to exhibit anti-bacterial activity against MRSA stains. **Methods:** GAGO was prepared with a GA loading of 933.67±0.04 mg/g. The anti-bacterial activity of GAGO was elucidated with disk diffusion method, CFU counting method and time-kill experiment, which was followed by HRTEM observation. **Results:** GAGO exhibited inhibition towards all bacteria strains employed. The anti-bacterial activity of GAGO was comparable to cefoxitin (CFX), at ≥ 150 µg/mL in MRSA, as well as MSSA. CA-MRSA was found to be less susceptible towards GAGO, with only 82.7% of inhibition observed in MRSA-pvl+ at the highest concentration employed (1000 µg/mL), while maintaining a comparable activity to CFX against MRSA-pvl-, at 500 and 1000 µg/mL. GAGO showed a significant delayed response towards CA-MRSA strains, with increased inhibition observed only upon 8h exposure, while a comparable activity to GA, was achieved at 24h. GAGO showed comparable activity with both GO and GA, against MRSA and MSSA as early as 2h of exposure. HRTEM micrographs of MRSA and MSSA exposed to GAGO revealed the apparent shrinkage of the cells, with loss of the coccal appearance, that leads to membrane destabilization and destructive extraction of the cell components. **Conclusion:** The collective data demonstrates that GAGO has the anti-bacterial activity towards different MRSA strains that may serve as an alternative to conventional antibiotics for multi-drug resistant bacteria. However, further study is warranted to develop this formulation for clinical applications.

Keywords: Multidrug Resistant, MRSA, Community-Acquired, Graphene Oxide, Gallic Acid

Lactobacillus plantarum LAB12 Reduced Severity Of Liver Pathology And Improved Intestinal Barrier In High-Fat Diet-Induced Non-Alcoholic Fatty Liver Disease Mouse Model

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ABSTRACT

Introduction: The association of non-alcoholic fatty liver disease (NAFLD) with gut dysbiosis raises the possibility of using probiotics to restore balance of gut microbiota and improve intestinal barrier in favour of a healthy liver. As such, this study aimed to assess the hepatoprotective effect of *Lactobacillus plantarum* LAB12 against liver pathology, imbalanced gut microbiota and leaky gut in high-fat diet (HFD)-induced NAFLD mouse model. **Methods:** Male C57BL/6J mice (7-week- old;10<n<11/group) were divided into five groups and fed with normal chow, normal chow supplemented with LAB12, HFD, HFD supplemented with LAB12 and HFD supplemented with vitamin E, respectively, for 20 weeks. For the intestinal permeability test at week-17, a 24-hour urine collection was performed following a gavage of lactulose and mannitol mixture after a 6-hour fasting. The urinary lactulose/ mannitol ratio was then determined using the high-performance liquid chromatography. At week-20, blood sampled from hepatic portal vein and cardiac puncture were obtained for determination of lipopolysaccharide (LPS) and alanine aminotransferase (ALT) levels, respectively. The livers were harvested and prepared for histological analysis using the total NAFLD Activity Score (NAS). The caecal and colonic contents were snap-frozen and kept at -80°C until 16S rRNA gene sequencing. **Results:** The supplementation of LAB12 significantly reduced ($p<0.05$) the total NAS and serum ALT of HFD-induced NAFLD group when compared to the HFD controls. Besides, HFD-fed mice supplemented with LAB12 were presented with improved gut barrier function with significantly reduced ($p<0.05$) lactulose/mannitol ratio and serum LPS when compared to the HFD controls. Interestingly, LAB12 supplemented normal control mice were presented with significantly increased bacterial gene richness ($p<0.01$) when compared to their normal control counterparts. **Conclusion:** The present findings implied that the reduced severity of liver pathology and improved intestinal barrier function in HFD-induced NAFLD mice supplemented with LAB12 could be associated with improved gut microbiota balance.

Keywords: Probiotics, Dysbiosis, Non-Alcoholic Fatty Liver Disease, Lipopolysaccharide, Lactulose/Mannitol Ratio

Lactobacillus plantarum LAB12 Conferred Atheroprotection *In Vitro* And *In Vivo*

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ABSTRACT

Introduction: Hypercholesterolaemia is a vital risk factor of atherosclerosis. As such, cholesterol lowering through pharmacological intervention and dietary changes remain the foundations of atherosclerosis management and treatment. However, these approaches appear to have limited maximum efficacy, typically impeded by low compliance in high-risk patients and drug-induced health consequences. Recent evidence on the beneficial effects of probiotics against hypercholesterolaemia and atherosclerosis raises the possibility of using functional food [i.e., lactic acid bacteria (LAB)] for atheroprotection. The present study had identified *Lactobacillus plantarum* LAB12 with promising cholesterol-lowering properties through a preliminary screening effort. Capitalising on these beneficial properties, this study went on to investigate the atheroprotective potentials of LAB12 in vitro and in vivo. **Methods:** For the in vitro study, the sub-toxic concentration of 24 hours LAB-fermented cell-free supernatant (CFS) against RAW264.7 and HUVEC were determined using the Sulforhodamine B assay. The LAB-derived CFS was also assessed against the oxLDL-induced foam cell formation, mitochondrial membrane potential (MMP) and antioxidant assays. The effect of LAB-derived CFS against oxLDL-induced endothelial dysfunction was explored using the monocyte-endothelial adhesion and MMP assays. For the in vivo study, the atheroprotective properties of freeze-dried LAB12 (LAB12-FD) was explored using male C57BL/6J mice fed with high-fat diet (HFD) for 20 weeks. **Results:** The LAB-derived CFS not only significantly ($p<0.05$) reduced oxLDL uptake, reduced MMP depolarisation and increased antioxidant activity in oxLDL-induced RAW264.7 cells, but also reduced monocyte adhesion and prevented MMP depolarisation in response to oxLDL-induced endothelial dysfunction. Daily consumption of LAB12-FD significantly ($p<0.05$) reduced total cholesterol content in the livers, reduced fatty streak formation and infiltration of macrophages in the aortas, as well as improved intestinal barrier in vivo. **Conclusion:** The present findings strongly implied that LAB12 could be used as a functional food for potential atheroprotection.

Keywords: Lactic Acid Bacteria, Probiotics, Cholesterol Lowering, Atherosclerosis, Atheroprotection

Practical And Economic Synthesis Of 2,3-O-Isopropylidene-D-Erythrose

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ABSTRACT

Introduction: 2,3-O-isopropylidene-D-erythrose has been a useful chiral precursor involved in the total synthesis of several natural products such as the leukotrienes, pyrrolizidine and indolizidine alkaloids. However, previous published protocols used expensive chemicals/reagents in the synthesis scheme. In this study, we synthesised D-erythrose from erythorbic acid with inexpensive and commercially available chemicals/reagents. **Methods:** D-erythrose was synthesised from published protocol with modification. Briefly, sodium erythorbate was treated with hydrogen peroxide to yield a mixture of D-erythronolactone, oxalic acid and sodium chloride. Further exposure to anhydrous acetone and p-toluenesulfonic acid monohydrate gave the acetonide form of D-erythronolactone. The corresponding acetonide was then partially reduced into the lactol in excellent yield by using sodium bis(2-methoxyethoxy-aluminium hydride. Purification of the products was carried out using the classical column chromatography Si-gel G60 (230-400 mesh, Merck). The 1H and 13C NMR spectra was registered in CDCl3 with Joel Resonance ECZ400S 400 MHz (1H) and 100 MHz (13C) using TMS as the internal standard. The specific optical rotation was determined using Anton Paar MCP 500 polarimeter. **Results:** 2,3-O-Isopropylidene-D-erythrose was obtained as colourless oil in the overall yield of 82% from the corresponding protected lactone. **Conclusion:** Selectively protected D-erythrose was easily prepared in substantial quantity as a mixture of enantiomers from the inexpensive chiral starting material. This may give a cheaper cost of production for clinically used drugs.

Keywords: Eythrose, Lactol, Acetonide Protection, Red-Al® Reduction

Potential Antimetastatic Effect of a 14-Deoxy-11,12-Didehydroandrographolide Analogue

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ABSTRACT

Introduction: Melanoma is considered as the most aggressive form of skin cancer. Onset of metastasis and the ability of melanoma to progressively rewire themselves make treatment against melanoma ineffective. As a result, prognosis of melanoma in the past decade has been poor. Hence, finding an effective melanoma treatment has become crucial. *Andrographis paniculata* (*A. paniculata*), commonly known as 'king of bitters', consists of labdane diterpenoids such as andrographolide (AG), 14-deoxy-11,12-didehydroandrographolide (DDAG) and neoandrographolide. These bioactive molecules and their analogues have exhibited varying degrees of anticancer activities both in vitro and in vivo experimental models of cancer. Several potential AG and DDAG analogues with outstanding anti-metastatic capabilities against melanoma were identified in our unpublished *in vitro* and *in vivo* results. Among these derivatives, 19-diacetyl-14-deoxy-11,12-didehydroandrographolide (SRS28), a DDAG analogue appeared to be the molecule with the most promising properties to be taken for further study. Therefore, the objective of the study is to further investigate the antimetastatic effect of SRS28 on metastatic melanoma. Methods: Cytotoxic effect of SRS28 against B16F10 melanoma cells were determined by conducting MTT assay. Effectiveness of different concentrations of SRS28 on reducing the total melanin content of B16F10 cells was also determined. The potential of SRS28 in preventing the migration of B16F10 cells was determined by conducting wound healing scratch assay and trans well migration assay **Results:** SRS28 was found to be non-toxic at a concentration range of 1-30 µM. No significant difference was observed between the control and SRS28 treated cells in reducing the extracellular or intracellular melanin content. The compound at 30 µM prevented the migration of B16F10 cells. **Conclusion:** This study showed the potential of SRS28 in preventing the migration of melanoma cells. Cell migration is a crucial step in metastasis dissemination and formation of metastasis.

Keywords: Metastasis, Melanoma, *Andrographis paniculata,* 14-Deoxy-11,12-Didehydroandrographolide

Purine Metabolites And Its Association With ENT2 Expression In Different Stages Of Colorectal Cancer Cell Lines

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ABSTRACT

Introduction: Colorectal cancer (CRC) remains the third common cancer occurred globally and the second leading cause of cancer mortality in the world. Purines are essential components of nucleotides in cell proliferation, hence altered purine metabolism has been linked to cancer growth. Increasing evidence have reported that equilibrative nucleoside transporter 2 (ENT2), a bidirectional transporter, mediates the uptake of purine nucleosides and other nucleobases. Hence, this study aims to determine the level of the rate-limiting enzymes and metabolites involved in the purine catabolism pathway and to relate the findings with the ENT2 expression level in different colorectal cancer stages. **Methods:** Xanthine oxidase (XO), hypoxanthine/xanthine and uric acid (UA) levels were measured in a panel of human CRC cell lines; SW480, HCT15 and HCT116, representing different CRC stages; Dukes' B, C, and D, respectively, and were compared to normal colon cell line; CCD-841CoN. RTq-PCR was performed to determine the level of ENT2 gene expression. **Results:** XO enzyme activity and UA levels were significantly decreased ($p<0.05$) in CRC cell lines compared to the normal cells. In contrast, hypoxanthine/xanthine concentrations and ENT2 expression were significantly higher ($p<0.05$) in all CRC cell lines as compared to normal cells. Hypoxanthine/xanthine concentration was 0.07, 0.096 and 0.12 μM in Dukes' B, C, and D stages of CRC. ENT2 expression level was 186, 471, and 123-fold higher in Dukes' B, C, and D stages of CRC, respectively. HCT116 (Dukes' D) showed the highest level of hypoxanthine/xanthine and the lowest level of ENT2 compared to other CRC stages. **Conclusion:** ENT2 is suggested to mediate the influx of hypoxanthine as indicated by the reduced XO activity and UA levels in CRC cells. Our findings implied that hypoxanthine may have a prognostic importance during CRC development. Further investigation is warranted to determine the role of ENT2 in CRC progression.

Keywords: Colorectal Cancer, Purine, ENT2, Hypoxanthine, Xanthine Oxidase

A New Look At The Anticancer Activities Of Novel Phenothiazine Derivatives

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ABSTRACT

Introduction: Cancer has now become a global problem. It is ranked the top leading cause of death worldwide after cardiovascular disease, tuberculosis and malaria combined. Although chemotherapy has improved survival of cancer patients, there is a need to discover and develop new potent antitumour agents with better selectivity and reduced side effects. **Methods:** This study has been prepared with the use of available internet search engines such as PubMed, Embase, Web of Science and Scopus, and on the basis of own scientific experience. **Results:** Phenothiazines are important class of heterocyclic compounds with wide spectrum of biological properties. Recent reports showed promising anticancer, antiplasmid, antibacterial, anti-inflammatory and immunosuppressive activities of classical and new phenothiazines. The modification of the phenothiazine structures with the pyridine ring leads to different pyridobenzothiazines and dipyridothiazines. Dipyridothiazine derivatives, amongst which 1,6-, 1,8-, 1,9-, 2,7- and 3,6-diazaphenothiazines were shown to possess interesting antiproliferative, anticancer, antioxidant and immunosuppressive activities. The compounds showed their anticancer action against various cancer lines of melanoma; leukemia; glioblastoma; and breast, colon, ovarian, renal, prostate, and lung cancers. The proteomic profiling studies showed that their most probable actions are through the intrinsic mitochondrial pathway of apoptosis, but in some cases the extrinsic (cell death receptor-dependent) route was also suggested. **Conclusion:** The present work highlights the importance of dipyridothiazines in the search for anti-cancer preparations.

Keywords: Dipyridothiazines, Anticancer Action, Mitochondrial Pathway Of Apoptosis

Long Non-Coding RNA KCNMA1-AS2-201 Regulates Cell Proliferation, Apoptosis, Migration And Sponges miR-1227-5p In SW1463 Colorectal Cancer Cell Line

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ABSTRACT

Introduction: Colorectal cancer (CRC) is one of the most common malignant cancers with high incidence and mortality. Aberrant expression of long non-coding RNA (lncRNA) is usually found in cancers and is associated with tumorigenesis by promoting malignant biological behaviours of tumour cells. LncRNA KCNMA1-AS2-201 was identified to be down-regulated in CRC by RNA sequencing. This study explored the undefined functions of lncRNA KCNMA1-AS2-201 in CRC. **Methods:** The lncRNA was in-vitro transcribed prior to transfection to overexpress KCNMA1-AS2-201 in SW1463 CRC cell line. 10 ng of KCNMA1-AS2-201 was transiently transfected into the cells in a 24-well plate and its expression was assessed by using real time qPCR. The potential functions of KCNMA1-AS2-201 in cell proliferation, apoptosis and cell migration were evaluated using MTT assay, Annexin V/PI staining and wound healing assay, respectively. The downstream microRNA (miRNA) targets of KCNMA1-AS2-201 were predicted using DIANA-LncBase v2 software and the interaction was verified by dual luciferase reporter assay by using KCNMA1-AS2-201/psiCheck-2 construct vector. **Results:** The expression of lncRNA KCNMA1-AS2-201 in 24hr post-transfected cells increased 29145 times compared with the control group. Overexpression of KCNMA1-AS2-201 inhibited the cell proliferation and migration from 24 to 72hr and improved the cells' apoptosis capacity. After 48hr of co-transfection of 13 pmol miR-1227-5p mimics with 200 ng KCNMA1-AS2-201/psiCheck-2 construct vector (containing lncRNA KCNMA1-AS2-201 potential binding site), the relative luciferase activity in wild type group decreased approximately half to that of the negative control. **Conclusion:** The lncRNA KCNMA1-AS2-201 regulated cell proliferation, apoptosis and migration of SW1463 CRC cells, and could sponge miR-1227-5p and its potential downstream target. This illustrates its potential as a therapeutic intervention target and prognostic marker for colorectal cancer. However, precise regulation mechanisms of these functions need to be explored further.

Keywords: Long Non-Coding RNA, Colorectal Cancer, Diagnostic Biomarker

Elucidation Of circRNA007* Functions In HCT116 And SW1463 Cell Lines

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ABSTRACT

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world. It is essential to identify some new molecular markers to raise the efficiency of CRC tumour diagnosis. Circular RNAs (circRNAs) are special endogenous non-coding RNAs molecules with a closed loop structure without 5′ to 3′ polarity and polyadenylated tail. Although there is an increased interest in circRNAs research worldwide, little is known about its relationship with CRC. Thus, this study aimed at elucidating the roles of circRNA in CRC, particularly the circRNA007*, which was found to be downregulated in CRC samples. **Methods:** circRNA007* was cloned into pcDNA3.1-CMV-circRNA-EF1-ZSGreen vector to construct the circRNA007* overexpressed plasmid, which was then transiently transfected into HCT116 and SW1463 cell lines. The expression of circRNA007* was assessed by quantitative real-time PCR at 24, 48 and 72h post transfection. Cell proliferation ability was assessed by using 3-(4, 5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Flow cytometry was performed to investigate cell apoptosis rate and cell cycle. The wound scratch assay was used to measure the migration ability of cells. **Results:** After 48h transfection, the circRNA007* expression increased more than thousand folds in circRNA007* overexpressed HCT116 and SW1463 cell lines as compared to the control cell. circRNA007* overexpression suppressed cell proliferation, migration as well as inducing apoptosis in both cell lines. In addition, the overexpression of circRNA significantly decreased the percentage of cells in S phase and increased the percentage of cells in G1 phase in HCT116 cell line. **Conclusion:** In our study, circRNA007* suppresses the progression of CRC cells, but the effects are more prominent in HCT116 cell line.

Keywords: Circular RNA, Colorectal Cancer, Biomarker

CRISPR Gene Editing: The Generation Of Genetically Engineered Breast Cancer Cells For Interrogating The L194F P53 Mutation

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ABSTRACT

Introduction: Breast cancer (BC) is the first leading cause of cancer-related deaths among women. Aggressive BC is commonly linked to the mutations in P53 tumour suppressor gene. The predominant P53 mutation is missense that can result in either loss of functions or acquisition of novel pro-oncogenic functions (gain-of-function mutation). Therefore, establishing robust in vitro models to interrogate mutated P53 molecular properties and functions is essential. In this study we embarked on generating three different in-vitro BC models by using CRISPR tool. **Methods:** For the first model, we performed pooled P53 targeting in T47D cells, which harbour L194F P53 gain-of-function mutation, by stably co-expressing the Cas9 and P53-targeting sgRNA using lentiviral transduction. The second model was the generation of complete P53 knockout T47D cells by transfecting the cells with a plasmid encoding for both Cas9 and P53 sgRNA, followed by single cell isolation, expansion, and screening for the isogenic knockout cells. Thirdly, we employed new CRISPR variant called Prime Editing (PE) to directly revert this T47D cells P53 mutation. **Results:** We have successfully established the first and second in-vitro BC models that can be subsequently utilized to study the L194F P53 molecular properties and roles in BC. However, upon screening the PE single cell-derived clones, we did not manage to obtain clones that harboured the desired reversion. **Conclusion:** The well-established, conventional CRISPR-based gene editing tool is highly efficient for both pooled gene targeting and generation of isogenic knockout cells. Yet, the PE efficiency was very low despite its promising mutation reversion application. Since we were editing a specific nucleotide, we did not have much flexibility to optimize our PE design because we had to meet the PE requirements. Nevertheless, this technology has recently been developed and there will soon be optimised protocols and updated knowledge available to improve the PE efficiency.

Keywords: Breast Cancer, Genetic Alterations, P53, CRISPR, Prime Editing

Preclinical Safety Study Of Allogeneic Wharton's Jelly-Derived Mesenchymal Stem Cells Delivered Via Systemic Route

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ABSTRACT

Introduction: Mesenchymal stem cells are unique adult cells with strong proliferative capacity, differentiation potential and more through the secretion of various functional metabolic. Current therapeutics that utilize chemical drugs for treatment of various chronic disease cannot efficiently recover functions and introduces side effects. Hence, MSC therapy becomes a high valuable novelty as it can successfully regulate inflammation and simultaneously facilitate regeneration. However, safety concerns for MSC remain due to conflicting reports for adverse and/or side effects. Hence, we conducted a safety study for the Wharton's Jelly derived mesenchymal stem cell (WJ-MSC) via intravenous route in an animal model. **Methods:** Male Sprague Dawley rats (300g) were randomized into treatment group that received 10x106 cells/kg of WJ-MSC or control group with equal volume of suspended saline. During the 12-week study period, the physical measurements and blood analysis (serum chemistry and whole blood profile) were performed during periods of Week 0, 2, 4, 8 and 12. The behaviour and mortality of animals were observed daily. Acute toxicity (n = 3) was performed during Week 2 and sub-chronic toxicity (n = 6 per group) during Week 12. The post-euthanasia assays included relative weight of organs, necropsy and histological staining of target metabolic organs. **Results:** The physical measurements, serum chemistry and whole blood profile was not statistically significantly ($p < 0.05$) from the IV infusion of WJ-MSC throughout the study. Similarly, relative organ weight and mortality did not report any significant outcomes from animals in the treatment group compared to control group. However, necropsy and histopathology revealed that the whole batch of rats, regardless of the group, reported minor inflammations in the lungs but only the treatment group appeared to have recovered with improvements over time. **Conclusion:** The intravenous administration of WJ-MSC was found to be safe with no adverse effects in the animal model.

Keywords: Mesenchymal Stromal Cell, Wharton's Jelly, Cell Transplantation, Toxicity, Rodent

Genome-edited Human Pluripotent Stem Cells (hPSCs) To Study The Functionality Of β_2-adrenergic Receptor Polymorphisms

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ABSTRACT

Introduction: Understanding molecular and functional mechanisms of disease is critical for developing new therapeutics. The beta-blockers (β-blockers) are the 4th most frequently prescribed medicine on the market. It widely used in treating cardiovascular and non-cardiovascular diseases but are influenced by genetic variation within the beta-adrenergic receptors (βARs). In ADRB2 locus, there are polymorphisms within the β_2AR, particularly at amino acid positions 16 and 27, that are potentially associated with cardiovascular disorders such as heart failure. The majority of the data available so far are based on the results of genetic association studies or models other than humans, which often lead to conflicting mechanistic explanations. **Methods:** To study the functionality of β_2AR polymorphism differences against a constant genetic background, CRISPR/Cas9 technology was used to genome-edit the HUES7 hESC line to produce 4 isogenic β_2AR variants (single amino acid codes: GE, GQ, RE, RQ). The isogenic variants were able to retain their pluripotency and differentiation capabilities into human Pluripotent Stem Cells-derived Cardiomyocytes (hPSC-CMs) whilst retaining responsiveness to β_2AR pharmacology. For the functional assessment, an initial phenotypic analysis was carried out by luciferase-based real-time cAMP (cyclic adenosine monophosphates) assay to estimate the differential response during acute and chronic agonist exposure to individual β_2AR variants. **Results:** It was found that RQ has the lower initial β_2AR density, GQ has the highest receptor desensitisation, Arg16 was resistant to desensitisation and GQ tended towards being the most downregulated. **Conclusion:** Since β_2AR is regulated by multiple complex processes and their regulation has essential effects on signal transduction, it is expected that these isogenic models will help to understand the mechanisms that underlie 'disease'. Moreover, the work shows how coupling hPSC-CMs with CRISPR/Cas9 technologies can be used to personalise diagnosis and identify new pathogenic variants in cardiovascular research.

Keywords: Genome-editing, CRISPR/Cas9, Human Pluripotent Stem Cells-derived Cardiomyocytes, Beta-adrenergic receptors, Polymorphisms

Reprogramming Of Mesenchymal Stem Cells Expanded Cord Blood-Derived CD34+ Cells To Human Induced Pluripotent Stem Cells

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ABSTRACT

Introduction: Cord blood (CB) is a valuable source of hematopoietic stem cells, but a low CD34+ cell count will be deemed unfit for cryo-preservation and will be discarded under the current practice. These rejected CB, especially those of unique properties, e.g., rare blood groups, can be recovered for therapeutic use by reprogramming them into human induced-pluripotent stem cells (hiPSCs). This project aimed to examine the effects of mesenchymal stem cells on CD34+ cell expansion and the outcome of reprogramming. **Methods:** CD34+ cells were isolated from cryo-preserved human CB and cocultured with MSCs for two days. The expanded CD34+ cells were reprogrammed using Gibco Episomal Reprogramming Kit. Pluripotency expression, karyotyping, and cell differentiation of the generated hiPSCs were assessed. **Results:** A lower doubling time of 25.1±2.3 h was observed in CD34+ cells in MSC coculture compared to 103.3±41.3 h in control ($p<0.0305$, n=6). The CD34 cell count was also increased from 1.5±0.2 x 105 to 9.7±0.1 x 105 cells in MSC coculture as compared to 2.7±0.3 x 105 cells in the control ($p<0.0001$, n=6). All isolated CD34+ cells were successfully reprogrammed to hiPSCs with an efficiency of 0.060±0.002% (n=21). Data showed no difference in reprogramming efficiency between CD34+ cells with or without MSC coculture (n=6). Reprogrammed hiPSCs also expressed TRA-1-60 (91.52±1.96%) and SSEA4 (85.3±6.14%) (n=2). Neither karyotypic abnormality nor differences in cardiomyocyte differentiation efficiency were found in both hiPSC- reprogrammed from freshly isolated CD34+ cells and expanded CD34+ cells in MSC coculture. **Conclusion:** The expanded CB-derived CD34+ cells in MSC coculture successfully increase the cell number sufficient for reprogramming. The generated hiPSCs showed no difference in reprogramming efficiency, expression of pluripotent markers, karyotype, and cardiomyocyte differentiation efficiency.

Keywords: Umbilical Cord Blood, Reprogramming, Induced Pluripotent Stem Cells

Neuroprotection Properties of *Hericium erinaceus* (Bull.:Fr.) Pers. Extract Against H_2O_2 assault - An *In-Vitro* Study

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ABSTRACT

Introduction: Current therapeutic approaches to neurodegenerative diseases are unsuccessful and often have undesirable side effects. As none can completely reverse nor cure neurodegenerative diseases, the focus has been on the screening of neuroactive compounds from natural sources. *Hericium erinaceus*, an edible medicinal mushroom known for health-promoting properties such as antimicrobial, anticancer, antioxidative effects, was used in this study to investigate the neuroprotective properties of its aqueous extract against oxidative stress caused by hydrogen peroxide (H_2O_2). **Methods:** *H. erinaceus* extract was prepared by macerating the freeze-dried mushroom in deionized water overnight at 15% w/v and boiled in a 90 C water bath for 30 min the following day. The mushroom precipitate was removed by filtration and the supernatant was freeze-dried for 48 hr to obtain the powdered extract. The half inhibitory concentration of H_2O_2 was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cell viability of *H. erinaceus* aqueous extracts on PC-12 cells after exposure to 150 µM H_2O_2 was determined using MTT assay under pre-, co-, and post-treatment models. The intracellular reactive oxygen species (ROS) percentage was evaluated using MUSE oxidative stress kit. **Results:** There was a significant increase in the percentage of viable cells in *H. erinaceus* extract pre-treated, co-treated and post-treated models as compared to 150 µM H_2O_2 treated cells by 28.5%, 21.4%, and 41.67% respectively. Preliminary results showed that intracellular ROS level was significantly reduced in *H. erinaceus* extract co-treated cells at 320 µg/ml by 52 % and in the post-treatment model. Extract at 160 µg/ml was able to bring down the ROS level by 34.5% compared to positive control. **Conclusion:** Reduction in ROS might be one of the mechanisms for H. erinacium extract to inhibit PC12 cell death that undergo H_2O_2-induced oxidative stress. These data indicate the neuroprotective activity of an aqueous extract of *H. erinaceus*.

Keywords: *Hericium erinaceus*, Hydrogen peroxide, Neuroprotection, Pheochromocytoma cells, Reactive oxygen species

Regeneration Of Dopaminergic Neurons In 6-OHDA-Lesioned Adult Zebrafish Involves Migration Of Dopaminergic Neurons From Olfactory Bulb And Telencephalon To Dienchephalon

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ABSTRACT

Introduction: Parkinson's disease (PD) is the fastest growing neurological disease worldwide. Unfortunately, there remains no cure for PD and current treatment modalities do not modify the disease's course. These limitations raise the need to focus on regeneration of dopaminergic neurons (DpN) as a potential approach of PD management. The process is, however, inefficient in mammals, making mammalian-based models unsuited for understanding of neuroregeneration. As such, adult zebrafish is greatly favoured for its close brain homology with human and neuronal self-renewal capability. Our previous findings suggested restoration of loss DpN by newly regenerated cells that may have potentially migrated from other brain regions of adult zebrafish. This study went on to investigate the proliferative phase of DpN regeneration through a time-based, double-pulse labelling method over 14 days post 6-OHDA lesion. **Methods:** Bromodeoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) were administered at early proliferative phase (day five, six, seven) and late proliferative phase (day 11, 12, 13) of neuroregeneration, respectively. The potential migration of proliferative neurons was tracked using immunostaining method whereby immunoreactive (ir) cells were identified through cell counting and brain mapping methods of both sagittal and coronal brain sections. **Results:** Whilst significantly higher ($p<0.05$) number of BrdU-ir cells were detected in both the olfactory bulb (OB) and pallium-subpallium border, significantly higher number of EdU-ir cells were found distributed in both the subpallium and preoptic area. Also, significant increase of BrdU-ir/ EdU-ir cells were detected in subpallium. Mapping of both sagittal and coronal brain sections indicated the presence of early proliferative cells (Brdu-ir) in the frontal area of the brain. Late proliferative (EdU-ir), however, occurred near the lesioned area. **Conclusion:** The present findings suggested the possible migration of proliferative DpN cells from OB and telencephalon to the lesioned site in diencephalon. This warrants further investigation of other distinctive phases of DpN regeneration.

Keywords: Neuroregeneration, Migration, Adult Zebrafish, 6-Hydroxydopamine, Double Pulse Chase

Protective Effect Of Mangostins Against Oxidative Stress-Induced Neurotoxicity

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ABSTRACT

Introduction: Oxidative stress has been observed in several neurodegenerative diseases such as Alzheimer's disease and brain injury. The excessive reactive oxygen species (ROS) in the neurons caused damage to the cellular components, leading to synaptic failure and neuronal death. *Garcinia mangostana* has been traditionally used in treating fever, inflammation and infections. Previous studies showed that the major secondary metabolite, the xanthones, possess neuroprotection, antioxidant and anti-inflammatory properties. In present study, the 3 major xanthones, α-, β-, and γ-mangostin were investigated for their protective effect against H_2O_2-induced neurotoxicity. **Methods:** SHSY-5Y neuroblastoma was pre- treated with different concentrations of mangostins for 24 hours and followed by 24 hours exposure to H_2O_2. The viability assay was assessed by MTT methods. Accumulation of ROS and mitochondrial membrane potential was determined using DCFDA and TMRE fluorescent dye, respectively. The apoptotic features of neurons were determined using Hoechst stain and PI. **Results:** Treatment with mangostins (0.05-1.5 μM) caused no cytotoxicity, in fact γ-mangostin showed significant proliferation of SHSY-5Y cells at all tested concentrations. Exposure of SHSY-5Y cells to H_2O_2 significantly reduced the cell viability and increased the accumulation of intracellular ROS. Pre-treatment with mangostins reduced the H_2O_2 induced cell death and accumulation of intracellular ROS, whereby β- and γ-mangostin showed higher protection compared to α-mangostin. On the other hand, cells pre-treated with α- and γ-mangostin had significantly lower apoptosis compared to cells treated with H_2O_2. In addition, only cells pre-treated with γ-mangostin showed significant reduction in the mitochondrial membrane potential. **Conclusion:** This study revealed the potential of mangostins from *G. mangostana* in prevention of neurotoxicity involving oxidative stress pathway whereby γ-mangostin showed higher protective effect compared to α- and β-mangostin.

Keywords: Neuroprotection, *Garcinia mangostana*, Mangostin, SHSY-5Y, Oxidative Stress

Preparation And Characterization Of Curcumin-Loaded Pluronic Nanoformulation (Nanocur) For Wound Healing Application And Its Toxicity Profile

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ABSTRACT

Introduction: Wound healing is a complex physiological response proceeding with three interrelated dynamics and overlapped phases. Different drugs and delivery systems have been extensively investigated, targeting the aforesaid phases of wound healing. However, current wound treatment requires frequent applications, making it less efficient with possible risk of infections. Curcumin (CUR), which can be found in turmeric, possesses the capability to boost wound healing processes, but the clinical development of CUR is often hampered by its rapid metabolism, poor absorption and low bioavailability. The purpose of the present study was to evaluate the effects of CUR loaded Pluronic F127 nanoformulation (NanoCUR) on wound healing applications in the aspect of wound healing and antioxidant properties, towards 3T3 murine fibroblast cells, as well as its toxicity profile in zebrafish embryos. **Methods:** NanoCUR was synthesized by thin film dehydration method, and characterized with DLS, FTIR, XRD, UV-Vis and FESEM analyses. The biological properties of NanoCUR were then evaluated through DPPH assay for its antioxidant property, its potential as wound healing treatment in 3T3 murine fibroblast cells, and toxicity in zebrafish embryos. **Results:** NanoCUR with the size of 25.35 ± 9 nm, and CUR loading of 18.19 ± 2.02 mg /g of NanoCUR was prepared, and DPPH assay revealed that NanoCUR has a significantly lower antioxidant activity compared to CUR. NanoCUR displayed delayed toxicity and exhibited concentration-dependent and time-dependent toxicity response. NanoCUR was also observed to generate a significantly low reactive oxygen species (ROS) compared to native CUR in ROS assay. Cells treated with NanoCUR at 10 μM showed improved wound healing activity in migration assay compared to CUR. **Conclusion:** The collective data in the present study provide a fundamental basis for the development of NanoCUR as a promising wound healing treatment with improved toxicity profile.

Keywords: Curcumin, Wound Healing, Nanocur, Pluronic F127, Toxicity

Factors Influencing The Skin Penetration Of Hydrogel Microneedles For Transdermal Drug Delivery

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ABSTRACT

Introduction: Hydrogel microneedles (MNs) are gaining increasing attention in the field of transdermal delivery of therapeutics. However, there is a discrepancy in the insertion methods of MN with different needle densities in previous studies. This study aims to determine the effect of MN insertion methods and needle density on the penetration of hydrogel MN into the skin for effective transdermal drug delivery. **Methods:** We have compared two of the most commonly adopted methods for MN application which are thumb pressure and impact-driven insertion in the penetration of hydrogel MNs. We have also investigated the effect of needle densities of hydrogel MN on the skin insertion efficiency and penetration depth. These optimised hydrogel MN application methods were tested in modified Franz diffusion cells for in-vitro transdermal delivery of caffeine, which served as a model drug. **Results:** A 100% penetration was recorded for both the applicator and combination of applicator and thumb pressure compared to only 11% for thumb pressure alone. The average diameter of micropores created by the applicator method was 62.94 µm, which was significantly lower than the combination of both applicator and thumb pressure MN application (100.53 µm). Based on the histological imaging, the penetration depth of hydrogel MN increased as the MN density per array decreased. With this approach, approximately 2.9 mg of caffeine was delivered in-vitro within 24 h by displaying a first-order release profile. **Conclusion:** The MN insertion methods and needle densities per array are important factors to enhance the penetration efficiency of hydrogel MN for transdermal drug delivery.

Keywords: Hydrogel Microneedle, Transdermal Drug Delivery, Microneedle Penetration, Caffeine

Size-Tunable *Sargassum polycystum* Mediated Synthesis Of Silver Nanoparticles And Its Production

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ABSTRACT

Introduction: A simple green synthesis method, involving the use of marine seaweed extract, was developed to synthesise silver nanoparticles (AgNPs). In this study, brown seaweed, *Sargassum polycystum* acted as the reducing and stabilising agent in the production of AgNPs. **Methods:** The effect of synthesis parameters on the size and production of AgNPs was assessed in this study by varying synthesis conditions such as concentration of seaweed extract, concentration of silver nitrate, temperature, and pH. The synthesised AgNPs were characterised by UV-Vis Spectroscopy, Dynamic Light Scattering (DLS), Fourier-transformed infrared spectroscopy (FT-IR), high resolution transmission electron microscope (HR-TEM), X-ray diffraction (XRD), and energy dispersive X-ray (EDX) spectroscopy. **Results:** The present findings indicate pH and temperature have the most impact on the synthesis of AgNPs, affecting the size of the synthesised AgNPs and the reaction completion time. Synthesised AgNPs at pH 11 at 25 C exhibited an intense narrow surface plasmon resonance (SPR) band at 414 nm and were 6.93 $\pm$ 0.49 nm in size as detected by DLS. **Conclusion:** The outcome of this study provided insights towards the importance of various synthesis conditions of AgNPs to produce the small sized NPs in a controllable manner.

Keywords: Silver Nanoparticles, Brown Seaweed, *Sargassum polycystum*

HPLC Method Development And Validation For The Determination Of Hydroxychloroquine In Human Whole Blood

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease in which the immune system attacks its own tissue. Prevalence of SLE in Malaysia is 43 per 100 000 individuals. Cutaneous manifestation occurs in about 75% of SLE patient while the prevalence of cutaneous lupus erythematosus (CLE) was 73.2 per 100 000 on 2006. Hydroxychloroquine (HCQ) has central role of treatment for SLE. HCQ prevents the flares occur and other manifestations related to antiphospholipid antibodies. The pharmacokinetics of HCQ cause great interindividual variability in blood concentration and makes its biological effect more complex. HCQ causes several side effects, major side effect is retinopathy. HCQ concentration in whole blood is important clinically. However, the current method of detection for HCQ in whole blood is inconvenient. **Methods:** In this study, a better and simpler high performance liquid chromatography (HPLC) method was developed. Reversed phase HPLC with fluorescence detector, C18 column used as stationary phase column and mobile phase consisted of 91.5% of phosphate buffer, 8.5% of acetonitrile, and 0.1% volume/volume triethylamine were used to analyse the HCQ in whole blood. Simple extraction method was carried out by adding in cold methanol and cupric sulphate into whole blood. **Results:** The method was validated with good precision, which the %RSD of intraday precision of retention time and peak area is < 2.7% and < 16.3% and interday precision is less than 14.1% except for concentration, satisfactory linearity, which the R^2 equal to 0.9996 and also good recovery which average %recovery is more than 87.15%. **Conclusion:** This method was successfully quantitated HCQ concentration in SLE patients. Preliminary samples collected found the HCQ concentration in patients to be 1469 ng/mL, 699 ng/mL, 2708 ng/mL, 1458 ng/mL, 630 ng/mL and 3247 ng/mL.

Keywords: Hydroxychloroquine, Reversed-Phase HPLC-FL, Whole Blood

Bespoke Music-Narration As Complementary Therapy: Reaching Disadvantaged Communities Through Translational Music-Medicine Research

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ABSTRACT

Introduction: WHO (2021) estimates that globally, 2.4 billion people are living with health conditions that would benefit from rehabilitation with 60%-70% of rehabilitation needs of people living in low and middle-income countries being unmet. There is a lack of innovative tools and personalised resources to empower disadvantaged communities in combating declining physical and mental health. The objective of this study is the creation and sharing of complementary therapies through interdisciplinary music-medicine collaboratives in humanising translational science-arts research using composed music and narration to promote health and wellness for communities affected by the COVID-19 pandemic. **Methods:** Multimethodologies comprising both qualitative and quantitative approaches were engaged. A methodological model incorporating a theoretical framework of translational research-in-practice was crafted to illustrate four dimensions of research planning and action that supported the needs of the end-user through the application of innovation from translational research. **Results:** A Bespoke Music-Narration web-based resource centre housing an armamentarium of uniquely-created audio-visual materials for complementary therapies resulted (www.bespokemusicnarration.com). It incorporates instructions for deep breathing exercises and progressive muscle relaxation techniques crafted in English and in Bahasa Malaysia enabling cultural inclusivity and on-demand access to professionally developed content for rural and disadvantaged communities in need. Initial evaluative outcomes of the resources included positive questionnaire feedback from twenty-nine healthcare workers who participated in a rehabilitation workshop using bespoke music and narration to promote relaxation. In another study, interviews conducted with participants of a clinical trial utilising further content from this website have resulted in complementary responses as to its rehabilitative effect. **Conclusion:** The construct of a web-based resource centre may provide an alternative approach to conventional means of rehabilitation. More studies in the efficacy of web-based resources using music in complementary therapy for clinical and home-based rehabilitation are needed.

Keywords: Bespoke Music-Narration, Complementary Therapy, Translational Research

Adolescents' Knowledge, Attitude And Risk Beliefs Towards E-Cigarette Use In Johor Bahru, Malaysia

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ABSTRACT

Introduction: To assess the knowledge, attitude, and risk beliefs towards e-cigarette use among adolescents and their association and difference across various socio- demographic characteristics. **Methods:** A cross-sectional study using validated questionnaires was conducted and enrolled 391 adolescents conveniently aged between 13 to 17 years who are studying in government secondary schools in Johor Bahru. **Results:** Most respondents were female (56.3%) Chinese (57.5%) with the age category of 16-17 years (53.7%). Currently, 11.5% of the respondents are a user of e- cigarette while 6.6% tobacco cigarettes. Surprisingly, 12.3% of adolescents answer correctly that e-cigarette usually contains nicotine, an addictive chemical. Overall, the respondents had a moderate knowledge (77.5%) about e-cigarette use with a mean score of 3.82 (+0.89). However significant association and the difference was found between e-cigarette use with supportive attitude (86.7%, $p<0.001$) and perceived less health risk of e-cigarette use (9.11 ±2.68, $p <0.001$). Susceptible adolescents towards e-cigarette use were more likely to support e-cigarette (63.1%, $p <0.001$) and perceived lesser health consequences (6.91 +3.12, $p=0.019$) of e-cigarette use as compared to non-susceptible. **Conclusion:** e-cigarette users possessed a moderate knowledge with a supportive attitude and perceived lesser risk as compared to non- e-cigarette users. Hence, collective efforts are required to raise awareness toward the e-cigarette through community awareness campaigns. Therefore, all authorities shall address promising avenues to cope with this issue.

Keywords: Electronic Cigarette, Adolescents, Knowledge, Attitude, Risk Beliefs

Comparative Retrospective Cohort Study On The Safety And Effectiveness Of Hydroxychloroquine In The Management Of COVID-19: A Malaysia Single Centre Experience

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ABSTRACT

Introduction: The outbreak of coronavirus disease (COVID-19) in December 2019 called for a rapid solution, leading to repurposing of existing drugs. Due to its immunomodulatory effect and antiviral properties, hydroxychloroquine (HCQ) has been used in early 2020 for treatment of COVID-19 patients. This study was conducted to evaluate the treatment outcome of HCQ monotherapy in Malaysia. **Methods:** A retrospective cohort study was conducted in COVID-19 ward in Hospital Kuala Lumpur (HKL), from March to April 2020. A total of 446 COVID-19 patients were recruited, only 325 patients were finally included for analysis. Statistical analysis was done using SPSS, with a significant value set at $p< 0.05$. **Results:** The median age of the patients were 36 (range 18-92). They were majority male, (n= 210, 64.6%), Malaysian (n=239, 73.5%) and Malay ethnicity (n=204, 62.8%). Ninety-one (28%) patients received HCQ monotherapy. HCQ monotherapy was associated with worse outcome (RR: 10.29, 95% CI 1.17 - 90.80). There was a significant difference in mean length of stay between those with and without HCQ treatment ($t323= 5.868$, $p< .001$, 95% CI 2.56 - 5.13). The average length of stay for HCQ treated group was 3.84 days longer than those without treatment. 6.6% of the patients receiving HCQ monotherapy encountered adverse drug effects. **Conclusion:** Similar to study reported worldwide, our study demonstrated that HCQ did not improve length of stay and the outcome of COVID-19 patients.

Keywords: Hydroxychloroquine, Treatment, Length of Stay, Outcome, COVID-19

Detection Of Circulating Neonatal Nav1.5 (Nnav1.5) Antigen In The Blood Of 4T1 Orthotopic Breast Cancer Mice Model And Breast Cancer Patients

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ABSTRACT

Introduction: Neonatal Nav1.5 (nNav1.5) is the alternative splice variant of Nav1.5 and its role in progression of metastasis is well established. Initially, the detection of nNav1.5 was performed on breast cancer tissues. However, this approach is highly invasive and expensive, therefore, we have attempted to detect the presence of circulating nNav1.5 in whole blood. **Methods:** A total of 16 female BALB/c mice was divided equally into two groups: control group (n=8) and 4T1 orthotopic mice models (n=8). 4T1 cells were injected at the 3rd mammary fat pad of the 4T1 mice group whereas PBS was introduced at the same site for the control group. After 42 days of tumour development, all mice were sacrificed. Blood samples and organs were collected. Organs were subjected to histopathological analysis. Simultaneously, 64 healthy participants and 64 breast cancer patients were recruited. Blood was withdrawn and collected. RNA extraction was performed on both animal and human blood using respective kits. RT-qPCR was performed using nNav1.5 and β-actin housekeeping genes on both mice and human samples. The relative mRNA expression of target genes was calculated using the $2^{-\Delta Ct}$ quantitative method for each individual data point. **Results:** Histopathological analysis revealed metastasis to the heart, lungs, liver, spleen, kidneys, and ribcages of 4T1 mice. nNav1.5 antigen was only detected in the whole blood of 4T1 mice and not in control mice. The $2^{-\Delta Ct}$ values ranged from 0.0001-0.0106. However, only five blood samples from the group of breast cancer patients exhibited the presence of circulating nNav1.5 antigen whereby the $2^{-\Delta Ct}$ values ranged from 0.0121-1.1728. Additionally, there was no detectable presence of nNav1.5 in the samples of healthy participants. **Conclusion:** The presence of circulating nNav1.5 could be detected in the whole blood of 4T1 orthotopic mice, but the expression could be too low in the blood of breast cancer patients.

Keywords: 4T1 Cell Line, Breast Cancer, Blood, Metastasis, Neonatal Nav1.5

Prognostic Analysis Of Inflammatory Markers In Peripheral Blood Of Oral Squamous Cell Carcinoma (OSCC) Patients

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ABSTRACT

Introduction: OSCC is the most common oral malignancy affecting the oral cavity. There is increasing evidence demonstrating the relationship between inflammation and carcinogenesis where haematological inflammatory markers have shown prognostic significance in the survival of OSCC patients. These inflammatory markers are being represented by neutrophils, lymphocytes, monocytes and platelets. Therefore, the aim of this study is to assess whether absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, absolute platelet count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte-ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) in peripheral blood have prognostic value in OSCC patients. **Methods:** Patients who were diagnosed with OSCC and underwent surgery at OMCS, Faculty of Dentistry, UM from December 1999 to December 2019 were included in this retrospective study. Information on patient's socio-demographic data, clinico-pathological data, peripheral blood tests parameters and survival status were collected from their records. ROC curves were generated and the haematological inflammatory markers with AUC value of >0.6 were selected for univariate analysis using the Kaplan-Meier method and were further analysed in a multivariate Cox regression analysis. **Results:** High pre-surgery NLR and post-surgery absolute monocyte count and PLR were associated with worse overall survival (OS). In addition, high pre-surgery NLR and post-surgery absolute monocyte count were also associated with worse disease-free survival (DFS) in this study. **Conclusion:** Pre-surgery NLR and post-surgery absolute monocyte count could potentially be the predictive factors for OS and DFS in OSCC patients.

Keywords: Oral Cancer, Prognostic Marker, Inflammation, Malaysia

Detection Of Novel Autoantibody Signatures As Potential Biomarkers In Prostate Cancer

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ABSTRACT

Introduction: Prostate cancer (PCa) is the third most prevalent malignancy in Malaysian men. Approximately half of these cases were detected late. While prostate-specific antigen (PSA) tests and digital rectal examinations are the gold standard for PCa screening, they cannot distinguish PCa from benign-prostatic hyperplasia (BPH). To this end, autoantibodies against tumour-associated antigens are emerging as promising biomarkers in the detection of cancers. This study aims to investigate serum autoantibody signatures as PCa specific biomarkers. **Methods:** A high-density protein microarray containing over 1600 immune-related human proteins to detect autoantibodies circulating in the sera of 30 patients with PCa and 30 controls with BPH was used. Putative markers were identified and ranked according to differential penetrance fold- change and high-frequency percentage between the two groups. Functional analysis for the top-ranked proteins was done using the feature selection method, unsupervised clustering approach, and functional annotation based on KEGG and gene ontology (GO). **Results:** A total of 27 positive interactions were detected in PCa patients' sera, but none in controls' sera. The protein-tyrosine kinase (PTK7), previously reported to be associated with increased PSA and Gleason scores, was expressed in most of the cases in this cohort. The protein function prediction analysis, which used machine learning algorithms, revealed a panel of 5 proteins, including PTK7, to be highly differentially expressed in PCa cases, with a ROC curve AUC of >.70. The current findings corroborate an earlier report on PTK7 overexpression as an independent predictor of PCa. Functional analysis of the top biomarkers revealed clusters related to RAS signalling, PI3k-Akt signalling pathway, and the MAPK signalling pathway. **Conclusion:** The preliminary findings of this study suggest that identifying autoantibodies could help distinguish PCa cases from BPH using a minimally invasive method, highlighting its potential role as a diagnostic and therapeutic target.

Keywords: Prostate Cancer, Biomarkers, Protein Microarray, Autoantibodies, Tumour-Associated Antigen

Andrographis paniculata Ethanolic Extract Prevents Airway Inflammation In House Dust Mite-Induced Asthma

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ABSTRACT

Introduction: Asthma is an intricate airway disorder characterized by allergic airway inflammation, obstruction and hyperresponsiveness. Considering the unmet medical need in asthma control, there is a need to develop new therapeutic agents. *Andrographis paniculata* (AP) has been used traditionally for treatment of various inflammatory diseases. However, the anti-asthmatic potential of *Andrographis paniculata* ethanolic extract ($APEE_{50}$) has not been investigated. The objective of this study was to explore the anti-asthmatic activity of $APEE_{50}$ in a house dust mite (HDM)-induced mouse asthma model. **Methods:** $APEE_{50}$ was prepared by decoction using 50% ethanol. Asthma was induced by sensitizing female balb/c mice with 100 µg i.n on day 0, then a daily challenge from day 7-11 with HDM. Mice were treated with 50, 100 or 200 mg/kg p.o of $APEE_{50}$, and 10 mg/kg of prednisolone (positive control) daily on day 7-11. On day 14, the animals were subjected to airway hyperresponsiveness test. BAL fluid, blood and lungs were collected for histology, cytokine analysis and gene expression studies. **Results:** $APEE_{50}$ significantly inhibited HDM-induced eosinophilia, neutrophil counts, IL-4, IL-5, IL-13 and eotaxin in BAL fluid. HDM specific IgE and IgG in serum and NF-kB p65 transcriptional activity in the asthmatic lungs were also assayed. Similarly, $APEE_{50}$ abrogated HDM-induced mucus production and airway hyperresponsiveness compared to the control group. Additionally, treatment with $APEE_{50}$ decreased Duox1 and increased Nfe212 and Hmox1 oxidative stress-related gene expressions and down-regulated all three Th2 related genes (IL-5, C-C motif chemokine 5, and 11). **Conclusion:** $APEE_{50}$ prevented HDM-induced asthmatic responses by down-regulating Th2 cytokines and reduced airway oxidative stress mediators, possibly via suppression of the NF-kB signalling pathway.

Keywords: *Andrographis paniculata*, Asthma, Cytokine, Inflammation

Evaluation Of Wound Healing Activity Of *Leucaena leucocephala* In Rats

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ABSTRACT

Introduction: *Leucaena leucocephala* is traditionally used to treat stomach pain and skin problems, including wounds. It is widely used as an effective and readily available treatment for various wounds. This study evaluated the wound healing properties of *Leucaena leucocephala* leaf extracts. **Methods:** Methanol, chloroform, diethyl ether, and ethyl acetate extracts of *Leucaena leucocephala* leaf was evaluated for its wound healing activity in albino rats using excision wound model. Animals were randomly divided into six groups of five each and group 1 served as untreated control, group 2 treated with standard (betadine ointment), group 3 treated with methanol, group 4 treated with chloroform, group 5 treated with diethyl ether and group 6 treated with ethyl acetate extracts. Rate of wound contraction were determined to assess the wound healing activity of the leaf extracts. **Results:** The group 3 methanolic extract showed a significant result and compare with control group and showed wound area closure of (100%) on day 14 when compare with control and other extracts groups. **Conclusion:** Our results highlight that *Leucaena leucocephala* methanolic extract could be the promising activity in treating wound healing.

Keywords: *Leucaena leucocephala*, Wound Healing, Wound Closure, Excision Model

Combining Pharmacophore-Based Screening And Molecular Dynamics Simulations In The Identification Of Novel Inhibitor With Dual Action On PDE1B And PDE10A

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ABSTRACT

Introduction: The inhibition of Phosphodiesterase 1B (PDE1B) has been proposed as a novel way to improve the cognitive function in many neurodegenerative and neuropsychiatric diseases, while; the inhibition of PDE10A has been proposed to alleviate the symptoms in Huntington's disease and Schizophrenia. Therefore, the present study aims to identify a dual PDE1B/PDE10A inhibitor as a potential drug candidate for the treatment of the cognitive symptoms of schizophrenia along with the positive and negative symptoms. **Methods:** A structure-based pharmacophore model for PDE1B and PDE10A was generated and validated using the test set and the decoy set method. The validated pharmacophore models were used as 3D queries in the virtual screening of Zinc database. The retrieved hits were filtered based on Lipinski's rule of 5, pharmacophore fit score, and the binding affinity for PDE1B and PDE10A. The stability of the ligands within the active site of the target proteins was studied using molecular dynamics simulations. **Results:** Zinc41306568 from Zinc database exhibited the highest affinity for PDE1B and PDE10A. The modelling studies demonstrated that Zinc41306568 interacted with the hydrophobic residues TYR74 and ILE223 and the P-clamp residues, LEU240, PHE244 and PHE276 in PDE1B. While in PDE10A, Zinc41306568 involved in hydrophobic interactions with LEU189, VAL232, ALA243, TYR247 and MET267, besides the common interactions with the P-clamp residues ILE246, PHE250 and PHE283. **Conclusion:** Dual inhibitors of PDE1B and PDE10A have not been reported in the literature; therefore, Zinc41306568 is considered the first dual inhibitor of PDE1B and PDE10A that has been identified by applying a computer-aided drug discovery approach. The newly identified inhibitor will be explored for further optimisation and evaluated in vivo for its antipsychotic-like effects.

Keywords: Molecular Dynamics Simulations, PDE1B and PDE10A, Schizophrenia, Structure-Based Pharmacophore, Virtual Screening

Effects Of Donepezil On The Structure Of Amyloid β-Peptide: A Molecular Dynamics Simulation Study

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ABSTRACT

Introduction: The accumulation of amyloid beta (Aβ) plays a crucial role in the onset and progression of Alzheimer's disease (AD), a chronic neurodegenerative disorder. In AD patients, abnormal interactions and misfolded $A\beta_{42}$ are being extensively explored as important pathogenic events. Donepezil, an acetylcholinesterase (AChE) inhibitor is FDA-approved drug for AD treatment. However, the binding mechanisms of donepezil with $A\beta_{42}$ monomer have not yet been clearly identified at the atomic level. We investigated the conformational dynamics and the binding interaction between donepezil with $A\beta_{42}$ by molecular dynamics (MD) simulation. This study provides significant insights in understanding detailed $A\beta_{42}$ peptide structural changes in the presence of the donepezil and their binding mechanisms. Methods: MD simulations for $A\beta_{42}$-APO (control) and $A\beta_{42}$-DPZ systems were performed using the GROMACS 5.1.4 package with the GROMOS96 54A7 force field for 100 ns. Both systems were solvated with a simple point charge (SPC) water model. The resulting trajectories were analyzed using the inbuilt GROMACS tools along with the visual molecular dynamics (VMD) and the dictionary of secondary structure of proteins (DSSP) program. Results: The $A\beta_{42}$-DPZ forms a stable complex with RMSD <2.0 E and showed less compact structure compared to $A\beta_{42}$-APO due to less folded shape. From DSSP analysis, donepezil significantly increased α-helix content while reduced the β-content and turn, suggesting that donepezil was successfully inhibiting the formation of β-sheet-rich structures and prevent the formation amyloid fibrils. Conclusion: This study showed that the donepezil binds to $A\beta_{42}$ monomer efficiently and forms a stable complex. Detailed Aβ structural changes upon loss of β-content in the presence of the donepezil are also revealed, which gives further insight at the atomistic level to understand the inhibitory functions of donepezil molecules used in AD treatment. These finding provide key insights into the inhibitory mechanism of donepezil against $A\beta_{42}$ aggregation in AD.

Keywords: $A\beta_{42}$ Peptide, Alzheimer's Disease, Donepezil, Acetylcholinesterase, Molecular Dynamics

Repeated Exposure To Mitragynine Induced Addiction And Alteration Of Cognitive Functions In Sprague Dawley Rats

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ABSTRACT

Introduction: Drug addiction changes the normal structure and functions of the brain, which may lead to memory deficit that requires urgent interventions. Mitragynine, the major indole alkaloid from *Mitragyna speciosa* has been used as a recreational drug and 'herbal high' preparation in Southeast Asia and Western countries due to its opium and coca-like effects. However, there are insufficient data regarding the effects of long-term exposure to mitragynine on addiction and cognitive behavior. Thus, in this study, effects of repeated exposure to mitragynine on withdrawal signs and cognitive functions were evaluated. **Methods:** Animals were treated (intraperitoneally) daily with mitragynine (1, 5, 10 and 30 mg/kg) for 14 days. Twenty-four (24) hours after the last dose, animals were examined for behavioral signs using global withdrawal scoring and the effect on cognitive behavior was determined using passive avoidance task. **Results:** One way ANOVA revealed that, mitragynine at the doses of 5, 10 and 30 mg/kg produced a significant ($P < 0.01$) and dose dependent increase in the behavioral "counted signs" and a non-dose dependent increase at all the doses tested (1, 5, 10 and 30 mg/kg) in "checked signs" (squeaking on touch and hostility on handling) as compared to control. A significant ($P < 0.01$) dose dependent increase in the mean aggregate withdrawal signs was found in all the doses tested when compared to vehicle. At the doses of 5, 10 and 30 mg/kg, mitragynine significantly ($P < 0.001$) reduced the step through latency as compared to control in passive avoidance task. **Conclusion:** Data from this study showed that mitragynine at high dose possessed abuse liability leading to cognitive decline. However, mitragynine at lower dose (1mg/kg) was found to be safe without cognitive decline. Hence, the use of mitragynine at high dose as a "herbal high" is justified.

Keywords: Mitragynine, Addiction, Cognitive Function, Withdrawal Scoring, Passive Avoidance Task

Bioenhanced Fraction Of *Clitoria ternatea* Ameliorates The Behavioral Performance And Hippocampal Neuroplasticity In Chronic Cerebral Hypoperfusion Rat Model

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ABSTRACT

Introduction: Vascular cognitive impairment is a broad concept that cover from mild cognitive impairment to vascular dementia. Global reduction of cerebral perfusion by carotid artery occlusion can result in transient or permanent ischemic which significantly caused cognitive decline. In the present study, effects of bioenhanced fraction from *Clitoria ternatea* root were investigated on behavioral performance and hippocampal neuroplasticity in rat model of chronic cerebral hypoperfusion (CCH). **Methods:** The *Clitoria ternatea* root fraction (CTRF) was prepared by flash column chromatography technique. The CCH rat model was established by permanently ligated two common carotid arteries (2VO) in rats. The rats were divided into five groups: Sham+veh.; 2VO+veh.; 2VO+CTRF (10, 20 and 40mg/kg). To study the effects of CTRF on behavioral performance, open field test (OFT), passive avoidance task (PAT) and Morris water maze (MWM) were conducted. An in vivo electrophysiological recording was used to study the effects of CTRF on hippocampal long-term potentiation (LTP). **Results:** The result of OFT shows insignificant effects following administration of CTRF on motor and exploratory behaviours. Interestingly, CTRF (40 mg/kg) exhibited the most significant increment in step-through latency in PAT and significant reduction in escape latency during five days training in MWM as compared to untreated rats (2VO+veh.). During probe trial session, CTRF (40 mg/kg) spent longer time in the target quadrant as compared to 2VO+veh. CTRF (40mg/kg) increased synaptic strength in hippocampal LTP. **Conclusion:** In conclusion, CTRF is able to alleviate the CCH- induced cognitive decline by improving the behavioral performance and hippocampal LTP. Hence, CTRF has a potential to be developed as a Smart Drug for the treatment of vascular dementia-related to cerebrovascular diseases.

Keywords: Vascular Dementia, Chronic Cerebral Hypoperfusion, *Clitoria ternatea*, Cognitive Functions, Long-Term Potentiation

Schisandra chinensis Extract Ameliorates Aβ-Induced Toxicity In Transgenic *Caenorhabditis elegans* And Chronic Cerebral Hypoperfusion-Induced Cognitive Impairment In Rats

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ABSTRACT

Introduction: Alzheimer's disease (AD), the leading cause of dementia in the elderly, is a neurodegenerative condition associated with accumulation of beta-amyloid (Aβ) in the brain. However, age-related vascular changes may accompany or even precede the development of Alzheimer's pathology thus may play a pathogenic role in the disease progression. In this study, two different model organisms were used to investigate the effect of *Schisandra chinensis* (SCH) extract in both amyloidogenic and non-amyloidogenic pathway. *S. chinensis*, a traditional Chinese medicine, has been indicated to have protective effect in neurological disease, the key neuroprotective mechanisms include antioxidant, suppression of apoptosis, anti-inflammation, and modulation of brain-derived neurotrophic factor (BDNF) related pathways. **Methods:** Transgenic *Caenorhabditis elegans* namely, GMC101 strains expressing human Aβ in muscle cell and CL2355 strains expressing human Aβ pan-neuronally, were used to study the protective effect of SCH (1, 2 and 4 mg/mL) against Aβ-induced pathologies, including paralysis and chemotaxis behaviours. In non-amyloidogenic pathway, chronic cerebral hypoperfusion was induced in male Sprague-Dawley by permanent occlusion of bilateral common carotid arteries (2VO). The rats were administered either a vehicle (sham group: water) or Schisandra-fortified milk formulation (100, 200 and 400 mg/kg BW/day, p.o.) for 28 days. Spatial learning and memory deficits were investigated using the Morris water maze (MWM) task. **Results:** The extract of SCH significantly delayed Aβ-induced paralysis in GMC101 strains. In addition, SCH significantly improved the Chemotaxis Index (CI) in CL2355 strains. The 2VO rats demonstrated significant learning and memory deficits as evidenced by increased latency time to reach the hidden platform and reduced target crossings in the probe trial in the MWM task. Treatment with SCH-fortified milk formulation significantly improved learning and memory impairments. **Conclusion:** Taken together, this study revealed that SCH supplementation has a beneficial role in amyloidogenic and non-amyloidogenic-targeted intervention for AD therapy.

Keywords: *Schisandra chinensis*, Beta-amyloid, *Caenorhabditis elegans*, Chronic Cerebral Hypoperfusion, Learning and Memory

Effects Of Dopamine Receptor Antagonist (SCH-23390) On The Acquisition And Expression Of Mitragynine-Induced Conditioned Place Preference In Rats

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ABSTRACT

Introduction: *Mitragyna speciosa* Korth or also known as kratom is a psychoactive plant indigenous to the Southeast Asia region which exhibits potential therapeutic values as an opioid substitute. Mitragynine (MG), the major alkaloid of *M. speciosa* Korth has been shown to contribute to the various opioid-like pharmacological effects. Previous studies lend support for the roles of the dopamine D1 receptor in the rewarding properties of various drugs of abuse, including opioid. However, the role of the dopamine D1 receptor in mediating MG reward has not been fully characterised. **Methods:** Using the conditioned place preference (CPP) model as a paradigm for measuring drug reward, the present study was conducted to evaluate the roles of the dopamine D1 receptor in the acquisition and expression of MG-induced CPP. Firstly, we examined the effects of the selective dopamine D1- type receptor antagonist SCH-23390 on the acquisition of MG-induced CPP. Rats were pre-treated systemically with a selective dopamine D1-type receptor antagonist (SCH-23390; 0, 0.1 and 0.3 mg/kg, i.p.) prior to MG (10 mg/kg, i.p.) conditioning sessions. Next, we tested the effects of the dopamine D1 receptor in the expression of MG-induced CPP when the rats were administered dopamine D1 receptor antagonist (SCH-23390; 0, 0.1 and 0.3 mg/kg, i.p.) prior to CPP test. **Results:** The results showed that SCH-23390 at 0.1 mg/kg dose was able to suppress the acquisition of MG-induced CPP. This effect was already evident for SCH-23390 at a dose of 0.1 mg/kg which, by itself, did not produce a conditioned place aversion (CPA) effect. In contrast, SCH-23390 at any doses had no effect on the expression of MG-induced CPP. **Conclusion:** The findings of the present study suggested the role of the dopamine D1-like receptor in mediating the acquisition of the rewarding effects of MG, but not the expression as indexed by CPP.

Keywords: Kratom, Mitragynine, SCH-23390, Conditioned Place Preference, Acquisition

Differential Gut Microbiota Composition Between T2DM And Healthy Controls

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM) is one of the most prevailing non-communicable diseases that causes high morbidity and mortality. Gut microbiota has recently been identified as one of the important contributing factors of this metabolic disease. In order to better understand the pathogenesis of T2DM in the context of gut microbiota, a case-control study was undertaken to assess the differential compositions of gut microbiota between T2DM patients and non-T2DM healthy controls. **Methods:** Ten participants (HbA_{1c}<5.6%) were recruited as control while another ten participants diagnosed with T2DM (HbA_{1c}>6.5%) as case. Both control and case were matched with similar age and BMI range. All participants lived in the same community and all were Malays. The blood samples collected were examined for glycemic parameters, inflammatory markers, antioxidants and oxidative stress markers. Faecal samples were collected and subjected to whole genome sequencing using the Illumina platform. **Results:** HbA_{1c} ($p<0.001$), fasting blood glucose (FBG) ($p<0.001$) and gut hormone glucose like peptide 1 (GLP-1) ($p<0.001$) were significantly higher in the T2DM group while insulin and IL-10 levels were significantly higher ($p<0.001$) in the control group. The sequencing results revealed that Actinobacteria phylum, specifically *Collinsella* spp. and its lineage as well as genus, Megasphaera, were significantly higher ($p<0.05$) in T2DM group while Proteobacteria phylum lineage showed otherwise. Also, increased Firmicutes and Actinobacteria together with their lineages were associated with increased HbA_{1c} and FBG but decreased insulin, IL-10 and superoxide dismutase (SOD) levels. On the other hand, increased Bacteroidetes and Proteobacteria lineages were associated with increased insulin, SOD and IL-10 levels but decreased malondialdehyde (MDA) and HbA_{1c} levels respectively **Conclusion:** The present findings indicated that increased Firmicutes and Actinobacteria together with their lineages could be a positive indicator towards T2DM condition whereas decreased Bacteroidetes and Proteobacteria lineages were linked to a better glycaemic response.

Keywords: Type 2 Diabetes Mellitus, Glycated Haemoglobin, Gut Microbiota, Metagenomics, Inflammation

The Diabetic Wound Healing Abilities of the Aqueous Ethanolic Stem Extract of *Tinospora cordifolia* in Streptozotocin-Nicotinamide Induced Diabetic Rats

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ABSTRACT

Introduction: Diabetic foot ulcer remains to be one of the main causes of non-traumatic amputation. However, there is still a lack of effective drugs in treating diabetic foot in clinical practice. Aqueous ethanolic stem extract of *Tinospora cordifolia* (AETC) is known to possess wound healing abilities, yet its effect on diabetic wound is unclear. This study aimed to investigate the diabetic wound healing potential of AETC. **Methods:** Wistar rats were induced diabetic using streptozotocin-nicotinamide diabetic model. Rats were induced with a single intravenous injection of 65 mg/kg streptozotocin after 15 min of 120 mg/kg intraperitoneal nicotinamide. Full thickness wound was then created using 8 mm biopsy punches on the dorsal of the rats. The rats were then divided into 5 groups based on the treatment provided. The 5 groups were normal control, diabetic control, positive control (600µg glibenclamide), AETC 250mg/kg and 500mg/kg. Treatment was provided for14 consecutive days. **Results:** Oral AETC possess pro-healing responses on diabetic wounds. The pro-healing responses of AETC could be credited to its ability to reduce inflammation, enhanced granulation tissue formation, increase angiogenesis, increase fibroblast proliferation, maturation and differentiation, increase rate of wound contraction and epithelization, and increased collagen deposition. **Conclusion:** Oral AETC exhibited significant pro-healing responses on diabetic wounds, which suggests its potential application in diabetic wound management.

Keywords: Diabetic Wound Healing, *Tinospora cordifolia*, Wound, Excision Wound, Diabetic Complication

Full PPAR-γ Agonist With Adiponectin Abrogates Oxidative Stress

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ABSTRACT

Introduction: Oxidative stress, associates with metabolic and anthropometric perturbations, leads to reactive oxygen species production and decrease in plasma adiponectin concentration. We investigated pharmacodynamically the pathophysiological role and potential implication of exogenously administered adiponectin with full and partial peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists on modulation of oxidative stress, metabolic dysregulation and antioxidant potential in streptozotocin induced Spontaneously hypertensive rats (SHR). **Methods:** Group I (n=6), (WKY) serve as normotensive control, whereas 42 male SHRs were randomized equally into 7 groups (n=6), group II: SHR control, groups III: SHR diabetic control, group IV, V and VI treated with irbesartan (30mg/kg), pioglitazone (10mg/kg) and adiponectin (2.5µg/kg), groups VII and VII received co-treatments as (irbesartan+adiponectin), (pioglitazone+adiponectin) respectively. Diabetes was induced using an intra-peritoneal injection of Streptozotocin (40 mg/kg). Plasma adiponectin, lipid contents, arterial stiffness with oxidative stress biomarkers were measured using an in-vitro and in-vivo analysis. **Results:** Diabetic SHRs exhibited hyperglycaemia, hypertriglyceridemia, hypercholesterolemia, increased arterial stiffness with reduced plasma adiponectin and antioxidant enzymatic levels including total superoxide dismutase (SOD), nitric oxide (NO), total anti-oxidative activity (TAC) and glutathione peroxidase (GSH), ($P<0.05$). Diabetic SHRs pre-treated with pioglitazone and adiponectin separately exerted improvements in oxidative stress biomarkers, i.e., malondialdehyde (MDA) with plasma antioxidant enzymatic activities, abrogated arterial stiffness, offset the increased production of reactive oxygen species and dyslipidaemic effects of STZ, whereas blood pressure values were significantly reduced in irbesartan treated groups only (all $P<0.05$). **Conclusion:** The combined treatment of exogenously administered adiponectin with full PPAR-γ agonist augmented the improvement in lipid contents and adiponectin concentration, restores arterial stiffness with antioxidant potential effects, indicating degree of synergism between adiponectin and full PPAR-γ agonists (pioglitazone).

Keywords: Streptozotocin, PPAR-γ Agonists, Adiponectin, Oxidative Stress, Arterial Stiffness

Glycosylated Sulfonylurea (2DGs) Activates AMPK/p38 MAPK/ GLUT 4 Pathway In L6 Skeletal Muscle Cell Line

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ABSTRACT

Introduction: Sulfonylureas have been used widely for close to 50 years in the battle against type 2 diabetes mellitus. Their ability to elevate insulin secretion by the pancreatic β cells reduces hyperglycemia and glycated haemoglobin (HbA1c) levels in these patients. However, side effects like hypoglycemia, cardiovascular mortality risks and weight gain have increased their caution for use, hence the need for continuous research to minimize or eradicate these side effects while maintaining or improving their activity and efficacy. Novel glycosylated sulfonylurea (2DGs) was developed by integrating an aryl sulfonamide with a glucosamine moiety. Previous research has shown that it has superior *in vitro* and *in vivo* antidiabetic activity to the current third-generation sulfonylurea, glimepiride. It also exhibits anti- AGE and antioxidant properties coupled with lipid metabolism regulation and reduction in cardiovascular and renal side effects. 2DGs activates insulin signalling via the insulin-dependent IRS/PI3K/PKC/AKT pathway. The current study seeks to evaluate glucose uptake via the insulin-independent pathway AMPK/p38Mapk/GLUT4 pathway. **Methods:** The rate of glucose uptake in normal differentiated ATCC grade L6 skeletal muscle cells were evaluated using the Promega 2NBDG Glucose Uptake Assay kit. Treatment was done with or without insulin, tolbutamide and 2DGS at different concentrations. The synergetic effect of insulin and 2DGs was also studied. Western blot and ELISA were done on the cell lysates to evaluate AMPK and p38Mapk phosphorylation. **Results:** A higher significant dose-dependent increase in glucose uptake and GLUT 4, AMPK and p38 Mapk expression and phosphorylation was observed in 2DGs compared to the tolbutamide treated and negative groups. A significantly higher synergetic effect in cells treated with insulin and 2DGs was also observed. **Conclusion:** 2DGs treatment increases glucose uptake via the activation of the AMPK/p38Mapk/GLUT 4 pathway in normal differentiated L6 Skeletal Muscle cells.

Keywords: Insulin Resistance, Glycosylated Sulfonylurea, AMPK, P38 Mapk, GLUT 4

Palmatine Regulate The Expression Of ER Stress-Associated Transducers In L6 Skeletal Muscle Cell Line

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ABSTRACT

Introduction: Endoplasmic Reticulum (ER) stress is a cellular condition induced by oxidative stress, which could lead to the accumulation of unfolded and misfolded proteins and subsequent activation of the unfolded protein response (UPR) signalling pathway. The UPR is activated by three major ER stress signalling transducers, PKR-like ER kinase (PERK, EIF2AK3), inositol requiring 1α (IRE1α), and activating transcription factor 6α (ATF6α), that are located on ER membrane. The UPR-induced apoptotic pathway reduces insulin translation, transcription and degradation of proinsulin mRNA, impaired insulin secretory pathway, β-cell apoptosis, and eventually lead to the development of Type 2 diabetes (T2DM). Plant alkaloid Palmatine has been previously reported to possess antidiabetic, antioxidant properties and reduce the up-regulation of chaperone proteins glucose regulatory protein 78 (GRP78), and calreticulin (CALR) protein in a streptozotocin (STZ)-induced diabetic rat model. This study aimed to evaluate inhibition of endoplasmic reticulum stress of Palmatine, through the modulation of PERK-EIF2α - IRE1α - ATF6α pathway in L6 Skeletal Muscle Cell Line. **Methods:** Cultured L6 rat skeletal muscle cells were treated with 5μg of Tunicamycin overnight to induce ER Stress. The cells were then treated with different concentrations of Palmatine for 24 hours. RNA and proteins from these cells were extracted to quantify the relative gene expression and determine the protein expression levels of PERK-EIF2α - IRE1α - ATF6α, respectively. The relative gene expression was quantified using quantitative reverse transcription polymerase chain technology (RT-qPCR) technique while ELISA was used to determine the concentration of these proteins. **Results:** The expression of these genes and proteins were downregulated in the Palmatine-treated cells as compared to the vehicle control. **Conclusion:** Palmatine showed potential in ameliorating ER stress through the inhibition of expression of PERK, EIF2α, IRE1α and ATF6α proteins.

Keywords: ER-stress, PERK, IRE1α, Palmatine, T2DM

ABPA Potentially Alleviates Insulin Resistance Via The Upregulation Of The Insulin-Dependent (IRS1-PI3K-GLUT4) Signaling Pathway In L6 Skeletal Muscle Cells

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ABSTRACT

Introduction: Insulin resistance (IR) that causes type II diabetes (T2D) impairs the insulin-dependent pathway that majorly involves the insulin receptor substrate 1 (IRS1) phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), and glucose transporter type 4 (GLUT4), which affects the metabolism of glucose. Existing antidiabetic agents have been associated with multiple adverse side effects and not completely efficient in the management of T2D. Hence, natural products such as herbal medications serve as an adjunct and complementary therapy in the management of T2D. ABPA is a combination of several herbal plants including *Gymnema sylvestre, Pterocarpus marsupium*, and *Terminalia chebula* that have been reported to be powerful antidiabetic agents individually. **Methods:** L6 skeletal muscle cells were cultured and differentiated into L6 myotubes under standard conditions. These cells were induced to be insulin-resistant prior to treating the cells with metformin (positive control) and ABPA at concentrations of 50 – 1000 µg/ml. The RNA and proteins from these cells were then extracted to quantify the relative gene expression and determine the protein expression levels of IRS1, PI3K, AKT and GLUT4, respectively. The relative gene expression was quantified using quantitative reverse transcription polymerase chain technology (RT-qPCR) technique while ELISA was used to determine the concentration of these proteins. **Results:** The ABPA- treated cells showed significantly higher relative gene expression levels of IRS1, PI3K, AKT and GLUT4 than the vehicle control. The ELISA analysis further confirmed the upregulation of these proteins in ABPA-treated cells with increased concentrations of these proteins observed. **Conclusion:** ABPA showed strong potential in ameliorating insulin resistance through the upregulation of IRS1, PI3K, AKT and GLUT4 proteins of the insulin-dependent pathway.

Keywords: ABPA, Insulin Resistance, IRS1-PI3K-GLUT4 Pathway, Type II Diabetes, RT-qPCR

Asymmetrical Curcumin Derivatives As Potential Basal-Like TNBC Anti-Cancer Agent: Synthesis, Biological Evaluation And Docking Analysis

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ABSTRACT

Introduction: Basal-like phenotype triple-negative breast cancer (B-TNBC) accounting for 50 -75% of all TNBC subtypes was associated with aggressive behaviour and poor prognosis. Previous studies showed that the symmetrical curcumin derivatives were able to suppress non-basal-like TNBC cells growth *in vitro*, but the effects of asymmetrical derivatives on B-TNBC are still unclear. **Methods:** Five asymmetrical compounds (a1-5) bearing different heterocyclic linkers were synthesised using the ultrasonic-aldol condensation method. Compounds were tested on B-TNBC derived HCC-1806 cells, and their selectivity was compared with non-B-TNBC derived MDA-MB-231 and non-cancerous cells (Beas-2B, BV-2, HEK-293 and pHME). The lead compound was further tested on cell cycle arrest, apoptosis and proteasome inhibition effect *in vitro*. Docking simulation was applied to explain the binding interactions of the lead compound with 20S proteasome β5 subunit active binding site. **Results:** Compound a5 [3-(methylbenzylidene)-5(4-hydroxy-3-methoxybenzylidene) dihydro-2H-pyran-4(3H)-one] exerted excellent growth inhibition on basal-like HCC-1806 (IC50 = 0.59±0.16 μM). When compared to non-basal-like MDA-MB-231 cells, a5 exhibited 7.34-fold more selective to HCC-1806. Furthermore, it displayed 1.29-, 1.69-, 6.49- and 6.61-fold selective to HCC-1806 cells over non-cancerous HEK-293, pHMEC, BEAS-2B and BV-2 cells, respectively. It arrested the G2/M phase cell-cycle progression, promoted apoptosis and inhibited the proteasome activity dose-dependently *in vitro*. Docking analysis revealed that compound a5 could form hydrophobic interactions with the hydrophobic residues in the binding pocket. It could also form hydrogen bonds via hydroxyl group with the Thr1 residue in the β5 subunit binding pocket. **Conclusion:** The present study suggests that asymmetrical curcumin derivative embedded with a heterocyclic linker could serve as a potential anti-cancer agent targeting B-TNBC.

Keywords: Asymmetrical, Curcumin, TNBC, Anti-Cancer

Gene Expression, Biochemical And Functional Analysis Of Stromal Interaction Molecule 1 Silencing In Acute Myeloid Leukaemic Cell Lines

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ABSTRACT

Introduction: Stromal interaction molecule (*STIM1*) was recently discovered to be a critical modulator of cell growth and survival in a variety of cancers. However, the role of *STIM1* in Acute Myeloid Leukaemia (AML) is still not fully understood. As such, the present study was undertaken to uncover the roles of *STIM1* in proliferation and survival of AML cells. Methods: *STIM1* role in proliferation and survival of AML cells was studied by using a dicer-substrate siRNA (dsiRNA)-mediated silencing approach in AML cell lines models, namely THP-1 and Kasumi-1 cells. The expression profile of targeted genes was assessed through RT-qPCR. Further assays on intracellular calcium, reactive oxygen species (ROS), cell proliferation and colony formation ability profiles were also studied. Results: In general, *STIM1* mRNA expression was found higher in THP-1 cells when compared to Kasumi-1 cells. The optimum *STIM1* silencing was achieved at 10 nM dsiSTIM1 for 24 hours in THP-1 cells and 20 nM dsiSTIM1 for 48 hours in Kasumi-1 cells. *STIM1* silencing yielded significant regulation of genes involved in proliferation, survival and apoptosis pathways. Furthermore, *STIM1* silencing also resulted in inhibition of critical AML cell lines functions such as intracellular calcium level, ROS level, proliferation, and colony formation abilities. Conclusion: The present findings suggest that the interplay between *STIM1*, calcium and ROS activities may be critical in AML functional pathways (proliferation, survival, and apoptosis). To support the present work, further comprehensive work on *STIM1* regularity role in AML is needed.

Keywords: *STIM1*, Acute Myeloid Leukaemia, Reactive Oxygen Species, Calcium Mediated Pathways, DsiRNA

In Vitro Anticancer Activity Of Andrographolide And Its Derivative And Tyrosine Kinase Inhibitors In Non-Small Cell Lung Cancer Cell Lines With EGFR And KRAS Mutations

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ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) has high prevalence of epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma (KRAS) mutation. Afatinib and osimertinib are second and third generation EGFR tyrosine kinase inhibitors (TKIs). Both agents target mutant EGFR and mutational resistance Thr790Met (T790M) in EGFR domain against first generation EGFR-TKI. Andrographolide and its derivative (SRJ09) are known to target KRAS mutation, however, it is unknown whether andrographolide and its derivative (SRJ09) have better activity in comparison to EGFR-TKIs (afatinib and osimertinib) in a panel of NSCLC cell line with various EGFR and KRAS mutational status. **Methods:** The *in vitro* growth inhibitory compounds were assessed in three NSCLC cell line: NCI-H1975 harbours the EGFR T790M resistance mutation and sensitizing KRAS mutation. A549 harbours KRAS G12S and H460 harbours Q61H mutational substitution which both cell lines have a wild-type EGFR. The compounds were tested against the cell lines at a concentration range of 0.01 – 100 μM for 72 hours followed by colorimetric assay by MTT assay. The absorbance was measured at 570 nm with spectrophotometer and results were expressed as the mean of triplicates as a percentage of control. **Results:** The average IC_{50} values of compounds against NCI-H1975: afatinib (10.5 μM), osimertinib (0.4 μM), andrographolide (3.5 μM) and SRJ09 (2.5 μM). The IC_{50} values of compounds against A549: afatinib (4.5 μM), osimertinib (3.0 μM), andrographolide (4.5 μM), SRJ09 (3.0 μM). The IC_{50} values of compounds against H460: afatinib (1.0 μM), osimertinib (0.6 μM), andrographolide (10.5 μM), SRJ09 (3.0 μM). **Conclusion:** The results showed that osimertinib displayed the highest selectivity against H460 and H1975. Afatinib was selective towards H460. Andrographolide was selective towards NCI-H1975 and A549. SRJ09 did not exhibit a pronounced selectivity. Overall, osimertinib had the highest potency and most pronounced selectivity.

Keywords: Non-Small Cell Lung Cancer, Tyrosine Kinase Inhibitor, Resistance Mutation, Andrographolide Derivatives

Dual Roles Of RUNX1 In Breast Cancer

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ABSTRACT

Introduction: The translocation with the ETO gene has highlighted RUNX1 as a vital gene for human development. The formation of RUNX1-ETO fusion protein represses the RUNX1 transcriptional activity, resulting in immature blood cells and development of acute myeloid leukaemia. RUNX1 has also drawn attention when it is implicated in human breast cancer pathogenesis. Mutations of RUNX1 have been identified in luminal breast cancer patients, implicating it as a tumour suppressor in ER-positive breast cancer. RUNX1 is also associated with breast cancer metastasis however there is no clear evidence emphasizes the subtypes of breast cancer in which this role is taking place. This warrants further analysis considering the deregulation of RUNX1 is mainly reported in ER-positive breast cancer. **Methods:** The effect of RUNX1 expression level on the survival of breast cancer patients was analysed by using Kaplan Meier plotter. This was followed by morphological analysis on MDA-MB-231 cells upon the knock-down of RUNX1 by shRNA. The phenotypic changes were observed in 3D culture and the expression of RUNX1 was assessed by Western blotting. **Results:** RUNX1 was associated with poor survival in ER-negative breast cancer patients however it prolonged the survival of ER-positive breast cancer patients. The metastatic MDA-MB-231 cells changed from stellate to round shape in the absence of RUNX1 and could not penetrate the extracellular matrix compared to control. This phenotype was rescued when RUNX1 was reintroduced into the cells. **Conclusion:** This study demonstrates that RUNX1 has pro-metastatic activity in triple-negative breast cancer. Our data also suggests that its role in breast cancer is cell-type dependent whereby it is an oncogene in ER-negative breast cancer and acts as a tumour suppressor in ER-positive breast cancer.

Keywords: RUNX1, Breast Cancer, Oncogene, Tumour Suppressor

Characterisation of Zerumbone-Superparamagnetic Iron Oxide Nanoparticle Co-Loaded Nanostructured Lipid Carriers

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ABSTRACT

Introduction: Zerumbone (ZER) is naturally occurring sesquiterpene, a phytochemical that was first isolated from rhizomes of Zingiber zerumbet (L.) Smith in 1960, showing significant medicinal values such as immunomodulatory, anti-inflammatory, antioxidant, antimicrobial, anti-proliferative, and anticancer activities. Poor solubility and bio-availability of ZER has been overcome by loading it into nanostructured lipid carriers (NLC) but clinical trials with this compound have been rarely reported due to lack of selectivity. Hence, loading of superparamagnetic iron oxide nanoparticle (SPION) in ZER-NLC could be a potential approach to achieve specific delivery in various treatments. Objective of this study is to characterise improved drug delivery system for ZER. **Methods:** ZER and SPION were loaded into NLC (ZER-SPION-NLC) using hot ultrasonication method. This formulation was characterised on particle diameter, polydispersity index (PDI), zeta potential, encapsulation efficiency, and loading capacity. DSC was performed to characterise state of ZER and lipid modification. FTIR was conducted to evaluate interactions between ZER, SPION and lipids used in NLC. **Results:** ZER-SPION-NLC has an average diameter of 140.32 ± 1.144 nm, PDI of 0.176 ± 0.015 and zeta-potential of -13.367 ± 0.608 mV. Loading capacity of ZER was found to be 20 ± 0.0002% while encapsulation efficiency of ZER was found to be 100 ± 0.0003%. DSC study revealed that ZER was dispersed in molecular form in NLC with crystallinity index below 20%. FTIR spectra revealed the absence of incompatibility between lipids and excipients used in preparation of ZER-SPION-NLC. **Conclusion:** ZER and SPION can be co-loaded into NLC. ZER-SPION-NLC could serve as a potential strategy to achieve specific delivery in various treatments via co-magnetic targeting.

Keywords: Zerumbone, Superparamagnetic Iron Oxide Nanoparticle, Nanostructured Lipid Carrier, Nanoparticle, Specific Targeting

A Systematic Review On Plant-Derived Exosome-Like Nanoparticles: Therapeutic Potential For Cancer Management

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ABSTRACT

Introduction: Cancer is a major cause of death. High global cancer incidence and death are caused by the adverse effects of chemotherapy and radiotherapy, cancer drug resistance, and lack of tailored drug delivery vehicles. Mammalian exosomes (EVs) can deliver diverse molecules to target cells; however, getting large amounts of EVs is challenging and may trigger host immunological reactions. Plant-derived exosome-like nanoparticles (PDENs) can address this difficulty because they contain biomolecules (lipids, proteins, and miRNAs) and are biocompatible, biodegradable, and highly abundant in nature. This systematic review was conducted to elucidate the potential role of PDENs in cancer management with their specific mode of action. **Methods:** A search was conducted through PubMed, Scopus, Web of Science, and the Cochrane Library (Wiley) databases, in addition to clinical trial registries. The search was not limited to time frame but English and Chinese language only. **Results:** After screening 426 search results, 14 articles published between 2015 and 2021 were chosen. PDENs were isolated from different plants, for example, grape, apple, ginger, garlic, lemon. Their structures and mode of cell-cell communication were similar to the EVs. *In vitro* evaluation of PDENs against multiple cancer cell lines (i.e., CAco-2, HeLa, B16F10 melanoma, breast, skin, gastric, colon, kidney, liver, and lung carcinoma cells) revealed inhibition of cancer cell proliferation and suppression of tumour growth through inhibition of TNF-α, activation of TRAIL-mediated apoptotic cell death, cell cycle arrest, or delivery of biomolecules to the target cells. PDENs were minimally cytotoxic to healthy cells. PDENs also reduced oral mucositis following radiation and chemotherapy for head and neck tumours. A few *in vivo* (rodents) and clinical findings were in line with *in vitro* results. **Conclusion:** This comprehensive review suggests that PDENs might be safe and effective anticancer therapeutic agents as well as carriers of exogenous biomolecules into human cells.

Keywords: Cancer, Extracellular Vesicles, Molecular Mechanism, Nanovesicles, PDEN

A Multimodal Nanoparticle-Based Drug Delivery System For Breast Cancer Treatment: The Thermo-Chemotherapeutic Approach

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ABSTRACT

Introduction: Methotrexate (MTX), a chemotherapeutic agent used in breast cancer treatment, has limited clinical application due to the poor water solubility, non-specific targeting and side effects. Recent years, magnetic hyperthermia becomes one of the most promising approaches in cancer treatment to kill cancerous cells as these cells are more susceptible to high temperature than normal cells. To further enhance the therapeutic outcome of MTX, a lipid-based drug delivery system was formulated to encapsulate both MTX and a hyperthermic-inducing agent, superparamagnetic iron oxide nanoparticles (SPIONs). **Methods:** This lipid-based homing system was formulated from nanostructure lipid carrier (NLC) by hot ultra-sonication method. The physicochemical characteristics such as particle size, polydispersity index, zeta potential, encapsulation efficiency of MTX, storage stability and hemo-compatibility of the nano-formulation were determined. The efficacy of this formulation was determined in MDA-MB-231 breast cancer cells via cell viability, cellular uptake and in vitro magnetic hyperthermic assessments. **Results:** This multi-modal therapeutic formulation was successfully formulated with ideal physicochemical characteristics such as size diameter around 210 nm, polydispersity index less than 0.1, negatively-charged surface, good encapsulation efficiency of MTX (73.1%), stability up to 3 months and hemo-compatible. The nano-formulation was cytotoxic towards MDA-MB-231 breast cancer cell line in a time-dependent manner with IC50 values of 137 µg/mL and 12 µg/mL at 48 and 72 hours, respectively. The formulation was internalized in the MDA-MB-231 cells via caveolae-mediated endocytosis in a time-dependent manner. Even though the encapsulation in NLC has impeded the hyperthermic behaviour of SPIONs, its weak superparamagnetism was sufficient to cause apoptotic cell death. **Conclusion:** MTX co-loaded with SPION in the lipid nanoparticle is a potential multi-modal therapeutic regimen for the treatment of breast cancer with enhanced efficacy of MTX. Nevertheless, further in vivo study is needed to understand the pharmacokinetics and magnetic hyperthermic effect of the formulation for clinical translation.

Keywords: Methotrexate, Superparamagnetic Iron Oxide Nanoparticle, Hyperthermia, Breast Cancer, Nanostructured Lipid Carrier

Evaluation Of SRJ23 And Hydroxyurea In Reducing The Viability Of Cell Line Isolated From Histiocytic Lymphoma

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ABSTRACT

Introduction: Histiocytic lymphoma is an aggressive non-Hodgkin lymphoma marked by the presence of histiocyte resembling cells but are typically of B or T lymphocyte in origin. Although rare, <0.5% of all lymphoid neoplasms, the treatment is usually toxic. Hydroxyurea (HU) is a cheap anticancer drug that suppresses DNA synthesis by inhibiting ribonucleoside diphosphate reductase and prevents cells from exiting the G1/S phase. SRJ23, a bicyclic lactone, is a novel semi-synthetic derivative of andrographolide (AGP). Its benzidine derivatives bind to transient pockets on Kirsten-Ras (K-Ras) and inhibit GDP-GTP exchange to induce apoptosis. We aim to evaluate the inhibitory effect of HU and SRJ23 separately on U937 cell line (isolated histiocytic lymphoma cells) and determine the efficacy of each treatment. **Methods:** MTT assay was used to assess the in vitro growth inhibition of HU and SRJ23 on U937 cell line. U937 cells were seeded and treated with four different concentrations (0.1, 1, 10, 100 μM) of HU and SRJ23. After 96 hours incubation, the MTT readings were determined using microplate reader at wavelength 590nm. Cell viability-inhibition values were plotted for the dose-response curves. **Results:** The dose-response curves showed the reduction of cell viability by HU and SRJ23 with IC50 >100 μM and 3.21 μM (p-value <0.0001), respectively. Both experiments were compared to the control (DMSO). **Conclusion:** From this study, IC50 of HU was very high and statistically not significant. SRJ23 on the other hand, have a significant inhibitory effect to histiocytic lymphoma in vitro and therefore, a potential novel therapy. We strongly recommend for future studies on SRJ23 in animal models, either alone or in combination with other chemotherapy.

Keywords: Histiocytic Lymphoma, SRJ3, Hydroxyurea, Cell Viability-Inhibitory

Synthesis And Anticancer Evaluation Of New Chalcone Derivative HQ-5BSA Towards MDA-MB-231 Breast Cancer Cells And HT-29 Colon Cancer Cell Lines

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ABSTRACT

Introduction: Chalcones are open-chain flavonoids widely present in edible plants that possess potential anti-cancer properties that kill cancerous cells by targeting multiple pathways. **Methods:** New chalcone derivative (E)-3-(5-bromo-2-hydroxyphenyl)-1-(2`-hydroxy-5`-methoxyphenyl)prop-2-en-1-one (HQ-5BSA) was synthesized through modification of rings A and B in flavokawain B while maintaining hydroxyl position at 2'. The compound was characterized using NMR, UV and IR. Cell viability assay was performed to investigate the anti-proliferative activity of the HQ-5BSA against triple-negative MDA-MB-231 breast cancer cells, HT-29 colorectal cells and non-cancerous human dermal fibroblast (HDF) cells. Morphological study and Annexin V/FTIC flow cytometry assay was performed by observing the nuclear and cytoplasmic morphological changes as well as the mode of cell death in HQ-5BSA-treated cells. Western blot analysis was conducted to determine the p53, caspase 3 and caspase 9 protein expression in both MDA-MB-231 and HT-29 cells. Finally, molecular docking was also performed to simulate the binding of HQ-5BSA towards Janus Kinase 2. **Results:** The study had synthesised a new chalcone derivative (HQ-5BSA) which exhibited potent activity towards MDA-MB-231 and HT-29 cells with IC50 value of 11.0 ± 0.9 and 8.8 ± 0.6 μM, respectively. Interestingly, this compound has low toxicity towards HDF cells (IC50 value 34.60 ± 6.06 μM) with selective index of more than 3. A reduction in cell population was observed when these two cell lines were treated with HQ-5BSA and the mode of cell death was confirmed as apoptosis by Annexin V/FTIC flow cytometry assay. HQ-5BSA-induced apoptosis may be associated with downregulation of mutant p53, activation of caspase-9 and caspase-3. It was found that the compound exhibited the stable interactions within the active site of Janus Kinase 2. **Conclusion:** The present study suggests that HQ-5BSA has the potential to be developed as anticancer agent against triple negative breast cancer and human colorectal cancers.

Keywords: Chalcone Derivative, Cancers, Apoptosis, p53, JAK2

The Anti-Angiogenic Effect Of F8268-A3, A Novel Endophytic Peptide: *In Vitro* Assessment and *In Silico* Target Prediction

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ABSTRACT

Introduction: Antiangiogenic therapy has emerged as a promising clinical anti-cancer strategy. The clinically approved anti-angiogenic agents, however, are only effective in a subset of the patients, and many who initially respond develop resistance over time. As such, continuing efforts are required for discovery of new angiogenesis inhibitors. F8268-A3 (patent number = US2011/0201642A1) is a novel endophytic peptide isolated and purified from a Malaysian endophytic fungus, Aspergillus sclerotiorum strain HAB10R12. Our in vitro study found F8268-A3 to inhibit tube formation by endothelial cells (HUVEC). Immunocytostaining indicated that F8268-A3 down-regulated VEGF and up-regulated TSP-1 in breast cancer cells (MDA468 and MCF7). The present study aimed to develop a machine learning algorithm to unveil the molecular events underlying the potential anti-angiogenicity of F8268-A3. **Methods:** A prediction model was built from bioactivity data of 23 angiogenic proteins and 7,293 active compounds (IC50/ EC50/ Ki/ Kd < 10 nM), using Random Forest as the classification algorithm and ECFP_4 as the chemical descriptor. The model was internally validated using 5-fold cross validation before being tested on F8268-A3. **Results:** The predictive model showed good performance with sensitivity and specificity values of 0.98 and 0.91 when rank 3 was used as a cut off. When F8268-A3 was subjected to the predictive model, the top 3 protein targets predicted were protein kinase C (PKC), VEGF2 and aminopeptidase N (ANPEP). The result of the predictive model showed that it is in line with the in vitro results whereby F8268-A3 was found to downregulate VEGF expression. **Conclusion:** The present findings showed that cheminformatics approach could aid in elucidating the molecular mechanism of novel compounds. Future work would include further validation through molecular docking to establish the specific binding interactions between F8268-A3 and VEGF2.

Keywords: Endophytic Fungus, Anti-angiogenicity, VEGF, TSP-1, *In Silico* Target Prediction

Two-Pore Channel 2 In MDA-MB-231 Breast Cancer Cells: An Aspect On The Proliferation And Chemosensitivity/ Chemoresistance

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ABSTRACT

Introduction: Two-pore channel 2 (TPC2) is a calcium channel that governs physiological functions in the endolysosomal system. Recent findings have demonstrated the implication of TPC2 signaling in cancer hallmarks and chemoresistance. However, the role of TPC2 in breast cancer and, its ability to modulate the sensitivity of breast cancer cells to chemotherapy remains to be elucidated. Here, we studied the role of TPC2 on the proliferation of MDA-MB-231 cells, an in vitro model of triple-negative breast cancer (TNBC), and its effect on doxorubicin-induced cytotoxicity in MDA-MB-231 cells. **Methods:** TPC2 expression in MDA-MB-231 cells was silenced using siRNA-mediated gene silencing. Successful knockdown (KD) of more than 80% was confirmed using real-time polymerase chain reaction (RT-PCR). We also look at the effect of TPC2 silencing on the mRNA expression of other calcium transporters at 96h post-transfection. The MTS assay was performed to assess the effect of TPC2 silencing on the proliferation of MDA-MB-231 cells. To study the effect of TPC2 silencing on doxorubicin-induced cytotoxicity, MTS assay was performed after the cells underwent subsequent TPC2 silencing and doxorubicin treatment. **Results:** According to our results, TPC2 siRNA efficiently KD TPC2 isoform and has no effect on TPC1 isoform. Further, the results indicated that TPC2 silencing doesn't have any effect on the expression of other key calcium transporters including TRPML1, PMCA1, and SPCA1. The results showed that TPC2 suppression does not influence the proliferation of MDA-MB-231 cells ($p>0.05$). However, its inhibition improved the susceptibility of MDA-MB-231 cells to doxorubicin and led to an increase in the cytotoxic activity of doxorubicin in comparison to the control ($p<0.05$). **Conclusion:** These findings suggest a possible role of TPC2 in TNBC chemosensitivity and chemoresistance which offers novel strategies to overcome therapeutic challenges in metastatic breast cancer. Besides, it suggests that TPC2 silencing has no effect on global Ca^{2+} signaling.

Keywords: TPC2, Breast Cancer, Calcium Signaling, Doxorubicin-Induced Cytotoxicity, Calcium Transporters

Spectroscopic Studies Of 10-Pyrimidyl-3,6-Diazaphenothiazine With Anticancer Activity

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ABSTRACT

Introduction: Phenothiazines exhibited promising anticancer activity against several cancer cell line (breast, ovarian, lung, colorectal, prostate, leukemia, melanoma, and renal). Among the diazaphenothiazines, 10-pyrimidyl-3,6-diazaphenothiazine had been reported to be effective in killing breast cancer cells, glioblastoma, melanoma and ovarian cancer cell line but less toxic against normal human fibroblast cells. This compound induces apoptosis through upregulation of pro-apoptotic genes such as BAX, p53 and CDKN1A and downregulation of anti-apoptotic genes such as Bcl-2 and H3. The aim of this project was to perform advanced spectroscopic studies of the antitumour active diazaphenothiazine for a better understanding of the pharmacokinetic properties that determine the achievement of the molecular target. **Methods**: In order to evaluate the spectroscopic properties of 10-pyrimidyl-3,6-diazaphenothiazine, UV-Vis, spectrofluorescence and circular dichroism techniques had been used. **Results:** An absorption spectrum of this phenothiazine derivative in the wavelength range between 240-500 nm was characterised by three distinct peaks (A_{285nm} = 0.3152, A_{337nm} = 0.0647 and A_{449nm} = 0.0734) and time-dependent studies proved that this compound is stable. Spectrofluorescence studies showed that 10-pyrimidyl-3,6-diazaphenothiazine did not fluoresce at the excitation wavelength of carrier proteins fluorophores (λ_{ex} 275 nm and 295 nm). Owing to the changes in the protein circular dichroism spectra, the influence of 10-pyrimidyl-3,6-diazaphenothiazine on secondary protein structure has to be taken into account. **Conclusion:** The promising results of both anticancer and spectroscopic studies prompted the undertaking of further pharmacokinetic studies in order to understand the possibility of binding with blood transporting proteins such as human serum albumin (HSA), a-1 acid glycoprotein (AGP) and human gamma globulin (HGG).

Keywords: Diazaphenothiazines, Anticancer Action, Pharmacokinetic Study

Analysis Of Antioxidative and Cytotoxic Effects of Crude Methanolic, Ethanolic and Acetone *Moringa oleifera* Leaf Extracts *In Vitro*

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ABSTRACT

Introduction: The burden of cancer disease is a global challenge. Although boundless improvements have been made in the cancer therapy to reduce the cancer prognosis, the non-selectivity and the resistance of drugs remain unsolved. This limitation exposes the patients to a long-term side effect and the mortality rate is distressing. However, it was reported that one third of common cancers are preventable. Hence, there is a constant demand for newer methods to prevent this disease. Following, medicinal plants are making a comeback due to their more tolerable and safer than the synthetic ones. Among thousands of plant species, *Moringa oleifera* were found to have medicinal value, with nearly all parts being reportedly effective against cancer. These intriguing properties encouraged us to perform a comparative analysis on antioxidant and anticancer activity of *Moringa oleifera* leaf using methanolic, ethanolic and acetone. **Methods:** The antioxidant capacity was measured by using DPPH radical scavenging assay and ABTS radical cation decolourization assay. The cytotoxic effect was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay against human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa) and human hepatoblastoma cancer cell line (HepG2). **Results:** The highest antioxidant activity (ABTS and DPPH) of M. oleifera leaf was found in methanolic (0.97 µg/ml±0.01 and 1.74 µg/ml±0.02) than ethanolic (0.54 µg/ml±0.02 and 1.18 µg/ml±0.03) when compared to acetone (0.33 µg/ml±0.02 and 0.85 µg/ml±0.03) extract. Varying cytotoxicity patterns were found whereby acetone extract (IC50:207.49 µg/ml±0.85, 193.73 µg/ml±1.83 and 233.46 µg/ml±2.89) exhibited relatively less potent cytotoxicity when compared to methanolic extract (177.95 µg/ml ±4.08, 178.78 µg/ml±1.2 and 195.42 µg/ml ±1.86), and ethanolic extract (198.13 µg/ml ±2.70, 175.21 µg/ml±1.61, and 207.41µg/ml±3.05) against Hela, MCF-7 and HepG2, respectively. **Conclusion:** These results point that the cytotoxicity of *M. oleifera* leaf could be associated with the antioxidant content, and that solvent selection plays an important role in both factors.

Keywords: Cancer, Medicinal Plant, *Moringa oleifera*, Antioxidant, Anti-Cancer

Ethanolic Extract Of *Moringa oleifera* (Lam.) Leaves Promote Proliferation Of Human Mesenchymal Stem Cells By Inhibiting Expansion-Mediated Apoptosis

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ABSTRACT

Introduction: The laboratory-based scaling up of mesenchymal stem cells (MSCs) is often hampered by many factors that affect proliferation, multilineage differentiation potentials, and abrogation of soluble factors secretion that deems necessary for their biological activities. These significantly impair their research and therapeutic potentials in regenerative medicine and drug discovery. In this study, we investigated the effect of 70% ethanolic extract of *Moringa oleifera* (MOETE) on the viability, proliferation, and apoptosis of MSCs. **Methods:** *Moringa oleifera* leaves were freshly harvested, extracted with 70% ethanol, lyophilised, and standardised for Kaempferol-3-O-glucoside and Quercetin. Human MSCs, generated from the umbilical cord, were characterised fully, and treated with different doses of MOETE (0.1, 1.0, 10.0 and 100.0 mg/ml) for 48 hrs in vitro. A cytotoxicity assay was conducted to determine the IC50 and safety doses of MOETE. The proliferation and apoptosis of MSCs were measured using fluorimetric assay and flow cytometer, respectively. **Results:** MOETE did not affect the viability of MSCs; only a slight decrease of viability was observed at the highest dose (100 mg/ml); Thus, IC50 is greater than 100 mg/ml. This finding was further supported by annexin-V/PI apoptosis staining, whereby MOETE (1 and 10 mg/ml) slightly decrease apoptosis induced due to culture expansion when compared with vehicle control. However, in corroborating with result of cell viability a slight increase in apoptosis was observed at MOETE 100 mg/ml. Interestingly, DNA staining revealed an increase in proliferation of MSCs, particularly at lower (1 and 10 mg/ml) doses. **Conclusion:** We conclude that MOETE improves the viability of MSCs and does not impair their viability at lower concentrations. These findings imply that MOETE enhance the in vitro proliferation of UC-MSCs by inhibiting expansion-mediated apoptosis at lower concentrations.

Keywords: *Moringa oleifera*, Mesenchymal Stem Cells, Cell Proliferation, Apoptosis, PB2

Derivation Of Human Midbrain Dopaminergic Neurons From Cord Blood-Derived Human Induced-Pluripotent Stem Cells *In Vitro*

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ABSTRACT

Introduction: Cell-replacement therapies may offer promising prospect for treating Parkinson's disease (PD). Human induced pluripotent stem cells (hiPSCs) offer possibility to generate functional midbrain dopaminergic (mDA) neurons. However, differentiation efficiency and expansion remain the challenges for subsequent scale-up and clinical applications. **Methods:** The episomal hiPSCs lines generated from cord blood CD34+ cells were obtained from Gibco. hiPSCs were cultured and maintained in mTesR medium. hiPSCs were seeded at 10,000/cm2 prior to differentiaton. Cell identity was assessed using immunocytochemistry and flow cytometric analysis. **Results:** Early neural induction was achieved via dual SMAD inhibition using SB431542 and Noggin. Co-stimulation of Wnt and sonic hedgehog signaling pathways, using glycogen synthase kinase-3β inhibitor (CHIR99021) at 0.75 μM and Shh-C24II at 200 ng/mL for day 9 showed greater ventral-midbrain patterning efficiency. FGF8b (100 ng/mL) stimulation between day 9 and day 16 was found to promote caudalization of ventral-midbrain progenitors, evident by the formation of FOXA2+/LMX1A+/OTX2+ cells by immunostaining at day 16 (n=7). The differentiation efficiency of FOXA2+/LMX1A+ was 93.3% and LMX1A+/OTX2+ was 94.7% (n=1). Further maintenance of the ventral-midbrain progenitors in the defined maturation medium induced the formation of early neuronal phenotype with distinct projection was observed at day 24. At day 37, the cells further matured into βIII-tubulin+ neurons which also expressed tyrosine hydrolase, the rate limiting enzyme involved in dopamine biosynthesis. **Conclusion:** mDA neurons can be efficiently and effectively derived from hiPSCs.

Keywords: Parkinson's Disease, Human Induced Pluripotent Stem Cells, Dopaminergic Neuron

Optimization Of Ventricular Cardiomyocyte Differentiation From Cord Blood-Derived Human Induced-Pluripotent Stem Cells

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ABSTRACT

Introduction: Chronic heart failure (CHF) remains the leading cause of morbidity and mortality worldwide. Significant reduction in viable cardiomyocytes in the failing heart could potentially be treated by cardiomyocytes replacement therapy. Human-induced pluripotent stem cells (hiPSCs) are the promising cardiomyocyte source, but the current differentiation method has yielded a heterogeneous population, which could lead to reduced therapeutic efficacy or increased risk of arrhythmias if transplanted into the heart. Hence, this study aimed to establish and optimize the differentiation of hiPSCs to derive ventricular cardiomyocytes (vCM). **Methods:** The cord blood CD34-reprogrammed hiPSC lines were obtained from Gibco. hiPSC were grown on Matrigel-coated plate in mTeSR medium for 3-4 days prior to induction of differentiation. Cardiomyocyte differentiation was stimulated through temporal modulation of Wnt signaling pathway. **Results:** Early cardiac mesodermal cell formation were induced through inhibiting Wnt signaling pathway using glycogen synthase kinase inhibition (CHIR99021) treatment. The optimal cell seeding density was increased with decreasing growth area in different plate format. Cell confluence which was ideal for initiation of differentiation was 70%-75%. Prolonged Wnt activation for 48 h of CHIR99021 stimulation reduces CM yield at day 7, as compared to 24h. High CHIR99021concentration (10μM) increased the yield of $Nkx2.5^{+}$/cTnTHuman Induced-Pluripotent Stem Cells, Ventricular Cardiomyocytes, Differentiation expressing cells at day 7 as compared to 5μM CHIR99021. Inhibition of Wnt signaling was comparatively more effectively using Wnt inhibitor IWP4 (5μM) than Dickoff-1 (DKK1) at 300 ng/ml. Dual-inhibition of Wnt and retinoic acid signaling at day 3 for 48 h, increased the $cTnT^{+}$ $MLC2v^{+}$ cells to 93.4%±0.029 at day 14, as confirmed by immunostaining and flow cytometric analysis. **Conclusion:** vCM can be efficiently and effectively generated from cord blood CD34 cells-derived human-induced pluripotent stem cells.

Keywords: Human Induced-Pluripotent Stem Cells, Ventricular Cardiomyocytes, Differentiation

Derivation Of Corneal Epithelial Like Cell From Cord Blood-derived Induced Pluripotent Stem Cells

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ABSTRACT

Introduction: Corneal epithelial cells serve as a potential source for transplantation. However, it has been challenging to use adult stem cell source for the clinical application. Human induced pluripotent stem cells (hiPSC) represent an infinite cell source for generating sufficient number of corneal epithelial cells through manipulating developmentally relevant signaling pathways. However, protocols that can reproducibly differentiate mature corneal epithelial cells from hiPSC of cord blood origin is still lacking. **Methods:** The hiPSC was bought from Gibco and differentiation methodologies from three published works were adopted with slight modifications. Early formation and differentiation efficiency of corneal epithelium was determined by using Immunocytochemistry and fluorescence microscopy. **Results:** Early ocular surface ectodermal patterning of hiPSCs was initiated primarily by modulating bone morphogenetic factor 4 (BMP4,) and retinoic acid (RA) signalling. Stepwise, temporal controlled stimulation using 25 ng/ml BMP4 (day 0), 1 μM RA (day 1) and 10ng/ml epidermal growth factor (day 2) showed more efficient derivation of ocular surface ectodermal fate than BMP4/RA co-stimulations method and the dual inhibitions of TGFβ and Wnt signalling method. The optimized protocol successfully derived differentiated corneal epithelial cells expressing the progenitor marker p63+ and the limbal epithelial stem cells markers ATP binding cassette transporters B5 (ABCB5+) and G2 (ABCG2+) cells at day 10. **Conclusion:** Cord blood-derived hiPSCs can be directed to differentiate into corneal epithelial like cells.

Keywords: Cord Blood-Induced Pluripotent Stem Cells, Corneal Epithelial Cells, Signaling Pathway, Small Molecules

Potential Wound Healing Properties Of Sabah Wild Plant, *Melastoma malabathricum* Leaf Extracts *In Vivo*

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ABSTRACT

Introduction: Sabah is known for its diverse biodiversity as well as its diverse cultures and customs. The natives claimed that *M. malabatricum* to be able to cure wound healing which we believe to be potentially attributed to its unique phytochemical constituents. The purpose of this study was to assess how different solvents (i.e., ethyl acetate, methanol, and hexane) would affect the extracted products. Additionally, the present study also aimed to identify the presence of phytochemical constituents in the plant through preliminary phytochemical screening. The current study went on to examine the wound healing properties of this plant in balb/c mice. **Methods:** The plant was extracted using maceration technique. Standard methods were used for the identification of flavonoid, tannins, phenol, terpenoid and saponin. A total of 12 mice were subjected to a 4 mm excision wound using a biopsy punch after proper sterilization and sedation. Topical application of the extracts was performed on each mouse for a total of 15 days. **Results:** Phytochemical screening revealed the presence of flavonoid, tannin, phenol, saponin and terpenoid in the extracts. The quickest recovery rate was seen in mice with topical application of hexane extracts, followed by ethyl acetate and finally methanolic extracts. Complete wound contraction was seen as early as 10 days on treated mice. The varying effects between the extracts might be related to the polarity value and strength differences of extract solvent. Furthermore, the concentration of phytochemical constituents may influence the wound healing speed. **Conclusion:** The extracts obtained from *M. malabathricum* may have the potential to increase the rate of wound healing in animals.

Keywords: Wound Healing, Melastoma, *In Vivo*

Immunomodulatory Effects Of The Aqueous Extract From *Trigonella feonum-graecum* L Seed On RAW 267.4 Murine Macrophage

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ABSTRACT

Introduction: *Trigonella feonum-graecum* L commonly known as fenugreek is known to be one of the oldest medicinal plants in which the seeds and leaves are used as a treatment in various ailments. There are various studies in which the leaves and seeds of fenugreek are extensively used to prepare extracts and powder for medicinal purposes. Macrophage plays an essential role in early innate immune response as phagocytic cells are the first line of defence against pathogens. The present study aims to examine the immunomodulation effect of different fenugreek extract preparation on RAW 267.4 murine macrophage. **Methods:** Three preparation of aqueous extraction was done using the maceration method on fenugreek crushed seeds (FCS), whole seeds (FWS) and germinated seeds (FGS). Cell viability of the macrophage cells after exposure to extracts was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The proliferative effect was also assessed through the same method at different time points. The phagocytic activity of the macrophage cells was evaluated using the fluorescein-labeled *Escherichia coli* and a trypan blue solution to quantify the effect of phagocytosis. **Results:** Fenugreek aqueous extract showed no toxicity to the macrophage cells at a concentration range of 0.1-10 mg/ml for all three extract preparations but is toxic in high concentration of 100 mg/ml. Results indicate an increase in proliferative activity of macrophages in a dose-dependent manner was observed for the FCS and FGS extracts only. All three extracts showed significant increase in phagocytic activity. **Conclusion:** Taken together this evaluation of the immunomodulatory properties of fenugreek aqueous extract demonstrate its immunostimulatory effect on murine macrophages.

Keywords: *Trigonella feonum-graecum* L, Macrophage, Immunomodulatory, Proliferation, Phagocytosis

Preclinical Development Of Treatment For Psoriasis

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ABSTRACT

Introduction: Psoriasis is a chronic inflammatory systemic condition, typically characterised by erythematic, skin lesions, or skin plaques that arise from epidermal keratinocytes hyperproliferation. Psoriasis affects approximately 1% to 3% of the global population. Existing treatments to manage psoriasis are associated with various adverse effects that may affect patient's quality of life. **Methods:** This review attempts to provide an overview of the compounds and extracts with anti-psoriatic activity conducted at the preclinical stage, in the last decade. This narrative review is based on literature search performed using Google Scholar, PubMed, ResearchGate, Science Direct, Wiley Online Library, Frontiers and Taylor and Francis between 2011 up to 2021. **Results:** A total of 66 agents with anti-psoriatic properties at the preclinical stage were reviewed and compared for their safety and efficacy. Among 66 compounds, 62 of them were pure compounds while the remaining 4 were herbal extracts. The anti-psoriatic effects of Isagarcinol (YDIS) and Quercitrin (YDHH) were comparable with the positive control (Cyclosporin) *in vitro* and *in vivo*. Besides, YDIS and YDHH, seem to possess better safety profile than Cyclosporin as both these compounds did not increase the total bilirubin, blood urea nitrogen (BUN) and serum creatinine level in animals. **Conclusion:** Preliminary antipsoriatic activity were conducted on various chemical compounds and herbal extracts in the last decade. In depth studies on YDIS and YDHH need to be further explored for their potential to be developed as a potential antipsoriatic drugs.

Keywords: Psoriasis, Anti-Psoriasis, Preclinical Studies, I*n Vitro, In Vivo*

Tocotrienol-Rich Fraction Induced The Expression Of Glutathione Peroxidase 1 In Mice Liver

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ABSTRACT

Introduction: Glutathione peroxidases are major antioxidant enzymes that scavenge hydrogen peroxide/organic hydroperoxides and protect cells from oxidative stress and electrophiles. Glutathione peroxidase 1 (GPx1) is the most naturally abundant and ubiquitous intracellular isoform. Tocotrienols are part of the vitamin E family and are believed to possess potent antioxidant activity. The objective of this study was to determine the effect of increasing doses of tocotrienol-rich fraction (TRF) supplementation on liver GPx1 gene expression. **Methods:** A total of 30 male ICR white mice were divided into five groups (n=6 for each group) and given treatment for 14 days through oral supplementation; three groups were administered TRF at doses of 200, 500 and 1000 mg/kg respectively, a positive control group administered 100 mg/kg butylated hydroxyanisole (BHA), and a control group where mice were only administered the vehicle (corn oil). On day 15, the mice were sacrificed and their livers isolated. Total RNA was extracted from the liver and quantitative real-time polymerase chain reaction (qPCR) assay was performed to analyze GPx1 gene expression. **Results:** TRF oral supplementation caused a significant dose-dependent increase in liver GPx1 gene expression, compared to controls. **Conclusion:** TRF oral supplementation for 14 days resulted in increased GPx1 gene expression in mice liver in a dose-dependent manner, with the highest expression seen in mice treated with 1000 mg/kg TRF.

Keywords: Vitamin E, Tocotrienol-Rich Fraction, Glutathione Peroxidase 1, Mice, Liver

Anti-Inflammatory And Anti-Hyperlipidaemic Activities Of *Myrmecodia platytyrea* Tuber: The *In Vitro* Studies

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ABSTRACT

Introduction: Inflammation plays a crucial role in the formation and progression of atherosclerosis and consequently on cardiovascular diseases (CVD). Hence, the need to find new therapy by regulating the inflammatory signalling pathways to prevent the progression of CVD. *Myrmecodia platytyrea* tuber (MP) has been traditionally used as a remedy in many diseases especially in inflammation-related diseases such as cancer and rheumatoid arthritis. Thus, this study was done to investigate the potential of this plant to inhibit inflammation that can improve the lipid profile. **Methods:** MP aqueous extract (MPAE) was produced by boiling ground fine powder of MP for 15 min. The filtrate was concentrated using a rotary evaporator and lyophilized. The phytochemical properties and antioxidant capacities of MPAE were determined via several assays. LPS-induced RAW246.7 cells were treated with MPAE and the cytokine levels were measured. In the lipotoxicity-induced WRL68 hepatocytes, after 24-h of incubation with MPAE, the lipid profile, malondialdehyde (MDA) level and the antioxidant enzymes activities were measured. **Results:** MPAE had a high flavonoid and phenolic content with significant free-radical scavenging and iron-chelating properties. The anti-inflammatory effect was observed in MP treated LPS-induced RAW246.7 cells via inhibition of TNF-α and IL-1β. The lipid profile of lipotoxicity-induced WRL68 hepatocytes was improved after MPAE treatment with reduction of MDA level and elevation of the antioxidant enzymes in the cells. **Conclusion:** MPAE was able to inhibit inflammation subsequently reduced LDL and triglyceride levels due to the antioxidants present in MPAE and can be developed further for the curative approach of many inflammation-related diseases.

Keywords: *Myrmecodia platytyrea*, Anti-Hyperlipidaemic, Anti-Inflammatory, Antioxidant

Spirulina Supplementation Upregulates Alkaline Phosphatase And Osteocalcin Genes In High-Fat Diet Induced Obese Rats

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ABSTRACT

Introduction: Obesity is generally caused by eating too much and moving too little. Consuming high amounts of energy, particularly fat and sugars, but do not burn off the energy through exercise and physical activity, much of the surplus energy will be stored by the body as fat. Obesity could lead to other non-communicable diseases such as bone disease, diabetes mellitus, cardiovascular, among others. Spirulina has been referred to as a super food and been reported to be rich in functional nutrient. This research aims to evaluate the protective effects of spirulina on the bone in high-fat diet induced obese rats. **Methods:** High-fat diet and high-fat emulsion (HFD+HFE) were administered via oral gavage to 18 six-week-old female Sprague Dawley rats (n=6) for 6 weeks to induce obesity, except for a normal group. Following four weeks of treatment, the diet-induced obese groups were administered orally daily with spirulina (S) at 300mg/kg while the normal and obese control groups were treated with equal volumes of 0.9% saline water. **Results:** It was found that spirulina significantly reduced body mass index (BMI) below the obese range (0.68g/cm2). The results show that spirulina modified biochemical bone marker; phosphate, calcium, and 25-OH vitamin D in the plasma, and maximum force and young's modulus, when compared to the obese control group ($p < 0.05$). It also showed upregulation of bone formation marker genes, Alkaline Phosphatase (ALP) and Osteocalcin (OCN), indicating bone protecting effect. **Conclusion:** Overall, spirulina shows effective weight reduction while augmenting bone formation and protecting the bone against bone fragility by HFD+HFE-induced obesity.

Keywords: Bone Fragility, Spirulina, Obesity, Alkaline Phosphatase, Osteocalcin

Probiotics And Antioxidant Activity Of *Lactobacillus* spp. Isolated From Kefir Samples In Malaysia

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ABSTRACT

Introduction: The gut is the key target for probiotics foods as it helps to balance the diet and metabolic pathways in human health, as well as modulation of the intestinal microflora. Kefir is a natural fermented drink comprising of probiotic microorganisms predominantly *Lactobacillus* spp. which makes up the major microbial population found in all kefir grains. Kefir consumption has been associated with many benefits to the general health, including as an anti-oxidative, anti-obesity, anti-inflammatory, anti-microbial and anti-tumour moiety. However, there are limited scientific data on the isolated *Lactobacillus* spp from Malaysian kefir on its probiotic potential and antioxidant activity. **Methods:** Thus, this study aims to evaluate the probiotic potential of isolated *Lactobacillus* spp. from Malaysian kefir such as acid and bile salt tolerances, adherence ability to the intestinal mucosa as well as the antioxidant capabilities. **Results:** Results indicated that the isolated *Lactobacillus* from kefir G maintained its survival rate within 3 hours of incubation at pH 3 and pH 4 at 98.0 ± 3.3% and 96.1 ± 1.7% of bacteria growth and exhibited the highest survival at bile salt condition of 0.3% and 0.5%. The same isolate also showed high adherence ability to intestinal cells about 96.3 ± 0.01%, Furthermore, the antioxidant analyses using TPC, TFC, FRAP and DPPH assay showed that the isolate from kefir G possessed high antioxidant activities. **Conclusion:** From these data, all *Lactobacillus* spp. isolated from Malaysian kefir may serve as a promising candidate for exploitation as probiotics starter culture since it exhibits potential probiotic properties and antioxidant activities.

Keywords: Foods, Probiotics, Antioxidant, *Lactobacillus*, Kefir

In Vitro Antioxidant And Antiglycation Effects Of *Centella asiatica* Leaves In Water Extract

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ABSTRACT

Introduction: Accumulation of advanced glycation end products (AGEs) is engaged in the development of high oxidative stress and evolution of diabetic complications. AGE is an irreversible compound that formed when non-enzymatic glycation occurs between reducing sugars and free amino groups of protein. *Centella asiatica* (pegaga) is a medicinal plant from family Apiaceae and has been used as a traditional treatment in wound healing, revitalizing nerves, gastrointestinal diseases, eczema, and skin treatment. Hence, this research was to investigate the antioxidant, antiglycation, and identified the phytochemical compounds in *C. asiatica* leaves (L.) water extract. **Methods:** The antioxidant activity of *C. asiatica* extract was evaluated through free radical assays, including 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), hydroxyl radicals (OH), nitric oxide (NO), and chelating capacity. Total phenolic content (TPC) of *C. asiatica* extract was evaluated. Antiglycation effects of *C. asiatica* extract were investigated through bovine serum albumin (BSA)-methylglyoxal (MGO), BSA-glucose, and MGO scavenging assay. **Results:** *C. asiatica* L. portrayed significantly ($p < 0.01$) better antioxidant effect with lower IC50 obtained compared to the positive control in DPPH, OH, and NO radical scavenging assay. Similarly, the IC50 of the *C. asiatica* L. was significantly ($p < 0.01$) lower than the standard quercetin, signifying that the plant extract demonstrated better antiglycation activity than the quercetin. The antiglycation activities of *C. asiatica* L. include the reduction the quantity of Amadori products, trapping reactive dicarbonyl intermediates, and free radical scavenging. At 100 µg/ml, a TPC value of 3.20 mg/GAE/g extract was identified and saponins had been detected in *C. asiatica* L. water extract. **Conclusion:** *C. asiatica* possessed effective antioxidant and antiglycation activities to reduce oxidative stress and inhibit AGE formation. Saponins may be the bioactive compounds that contributed to antiglycation activities. Therefore, *C. asiatica* can be used as a natural supplement to prevent diabetes complications and other chronic diseases in future.

Keywords: *C. asiatica* L., Advanced Glycation End Product, Methylglyoxal, Antioxidant

Exploring Mental Wellness Of Academicians And Their Perspectives On The Use Of Digital Technology Associated With Mental Health In Klang Valley, Malaysia

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ABSTRACT

Introduction: Mental wellness relates to a state of positive mental health that involves a balance between emotional, psychological and social well-being. This study aims to explore the positive mental health (PMH) of academicians, its association with the socio-demographic factors and the usage of digital technology to support mental health. **Methods:** A cross-sectional mixed method study was conducted among university academicians in Klang Valley, Malaysia. A total of 388 academicians completed the online survey which included the multidimensional 19-item PMH instrument and qualitative questions exploring the academicians' perspective on the use of mobile mental health apps. **Results:** The mean total PMH score was 4.65 ± 0.68. The total PMH and domain specific scores showed significant differences with age, ethnicity, marital status, qualification, academician rank and teaching experience ($p<0.05$). Significant associations were also found for faculty and organization with spirituality, working hours with global affect and spirituality, citizenship with general coping and spirituality domains, respectively. Older academicians, long years of service and senior academic ranking had higher PMH scores. Academicians with longer working hours showed significantly lower scores for spirituality and global affect domains. Majority of academicians (83.7%) demonstrated poor awareness towards the use of mobile apps for supporting mental health but a small group (18.3%) specified it was crucial for emotional need or support. The most common perceived benefit of mobile mental health apps was convenience (22.7%), whereas the major disadvantage was lack of human interaction (46.9%). Most academicians suggested that mobile apps should have extra features such as updated information and human support. **Conclusion:** Academicians in Malaysia have relatively higher PMH scores. Higher learning institutes may need to consider fostering the psychosocial aspects of PMH and increase the awareness of mobile apps' usage especially among younger academicians to ensure both mental wellness and work efficiency.

Keywords: Mental Health, Academician, Mental Health App, Perspective, Digital Technology

The Effects Of Mitragynine (Kratom) Early Exposure On Cognitive Behaviours In Adolescent Rats

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ABSTRACT

Introduction: Kratom consumption has spread to a younger population despite little is known about its cognitive effect. Here we investigated the changes in cognitive behaviour of adolescent rats after early exposure to kratom or its main alkaloid, mitragynine. **Methods:** Adolescent male Sprague-Dawley rats (postnatal day, PND 31) were treated with vehicle (p.o.), morphine (5 mg/kg, i.p.), kratom juice (30 mg/kg, p.o.) or different doses of mitragynine (3, 10 or 30 mg/kg, p.o.) for 15 consecutive days. Then the cognitive behaviours were assessed using social interaction, spontaneous social behaviour, and Morris water maze tasks. **Results:** Social interaction showed that morphine and mitragynine at all doses induced a significant increase in non-anogenital exploration. Similar effects were seen in tail manipulation for mitragynine (10 mg/kg). In spontaneous social behaviour, grooming partner, anogenital exploration, non-anogenital exploration and entire social behaviour were significantly reduced in kratom juice-treated group relative to control. However, kratom did not affect the spatial learning and reference memory in Morris water maze task. **Conclusion:** These findings generally suggest that early adolescent kratom exposure might impair certain cognitive domain, but further investigation is warranted to ensure if the impairment prolongs into the adulthood.

Keywords: Mitragynine, Kratom, Cognitive Behaviour, Adolescent Rat

Suppressive Activity Of Standardised Extract Of *Centella asiatica* (SECA) And Its Fractions Against Lipopolysaccharides (LPS)- Induced Activation Of BV2 Microglial Cells

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ABSTRACT

Introduction: Alzheimer's disease (AD) is known to be an age-related progressive neurodegenerative condition. Neuroinflammation plays a role in AD progression which characterized by activation of microglial cells in the brain. Recently, there is a growing interest in natural products with anti-inflammatory agents to develop alternative therapeutic agents for AD. In this study, we utilized standardized extract of *Centella asiatica* (SECA) and its fractions (hexane, dichloromethane (DCM), ethyl acetate (EA) and water) on lipopolysaccharides (LPS)-induced activation of BV2 microglial cells. The SECA was standardized for its major phytochemical constituents which are madecassosides and asiaticosides, along with their triterpenic metabolites, medecassic acid and asiatic acid. **Methods:** SECA was fractionated by solvent partitioning method using four different solvents (hexane, DCM, EA and water). SECA and its fractions was evaluated for their suppressive activity against LPS-induced activation of BV2 microglial cells by measuring the level of nitric oxide (NO) using Griess assay. **Results:** Our results showed that SECA, hexane and DCM fractions reduced the production of NO in dose-dependent manner and achieved the basal level of NO (negative control) at the highest concentration (100 µg/ml). **Conclusion:** SECA and its fractions, particularly hexane and DCM, exhibit their suppressive activity by attenuating the production of NO in LPS-induced BV2 microglial cells.

Keywords: Alzheimer's Disease, Microglial Cells, Neuroinflammation, *Centella asiatica*, Anti- Inflammatory

Kainate-Induced Excitotoxicity In NSC-34 Motor Neuron-Like Cells

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ABSTRACT

Introduction: Glutamate toxicity is implicated in the pathogenesis of motor neuron degeneration such as amyotrophic lateral sclerosis and spinal cord injury. Investigations on glutamate toxicity have mainly been focusing on ionotropic NMDA and AMPA receptors. However, little is known about neuronal degeneration caused by excitotoxicity of kainate receptor. A hybrid cell line, NSC-34 is fusion of mouse spinal cord neurons with neuroblastoma cells. It is commonly used to study the pathophysiology of motor neurons. Therefore, the aim of this study is to investigate the role of kainate receptors in mediating excitotoxicity in motor neuron-like cells, NSC-34. **Methods:** NSC-34 cells were differentiated by serum deprivation and treatment with all-trans retinoic acid. Differentiated NSC-34 cells were exposed to kainate receptor agonist, kainic acid at various concentrations. Apoptotic activity was assessed using Annexin V staining and propidium iodide. Expression of the apoptotic and injury markers were measured using quantitative polymerase chain reaction (qPCR). **Results:** Findings indicate that differentiated NSC-34 cells responded to high concentrations of kainic acid. There is an increased number of cell death as the concentration of kainic acid increased. **Conclusion:** High concentrations of kainic acid trigger excitotoxicity of kainate receptor. Therefore, this suggests that kainate-induced excitotoxicity has a potential role in motor neuron degeneration.

Keywords: NSC-34, *In-Vitro* Model, Neuronal Injury, Kainate, Glutamate Excitotoxicity

Do Hamster Models Develop Neuroleptospirosis After Experimental Leptospira Infection?

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ABSTRACT

Introduction: Leptospirosis is an emerging zoonotic infection caused by pathogenic *Leptospira* and continues to pose a challenge for public health experts due to the high morbidity and fatality rates. Neurological disorders such as intracranial haemorrhage and cerebellar ataxia have been reported in patients infected with *Leptospira interrogans*. Unfortunately, neuroleptospirosis is frequently underdiagnosed due to a lack of understanding on the pathophysiology and its diverse clinical presentation. The *Leptospira interrogans* ST238 strain was recently isolated in Malaysia and showed high virulence with haemorrhage in the lungs of infected Golden Syrian hamster models, but no information on the neuropathogenicity. Therefore, this study investigates the neuropathogenic changes observed in infected Golden Syrian hamster models to validate the animal model for future neuroleptospirosis study. **Methods:** Golden Syrian hamsters (n=21) were intraperitonially infected with 0.5 ml (2 x 108 leptospires/ml) of *Leptospira interrogans* strain ST238 per hamster. The hamsters (infected, n=21 and control, n=7) were observed daily and euthanised at random from 1st to 7th day post-infection, unless clinical signs of infection were present. The clinical scores of the hamsters were recorded. Brain samples were collected and sectioned into multiple coronal and sagittal planes for histopathological analysis. **Results:** A mortality rate of 19.05% and morbidity rate of 85.71% were observed in the infected hamster group. No significant pathological changes were identified in brain tissues of both the control (n=7) and infected (n=17) groups. However, extravasation of red blood cells in focal areas (+1) and congested blood vessels (n=4) were observed in brain tissues of infected hamsters. **Conclusion:** The findings from this study reveal a possible neuroleptospirosis development in hamsters following infection with *Leptospira interrogans* (ST238), providing new information about the disease's pathogenesis and the potential use of Golden Syrian hamsters as an animal model to study the disease.

Keywords: Neuroleptospirosis, Hamster Model, *Leptospira* Infection

In Vitro Antagonistic Effect of *Lactobacillus plantarum* FT 5 against Periodontal Pathogens

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ABSTRACT

Introduction: Dysbiosis of the oral microbiome is associated with a variety of diseases including periodontal disease. The disease is caused by the colonisation of periodontal pathogens and could predispose individuals to systemic disease. Probiotics, a beneficial live microorganism are reported to possess antimicrobial effects against pathogens. Thus, the objective of this study is to investigate the antimicrobial activity of a probiotic and its mechanism against periodontal pathogens. **Methods:** *Lactobacillus plantarum* FT 5 was isolated from local fermented food and is a private collection. *Lactobacillus plantarum* FT 5 overnight culture was centrifuged, and filter sterilised to obtain its cell- free supernatant (CFS). For the antimicrobial test, the disc diffusion assay was carried out against three periodontal pathogens: *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. Sterile MRS broth acted as negative control while a commercial probiotic, Lactobacillus rhamnosus ATCC 7469 CFS and 0.2% chlorhexidine served as the positive control. To assess for organic acid production, the CFS was neutralised to pH 6.5 and subjected to disc diffusion assay. The untreated CFS served as a control. All tests in the study were repeated three times in triplicates. **Results:** Lactobacillus plantarum FT 5 showed a significant inhibition activity against *A. actinomycetemcomitans* (11.22±0.19mm), *F. nucleatum* (10.33±0.25mm) as well as *P. gingivalis* (10.89±0.67 mm) compared to negative controls ($p<0.05$). Furthermore, the inhibition against *A. actinomycetemcomitans* is higher than that of positive control, *L. rhamnosus* ATCC 7469 (10.68±0.48mm). Interestingly, the neutralised CFS did not show any antimicrobial activity against periodontal pathogens suggesting the production of organic acid as an inhibitory substance. **Conclusion:** *L. plantarum* FT 5 exhibits inhibitory properties towards selected periodontal pathogens and the antagonistic activity may be due to the production of organic acid.

Keywords: Probiotics, Periodontal Pathogens, Periodontal Disease, Oral Health

Optimisation Of PEI Transient Transfection Parameters On WRL 68 Cell Line

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ABSTRACT

Introduction: Transfection is one of the techniques used to introduce foreign nucleic acids into a cell. Commercially available transfection reagents are costly. Polyethyleneimine (PEI) is an inexpensive polymer that has been used for transfection by condensing DNA into polyplexes to promote gene delivery into cells, but it requires optimisation for higher transfection efficiency. To achieve efficient PEI-mediated transfection and overexpression of genes into cells, a variety of parameters must be optimised. **Methods:** The MTS assay was performed on WRL-68, HepG2, A549 and MCF-7 cell lines to evaluate the cytotoxicity of PEI. The CYP2C9 gene was transiently transfected into WRL 68 cells using PEI, and the cell density, DNA concentration, and transfection time were optimized for the best efficiency. ImageJ was used to analyze fluorescence images to measure the mean fluorescence intensity. WRL 68 was also transfected using commercially available jetPRIME® as comparison. **Results:** The IC50 values of PEI were 13.08 ± 0.52μM, 15.80 ± 1.48μM, 46.36 ± 1.28μM and 45.12 ± 0.00μM for WRL-68, HepG2, A549 and MCF-7 respectively. The highest transfection efficiency and expression were achieved from the combination of 3μg plasmid DNA, 2.8μM PEI and 10.5mM NaCl with 48 hours incubation. Comparison with commercial transfection reagent, jetPRIME® showed PEI has 27.9% lower transfection efficiency, but there was significant toxicity observed in the cells transfected with jetPRIME®. Thus, PEI is a better option due to its lower toxicity with acceptable efficiency. **Conclusion:** The parameters for an inexpensive PEI-mediated transfection have been optimised using CYP2C9 gene and WRL 68 host cells. The method is practical for use in cell-based enzyme studies.

Keywords: Polyethylenimine, Transfection, WRL 68, Overexpression

WRL 68 With Overexpression Of CYP3A4*1, CYP3A4*4 And CYP3A4*18 As *In Vitro* Tools For Drug Metabolism Study

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ABSTRACT

Introduction: The 'gold standard' primary hepatocytes are widely used in the in vitro assessments of CYP450-mediated drug metabolism. However, donor-to-donor variation in the metabolic activity of hepatocytes could decrease the reproducibility of the results. Single nucleotide polymorphisms of CYP450 enzymes are also an important consideration in the selection of in vitro tools for metabolism studies. This study aims to develop in vitro models using WRL 68 cells overexpressing variants of the CYP3A4. The models would serve as efficient tools for studies of drug metabolism and drug interactions. **Methods:** Point mutations were introduced Into CYP3A4-expressing plasmid using site-directed mutagenesis. Mutations were confirmed by DNA sequencing. Polyethylenimine was used as the transfection reagent for gene delivery. Overexpression of CYP3A4*1, CYP3A4*4 and CYP3A4*18 in the transiently transfected cells were measured using quantitative real-time PCR and confirmed by Western blot analysis. The expression of CYP450 reductase and cytochrome b5 in WRL 68 were also measured after transfection. Testosterone was used as a probe drug to evaluate the kinetic parameters of all enzyme variants. **Results:** Comparable transfection efficiencies and levels of overexpression were observed between cells expressing wild-type and variants of CYP3A4. In addition, the expression of CYP450 reductase and cytochrome b5 expression, which are vital for CYP3A4 catalytic activity, was found to remain intact after CYP3A4 variants overexpression. These models were also evaluated for testosterone 6β-hydroxylation activity (CYP3A4*1: Km: 20.60 ± 0.10 μM; Vmax: 43.23 ± 1.21 nM/minutes/mg total protein, CYP3A4*18: Km: 13.82 ± 0.28 μM; Vmax: 53.33 ± 0.14 nM/minutes/mg total protein, CYP3A4*4: Km: 44.64 ± 2.97 μM; Vmax: 34.80 ± 0.79 nM/minutes/mg total protein). CYP3A4*18 has higher activity and CYP3A4*4 has lower activity when compared to wild-type. **Conclusion:** WRL68-CYP3A4*1, WRL68-CYP3A4*4, WRL68-CYP3A4*18 models were developed to be a useful tool for in vitro drug metabolism and interaction studies involving CYP3A4 SNP variants.

Keywords: WRL 68, Transfection, Overexpression, CYP3A4, Single Nucleotide Polymorphism

Salmonella spp. Induced Abdominal Aorta Mycotic Aneurysm

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ABSTRACT

Introduction: *Salmonella* spp. usually presents as gastroenteritis and are often self-limiting. A mycotic aneurysm is a lobulated saccular outpouching of the blood vessel wall communicating with the lumen, due to a localized, abnormal, weak spot in the arterial wall due to infection. A mycotic aneurysm carries a risk of mortality due to rupture. **Case summary:** A 65-year-old man who was an ex-smoker with underlying diabetes mellitus presented with fever for two months, associated with left lower limb paraesthesia. On physical examination, he had a high-grade fever, but other vital signs were stable. There was an abdominal mass that was pulsatile and expansile. All of his peripheral pulses were palpable with good Doppler signals. His blood culture grew *Salmonella* spp. He was diagnosed with Salmonella septicaemia with CIA aneurysm and started on IV Ceftriaxone 1g once a day. However, repeat CTA (2 weeks after first imaging) showed an increase in the size of the left CIA aneurysm measuring 9.0 x 6.6 x 8.2 cm with a retroperitoneal collection extending to the pelvis. He was transferred to a vascular unit and subsequently underwent exploratory laparotomy, aneurysmectomy and distal aortic ligation with left axillobifemoral artery bypass. He was given IV Ceftazidime for six weeks and recovered well. **Conclusion:** This case highlights the clinical importance of *Salmonella* spp. as an etiologic agent in a mycotic aneurysm.

Keywords: *Salmonella*, Mycotic Aneurysm

Determining The Enhancing Effects Of PCR Additives On Long-Range PCR

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ABSTRACT

Introduction: Long-range polymerase chain reaction (PCR) is used to amplify large DNA fragments >5 kb. Despite its widespread application, the performance of the technique is limited by low product yields. Many PCR additives are known to enhance the performance of 'short' PCR, but their potential benefits for long PCR have not been systematically ascertained. Hence, this study was aimed at determining the effects of different PCR additives on the performance of long-range PCR. **Methods:** Six PCR additives (betaine, bovine serum albumin (BSA), glycerol, dithiotreitol (DTT), Taq DNA ligase, and trehalose) were chosen based on their modes of action and added at various concentrations to PCR mixtures amplifying 10 kb fragments from *CYP2C19* and 6.6 kb fragments from *CYP2D6*. Different commercial enzyme kits, namely AtMax Taq DNA polymerase, KAPA Long-Range Hotstart DNA polymerase, and PrimeSTAR GXL DNA polymerase, were also compared. The resultant products were analysed by agarose gel electrophoresis. **Results:** Betaine, DTT, and trehalose were found to be PCR-enhancing at certain concentrations and across experimental setups. These additives gave the highest level of amplification of the target band and showed consistent trend of results when tested with different enzyme kits while amplifying the DNA fragments from *CYP2C19* and *CYP2D6*. In contrast, BSA, Glycerol and Taq DNA ligase were found to have no substantial effect on the performance of long PCR. **Conclusion:** In this study, we identified several PCR additives that were effective at enhancing the amplification of large DNA fragments from the *CYP2C19* and *CYP2D6* genes. The information from this study may serve as a reference to overcome some of the challenges in amplifying large DNA fragments and to increase the yield of specific PCR products.

Keywords: Long-Range Polymerase Chain Reaction, PCR Additives, *CYP2D6, CYP2C19*

A Modified Method For Extraction And Nested RT-PCR For Detection Of SARS-CoV2 From Saliva

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ABSTRACT

Introduction: The COVID-19 pandemic caused by SAR-CoV-2 virus has had a significant influence on human health and safety. For the laboratory diagnosis of COVID-19, molecular diagnostics have been developed, mostly employing platforms such as real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR). It is vital to note that a laboratory capacity, in terms of set-up, equipment, competency, capability, and most importantly, financial viability, is critical in adopting or developing of detection method(s). The aforementioned elements will decide the strategy that will be utilised to depict the real-world testing scenario. Saliva was chosen as the sample type since it is similar to the over-the-counter commercial saliva-based COVID-19 rapid test kit (RTK) in terms of ease-of-use. **Methods:** We used a nested reverse-transcription polymerase chain reaction (RT-nPCR) and tested its applicability as an alternative to RT-qPCR. For total nucleic acid (TNA) isolation, a salting-out process was adopted in conjunction with a single-tube RT-PCR step followed by nested PCR. **Results:** Through the identification of N, Orf1ab, and MF genes, TNA isolated using this approach can detect the presence of SARS-CoV-2 from saliva samples. **Conclusion:** Saliva can be utilised to detect COVID-19 according to our findings utilising this modified approach.

Keywords: COVID-19, Nested RT-PCR, Total Nucleic Acid, Saliva, Salting-ut Procedure

Development Of Quadrupole Time-Of-Flight Liquid Chromatography Mass Spectrometry (QTOF-LC/MS) Method For Colistin In Human Plasma

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ABSTRACT

Introduction: Colistin is a last resort's antibiotic for the treatment of carbapenem- resistant infections due to a lack of effective and safe therapy options. Colistin has a narrow therapeutic window. Measurement of plasma drug concentrations is crucial in ensuring levels are within a targeted therapeutic range for dose optimization. The purpose of this study was to develop analytical method for colistin quantification in human plasma. **Methods:** Reversed-phase chromatography was performed on a Zorbax SB-C8 column maintained at 40°C using gradient elution with water and acetonitrile, both containing 0.1% formic acid. The total analysis time, including column equilibration was 18 min per injection. Detection was performed with quadrupole time- of-flight (QTOF) analyser, using electrospray ionization (ESI) operated in the negative mode and the precursor–product ion pairs was identified at m/z 1123.7300, 1079.7032 for colistin A, *m/z* 1109.7151, 1065.6886 for colistin B and *m/z* 1157.7159, 1113.6889 for polymyxin B (internal standard). Colistin A, colistin B and polymyxin B were extracted from plasma using a nonacidic solid phase extraction (SPE) procedure. **Results:** The retention times for colistin A was 4.003 min, colistin B was 3.81 min, and polymyxin B was 4.047 min. The lower limit of quantification (LLOQ) was 0.5mcg/ml with the coefficients of variation (CV) <10%. For plasma pre-treatment, the extraction recovery ranges 78-95% (CV <10%) and matrix effects ranges 40-50% (CV <10%) for analytes with different concentration. **Conclusion:** The optimum chromatographic condition for separation and detection of colistin using quadrupole time-of-flight liquid chromatography mass spectrometry (QTOF-LC/MS) was established.

Keywords: Colistin, High Performance Liquid Chromatography, Mass Spectrometry, QTOF-LC/MS, Human Plasma

P-Glycoprotein Inhibition Cannot Explain Predominantly Efflux At Steady-State Of Mitragynine Brain Uptake

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ABSTRACT

Introduction: Mitragynine, a major compound isolated from the leaves of *Mitragyna speciosa* Korth. (kratom), acts as a mu-opioid receptor agonist and may be developed as a treatment for chronic pain and opioid addiction. Earlier work established that mitragynine was predominantly efflux at steady-state in rat brain, with an unbound brain-to-blood partition coefficient (Kpuu) of 0.091. Most CNS drugs are limited in their cellular uptake and brain accumulation by the P-glycoprotein (P-gp) efflux pump. The purpose of this study was to determine the involvement of P-gp-mediated efflux across the blood-brain barrier (BBB) in rats using P-gp inhibitor verapamil. **Methods:** The transport of radiolabelled mitragynine (3H-mitragynine) across the rat BBB was measured with or without verapamil, a well-known P-gp inhibitor by bilateral *in-Situ* brain perfusion technique. By comparing the difference in brain transport achieved with or without verapamil, it is possible to determine the P-gp-mediated efflux component of mitragynine across BBB using this technique. Cerebral vascular volume and BBB integrity was determined by co-perfusion with radiolabelled sucrose (14C-sucrose), a non-permeating BBB marker in all experiments. **Results:** 14C-Sucrose perfusion indicated that vascular space was within a normal range (~2% brain volume) in all the studies, indicating that the BBB integrity remained intact. The volume of distribution of 3H-mitragynine in the brain was approximately 9-fold than that of 14C-sucrose, indicating that mitragynine was transported across the BBB under physiological condition. Bilateral *in-situ* brain perfusion in rat demonstrated that the transport co-efficient, Kin of mitragynine perfused without verapamil (0.2262±0.1003 mL/min/g brain) was no different with Kin of mitragynine perfused with verapamil (0.1756±0.0957 mL/min/g brain). **Conclusion:** This result implies that P-gp had no influence on the uptake of mitragynine in rat brains. Additional research is required to elucidate the role of other key efflux pumps, including Multi-Drug Resistance Proteins (MRPs) and Breast Cancer Resistance Proteins (BCRPs).

Keywords: Mitragynine, *Mitragyna speciosa* Korth., Blood Brain Barrier, *In-Situ* Brain Perfusion, P-gp Efflux Pump

Potential Involvement Of Opioid Receptors In Exerting The Antinociceptive Activity Of *Uncaria attenuata*

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ABSTRACT

Introduction: *Uncaria* species is a type of climbing vine found across Southeast Asia, East Asia, and South America. The leaf and hook (claw-like) parts of *Uncaria* plants have traditionally been used to treat pain, neurological disorders, hypertension, stroke, rheumatism, and other ailments. Chemically, the *Uncaria* species is rich in indole and oxindole alkaloids. Methods: In this study, we investigated the potential antinociceptive activity of a rare Malaysian *Uncaria* species – *Uncaria attenuate* (UA) on rats using the hot plate method. The alkaloid profile of UA was analysed using various chromatographic techniques and spectroscopic methods. Results: In the hot plate test, UA methanolic leaf extract (500 mg/kg) and alkaloid leaf extract (100 mg/kg) given orally demonstrated suppression of pain sensation when compared to control animals. This is evident as reduced pain effect encountered over a substantial latency period. The antinociceptive activity of methanolic and alkaloid UA leaf extracts were significantly reversed in animals pre-treated with a non-selective opioid antagonist (naloxone) (2 mg/kg i.p.). Extensive chromatographic analyses revealed that the major alkaloid marker of UA is a monoterpene oxindole alkaloid. Conclusion: These findings suggest that methanolic and alkaloid extracts of UA leaves as antinociceptive agents. This effect is likely mediated by a mechanism involving interactions with the central opioid receptors. Malaysian plant *Uncaria* species can hold prospective as a herbal plant for pain treatment.

Keywords: *Uncaria attenuata*, Leaf, Alkaloid, Hot Plate, Opioid Receptors

Antibiofilm Activities Of Postbiotics Against *Candida* Species

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ABSTRACT

Introduction: A plethora of studies have been orchestrated to investigate the antagonistic effects of cell free supernatants (CFS) of *Lactobacillus* strains against *Candida* species. The mechanism of action has been attributed to microbial excretes such as hydrogen peroxide, antimicrobial proteins/bacteriocins, short chain fatty acids and organic acids. Particularly, there has been reports of the antibiofilm disruption capabilities of the metabolites produced in the CFS against fungal species. These metabolites are widely termed as postbiotics and are strain dependent. Therefore, this study is to investigate the antibiofilm prowess of the CFS produced by an indigenously isolated vaginal *Lactobacillus* strain against *Candida albicans* and *Candida glabrata*. The present study aims to elucidate the antibiofilm effect of the strain *Lactobacillus* 29A (L29A) CFS/postbiotics on the morphology of C. albicans and C. glabrata biofilms and to characterise the contents of the CFS/postbiotics. Methods: Preformed biofilms of C. albicans and C. glabrata were treated with the CFS of L29A and visualised by scanning electron microscopy (SEM). Subsequently, gas chromatography-mass spectrophotometry (GC-MS) was also employed to identify the metabolites in the CFS which confer the biofilm disruptive activities. Results: The CFS produced by L29A strongly disrupted the biofilms of C. albicans and C. glabrata reference and clinical strains, respectively. The metabolites identified in the CFS by GC-MS include organic acids, fatty acids, amino acids (proteins/bacteriocins), alcohol, alkanes, alkaloids, aldehydes, phenols and some aromatic compounds. Conclusion: Therefore, the CFS/postbiotics of L29A showed potent biofilm disruption properties against *Candida* species. They consist of a diversity of various organic compounds. This preliminary study suggests that the CFS/postbiotics of L29A can serve as a source to search for potential biofilm inhibitor and disruptor agents against pathogenic *Candida* species.

Keywords: Postbiotic, Antibiofilm, *Candida*, *Lactobacillus*, GC-MS

Antibacterial Activity Of *Salvadora persica* Against *Porphyromonas gingivalis* And *Streptococcus mutans*

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ABSTRACT

Introduction: *Porphyromonas gingivalis* (*P. gingivalis*) and *Streptococcus mutans* (*S. mutans*) are Gram-negative and Gram-positive bacteria, respectively. They are most implicated in periodontal disease. *Salvadora persica* or miswak is very popular as an effective tool used in Africa, South America, Middle East and Asia for oral hygiene. It is presently acknowledged as significantly due to the presence of biological active compounds that inhibited the growth of bacteria. Thus, the main objective of this study is to determine the antibacterial activities of *S. persica* against *P. gingivalis* and *S. mutans*. **Methods:** Sequential extractions of *S. persica* were conducted using five types of different solvents (n-hexane, dichloromethane, acetone, ethanol and methanol). The antibacterial activities were performed by disc diffusion assay (DDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and biofilm assay against *P. gingivalis* and *S. mutans*, using chlorohexidine (positive control) and dimethyl sulfoxide (negative control). **Results:** There are significant differences between different types of *S. persica* extracts and diameter of inhibition zone for *P. gingivalis* and *S. mutans* ($p < 0.001$). When compared between 0.2% chlorohexidine and *S. persica* extract, chlorohexidine has superior effect against *P. gingivalis* and *S. mutans*. **Conclusion:** As an alternative to chlorohexidine, *S. persica* extract can be recommended for oral hygiene because it has antibacterial properties that can inhibit the growth of oral pathogen.

Keywords: *Salvadora persica, Porphyromonas gingivalis, Streptococcus mutans*

Antimicrobial And Cytotoxic Activities Of *Sansevieria trifasciata* Leaf Extracts *In Vitro*

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ABSTRACT

Introduction: *Sansevieria trifasciata*, also known as mother-in-law's tongue, belongs to the family Agavaceae. It is a native to tropical West Africa. The plant possesses a broad range of therapeutic properties including antidiabetic, analgesic, antipyretic, thrombolytic, anti-allergenic, anti-anaphylactic and antioxidant activity. The main objectives of this study were to screen for the phytochemical constituents of *S. trifasciata*, to determine the antimicrobial activity of its leaf extracts against six bacterial strains (i.e., *Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*), and to assess the cytotoxic activity of its ethanolic extract against two cell lines. **Methods:** Different concentrations (5, 10 and 20 mg/mL) of plant extracts (petroleum ether and ethanol) were tested against microorganisms using the agar well diffusion method. Two-fold serial dilution method was used to determine the minimum inhibitory concentration (MIC). The cytotoxic study was performed using (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) (MTT) assay against human colorectal cancer cell (HCT-116) and primary colon epithelial (PCE) cell lines. **Results:** Phytochemical screening revealed the presence of alkaloids, terpenoids, tannins, glycosides and saponins. MIC of *S. trifasciata* leaf extracts indicated a broad spectrum of antimicrobial activity against the tested microorganisms between the concentrations of 0.156 to 5 mg/mL. The standard antibiotic, tetracycline yielded a MIC of 0.05 mg/mL. The most susceptible bacterial strains to ethanolic and petroleum ether extracts were *Proteus vulgaris* and *Staphylococcus aureus*, respectively. Different concentrations (7.8 - 1000 µg/mL) of ethanolic extract resulted in a lower IC_{50} value in HCT-116 than that of PCE. The IC_{50} values for HCT-116 and PCE after 72 hrs of incubation was 10.00 µg/mL and 92.9 µg/mL, respectively. The standard control, fluorouracil yielded an IC_{50} value of 10 µg/mL. **Conclusion:** Both ethanolic and petroleum ether extracts exhibited significant antimicrobial activity. Ethanolic extract of *S. trifasciata* showed selective cytotoxic activity against HCT-116.

Keywords: *Sansevieria trifasciata*, Minimum Inhibitory Concentration, Antimicrobial Activity, Cytotoxic Activity, MTT Assay