2nd International Anatomical And Biomedical Scientific Conference 2017

1st - 2nd August 2017

Venue:
FACULTY OF MEDICINE AND HEALTH SCIENCES
Universiti Putra Malaysia

"Research Advances in Health Sciences"
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The International Anatomical and Biomedical Scientific Conference (IABS) was first held in 2015. It was held from 18th – 20th August 2015 at Faculty of Medicine and Health Sciences. The theme of the conference was From Cell towards Translational Medicine. It covered diverse topics from cutting-edge biomedical research and development to clinical applications. Prominent speakers were from University of Edinburgh, United Kingdom, University of Adelaide, Australia and various institutes in Malaysia. The conference was successful thus it was planned to have a biannual IABS for the good development of Health Sciences.

International Anatomical and Biomedical Scientific Conference 2017 (IABS 2017) is the second conference, and it has now established itself as the premier conference dedicated to Anatomy and Biomedical Science. This conference is a multidisciplinary conference that will provide an opportunity for the postgraduate students, academicians, researchers and scientists to showcase their latest findings and to disseminate their valuable research at international level. It will provide the update on the latest and exciting research advances in various health sciences setting. Topics to be covered are Anatomy, Metabolomics, Nanomedicine, Natural Products and Drug Discovery, Neuroscience, Nutrition and Dietetics, Pharmaceuticals and Nutraceutical, Regenerative Medicine and Cancer. It will provide an excellent opportunity for international networking between researchers and students as well. Prominent speakers are National Central University Taiwan, Chinese University of Hong Kong, Universiti Sains Malaysia, Universiti Malaya, Universiti Kebangsaan Malaysia, International Medical University and Universiti Putra Malaysia.
Assalamualaikum Warahmatullahi Wabarakatuh

On behalf of the Organising Committee, it is my pleasure to welcome you to the 2nd International Anatomical and Biomedical Sciences Scientific Conference 2017 (IABS2017). The IABS 2017 will be held at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.

In this meeting, we have chosen the theme ‘Research Advances in Health Sciences’, where we hope to cover diverse topics from cutting-edge biomedical research and development to clinical applications. More specifically the symposia will encompass Anatomy, Neuroscience, Pharmaceutical, Natural Product, Nanotechnology, Stem Cell, Infectious Disease and Clinical Trial. With many research activities now taking on a global dimension, it is necessary to discuss on the best approaches towards inculcating the best research practises.

We have with us today, exciting and high-profile scientists, who will be delivering lectures in plenary/keynote session, some will be presenting in the invited lecture slots, depending on each specialities. We are very pleased to hold this conference for everyone, especially the young scientists and post graduate students to share the same platform to meet, distributing and sharing ideas, communicating their thoughts and information, in order to enhance their research skills and expanding knowledge. I hope that by being able to be with eminent speakers in their respective fields, they are able to inspire and motivates our delegates to be creative and innovative in their career. Besides, this is the time to get to know everyone and extend the research networking. Hence, we invite you to take full advantage of many opportunities offered to you by IABS 2017.

I would like to take the opportunity to thank the members of Organising Committee, for their hard work and effort in planning and coordinating this event. Without their effort, the conference could not have been materialised. I would also like to thank the management of the faculty, and Research Management Centre, UPM for their support in making this conference possible.

In addition, we have received grant support from various industries, in terms of materials and monetary, which without them, the organisation of this conference is unlikely.

Finally, I encourage delegates to participate actively in the interesting discussions over the next four days. I wish everyone a successful and fruitful conference.

Thank you.

Assoc. Professor Datin Dr. Sharida Fakurazi
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
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<td>Speaker: Professor Datuk Dr Asma Ismail (Universiti Sains Malaysia, Malaysia)</td>
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<td>PLENARY TALK: REGENERATIVE MEDICINE</td>
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<td>Speaker: Professor Dato’ Dr. Ruszymah Hj. Idrus (Universiti Kebangsaan Malaysia, Malaysia)</td>
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<td></td>
<td>Title: Advances in regenerative medicine teaches the body to rebuild damaged parts</td>
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<td>Title: My research journey with the drumstick tree</td>
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<td>Title: Blocking smooth muscle cell proliferation with a synthetic geranyl acetophenone</td>
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<td>Title: Current status of clinical trials of human pluripotent stem cells and culture and differentiation of human pluripotent stem cells on biomaterials having nanosegments</td>
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<td>1030</td>
<td><strong>PLENARY TALK: PHARMACEUTICAL &amp; NUTRACEUTICAL</strong>&lt;br&gt;Speaker: Professor Dr. Yuen Kah Hay (Universiti Sains Malaysia, Malaysia)&lt;br&gt;Title: Challenges in generic drug development</td>
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<td>1130</td>
<td><strong>OA1: Image driven pharmacokinetics using molecular imaging for cancer nanotherapeutics</strong>&lt;br&gt;Mohd Janib, S. N.; Wan Kamal, W. H. B.; Ahmad Fadzil, M. F.; Abdul Hamid, S. S. and Ng, Y.&lt;br&gt;Malaysian Nuclear Agency, Malaysia</td>
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<td><strong>OA2: Acute toxicity of intravenous administered thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) in Sprague Dawley rats</strong>&lt;br&gt;Seiful Yazan, L.; Mohd Azlan, S. N.; Zakariah Ansar, F. H. and Mohd Ali, R.&lt;br&gt;Universiti Putra Malaysia, Malaysia</td>
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<td><strong>OA4: In silico and in vitro investigations of α-glucosidase inhibitory activity of phenolic constituents of Tetracera indica</strong>&lt;br&gt;Ahmed, Q. U.; Alhassan, M. A.; Khatib, A. and Sarian, M. N.&lt;br&gt;International Islamic University Malaysia, Malaysia</td>
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<td><strong>PLENARY TALK: NUTRITION &amp; DIETETICS</strong>&lt;br&gt;Speaker: Assoc. Prof. Dr. Hamid Jan Jan Mohamed (Universiti Sains Malaysia, Malaysia)&lt;br&gt;Title: Vitamin D: Beyond the skeletal system</td>
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<td><strong>OC2: Joint pain and function in osteoarthritis: Exploring the role of different dietary interventions</strong>&lt;br&gt;Othman, Z. A.; Noordin, L.; Wan Ghazali, W. S.; Omar, N. and Mohamed, M.&lt;br&gt;Universiti Sains Malaysia, Malaysia</td>
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<td><strong>OC3: The effect of dietary fibre on colon cancer risk</strong>&lt;br&gt;Othman, Z. A.; Noordin, L.; Wan Ghazali, W. S.; Omar, N. and Mohamed, M.&lt;br&gt;Universiti Sains Malaysia, Malaysia</td>
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<td><strong>OC4: The potential of garlic as a modulator of cardiovascular health: A systematic review and meta-analysis</strong>&lt;br&gt;Othman, Z. A.; Noordin, L.; Wan Ghazali, W. S.; Omar, N. and Mohamed, M.&lt;br&gt;Universiti Sains Malaysia, Malaysia</td>
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<td>OC2: Bioassays activity and FT-IR analysis of <em>Clinacanthus Nutans</em> (Burm F.) Lindau leaves extracts</td>
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<td>OD2: Identification of stem cell antigen-1 (Sca-1) on endothelial cells.</td>
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<td>OC3: Evaluation of antioxidant activity of <em>Momordica Charantia</em> using LC-MS metabolomics approach</td>
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<td>OC4: Curcumin analogues inhibit oxidized-1-palmitoyl-2-arachydonoyl-sn-glycero-3-phosphorylcholine-induced proinflammatory chemokines and XBP-1 activation in U937 macrophages</td>
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<td>OC5: Molecular insight of a geranyl acetophenone in IgE-mediated mast cell activation of allergy</td>
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<td>OC6: Antinociceptive activity of <em>Dicranopteris linearis</em> leaves methanol extract and its partitions: Elucidation of the mechanisms of action</td>
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<td>OD5: Amelioration of hypercholesterolaemia-induced aortic and hepatic histopathological changes with roselle</td>
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<td>OD6: <em>Clinacanthus Nutans</em> aqueous extract protects against systemic anaphylaxis via the IgG pathway</td>
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<td>OD7: Inhibitive effect of cardamomin upon proliferation of serum induced human bronchial smooth muscle cells</td>
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Coffee and tea
Award Ceremony
Prof. Datuk Dr. Asma Ismail is a woman of many firsts. She currently serves as first woman Vice-Chancellor (VC), Universiti Sains Malaysia (USM) and first woman VC of Universiti Sains Islam Malaysia (USIM) in 2012; making her the first woman appointed twice as VC in two public universities. She served as the country’s first woman Director-General of Higher Education and is currently the first woman President of Academy of Sciences Malaysia (2016-2019).

Prof. Datuk Dr. Asma Ismail’s expertise is in rapid diagnostics for infectious diseases. With her BSc in Biology from University of Nevada, Reno (UNR), MA (Microbiology) from Indiana University, Bloomington and PhD (Cellular and Molecular Biology) from UNR, Prof. Datuk Dr. Asma Ismail made scientific discoveries that led to attainment of 13 patents and commercialization of rapid diagnostic test for typhoid called TYPHIDOT which was advocated by WHO. As a researcher, she published 131 papers, received more than 213 awards and recognitions, presented more than 371 papers including 294 invited talks/plenaries and 31 keynotes both at national and international levels. She was elected to The Academy of Sciences Malaysia in 2003, The Academy of Sciences for the Developing World (TWAS) in 2010 and The Islamic World Academy of Sciences in 2016.

Her landmark contributions to Malaysia’s higher education system include establishment of the prestigious National Academic Award, 5 Research Universities in Malaysia and co-helmed development and implementation of The Malaysian Education Blueprint (higher education) 2013-2025.

For these and other achievements, she received an Honorary Doctor of Science from University of Glasgow in 2013, Indiana University’s Thomas Hart Benton Mural Medallion in 2015 and Honorary Degree Doctor of the University, Keele University in 2017.
PROFESSOR DR. AKON HIGUCHI  
National Central University, Taiwan

Professor Akon Higuchi is a Chair Professor from Department of Chemical & Materials Engineering, National Central University, Taiwan. Apart from that, he has also been appointed as Adjunct Professor at Kyoto Institute of Technology (Kyoto, Japan), special researcher in Riken (Japan) and also as visiting Professor of King Saud University (Saudi Arabia). He has received numerous awards and recognitions as well as involve in a number of international bodies and institutions; among them from Royal Society of Chemistry, England (Fellow), Taiwan Chemical Engineering Society (Fellow), Royal Society of Chemistry Japan, The Asia Pacific Tissue Engineering and Regenerative Medicine International Society and Chemical Society of Japan. Currently, he has been appointed as editorial and advisory board for various journals that cover chemistry, tissue engineering, stem cells and cell biology.

Prof Akon is renowned for his expertise that covers various aspects of biomedical research including biomaterials, stem cell engineering, biomedical membranes and bio-separation. His research field includes preparation of nano brush-grafted surface, culture of stem cells on biomaterials under xeno-free conditions, reprogramming of human somatic cells into induced pluripotent stem cells (iPSCs), and isolation of adipose-derived stem cells from human fat tissue by membrane filtration method.

Prof Akon expertise and research interest opens up a number of potential applications in biomedical research. Among them include isolation of stem cells from human tissue, human pluripotent stem cells (embryonic stem cells and iPSCs) and adult stem cell culture and differentiation for clinical application, and generation of human iPSCs in safety conditions.

Abstract

Current Status of Clinical Trials of Human Pluripotent Stem Cells and Culture and Differentiation of Human Pluripotent Stem Cells on Biomaterials Having Nanosegments

Akon Higuchii, S. Suresh Kumar3

1Department of Chemical & Materials Engineering, National Central University, Taoyuan 32001, Taiwan.
2Nano Medical Engineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-098, Japan
3Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Human pluripotent stem cells (hPSCs) hold excellent promise for regenerative medicine and drug discovery from their high differentiation ability into any kind of cell types in human tissues. However, clinical application of hPSCs is currently extremely limited compared to the clinical application of adult stem cells. Currently, hPSCs, especially human embryonic stem cells (hESCs) are starting to use for the treatment of the patients with myocardial infarction, ocular disease, diabetes (1 trial), and spinal cord injury (2 trials). Ocular diseases are the first human trials of hPSCs and Phase I/II trials showed promising safety results as well as some possible efficacy. The current clinical trials evaluating hPSC-based therapies predominantly targeting on treatment of retinal degeneration in the eye. This is because eye tissue has an immunoprivileged nature (tolerating characteristics of foreign antigens and non-histocompatible cells; almost no immune response to foreign materials). It can be possible to visualize the internal tissue through lens after transplantation of the cells. Age-related macular degeneration (AMD) and Stargard macular dystrophy are the progressive degradation of light-sensing photoreceptor cells and their supportive retinal pigment epithelium (RPE). Preclinical investigation showed the safety and efficacy of hPSC-derived RPE in animal models. Several clinical trials are now ongoing for hPSC-derived RPEs for AMD and Stargard macular dystrophy.

The current status of clinical trials of hPSC-derived RPE for ocular diseases is reviewed in this study. Furthermore, hPSC culture on biomaterials grafted with nanosegment are discussed for future clinical usage of hPSCs for treatment of ocular and other disease. Establishing cultures of human embryonic (ES) and induced pluripotent (iPS) stem cells in xeno-free conditions is essential for producing clinical-grade cells. Development of cell culture...
Biomaterials for human ES and IPS cells is critical for this purpose. We designed several structures of oligopeptide-grafted poly (vinyl alcohol-co-itaconic acid) hydrogels with optimal elasticity, and prepared them in formations of single chain, single chain with joint segment, dual chain with joint segment, and branched-type chain. Oligopeptide sequences were selected from integrin- and glycosaminoglycan-binding domains of the extracellular matrix. The hydrogels grafted with vitronectin-derived oligopeptides having a joint segment or a dual chain, which has a storage modulus of 25 kPa, supported the long-term culture of human ES and IPS cells for over 10 passages. The dual chain and/or joint segment with cell adhesion molecules on the hydrogels facilitated the proliferation and pluripotency of human ES and IPS cells. We will also discuss cardiomyocyte differentiation of human ES cells on biomaterials immobilized with several extracellular matrices.

References

1. Akon Higuchi,* S. Suresh Kumar, Qing-Dong Ling, Abdullah A. Alarfaj, Murugan A. Munusamy, Kadarkarai Murugan, Shih-Tien Hsu, Giovanni Benelli, Akihiro Umezawa, Polymeric design of cell culture materials that guide the differentiation of human pluripotent stem cells, Prog. Polym. Sci., (2016) in press. <SCI, Impact factor= 27.184>


Professor Dato’ Dr Ruszymah Bt Hj Idrus is the pioneering researcher in the field of Tissue Engineering and Regenerative Medicine in Malaysia. She is the Professor of Physiology at the Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM). She has been the founder Head of the Tissue Engineering Center at UKM Medical Center. She is also the founder President of the Tissue Engineering Society of Malaysia (TESMA) and a Council Member of the Tissue Engineering and Regenerative Medicine International Society-Asia Pacific Chapter (TERMIS-AP) when it was first initiated.

Her main area of research is cell therapy and tissue engineering for clinical applications including skin, cartilage, bone, nerve, cornea, respiratory epithelium etc. She was instrumental in forming and expanding the Tissue Engineering Research Group and now spearheading the research team in UKM. The group’s programme is at the clinical interphase, with ‘MyDerm’ going and more stem cell treatments planned for otolaryngology and orthopaedic repair. In the span of 15 years, a voluminous amount of research work was produced. She is running numerous research projects granted by the university, MOSTI, MOHE and IAEA with post-graduate and under-graduate students from multidisciplinary background. With her vibrant personality, she has mentored and trained many post-graduate students; many have become credible researchers themselves. She is dedicated at producing the next generation of researches to perpetuate this field in Malaysia.

Abstract

Advances in Regenerative Medicine Teaches the Body to Rebuild Damaged Parts

Prof. Dato’ Dr. Ruszymah Bt Hj Idrus

Regenerative Medicine may one day revolutionize the treatment of ailing or damaged cells, tissues or organs. Researchers are identifying the ‘starter kit’ that can either be bioactive compounds, proteins, cells, tissues or part of an organ, to allow the body to take over from there. The ‘starter kit’ could stimulate body's own repair mechanisms to heal diseased tissues. In this case the disease can be cured, where most treatment only treats the symptoms but not curing the disease.

MyDerm™ a biological skin substitute was successfully produced for the treatment of full thickness wounds resulting from burns and trauma. This bilayered tissue engineered skin is made from patients’ own skin cells. This will be an alternative to split skin graft (SSG) that causes bigger donor site morbidity. This fully autologous skin substitute has completed its Phase I/IIa human trial and the results were very promising. Another product going to pre-clinical trial is the application of Platelet-Rich-Plasma (PRP) gel with keratinocytes and fibroblasts to aid in the regeneration of full-thickness wound healing.

Osteoarthritis is a major degenerative joint disease and to date there is no definitive treatment to stop the degenerative changes from progressing and reverse the damage. Mesenchymal stem cell based therapies; adipose stem cells and bone marrow stem cells hold promises as treatment options. Osteoarthritic sheep receiving single dose of autologous chondrogenic induced stem cells showed regenerated de novo cartilage. These improved in structure at the fourth month, with the greatest maturity in appearance seen at the eighth months post implantation.

We have engineered the cornea using either limbal stem cell or bone marrow stem cell with fibrin or amniotic membrane as the biomaterial. These two applications prove viable in our animal model. Work on peripheral nerve engineering is also showing encouraging results. We are also differentiating mesenchymal stem cells to cardiomyocytes with the vision of finding the cure for heart failure.

Regenerative Medicine attempts to change the course of chronic diseases and will regenerate tired and failing organs. Given time, effort, as well as passion, and funding this technology will probably provide potential cure for many diseases.
Professor Dr. Murali D Kuppusamy Naidu is currently the Head of Department of Anatomy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. He obtained his BDS and MMedSc from University of Malaya and PhD from University of Cambridge, United Kingdom.

His area of specialization is Anatomy and Neuroscience. Recently, he has published his work in various journals including Frontiers in Pharmacology, PLOS One and Scientific Reports. Some of the publications include the antipsychotic-like effect of Mitragyna speciosa in mice, the neurite outgrowth stimulatory effects of uridine from Pleurotus giganteus and the neuritogenic activity of Lignosus rhinocerotis (Cooke) Ryvarden via MEK/ERK1/2 signaling pathway in PC-12 cells.

Abstract

Major Challenges in Teaching and Learning Anatomy

Professor Dr Murali Naidu

It cannot be denied that a proper knowledge in Human Anatomy is vital for any doctor, dentist, surgeon and other medically related professions. Anatomy is not only a visual science, but it is also a language and backbone of medicine. Over the many years various methods of teaching and learning anatomy were used together with full body dissection, prossection, dry and multi medium aids. Various curriculum such as traditional, integrated, system-based, problem-based, case-based etc were used in many medical, dental, and biomedical schools to attain the best anatomy learning outcomes. Many challenges are encountered along the way, including student, teacher, assessment, resources, organizational and society related issues. In this talk the main challenges are listed and discussed.
Dr Sharida Fakurazi, is an Associate Professor in Universiti Putra Malaysia, where she started her teaching and research career in this university back in 2001, after graduating from Imperial College of Science, Technology and Medicine in Biochemical and Molecular Toxicology. Her initial training was in Pharmacology which was in University of Dundee, Scotland, United Kingdom.

Dr Sharida believes that teaching and learning are processes which occurs throughout life. Looking throughout life dimension, her philosophy towards teaching is largely influenced by life experience. Being a person and also an educator, she is intrinsically and extrinsically motivated to inquire, infer, and interpret; to have a reflective thinking ability, able to become critical and creative; and has the ability to make final analysis utilising the knowledge and skills to become effective decision makers. She was once before engaged in post such as the Head of Department and Head of Laboratories, which has earned her more than 8 years of management experience.

Her research interest focuses in development of plant products specifically looking at hepatoprotective, wound healing, anti-inflammatory and anticancer properties. In addition, Dr Sharida is also looking into the development of drug nanodelivery system particularly looking into drug uptake, absorption and cytotoxicity. Embarking into research work is fairly very exciting especially when the research findings are able to be utilised by the general publics and nations. Her viewpoints about research is when the work contributes to at least, advancement of knowledge or importantly when, the findings and innovations are able to generate income to the institution or having economic impact, or contributes to societal wellbeing. She believes the most important commodities would be high quality students and candidates that an institution produces have reached a certain standards and morals. The ultimate goal of research activities is to create an ecosystem to train a student to be able to buckle down, explore imperative avenue within limited resources, and not compromising quality and principles. Having those in mind, Dr Sharida has continuously taken the initiative to publish her findings in local and internationally reputable journal and present her work in conferences and symposiums.
PROFESSOR DR. DAUD AHMAD ISRAF ALI
Universiti Putra Malaysia, Malaysia

Daud Israf is a Professor of Biomedical Science at the Faculty of Medicine & Health Sciences, Universiti Putra Malaysia. He has extensive experience in research on the immunopharmacology of anti-inflammatory compounds and extracts. He is currently interested in understanding the molecular mechanism of airway remodelling in asthma and the use of novel compounds in its prevention and reversing the condition.

Abstract

Blocking Smooth Muscle Cell Proliferation with a Synthetic Geranyl Acetophenone

Prof. Dr. Daud Ahmad Israf Ali

Smooth muscle proliferation is an integral component of tissue remodelling in chronic inflammatory airway disease. Airway remodelling in asthma is irreversible and remains a major challenge in the management of this disease. We have shown suppression of airway remodelling in a murine model of allergic asthma following oral dosing with tHGA, a synthetic geranyl acetophenone. In this presentation, we show the effects of tHGA upon proliferation and migration of human airway smooth muscle cells and demonstrate the molecular target of this compound.
Dr. Ivan Yap Kok Seng received his PhD in Chemistry from Imperial College London. He is currently the Head of the Centre for Translational Research, Institute for Research, Development and Innovations at the International Medical University, Malaysia. He also heads the School of Pharmacy and Health Sciences.

He received numerous awards including the Overseas Research Scholarship 2002 – 2005, the E de Barry Barnett Prize (Chemical Sciences) for Outstanding Achievement in 2001 and the British Scholarship Scheme in 1998.

His research interests include areas of metabonomics and chemometrics, human health, gut microbial-mammalian metabolic interactions, nutritional interventions, environmental toxicology, parasitology and diseases, large-scale epidemiological research in cardiovascular diseases and application of metabonomics in epidemiology. Dr Ivan is also the recipient of numerous private and public research grants and has authored over 30 articles in prominent journals.

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**Abstract**

**Systems Biology in Biomedical Research**

*Dr. Ivan Yap Kok Seng*

Translational research involves a holistic understanding into human health incorporating global systems biology which attempts to integrate information from the genome, proteome, metabolome, metagenome as well as their interactions with the environment. Such complex biological and interactive structures within the host with the environment and population sociodemographics necessitate the use of advanced technology and expert machine learning tools that allow scientists and clinicians to tease out relevant information that are easily interpreted and improve patient point of care. This talk will cover the application of integrated metabonomics and metagenomics in biomedical sciences research. The results from these studies indicated that integrated systems biology together with extensive multivariate statistical analyses assisted in extraction of useful biochemical information, which helps illuminate the mechanistic pathways linking dietary pattern with risk of cardiovascular disease and parasitic infections and highlighted the involvement of the gut microbiota in disease pathology.
Plenary Speakers

PROFESSOR DR. YUEN KAH HAY
Universiti Sains Malaysia, Malaysia

Prof Yuen Kah Hay obtained his B.Pharm.(1st class Hons) and M.Sc. from Universiti Sains Malaysia and Ph.D. from University of London. He has been lecturing at the School of Pharmaceutical Sciences, Universiti Sains Malaysia for 37 years. To date he has successfully supervised over 60 postgraduate students at the master's and Ph.D levels, and published over 130 research papers in international journals and another 20 in regional and local journals.

He has been a consultant to a local pharmaceutical company, Hovid Bhd since 1992, where he leads a team in drug formulation and product development. He is also currently heading a BE center in conducting bioequivalence studies for the pharmaceutical industry and also the lead investigator of several clinical studies investigating the neuroprotective effects of palm vitamin E tocotrienols. He has retired from the university on 31st December 2016, but stayed on as honorary professor and has been appointed as executive director of Hovid Bhd since 2nd January 2017.

Abstract

Challenges in the Development of Generic Drug Products

Prof. Dr. Yuen Kah Hay

Formulating a generic tablet product today has become very complex compared to yesteryears. In the past, a generic product has just to fulfil certain established pharmacopeia requirements but today it must also be shown to be bioequivalent to the innovator product. Many factors can influence drug absorption/bioavailability and hence formulating a bioequivalent generic product has become a huge challenge. Moreover surrogate measures such as dissolution and disintegration may not be sufficiently predictive of in vivo performance. The introduction of combination products containing 2 active ingredients has caused further complications. Achieving bioequivalence with a single molecule is already a big challenge, to attain bioequivalence with 2 molecules in a single tablet is much more difficult. There is also the issue of suprabioavailability. A generic product must not be inferior but neither can it be better than that of the innovator. If it is better formulated with superior bioavailability than the innovator product, then it will fail the acceptance criteria for bioequivalence. Generic products can only be launched upon expiry of the innovator molecule patent. However, innovator companies can also file additional patents to prevent entry of generic products into the market. These patents can cover for example, product formulation, crystal form of the drug and dosage regimen. In this presentation, all the above issues will be discussed and illustrated with real examples as well as measures to counter them.
Professor Rozita Rosli currently heads the UPM-MAKNA Cancer Research Laboratory at the Institute of Bioscience, UPM where she initiated the translational genomics research program which focuses on application of new molecular technologies in the identification of genetic and epigenetic alterations of various pathways of carcinogenesis or the cancer microenvironment of common cancers, especially that of the Asian-specific genetic variation. She previously served as Deputy Dean for Research and Graduate Studies (200–2010) at the Faculty of Medicine and Health Sciences, UPM. She received her tertiary education in the United States, obtaining her undergraduate degree in Biology from Purdue University, her Masters and doctoral degree in 1986 and 1994 respectively from Ball State University where her laboratory skills in molecular biology was honed. She subsequently pursued her post-doctoral training at the Indiana University School of Medicine in the area of Hematology/Oncology where her interest in cancer research developed.

Her current research interest mainly lies in the application of genomics and proteomics as tools in understanding and combating genetic diseases, especially cancer as well as infectious diseases. Together with her graduate students, her work has led to the publication of more than a hundred and thirty research articles, the development of DNA vaccines, molecular diagnostic tools, the production of therapeutics from natural products and a number of related patents.

**Abstract**

**Breast Cancer and Lymphedema: Understanding the Connection**

*Professor Dr Rozita Rosli*

Breast cancer is the most common cancer diagnosed in women globally, and in Malaysia. Although the incidence of breast cancer has steadily increased over the last couple of decades, the overall five-year survival rate has also increased. This increase in the number of survivors gives rise to more patients with treatment-related side effects and one of these is breast cancer-related lymphedema (BCRL). In general, the incidence of BCRL varies depending on methods of measuring the lymphedema and different treatments for breast cancer such as axillary dissection and post-surgery radiotherapy to the breast and axilla. In Malaysia, the incidence and risk factors of BCRL is unknown. Lymphedema occurs when the draining of lymphatic fluid ceases to work, and fluids accumulate in the tissue resulting in swelling of the affected area. Lymphedema may occur within days and up to 30 years after breast cancer treatment. Despite the morbidity and costs of lymphedema, the mechanisms that regulate its development remain largely unknown. It remains unclear for instance why some patients develop lymphedema and others who are identically treated do not. This lecture will highlight the relationship between breast cancer and lymphedema and draw on several studies to understand the connection. Understanding the cellular and molecular mechanisms underlying the lymphatic development of BCRL may contribute towards a strategy for early identification and risk stratification of patients likely to develop lymphedema and is envisioned to facilitate targeted therapies that would improve the quality of life of these breast cancer survivors.
Dr Hamid Jan is Associate Professor at the Nutrition and Dietetics Programme, School of Health Sciences, Universiti Sains Malaysia (USM). He joined this institution in year 2007, soon after completing his PhD (Nutrition) at University of London, United Kingdom. He holds a Masters Degree in Nutrition from Universiti Kebangsaan Malaysia and Bachelor Degree in Nutrition and Community Health from University Putra Malaysia. He received extensive training on laboratory skills from the Diploma in Medical Laboratory Technology Program at USM.

The diversity of his education background contributed towards the creation of several interesting research related to nutrition and disease in Malaysia. He initiated the first pregnancy cohort in Malaysia named the USM Pregnancy Cohort Study which started in year 2009. This pregnancy cohort is aimed at investigating the role of maternal nutrition, oxidative stress and adipokines in the development of obesity and diabetes. Preliminary findings of this study are available in several local and international journals. In addition to teaching undergraduate students, he has successfully graduated 2 PhD and 10 Master (MSc) students and currently supervising 7 PhD and 2 MSc students. Dr Hamid is a Fellow Member of Nutrition Society of Malaysia and Life Member of the Malaysian Association for the Study of Obesity since 2008, and Member of the Malaysian Endocrine and Metabolic Society.

He is actively involved in obesity and non-communicable diseases prevention/management activities by giving public lectures on healthy lifestyle to communities and researchers. He also works closely with the Ministry of Health Malaysia by being committee member of the Technical Working Group for Nutrition Guidelines. He was one of the editorial board member and author of the Malaysian Dietary Guideline for Children and Adolescence.

**Abstract**

**Vitamin D: Beyond the Skeletal System.**

*Hamid Jan B. Jan Mohamed*

Vitamin D is once named as vitamin of the millennium. The dominant function of Vitamin D in its hormonal/active form (calcitriol or 1,2,5-dihydroxyvitamin D) is with the skeletal system. However, it is noteworthy that the vitamin D receptor (VDR) is present in the nucleus of many tissues that are not involved in the regulation of calcium and phosphate metabolism. For example, the VDR has been clearly described in epidermal keratinocytes, in activated T cells of the immune system, in antigen-presenting cells, in macrophages and monocytes, and in cytotoxic T cells. Hence, with the discovery of VDR on non-skeletal tissue systems, lots of research has been focused at the non-skeletal chronic disease outcomes such as diabetes, cancer, cardiovascular disease and metabolic syndrome. Vitamin D is a unique vitamin as the only nutrient that can be activated by non-dietary source which is the sunlight. Despite the presence of abundant sunlight in Malaysia, high prevalence of vitamin D insufficiency has been reported among Malaysian population. This presentation will share current status of vitamin D among Malaysians, findings of the Universiti Sains Malaysia (USM) Monsoon Study and literatures on the association between vitamin D and chronic diseases such as cancer and metabolic syndrome.
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Comparison of anxiolytic effects of the homeopathic complex Vita-C 15 with *Aconitum Napellus* versus diazepam in the acutely stressed C57BL6 mice

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Anxiety, phobias and stress are the main mental health problems among the Malaysian population, with global prevalence varying from 8% to 18%. Even so, less than 30% who suffer these disturbances seek treatment. The objective of this study is to evaluate and compare the anxiolytic effects of *Aconitum napellus* and Homeopathic complex Vita-C 15 in the acutely stressed C57BL6 mice by using the fecal and serum corticoid test, and open field test (OFT). A double blinded randomized controlled study was conducted at SPF animal facility of Brain Research Institute Monash Sunway (BRIMS) - Jeffrey Cheah School of Medicine and Health Sciences of Sunway University Malaysia. All the animals were acclimatized to constant laboratory conditions for 14 days before starting the experiments. The treatments were carried out over 7 days. 48 male C57BL6 mice (n=6), 4-5 weeks of age were used. They were randomly selected and divided into two groups. Group I was the healthy control group of mice which were not exposed to acute stress. Group II (stress group); comprise of mice expose to acute restraint stress. Prior to restraint stress, the treatments given were *Aconitum napellus* 30 cH, Homeopathic complex Vita-C 15, diazepam, and placebo. Then the results were evaluated by fecal and serum CORT test and open field test by comparing the anxiolytics effects between pre-test and post-test. The results showed higher levels of serum CORT and a significant increase in FCM than CON animals in acutely stressed animals on Day 7 (p<0.05). Acutely stressed animals demonstrated treated mice spent more time in the center had more entries into the center of the open field (p<0.001) and more active as measured in distance traveled in the center and traveled greater distance overall (p<0.001). Thus research into prevention and supportive therapies is necessary and beneficial for this disorder.

**Keywords:** Anxiolytic, homeopathic complex, *Aconitum Napellus*, diazepam, acutely stressed C57BL6 mice
OA2

Acute toxicity of intravenous administered thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) in *Sprague dawley* rats

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Thymoquinone (TQ), a major active compound of *Nigella sativa*, has been reported to exhibit wide range of therapeutic benefits such as anticancer, anti-inflammatory and antioxidant. However, its hydrophobic properties lead to poor bioavailability and solubility that affect its effectiveness in clinical use. In our previous studies, TQ has been encapsulated in a nanostructured lipid carrier (NLC) (referred as TQ-NLC), which is believed to enhance its solubility and efficacy. Nanoparticles are usually administered via intravenous injection to ensure systemic delivery of the drug for systemic action. This study determined the acute toxicity of intravenous administered TQ-NLC in *Sprague dawley* rats. Briefly, ten female *Sprague dawley* rats of 140-280 g in weight and age of 10 weeks were randomly assigned into two groups (n=5), which were the control group and treatment group at one fixed dose of TQ-NLC (25 mg/kg body weight). The control group received normal saline. The treatments were via intravenous administration on the first day only. The rats were monitored for 14 days for any changes that deprived from normal physical conditions including behaviour and mortality. The body weight was measured every 3 days. Upon completion, the rats were sacrificed and important organs such as the liver, lung, spleen, kidney and heart were collected for histological analysis. Blood was also collected for hematological analysis and biochemical analysis of liver and kidney functions. Based on the data, a single intravenous administration of 25 mg/kg of TQ-NLC did not cause mortality but resulted in inflammation of the rats’ tails. There were no significant changes in the body weight and organ-to-body weight ratio of the group treated with TQ-NLC as compared to the control (P>0.05). There was no sign of behavioral abnormality in the TQ-NLC-treated rats. There were no significant changes (P>0.05) in the hematological and biochemical profiles except for a decrease in the AST level in the rats treated with 25 mg/kg of TQ-NLC compared to the control group (P<0.05). The intravenous administration of TQ-NLC did not cause toxicity to the rats.

**Keywords:** Thymoquinone-loaded nanostructured lipid carrier, acute toxicity
OA3

Enhanced antigen presentation and co-stimulatory molecule expression of mice bone marrow derived-dendritic cells using silica colloid and iron oxide nanoparticles

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Nanoparticles (NPs) have been utilized to target dendritic cells (DCs), the sentinels or immune response, as they are capable of activating bone marrow derived-dendritic cells (BMDCs) that lead to DC maturation, however the precise physiological mechanisms involved are unclear. In this study, we have synthesized and characterized two types of NPs namely silica colloid (SiO₂) and iron oxide (Fe₃O₄) NPs, to elucidate the morphology and activation status of BMDCs upon exposure to NPs and also to determine the uptake and intracellular localization of both NPs by BMDCs. The spherical SiO₂ and Fe₃O₄ NPs synthesized have shown effective internalization of these NPs into the enclosed membrane of BMDCs, observed through scanning electron microscopy. Flow cytometry analyses showed increased expression of DCs surface markers; namely MHCII, CD86 and CD11c in the BMDCs that were exposed to Fe₃O₄ NPs compared to the BMDCs without NPs exposure (P<0.0001), indicating activation status. Furthermore, the intracellular localization of NPs into the membrane of BMDCs was confirmed by confocal microscopy. Whilst these results revealed several promising aspect of SiO₂ and Fe₃O₄ NPs as a future candidate for an effective therapeutic applications, these findings warrant further research before they can be integrated into the development of future drug delivery agent, vaccine and immunotherapeutic treatments.

Keywords: Nanoparticles, dendritic cells, maturation, T cells, cytokine
**OA4**

*In silico and in vitro* investigations of alpha-glucosidase inhibitory activity of phenolic constituents of *Tetracera indica*

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*Tetracera indica* leaves are used in traditional medicine for the treatment of diabetes mellitus in Malaysia. This study was aimed at investigating the phytochemical constituents present in *T. indica* leaves and their activity against digestive enzyme (i.e., alpha-glucosidase) which may be part of the mechanism of the antidiabetic effect of the plant. Methanol extract from the leaves of *T. indica* which demonstrates significant alpha-glucosidase inhibitory activity was separated and purified by silica gel, ODS and sephadex LH-20 column chromatographies, the structures of the isolated compounds were identified by UV, 1H-NMR, 13C-NMR and MS spectroscopic techniques. Five aglycones of flavonoids were isolated from the *T. indica* methanol leaves extract, and these compounds were identified as 5,7-dihydroxy-8-methoxyflavone, 5,7,8-trihydroxyflavone, 3′,4′,3,5,7-pentahydroxyflavone, 5-hydroxy-7-methoxyflavone and 4′,3,5,7-tetrahydroxyflavone. The isolated compounds show moderate to strong *in vitro* inhibitory activity against alpha-glucosidase. *In silico* studies using molecular docking show the mode of interactions of the isolated compounds with the active site residues of alpha-glucosidase and their binding energies. The results of this study reveal that alpha-glucosidase inhibition is one of the primary modes of action of *T. indica* antidiabetic effect that further support the traditional usage of *T. indica* leaves in the management of diabetes in Malaysia.

**Keywords:** *Tetracera indica*, phytochemical constituents, alpha-glucosidase, *in vitro*, *in silico*
Osteoarthritis (OA) is a progression of degenerative joint disease which can cause disability. Previous study shown that thickness of subchondral bone plate (Sbp), trabecular bone (Tb) and calcified cartilage (CC) increases along the progression of OA. However, none of these studies provide evidence of their association, especially during early stage of OA. Thus, this study aims to determine the association between the CC and Sbp thickness during the development of OA in Dunkin Hartley (DH) guinea pigs. Micro-computed tomography (Micro-CT) analyser software was used to measure the Sbp thickness (Sbp Th) in medial and lateral side of DH tibia at 10, 20 and 30 weeks of age. Histological analysis was used to determine the thickness of CC, uncalcified cartilage (unCC) and total articular cartilage (AC), and assess severity of the AC degeneration. The results showed that the Sbp [Medial, p = 0.001; Lateral: p = 0.001] and CC [Medial, p = 0.013; Lateral, p = 0.005] was thicker, but the unCC [Medial, p = 0.004; Lateral, p = 0.004] was thinner, in the medial than lateral side of DH tibia at all time points. In the medial side, a significant increase of Sbp Th and CC Th was observed between 10 and 20 weeks of age [(Sbp Medial, p = 0.01; Sbp Lateral, p = 0.01), (CC Medial, p = 0.045; CC Lateral, p = 0.014)]. In contrast, the unCC thickness was decreased with ageing. Since these changes were significantly associated with the AC degeneration, it may suggest that these events play an important role during the initiation and progression of OA.

Keywords: Osteoarthritis (OA), subchondral bone plate (Sbp), articular cartilage (AC)
OB2

A study on anthropometric measurement of the foot amongst undergraduate students

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The foot anthropometric data provides highly important information for anatomist, forensic scientist, physical anthropologist, health science, sports science and medical science professionals and also footwear industrial personnel. The anthropometric variations not only depend on genetic inheritance, but also differs based on environment, geographical demarcations, ethnicities and cultures. The study aims to determine the differences in the foot dimensions between right and left foot of each gender, the relationship of foot dimension with height, body weight and the foot dominance with genders. The study included 227 undergraduate Malaysian students of AIMST University, age ranged from 18-25 years. A total of ten parameters were taken that includes height, weight, dominant foot, foot length, arch length, foot breadth, heel breadth, heel-ankle circumference, mid-foot circumference and metatarsophalangeal joint circumference. All the measurements were carried out by using standard equipment, techniques and procedures. All the data were analyzed with SPSS trial. The socio-demographic data and the foot dimensions of the participants were described with descriptive analysis. The results showed that there were significant differences in some of the foot dimensions between right and left foot of each gender, significant correlation between all foot dimensions with height and weight, but no association between gender and the dominant leg, significant bilateral foot asymmetry and sexual differences for some parameters among with majority of them presented with the right foot dominance. The study showed a strong relationship between stature and body weight with foot dimensions and no association between foot dominance with gender. The anthropometric data obtained not only help to establish the individual profile of the university student but also it will be of great value in practical applications this field.

Keywords: Anthropometric, foot, gender
Chronic photoperiod disruption does not increase sensitivity to transient focal cerebral ischaemia in spontaneously hypertensive rats

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Photoperiod disruption (PD), which occurs during shift work, is associated with adverse health consequences (Vyas et al., 2012) that have the potential to affect stroke outcome (McCabe et al., 2009). We have shown that PD in young healthy rats did not alter sensitivity to permanent focal cerebral ischaemia (Ku Mohd Noor et al 2016). In humans, stroke typically occurs as a result of the cumulative effects of pre-existing risk factors (i.e. hypertension, diabetes) therefore we investigated the consequences of PD on sensitivity to experimental stroke in the presence of pre-existing hypertension. Adult male spontaneously hypertensive (SHR) rats were housed singly under two different dark light cycle conditions (n=20 each). Controls: standard 12:12 light dark cycle for 9 weeks, PD: 6 hour phase advance of light on time every 3 days for 9 weeks. Locomotor activity was monitored continuously by cage-top infrared movement sensors. At the end of the 9 week protocol all rats underwent transient middle cerebral artery occlusion (tMCAO; 30 min). Acute diffusion weighted imaging (DWI) was carried out at 25 min post-MCAO with reperfusion at 30 min. Day 7 infarct was evaluated by T2 MRI. Data presented as mean ± SD. Initial lesion volume at 25 min post-MCAO was not statistically different between groups (288.4 ± 35.8 mm³ vs 280.6 ± 52.9 mm³) and similarly final infarct volume at day 7 was not different (191.0 ± 70.0 mm³ vs 172.3 ± 73.6 mm³). Reperfusion resulted in significant tissue salvage when assessing the reduction in lesion volume from 30 min to day 7 (33 ± 25% vs 37 ± 27%) however this was not significantly different between groups. Photoperiod disruption in the presence of pre-existing hypertension, a known stroke co-morbidity, does not influence sensitivity to transient cerebral ischaemia or acute lesion growth.

Keywords: Stroke, animal model, circadian rhythm, hypertension, focal cerebral ischaemia.
OB4

Antioxidant effects of some selected flavonoids: A structure-activity relationship based study

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To investigate the antioxidant and radical scavenging activities of some selected flavonoids with respect to identify key positions responsible for antioxidant effects as well as the effect of derivatisation on the antioxidative effects. Antioxidant potential was evaluated using different sets of assays viz., rapid test by dot blot, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, ABTS+ radical cation scavenging, ferric reducing antioxidant powder (FRAP) and xanthine oxidase inhibitory (XOI) assays. It was determined that the total number and the configuration of hydroxyl group play an important role in regulating bioactivity of flavonoids in scavenging DPPH radical, ABTS+ radical cation and FRAP assays. Presence of catechol and the absence of C-2-C-3 double bond as well as ketonic group at C-4 reduced the xanthine oxidase inhibitory activity. Methylation and acetylation of hydroxyl groups at particular positions were also found to decrease the in vitro bioactivity of flavonoids. The results of this study will further help to understand the role of flavonoids as natural antioxidants which might facilitate in the development of nutritional products and semi synthetic analogs that retain substantial antioxidant capacity with minimal adverse effects.

Keywords: Flavonoids, antioxidant activity, derivatisation, structure activity relationship
Beet bread improves cardiovascular disease risk factors and oxidative stress status in a high fat diet-induced obese animal model

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To determine the effects of beet bread on cardiovascular disease risk factors and antioxidant status in high fat diet (HFD)-induced obese rats. Thirty-two (32) male Sprague-Dawley rats were divided into 4 groups (n=8/group) i.e. normal (on normal diet), HFD (on HFD), HFD+BB (on HFD and beet bread at 0.5 g/kg/day orally) and HFD+O (on HFD and orlistat at 10 mg/kg/day orally) groups. After 6 weeks, Lee index and serum lipid profiles were measured. Aorta was dissected for assessments on oxidised low-density lipoprotein (ox-LDL) level, antioxidant enzymes activities and the presence of atherosclerotic plaque. Lee index and levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and ox-LDL were significantly higher while activities of glutathione peroxidase (GPx) and catalase (CAT) were significantly lower in HFD group compared to normal group. However, Lee index and levels of TC, LDL-c and ox-LDL were significantly lower while activities of SOD and GPx were significantly higher in HFD+BB compared to HFD group. Levels of LDL-c and ox-LDL were significantly lower while SOD and GPx activities were significantly higher in HFD+O group compared to HFD group. Meanwhile, atherosclerotic plaque was present in HFD group only. This study suggests that beet bread supplementation at 0.5 g/kg/day for 6 weeks significantly protects against obesity, oxidative stress and atherosclerotic plaque formation possibly by improving antioxidant enzymes activities and its hypolipidaemic action in HFD-induced obese rats. This may indicate the potential use of beet bread in reducing the risk of cardiovascular diseases which needs further study as well as to determine its exact mechanism of action.

Keywords: Beet bread, cardiovascular disease risk, antioxidant
Bioassays activity and FT-IR analysis of *Clinacanthus Nutans* (Burm F.) Lindau leaves extracts

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The present study was designed to investigate the antidiabetic and antioxidant activities of the different solvents extracts of *Clinacanthus nutans* (Burm. F) Lindau leaves through different bioassays as well as to identify the functional group(s) responsible for the particular bioactivity through Fourier transform infrared (FT-IR) spectroscopy. Mature leaves of *C. nutans* were collected, oven dried at 40°C, powdered, and extracted in 80% hydro-methanol to obtain crude extract. This extract was then subjected to liquid to liquid partition to obtain hexane, ethyl acetate, butanol and aqueous extracts. All the extracts were then analysed for their bioactivity through various bioassays which include anti-oxidant (DPPH and FRAP) assay, xanthine oxidase and α±-glucosidase inhibitory assays. FT-IR analyses for the qualitative identification of bioactive compounds was then carried out to all the extracts. Bioactivity analyses of the plant’s leaves extracts on anti-oxidant (DPPH and FRAP) assay, xanthine oxidase and α±-glucosidase inhibitory assays revealed that the hexane extract was highly active against α±-glucosidase. Meanwhile ethyl acetate exhibited average activity in DPPH scavenging and xanthine oxidase inhibitory assays. Highest FRAP value was exhibited by ethyl acetate extract compared to others. Interestingly, FT-IR spectra analyses of each extract confirmed the presence of different functional groups that may have contributed to the various biological activities. The results of the present study produced the FTIR spectrum profile for the vulnerable medicinally important plant *C. nutans* (Burm. F) Lindau that further confirms its medicinal values. Hence, *C. nutans* leaves extract is medicinally potent that may serve essentially in the development of new pharmaceuticals for natural plant-based medicine.

**Keywords:** *Clinacanthus nutans* (Burm. F) Lindau, DPPH, FRAP, xanthine oxidase, α±-glucosidase, FT-IR
Evaluation of antioxidant activity of *Momordica Charantia* using LC-MS metabolomics approach

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The present study was designed to identify the significant biomarkers from *M. charantia* that possess antioxidant effect using liquid chromatography-mass spectrometry (LC-MS) based metabolomics approaches. Initially, the fruit was extracted by soaking in different solvents with different concentrations of ethanol in water (0, 20, 40, 60, 80, 100%, v/v). Then, the extracts were tested for antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assays (FRAP). LC-MS based metabolomics approach correlated with multivariate data analysis was applied to profile the bioactive compounds present in the extract. The 80% ethanol extract showed the highest inhibitory activity on DPPH and FRAP. LC-MS based metabolomics approaches helped to identify several antioxidants in this extract such as ascorbic acid, margarolic acid, brevifolin carboxylic acid, quercetin 3-O-glycoside, kuguacin H, cucurbitacin E, 3-malonylmomordicin I, goyaglycoside G. In the current study, LC-MS based metabolomics approach was found to be effective in identifying the particular secondary metabolites of *M. charantia* fruit that could be responsible for its antioxidant effect. This finding might further help to validate natural medicines based on *M. charantia* fruit.

**Keywords:** *Momordica charantia*, LCMS, metabolomics, antioxidant, multivariate data analysis.
OC4

Curcumin analogues inhibit oxidized-1-palmitoyl-2-arachydonoyl-sn-glycero-3-phosphorylcholine-induced proinflammatory chemokines and XBP-1 activation in U937 macrophages

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The present study aimed to investigate the effects of curcumin analogues against oxidized palmitoyl-arachidonoyl phosphatidylcholine (OxPAPC)-induced proinflammatory chemokines and XBP-1 protein expression in PMA-differentiated U937 macrophage. Concentrations of curcumin analogues that cause more than 90% cell viability were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Effect of curcumin analogues on OxPAPC-induced interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) were carried out using enzyme-linked immunosorbent assay. Moreover, the effect of analogues on OxPAPC-induced X-box binding protein-1 (XBP1) expressions were analysed by western blotting. Curcumin analogue, 2,5-bis[4-(methylaminoethanol) benzylidene] cyclopentanone), significantly inhibited OxPAPC-induced IL-8 production with 73.46 ± 2.00% and exhibited the lowest IC50 value of 7.33 ± 2.81 µM. On the other hand, (1Z,4Z)-1-5-bis[4(tert-butyl) phenyl]-1,4-pentadien-3-one significantly inhibit OxPAPC-induced MCP-1 production (66.76 ± 1.71%), while the lowest IC50 value was achieved by compound 2,5-Bis(2,3-dimethoxybenzylidene) cyclopentanone) (12.98 ± 1.97 µM). All curcumin analogues tested, except (1Z,4Z)-1-5-bis[4(tert-butyl) phenyl]-1,4-pentadien-3-one, inhibited OxPAPC-induced XBP1 protein expressions in a concentration-dependent manner. These results suggest that curcumin analogues may inhibit proinflammatory chemokines and XBP1 signaling pathway, both of which are important in the pathogenesis of atherosclerosis.

Keywords: Curcumin analogues, OxPAPC, chemokines, XBP1, atherosclerosis
Molecular insight of a geranyl acetophenone in IgE-mediated mast cell activation of allergy

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Mast cells are important effector cells of the innate immune system. Upon activation, there will be a complex interaction between LAT and PI3K axis signalling pathways causing the secretion of important proinflammatory mediators that contributes to allergy reactions. We have previously reported that tHGA, an active compound originally from a local shrub known as Melicope ptelefolia, exerts anti-allergic effects by attenuating IgE-mediated mast cell degranulation in both cellular and animal models. However, the underlying mechanism associated with the mast cell stabilizing effect of tHGA still remains unknown. This study aims to dissect the mechanism of action of tHGA in in vitro model of IgE-mediated mast cell activation as well as to identify the possible molecular target. RBL-2H3 cells were sensitized overnight with IgE followed by pre-treatment with tHGA for 20 minutes and challenged with DNP-BSA for 10 TO 15 minutes. The protein expression of signalling molecules including LAT, Syk, PLCγ, MAPKs, arachidonic acid-associated enzymes, PI3K and NFκB were analyzed by using Western Blot. The gene of speculated molecular target, LAT, was knockdown using short interfering RNA (siRNA). The inhibitory effect of tHGA on siRNA-treated cells were assessed by measuring the release of histamine, prostaglandins D2 and interleukin-4 by using ELISA or EIA kits. Pre-treatment of tHGA (1.25, 5 and 20 µM) significantly decreased the protein expression of all signalling molecules located in LAT axis pathway without affecting the upstream signalling molecule of Syk. On the other hand, tHGA only exhibited partial inhibitory effects in PI3K axis pathways. The findings collectively indicated that tHGA specifically targets LAT. This speculation was further confirmed by the findings that tHGA failed to decrease the release of all mediators in LAT-deficient challenged cells. LAT plays an important role in the mast cell stabilizing effect of tHGA. Thus, it may have the potential to be developed as a mast cell stabilizer which specifically targets LAT.

Keywords: tHGA, IgE-mediated mast cell activation, allergy, RBL-2H3, siRNA
Antinociceptive activity of *Dicranopteris linearis* leaves methanolic extract and its partitions: Elucidation of the mechanisms of action

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Despite rapid advancements in the field of pain and the development of various treatments for pain, the clinical efficacy and tolerability of conventional analgesics can be overshadowed by unwanted side effects. Thus, as an attempt to contribute to the drug discovery and development of new analgesics, this study was carried out to investigate a neglected fern called *Dicranopteris linearis* or locally known to the Malays as “Resam”. It is a plant that has been used in the Malays’ traditional medicine to reduce body temperature and to control fever. Scientifically it has been reported to have antioxidant, antiproliferative, anti-inflammatory and other beneficial pharmacological activities. The objective of this study was to investigate the antinociceptive activities of *Dicranopteris linearis* leaves crude methanolic extract and its partitions and more importantly to elucidate the possible mechanisms of action involved. *Dicranopteris linearis* leaves was extracted using methanol and further partitioned with petroleum ether, ethyl acetate and aqueous successively. Three antinociceptive models for mice were used in this study which were acetic acid-induced abdominal writhing test, formalin test and hot plate test (n=6). The most effective partition was found to be petroleum ether partition and it was also found to be involved in all different pathways tested which were capsaicin-induced paw licking test, glutamate-induced paw licking test, bradykinin-induced paw licking test, involvement of Protein Kinase C (PKC) test, involvement of opioid system test and involvement of L-Arginine/NO/cGMP pathway test. Results were obtained after being analysed using one-way ANOVA followed by Tukey’s test, and a value of $p \leq 0.05$ was considered as significant. As conclusion, the methanol extract and petroleum ether partition of *Dicranopteris linearis* were shown to possess antinociceptive activities and the mechanisms of action involved were also successfully determined.

**Keywords:** *Dicranopteris linearis*, antinociceptive, analgesics
The protective role of vitamin C on endothelial dysfunction in a rat model of REM sleep deprivation

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This study investigated the protective role of an antioxidant vitamin C in endothelial dysfunction in a rat model of REM sleep deprivation. Forty (40) male Sprague Dawley rats were equally divided into 5 groups: free-moving control rats (FMC), 72-h REM sleep-deprived rats (REMsd), REMsd pretreated with vitamin C (RVC), FMC pretreated with vitamin C (FVC) and tank control rats (TC). The rats were deprived of REM sleep using the inverted flowerpot technique. Plasma levels of oxidative stress markers (superoxide dismutase; SOD, glutathione reductase; GR, total antioxidants capacity; TAC and malondialdehyde; MDA) were measured using ELISA kits. The thoracic descending aortas were harvested and subjected to in vitro functional myograph study. Histological assessment of the thoracic descending aorta was also evaluated using scanning electron microscope (SEM). In REMsd rats, plasma levels of SOD were significantly lower compared to other groups. However, the results demonstrated no changes in other markers (TAC, GR and MDA). In myograph study, there was a significant impairment in endothelium-dependent relaxation with vasomotion observed in REMsd aortic ring, but not in other groups. In SEM study, the endothelium of the thoracic descending aortas in REMsd group demonstrated a rough surface, widening of the intercellular clefts and derangement of endothelial cells. However, the endothelium of rats from other groups was smooth with a regular arrangement of the endothelial cells. Administration of vitamin C has reduced the alteration in SOD and endothelium structure and function in a rat model of REM sleep deprivation suggesting of its protective role in the mechanism of endothelial dysfunction in REM sleep deprivation.

Keywords: REM sleep deprivation, oxidative stress, vitamin C, antioxidants, endothelial dysfunctions
Zerumbone protects against house dust mite-induced airway epithelial barrier disruption by preserving junctional permeability and localization

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Human airway system is lined by epithelial cells attached to each other by junctional system. This epithelial barrier can be disrupted by common allergen such as house dust mites (HDM) and consequently resulting in airway diseases such as asthma. Zerumbone was found to possess anti-asthmatic effect by modulating Th1/Th2 cytokines. However, there is yet study done to assess whether zerumbone protects the epithelial barrier from junctional disruption before the allergen invades into the immunological barrier of the airway system. To investigate the effect of zerumbone on HDM-induced airway epithelial barrier disruption. A human bronchial epithelium 16HBE14o-cell line, which possesses characteristics of in vivo human airway lining, was co-treated with 100 µg/mL HDM and three concentrations of zerumbone (6.25 µM, 12.5 µM and 25 µM) for 24 hours. Transepithelial electrical resistance (TEERµ) assay and FITC-Dextran permeability assay were carried out to study the effect of zerumbone on HDM-induced junctional integrity and permeability of the epithelial monolayer respectively. The localization of junctional proteins, occludin and ZO-1, was studied by using immunofluorescence (IF) while the protein and gene expression were studied by immunoblotting and Real Time-Polymerase Chain Reaction (qPCR) respectively. This study has proven that zerumbone preserves both HDM-induced junctional integrity and permeability by maintaining the localization of occludin and ZO-1 without affecting both proteins and gene expression. Zerumbone possesses protective effect on HDM-induced airway epithelial barrier disruption by preserving the junctional permeability and localization without affecting the junctional protein and mRNA expression.

Keywords: Tight junction, epithelial cells airway system, zerumbone, house dust mites, 16HBE14o-cell line
OD1

Promising anti-cancer activity of protocatechuic acid-zinc aluminium nanocomposite in N-nitrosodiethylamine/phenobarbitol-induced liver cancer in mice

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One of the major focuses in current cancer chemotherapy is the search for anti-cancer agent which selectively kills malignant cells and undamaging to the healthy neighbouring cells. Natural product provides relevant resources in anti-cancer drug discovery. One of most popular source of anticancer drug is from Pacific Yew tree to produce Paclitaxel (Taxol®) which has been widely used commercially to treat Kaposi sarcoma, breast, non-small cell lung, and ovarian cancer. However, the physicochemical properties of these compounds limit its uptake and bioavailability in cancerous cells. To counteract this issue, we have introduced a nanocarrier system namely, zinc-aluminium layered double hydroxide (ZnAl-LDH). In the present study, the intercalation of protocatechuic acid with zinc/aluminium-LDH (PCA-ZnAl) exhibited apparent cytotoxicity in diethylnitrosamine/phenobarbitol (DEN/PB)-induced hepatocellular carcinoma (HCC) in male Balb/c mice. Following induction, confirmation and treatment with PCA-ZnAl, samples were collected and analysis was made. Treatment duration of 4 weeks has significantly attenuated liver damage in animals induced with DEN/PB. There was restoration in hepatocytes morphology and it was comparable to the normal control group. Deterioration of hepatocellular histology and significant increment of ALT, AST and ALP enzymes were observed in induced group which was not treated with the nanoparticles. The level of liver damage was indicated by biochemical and histopathological analysis, and interestingly cancer marker level α±-fetoprotein was reduced from 512.05±40.83 ng/ml to 290.23±20.52 ng/ml and the histopathological features of the liver obtained from PCA-ZnAl treated mice were almost similar to normal. In this study, the synthesized developed PCA-ZnAl displayed higher potential anticancer activity compared to a commercial anticancer drug, doxorubicin in DEN/PB-induced HCC in our clinical model mice. Taken together, these observations suggested that the new PCA-ZnAl nanohybrid is able to prevent the progression of liver damage induced by DEN/PB.

Keywords: Protocatechuic acid, zinc-Aluminium nanocomposite, liver cancer
Identification of stem cell antigen-1 (Sca-1) on endothelial cells

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Stem cell antigen-1 (Sca-1) is a member of the Ly-6 family and phosphotidylinositol-anchored glycoprotein, found on the surface of several murine marrow stem cell subtypes, including, hematopoietic stem cells (HSCs), mesenchymal stem cells and multipotent adult progenitor cells, Hoechst side population (SP) cells. Sca-1 expression is also present in the parenchyma of nonhematopoietic tissues. Cardiac stem cells also expressed Sca-1. This study is to localize the Sca-1 expression in endothelium cells. Five to six-week-old male C57BL/6J mice were used in this study. Messenger RNA and Sca-1 protein were detected in bone marrow, lung and liver tissues by RT-PCR, and western blotting. Magnetic cell sorting and flow cytometry was done to identify the CD31 and Sca-1 positive cells in the heart. Electron microscopy, immunohistochemistry, immunofluorescence staining and in situ hybridization were done to identify the Sca-1 protein and RNA expression in endothelium. RT-PCR and Western blot analysis showed that Sca-1 was also expressed in adult murine heart. In situ hybridization of Sca-1 demonstrated that, Sca-1 mRNA is ubiquitously expressed in cardiac endothelial cells. Sca-1 immunohistochemistry revealed circular and linear staining patterns consistent with that of blood vessels. These Sca-1 positive cells coexpressed endothelial cell markers platelet and endothelial cell adhesion molecule 1 (PECAM-1) and Von Willebrand factor (VWF). By immunofluorescence staining, endothelial cells express both Sca-1 and oxytocin receptors. By combination of magnetic cell sorting and flow cytometry: about 2/3 of Sca-1 cells are endothelial cells and almost all the endothelial cells express Sca-1. These results indicated that Sca-1 antigen is a useful marker for endothelial cells in cardiac tissue and ubiquitously expressed in cardiac endothelium.

Keywords: Sca-1, heart, endothelium
Dual proliferative effects of Trigona honey on bone marrow-derived mesenchymal stem cells and MCF7 breast cancer cells


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Stem cells and cancer cells share a list of very similar molecular markers mainly due to their ability to self-renew. Previous data from our group revealed that certain foods especially honey, promote the proliferation of human bone marrow-derived mesenchymal stem cells in vitro. As both cells share some similarity, it is reasonable to evaluate this proliferation promoting effect in a cancer cell model. Hence, this study aimed to investigate the in vitro proliferative effect of Trigona honey on human bone-marrow derived mesenchymal cells (BM-MSCs) and the human breast carcinoma cell line, MCF7. BM-MSCs and MCF7 were cultured in basal medium, DMEM, containing 10% fetal bovine serum (FBS), 1% antibiotic antimycotic and 1% glutamax. Upon 80% confluence in 96-well plates, old media was replaced with basal media with 2% FBS and enriched with different percentages (v/v) of Trigona honey; 0.097, 0.195, 0.39, 0.78, 1.56 and 3.125. We measured the proliferation of BM-MSCs and MCF7 after 24 and 72 hours using MTT assays. Relative viability of the cells after normalisation with negative control (medium without honey) denote the proliferative properties of BM-MSCs and MCF7 after treatment. Our results suggest that when comparing both types of cells, MCF7 was more susceptible towards Trigona honey treatment. At increasing concentrations specifically between 0.39% and 1.56% there was a striking decrease in the viability of the MCF7 cells. On the other hand, at these concentrations BMMSC viability was comparatively more stable. These findings were based on a sample size of 3, hence more samples need to be tested to validate the results and its significance. However, through these results, we speculate that BM-MSCs and MCF7 respond differently towards honey treatment due to their cellular properties.

Keywords: Stem cell, Trigona honey, BM-MSC, MCF7, proliferation
Response of human karyopherin alpha 2 (Kpna2) promoter to oxidative stress

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Nuclear-cytoplasmic shuttling lies at the heart of the response to oxidants: its key regulator Nrf2 is predominantly cytoplasmic in naive cells but upon oxidative insult, translocates to the nucleus and activates genes encoding cytoprotective enzymes to eliminate oxidants. Other components of the transcriptional response to oxidative stress also translocate to the nucleus in a temporal manner during the stress response: Fyn kinase phosphorylates nuclear Nrf2 and triggers its translocation back to the cytoplasm for degradation while Bach1 competes with Nrf2 to down-regulate certain promoters. The karyopherin α± family of adapter proteins (6 in man) form a molecular bridge between these nuclear cargoes and the nuclear import machinery. The aim of this study was to delineate the molecular mechanism underlying transcriptional regulation of human karyopherin α±2 (KPNA2) gene upon oxidative stress. Using a reporter gene assay in the human hepatoma cell line Huh7, preliminary experiments demonstrated that the KPNA2 gene was responsive to oxidative stress inducers. In-depth analysis including promoter deletion, site directed mutagenesis and electrophoretic mobility shift assay revealed that the down-regulation of human KPNA2 expression by Wy-14,643 was elicited not by its cognate nuclear receptor, PPAR α ± but most likely via Nrf2 through the oxidative stress signaling pathway. We postulate that nuclear transport is disrupted by prolonged oxidative insult and that this will have a feedback effect on the localisation of Nrf2 and its co-regulators.

Keywords: Nucleocytoplasmic shuttling, human karyopherin α ±2 (KPNA2), oxidative stress
Amelioration of hypercholesterolaemia-induced aortic and hepatic histopathological changes with roselle

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The present study is to evaluate the improvement in liver and aorta histopathological of obese-hypercholesterolemic (OHC) rats treated with 4-weeks Roselle aqueous extract (RAE). Twenty-four Sprague-Dawley (SD) rats (200-250 g) were induced to be OHC with high fat diet (4% cholesterol) over 6 weeks. RAE (300 mg/kg) and control drugs (Orlistat as anti-obesity and atorvastatin as hypocholesterolemic drugs) were orally administered for 4 weeks (sub-acute) after OHC induction. The liver and aorta was dissected out and evaluated with histopathological studies including hematoxylin and eosin staining (H&E stain) and scanning electron microscope (SEM). H&E stained slide of the untreated-OHC rat's liver showed significant steatosis (acute fatty liver stage). In RAE treated-OHC rats, the liver structure was near-normal as in normal group. Disorganized tunica media layer was observed in the aorta of untreated-OHC rats. The aorta of RAE treated-OHC rats showed no morphological degeneration. In SEM, the liver surface of untreated-OHC rat appeared rough with fat cells, however it showed normal-like surface in RAE treated-OHC rats. There were numerous fat cells, lymphocytes and fibrin on the inner surface of untreated-OHC rat's aorta. In the RAE treated-OHC rat's aorta, the appearance is comparable to aorta of normal group. Roselle aqueous extract improved the histopathological changes of the liver and aorta secondary to obese-hypercholesterolaemic state.

Keywords: Roselle, hypercholesterolemia, histopathological, liver, aorta
Clinacanthus Nutans aqueous extract protects against systemic anaphylaxis via the IgG pathway

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Allergy is a hypersensitive reaction of the immune system against any foreign particle. World Health Organisation (WHO) reported that cases of allergic diseases such as rhinitis, asthma, eczema, food allergies, and anaphylaxis are rising especially in the last two decades. Anaphylaxis can be mediated via the classical pathway involving immunoglobulin (Ig) E and mast cells while IgG and macrophages are involved in the alternative pathway. Clinacanthus nutans (C. nutans), commonly known as ‘Sabah Snake Grass’ or ‘Belalai Gajah’ in Malaysia is a native plant found also in Thailand and Indonesia. It has been used traditionally to treat insect and snake bites, and skin rashes. As skin rash is a symptom of allergy, this study aims to elucidate the anti-allergic property of C. nutans aqueous extract in an anaphylaxis model and also whether its protection is exerted via IgE or IgG pathway. The anti-allergic property of C. nutans was assessed by measuring pre-formed mediators and cytokines involved in allergy through in vitro IgE-mediated mast cell degranulation and in vivo ovalbumin (OVA)-induced active systemic anaphylaxis (ASA), IgE- and IgG-mediated passive systemic anaphylaxis (PSA). The in vitro results showed that C. nutans aqueous extract could not reduce the release of histamine, interleukin-4 (IL-4) and leukotriene C4 (LTC4) in IgE-PSA at 500 mg/kg and 2000 mg/kg. The amount of IgE also was not reduced in OVA-ASA at all concentrations tested. Interestingly, there was significant reduction in the release of IgG, platelet activating factor (PAF) and IL-6. This was further confirmed in the IgG-PSA model where reduction of IL-6, no clinical anaphylaxis symptoms and stable rectal temperature were recorded at 2000 mg/kg. C. nutans aqueous extract may possess anti-allergic property and it is exerting its protection on anaphylaxis via the IgG-mediated pathway.

Keywords: Allergy, Clinacanthus nutans, OVA-ASA, IgE-PSA, IgG-PSA
Inhibitive effect of cardamonin upon proliferation of serum induced human bronchial smooth muscle cells

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Airway remodeling is defined as structural changes in the airways and is believed to be a major pathophysiological change observed in asthma. It is characterized by an increase in pulmonary smooth muscle mass contributing to airway narrowing and eventually airflow obstruction. Inhaled corticosteroids are less effective in preventing chronic structural changes in severe asthmatic patients. Cardamonin is a chalcone analog that exhibits anti-inflammatory and anti-proliferative properties. It has been reported to inhibit proliferation of aortic and vascular smooth muscle. However, its effects upon bronchial smooth muscle has not been determined. Cardamonin was assessed to determine whether it can prevent bronchial smooth muscle cell proliferation. The anti-proliferative effect of cardamonin was evaluated by assessing cellular proliferation, DNA synthesis, Ki-67 gene expression, and cell cycle disruption following induction of human bronchial smooth muscle cells (HBSMCs) with 5% Fetal Bovine Serum. Cardamonin demonstrated a dose-dependent inhibition of cell proliferation, Ki-67 gene expression, DNA synthesis and cell cycle at G1 phase. Cardamonin has the potential to suppress airway remodeling in asthma by inhibiting HBSMCs proliferation.

Keywords: Asthma, proliferation, human bronchial smooth muscle cells, serum, cardamonin
The effect of dental pulp stem cells in MPTP induced PD mice model

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Parkinson’s disease (PD) is an age related neurodegenerative disease that progressively damages the motor system following deterioration of dopaminergic neurons within the substantia nigra (SN). Unfortunately, the current available treatment options neither pharmacological nor neurosurgical are efficient in arresting the progression of the neurodegenerative processes. Stem cell based therapeutics, on the other hand, have offered promising hope for almost all forms of neurodegenerative diseases. Nevertheless, the optimization of stem cell efficacy requires delivery of the implanted cells to the site of neurological injury without significant effect on cell viability and function. Amongst the stem cells that has been unravelled and explored extensively over the past few years, dental pulp stem cells (DPSCs) has been proposed due to its excellent differentiating abilities and non-immunogenic properties. We first demonstrated the in vitro differentiation capacity of DPSCs towards dopaminergic like neurons before evaluating their neuroprotective abilities in MPTP-induced mice. Transplantation via intranasal was performed with behavioural assessments being evaluated every fortnight to measure olfactory and sensorimotor function. Tyrosine hydroxylase (TH) immunofluorescence was used to evaluate MPTP neurotoxicity in SN neurons. It was apparent that the behavioural parameters began to improve significantly corresponding to the tyrosine hydroxylase (TH) immunostaining in SN as early as 4 weeks post-transplantation (p<0.05). A significant amount of restoration was also recorded among the dopaminergic neurons of SN in the MPTP-treated mice after 4-weeks post-transplantation. Based upon our results, we have a strong reason to believe that the intranasal delivery of DPSCs optimized the therapeutic benefit of DPSCs in the PD mice model.

Keywords: DPSCs, MPTP, neuroprotective, intranasal, PKH26
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Improvement of diabetic wound healing by topical application of Vicenin-2 hydrocolloid film on Sprague dawley rats

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Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to lack of insulin or cells resistant to insulin. Impaired wound healing is one of the debilitating complications due to diabetes and leads to significant morbidity. It was reported that, the risk of developing diabetic foot ulcer for diabetics is between 15-25% during their lifetime and almost 85% of limb amputation is due to non-healing ulcer. Unhealed and gangrenous wound destroys the structural integrity of skin which acts as protective barrier to prevent invasion of external noxious agents to the body. Vicenin-2 (VCN) has been reported to contain prospective anti-diabetic, anti-oxidant, anti-inflammatory properties, and found to be able to enhance cell proliferation and migration. Sodium alginate (SA) is a natural polysaccharide possesses gel forming properties, with biodegradable and biocompatible characteristics. Therefore, the objective of this project is to develop a wound dressing based SA containing VCN to be tested on diabetic wound inflicted model. Initially, a prototype formulation is developed and screening via Scratch Test assay. Preliminary results suggested that VCN has promising effects on wound healing. The physicochemical properties are determined from the formulation, including verifying the drug content before proceeding with in vivo diabetic animal model. Wound is inflicted in streptozotocin (STZ) induced male Sprague Dawley rats, following verification of sustained hyperglycemic condition. Subsequently, the relevant group is treated with the developed formulation during the study duration, while the control group is treated with the appropriate dose vehicle. Wound tissues are collected when the study is terminated. Appropriate wound assessments are conducted to substantiate the observation that we have previously made, to confirm the efficacy of wound healing in the in vivo animal model.

Keywords: Vicenin-2, sodium alginate, hydrocolloid film, diabetic wound healing
P02

**Conditioned medium derived rat amniotic fluid stem cells accelerates wound healing in diabetic human dermal fibroblast cells**

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Numerous reports have been published on the substantial potential of conditioned medium (CM) isolated from stem cells improves various disease conditions. Some studies reported the beneficial effects on stem cell-derived secreted factors demonstrated to have many beneficial therapeutic effects in various diseases, such as neurodegenerative diseases, cancers, and heart failure. This study was conducted to yield and investigate the potential of CM or secretome derived from rat amniotic fluid stem cells (rAFSCs) and tested on in vitro wound healing model. The CM was prepared from rAFSCs; where the appropriate number of stem cells required was optimized either in the presence of Leukaemia Inhibitory Factor (LIF+) or absence of LIF (LIF-). Pluripotency of the cells is verified by observing the level of octamer-binding transcription factor 4 (OCT4) and NANOG. The presence and level of growth factors in the CM was identified and confirmed using Enzyme Linked Immunosorobent Assay (ELISA) and Western blotting technique. To substantiate the efficacy of CM, Scratch test assay and CCK-8 assay were conducted to observe effective cell migration and proliferation. The ELISA data have proven that the concocted CM contains vascular endothelial growth factor (VEGF), transforming growth factor beta-1 (TGF-α²), Interleukin 6 (IL-6), Interleukin 1 beta (IL-1α²) and Tumor necrosis factor alpha (TNF-α±). Meanwhile there has been an insignificant upregulation of VEGF and TGF-α² in CM regardless to the presence of LIF. These results was substantiated with the observation made in Scratch Test Assay and CCK-8 assay when CM LIF+ and LIF-significant accelerated wound closure; increased cellular activity and proliferate compared to the cells when incubated with non-CM. Our observation provided significant evidence on the potential of CM derived from rAFSCs and warranted further studies in understanding and development of CM to enhance wound healing.

**Keywords:** Rat amniotic fluid stem cells, conditioned medium, diabetic wound healing, growth factor, cytokines
Enhanced anti-inflammatory potential of protocatechuic acid intercalated with zinc/aluminum-ldh (Zal) on LPS-stimulated macrophages (Raw 264.7)

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Inflammation is a normal body respond towards infections or damaged tissues. Prolonged activation of inflammatory response is considered as the major cause of most chronic diseases. Protocatechuic acid (PCA) is a compound reported to have various pharmacological activities including anti-inflammatory action. However, conventional usage of PCA is restricted due to rapid elimination from the body, limiting effective utilization of the compound. Zinc/aluminum layered double hydroxide (ZAL) is a drug delivery system to which PCA was intercalated (PCA-ZAL) to specifically tailored to diseased tissue, increasing effective delivery of PCA. In this study, we are assessing the anti-inflammatory capacity of PCA intercalated to ZAL in LPS-stimulated macrophage (RAW 264.7). Cell viability following incubation with various concentrations of nanoparticles was assessed using MTT assay with relevant controls were included. The level of nitric oxide (NO) was determined using Griess reagent while inflammatory cytokines and prostaglandin E2 (PGE2) were measured via ELISA. Additionally, protein expression of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2), and nuclear factor kappa B (NF-κB) were detected via Western blot. Results demonstrated that PCA-ZAL significantly inhibited the production of NO, pro-inflammatory cytokine (IL-6, IL-1α, and TNF-α) and protein expression of iNOS, COX2, and NF-κB. The inhibition of inflammatory markers by PCA-ZAL was in most cases found to be similar to the level of reduction shown by non-intercalated PCA and in some cases better than positive control (dexamethasone). The anti-inflammatory cytokine IL-10 production was increased at 2.5 µg/ml PCA-ZAL while PCA did not increase IL-10 at any concentration. However, no significant effect was observed with the carrier system (ZAL). In conclusion, the intercalation of PCA with ZAL has improved the activity of the compound. Nanocarrier ZAL did not display any significant anti-inflammatory activity but rather served as a drug delivery vehicle.

Keywords: Inflammation, protocatechuic acid, zinc/aluminum layered double hydroxide, macrophage (RAW 264.7)
In-vitro comparison of anticancer activity between pure gallic acid and gallic acid - iron oxide coated with polyethylene glycol nanoparticles

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Cancer poses one of the biggest health threats in the world. A cancer report by the World Health Organization in 2012 claimed that lung cancer, breast cancer and colorectal cancer are the most common fatal cancers globally. Although existing cancer therapy protocols have shown to help cancer patients combat the disease, side effects of these therapies are detrimental to the body due to the affected healthy normal cells surrounding the malignant tumours, hence calling the need for a more specific and sustained delivery of effective anticancer drug. Gallic acid is a polyphenols that can be found in plants and foods such as blueberries and grape seeds. It has been reported to possess anticancer properties due to its cytoprotective activity and antiproliferative activity against cancer cells in dose-and-time-dependent manner. Here, we employ nanotechnology which enables delivery of chemotherapeutics directly to the cancer tissues while minimizing undesirable toxicity to the rest of the body. Gallic acid-iron oxide coated with polyethylene glycol (FGPEG) nanocomposite is developed, synthesized and characterised to ensure a more specific targeted gallic acid delivery and to increase the circulatory half-life respectively. The anticancer activity between pure gallic acid (GA), the nanocomposite (FGPEG) and iron oxide-PEG (FPEG) nanocarriers were screened against human lung cancer cells (A549), human breast cancer cells (MCF-7), human colon cancer cells (HT-29) and normal fibroblast cells (3T3) using the 3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay at 24, 48 and 72 hours and subsequently, the apoptotic activity between pure gallic acid (GA) and the nanocomposite (FGPEG) on human colon cancer (HT-29) cell line at 72 hours was observed using acridine orange propidium iodide (AO/PI) double staining. The IC₅₀ values of GA alone were 14.52 µg/mL for HT-29, 21.35 µg/mL for MCF-7 and 56.49 µg/mL for A549 cell line while FGPEG resulted in lower IC₅₀ values of 4.85 µg/mL for HT-29, 7.28 µg/mL for MCF-7 and 37.49 µg/mL for A549 cell line. Under fluorescence microscopy, treated cells had typical morphological changes that implicate apoptosis such as DNA fragmentation, membrane blebbing, and apoptotic body formation, which were evidenced by the visual bright green and orange colour along with the indication of dead necrotic cells which were stained bright red. AO/PI staining of HT-29 showed that the cells treated with FGPEG underwent remarkable morphological changes in apoptotic bodies compared to cells treated with pure gallic acid (GA) in all three drug concentrations (2,4 and 8µg/mL). Although both GA and FGPEG demonstrated cytotoxicity effect in a time and dose dependent manner in all cancer cell lines and were not toxic to 3T3 cells, FGPEG was more effective in inhibiting the growth of cancer cells than pure GA which displayed less cytotoxicity due to larger IC₅₀ values. Among the three cancer cell lines, HT-29 was the most responsive to FGPEG with the lowest IC₅₀ while A549
was the least responsive to the nanocomposite with the highest IC$ _{50} $. Our results demonstrate the advantage of polymeric magnetite nanoparticles in anticancer drug delivery over pure drug alone, forming a compelling justification for the utilization of this design as a platform for a safer, more specific and sustained drug delivery.

**Keywords:** Gallic acid, anticancer, superparamagnetic, dose-and-time-dependent, targeted drug delivery, nanoparticle
Cytotoxicity screening of functionalized graphene oxide designed for drug nanodelivery system

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Graphene oxide (GO) is one-atom-thickness, two-dimensional carbon nanomaterial contains reactive chemical functionalities such as hydroxyl, carbonyl, carboxyl, and epoxy group, which enable biochemo-functionalization, biocompatibility, and greater potentials biomedical application including drug and gene delivery. The objective of this work is to screen the cytotoxicity activity against cancer cell lines of functionalized GO nanocomposites loaded with protocatechuic acid (PCA) and chlorogenic acid (CA). The nanocomposites are coated with polyethylene glycol (PEG; GO/PEG) and chitosan (GO/CHI) biopolymer loaded with PCA and CA. The nanocomposites were then screened against normal 3T3 fibroblast cell lines, HepaRG hepatoma cell lines, Hep G2 hepatocellular carcinoma and HT 29 colorectal adenocarcinoma cell lines. Cytotoxicity experiments demonstrated that (Graphene Oxide with polyethylene glycol loaded Protocatechuic acid) GO/PEG/PCA mediated the lowest IC50 (29.84 ug/ml) compared to free PCA which was observed in HepG2. The viability of normal cells was not being influenced by all nanocomposites tested. In parallel, the nanocarriers were not affecting the cancer as well as normal cells. Cytotoxicity effect was also seen with PCA or CA alone in cancer cell lines, however, the respective nanocomposite showed a better activity. Our observations have suggested that GO/PEG/PCA is a suitable candidate to be further developed towards as a promising anticancer agent for hepatocellular carcinoma.

Keywords: Graphene oxide, polymer, protocatechuic acid, HepG2, hepatocellular carcinoma
Phytochemical screening and antioxidant activities of *Erythroxylum cuneatum* leaf extracts

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*Erythroxylum cuneatum* is locally known as ‘Chinta mula’, is known to have anti-inflammatory, iron-chelating, anticancer, and antifever properties. This study identified the active phytochemicals and antioxidant properties in various extracts derived from the dried leaves of *E. cuneatum*. Our pilot study has found that EC able to reduce inflammation in carrageenan-induced paw edema test. Soxhlet extraction was used to extract EC dried leaves in various solvents such as ethanol, acetone, hexane and aqueous. The tests of phytochemical screening included extracts in all solvents. The antioxidant activity was determined by measuring total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and hydrogen peroxide scavenging activity. The phytochemical screening revealed the presence of phenolic compounds, namely flavonoids, tannins and total phenols. Alkaloids and saponins were also detected. The antioxidant activity of the examined extracts varies depending on the solvent used. Generally, acetone extract showed highest total phenolic content with a value of 2228 µg GAE/g and highest DPPH radical scavenging activity with IC50 of 1020.00 µg/mL compared to the standard ascorbic acid of 304.44 µg/mL. Ethanol extract exhibited high hydrogen peroxide scavenging activity with IC50 of 83.09 µg/mL. This study showed that EC acetone extract contains highest phenolic compounds and ethanol and acetone extracts are a potential source of natural antioxidants.

**Keywords:** *Erythroxylum cuneatum*, phytochemicals, antioxidant activity
**Introduction**

*In vitro* antiproliferative activities of *Dicranopteris linearis* leaves against human breast cancer cell line (MDA-MB-231)

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To screen the cytotoxicity effect of *D. linearis* crude extracts in inhibiting the growth of a panel of cancer cell lines and to evaluate the antiproliferative activities of *D. linearis* crude extract that showed the most potent cytotoxic effect. The leaves of *D. linearis* were collected from its natural habitat in Universiti Putra Malaysia, Serdang. Screening of cytotoxicity activity of the methanolic extract (MEDL) and petroleum ether extract of *D. linearis* (PEEDL) against a series of human cancer cell lines and normal mouse fibroblast cells (3T3) were achieved by using the MTT cytotoxicity assay. Evaluations of the antiproliferative activity of *D. linearis* extract were achieved by performing the morphological analysis using phase contrast microscope, AO/PI viability assay, cell cycle analysis and Annexin V apoptosis assay. Statistical analysis was performed using GraphPad Prism version 6.0 software. Significance difference was evaluated using Tukey’s multiple comparison test, and p < 0.05 was considered significant. Our data indicated that MEDL showed the most significant cytotoxicity effect against MDA-MB-231 cells at IC₅₀ of 22.4 µg/mL. MEDL also showed selective cytotoxic activity against the proliferating cancerous cell and did not harm the normal mouse fibroblast cells (3T3). Phase contrast and fluorescence microscopy examination showed MEDL extract is able to induce apoptosis in MDA-MB-231 cells. Cell cycle analysis revealed that MEDL induced S phase cell cycle arrest in MDA-MB-231 cells effectively after 72 hours incubation. Early apoptosis induction in MDA-MB-231 cells was confirmed by Annexin V-FITC and PI staining. Significant increased in apoptotic cells were detected after only 24 hours of treatment with MEDL with 15.1% cells undergoing apoptosis, and the amount of the apoptotic cells escalated to 18.3% as the incubation period with MEDL were prolonged to 48 hours. These findings suggested that MEDL has the potential as a potent cytototoxic agent against MDA-MB-231 cancer cell line.

**Keywords:** *Dicranopteris linearis*, *in vitro* antiproliferative activity, MDA-MB-231, apoptosis, cell cycle arrest
The rate of remodelling in the subchondral bone plate and trabecular bone of tibia during development of osteoarthritis

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Osteoarthritis (OA) is a degenerative joint disease that mostly affects people aged 65 years old and above. In the knee OA, several joint changes were observed and among these include articular cartilage (AC) degeneration, and subchondral bone (Sb) remodelling. Despite extensive experimental and clinical studies, the role of bone remodelling in the two sub-regions of Sb, i.e. the subchondral bone plate (Sbp) and trabecular bone (Tb), during the initiation and development of OA has yet to be determined. Hence, this study aims to determine the rate of Sbp and Tb remodelling in Dunkin Hartley (DH) guinea pigs at 10, 20 and 30 weeks of age. To achieve this aim, the Sb remodelling parameters and AC degeneration were assessed in the medial and lateral side of the tibia by using histomorphometric and histological techniques, respectively. The differences and association of Sb changes between the sides, sub-regions of Sb and time points were analysed using statistical analyses. The data showed that the AC degeneration was more pronounced in the medial than the lateral side, and worsen with ageing. At 30 weeks of age, the percentage of mineralizing surface (MS) of Sbp was higher in the former than the later (medial: 4.17±0 %, lateral: 4.17±0 %). In contrast, the MS, mineral apposition rate (MAR) and bone formation rate (BFR) of Tb was decreased at the final time point and significantly greater in the lateral than the medial side of the tibia. Taken together, the above findings suggest that the decrease of Tb remodelling was due to stress-shielding effects as a result of Sbp thickening and hypermineralization.

Keywords: OA, AC, Sbp, Tb, MS, MAR, BFR
Prevalence of musculoskeletal discomfort among female wearing different heights and shapes of heel shoes in Kuantan

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The trend of wearing high heels is not restricted only to adult females but also involved females at all ages. In fact, women preferred to wear high heels for some reason although many studies found that this habit can cause physical and functional abnormalities to the lower and upper limbs. The trend of wearing high heels is not only important as a part of clothing but also reflects their personality, confidence and professionalism. Nevertheless, the past shoe wearing behavior may develop musculoskeletal discomfort (MSD) among heel shoes wearers. Thus, this study aims to determine the prevalence of MSD and its association with the risk factors, shoe designs and past shoe behaviors among female wearing heel shoes in Kuantan, Pahang. A cross sectional study was performed and subjects were recruited by using a purposive sampling. A self-administered questionnaire was given, and the height and shape of the heels were measured. The prevalence of MSD was calculated and association with the risk factors, shoe designs and past shoe behaviors were determined. The data showed that the overall prevalence of MSD among female wearing heeled shoes was 67.1%. The prevalence was slightly higher in the high-heel (68.2%) than the low-heel group (66.7%). Surprisingly, the prevalence of MSD among female wearing dress shoes (68.0%) and wedges (67.3%) was greater than those who worn stilettos (50.0%). However, none of the shoe designs showed significant different between the groups. Among these participants, they felt uncomfortable in the lower leg and ankle, and apart of all the factors examined, there was significant association between MSD with duration of wearing heel shoes (r = 0.223, p-value= 0.049). Since prolonged wearing high heels can interfere walking ability, public awareness campaigns should stress on the important of wearing the right shoe for the right activity.

Keywords: musculoskeletal discomfort, heel shoes, risk factors, past shoe behaviors, shoe designs
Anti-hyperalgesic effect of *Ficus deltoidea* extract in nitroglycerin-induced model of migraine

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The association between the clinical use of nitroglycerin (NTG) and migraine suggests NTG as a model trigger for migraine. NTG-induced hyperalgesia in rodents has been extensively used as a migraine model for pre-clinical research. *Ficus deltoidea* (FD) or locally known as “Mas Cotek” is commonly used in the Malay traditional medicine as a pain reliever including headaches. We perform present study to investigate anti-hyperalgesia activity of *Ficus deltoidea* aqueous extract in NTG-induced animal model of migraine. We assess the anti-hyperalgeisa activity of NTG-induced animal model of migraine through hot plate latency test and formalin induced paw licking test. The extract significantly (N=8, P<0.001) prolonged response latency time to heat stimulus in the hot plate test when administered intraperitoneally at 100 and 200mg/kg starting from 60 min until 180 min after NTG administration. In the formalin test, the extract significantly (N=8, P<0.001) increased licking latency in phase II of formalin test, 4 hours after NTG administration, at doses of 100 and 200mg/kg. These findings provide evidence that FD extract exhibit anti-hyperalgesia activity in NTG-induced hyperalgesia and potentially possess anti-migraine property for future therapeutic use.

**Keywords:** Anti-hyperalgesia, migraine, *Ficus deltoidea*, hot-plate latency test, formalin induced paw licking test
Ethnomedicinal survey of medicinal plants used to treat diabetes in Bangi, Selangor, Malaysia

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Synthetic antidiabetic drugs have been reported to exhibit deleterious effects and have failed to alter the course of diabetic complications. Traditional medicinal plants possessing antidiabetic effects can be a valuable source for the development of safer oral hypoglycemic agents. Hence, the aim of this ethnomedicinal survey was to document the ethnopharmacological use of medicinal plants for the treatment of diabetes in Bangi, Selangor, Malaysia. The field survey was performed from November 2013 through December 2014. Face-to-face interview and questionnaires were used to assemble information regarding the participants, local name of the plant, origin, parts used and method of herbal remedy. Citation index (CI) for each species mentioned and relative citation index (RCI) were calculated. Thirty species belonging to twenty-five families and twenty-nine genera were recorded. 63.3% of these species were native while 36.7% were cultivated. The most frequently used part was leaves. Decoction was the most common herbal preparation. Hibiscus rosa-sinensis has the highest citation index (CI) followed by Psidium guajava and Cymbopogon citratus with 0.09, 0.08, and 0.07, respectively. Three plants viz. Polyalthia bullata, Rourea concolor and Smilax myosotiflora have never been scientifically validated for their traditional use as antidiabetic agents. Plant species for diabetes treatment varied in every village due to the differences in rate of industrialization, urbanization and environmental degradation. This survey has successfully recognized the plants most commonly used by local practitioners in Bangi community to treat diabetes. Research studies on P. bullata, R. concolor and S. myosotiflora might furnish a new class of safe antidiabetic agents.

Keywords: Diabetes, ethno-medicinal plants, citation index, Bangi, Malaysia
P12

Evaluation of 5α-reductase inhibition activity of Pueraria mirifica on prostate hyperplasia

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5α-reductase is the enzyme that responsible to reduce the androgen testosterone into dihydrotestosterone (DHT), the more potent androgen. Overexpression of DHT may lead to disorders such as benign prostate hyperplasia (BPH), prostatic cancer, acne and baldness. The aim of this study is to determine the inhibition activity of 5α-reductase by Pueraria mirifica (PM) extract on hyperplasia of prostate induced by the subcutaneous administration of testosterone in rats. PM was extracted with water. In vitro studies were performed to determine the 5α-reductase inhibitory potential of the extract. Five groups containing six rats per group were created for this study. Prostate hyperplasia was induced by administration of testosterone (3mg/kg BW, s.c.) for 30 days in all group except the vehicle-treated group. Simultaneous administration of PM extract (100 or 1000 mg/kg BW) was conducted. Finasteride, 5α-reductase inhibitor standard was used as positive control. The weight of the rats was recorded. On day 30, rats were euthanised; prostates were dissected out and weighed. The blood was collected for serum dihydrotestosterone (DHT) level determination. The water extract showed a 5α-reductase inhibitory activity with increased NADPH concentration (22.68± 0.38 µg/mL) after 30 minutes, while finasteride the NADPH concentration increased to 33.79 ± 0.52 µg/mL. Contrary with testosterone control showed a decrease NADPH concentration, showed that testosterone were reduced to more potent form, DHT. Further in vivo study showed that testosterone administration significantly increases the P/BW ratio except in vehicle-treated rats. The PM extract (1000mg/kg, p.o.) showed a significant inhibition in P/BW ratio increment, similar to finasteride. The testosterone administration significantly increases the serum DHT level, and this increase in serum DHT was significantly inhibited in rats supplemented with PM extract, similar to the finasteride supplemented rats. Water extract of PM showed an inhibition activity of 5α-reductase, simultaneously prevent testosterone-induced hyperplasia of the prostate in the rats.

Keywords: 5α-reductase, benign prostate hyperplasia, dihydrotetosterone, Pueraria mirifica
Bone histomorphometric study in glucocorticoid-induced osteoporotic rats treated with *Piper sarmentosum*

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Osteoporosis is a systemic skeletal disease causing reduced bone mineral density and deterioration of the bone micro-architecture which increases the risk of fractures. Glucocorticoid-induced osteoporosis is the most common cause of secondary osteoporosis. Recent studies showed that glucocorticoid excess decreased osteoblasts proliferation and biosynthetic activity, whereas the lifespan of osteoclasts increased. Bone histomorphometry or quantitative histology allows the study of bone structure and remodeling parameter at three levels: cell, remodeling unit and tissue levels. Osteoporosis is characterized by reduced bone volume (BV/TV) and disruption of trabecular architecture, by measuring trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). Increased bone resorption and increased bone formation were revealed by osteoclast surface (Oc.S), osteoblast surface (Ob.S), osteoid surface (OS/BS), mineralizing surface (MS/BS) and bone formation rate (BFR). *Piper sarmentosum* (Ps) extract is known to possess antioxidant and anti-inflammatory activity. In this study, we determined the aqueous effects of *Piper sarmentosum* leaves on structural, static and dynamic histomorphometry in the bone of glucocorticoid-induced osteoporotic rats. Three-month old male Sprague-Dawley rats (250-300g) were adrenalectomized to remove the main source of circulating glucocorticoids. These animals were induced with Dexamethasone 120µg/kg body weight/day. Treatment with water-based *Piper sarmentosum* leaf extract was given for 2 months at 125mg/kg body weight. Following sacrifice, the left femora were taken for bone histomorphometry. The results showed that *Piper sarmentosum* leaf extract was able to significantly prevent the glucocorticoid-induced osteoporotic changes in bone volume, trabecular thickness, trabecular number, trabecular separation, osteoclast surface, osteoblast surface, osteoid surface, mineralizing surface and bone formation rate (p<0.05). The results showed that *Piper sarmentosum* leaf extract was able to prevent changes in bone structural histomorphometry and bone remodeling due to long-term glucocorticoid. Thus *Piper sarmentosum* may have the potential to be used as prophylaxis against osteoporosis and fractures in patients on long-term glucocorticoid treatment.

**Keywords:** Osteoporosis, glucocorticoid, histomorphometry, *Piper sarmentosum*
Cancer is among the leading cause of death worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012. In Malaysia, it is the third common cause of death with breast, colorectal and lung being the most common cancers reported from 2007-2011. Baeckea frutescens of the family Myrtaceae or also known as Cucur Atap is a medicinal plant that has been used in traditional medicine is known to possess antibacterial, anti-pyretic and cytoprotective properties. In this study, we investigated the cytotoxic activity of nine Baeckea frutescens leaves extracts against human breast cancer (MCF-7) and mammary epithelial breast (MCF10A) cell line. The extracts were prepared using Soxhlet apparatus for ethanol and hexane extracts while the water extracts were freeze-dried. In vitro cytotoxic activity of Baeckea frutescens extracts of various concentrations (20 to 160ug/ml) at 24, 48 and 72 hour time points were studied using MTT assay. After 24 and 48 hours exposure, IC_{50} of these extracts were unable to determine. All extracts showed IC_{50} values ranging from 10 to 127ug/ml in MCF-7 at 72 hours exposure. Hexane extract showed the lowest IC_{50} value (10ug/ml), indicating its potent cytotoxic activity. The IC_{50} obtained from MCF-7 were also tested on MCF10A. The results showed 80% of cell viability which indicates the selective cytotoxicity of hexane extract towards MCF-7 cancer cells. Hence, this plant can be used to discover bioactive natural products that possess anticancer activity and may serve as leads in the development of new pharmaceuticals research activities.

**Keywords:** Cancer, breast cancer, Baeckea frutescens, cytotoxicity
Study of antioxidant properties of standardised *Lawsonia inermis* aqueous extracts and their possible application as anti-ageing agents

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*Lawsonia inermis* (or locally known as henna) has been used since ancient time as a cosmetic agent to beautify skin, hair and nail without known side effects, and also as a healing modality in Ayurveda and Islamic medicine. Scientifically, it has been reported to possess a wide range of pharmacological properties such as anti-carcinogenic, anti-inflammatory, analgesic, antipyretic and wound healing. The link between inflammation, oxidation and proliferation processes with the plant extract have been generally acknowledged. Antioxidants, such as phenolic compounds and flavonoids, are molecules that can inhibit the oxidation processes, such as in skin aging. Plant that is rich in antioxidant content is expected to show good anti-aging property due to its radical scavenging and reducing abilities. This study is aimed to examine the total phenolic (TPC) and flavonoid (TFC) contents, as well as the antioxidant properties of *L.inermis* standardised hot (LIHAE) and cold (LICAE) aqueous extracts. The elastase inhibitory activity was also investigated to determine the anti-skin aging property of the extracts. All assays were carried out by using standardised protocols. Statistical result was analysed by using one way ANOVA with Dunnet test as pos-hoc test which P≤0.05 be considered as significant. As a result, the analysis of TPC and TFV contents of both LIHAE and LICAE showed significant differences (P≤0.05), where the LIHAE exhibited higher antioxidant content compared to the LICAE. For analysis of the DPPH radical scavenging capacity, no significant differences were detected, where both LIHAE and LICAE showed equal activity of scavenging free radicals. For β-carotene bleaching assay, there are significant differences were shown among LICAE, LIHAE and the standard (BHT), with LIHAE exhibited the best antioxidant activity. In elastase inhibitory activity screening assay, both LICAE and LIHAE showed significantly better elastase inhibition rate at lower concentrations AS compared to positive control (ECGC). In conclusion, both LICAE and LIHAE contain good flavonoid and phenolic contents and possessed excellent antioxidant properties, with LIHAE exhibited higher antioxidant properties. Both rich antioxidant extracts also showed excellent elastase inhibitory assays which highlighted their potential as promising anti-aging agents.

**Keywords:** *Lawsonia inermis*, antioxidant, anti-aging
Expression of beta defensin genes in frozen thawed human corneal epithelial cell line

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Human beta defensin (hBD) are important host defense molecules at the ocular surface. In addition to their antimicrobial activities, hBD may also act as regulatory factors in recruiting and activating immune cells. Only hBD1 - hBD4 have been well characterised. To date, the complete profile of the beta defensin genes (DEFB) expression in normal human corneal epithelial cell has not been established. This study is aimed to explore a spectrum of DEFB expression in human corneal epithelial cell line (HCE-2). Total RNA was extracted from frozen thawed HCE-2. The RNA was reverse transcribed into cDNA. The expression of 10 DEFB (DEFB1, DEFB4A, DEFB103, DEFB104, DEFB105, DEFB106, DEFB109, DEFB123, DEFB126 and DEFB127) were analysed using PCR and gel electrophoresis. DEFB1 and DEFB103 were the only DEFB found constitutively expressed in the frozen thawed HCE-2. These findings suggest that corneal epithelium constantly produce hBD1 and hBD3, which presumably provide the baseline defense against infection. Further investigation on the expression of these genes when HCE-2 stimulated with proinflammatory cytokines would help in better understanding of the ocular surface defense mechanism.
Assessments of colonization and survivability of community-acquired *Staphylococcus aureus* in peritonitis

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*Staphylococcus aureus* (*S. aureus*) have ability to cause a wide range of human diseases. The ability of *S. aureus* to adhere and invade within host cells is an important step in the immune response activation. Peritonitis is one of the results of *S. aureus* infection which caused potentially fatal inflammation at the peritoneum, an abdomen lining. Furthermore, it was documented that *S. aureus* is the frequent cause of peritonitis in individuals with continuous ambulatory peritoneal dialysis and end-stage renal disease. However, little is known about the ability of community-acquired *S. aureus* (*CA-SA*) isolates to survive and overcome peritoneal-derived cells phagocytosis, thus leading to peritonitis. In this study, the survival ability and pathogenicity of CA-SA isolates were assessed in vitro and in vivo with comparison to ATTC-*S. aureus* (ATCC-SA). For in vitro study, peritoneal-derived cells isolated from C57BL/6 mice were infected with either ATCC-SA or CA-SA and cultured onto TSA agar at 37°C overnight. Bacterial viable counting was performed the next day. The viable colony was counted and calculated to determine its colony-forming unit (CFU). The results suggest that CA-SA is able to invade and survived within peritoneal-derived cells and no significant difference observed when compared to ATCC-SA. For in vivo study, mouse were assigned into 3 groups and injected intraperitoneally with 200µL of sterile DPBS, ATCC-SA (10^8 CFU) or CA-SA (10^8 CFU), respectively. Observation was performed daily, from 0 hour to 120 hours post-inoculation of bacterial. Mouse were euthanized after 120 hours or immediately when it is found dead and organs of interested were isolated and process for bacterial colony count. All, but one (CA-SA, 66.6%), mice survived and did not develop peritonitis after injected with lethal dose of *S. aureus*. Although the CFU of bacteria in organs isolated from CA-SA-infected mouse were slightly higher than ATCC-SA, they were not statistically significant when compared to control. Taken together, the present study demonstrated that CA-SA can survive within peritoneal-derived cells and sub-lethal dose of CA-SA not cause fatality in mice model of peritonitis.

**Keywords**: Community-acquired *Staphylococcus aureus*, peritonitis, peritoneal-derived cells
Cocoa polyphenol is rich with Flavan-3-ol, procyanidin, epicatechin and catechin. There are three major classes of anti-inflammatory drugs namely non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammation drugs (SAIDs) and opioids. However, these drugs produced undesirable adverse effects such as gastrointestinal damage and respiratory depression. In effort to find an alternative to this problem, the effect of cocoa polyphenol was evaluated on in vitro anti-inflammatory model using Raw 264.7 cells. The main objective is to determine the anti-inflammatory activity of cocoa polyphenol co-treated with LPS induced in the Raw 264.7 cells. Specific objectives are (1) to determine cytotoxicity in Raw 264.7 cells treated with different cocoa polyphenol dose groups using methylene blue, (2) to determine the Reactive Oxygen Specie (ROS) level in cells with different cocoa polyphenol dose groups and (3) to determine the nitric oxide release by Raw 264.7 cells with different cocoa polyphenol dose groups. Inflammation was induced in Raw 264.7 cells (2 x 10^5 cells/ml) with 10µg/ml lipopolysaccharide (LPS) co-treated with cocoa polyphenol extracts at different concentrations of 30.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml for 24, 48 and 72 hours. The extracts were dissolved in 0.1% DMSO and DMEM. There were one negative control (uninduced and untreated cells), one group of induced untreated cells and one positive control (aspirin). All groups were in triplicate. The cell viability was obtained using methylene blue staining to determine the cytotoxicity of cocoa polyphenol. For 2',7'-dichlorofluorescein diacetate (DCF-DA) assay, the cells were treated with the same concentration of cocoa polyphenol extracts to measure ROS level at 24, 48 and 72 hours. For Griess assay, the media mixed with Griess reagent to measure Nitric oxide. Data collected was analyzed using one way ANOVA as statistical approach by using GraphPad Prism. The cocoa polyphenol was not cytotoxic towards Raw 264.7 cells. Raw 264.7 cells induced with LPS produced stronger DCF signals than cells co-treated with LPS and cocoa polyphenols (concentration range from 30.25µg/ml to 1000µg/ml). The co-treated groups resulted in a marked reduction in DCF fluorescence, indicating an inhibitory effect of cocoa polyphenol on intracellular ROS production. Nitric oxide decreased when the concentration of cocoa polyphenol doses increased. Cocoa polyphenol extracts reduced inflammatory activity towards LPS induced of Raw 264.7 cells by reducing intracellular Reactive Oxygen Species produced and reducing Nitric oxide level. This study concludes that co-treatment of cocoa polyphenol reduced the anti-inflammatory activity towards LPS-induced Raw 264.7 cell.

**Keywords:** Cocoa polyphenols, inflammation, lipopolysaccharides, RAW 264.7 macrophage cells
Screening and group 1 assessment of Torque Teno Virus (TTV) isolated from healthy blood donor in Malaysia

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Torque Teno Virus (TTV) is a small, single-stranded DNA virus of ~3.8kb, which was first identified in hepatitis patient of unknown aetiology. Several studies have been conducted in order to evaluate the pathological aspect of TTV, and it has been suggested to be linked with several types of diseases such as hepatitis, respiratory diseases, cancer, haematological and autoimmune disorder. However, it has also been reported to be prevalent among general population worldwide. To screen the prevalence and genotypic characteristics of TTV isolated from healthy blood donor in Malaysia. Plasma samples were collected from 34 healthy blood donors in Hospital Tengku Ampuan Afzan, Kuantan, Pahang from April to June 2017. Genetic material was extracted, and the presence of TTV DNA and its genotype was assessed by Polymerase Chain Reaction (PCR) using primer derived from untranslated region (UTR) (UTR-PCR) and N22 region (N22-PCR) of TTV genome, respectively. Initial screening showed TTV DNA was detected in 14 out of 34 samples (41.2%) using UTR-PCR, and 3 out of 6 samples (50%) using N22-PCR. This finding suggest that healthy individuals also are infected with TTV. To fully evaluate the prevalence of TTV and its genotype in Malaysia, further studies are required with larger sample size which involves a wider range of studied population and taking into considerations of the sociodemographic factors, geographical location and health status as well.

Keywords: Torque Teno Virus (TTV), Polymerase Chain Reaction (PCR), N22 region
A cadaveric study of the anatomical variations of the axillary artery among cadavers in Universiti Putra Malaysia

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Axillary artery is a direct continuation of the subclavian artery which begins at the outer border of the first rib and ends at the inferior border of teres major muscle. The artery is crossed by pectoralis minor muscle superficially which divides it into first, second and third parts. The first part gives off superior thoracic artery. Lateral thoracic artery and thoracoacromial artery arise from the second part; while the subscapular artery, anterior and posterior circumflex humeral arteries arise from the third part of the axillary artery. To study the anatomical variations of the axillary artery among cadavers in Universiti Putra Malaysia. This is an analytical cross-sectional study involving 22 cadavers at FPSK, UPM. The variations in the formation, course and distribution of axillary artery on the dissected cadavers were photographed and recorded. Total incidence of variant branching pattern of axillary artery was 32 out of 44 limbs (72.7%). Greater numbers of variations were noticed in the right limbs (45.5%) compared to the left limbs (31.8%) for both first and second part of axillary artery. The variation of branching pattern of third part of axillary artery was observed in 12 cases on the right side and 12 cases on the left side (54.5%). There is no consistency between variations of axillary artery with the side of upper limb. However, the knowledge on axillary artery variations is important in dealing with surgery around the axilla.

Keywords: Anatomical variations of axillary artery, side of upper limb
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A cadaveric study on the anatomical variations of the musculocutaneous nerve among cadavers in University Putra Malaysia

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The musculocutaneous nerve, arising from the lateral cord of brachial plexus containing the spinal roots of C5, C6 and C7. However, there have been reported cases on findings of musculocutaneous nerve variations which complicate the surgical procedures which will result in increasing the risk of compression and injury of the nerve. Therefore, this study aims to determine the anatomical variations of musculocutaneous nerve and its association with the side of limb. Method: An analytical cross-sectional study was conducted among 22 cadavers. Dissections were carried out and data were recorded. The associations between variation and side of the limb were analyzed using McNemar’s test. The normal type of origin account for 86.36% (left) and 90.91% (right). Absent type was spotted on right and left limbs with percentage of 9.09% each. The combining type was found only in the left limb that accounts 4.55%. No communications were seen with median nerve in 50.00% (left) and 81.82% (right). There were 36.36% of communications with median nerve seen on the left side only. Musculocutaneous nerve arises from median nerve in 9.09% on the left side and 4.55% on right side and they were absent in 4.55% (left) and 13.64% (right). The communication with median nerve was proximal to coracobrachialis muscle in 13.64% (left) and 9.09% (right) while the communication was distal to the muscle in 27.27% of left limb only. Even though variations were spotted on the right limb, there is no significant association between variation and the side of limb. However, the knowledge of musculocutaneous nerve variation is important to practitioners in dealing with surgery around the nerve to prevent complications.

Keywords: Musculocutaneous nerve variations, associations, side of upper limb
Porcupine bezoar: *In vitro* antioxidant and anti-proliferative effects

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Porcupine bezoar (PB) is a mass of undigested organic and inorganic materials formed within the gastrointestinal tract of the porcupine. PB has been claimed to possess medicinal properties to treat different types of diseases including cancer. However, these traditional claims are yet to be scientifically ascertained and properly validated. Hence, this study was aimed to evaluate antioxidant and anticancer activities of PB through different biological assays. Powdered PB was sonicated with double distilled water to get aqueous extract (PBA) which was initially screened for its phenolic content, flavonoid content and anti-oxidant potential using total phenolic content (TPC), total flavonoid content (TFC) and diphenyl-2-picrylhydrazyl (DPPH) assays, respectively. Later on, in vitro anti-proliferative effect of PBA was evaluated against A375 (Skin Malignant Melanoma) and HGF-1 (normal cell). PBA was found to contain low level of phenolic compounds and devoid of flavonoids. However, the DPPH assay showed low IC\textsubscript{50} value indicating PBA’s potent anti-oxidant characteristic. Moreover, the PBA displayed low IC\textsubscript{50} value and also showed significant anti-proliferation pattern at 24, 48 and 72 hours exposures against A375 and HGF-1 cell lines. The results of this study suggest that PB is medicinally potent in nature due to its strong antioxidant and anti-proliferative effects and could play an important role to cure different kinds of cancers.

**Keywords:** Porcupine bezoar, antioxidant activity, anti-proliferative activity, A375 and HGF-1 cell lines
Protective effect of aqueous extract of 7 different herbs against hydrogen peroxide (H₂O₂) toxicity on normal mouse fibroblast cell (3T3) line

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Traditionally, 7 different herbs (Etlingera elatior, Cymbopogon nardus, Melastoma malabathrium, Blumea balsamifera, Vitex trifolia varnegundo, Micromelum pubescens, Flemingia strobilifera) has been used to bathe a postpartum women. This aromatic bath product is considered a diaphoretic which would help to reduce swelling, speed healing of vaginal soreness, and remove toxin from body. Furthermore, it also can keep body freshness and serve as a therapy to reduce stress. However, there are lack of study on these herbs whether they can protect us from oxidative stress which can cause toxicity. Thus, this study was aimed to determine the protective effect of aqueous extract of 7 different herbs against hydrogen peroxide and also to compare the level of protection among them. In this study, the aqueous extract of 7 different herbs was prepared according to standardized protocol for aqueous extraction. Next, protective effect level was evaluated in normal mouse fibroblast cell (3T3) by using MTT assay with 6 different concentration which are 9.375µg/ml, 18.75µg/ml, 37.5µg/ml, 75µg/ml, 150µg/ml and 300µg/ml for 24 hours, 48 hours and 72 hours which then were induced by hydrogen peroxide for 4 hours before MTT reagent was added. Media without plant extraction was used as negative control in this experiment. The data was analysed using one way ANOVA followed by Dunnett test. All the 7 different herbs showed their potential to protect the normal mouse fibroblast cell (3T3) against toxicity induce by hydrogen peroxide at certain concentration in different time frame. As the conclusion, these 7 different herbs were suggested to be safe as herbal bath which also may protect us from oxidative stress.

Keywords: Etlingera elatior, Cymbopogon nardus, Melastoma malabathrium, Blumea balsamifera, Vitex trifolia varnegundo, Micromelum pubescens, Flemingia strobilifera: protective effects, normal mouse fibroblast cell (3T3)
The modulation of stathmin activity in transforming growth factor-β-mediated bronchial epithelial-mesenchymal transition

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Airway remodelling is a key process underlying asthma pathogenesis. Epithelial-mesenchymal transition (EMT) is a feature of airway remodelling that underlies the loss of epithelial cell-cell contact and differentiation into mesenchymal-like cells. These changes involve the dysregulation of microtubule dynamics. Stathmin is a microtubule destabilizer whose activity is negatively regulated by the phosphorylation of its serine residues, specifically Ser16, Ser25, Ser38 and Ser63. This study aims to investigate the modulation of stathmin in a cellular model of bronchial EMT. Briefly, human bronchial epithelial cells (BEAS-2B) will be stimulated with a known EMT inducer, transforming growth factor β (TGF-β), alone or together with the TGF-β inhibitor, SB431542 for 48 hours. EMT induction will be confirmed by morphological analysis and protein expression analysis for common epithelial or mesenchymal markers. The modulation of stathmin activity will be assessed by examining the phosphorylated stathmin and acetylated tubulin expression by immunoblotting, as well as the microtubule structure by immunostaining. Our preliminary study demonstrates that TGF-β-induced cells showed morphological changes from cobblestone to spindle form, with significantly higher radius ratio and lower expression of E-cadherin, a mesenchymal marker, compared to non-induced cells. In the presence of SB431542, the cells showed intermediate morphological changes, with lower radius ratio compared to induced cells and retained E-cadherin expression. The expression of phospho-stathmin (Ser16) and phospho-stathmin (Ser38) was significantly lower in induced cells compared to non-induced cells, and this was inhibited in the presence of SB431542. These observations suggest that alteration in stathmin phosphorylation may regulate the EMT process in bronchial remodelling. Further studies are required to determine whether total stathmin expression and other phosphorylation sites are affected, and to determine the association between these alterations and microtubule reorganization in bronchial epithelial cells. Increased understanding of the mechanisms involved in bronchial EMT will allow the development of better therapeutic options.

Keywords: Epithelial-mesenchymal transition, stathmin, airway remodelling, asthma
Histopathological studies in an adverse effect of dengue virus infection on liver and blood vessels of BALB/C mice

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Dengue virus (DENV) has become a burden to the economy of various countries, particularly the subtropical and tropical regions. Dengue virus infection causes clinical manifestations such as plasma leakage, bleeding with endothelial cells (ECs) –cell loosening and damaging of the liver. Liver and ECs plays a major role in mammals. Liver processes the nutrients, provides them to other body sites, removes toxic constituents from the blood and neutralizes the circulatory system. While ECs aid in gas exchange, fluid filtration in glomerulus and homeostasis. These function are dysregulated during DENV infection and damages other regions of the body. In our current research, the BALB/c mice were intraperitoneally injected with DENV-2. The liver and blood vessels tissues were processed and analyzed for histological alteration. The histopathology of liver of DENV-2 infected BALB/c mice exhibited marked increase of mononuclear cells infiltration, loss of cell integrity, and widening of the sinusoidal spaces. Apoptosis and necrosis were profuse. The areas of hemorrhage, macro- and microvascular steatosis were also noted. The characteristic of viral infection, for example the cytopathic effects, vacuole formation, and intracellular edema collectively results in lobular and sinusoidal collapse in the liver. The blood vessels of DENV-2 infected mice exhibit distorted endothelium lining, disturbed smooth muscle, and elastic laminae. Vascular disarrangement were also observed. In conclusion, the result present in this study may explain the severe pathological illness observed in DENV-infected individuals and could help in the development of treatment of complications due to dengue.
Establishment of a chemically-inducible retinitis pigmentosa rat model for stem cell therapy of retinal disorders

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Retinitis pigmentosa is a complex genetic disorder that is characterized by mutations in the retinal pigment epithelium and photoreceptors, which eventually causes retinal degeneration and blindness. Hence, utilizing a good animal model is crucial for the development of novel therapies to treat retinal degeneration. Stem cell therapy is one such promising method that is quickly gaining popularity around the globe. Sodium iodate (NaIO₃) is an oxidative chemical that induces retinal degeneration by damaging both the retinal pigment epithelium and photoreceptors. In our study, we induced retinal degeneration in Sprague-Dawley rats through systemic administration of NaIO₃ in several doses up to 80 mg/kg. We showed that within 48 h, the model suffered from blindness by way of electroretinography (ERG). After enucleating the eyes, we were able to observe lesions in individual layers of the retina using histology and immunohistochemistry. Furthermore, we were able to correlate the intensity of damage with the increase in dose through the TUNEL assay. Finally, we showed that systemically administered human Wharton’s jelly mesenchymal stem cells were detectable in the damaged ocular tissue after 24 h using immunohistochemistry. From this study, we hope to show that this model can be utilized along with novel stem cell therapies to treat retinitis pigmentosa and other such diseases.
Craniofacial anthropometric database of the normal healthy population is always necessary that may be required for scientists or for plastic and reconstructive surgeons. For the reconstruction of facial deformities of genetic origin or post traumatic and for the identification of an unknown individual missing or dead, craniofacial anthropometric normative database is the only reliable reference. To use the norms of the given population as reference data, craniofacial anthropometry was brought to clinical practice so that the deviation from the normal can easily be quantified. Even though various anthropometric researches have been done extensively in this field, but not all the three Malaysian ethnic groups were studied together and compared. So, in this study, a total number of 400 Malaysian students were included who belong to the three ethnic group of Malaysia namely Malay, Chinese and Indians. The age range was between 18-25 years and 17 parameters were measured from them. The collected data was then processed statistically in independent sample t test by IBM SPSS Statistics Package 22. The extracted statistical data was then compared with the previous relevant studies. The result has clearly shown major facial characteristics of different ethnic groups and enriched the database with normal range of facial measurements. Comparative study shows significant differences between male and female Malaysians of individual ethnic groups and maximum parameters showed smaller measurements for female than the male. Compared discussions have also concluded that Malays are structurally identical in many facial parameters with that of Singaporean Chinese. Whereas, Malaysian Chinese have shown different facial values than Singaporean Chinese. Contrary to that, there was no difference found among Malaysian Indians and the Indians residing in India. This comparative data establishes that Malay and Chinese ethnic groups residing in Malaysia originated from the Mongolians but still there are some demographic effects available on the anthropometric database of Chinese people. Indians living in Malaysia are descendant of Indians from India.

Keywords: Craniofacial, anthropometry, anatomy, Malaysia, ethnicity
**P28**

**In-vitro release profile of anti-periodontitis doxycycline hyclate emulsion**

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Periodontitis, a biofilm infection affecting the gum and teeth, has a high prevalence and has no efficient treatment to date. In this study, doxycycline hyclate, a semi synthetic derivative of tetracycline was used as the active pharmaceutical ingredient (API) to formulate an anti-periodontitis emulsion. Different amount of lecithin and hydroxypropylmethylcellulose (HPMC), both acting as surfactants, were used in the formulations and tested for drug release profile. The in-vitro drug release profile was conducted using USP Apparatus 1 with PBS of pH 6.8 under sink condition. A predetermined time point $t_0$ was set up to take out the solution from the vessel for quantification. In order to quantify the in-vitro performance, a method validation was completed using UV-spectrophotometry following the requirements listed under ICH Q2 (R1) guidelines. A standard calibration curve with a good linearity, $R^2=0.9973$ was established. LOD and LOQ were in the range of 0.009 and 0.027 $\mu$g/mL respectively, whereas the slope of the standard curve was 0.051. The recovery rate for intra-day was from 98.27 to 101.39%, and from 100.85 to 102.57% for inter-day. The RSD was 1.6. It was found that lecithin-added formulation showed complete drug release after 5 hours. In contrast, lecithin+HPMC-added formulation gave a total release after 2 hours. Thus, the lecithin-added formulation showed a slower release profile compared to the lecithin+HPMC-added formulation and may be the formulation of choice for future testings.

**Keywords:** Periodontitis, doxycycline, release profile, emulsion, dentistry
P29

Digital photography: An effective touch-less dermatoglyphic analysis system

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Dermatoglyphics or epidermal ridge pattern on fingers, palm, and soles is claimed to be an important diagnostic tool for some diseases especially the diseases with unknown cause and apparently spontaneous pathogenesis. To address the finger, hand, individual, gender, regional as well as population variability of dermatoglyphics in diagnosis, a baseline data is essential for that population. Currently touch-less fingerprint obtaining systems are being more popular than touch-based systems. In touch-less recognition system, acquisition of fingerprint can be based on one or multiple digital cameras, webcams which might have possibilities of reflections, noise and complex background. Recent studies have proposed various software and hardware systems to address these issues.

Keywords: Dermatoglyphic, digital photography, touch-less
The effect of Orthosiphon Stamineus Benth (Misai Kucing) on Glut4 translocation in skeletal muscle of diabetic rats

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Glucose homeostasis is dependent on glucose uptake into muscle and adipose tissue which in turn regulated by the insulin and glucose transporters. In this case, GLUT4 acts as the insulin-responsive glucose transporter, promoting the uptake of glucose from the circulation into muscle and fat. One of the plants that have been reported to have potential anti-diabetic activity is Orthosiphon stamineus (O. stamineus) Benth which is popular among Malaysian. Therefore, the aim of this study is to determine the effect O.stamineus Benth extract on GLUT4 translocation in the skeletal muscle of type II diabetic rats using immunofluorescent technique. For this study, male Wistar rats aged 10 weeks (180-220g) were divided into (1) normal control group (received vehicle), (2) positive control group (diabetic rats treated with 5mg/kg metformin), (3) negative control group (diabetic rats which did not receive any treatment) and (4) treated group (diabetic rats treated with 1g/kg O.stamineus Benth leaves aqueous extract). The rats were treated daily 14 days. Fasting blood glucose level and serum insulin were measured. At the end of the treatment, the rats were sacrificed and soleus muscles were dissected, fixed in 10% buffered formalin and processed for immunofluorescent technique. Our findings showed significantly reduced (p<0.01) mean fasting glucose concentration was in the positive control and treated groups, while the positive control group did not show any glucose reduction. The relative pancreas weight and serum plasma concentration were similar in all groups. In the diabetic rats, the GLUT4 translocation activity was decreased and there were relatively more GLUT4 detected in the muscular cytoplasm than at the plasma membrane as compared to the normal group. In the diabetic rats treated with O.stamineus Benth, improvement in the translocation activity was observed. Thus, our findings suggest that O.stamineus Benth may have the potential in the treatment of Type II Diabetes Mellitus.

Keywords: Diabetes Mellitus, Orthosiphon stamineus (O.stamineus) Benth, GLUT4, immunofluorescent technique
Evaluation of the quality of single best answer questions (SBAQs) by item analysis in endocrine system among medical students in Cyberjaya University College of Medical Sciences (CUCMS)

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SBAQs is a form of Multiple Choice Questions (MCQs) used extensively to assess student performance due to its reliability. One powerful technique available for guidance and improvement of instruction is the test item analysis. To evaluate the quality of questions used in Endocrine System including Anatomy and Physiology via item analysis and their interrelationship. This study was conducted among 100 year 1 medical students in CUCMS. A total of 30 SBAQs from the Mid-Course Assessment (MCA) and 35 SBAQs from the End-of-Course Exam (EOC) were analysed. Each question had a stem and 4 answer options with one best answer and three distractors. The Difficulty Index (DIF I), Discrimination Index (DI) and Distractor Effectiveness (DE) were the parameters used in this study. An ideal item/question will be the one which has average DIF I between 31 and 60%, high DI 0.25 and maximum DE (100%) with three functional distractors. The data was recorded as mean ± standard deviation via Microsoft Excel. The relationship between the DIF I and DI was further analysed via Pearson correlation. The mean score for DIF I was found to be appropriate (0.66 ± 0.12) for MCA and difficult for EOC exam (0.23 ±0.03). Excellent DI was achieved in MCA (0.57 ± 0.13) and satisfactory for the EOC exam (0.24 ±0.23). A total of 90 and 105 distractors were analysed overall; 47% and 74% distractors were noted to be effective in the MCA and EOC, respectively. Pearson correlation showed that DI correlates poorly with DIF I (r= -0.413, p<0.05) and (r=-0.136). In the present study, majority of the questions are in acceptable range. Items having appropriate difficulty and high discriminating power with functional distractors should be incorporated in future to improve the quality of the test leading to a development of a standard question bank.

Keywords: SBAQs, item analysis, difficulty index, discrimination index, distractor effectiveness
P32

Fabrication of graphene oxide film supports in vitro proliferation of Wharton’s jelly derived mesenchymal stem cells

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Wharton’s jelly-derived Mesenchymal Stem Cells (WJ-MSCs) have drawn much attention for being more primitive representing an early-stage mesenchymal-like stem cells and easily collected without ethical restrictions. In order to ‘drive’ stem cell to develop into functional organs, researchers applied various tissue engineering and biomedical strategies to design and manipulate nanoscale cell culture scaffolds to provide a unique physical framework, comparable to natural extracellular matrix (ECM), for stem cell. Graphene oxide is an artificial substrate that is attracting a lot of attention in biomedical field due to its nanoscale topography which can significantly influence the characteristic of stem cells by enhancing cell adhesion, proliferation, and differentiation. Therefore, graphene oxide is a potential scaffold which is useful in tissue engineering. Current research, we aimed to evaluate the biocompatibility of synthesized graphene oxide in WJ-MSCs. Graphene oxide was synthesized using modified Hummers method and characterized by ultraviolet visible spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and scanning electron microscope (SEM). Applying drop-casting method, different concentrations of GO were successfully coated on glass substrate for GO films fabrication. Following, WJ-MSCs were cultured on GO films for 5 days. The cell survival assay (MTT) demonstrated that WJ-MSCs proliferate on the films coated with lower concentration of GO. These cells possessed similar proliferation efficacy and comparable to the glass (uncoated GO substrate). In addition, the GO film proved to be a suitable environment for the time-dependent viability of WJ-MSCs. In conclusion, lower concentration of GO film was found to be non-toxic when exposed to WJ-MSCs and as a potential substrate for the adhesion and proliferation of WJ-MSCs.

Keywords: Graphene oxide, Wharton’s jelly mesenchymal stem cells, proliferation
High-throughput mass spectrometry based metabolomics: Revealing Sc11-F5 isolated from Sinularia Sp. triggering up-regulation of pro-apoptosis metabolites in Hep 3B

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Marine natural products have been demonstrated inheriting anti-cancer, anti-viral and anti-inflammatory properties which are beneficial to the pharmaceutical industry. As a result of the advent of comprehensive analytical platforms and sensitive instruments, classical labour intensive natural product chemistry evolves to now-a-day’s high-throughput screening. Soft coral is among the marine invertebrates inheriting vast number of anti-cancer compounds. High-throughput metabolomics is a platform which enables researchers to profile and identify metabolites. Throughout chemometric analysis, comparison could be made between subjects. The outcomes allow researcher to map the putative metabolic pathway within the biological sample and understand the response of the biological system towards the newly discovered compound. The aim of current study is determining efficiency of SC11-F5 against hepatocellular carcinoma Hep 3B and its cell death triggering mechanisms using mass spectrometry based metabolomics. 12 soft corals were collected from the North coastal area of Sabah via SCUBA diving. Modified Bligh and Dyer extraction protocol was applied throughout the collected soft corals. The anti-cancer potential of each extracts was screened against the hepatocellular carcinoma (Hep 3B). The most effective crude extract (SC 11) was further purified using chromatography technique. Eight fractions were acquired and each fraction was tested against the cancer at different concentrations (25, 50 and 100 µg/mL) for 48 h. SC11-F5 was found to be the most effective in inducing apoptosis in Hep 3B. Lethal dose at 50% of total cell survival (LD50) was determined at 61.93 µg/mL. Following, cell metabolomics was applied at LD25 against SC11-F5. Chemometric analysis revealed a total of 689 metabolites were significantly (p-value < 0.001) perturbed. Among them, triacylglycerol and sphingolipids including ceramides species were up-regulated in SC11-F5 treated cell samples. These metabolites were found to have correlation to the programmed cell death. To conclude, SC11-F5 has cytotoxicity effect towards Hep 3B cell line.

Keywords: Soft coral, metabolomics, LD50
Isolation and characterization of anti-lipase compounds from *Carica papaya* Linn.

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Increase of obesity and associated complication has gain the society attention. Such metabolic disorder induced complications drained numerous public resources as lots of medical attentions need to be input onto obesity patients. Previously, numerous phytochemicals had been discovered from the local plants/herbs to overcome obesity. In this study, we aimed to determine anti-lipase compounds which could be isolated from *Carica papaya* Linn. Throughout the study, we compared different isolates from various parts of papaya including leaves, peels, flesh and seeds against pancreatic lipase. Two major strain of papaya, *C. papaya* L. var Eksotika and var Sekaki were selected. Freeze dried and fine grinded papaya parts were extracted using modified Folch extraction protocol. Inhibitory effect of different extracts on pancreatic lipase activity against its substrate p-nitrophenyl butyrate were evaluated. The fractions which possessed as the highest inhibitory efficiency against pancreatic lipase was chosen for further purification and isolation using column chromatography followed by preparative thin layer chromatography. Successfully isolated compounds which possess the highest anti-lipase activity will undergo structure elucidation using nuclear magnetic resonance and high-resolution mass spectrometry. From the acquired results, unripe *C. papaya* exhibited higher lipase inhibitory compared to ripen. The peels, flesh and seeds from Eksotika and Sekaki were shown higher lipase inhibitory against porcine pancreatic lipase at 0.50 units/mg. Eksotika variety extracts demonstrated higher inhibitory percentage (81.32±6.00 %) than Sekaki variety, where the result was comparable to inhibitor, Orlistat (88.15±2.26 %). Therefore, unripe Eksotika papaya's flesh was chosen for further purification and isolation. Eight fractions were successfully obtained and test on pancreatic lipase inhibition assay. The most effective fraction was further fractionated using preparative thin layer chromatography and profiled using LC-QTOF-MS, while structure elucidate using NMR. Anti-lipase compounds isolated from *C. papaya* flesh can substituted the pharmaceutical anti-obesity drug, Orlistat that may have numerous side effects.

**Keywords:** *Carica papaya* Linn., orlistat, pancreatic lipase inhibitor, *in vitro* assay
Effects of palm tocotrienol on metabolic syndrome in male rats

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This study aimed to investigate the effects of palm tocotrienol on metabolic syndrome (MetS) parameters in male rats. Male Wistar rats were divided into five groups: baseline (BL), normal (N), metabolic syndrome (MetS), and MetS treated with 60 mg/kg palm tocotrienol (MetS+60 pT3) or 100 mg/kg palm tocotrienol (MetS+100 pT3) groups. The baseline group was sacrificed at the onset of study. The normal group was given standard rat chow. The MetS groups were given high-carbohydrate high-fat (HCHF) diet, comprises of fructose, sweetened condensed milk, ghee, Hubble Mendel and Wakeman salt mixture, and powdered rat food. Diet regimen was assigned for a period of 20 weeks. Palm tocotrienol (60 or 100 mg/kg) was given via daily oral administration for 12 weeks starting from week 8. At the end of the end of study, all rats were subjected for the analysis of MetS parameters (including the measurement of abdominal circumference, blood pressure, blood glucose, and lipid profile). Our results indicated that both systolic and diastolic blood pressure was normalised in rats fed with HCHF diet supplemented with 60 or 100 mg/kg palm tocotrienol (P<0.05). Supplementation of 60 mg/kg palm tocotrienol improved fasting blood glucose and lipid profile (reduced triglyceride and total cholesterol levels) in MetS rats. Meanwhile, higher dose of palm tocotrienol (100 mg/kg) improved fasting blood glucose, glucose tolerance, and lipid profile (reduced triglyceride, total cholesterol, and increased HDL cholesterol levels) in MetS rats compared to non-treated MetS rats (P<0.05). Palm tocotrienol exerts potential dose-dependent effects in improving the medical conditions associated to MetS.

Keywords: Tocotrienol, obesity, hyperglycaemia, hypertension, dyslipidaemia
Regulation of aldosterone and cholesterol-related genes by Micro-RNAs in the H295R adrenocortical carcinoma cell line

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Adrenocortical carcinoma (ACC) is an aggressive tumour with 50% survival rate within 5 years; the survival rate decreases sharply to 35% in those unable to undergo surgery. Up to 40% of patients with ACC present with Cushing’s syndrome. The most common clinical signs are hypertension, central obesity, muscle wasting, hirsutism and acne. Most present with inappropriately high levels of cortisol and aldosterone. Aldosterone is a mineralocorticoid hormone secreted from the zona glomerulus of the adrenal gland. The final stage of its production is catalysed by aldosterone synthase, encoded by the CYP11B2 gene. Recently, studies have shown that CYP11B2 is partly regulated by microRNA (miRNA), which are small, non-coding, single stranded RNA that negatively modulate gene expression at the post-transcriptional level. Previously, we showed that miRNAs directly regulate CYP11B2 expression and, consequently, aldosterone levels. Here, we investigated the miRNA profiles of an adrenocortical carcinoma cell line (H295R), in its basal non-stimulated state and also following 24-hr stimulation of aldosterone production using either 100nM angiotensin II (AngII), 1mM dibutyryl cyclic AMP (dbcAMP) or 20mM potassium chloride (KCl). The miRNA profiles of the stimulated groups (each n=3) were generated by microarray and differentially-expressed miRNAs relative to basal were identified. Subsequent bioinformatic investigation using Ingenuity Pathway Analysis (IPA) identified putative target genes of the differentially-expressed miRNAs. Quantification of selected miRNAs and mRNAs was validated by qPCR. Each treatment (AngII, dbcAMP and KCl) significantly increased CYP11B2 mRNA levels relative to basal. miRNAs that were differentially-expressed following stimulation included miR-17-5p, miR-101-3p and miR-1207-5p. IPA analysis showed that 2 genes important to steriodogenesis were predicted targets of miR-17-5p and miR-101-3p (targeting ABCA1) and of miR-1207-5p (targeting LDLR). Transfection of H295R cells with pre-miR-17 resulted in reduced ABCA1 mRNA, supporting its status as a miRNA target. In conclusion, we have shown H295R miRNA profiles, as generated by microarray, are significantly altered under 3 aldosterone-stimulating conditions, and that bioinformatic and in vitro analysis supports an effect on steriodogenesis. These results suggest that a mechanism regulated by miRNA and affecting cholesterol levels might have an impact to aldosterone homeostasis. miRNAs may therefore have an important role in future diagnosis and treatment of ACC.

Keywords: Adrenocortical carcinoma, aldosterone, hypertension, CYP11B2, microRNA
The origin of gamma band EEG signals during reading

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In this study EEG data was obtained from 26 undergraduate students during a lexical decision task. Using this data we then analysed the EEG gamma band signals and have shown how they are caused by eye saccades. We found that at the onset and progression of a saccade, gamma band EEG signals spontaneously appear in the EEG signals. These gamma band EEG signals terminate when the saccade terminates. To obtain the gamma band EEG signals, we have performed time-frequency analysis using intrinsic mode decomposition and the Hilbert-Huang transform (HHT). From this analysis we are able to track the onset, progression and termination of specific frequency bands in the EEG data. Since the gamma band EEG signal was found to be matched to the eye saccades, we conclude that these Gamma band is caused eye saccades.

Keywords: Reading, EEG, gamma band, Hilbert-Huang transform, Saccades
Spine and whiplash injuries in motor vehicle crashes

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Spinal injury is one of the most devastating traumas that can be sustained in a motor vehicle crashes. In rear impact vehicle collision, one of the most common injuries is whiplash injury. The primary goals of the study were to identify the prevalence of whiplash injuries among car occupants in Malaysia and the factor associate with the risk of whiplash injury. The data were collected retrospectively from closed files of the third-party bodily injury (TPBI) insurance claims database for the period 2013-2015. Injury to the body region and severity coding used in this study are based on Abbreviated Injury Scale (AIS), updated version 2008. 88 (8.55%) out of 1029 occupants are identified with spinal injuries. The cervical region is the commonly injured region with the highest percentage at the C5 and C6 section. There are two crash factors were found to associate significantly with the outcome of spine injury which are road type and impact mode. The occupants who were involved in a collision that occurred on the highway were twice more likely to get spine injury compared to those whose collision occurred on the non-highway. Other than that, occupants who were involved in a rear impact collision were four times more likely to get spine injury compared to those who involved in the frontal impact collision.

**Keywords:** Spine, injury, whiplash, crash, traffic
Liver sinusoidal endothelial cells (LSEC) isolation from rat liver

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Liver perfusion has been the standard method to digest and isolate liver cells including liver sinusoidal endothelial cells (LSEC). Poor cannulating skills through portal vein results in a waste of animal resource. Familiarization of both liver perfusion technique and adhering strictly to aseptic technique during handling cells ensure high cell yield, minimum morphology disruption and cell contamination. We presented a method of liver perfusion procedure followed by for the isolation of LSEC. The study was conducted with the approval of IACUC committee. Seven Sprague Dawley rats underwent these procedures under anaesthesia. Liver perfusion was done as previously described. Briefly, LSEC were isolated by liberase enzyme perfusion of the liver, isopycnic sedimentation in a two-step Percoll gradient and selective adherence. The purification and cultivation of LSEC was evaluated by light and electron microscopy. Purity and viability of LSEC after selective adherence was 80.5 ± 3.5% and > 95 % respectively. The average concentration of the cells ranged from 32 - 75 x 10^6 per 400 g rat. After 8 hours of culture, LSEC monolayers were contaminated with less than 5% of other cells. This method is reliable and reproducible for isolation of LSEC to enable the study of structure and function of these cells in vitro. However, improvement on the perfusion skills and isolation technique are vital to ensure better cell purity.

Keywords: Liver sinusoidal endothelial cell, liver perfusion, cell isolation, cell culture, Percoll
Antioxidant contents and activities of *Pithecellobium jiringa* (jering) seeds

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*Pithecellobium jiringa* (jering) is one of the traditional Malaysian vegetables and commonly eaten as ‘ulam’. The aim of this project was to investigate antioxidant content and antioxidant activity of *Pithecellobium jiringa* (jering) seeds extracts. *P. jiringa* seeds were extracted using three different extraction solvents; hot aqueous, cold aqueous and 70% ethanol. Five different antioxidant assays, namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), β-carotene bleaching and phosphomolybdenum were carried out for determination of antioxidant activity of *P. jiringa* seeds while Folin-Ciocalteu and aluminium chloride colorimetric assays were used to determine the antioxidant content of *P. jiringa* seeds for total phenolic content (TPC) and total flavonoid content (TFC), respectively. The antioxidant assays revealed 70% of ethanol extract of *P. jiringa* seeds showed the highest DPPH scavenging activity (EC₅₀ of 161.67 µg/mL), FRAP (6.67 ± 2.03 mmol/g Fe²⁺) and phosphomolybdenum (14.36 ± 0.43 mg/g ascorbic acid). In addition, the 70% ethanol extract of *P. jiringa* seeds possessed the highest total phenolic content (16.37 mg GAE/g DW) and total flavonoid content (13.76 mg QE/g DW). The hot and cold aqueous extracts of *P. jiringa* seeds with moderate amounts of TPC and TFC exhibited the highest antioxidant potential in ABTS (0.28 ± 0.01 mM/g trolox) assay while the hot aqueous extract of *P. jiringa* seeds showed the highest inhibition of β-carotene bleaching activity (79.09%). In conclusion, *P. jiringa* (jering) has the potential as a natural source of antioxidant and can be further explored for its health benefits.

**Keywords:** *Pithecellobium jiringa*, antioxidant activity, total phenolic content, total flavonoid content
Antioxidant properties of MD2 pineapple [*Ananas Comosus L.*] parts as source of natural antioxidant

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Pineapple’s (*Ananas comosus* L.) flesh is usually eaten fresh while peel and core are discarded as waste. Nevertheless, these by-products are believed to contain good amount of antioxidants. This study aimed to investigate antioxidant properties (content and activities) of different parts (peel, flesh, core and mixture of the three) of MD2 pineapple extracted using 80% ethanol. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using Folin-Ciocalteu and Aluminium Chloride colorimetric methods, respectively while antioxidant activities were estimated by performing DPPH free radical scavenging and ferric ion reducing antioxidant power (FRAP) assays. TPC of samples ranged from 6.14 ± 0.07 to 8.31 ± 0.28 mg GAE/g DW while TFC ranged from 2.63 ± 0.14 to 5.46 ± 0.26 mg QE/g DW with pineapple peel possessed the highest value. There were significant differences among samples (p<0.05) for TPC, but for TFC flesh and core showed no significant different. EC$_{50}$ values obtained from DPPH assay ranged from 37.25 ± 2.70 to 54.68 ± 3.65 ug/ml, with no significant differences among samples (p<0.05) except between peel and flesh. Generally, peel has the highest scavenging activity compared to others. FRAP values of samples ranged from 1.033 ± 0.53 to 1.25 ± 0.25 mM Fe$_2$+/g DW with peel presenting the highest activity, significantly. Pearson correlation revealed significant correlation between TPC, TFC with EC$_{50}$ and FRAP values (p<0.05). In summary, MD2 pineapple peel possessed the highest antioxidant content and activity thus potentially considered as new source of natural antioxidants for prevention of NCDs.

**Keywords:** Pineapple skin, antioxidant, phenolics, flavonoids, natural
Electron microscopic changes of the liver following exposure to organic arsenic

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There has been a growing concern over the toxicity of organic arsenic to human body since recent studies indicated these compounds might be at least as toxic as inorganic arsenic. Liver is the primary site of metabolism of arsenic. Therefore it is highly susceptible to the adverse effects of both organic and inorganic arsenic. We aimed to identify any morphological changes in the liver associated with organic exposure on scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Our study employed monosodium methylarsonate (MSMA) to investigate the morphological changes of the liver on scanning electron microscope following methylated organic arsenic exposure. A total of fifty-five male Sprague Dawley rats were divided into five groups (n=11) according to the dosage of MSMA given. Control group were given distilled water for 16 weeks and the treatment groups were treated with daily oral MSMA for the same period of time. The treatment groups were T1, T2, T3 and T4 and the treatment doses were 42.13, 63.20, 126.4 and 210 mg/kg body weight respectively. Individuals from groups T3 and T4 experienced severe diarrhea and drastic weight reduction, and therefore were discontinued from this study. At the end of week 16, the remaining rats were sacrificed and their liver tissues were harvested for SEM and TEM studies. SEM revealed an increase in the number of bulblike structures called blebs in dose-dependent manner in treatment groups compared to control group. There were also serpentine filopodia observed located near blebs in treatment groups but none of them were present in control group. TEM revealed reduction in the number of mitochondria and rough endoplasmic reticulum in treated groups compared to control rats. MSMA exposure caused apoptotic-related morphological changes in the hepatocytes on SEM and alterations in organelles on TEM.

Keywords: Organic arsenic, liver, electron microscopy
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