3rd International Anatomical and Biomedical Scientific Conference (IABS) 2022

Siti Khadijah Adam*, Yong Yoke Keong, Che Norma Mat Taib, Mohamad Aris Mohd Moklas, Sharida Fakurazi, Hasiah Ab. Hamid, Mohd Khairi Hussain, Nurul Hayati Mohd Zainal and Azmah Sa’at

*Correspondence: sk.adam@upm.edu.my

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ABSTRACT

Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, partnered with Universitas Brawijaya, Indonesia to host the virtual 3rd International Anatomical and Biomedical Scientific Conference (IABS) 2022 on 22nd – 24th November 2022. With the theme ‘Rejuvenating health sciences towards inclusive and sustainable ecosystem’, this platform is one of the best avenues that cultivates sharing and exchanging scientific endeavours among scientists, clinicians, postgraduate students and academicians involved in the field of anatomy, health sciences and relevant disciplines. The goal of the Special Issue is to revitalise the body of knowledge in health sciences to enhance and offer a sustainable and inclusive ecosystem now that the COVID-19 pandemic has transitioned to the endemic stage globally. Submissions that conceptually, experimentally, or theoretically analyse and explain some of the major problems with achieving the objectives. In light of this, this Special Issue of the conference proceedings aims to integrate practitioner and academic viewpoints. This special issue, in particular, can advance the theory and synthesis of the selected topic. Additionally, it can present fresh findings from experiments, drawing attention to the effectiveness of techniques now employed by subject-matter experts.

Keywords: Anatomy; biomedical sciences; neuroscience; natural products; non-communicable diseases

ORGANIZED BY:

Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.
Universitas Brawijaya, Indonesia.
LEAD GUEST EDITORS

Dr. Siti Khadijah Adam
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: sk.adam@upm.edu.my

Associate Professor Dr. Yong Yoke Keong
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: yoke_keong@upm.edu.my

GUEST EDITORS

Associate Professor Dr. Che Norma Mat Taib
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: chenorma@upm.edu.my

Associate Professor Dr. Mohamad Aris Mohd Moklas
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my

Professor Datin Dr. Sharida Fakurazi
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: sharida@upm.edu.my

Dr. Hasiah Ab. Hamid
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
Email: hasiah@upm.edu.my

Dr. Mohd. Khairi Hussain
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
Email: khairi@upm.edu.my

Dr. Nurul Hayati Mohamad Zainal
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: mz_nurul@upm.edu.my

Dr. Azmah Sa’at
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: azmahsaat@upm.edu.my

ABSTRACTS

All presented abstracts are listed from Page 3 to 74.
Subacute 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) treatment models pre-symptomatic and symptomatic stages of Parkinsonism in mice

Musa Mustapha1,2, Che Norma Mat Taib1,*, Sharida Fakurazi1, Mohamad Aris Mohd Moklas1 and Noor Azuin Suliman3

1Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
2Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, 810211, Zaria, Nigeria.
3Faculty of Medicine and Health Science, Universiti Sultan Zainal Abidin (UniSZA), Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: chenorma@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

A compelling animal model is required for a heterogeneous disease like Parkinson’s disease (PD) to understand the underlying mechanisms better and create therapies. MPTP, a neurotoxin, is widely used to replicate the pathophysiology of PD in rodents; however, there is no robust consensus on the best and most reproducible model that recapitulates the pathologic phases of Parkinsonism. Furthermore, susceptibility to the subacute regime of MPTP among mice strains still needs to be confirmed. Thus, this study aimed to find an accurate and reproducible mice model of presymptomatic and symptomatic Parkinsonism using a subacute MPTP regime in Balb/c and C57bl/6 mice. 13 weeks old male Balb/c and C57bl/6 mice (27 g), divided randomly into three groups were injected (intraperitoneal) with 0.9% normal saline (0.01 ml/g), 15 mg/kg and 30 mg/kg MPTP-HCL respectively for five consecutive days. Weight was monitored during the treatment. Behavioural tests, biochemical and morphological studies were conducted. MPTP induced weight gain and weight loss in Balb/c and C57bl/6 mice, respectively (p<0.05). Behavioural tests such as open field, pole, catalepsy and traction revealed severe impairment in locomotor activity in C57bl/6 mice. This motor dysfunction is suggestive of insufficient compensatory response as demonstrated by the suprathreshold depletion of striatal dopamine (>70%) and striatal/nigral neuronal degeneration (>50%), especially in the 30 mg/kg MPTP-HCL group. However, in the Balb/c mice, behavioural motor hyperactivity was evident because of the active dopamine compensatory recovery overshoot mechanisms. This hyperactivity was reflected in the subthreshold dopamine depletions (<70%) and striatal/nigral neuronal degeneration (<50%) also in the 30 mg/kg MPTP-HCL group. The subacute MPTP dose regimen modelled presymptomatic and symptomatic Parkinsonism in Balb/c mice and C57bl/6 mice, respectively. Thus, these models could be used to learn more about the mechanisms behind MPTP-induced neuroplasticity and neurodegeneration, which has the potential to be an excellent tool for translational medicine.

Keywords: Balb/c mice; C57bl/6 mice; MPTP; pre-symptomatic and symptomatic Parkinsonism
Lipopolysaccharide-induced acute sickness behaviour in rats with chronic cerebral hypoperfusion: A model for delirium

Hui Phing Ang¹, Muhammad Luqman Nasaruddin², Wan Ashraf³, Suzana Makpol² and Hashim Embong¹,*

¹Department of Emergency Medicine, Faculty of Medicine, UKM Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia
²Department of Biochemistry, Faculty of Medicine, Level 17 Preclinical Building, UKM Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia
³Department of Medicine, Faculty of Medicine, UKM Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia

*Correspondence:
Department of Emergency Medicine, Faculty of Medicine, UKM Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia
Email: hashimembong77@ukm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

To date, a feasible animal model to mimic the condition that gives rise to clinical delirium has never been validated. This study attempted to develop an animal model of delirium in a rat-based simulation of a chronic cerebral hypoperfusion state and investigated the effects of lipopolysaccharide (LPS) - induced delirium in surgically altered rats. Eight weeks after a permanent bilateral common carotid artery ligation (BCCAL), we exposed the rats to systemic or direct LPS injection via intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) respectively. The rats were divided into the following experiment groups: 1) post BCCAL with i.p. saline (control), 2) post BCCAL with i.p. LPS, 3) post BCCAL with i.c.v. LPS, 4) sham with i.p. LPS and 5) sham with i.c.v. LPS. Each group consisted of 10 male rats. To elucidate the LPS-induced delirium, three behaviour tests consisting of a buried food test (BFT), open field test (OFT) and Y-maze test were performed at baseline, 24-hour, 48-hour, and 72-hour after LPS injection. Overall, these data suggest that BCCAL rats exposed to systemic LPS administration lead to a global neurocognitive deficit, as shown in the BFT, OFT and Y-maze test. These behaviour impairments were acute and fluctuating, consistent with delirium-like behaviour. However, the effects of LPS in the OFT and Y-maze were indistinguishable between the BCCAL and sham rats. This model successfully demonstrated acute and transient instability in mental function typical of delirium following induction of systemic inflammation in a chronic hypoperfused brain. Further study in the specific metabolic pathway relating to acute delirium may lead to the discovery of specific biomarker diagnostic markers of delirium.

Keywords: Acute; delirium; neurodegenerative diseases; dementia and neurocognitive disorders
Riddle-based games and gamified digital case studies as an interactive online learning tools in pharmacology and toxicology

Sharifah Sakinah Syed Alwi, Tabassum Tahia, Muhammad Amirul Hakim Yusri and Manraj Singh Cheema*

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

*Correspondence:
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia.
Email: manraj@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

All educational institutions were forced to implement online teaching and learning as a result of the COVID-19 epidemic, which has drastically altered the higher education landscape. The sudden adjustments don't help pupils comprehend a subject matter better or become more motivated to learn. Therefore, cutting-edge learning systems that use gamification and technical components to deliver these rich experiences are required. Designing and evaluating riddle-based games and digital case studies (RBGDCS) with interactive gaming features was the goal in order to improve student learning gains in pharmacology and toxicology (P&T). First, storyboards were conceptualised and built using P&T-related themes. Then, they were digitalised by adding characters, graded quizzes, and digital photos and videos using Articulate Studio 360 and PowToon. Then, using the ASPIRE framework and storyboard criteria, the flow and running were verified. In order to gauge the respondents' opinions and attitudes on the RBGDCS and learning gains, a series of open-ended questionnaires with a Likert scale was created and distributed. Seven digital case studies and a riddle-based game were successfully developed and validated. The questionnaire helps determine if riddle-based games and digital case studies are effective in making learning simpler, enjoyable, and engaging. In summary, RBGDCS were successful in enhancing comprehension and improving learning gains because they were motivating, engaging, and demanding and had the ability to aid students in acquiring the necessary abilities.

Keywords: COVID-19; digital case study; digital education; e-learning and riddle-based game
Rutin ameliorates lipopolysaccharide-induced depressive-like behaviours in mice models via inhibition of neuroinflammation

Saeed Mustapha1,2,4, Rabiu Abdussalam Magaji2, Mohammed Magaji3, Ibrahim Bako Gaya2, Yusuf Yusha’u2, Mohamad Zulfadli Mehat4, Che Norma Mat Taib4 and Mohamad Aris Mohd Moklas4,*

1Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Dutse, 7156, Jigawa State, Nigeria.
2Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, 810107, Zaria, Nigeria.
3Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, 810107, Zaria, Nigeria.
4Department of Human Anatomy, Faculty of Medicine and Health Science, University Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Science, University Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Depression is a common illness that affects millions of people in the world. Rutin is a flavonol, and flavonols stand out due to their antioxidant, anti-inflammatory, neuroprotective, and antidepressant effects. Inflammation is associated with oxidative stress and the brain is highly sensitive to changes in redox conditions. There is a paucity of data on the effects of rutin on lipopolysaccharides (LPS)-induced depression. This study aimed at investigating the protective roles of rutin on depressive-like behaviours in the LPS model of depression in mice, focusing on inflammation and oxidative stress. Thirty-six (36) Swiss Albino mice were randomly divided into six groups (n = 6). All treatments were administered intraperitoneally for 21 days, except LPS which was administered on the 21st day only: group I served as the normal control (normal saline 10 ml/kg), group II received LPS 0.5 mg/kg only, group III received fluoxetine 20 mg/kg + LPS 0.5 mg/kg, group IV received rutin 25 mg/kg + LPS 0.5 mg/kg, group V received rutin 50 mg/kg + LPS 0.5 mg/kg, and group VI received rutin 100 mg/kg + LPS 0.5 mg/kg. Mice’ behaviours were evaluated through a tail suspension test (TST), followed by assessments of MDA, IL-6, TNF-α, and BDNF. Rutin improved behaviourial despair induced by LPS by significantly reducing immobility time in the TST. It also prevented the increase of inflammatory cytokine IL-6 in the hippocampus due to LPS. Moreso, rutin prevented an increase in brain-derived neurotrophic factor (BDNF) by LPS. These findings suggest the potential use of rutin to prevent LPS-induced depressive symptoms, through its anti-inflammatory activities.

Keywords: Rutin; depression; lipopolysaccharide; flavonoids and inflammation
Synthesis, characterisation, and toxicity study of gallic acid-loaded graphene oxide (GAGO) nanoformulation for anti-inflammation

Suhaili Shamsi1,*, Nur Hazwani Norjoshukrudin1, Seri Narti Edayu Sarchio2 and Faizah Md Yasin3,4

1Laboratory of Animal Biochemistry and Biotechnology, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
2Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
3Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
4Institute of Nanoscience and Nanotechnology (ION2), Faculty of Engineering Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Laboratory of Animal Biochemistry and Biotechnology, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Email: sh_suhaili@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Bacterial infection is one of the factors that contribute to inflammation in our body. When inflammation occurs, macrophages will be recruited to the site of injury and secrete pro-inflammatory cytokines. Although antibiotic is often used to treat bacterial inflammation, it is not effective to treat the inflammation that is caused by multidrug-resistant bacteria. Gallic acid-loaded graphene oxide (GAGO), a simple nanocomposite formulation has been shown to have anti-bacterial effects towards multi-drug resistant bacteria such as multi-drug resistant Staphylococcus aureus (MRSA), but its effect on inflammation and macrophage cells is unknown. The purpose of this study was to evaluate the toxicity and interaction of GAGO nanoformulation in a cellular in vitro model for an anti-inflammation application. In the present study, GAGO was synthesised and characterised with a series of investigations by UV-Vis, FTIR, TEM, Raman, and DLS analyses. The biological properties of GAGO were then evaluated through MTT assay in 3T3 murine fibroblast cells for its cytotoxicity profile and its potential as an anti-inflammation treatment in RAW 264.7 macrophage cells by evaluating the level of both TNF-α and IL-6 using cytometric beads array (CBA) method. A stable GAGO nanoformulation with a drug loading of 0.241 ± 0.052 g/g of GAGO and a loading efficiency of 28.92 ± 6.204% was successfully prepared and characterised. GAGO exhibited an improved toxicity profile against 3T3 murine fibroblast cells, with lower LC50 values at all time points (24-72h), when compared to both GO and GA alone. The level of IL-6 produced in LPS-induced RAW 264.7 macrophage cells that were treated with GAGO was found to be lower compared to the untreated macrophage cells, meanwhile, the level of TNF-α produced in LPS-induced RAW 264.7 macrophage cells showed no significant decrease compared to the untreated cells. Taken together, the data acquired from the present study provide a vital fundamental basis for the development of GAGO as a promising treatment for inflammation.

Keywords: Graphene oxide; GAGO nanocomposite; inflammation and toxicity
Augmented reality of brain: A metaverse approach for teaching & learning

Nur Izah Ab Razak1,*, Sufi Firdaus Fakhrurrazey2, Rahmita Wirza O.K Rahmat2

1Human Physiology Unit, Department of Human Anatomy, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
2Department of Multimedia, Faculty of Computer Science and Information Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Human Physiology Unit, Department of Human Anatomy, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: nurizah@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Augmented Reality (AR) for the brain via mobile devices (i.e., phone and tablet) is a novel method for anatomy and physiology teaching & learning (T&L) in Malaysia. AR is a real-time visualisation technique that superimposes a computer-generated image on a user’s view of the real world. In comparison to traditional learning methods, this 3D technology will allow educators & learners (E&L) to learn and assess their brain knowledge more effectively. The complexities and dynamic nature of T&L in brain subjects necessitate a clear and precise depiction to effectively deliver the subject. During the COVID-19 pandemic, E&L have very limited access to traditional face-to-face T&L methods. This conventional method typically employs standard two-dimensional (2D) sources such as lecture slides, whiteboards, medical books reference, internet websites and other traditional methods. The brain AR was developed using normal brain anatomy and physiology based on several verified resources. Image modelling, mapping, texturing, animation, and rigging are all part of the method. The AR programming made use of Unity, an AR development software. The user interface for this application was designed using Adobe Illustrator CS6. Many studies have shown that the three-dimension (3D) AR method is advantageous because it can help both E&L and T&L in the brain using a 3D approach. It is proposed that this method can effectively increase engagement and improve understanding of brain knowledge.

Keywords: Brain; anatomy; physiology; augmented reality and metaverse
The effect of pleasure-eating towards dopamine secretion and saccharin intake in vivo model

Zakaria Toumi*, Mohamad Aris Mohd Moklas and Nurul Huda Mohd Nor

Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Email: zakichan789@gmail.com
DOI https://doi.org/10.28916/lismb.6.1.2022.108

Abstract

Pleasure-eating is a non-homeostatic overeating and a negative reinforcement for compensating pleasure deficiency that is activated by dopamine, known for positive motivation towards subjective needs. The objective of the study was to identify the relationship between pleasure deficit, high dopamine secretion, and overeating of palatable food by focusing on beta-endorphin as a pleasure neurotransmitter; dopamine as a motivation neurotransmitter; and overconsumption of saccharin as an eating reaction. ELISA method was used for dopamine measurements during hunger and fullness, before tasting saccharin, and after tasting saccharin in rats’ striatum homogenate, while it was used for Beta-endorphin before and after tasting liquid saccharin in rats’ striatum homogenate to assess their contributions in emotional eating. Food preference was also observed in rats during food deprivation and satiety by using two choices of food, Mazuri high-fat diet that contains fats without sweet taste represents the caloric part and liquid saccharin as a non-nutritive sweetener that contains sweet taste without any calories represents the pleasure part. Here, two different choices of food with opposite properties were used to compare between caloric and pleasure needs. Results showed that lower beta-endorphin and dopamine levels were released in groups without saccharin treatment, compared to those with higher beta-endorphin and dopamine levels after saccharin treatment. While for the food preference test, all hungry rats chose Mazuri high-fat diet to compensate for their caloric needs, on the other hand, all full rats chose liquid saccharin to compensate for their pleasure needs. This suggested that the non-homeostatic pleasure deficiency condition stimulates striatum dopamine secretion that leads to the consumption of non-nutritive liquid saccharin to compensate for the non-homeostatic pleasure deficit.

Keywords: Dopamine; motivation; pleasure eating; striatum and lack
Multiplex detection of respiratory viruses using the Luminex xTAG® technology among patients with acute respiratory illnesses

Thevambiga Iyadorai1, Kim Tien Ng2,3,4, Xiang Yong Oong1, Nik Sherina Hanafi5, Adeeba Kamarulzaman2, Sin How Lim6 and Kok Keng Tee1,*

1Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
2Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
3Infectious Diseases Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
4Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Singapore.
5Department of Primary Care Medicine, Faculty of Medicine, University of Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
6Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.

*Correspondence: Department of Medical Microbiology, University of Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia
Email: k2tee@um.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Respiratory viruses are known to cause severe respiratory illnesses, especially in high-risk populations. However, few modern technologies can accurately determine the specific type/subtype of respiratory viruses causing the illness. In the present study, we applied the technology of xTAG Respiratory Virus Panel (RVP) FAST multiplex reverse transcription polymerase chain reaction (RT-PCR) assay in order to detect multiple types/subtypes of respiratory viruses: influenza A and B viruses, respiratory syncytial virus (RSV), parainfluenza viruses 1–4, adenovirus (ADV), human metapneumovirus (hMPV), human coronavirus (HCoV), enterovirus/rhinovirus, and human bocavirus (HBoV) in a single test. In this study, a total of 3935 outpatients presented with common cold symptoms related to respiratory tract infection were recruited at the University of Malaya Medical Centre, Kuala Lumpur, Malaysia from 2012-2014. Nasopharyngeal swab samples were acquired and the total nucleic acids were extracted using NucliSENS easyMAG automated nucleic acid extraction system. Then, the presence of respiratory viruses was detected using the xTAG RVP FAST multiplex RT-PCR assay. Our findings showed that more than half of the samples (51.0%, n=2008) were positive for respiratory viruses. Among these samples, 47.2% were positive for the presence of enterovirus/rhinovirus, followed by influenza A (13.7%), influenza B (8.7%), RSV (4.4%), HCoV OC43 (4.1%), hMPV (4.1%), parainfluenza virus 3 (3.5%), HCoV NL63 (3.2%), ADV (2.3%), HCoV 229E (2.1%), HCoV HKU1 (2.1%), parainfluenza virus 4 (2.1%), parainfluenza virus 1 (1.7%), parainfluenza virus 2 (0.5%) and HBoV (0.3%). Additionally, 85 patients (4.2%) had double infections while three (0.2%) had triple infections. These results highlight the efficiency and importance of using Luminex xTAG® technology in detecting the specific type/subtype of respiratory viruses in patients presented with common cold symptoms.

Keywords: Respiratory viruses; xTAG Respiratory Virus Panel (RVP); multiplex reverse transcription PCR and Luminex® 200™
Curcumin-loaded cockle shell-derived calcium carbonate nanoparticles: A promising candidate for therapeutic treatment of hepato-renal impairments in lead-induced rats

Maryam Muhammad Mailafiya¹, Kabeer Abubakar¹, Abubakar Danmaigoro², Samaila Musa Chiroma³, Ezamin Abdul Rahim⁴, Mohamad Aris Mohd Moklas⁵* and Zuki Abu Bakar Zakaria⁶

¹Department of Human Anatomy, College of Medical Sciences, Federal University of Lafia, P.M.B 146, Akunza, Lafia, Nasarawa State, Nigeria.
²Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usman Danfodiyo University, P.M.B 2346, Sokoto, Nigeria.
³Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Maiduguri, 600230, Maiduguri, Borno State, Nigeria.
⁴Department of Radiology, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
⁵Department of Human Anatomy, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
⁶Department of Preclinical Sciences, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Lead toxicity affects the liver and kidney which could result in hepato-renal dysfunctions. Recently the use of curcumin as an alternative therapy for heavy metal-induced toxicity is attracting nucleus attention. Despite its remarkable pharmacological activities, it remains clinically constrained due to its poor bioavailability. Nanomedicine unravelled promising potentials in drug delivery and has brought forth the use of cockle shell-derived calcium carbonate nanoparticles (CSCaCO₃NP) to enhance effective drug delivery. Thus, this study aimed at evaluating the therapeutic effect of curcumin-loaded CSCaCO₃NP (Cur-CSCaCO₃NP) on lead-induced hepato-renal toxicity in rats. Synthesis of CSCaCO₃NP was conducted using a simple top-down method and was further characterised using standard analytical techniques. Further, 36 Sprague-Dawley rats were randomly assigned into five groups. All groups contained six rats each apart from group A (12 rats). All groups apart from group A were orally administered a flat dose of 50mg/kg of lead for four weeks. Six rats from groups A and B were euthanised for toxicity confirmatory tests at week four. Oral administration of curcumin (100mg/kg) for group C and Cur-CSCaCO₃NP (50mg/kg and 100mg/kg) for group D and E respectively, commenced at week 5 and all rats were euthanised at the 8th week. Body and organ weights were measured. In addition, biochemical and histological analyses were performed. A spherical-shaped CSCaCO₃NP with a surface area of 14.48±0.1 m²/g, mean diameter of 21.38±2.7 nm and zeta potential of -18.5 mV was synthesised. Significant (p < 0.05) biochemical and histological alterations in lead-induced rats were observed. However, Cur-CSCaCO₃NP treatment demonstrated an enhanced therapeutic effect through significant (p < 0.05) improvements of the aforementioned changes when compared to free curcumin treatment. Findings from this study hold great prospects for Cur-CSCaCO₃NP as a novel approach for the effective treatment of lead-induced hepato-renal impairments.

Keywords: Hepato-renal injuries; cockle shell; curcumin; nanoparticles and drug delivery
REST dysregulation in the Down syndrome Ts1Cje mouse brain

Xin-Jieh Lam¹, Chong-Teik Lim², Arthini-Arumugam Crystal², Tan Huang², Norhazlin Jusoh², Pike-See Cheah¹ and King-Hwa Ling²,*

¹Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
Email: lkh@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Down syndrome (DS) is a common genetic disorder caused by the presence of an extra full or partial human chromosome 21. Individuals with DS typically have poor neurological development that leads to neuropsychiatric manifestations. Repressor element-1 silencing transcription factor (REST) is a key epigenetic neuronal gene expression regulator. REST nuclear translocation is essential for binding to target genes and exerting functional repression. Previous studies reported REST dysregulation in human and mouse DS models. However, the results across studies are inconsistent and each study only covered part of the developmental stages. Therefore, we performed a comprehensive REST expression profiling and subcellular localisation analysis to determine its association with DS using the Ts1Cje mouse model. Brain tissues from various brain regions in wildtype and Ts1Cje mice were procured at selected embryonic and postnatal time points throughout development. qPCR was performed to analyse the RNAs from the tissues and cultured neurospheres for the comprehensive Rest expression profiling, while Western blot (WB) and immunohistochemistry (IHC) were performed to analyse REST protein expression and its subcellular localisation between wildtype and Ts1Cje. From the qPCR analysis, it revealed the Rest expression is significantly downregulated in Ts1Cje neural progenitors compared to wildtype, leading to potential precocious maturation and stem cell pool depletion in Ts1Cje models. Overall, Rest expression in Ts1Cje declines in the cerebellum and hippocampus over the development. Moreover, WB analysis showed significantly downregulated REST expression in Ts1Cje against wildtype, while IHC depicted REST is consistently perinuclear marginalised in Ts1Cje cortical neurons but nuclear translocated in wildtype cortical neurons, indicating loss of functional nuclear REST repression in ageing Ts1Cje mouse brains, thereby resulting in loss of REST neuroprotection. In conclusion, the comprehensive REST expression profiling implicated REST dysregulation and loss-of-nuclear REST-function in DS neuropathology related to early cell fate determination and stress resilience during ageing.

Keywords: Down syndrome; REST; spatiotemporal; nuclear translocation and Ts1Cj
Epidemiology and risk factors for disease progression in Malaysian patients with IgA nephropathy

Aminat Suleman-Alabi¹, Fauzah Abd Ghani², Nor Fadhlina Zakaria³ and Rafidah Hod¹,*

¹Human Anatomy Department, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³Department of Internal Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Human Anatomy Department, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: rafidahhod@upm.edu.my
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Abstract

IgA nephropathy (IgAN) is a major cause of primary glomerulonephritis worldwide, and it is also the foremost primary glomerular disease leading to chronic kidney disease and ESRD. The Malaysian renal biopsy registry reports that IgAN accounts for 23.3% of renal biopsies. We, therefore, evaluated the epidemiology and risk factors for disease progression in Malaysian patients with IgAN. The study was approved by the Malaysian Ministry of Health NMRR-ID-22-01020-ZFU (IIR). Out of 175 patients with renal biopsy-proven IgAN diagnosed from March 2012 to April 2022, eighty-one adult patients with primary IgAN with available baseline data with at least 8 glomeruli seen in histology were involved in this retrospective study. The patients were divided into two groups according to eGFR at the end of a median follow-up period of 2(3.4) years; progressors (those with eGFR <15 ml/min or 50% decline in eGFR from point of renal biopsy) and non-progressors. There were more female (72.9%) patients. The mean age at histologic diagnosis was 33.01 ± 10.01 years. At the end of the study, 21 (25.9%) patients reached the composite outcome, 17 (21.0%) had ESRD and 4 (4.9%) had a 50% decline in renal function. Renal function represented by serum creatinine was significantly higher and eGFR was significantly lower among progressors (P < 0.001) at the time of renal biopsy. Compared with the non-progressors mean UPCI and uric acid were significantly higher (P < 0.05) among progressors. According to the Oxford evaluation, the proportion of T1-2 (90.5%) and C1-2 (63.2%) was significantly increased (P < 0.001) and the proportion that had a MEST-C score >3 was significantly higher (85.7%, P = 0.02) among patients with progressive disease. Elevated serum creatinine, reduced eGFR and MEST-C score >3 are independent risk factors for disease progression among Malaysian IgAN patients.

Keywords: IgA nephropathy; disease progression; risk factors; ESRD and Malaysia
Antinociceptive mechanisms of oleuropein from *Olea europaea* L. fruits: An *in vivo* and *in silico* study

Hidayat Ur Rahman¹,²,*, Muhammad Hamdi Mahmood¹, Najm Us Sama³, Mohd Razip Asaruddin⁴ and Mohammed Safwan Ali Khan⁵

¹Faculty of Medicine & Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Malaysia.
²Department of Clinical Pharmacy, College of Pharmacy, Jouf University, Sakaka, Aljouf-72341, Saudi Arabia.
³Faculty of Computer Science & Information Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Malaysia.
⁴Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Malaysia.
⁵Department of Pharmacology School of Medicine, Nazarbayev University, Nur-Sultan 010000, Kazakhstan.

*Correspondence:
Faculty of Medicine & Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Malaysia.
Email: hidayat@ju.edu.sa
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

In recent years, pain has been a major source of concern. It is usually treated with nonsteroidal anti-inflammatory drugs and opioid analgesics, which come in various forms. The adverse effects of these drugs include gastrointestinal bleeding, peptic ulcers, renal failure, cardiovascular difficulties, increased treatment costs, and severe economic losses. Consequently, the search for the ideal painkiller continues. Recent emphasis has been placed on discovering safe and effective drugs derived from natural sources. Olives are among the most important natural resources in the Middle East. Olive (*Olea europaea* L.), a member of the Oleaceae family, is abundant and widely available, and humans use its fruits and oil. Olive contains flavonoids, secoiridoids, glycosides, phenolic compounds such as tyrosol and its derivatives, galactolipids, triacylglycerols, and fatty acids. Oleuropein is one of the principal olive components that has powerful anti-inflammatory properties. A recent study indicates that oleuropein may be used to treat cancer, inflammation, neurological and cardiovascular disorders. In a recent study, oleuropein-rich extract demonstrated antinociceptive activity in an acetic acid-induced writhing model. However, the mechanisms are still unreported. This led us to design the present investigation to unravel the molecular mechanisms in the antinociceptive action of oleuropein.

**Keywords:** Antinociceptive mechanisms; olive; oleuropein; *in vivo* and *in silico*
The therapeutic effect of delta-9-tetrahydrocannabinol against D-galactose and aluminium chloride in Alzheimer’s induced rat model: Behavioural and histological changes in the hippocampus

Fatin Nadzirah Zakaria1,2, Mohamad Taufik Hidayat Baharuldin3, Che Norma Mat Taib1, Hasiah Ab Hamid1, Hafizah Abdul Hamid1 and Mohamad Aris Mohd Moklas1,*

1Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
2Department of Basic Sciences, Faculty of Health Sciences, Universiti Teknologi Mara Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.
3Faculty of Medicine and Defense Health, National Defense Universiti of Malaysia, 57000 Kem Perdana Sungai Besi, Federal Territory of Kuala Lumpur, Malaysia.

*Correspondence: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Email: aris@upm.edu.my
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Abstract

Alzheimer’s disease (AD) is a serious multifactorial form of dementia characterised by memory loss, cognitive decline and neurodegeneration. D-galactose (D-gal) is a senescence agent, while aluminium chloride (AlCl3) is a neurotoxin linked to the pathogenesis of AD. Delta-9-tetrahydrocannabinol (Δ9THC) has evolved as a therapeutic compound in the biomedical field, particularly for neurodegenerative diseases. In regard to this claim, this study has been designed to explore the potential therapeutic effect of Δ9THC on cognition and brain histology in D-gal + AlCl3-induced Wistar rats. In this study, male albino Wistar rats were exposed to 60 mg/kg D-gal intraperitoneally and 200 mg/kg AlCl3 orally, once daily for 10 consecutive weeks. After 10 weeks, Δ9THC at 1.5 mg/kg was administered for 28 days in the treatment phase. 1 mg/kg donepezil was used as a positive control measure. The performance of the rats was evaluated through behavioural assessment; Morris Water Maze (MWM). Nissl staining was used to determine the survival of hippocampus CA3 pyramidal cells while immunohistochemistry was performed to determine the glial fibrillary acidic protein (GFAP) marker for neurogenesis activity. The results revealed that D-gal + AlCl3 could significantly impair behaviour and cognitive function besides causing neurodegeneration by damaging CA3 pyramidal neurons in rats. It also caused a marked decrease in GFAP+ cells in the hippocampus. Conversely, treatment of Δ9THC alleviated the cognitive impairment by improving spatial learning and memory, ameliorating pyramidal cell loss and preventing morphological aberrations in the CA3 region of the rat’s hippocampus. In addition, Δ9THC also enhances the neurogenesis activity by increasing GFAP+ cells marker. Resultantly, the combination of behavioural and histological results suggests that Δ9THC could have well potential therapeutic in the D-gal + AlCl3-induced rats’ model of AD. Enhancing neurogenesis represents another promising strategy that may ameliorate AD-associated cognitive deficit.

Keywords: Alzheimer’s disease; Δ9THC; hippocampus; cognitive impairment and brain histology
The future approach of using muscle tissues as indicative of post-mortem interval in temperate climate

Nur Iylani Ramlee, Mohamad Aris Mohd Moklas* and Che Norma Mat Taib

Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Muscle tissues provide a locomotive advantage for heat, circulation, and protection for any living organism. Forensic science is another branch within the world of anatomy to help to determine, identify and investigate any parts of the human body to identify sex, time, and cause of death but also poses many challenges. The most arbitrary challenge influencing the time of death is the changes in temperature as it generally affects biological balances within an ecosystem. The prospect of using muscle tissues creates an ample opportunity for determining the time since death due to the physiological changes that occur. Here, muscle tissues from mice were harvested for their cardiac, skeletal, and small intestine for western blotting. Histological staining showed the structure of muscle tissues throughout their decomposition stage. We aimed to explore various biomarkers in muscle tissues to establish a chronological time frame via muscle degradation. A standard representation of selected protein markers cTnT, tropomyosin and MHC11 were established for 16, 26 and 30 degrees Celsius. Muscle sampling began from 0, 3rd, 6th, 9th, 12th and at every 12th hour consecutively until sampling remained obsolete. Subsequently, the reliability of protein markers was tested where muscle tissues were randomly harvested based on the already selected time points in burial grounds and submersion underwater, respectively. The breakdown of muscle tissue observed was not proportional over time albeit the varying temperature despite histological stains showed the result of muscle deterioration. Instead, fluctuation of protein markers was observed at several time points while some remained undetected regardless of time. The prospect of muscle in anatomy has given ample opportunities in forensic studies.

Keywords: Muscle tissues; post-mortem interval and protein markers
Nicotine impairs the anti-contractile function of perivascular adipose tissue

Afifah Zahirah Abd Rami1, Amilia Aminuddin1, Adila A. Hamid1, Mohd Helmy Mokhtar1, Norizam Salamt1, Zanariyah Asmawi1, Fadhullah Zuhair Japar Sidik2, Aini Farzana Zulkefli1 and Azizah Ugusman1,*

1Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Cheras, 56000 Kuala Lumpur, Malaysia.
2Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Cheras, 56000 Kuala Lumpur, Malaysia.

*Correspondence: Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Cheras, 56000 Kuala Lumpur, Malaysia.
Email: dr.azizah@ppukm.ukm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Nicotine is a toxic substance found in cigarette smoke that leads to impaired vasorelaxation, hypertension and atherosclerosis. Perivascular adipose tissue (PVAT) exerts an anti-contractile effect on the underlying vessels via the release of adipokines. The anti-contractile function and adipokine profile of PVAT is impaired in diseases such as hypertension and metabolic syndrome. Adiponectin is one of the adipokines that mediates PVAT’s anti-contractile function. However, nicotine’s effect on PVAT’s anti-contractile function and adiponectin release is unknown. Hence, this study aimed to determine the effect of nicotine on PVAT’s anti-contractile function and adiponectin release. Sprague-Dawley rats were divided into three groups: negative control, nicotine, and positive control (nicotine + telmisartan). Following 21 days of treatment, PVAT’s anti-contractile function was assessed via wire myography using the rats’ aortic rings, while adiponectin levels in the PVAT were measured using ELISA. PVAT-intact aortic rings of nicotine-induced rats showed a higher contractile response to phenylephrine ($P<0.05$), and reduced vasorelaxation to acetylcholine ($P<0.05$) compared to the control group. Besides, nicotine decreased PVAT’s adiponectin level ($P<0.05$). Treatment with telmisartan reversed both the impaired vasoreactivity and adiponectin release induced by nicotine ($P<0.05$). In summary, the findings suggest that nicotine impairs the anti-contractile function of PVAT by reducing PVAT’s adiponectin release.

Keywords: Perivascular adipose tissue; nicotine; anti-contractile; telmisartan and adiponectin
Neural alpha phase synchronisation mediates the connectivity between the cerebrum and cerebellum during receptive listening to rhythmic Quranic recitations

Nurfaizatul Aisyah Ab Aziz¹, Nur Syairah Ab Rani¹, Nidal Kamel² and Muzaimi Mustapha¹,*

¹Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
²Centre for Intelligent Signal and Imaging Research (CISIR), Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia.

*Correspondence: Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Email: mmuzaimi@usm.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Neuronal oscillations coordinate the temporal and spatial signals that facilitate the neural network in managing distinct neural processes into a higher cognitive function. Alpha oscillation (8-13Hz) is the focus of most studies studying the effect of listening to Quranic recitations, based on the notion that its presence indicates the relaxation effect. This study explores the alpha phase synchronisation in finding the neural correlate of different styles of rhythmic Quranic recitations. Alpha brainwave responses from the simultaneous magnetoencephalography (MEG) and electroencephalography (EEG) (M/EEG) were recorded from twenty-nine (29) healthy participants (n = 14 Muslim, 15 = non-Muslim). They were subjected to three different modes of recitations (Ayatul Kursi), namely Murattal ‘Asim, Murattal Susi and Tarannum Asli. The MEG data whole brain source-level functional connectivity was quantified using Phase Locking Value (PLV) and subjected to a non-parametric permutation test. During receptive listening to Murattal ‘Asim style, there was increased connectivity between the right superior frontal gyrus (SFG) and the left cerebellum. While during receptive listening to Murattal Susi, there was functional connectivity between the left inferior parietal gyrus (IPG) – right cerebellum. The functional connectivity between these regions showed the increased language processing of the Quranic recitation that entrained the synchronisation of the alpha oscillation. Moreover, there was an increased alpha phase synchronisation between the right angular (part of DMN regions) and left cerebellum during receptive listening to Tarannum Asli style, indicating the role of alpha oscillations in protecting internal information by gating out sensory input. These results suggest receptive listening to rhythmic Quranic recitations engages the synchronisation of alpha oscillations that mediate the cerebrum and cerebellum neural networks responsible for language perception.

Keywords: functional connectivity; Quranic recitation; alpha brainwave; magnetoencephalography (MEG) and electroencephalography (EEG)
Current trends in essential oils research as a neuroprotective agent in Alzheimer's disease

Mohammad Adi Mohammad Fadzil1,*, Ahmad Zuhairi Abd Rahman2, Devi Nair Gunasegavan Rathi1 and Aswir Abd Rashed1

1Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, No.1, Jalan Setia Murni U13/52, Seksyen U13 Setia Alam, Shah Alam 40170, Malaysia.

*Correspondence:
Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Ministry.
Email: adifadzil@moh.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Despite the increase in life expectancy, neurological diseases, particularly Alzheimer's disease, is undoubtedly one of the most incapacitating illnesses of the elderly. Essential oils are being investigated extensively all around the world for their potency to prevent neurodegeneration and anti-ageing properties. The goal of this review is to assess essential oils' efficacy as neuroprotective treatments for Alzheimer's disease. Using key phrases "essential oil" crossed with "Alzheimer's disease (AD)," relevant papers were collected from three databases (Google Scholar, Web of Science, and PubMed) between the years of 2018 and 2022. Approximately, 36 genera have been studied in vitro (22 articles), in vivo (18 articles), a combination of in vitro and in vivo studies (3 articles) and clinical trials (1 article) to assess the potential of these plants as neuroprotective agents. Lamiaceae is the most studied plant family and displayed the ability as anti-cholinesterase, neuroprotective, antioxidant, and neuritogenic activities. Although only one human study was obtained as part of our search, we strongly believe that the presence of essential components in essential oils could play significant roles in efforts towards the prevention and treatment of neurodegenerative disorders such as Alzheimer's disease.

Keywords: Essential oils; neurodegenerative; Alzheimer's disease; in vitro and in vivo
Amelioration of Morris Water Maze target quadrant outcome in endocannabinoid-glial cell modulation in Alzheimer’s disease animal studies

Mohd Amir Kamaruzzaman\textsuperscript{1,2}, Razif Abas\textsuperscript{2}, Sharmili Vidyadaran\textsuperscript{3}, Mohamad Taufik Hidayat Baharuldin\textsuperscript{4} and Mohamad Aris Mohd Moklas\textsuperscript{2,*}

\textsuperscript{1}Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia.
\textsuperscript{2}Department of Human Anatomy, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
\textsuperscript{3}Department of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
\textsuperscript{4}Department of Anatomy, Faculty of Medicine & Defence Health, Universiti Pertahanan Nasional Malaysia, Sungai Besi, 57000 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia.

*Correspondence: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Alzheimer’s disease (AD) is a neurodegenerative condition prevalent worldwide and is clinically characterised by increasing memory loss and the gradual decline of cognitive performance. This systematic review and meta-analysis observe how endocannabinoids modulate glial cells to affect cognition in animal AD models. A search was conducted on the EBSCOhost platform and Scopus electronic databases until June 2022. Search terms were “Alzheimer’s disease”, “endocannabinoid”, “glial cells”, “cognition” and “animal”. In total, 1498 were found, but only ten were included. The meta-analysis demonstrated a statistically significant benefit of endocannabinoid modulation on glial cells toward spatial memory of rodents displayed in the target quadrant of the Morris Water Maze test (standardised mean difference [SMD] = −2.49 (95 % CI: −3.66 to −1.32, P < 0.0001) in experimental models of AD. It could be suggested that endocannabinoids would influence the alteration of glial cells toward an alternative state which is beneficial for neuroprotection. In conclusion, endocannabinoid-mediated glial cells attenuate cognitive deficits in animal AD models. This study may support further studies to translate cannabinoid treatment of AD in humans. The revised data, although not entirely conclusive, seem to suggest that glial cell changes induced by endocannabinoids promote improvement in cognitive function and neurogenesis.

Keywords: Endocannabinoid; glial cells and target quadrant
Antidepressant-like effect of "NevGro Forte" in chronic unpredictable mild stress (cums) model of depression in rat

Zakirah Zainal Abidin¹, Juwita Junit¹, Syntyche Sing Seow Ling², Guat Cheng Poh² and Muhammad Danial Che Ramli¹,*

¹Department of Diagnostic and Allied Health Sciences, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia.
²Ganofarm Sdn. Bhd., 01-01, Skypod Square, Persiaran Puchong Jaya Selatan, Bandar Puchong Jaya, 47100 Puchong, Selangor, Malaysia.

*Correspondence: Department of Diagnostic and Allied Health Sciences, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia. Email: muhddanial.cheramli@msu.edu.my

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Abstract

Depression is a leading cause of disability worldwide and is a major contributor to the overall global burden of disease which affects almost 320 million individuals globally left untreated with depression that has the highest prevalence. Despite the fact that there are multiple conventional antidepressant types available, patients are still left untreated due to the inadequate pharmacological effectiveness that accounts for such a predicament as well as the high rate of remissions, side effects, and patient non-compliance. Lignosus rhinocerotis, Hericium erinaceus and Ganoderma Lucidum which are formulated into NevGro® forte have been reported to produce a therapeutic potential for treating depression. Sprague Dawley rats subjected to chronic mild stress (CMS) protocol were given 4-week oral NevGro® forte treatment. Animals were consecutively screened for depressive-like behaviours through behavioural assessments. Histological analysis was performed to probe the role of neurogenesis in mediating the therapeutic effect of NevGro® forte. Fluoxetine was orally administered to validate the neurogenesis-dependent mechanism of the NevGro® forte. The final outcome of the studies exhibited that 4 weeks of NevGro® forte treatment ameliorated depressive-like behaviours in rats subjected to 56 days of chronic mild stress (CMS). Further, histological studies also revealed decreased degeneration characterised by eosinophilic pyknotic neurons and increased thickness of the pyramidal layer in the hippocampus. Therefore, this research shows a positive outcome of using NevGro forte in ameliorating depressive symptoms.

Keywords: Depression; neurogenesis; antidepressant and Lignosus rhinocerotis, Hericium erinaceus and Ganoderma lucidum
Protective effect of *Clitoria ternatae* on the 6-hydroxydopamine-induced rat model of Parkinson's disease

Hani Qistina Dayana Avelino S. Cavan and Muhammad Danial Che Ramli*

Department of Diagnostic and Allied Health Sciences, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia.

*Correspondence:*  
Department of Diagnostic and Allied Health Sciences, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia  
Email: muhddanial.cheramli@msu.edu.my  
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Parkinson's disease (PD) is the second most frequent age-related neurodegenerative disorder for which there is no cure. The brain is damaged in PD as a result of excessive accumulation of misfolded α-synuclein, which is found in intra-cytoplasmic inclusions called Lewy bodies. Therefore, we performed a thorough behavioural analysis including motor, emotional and cognitive dimensions, of the 6-hydroxydopamine-induced rat model of PD. In the present study, the extract of *Clitoria ternatae* (CT) leaves was evaluated for its neuroprotective ability against neurodegenerative disorders. Rats' models were induced with rotenone (2 mg/kg) for 28 days before administering the extract of CT leaves as a treatment for 28 days. Rotenone is a pesticide that is commonly used to model PD that mimics and elicits PD-like symptoms, such as motor and cognitive decline. We conducted behavioural studies which includes an open field test, forced swim test and beam walking test. Rat models were subdivided into three different doses of CT from 150 mg/kg, 250 mg/kg and 500 mg/kg. The beam walking test resulted in an insignificant difference among three different dosages with an average time to cross beam of 8-10 seconds. The open field was the test used to assess exploratory activity. Exploratory activity did not differ between each dosage, with no differences found in the total distance travelled, number of ambulatory episodes and number of rearing. Administration of CT extraction as a treatment did not affect the ability of the models to increase the immobility time in the forced swim test. Overall, these behavioural studies are suitable to test motor and cognitive skills, sensory-motor function and emotional behaviour of PD-like behaviour of rat models. We found that a minimal dose of CT had shown a similar effect as the highest dosage. The extract of CT leaves proved to be effective in reducing the PD-like behaviour of rat models.

**Keywords:** Parkinson's disease; *Clitoria ternatae*; behavioural studies; rotenone and rat model
Chemical profiling and antioxidant studies on the leaf of *Breonadia salicina* Hepper and J. R. I. Wood (Rubiaceae)

Uwaisu Iliyasu¹,²,⁴, Hajara Ibrahim¹, Umar Katsayal Adamu¹, Sani Ismaila³, Kim Wei Chan⁴ and Ahmad Faizal Abdul Razis⁵,⁶,*

¹Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Nigeria.
²Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences Kaduna State University, Kaduna, Nigeria.
³Department of Basic Veterinary Sciences, School of Veterinary Medicine, University of the West Indies, Trinidad and Tobago.
⁴Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
⁵Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
⁶Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:*
Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Email: madfaizal@upm.edu.my

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Abstract

This study evaluated the antioxidant properties of the leaves of *Breonadia salicina* and the profiling of its chemicals. Ethnically, the leaves of the plant are widely used for the treatment of cancer, gastrointestinal diseases, fevers, headaches, arthritis, diabetes, inflamed wounds, and ulcers. The plant is an evergreen growing along riverbanks, streams and river tributaries, belonging to the family Rubiaceae. Collection and identification of the plant, extraction and fractionation of the sample collected, then antioxidant evaluation using DPPH and ABTS methods, and finally chemical profiling of the sample using LC-MS/MS. Antioxidant equivalence of the leaf extracts/fractions of the plant at R² value of 0.9938 and standard equation (y = 0.9891x - 1.996) was found to be highest at 281.7 ± 0.8mg Trolox Equivalent and lowest at 118.7 ± 2.7 mg Trolox Equivalent. The LC-ESI-MS/MS of the sample identified 22 compounds with their structures, belonging to different classes including flavonoids, glycosides, phenolic acids, triterpenoids, amides and sulphonamides. The identified compounds are of medicinal importance and are undoubtedly responsible for the antioxidant and anticancer properties of the plant. The good antioxidant values obtained revealed possible justification for the use of the plant in the treatment of many diseases that are known to respond to antioxidation.

**Keywords:** *Breonadia salicina; Rubiaceae; antioxidant; flavonoids and phenolic acids*
Occurrence of *Klebsiella pneumoniae* K1/K2 serotypes in ESBL and non-ESBL clinical isolates

Nurul Syazrah Anuar¹, Siti Norbaya Masri², Mohd Nasir Mohd Desa¹, Nur Afiza Aziz³, Hazmin Hazman¹ and Nurshahira Sulaiman¹,∗

¹Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

²Department of Medical Microbiology, Hospital Pengajar UPM, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

³Microbiology Unit, Department of Pathology, Hospital Sultanah Aminah, Jalan Persiaran Sultan Abu Bakar, 80100 Johor Bahru, Johor, Malaysia.

*Correspondence:
Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Email: nurshahira@upm.edu.my

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Abstract

*Klebsiella pneumoniae* is an opportunistic pathogen responsible for many severe hospital-acquired infections including pneumonia, liver abscesses, urinary tract infections, bacteraemia and meningitis. Infections caused by extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* strains have been extensively reported to contribute to higher mortality rates, clinical complication; and longer and more costly hospital stay due to limited treatment options. The emergence of hypervirulent *K. pneumoniae* strains is commonly associated with K1 and K2 capsular serotypes. Importantly, K1 and K2 serotypes were found to be serotype-specific with mucoviscosity-associated gene A (*magA*) and K2 capsule-associated gene A (*K2A*), respectively. The study aims to determine the occurrence of K1 and K2 serotypes in ESBL and non-ESBL producing *K. pneumoniae* clinical isolates in Hospital Pengajar Universiti Putra Malaysia, Serdang, Selangor and Hospital Sultanah Aminah Johor Bahru, Johor. A total of 194 *K. pneumoniae* isolates were collected from both hospitals. A string test was carried out to observe the formation of a viscous string of hypervirulent *K. pneumoniae* strain with more than 5 mm in length. An antibiotic disc diffusion test was conducted to classify isolates possessing ESBL-producing phenotype. The presence of *magA* and *K2A* genes was detected using multiplex PCR. Isolates possessing ESBL phenotype and K1/K2 serotypes were selected for DNA sequencing and multilocus sequence typing (MLST) analysis. Phylogenetic analysis was carried out to observe the genetic association between the K1 and K2 serotypes among the ESBL-producing isolates. A total of 12 out of 89 ESBL-producing *K. pneumoniae* isolates were detected positive for *magA* or *K2A* genes. Following phylogenetic analysis, two distinct clusters were observed. Seven different sequence types (ST) were identified by MLST analysis with ST23 being predominantly associated with the K1 serotype. Meanwhile, K2 serotype is widespread among ST14, ST65, ST86, ST628, ST657 and ST792.

**Keywords:** Klebsiella pneumoniae; extended-spectrum β-lactamase and K1/K2 serotypes
Predictors of non-communicable disease management behaviour and mediating effects of attitude among support staff in selected ministries in Putrajaya, Malaysia

Azrin Shah Abu Bakar¹, Haliza Abdul Rahman¹,²,*, Hayati Kadir Shahar³,⁴, Ahmad Iqmer Nashriq Mohd Nazan⁴ and Khairuddin Idris¹

¹Institute for Social Science Studies, Putra InfoPort, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³Malaysian Research Institute on Ageing (MyAgeing™), Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
⁴Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Institute for Social Science Studies, Putra InfoPort, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia & Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: dr.haliza@upm.edu.my
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Abstract

Non-communicable disease (NCD) is a growing health issue and threatens many lives around the world including in Malaysia. The lower-income group are more vulnerable to this problem. In this regard, it would be useful to understand the factors that affect non-communicable disease management behaviour. The present study sought to examine the relationship between the individual factor, interpersonal factors, organisational factors, community factors, societal factors and non-communicable disease management behaviour as well as the mediating role of attitude in these relations. This is a cross-sectional study consisting of 280 support staff selected using a random cluster sampling method from eight ministries located in Putrajaya. Data were collected through a self-administered questionnaire distributed through email. Data were analysed using descriptive statistics, Pearson Correlation analysis, regression analysis and PROCESS macro in SPSS. The findings of Pearson correlation analysis demonstrated individual factors (knowledge) ($r=0.241$, $p = 0.000 <0.05$), interpersonal factors (family and friends) ($r=0.671$, $p = 0.000 <0.05$), organisational factor (workplace) ($r=0.495$, $p = 0.000 <0.05$), community factors (neighbourhood, local community, community organisation and health services) ($r=0.632$, $p = 0.000 <0.05$), societal factors (health policy and mass media) ($r=0.648$, $p = 0.000 <0.05$) and attitude ($r = 0.373$, $p = 0.000 < 0.05$) have a significant positive relationship with non-communicable disease management behaviour. From the multiple regression analysis, this study found that individual factors ($b = 0.362$, $p = 0.031 < 0.05$), interpersonal factors ($b = 0.377$, $p = 0.000 < 0.05$) and societal factors ($b = 0.386$, $p = 0.000 < 0.05$) statistically and significantly predict the non-communicable disease management behaviour. Mediation analysis found that attitude significantly mediated the relationships between individual factors, interpersonal factors, organisational factors, community factors, societal factors and non-communicable disease management behaviour. Hence, policymakers and government may utilise this study to develop health programmes or campaigns to enhance non-communicable disease management behaviour.

Keywords: Non-communicable disease management behaviour; mediating effect and support staff
Clitoria ternatea as a treatment for memory deficits in the zebrafish model of Alzheimer's disease: A neurobehavioural study

Nur Arissa Syazwani Md Nazrey¹, Muhammad Danial Che Ramli¹,*, Mahathir Uzid¹, Nur Batrisyia Nizam¹, Nadhratun Nabila Mazlan¹, Nurshafiqah Najihah Mohd Mizan¹ and Muhamad Hussin²

¹Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia. 
²Toxicology and Pharmacology Unit, Herbal Medicine Research Centre, Institute of Medical Research, 50588 Kuala Lumpur, Malaysia.

*Correspondence: 
Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia. 
Email: muhddanial_cheramli@msu.edu.my

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is currently affecting 40-50 million people worldwide. It is generally recognised from its main symptom dementia, in which the patient undergoes a rapid decline in their cognitive memory. Recent studies have shown that medicinal plants such as Clitoria ternatea equipped with antioxidant properties have high potential in treating Alzheimer's disease. The study was conducted using an aluminium chloride induced zebrafish model of AD for 28 days. The treatment dose of 4.34 mg/L was then given for 14 days. The behaviour of the zebrafish was evaluated through memory testing by using a T-maze test and a novel tank diving test. Histological studies were also performed. 50% of the zebrafish tested showed improvement in memory through the T-maze test after treatment with Clitoria ternatea extract. Zebrafish treated with Clitoria ternatea extract also shows a decrease in anxiety in the novel tank diving test. A significant increase in the number of Purkinje cells was also observed from the histological study of the zebrafish model brain after being treated with Clitoria ternatea extract. Nucleus elongation of oligodendrocytes from the aluminium chloride-induced zebrafish model of AD was improved when treated with the Clitoria ternatea extract. The results obtained suggest that the Clitoria ternatea extract exhibits strong potential for treating the aluminium chloride induced zebrafish model of AD.

Keywords: Alzheimer’s disease; Clitoria ternatea; zebrafish model; neurodegenerative and treatment
Attenuation of type 2 cytokines in ovalbumin-induced allergic asthma mice via intranasal administration of *Lignosus rhinocerotis* polysaccharide fraction

Bushra Solehah Mohd Rosdan¹, Ruzilawati Abu Bakar², Sabreena Safuan¹ and Nurul Asma Abdullah¹,*

¹School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
²School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

*Correspondence:
School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Email: nurulasma@usm.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Asthma is a respiratory disorder characterised by chronic airway inflammation mainly associated with type 2 cytokines including interleukin-4 (IL-4), IL-5, and IL-13 leading to eosinophilia, hyperresponsiveness, excessive mucus secretion and immunoglobulin E (IgE) production. *Lignosus rhinocerotis* (LR), is a medicinal mushroom that is effective as a prophylactic and therapeutic agent of inflammatory diseases including allergic asthma. However, there are limited studies on the polysaccharide fraction of LR (LRP) effect on the allergic asthma model. In this study, the total carbohydrate content of LRP was determined using a phenol-sulphuric acid method and its chemical compound was characterised via Fourier-transform infrared spectroscopy (FTIR). Subsequently, the asthmatic features of type 2 cytokines were investigated in the ovalbumin (OVA)-induced airway inflammation mouse model via intranasal administration of LRP. Asthma condition was established by sensitising the mice on day 0 and 14 via intraperitoneal injection of OVA and aluminium hydroxide adjuvant. The challenge and treatment were conducted for seven consecutive days from day 21 to 27 with 1% aerosolized OVA and LRP. After 24 h, the bronchoalveolar lavage fluid (BALF) was collected and the level of type 2 cytokines was determined. Interestingly, the FTIR spectrum suggested the presence of typical polysaccharide characteristics with the absorption bands of O-H, C-H and C-O groups. Besides, the result also demonstrated that LRP significantly reduced the level of IL-4, IL-5, and IL-13 in OVA-sensitised asthma in coherence with the histopathological analysis of the lung tissue. In conclusion, these findings suggest that LRP could demonstrate a promising therapeutic effect for the management of allergic asthma.

Keywords: Allergy asthma; cytokines; *Lignosus rhinocerotis* and polysaccharide
A facet of vaginal agenesis by isolation and characterisation of vaginal epithelial cells

Lih Yuan Too¹, Nadiah Sulaiman¹, Nur Azurah Abdul Ghani², Manira Maarof¹, Rabiatul Adawiyah Razali¹ and Muhammad Dain Yazid¹,*

¹Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia.
²Department of Obstetrics and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia.

*Correspondence:
Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia.
Email: dain@ukm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Vaginal agenesis is a congenital disorder commonly seen in Mayer-Rokitansky-Küster-Hauser (MRKH) women whereby the vagina is absent. The aetiology of the disorder remains unknown but has been shown associated with failure in the development of the Mullerian duct. The surgical procedure of creating a new vagina (neovagina) and subsequently anastomosing the uterus to the vagina is the only solution. Thus, the use of in vitro cultured vaginal mucosa has emerged as a novel technique to epithelize the new vaginal wall. However, the potentiality and feasibility of the patient’s cells are still unknown. In this study, we aimed to establish a suitable isolation procedure and characterise vaginal epithelial cells (VECs) from vaginal mucosa biopsy of obstructed hemi-vagina with ipsilateral renal agenesis (OHVIRA) patients. Vaginal mucosa tissues were harvested from the vaginoscopic incision of non-menopausal, OHVIRA patients aged 12 to 30 years. VECs were isolated using two different enzymes: 0.6% collagenase type I enzyme and 0.25% trypsin-EDTA. The cells were cultured in Epilife medium supplemented with 60µM calcium and Human Keratinocyte Growth Supplement (HKGS) prior to proliferation analysis. The VECs were characterised via immunofluorescence technique along with VK2/E6E7 cell line as a control. As result, VECs were successfully isolated from vaginal mucosa biopsy using 0.6% collagenase type I enzyme based on cell attachment and morphology in a culture medium. Population doubling time (PDT) analysis showed that primary VECs had a significantly lower PDT (mean PDT = 39.05 hours) as compared to VK2/E6E7 cell line (mean PDT = 44.61 hours) (P<0.05). Both cells expressed cytokeratin and vimentin indicating epithelial and mesenchymal phenotypic properties, respectively. In conclusion, vaginal mucosa biopsy from OHVIRA patients consists of both epithelial and mesenchymal cells which can be cultured stably and used as a VECs study model to further understand the development of MRKH and regulate vaginal epithelialization to ensure a better surgical outcome.

Keywords: Vaginal agenesis; vaginal epithelial cells; epithelial-mesenchymal transition; cytokeratin and vimentin
Combating intimal hyperplasia with hydroxytyrosol: An in vitro analysis

Ubashini Vijakumaran1,*, Muhammad Dain Yazid1, Mohd Ramzisham Abdul Rahman2, Hairulfaizi Haron2, Nur Ayub Md Ali2, Muhammad Ishamuddin Ismail 2 and Nadiah Sulaiman1

1Center of Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Kuala Lumpur, Malaysia.
2Department of Surgery, Hospital Canselor Tuanku Mukhriz, Universiti Kebangsaan Malaysia Medical Centre, 56000 Kuala Lumpur, Malaysia.

*Correspondence:
Center of Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Kuala Lumpur, Malaysia.
Email: ubashini.shini@gmail.com
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Intimal hyperplasia (IH) is a vascular thickening that occurs due to excessive proliferation and migration of smooth muscle cells (SMCs), often in response to a traumatic stimulus. IH decreases the patency of bypass graft post coronary artery bypass graft surgery (CABG) and threatens the angioplasty procedure. Therapies employing antiproliferative drugs have inhibited SMCs proliferation but concurrently impede re-endothelialisation. Hydroxytyrosol (HT) is an olive-derived polyphenol. Its effect on IH attenuation has not been extensively studied. Therefore, we aim to study the effect of HT on endothelial cell proliferation and SMC inhibition in vitro before its application in an ex-vivo IH model. We isolated ECs and SMCs from the saphenous veins of patients undergoing CABG surgery. The half-maximal inhibitory concentration (IC50) of HT-treated SMC is 300 μM. EDU assay detected 40 μM up to 320 μM significantly (p<0.05) decreases PDGF-BB induced SMCs proliferation. A dose of 20 μM-80 μM suppressed the wound closure and migration of PDGF-induced SMCs by 54% (p<0.001). HT downregulated vimentin, a synthetic phenotype marker in PDGF-BB-induced SMCs. Besides, up to 50 μM HT could maintain EC proliferation. VEGFR2 protein, an angiogenesis marker, was highly expressed in HT-treated EC. Hydroxytyrosol also salvages TNF-α induced cytotoxicity and apoptosis in ECs. Following this, we also established the IH ex-vivo model by mechanically scraping the ECs in in-vitro artificial conditions where the intimal media thickness was significantly higher than the native vessel. Therefore, these preliminary findings enable the study of HT combinatory effect on both endothelial cells and smooth muscle cells in an ex-vivo intimal hyperplasia model to be further elucidated soon.

Keywords: Intimal hyperplasia; olive; hydroxytyrosol and cardiovascular disease
**Centella asiatica** improves the cognition, memory and neuronal changes in the hippocampus of aluminium chloride and D-galactose induced male albino Wistar rats

Vishnumukkala Thirupathirao¹, Saravanan Jagadeesan², Samaila Musa Chiroma³, Mohamad Taufik Hidayat Baharuldin⁴, Sharmili Vidyadaran⁵, Nurul Huda Mohd Nor⁶ and Mohamad Aris Mohamad Moklas*⁶

¹Department of Human Biology, School of Medicine, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
²Department of Human Anatomy, School of Medicine, Taylors University (Lakeside Campus), 47500 Subang Jaya, Selangor, Malaysia.
³Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Maiduguri, Maiduguri, Nigeria.
⁴Department of Physiology, Faculty of Medicine and Defence Health, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia.
⁵Immunology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
⁶Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

**Abstract**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder presenting with loss of memory, cognitive impairment, personality alteration, and behavioural changes in the affected persons. The effects of the current pharmaceutical compounds to treat AD have been limited by their short duration of action and toxicity. **Centella asiatica** (CA) is a medicinal herb which has been used in traditional medicine as a neuroprotective agent. An aqueous extract of CA was used in this experiment. Male albino Wistar rats were randomly divided into six groups of eight rats each group. The groups include the control group, model group, positive control group, and three doses of treatment groups. The control group was administered distilled water, all the other groups were administered AlCl₃ (200 mg/kg/oral) and D-gal (60 mg/kg/i.p). In addition, the positive control group was also administered donepezil 1 mg/kg orally, while the CA was administered in dosages of 100, 200 and 300 mg/kg/orally daily for 10 weeks. At the end of 10 weeks, behavioural assessments (open field test, novel object recognition test, modified elevated plus maze) of the rats were performed after which the rats were euthanised and histological analysis (Nissl's staining) of their brains (cerebral cortex and hippocampus) were carried out. The results revealed that the model group rats exhibited a significant cognitive impairment in both spatial and non-spatial learning and memory tests, which was also associated with marked neuronal loss (p <0.05). Notwithstanding, CA at the dosage of 300 mg/kg attenuated these aforementioned changes in the rats. These findings provide scientific evidence to support the use of CA as a safe and effective medicinal plant to consider in the fight against AD.

**Keywords:** Alzheimer's disease; Centella asiatica; behaviour and hippocampus
Limestone-based hydroxyapatite-hyaluronic acid (HA/HYA) composite: Effects on physico-chemical, morphological properties and protein adsorption

Zatil Hanan Yahaya1, Mior Muhamad Akmal Mior Muhammad Jamil2, Akmal Azman2, Masfueh Razali1,*, Nurulhuda Mohd1 and Mariam Jameelah Ghazali3

1Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
2Oral Health Division, Ministry of Health Malaysia, 62590 Putrajaya, Malaysia.
3Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

*Correspondence:
Unit of Periodontology, Department of Restorative Dentistry, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
Email: masfuah@ukm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Bone tissue engineering in dental applications is gaining more interest, especially for periodontal bone grafting. Hydroxyapatite (HA), a calcium-based biomaterial, abundantly found in Malaysian limestones, consists of similar composition to human bone and is widely used as a bone graft in the replacement and reconstruction of alveolar bone. However, HA alone possesses a weak degradation rate, thus disrupting its regenerative potential as an alloplastic bone graft. In overcoming HA’s weak regenerative ability, natural polymers such as hyaluronic acid (HYA) have shown to improve the osseointegration and overall structure of the bone graft composite. This study aims to evaluate the porosity, surface morphology and protein adsorption of limestone-based hydroxyapatite/hyaluronic acid (HA/HYA) composites. These mixtures were freeze-dried and cross-linked prior to the assessment of porosity and density. The morphology and pore configurations of the composite scaffolds were examined through field emission scanning electron microscopy, while the water displacement test was used to evaluate the porosity and density. Surface morphology indicated the presence of pores and needle-like apatite crystals, which denotes HA and HYA in the composite. For the porosity test, 30% HA composition showed a result of high porosity and density suitable for bone regeneration. Protein adsorption on HA/HYA composite was determined and its performance showed similar outcomes with pure HA. In conclusion, HA/HYA composites showed acceptable porosity with a good protein adsorption ability for the development of synthetic bone grafts.

Keywords: Bone graft; nanohydroxyapatite; hyaluronic acid; porosity and protein adsorption
Cytotoxicity and genotoxicity studies of selected medicinal mushrooms extracts using Chinese hamster lung fibroblast cell line (V79)

Nur Shahirah Nasir1,*, Farida Zuraina Mohd Yusof2, Nor Fadilah Rajab3 and Muhamad Zakuan Abdullah4

1Faculty of General Study and Foundation, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.
2Faculty of Applied Science, Department of Biology, University Teknologi Mara, 40450 Shah Alam, Selangor, Malaysia.
3Faculty of Allied Health Science, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
4Department of Medical Science & Technology, Faculty of Health Sciences, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.

*Correspondence: Faculty of General Study and Foundation, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.
Email: shahirah@ucmi.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Medicinal mushrooms are well-known for their health and nutritional benefits. However, undesirable effects have been reported in animals fed with these types of edible mushrooms. For health and safety reasons, it is necessary to evaluate the toxicity of medicinal mushrooms, especially those that have been artificially cultured in recent decades. The aim of this study was to identify the potential toxicity (cytotoxicity and genotoxicity) in various concentrations of Agaricus blazei, Grifola frondosa and Hericium erinaceus, which are commonly used in Asian and also Malaysia for medicinal treatment. In this study, screening of phytochemical compounds, evaluating the cytotoxicity effect through cell viability-MTT assay, observing the morphological changes of cells and analysing the genotoxic activity by using Comet Assay were done to assess the potential toxicity. All toxicity tests were evaluated against Chinese hamster lung fibroblast cell line (V79) using two different solvents; methanol and aqueous in five different concentrations of the extracts (0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml). Phytochemical screening of bioactive compounds in mushrooms showed that the extracts contain alkaloid, antraquinone, terpenes and phenol. The cytotoxicity effect (MTT assay) demonstrated that all extracts showed low cytotoxicity effect at the maximum concentration of 2 mg/ml. Only aqueous extract of A. blazei displayed the highest cytotoxicity effect (IC50 - 1.7 mg/ml) compared to other extracts. Cell morphology did not show any sign of apoptotic and cell injury when exposed to mushroom extracts at the concentrations of 1 mg/ml and 2 mg/ml during 24 hours treatment. On the other hand, genotoxicity activity for A. blazei, G. frondosa and H. erinaceus in V79 cell results revealed that DNA damages were detected during Comet Assay test at concentrations of 1 mg/ml and 2 mg/ml. It can be concluded that these three selected medicinal mushrooms had caused toxicity to normal cells as demonstrated by V79 cell line (Chinese hamster lung) at the concentration of 1 to 2 mg/ml.

Keywords: Cytotoxicity; genotoxicity; Chinese hamster lung fibroblast cell line (V79); cell viability-MTT assay and Comet Assay
Determination of ideal age and harvesting time of *Moringa oleifera* plant for optimum polyphenols content

Yogespiriya Shreeraman¹, Intan Safinar Ismail¹,², Wee Kian Yeo ⁴, Sandra Maniam³, Yoke Mun Chan ⁵ and Sharida Fakurazi¹,³, *

¹Laboratory of Natural Medicine and Product Research, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
⁴Division of Research and Innovation, National Sports Institute of Malaysia, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
⁵Department of Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:*
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Email: sharida@upm.edu.my
DOI https://doi.org/10.28916/lasm.6.1.2022.108

Abstract

The need and demand for plant-based dietary supplements has escalated all-over the world, particularly when awareness about healthy living has grown within the society. Intake of dietary supplement would at least impart some form of extra diet component to an individual, ensuring that the person is equipped with sufficient dietary needs. To ensure a high quality and proven efficiency of product, the production of dietary supplements must be sustainable and standardised from the planting stage throughout harvesting time. In this study, leaves of *Moringa oleifera* (MO) were harvested from a geographical location 3°23’27.2”N 101°26’57.5”E in the morning (8-10 am) and evening (3-5 pm) from trees of different ages such as 1-2, 5-6 and 9-10 years, indicated by the cultivator. Leaves were air dried, powdered, sieved and extracted with 70 % ethanol in distilled water, which was then followed by ultrasonication method. Total phenolic content (TPC) and total flavonoid content (TFC) will be determined from the prepared extract. Moreover, the extracts will be analysed by HPLC-DAD to quantify the targeted compounds. The percentage of antioxidant activity (DPPH assay) also will be measured to support the findings. Analysis will be conducted from different groups of extract to compare the level of phenolics and flavonoids and the content of targeted active compounds. It is predicted that leaves obtained from trees aged 9-10 years, harvested in the late afternoon will bear the highest percentage of phenolics and flavonoids content with optimal detections of relevant antioxidant compounds. The leaves obtained from *Moringa oleifera* of this age at the given time have sufficient exposure to sunlight, which allow the most favourable condition for the synthesis of phenolic compounds.

**Keywords:** *Moringa oleifera; extraction; harvesting time; plant age and polyphenols*
Comparison study on histopathological changes of the gastrointestinal tract after treatment of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) between BALB/C and C57BLK/6 mice

Nur Hidayah Saubari¹, Tengku Azam Shah Tengku Mohamad¹ and Che Norma Mat Taib²,*

¹Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Email: chenorma@upm.edu.my

Abstract

The neurotoxin 1-methyl,4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is used in rodents to induce experimental parkinsonism. Parkinson’s disease (PD) can give effects both and non-motor symptoms as well as various gastrointestinal symptoms. Administration of neurotoxin (MPTP) in this study to BALB/c and C57BLK/6 is to create a PD model and understand the effects of MPTP on the gastrointestinal tract. It is still controversial and not clear since previous studies showed MPTP works well in C57BLK/6 but still there are findings showing it also can cause effects on BALB/c. This study aims to compare the effects of MPTP on the gastrointestinal tract between two inbred-mice strains. A total of 32 adult males of BALB/c and C57BLK/6 were randomly divided into four groups, with each group comprised of four mice. The four groups were administered with normal saline, 15mg/kg, 30mg/kg, and 60mg/kg of MPTP respectively. After five days of treatment, the stomach, ileum, and colon were collected and fixed in formalin, prior routine histological techniques. Each photomicrograph taken was examined and scored based on three different categories of necrosis. The results yielded increasing doses of MPTP can cause enhancement of necrosis formation in the stomach, ileum, and colon of C57BLK/6 compared to BALB/c. It is proven by three different categories of necrosis that showed increasing trends in graph means and histopathology examination of organs were more severe in C57BLK/6 in comparison to BALB/c. MPTP also can cause toxicity effects in BALB/c although it has the characteristics of resistance. However, it is only reasonable to see the effects of MPTP histologically right after the administration of MPTP. In short, the present study signifies that MPTP can cause effects of toxicity in both strains including BALB/c; however, necrosis formation is enhanced with increasing doses of MPTP in C57BLK/6 compared to BALB/c.

Keywords: Parkinson’s disease (PD); 1-methyl,4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); BALB/C; C57BLK/6; gastrointestinal tract and necrosis
Neuroprotective effects of *Centella asiatica* on the expression of apoptotic markers, BAX and BCL-2 in AlCl₃ / D- galactose induced Alzheimer’s like male albino Wistar rat model

Tiriishan Reedy Santhira Sekaran¹, Thirupathi Vishnumukkala²³, Tengku Azham Shah Tengku Mohamad¹ and Mohamad Aris Mohd Moklas²,*

¹Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³PURSI School of Medicine, Perdana University, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: aris@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Neuronal apoptosis due to the accumulation of amyloid β peptide (Aβ), is a suspected cause of neurodegeneration in Alzheimer’s disease (AD). There are presently no effective treatment options available for a vast majority of AD patients. Several studies suggest the plant *Centella asiatica* (CA) as a potential therapeutic candidate for AD considering its ability to prevent cognitive deterioration and neuron apoptosis by reducing the beta-amyloid toxicity. However, little is known regarding the effects of CA on the expression of mitochondrial apoptotic markers in relation to beta-amyloid toxicity. This study aims to determine the effects of CA on the expression of mitochondrial pro-apoptotic marker BAX and anti-apoptotic marker BCL-2 in D-galactose and aluminum chloride-induced Alzheimer’s-like albino Wistar male rat model. The rats were randomly distributed into six groups with eight rats in each: 1 control group, 1 model group (D-galactose + AlCl₃), 1 positive control group (1 mg/kg/ I.p Donepezil), 3 treatment groups (CA100, 200 and 300 mg/kg). The dosages of CA were administered along with the model dosage, and the control group received an equivalent volume of distilled water and normal saline for the same period. The rats were euthanised by decapitation, and their brains were excised. ELISA was used to measure the apoptotic markers’ concentration in the brain tissues. Protein expression analyses of rat brains revealed a significant increase in the BCL-2 level and a significant decrease in BAX level in the CA200 and 300 mg/kg group compared to the model group. These results show that the extract of CA at 200 and 300 mg/kg can provide neuroprotective effects by inhibiting apoptosis through increased BCL-2 and reduced BAX expression. However, no significant difference is observed between these two groups indicating that CA 200 mg/kg is the most effective dose to reduce neuron apoptosis in this AD-induced rat model.

Keywords: *Centella asiatica*; Alzheimer’s disease; apoptosis; BAX and BCL-2
Abstract

Benign prostatic hyperplasia (BPH) is the most prevalent prostate disease in elderly men, characterised by abnormal development of prostatic epithelial and stromal cells. Despite the wide range of pharmaceutical interventions accessible, the present treatments for BPH are not without side effects, hence there is a need for a new alternative option such as phytocompound. Isoflavones daidzein has a chemical similarity to mammalian oestrogen. Daidzein showed anti-androgenic activity by inhibiting the 5-alpha reductase enzyme that leads to the reduction of DHT conversion from testosterone. In this study, we examined the effects of daidzein on the BPH animal model. The rats were divided into five groups randomly: sham, BPH-induced without treatment (BPH group), and three BPH-induced with treatment groups that received daidzein (10 and 100 mg/kg) and finasteride (1 mg/kg) as a positive control, intragastrically for 30 days. BPH was induced by daily subcutaneous injection of testosterone propionate (3 mg/kg) daily. The prostatic index and prostate histomorphometry were performed. The BPH group had significantly higher prostatic weight which contributed to a higher prostatic index (9.49 g/g) compared to the sham (3.85 g/g) group. However, the treatment of 10 mg/kg and 100 mg/kg daidzein, and finasteride significantly reduced the increase in prostatic index (5.39 g/g, 5.09 g/g and 4.92 g/g, respectively) compared to BPH group. The serum dihydrotestosterone level also showed a significant lowering effect with daidzein treatment. Additionally, daidzein treatment showed a restoration in prostate histomorphology and significantly reduced the histo-score compared to BPH group. The prostatic acini presented with tubular luminal that ranged from regular to villous with significantly fewer involution projections. In this study, daidzein demonstrated anti-BPH activity that was comparable to finasteride, the commercial anti-BPH medicine in our BPH rat model. Together, these findings suggested that daidzein has the ability to prevent the progression of BPH induced by testosterone.

Keywords: Benign prostatic hyperplasia; dihydrotestosterone; prostatic index; histomorphology and daidzein
Dysregulation of REST targeted genes in Down syndrome human induced pluripotent stem cell-derived cerebral organoid

Norhazlin Jusoh¹, Norshariza Nordin¹, Pike-See Cheah² and King-Hwa Ling¹,*

¹Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: lkh@upm.edu.my
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Abstract

One in every 800 live births is affected by Down syndrome (DS), which is caused by chromosome 21 trisomy. Although the cognitive impairments in DS are caused by abnormalities in cortical development, the underlying molecular mechanism affecting dysregulation of prenatal neurogenesis is unclear. RE1-silencing transcription factor (REST) is a negative regulator of neuronal gene expression during embryogenesis and neurogenesis. It was downregulated in the human DS brain cortex and linked to DS neuropathology. As a result, this study sought to link REST targets to differentially expressed genes (DEGs) in DS vs control hiPSC-derived cerebral organoids. Datasets containing DEGs of DS vs control cerebral organoids were obtained from the public domain. Overlapping genes between REST targets and both DEGs were identified using Venny 2.1. The REST-targeted DEGs were compared to REST targets and DEGs for overlapping genes using hypergeometric distribution testing. Toppgene Suite (https://toppgene.cchmc.org/) was applied to determine the Gene Ontology of REST-targeted DEGs using a p<0.05 cut-off with FDR correction. In DS vs control cerebral organoids, 195 DEGs were discovered. From the DEGs, there were 113 DEGs as REST targeted in the DS cerebral organoids, with 58 down- and 55 upregulated genes. The forebrain development pathway was significantly enriched in REST-targeted DEGs with the highest enriched upregulated genes of DS cerebral organoids. The presence of REST targets in DS cerebral organoid DEGs demonstrates that REST may be dysregulated in DS brain development or function, particularly in central nervous system development and neuropeptide-mediated synaptic development, which are essential for learning and memory.

Keywords: REST; Down syndrome; cerebral organoid and neurogenesis
Identification of the hub genes and the role of rest in human Down syndrome neural cells

Tan Huang1, Pike-See Cheah2, Sharida Fakurazi2 and King-Hwa Ling1,*

1Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
2Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: lkh@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Down syndrome (DS) is the most common chromosomal disorder characterised by intellectual disability (ID) with neurogenic-to-gliogenic shift affecting the morphology and function of neurons and astrocytes. Repressor Element-1 Silencing Transcription factor (REST) is a transcription repressor that acts as the key initiator of epigenetic neural gene-expression modification. We download the microarray data of GSE84887 (Neural Progenitor Cells, NPC), GSE48611 (Neuron), and GSE42772 (Astrocyte) from NCBI Gene Expression Omnibus (GEO) and performed differential gene expression (DEG) analysis with R limma package. REST target genes of the human were obtained from GTRD (http://gtrd.biouml.org) and compared with DEGs for any significant representation. REST-targeted DEGs were performed Gene Ontology analysis using the R clusterProfiler package. STRING (http://string-db.org) was used to induce the PPI network. Then cytoHubba plugin of Cytoscape was employed for Hub gene screening. We found a total of 482 of 707 DEGs in the NPC were REST target genes involved in neural cell differentiation and development, axon guidance and the TNF signalling pathway. 81 of 136 DEGs were REST target genes involved in the cell cycle, glycosylphosphatidylinositol anchor protein biosynthesis and JAK-STAT signalling pathway. 77 of 110 DEGs were REST target genes mainly involved in the biosynthesis and metabolic processes of lipids and growth hormones. We verified a total of 15 Hub genes (NPC: BMP4, CDH1, MYC, CDH5, NCAM1; neuron: BACE2, DONSON, CAT, MRPL39, SH3BGR; astrocyte: AGRN, APP, LDLR, POSTN, P4HB). 12 of these 15 genes were subsequently verified to be REST target genes. Dysregulation of REST leads to dysfunction of lipid metabolism, TNF and JAK-STAT signalling pathway and impair the phenotype, function and development of NPC, neuron, and astrocyte. DEGs in DS neural cells were significantly targeted by REST, suggesting the critical role of REST throughout the development of DS neuropathology. We determined REST may be a potential therapeutic target for DS.

Keywords: Down syndrome; REST; NPC; neuron and astrocyte
Abstract

COVID-19 is a contagious disease caused by a coronavirus strain. During the outbreak of COVID-19, anxiety, anticipation and lack of knowledge in the previous experience on severe acute respiratory syndrome and the H1N1 pandemic have had a substantial impact on significant public health behaviours. This includes wearing a face mask in public spaces. We investigate the prevalence of face mask usage and its associated factors during COVID-19 amongst students and staff of the Faculty of Medicine and Health Sciences (FMHS), Universiti Putra Malaysia (UPM). Using a cross-sectional study, 220 respondents participated in an electronic version of a self-administered questionnaire. Analyses were performed using the Chi-square test, IBM SPSS Statistics 25, with statistical significance at p<0.05. Out of the 220 respondents, 197 (89.5%) reported that they wore face masks all the time. The majority of the respondents (90.3%) had a high level of precautionary measures against COVID-19, 89.6% selected the internet as their source of information and 90.9% of the respondents had a good attitude level towards measures against COVID-19. There were significant associations between hygienic practices (p=0.001), source of information (internet)(p=0.025), and attitude towards COVID-19 (p=0.001) with face mask usage. In conclusion, this study demonstrated a high prevalence (89.5%) of face mask usage during the COVID-19 outbreak amongst students and staff of FMHS, UPM.

Keywords: COVID-19 pandemic; face mask; lack of knowledge and hygienic practice
Preclinical medical students’ academic performance variation in association with gender, ethnicity and basic enrolment requirement intake

Debby Marissa Lang¹, Shahadatunnur Najad Shahbudin¹, Prashant Vimalanathan¹, Nurul Huda Mohd Nor² and Nurul Hayati Mohamad Zainal²,*

¹Third Year Doctor of Medicine (MD) Programme, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor.
Email: mz_nurul@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

There are various basic enrolment intake types accepted by the Medical School of Universiti Putra Malaysia (UPM) including the Sijil Tinggi Persekolahan Malaysia (STPM), matriculation and Asasi Sains Pertanian (ASPER) Programme. This study aims to determine the relationship between academic performance variation of preclinical medical students in UPM with socio-demographic factors of gender and ethnicity, and different types of basic enrolment intake such as STPM, matriculation or ASPER. A retrospective cohort study was conducted using secondary data from the Deputy Dean's Office (Academic of Medicine) Faculty of Medicine and Health Sciences (FMHS). The information obtained includes the gender, ethnicity, basic enrolment intake, and examination results of Package 1 to 9 and the Professional Exam 1 of 3 cohorts of students (total n=308). Their identities and year of enrolment were kept anonymous. Data were analysed using IBM Statistical Package for the Social Science (SPSS) v26.0 using Chi-square or Fisher's Exact test (significant association if p<0.05). There was no significant association between the gender of students and the academic performances of the preclinical medical students. The Chinese group had a significant association with good academic performance, while the Malay group had an association with poor academic performance. Furthermore, there were significant associations between STPM intake and good academic performance for Cohort 1 in Package 1 (p=0.007), 2 (p<0.001), 5 (p=0.007), 6 (p=0.012), 7 (p=0.006), 8 (p=0.002), and for Cohort 2 in Package 1 (p=0.049), 6 (p=0.031) and 9 (p=0.049). However, no significant association was seen between the basic enrolment intake and the academic performances of the preclinical medical students for Cohort 3. In conclusion, STPM graduates outperformed students from other basic enrolment intakes in terms of academic achievement. Academic performance was also significantly associated with factors such as Chinese and Malay ethnicity in the Medical School of UPM.

Keywords: preclinical medical students; academic performance; socio-demographic factors and basic enrolment intake
Anti-inflammatory properties of *Abelmoschus esculentus* (L.) extracts on tumour necrosis factor-alpha-induced SW982 cell lines

Veshalini Kasiraja¹, Daryl Jesus Arapoc², Zainah Adam², Rosniza Razali², Noor Azuin Suliman¹,* and Noor Azlina Abu Bakar¹

¹Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, 20400 Kuala Terengganu, Terengganu, Malaysia.  
²Medical Technology Group, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.

*Correspondence:  
Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, 20400 Kuala Terengganu, Terengganu, Malaysia.  
Email: azuinsuliman@unisza.edu.my
DOI https://doi.org/10.28916/lismb.6.1.2022.108

Abstract

*Abelmoschus esculentus* (*A. esculentus*) commonly known as okra, exhibits beneficial effects against multiple chronic diseases. Therefore, this study aimed to evaluate the anti-inflammatory effects of *A. esculentus* extracts on tumour necrosis factor-alpha (TNF-α)-induced synovial sarcoma cell line (SW982). The *A. esculentus* fruits were extracted sequentially to acquire hexane (HE), chloroform (CE), methanol (ME), and aqueous extracts (AE). The inflammatory model was acquired by inducing 10 ng/mL of TNF-α onto SW982 cell lines. Methotrexate was used as a positive control for this study. The cytotoxicity of the extracts on inflammatory induced-SW982 was determined by using an MTT assay. The anti-inflammatory effects of the extracts were assessed by measuring the concentrations of intracellular calcium ions ([Ca²⁺]) and nitric oxide (NO). Despite of no significant difference observed on other *A. esculentus* extracts, HE showed significant cytotoxicity on normal SW982 cells at 24, 48, and 72 hours and on TNF-α-induced SW982 cells at 48 hours of treatment. To exert their anti-inflammatory effects, ME, AE and methotrexate were reported to significantly decrease the concentrations of [Ca²⁺] and NO as the treatment concentration increased. A decreased [Ca²⁺] was suggested to diminish the nuclear factor of activated T cells and inhibit the production of inflammatory cytokines. The NO scavenging or its inhibitory characteristics of *A. esculentus* extracts and methotrexate was postulated to be the reason for the reduction in NO synthesis. These characteristics have the potential to suppress the propagation of inflammatory action by NO. This study demonstrated that *A. esculentus* was postulated to have promising anti-inflammatory effects in treating chronic inflammatory diseases.

**Keywords:** *Abelmoschus esculentus*; anti-inflammatory; SW982 and TNF-α
Effects of mitragynine administration on the non-neural organs of mice – histological analyses

Nor Aini Lubis Mhd Zain¹, Nurfariesha Md Hashim², Hafizah Abdul Hamid¹, Muzaimi Mustapha³ and Muhammad Zulfadli Mehat¹,*

¹Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kota Bharu, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: m_zulfadli@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Mitragynine (MG) is the major alkaloid contained in the psychoactive substance kratom (Mitragyna speciosa Korth.) This alkaloid acts as both a mu-opioid receptor agonist and a kappa-opioid receptor antagonist, eliciting anti-depressant effects in animals and humans. MG also possessed anti-nociceptive and psychostimulant effects and has been used as a substitute for opioid withdrawal symptoms in a dose-dependent manner. Studies showed that MG metabolism is predominantly mediated by the CYP3A4 enzyme, with minor contribution by CYP2D6, CYP2C9 and CYP2C19 in the liver and before renally excreted through urine. In this study, we presented the histological analyses of non-neural organs (liver, kidney and heart) in adult male Swiss albino mice; n≥6, post 28 days of treatment, using hematoxylin and eosin staining. Any morphological changes and severity observed among these six treatment groups: (1) negative control (no treatment), (2) tween-20 (vehicle), (3) low-dose mitragynine, MGL (1-4 mg/kg, i.p.), (4) high-dose mitragynine, MGH (5-25 mg/kg, i.p.), (5) morphine (5 mg/kg, subcutaneous), and (6) tetrahydrocannabinol (THC, 2 mg/kg, i.p.) were compared. The control and tween-20 treated groups displayed a normal histological structure of renal corpuscles, hepatic architecture, and cardiac muscle structures with cross-striations. High-dose administration of MG caused multiple kidney injuries and showed aggregated hepatic inflammatory responses and damaged myocytes architecture with loss of cellular organization. However, at low-dose MG administration, these alterations were less prominent or non-existence. Treatment with morphine and THC resulted in significant morphological changes in all three organs comparable to the high-dose MG group, indicating that both compounds are toxic and possess lethal damage. In conclusion, the present study suggests that mitragynine possesses beneficial properties at a lower dose but is detrimental at a higher dose on the non-neural organs.

Keywords: Kratom; mitragynine; morphine; tetrahydrocannabinol and hematoxylin & eosin staining
Radiolabelling of kanamycin with technetium-99m for infection imaging

Daryl Jesus Arapoc1,*, Wan Hamirul Bahrin Wan Kamal1, Rosniza Razali1, Zainah Adam1, Azfar Hanif Abd Aziz1, Abang Abdul Rahim Ossen1, Nurmaizah Mohammad Shafie1, Veshalini Kasiraja2 and Mohd Kamal Nik Hassan3

1Medical Technology Group, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.
2Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, 20400 Kuala Terengganu, Terengganu, Malaysia.
3Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia.

*Correspondence:
Medical Technology Group, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.
Email: daryl@nm.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Infectious diseases were responsible for the death of almost 9 million people worldwide every year. Those infection deaths occur because of late identifications of main infections which occur in very deep parts of the body (deep-seated infection). Kanamycin is an antibiotic belonging to aminoglycosides, its nature as a broad-spectrum antibiotic allows it to bind to Gram-negative and Gram-positive bacteria by binding to the bacterial ribosome. Technetium (Tc-99m) is an isotope commonly used in many medical diagnostic imaging scans. Tc99m is a gamma emitter with a principal photon energy of 140 keV and a physical half-life of 6.04 hours. This study aimed to radiolabel kanamycin with technetium-99m. Direct labelling was done by adding 5mg of kanamycin and 30 ug/ml of SnCl2 with Tc99m solutions. This reaction occurred at pH7 with a 30 minutes incubation period at room temperature. Radiochemical purity and stability of the Kanamycin-Tc99m were determined by ascending paper chromatography with 1M NaOH and acetone as mobile phase. A biodistribution study was done by injecting Kanamycin-Tc99m solution into AZK nude rats and observed for two hours using a Gamma camera. From this study, kanamycin was successfully radiolabelled with Tc99m at >95%. Besides that, the biodistribution study also showed that Kanamycin-Tc99m was excreted normally from the model. These results show that Kanamycin-Tc99m radiopharmaceutical has a potential application for infection diagnosis. Further studies using the inflammatory model are suggested.

Keywords: Kanamycin; technetium-99m; infectious disease; gamma ray and radiolabeling
Effects of mitragynine on bone mineral density in mice

Michelle William Santhiagu¹, Norazlina Mohamed², Hafizah Abdul Hamid¹, Muzaimi Mustapha⁴ and Muhammad Zulfadli Mehat¹,*

¹Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Pharmacology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
⁴Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: m_zulfadli@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The emerging ‘legal high epidemic’ is strongly tied to the opioid epidemic as opioids are a widely accessible new psychoactive substance (NPS). Mitragynine, the major alkaloid of Kratom has psychoactive and analgesic properties. Due to its strong affinity to opioid receptors, it is categorised as an NPS. Mitragynine being a highly regulated compound is known to cause addiction. However, its effect on the skeletal system remains elusive. Osteoporosis is increasingly becoming identified as a side effect of chronic usage of ‘legal highs. This study aimed to investigate if Mitragynine addiction causes an alteration in bone mineral density (BMD). Four groups of mice were treated with a low dose of Mitragynine, a high dose of Mitragynine, morphine and Tetrahydrocannabinol (THC) respectively. Negative control and vehicle control were also included in the study. Mitragynine was administered intraperitoneally with an increment of dosage over a 28-days period to mimic human Kratom consumption. The left femur of the mice was subjected to an ex-vivo Dual Energy X-ray Absorptiometry (DEXA) scan at the end of the treatment period to compare the BMD among the treatment groups. The trend of the BMD observed was as follows: Mitragynine low dose > Mitragynine high dose > Control > Morphine > Vehicle > THC. One-way ANOVA showed there were no statistically significant differences in femur BMD across the six groups of mice as p>0.05. In a world where opioids and psychoactives are altering bone metabolism, this provides a new pathway to utilise a safer alternative for humans since the femur BMD in Mitragynine treated group was not far different from the control. Mitragynine can be used as a possible replacement for the current opioid if given in the right dosing regimen. It has been shown to increase BMD, but more research is needed to prove this theory.

Keywords: Mitragynine; Mitragyna speciosa; Dual Energy X-Ray Absorptiometry (DEXA/ DXA); bone mineral density and bone metabolism
Chemical profiling, total phenolic content, antioxidant and antidiabetic activities of selected medicinal plant species

Firdaus Kamarulzaman*, Fauziah Abdullah, Ihsan Safwan Kamarazaman, Mohd Hafidz Hadi Abdullah, Juliza Mohamed, Nik Musa’adah Mustapha and Sui Kiong Ling

Natural Products Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.

*Correspondence:
Natural Products Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
Email: firdauskamarul@frim.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Medicinal plants have continuously been the subject of interest for the discovery of new therapeutic agents. In this study, the crude leaf extract of selected eight medicinal plant species was prepared using methanol and water. The total phenolic content of the 16 samples was determined using Folin-Ciocalteu reagent and chemical profiling was performed by HPLC. All extracts were tested for antioxidant activity using a DPPH scavenging assay, while the antidiabetic activity was tested using α-amylase and α-glucosidase inhibition assays. Results showed that both methanol and aqueous leaves extracts of *Aquilaria malaccensis*, *Mitragyna speciosa*, *Terminalia catappa* and *Barringtonia asiatica* exhibited high inhibition (>80%) against free radical scavenging activity at 100 µg/ml. The high antioxidant activity shown by the extracts was related to their high phenolics content. For antidiabetic activity, methanol leaves extract of *A. malaccensis*, *M. speciosa*, *B. asiatica* and *Leucaena leucocephala* exhibited high inhibitory activity against both carbohydrate digestive enzymes at 100 µg/ml. The aqueous extracts on the other hand demonstrated a selective inhibition on α-amylase and α-glucosidase. The composition of different phytochemical compounds in the methanol and aqueous extracts may contribute to the different levels of biological activities of the plant samples. Further analysis of the major compound is needed to elucidate the mechanism of action of the antioxidant and antidiabetic activities of the potential medicinal plants.

Keywords: Medicinal plants; total phenolic content; antioxidant; antidiabetic and therapeutic agent
Alignment of Lokomat robotic therapy data with standard clinical gait data

Mohd Razali Sapiee¹,²,³, Mohammad Hamiruce Marhaban², Asnor Juraiza Ishak² and Muhammad Fahmi Miskon¹

¹Centre for Robotics & Industrial Automation (CeRIA), Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, 76100 Durian Tunggal, Melaka, Malaysia.
²Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³Correspondence: Faculty of Electrical & Electronic Engineering Technology, Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, 76100 Durian Tunggal, Melaka, Malaysia.
Email: mohd.razali@utem.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Gait, a human walking pattern is a repeated action of two legs in alternate movement. A single leg has to follow a gait cycle consisting of seven walking phases. A specific joint position profile for a normal person starts from a leg heel strike until the instant before the next heel strike of the same leg. A person with walking disabilities will have a profile that deviated from the normal one. Thus, the Lokomat therapy robot, a lower limb exoskeleton robot is used for walking rehabilitation for patients suffering from walking disabilities. Trauma or neurological problems in the central nervous system may result in walking disabilities. Clinical gait analysis (CGA) uses a force plate to determine heel strike thus the start of the gait cycle. But no force plate is used to determine heel strike in Lokomat. Using the Lokomat, hip joint position data recording from therapy sessions starts at Gait Index (GI) 0 which is at a different phase from heel strike. Heel strike needs to be determined when the knee anatomical joint flexion is minimum. Thus, the hip profile is aligned at GI 345 at the heel strike. Therefore 0% gait cycle starts at GI 345 and ends at GI 1345 (100% gait cycle). From this, the Lokomat profile data has been slightly aligned following the standard CGA profile although the GI value is not quite accurate as it is only an interpretation of heel strike by Hocoma, the manufacturer. The work has been approximated based on knee joint flexion and extension information. The exact GI value during heel strike needs to be determined so that the profile can accurately follow the standard profile. The information from recorded data from Lokomat therapy sessions can be utilised by many physiologists once the data profile can be standardised and normalised to follow the CGA data profile.

Keywords: Lokomat rehabilitation robot; walking therapy; clinical gait database and gait cycle
Antibacterial and cytotoxic properties of rare actinobacteria, *Barrientosiimonas humi* gen. nov., sp. nov. 39T from Antarctica

Sin Yee Chong1, Chiann Ying Yeoh1, Aida Azrina Azmi2 and Yoke Kqueen Cheah1,2,*

1Unit of Molecular Biology and Bioinformatics, Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
2Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

*Correspondence:
Unit of Molecular Biology and Bioinformatics, Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
Email: ykcheah@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Rare actinobacteria, from unexplored or less explored extreme environments riches in bioactive secondary metabolites. The extreme environment starts gaining more attention from the public for the discovery of new microbial secondary metabolites. The bioinformatics approach assists the virtual screen to identify and validate the potential source of drug molecules and minimise the number of possible chemical compounds to be investigated. *Barrientosiimonas humi* (*B. humi*) is the rare actinobacteria that is isolated from the less explored extreme environment, the upper topsoil layer of Antarctic soil in Barrientos Island. This research manages and analyses the genetic makeup of *B. humi* from its high-quality draft genome. *B. humi* was identified to have antibacterial and anticancer properties by the three computational genome mining approaches, including antiSMASH 5.0.0, BAGEL 4 and DeepBGC v0.1. There were a total of 52 biosynthetic gene clusters (BGCs) predicted involving secondary metabolites or natural product biosynthesis of *B. humi*. The presence of BGCs from *B. humi* assembled sequences indicated *B. humi* had an outstanding potential to produce secondary metabolites. These annotated genome sequences evident *B. humi* was capable of producing antibacterial, antifungal, cytotoxic and inhibitor bioactive compounds. The preliminary screening analysis revealed the ethyl acetate extract isolated from *B. humi* possessed significant antibacterial and anticancer activities against HT-29 colorectal cancer cells. The ethyl acetate extract of *B. humi* also demonstrated a significant cytotoxic effect against MCF-7 and MDA-MB-231 breast cancer cells. The results concluded that *B. humi* had attractive antibacterial and cytotoxic bioactivities, indicating *B. humi* is a potential candidate for new and improved antibiotics and cancer drugs. This research serves as an alternative treatment option for antimicrobial resistance (AMR) and cancer. It acts as a significant approach which is a complementary strategy in response to dealing with the global need for new antibiotics and anticancer drugs in all parts of the world nowadays.

**Keywords:** Actinobacteria; antibacterial; anticancer; *Barrientosiimonas humi* and cytotoxic
Anatomical justification of a new method of one-time three-step thread facelift

Sergey Dydykin¹, Yuriy Vasil’ev¹, Olga Zhukova², Eliška Kubíková³, Natalia Markova⁴, Marina Kapitonova⁵*, Alena Khlebnikova⁶ and Artem Mirontsev¹

¹Department of Topographic Anatomy and Operative Surgery, Sechenov University, Trubetskaya str. 8-2, 119991 Moscow, Russia.
²RUDN Peoples’ Friendship University of Russia, Miiklukho-Maklaya str. 6, 117198 Moscow, Russia.
³Anatomical Institute of Comenius University, Špitálska 24, 81108 Bratislava, Slovakia.
⁴S.R.O."Medical Esthetic", Ladova 2045/1, 128 00 Nové Město, Czechia.
⁵Department of Basic Medical Sciences Anatomy, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
⁶"Dokma Line" Clinic, Marshala Tchuikova str.24, 400066 Volgograd, Russia.

*Correspondence:
Department of Basic Medical Sciences Anatomy, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
Email: kmarina@unimas.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Minimally invasive correction of age-related ptosis of the facial soft tissues is a complicated and not fully resolved problem of modern cosmetology. Most papers describe the isolated correction of the upper, middle, or lower third of the face and the submental zone of the neck, while descriptions of the simultaneous fixation of the soft tissues of the head and neck and its anatomical rationale are missing. The aim of the study is the anatomical justification of an easy-to-perform, minimally invasive method of fixation of the face and neck soft tissues for better correction and prevention of age-related ptosis. A total of five male and seven female cadavers of people who died from causes not related to the pathology of the head and neck were used in the study. In an anatomical experiment, thread lift was performed simultaneously in the area of the face (upper, middle, and lower thirds) and neck. Surgical threads with counter-directed incisions were inserted through skin punctures in the frontal-aponeurotic and temporal regions of the face, passing them through the ligaments and septa with a dense fibre structure, the superficial fatty bags, with the ends brought to the surface of the skin and additionally fixed with knots. The five ligamentous structures most important for the thread-lift were identified and used as fixation points and for the placement of the most effective vectors of threads implantation, such as the inferior temporal septum, zygomatic, bucco-maxillary, mandibular, and platysmo-auricular ligaments. The proposed method has several advantages: minimally invasive, highly efficient, less traumatic, allowing soft tissue fixation using only local anaesthesia, and providing sex- and age-related protocol. The one-step method of the face and neck soft tissue fixation provides reliable correction of age-related ptosis and may serve as a reasonable model for subsequent use in a clinical setting.

Keywords: Face; neck; ligament; ptosis and thread lift
Incidence of bacterial and fungal infection among patients admitted for treatment of haematological malignancies in a teaching hospital

Najihah Hussein¹, Chee Chiat Liong¹, Sun Tee Tay², Reena Rajasuriar¹, Chandramathi S Raju², Thevambiga Iyadorai², Gin Gin Gan¹ and Chin Sum Cheong¹,*

¹Department of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
²Department of Medical Microbiology, University of Malaya, 50603 Kuala Lumpur, Malaysia

*Correspondence:
Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
Email: chinsum@gmail.com
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Bacterial and fungal infections are the leading causes of morbidity and mortality in patients with haematological malignancies due to their immunocompromised condition. The incidence of infections for various high-risk chemotherapy regimens differs among countries. In the present study, we aim to report the incidence of bacterial and fungal infections in adult patients with haematological malignancies undergoing treatment at the University Malaya Medical Centre. The demographic data, febrile episodes during admission and microbiological diagnoses were extracted and analysed from March 2020 to June 2022. A total of 127 admissions, involving patients (median age: 52 years old) who underwent therapies (88 chemotherapy treatments and 39 allogeneic hematopoietic transplantations) for haematological malignancies were included in the analysis. These patients were diagnosed with acute myeloid leukaemia (AML) (63.8%), acute lymphocytic leukaemia (ALL) (15.7%), multiple myeloma (9.4%), Hodgkin lymphoma (5.5%), and non-Hodgkin lymphoma (5.5%). 92.9% of the admissions had neutropenia ranging from 1 to 102 days (mean: 13.27 days), with more than half (71.7%) having neutropenia for more than 7 days. A total of 87 admissions (68.5%) had febrile episodes: 53 (60.9%) had documented bacterial infections while 14 (16.1%) had fungal infections but only 8 were proven. Among them, 64.2% had a single bacterial infection while 35.8% had multiple bacterial infections. The predominant bacterial species isolated were *Klebsiella pneumoniae* (17.7%) and *Escherichia coli* (15.2%). As for fungal infection, *Candida spp.* (100%) were the predominant fungi isolated. AML patients with neutropenia for more than 7 days (64.2%) were at a higher risk of bacterial and fungal infections. This prospective study provides information on the current incidence of bacterial and fungal infections amongst Malaysian patients with haematological malignancies undergoing treatment. The findings from this study highlight the potential risk factors and provide a useful perspective in establishing guidelines for treatments.

**Keywords:** Haematological malignancies; bacterial infection and fungal infection
Effects of Zerumbone on hypoxia-induced HCT116 colon cancer cell invasion

Fathin Dalila Aminuddin, Nur Fariesha Md Hashim, Nurul Akmaryanti Abdullah and Noraina Muhamad Zakuan*

Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Email: noraina@upm.edu.my

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Abstract

Colon cancer is the fourth leading cause of death by cancer throughout Asia by 2020. These deaths often occur in patients with metastatic stages, where their survival rate is greatly reduced compared to early-stage cancer patients. There had been a persistent increase in disease reoccurring despite having tumour resection, caused by the patients developing drug resistance against radiotherapy due to hypoxia. Zerumbone (ZER), a phytochemical is known for its antibacterial, antioxidant and anticancer properties. In addition, ZER is also known to possess therapeutic and chemopreventive properties in the treatment of cancer metastasis. Generally, this study is to determine the effects of ZER on the invasiveness of hypoxia-induced colon cancer cells (HCT116). HCT116 cells were cultured and two assays were performed: MTT assay was performed to determine the cytotoxic effects of ZER towards HCT116 cells, while Transwell invasion assay was carried out to determine the effects of different concentrations of ZER in the background of normoxic and hypoxic condition on the invasiveness of the HCT116 colon cancer cells. IC20 and IC50 were determined using MTT assay. Under the normoxic condition, IC20 and IC50 were 16 and 35 µM respectively, while under the hypoxic condition, the values are 20 and 44 µM, respectively. Based on the statistical analysis obtained, at 50 and 100 µM, there was a significant decrease in cell viability in both normoxic and hypoxic backgrounds, both 24 and 48 hours of incubation. IC50 of ZER reduced the number of invasive cells and the concentration was significantly reduced to half its initial concentration (p<0.001) at 48 hours of incubation, showing it is time-dependent. In conclusion, ZER has cytotoxic and inhibitory effects on hypoxia-induced human colorectal carcinoma cell invasion, thus, reducing metastasis under hypoxic background.

Keywords: Colon cancer cells; invasion; metastasis and Zerumbone
Effects of Zerumbone on invadopodia formation of hypoxia-induced MDA-MB-231 breast cancer cells

Nur Yasmin Muhammad Fauzi, Noraina Muhamad Zakuan, Nurul Akmaryanti Abdullah and Nur Fariesha Md Hashim*

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: nurfariesha@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The hypoxic tumour microenvironment and its function in the progression of cancer have been the focus of recent studies. Extremely aggressive cancer cells are thought to form invadopodia, which are protrusions of actin-rich plasma membranes that penetrate the extracellular matrix (ECM) and instigated metastasis. Metastatic breast cancer greatly adds to the death rate of breast cancer patients since it is often incurable via therapy, although recent studies are on the verge of slowing the incidence of breast cancer mortality. Zerumbone is an anti-inflammatory compound isolated from the Zingiber zerumbet rhizomes and was recently discovered for its anticancer effects in breast cancer. To test its effect on invadopodia formation in hypoxia-induced MDA-MB-231 cells, we first employed an MTT assay to determine Zerumbone's cytotoxicity against normoxic and hypoxic MDA-MB-231 cells. Following this, an invadopodia assay, also known as gelatin degradation assay was performed to investigate the invadopodia-forming ability of breast cancer cells after Zerumbone treatment. The two-way analysis of variance (ANOVA) was used to analyse the data, followed by a Tukey post hoc test. MTT assay results showed that the IC20 values for MDA-MB-231 cells treated with Zerumbone under normoxic conditions are 53μM and 45μM at 24 and 48 hours respectively. Meanwhile, the IC20 values for MDA-MB-231 cells treated with Zerumbone under hypoxia conditions are 58μM and 6μM at 24 and 48 hours, respectively. Zerumbone was shown to significantly increase the cytotoxicity effects in a dose and time-dependent manner with the IC20 observed at two different incubation periods. In this study, we found that the percentage of cells forming invadopodia after being treated with Zerumbone decreased significantly under both normoxic and hypoxic conditions. In conclusion, Zerumbone exhibits cytotoxic effects on hypoxia-induced MDA-MB-231 cells and also reduces the capability of hypoxia-induced MDA-MB-231 cells to form invadopodia.

Keywords: Breast cancer; invadopodia; hypoxia; Zerumbone and MDA-MB-231
Effect of 1-methylpropyl 2-imidazolyl disulfide (PX-12) on the invadopodia formation of hypoxia-induced HCT116 human colorectal cancer cells

Sharah Muhammad Shan, Nurul Akmaryanti Abdullah*, Noraina Muhammad Zakuan and Nur Fariesha Md. Hashim

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence: Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: nurulakmar@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Colorectal cancer is one of the most common solid tumours in both women and men reported by The American Cancer Society. Cancer metastasis is a multi-step process which includes local infiltrations of cancer cells into adjacent tissues, invasation of cancer cells into the vessels system, followed by extravasation and colonisation of secondary sites. Extravasation is the key role in cancer metastasis that allows cancer cells to migrate and invade via protrusive structures known as invadopodia. The ability of invadopodia to degrade the extracellular matrix allows the migration of primary tumours into secondary sites. Several studies have evaluated the role of 1-methylpropyl 2-imidazolyl disulfide (PX-12), a thioredoxin inhibitory drug on cancer migration and invasion under normoxic conditions, however, little is known on the effects of PX-12 on tumour cell invasion and invadopodia formation in colorectal cancer cells under hypoxic condition. This study aims to evaluate the cytotoxic effects and invadopodia formation of PX-12 in HCT116 human colorectal cancer cells in normoxic and hypoxic conditions. HCT116 cells were seeded into a 96-well plate prior to 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay to determine the inhibitory concentrations of PX-12. For gelatin degradation assay, HCT116 cells were treated with IC_{20} of PX-12 for both normoxic and hypoxic conditions and the cells were re-seeded onto gelatin-coated coverslips further stained with Rhodamine to observe the invadopodia formation. The IC_{20} values of PX-12 for HCT116 cells at 24 and 48 hours were ranging from 1.5μM to 4μM while IC_{50} values ranged from 11μM to 14μM in both conditions. The gelatin degradation assay showed that PX12 at IC_{20} concentration significantly reduced the number of cells forming invadopodia suggesting that low concentrations of PX-12 (1.5μM to 4μM) were sufficient to reduce colorectal cancer cell invasion. The present study suggests that PX-12 can potentially be an anti-invasive drug candidate for colorectal cancer.

Keywords: Colorectal cancer; metastasis; hypoxia; invadopodia and thioredoxin
Effects of *Piper sarmentosum* fraction on the obesity markers in the 3T3L1 cell culture

Fairus Ahmad1,*, Ima Nirwana Soelaiman2, Khairana Husain3, Farihah Hj Suhaimi1 and Norzana Abd Ghafar1

1Department of Anatomy, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaakob Latif, Bandar Tun Razak, Cheras 56000, Kuala Lumpur, Malaysia.
2Department of Pharmacology, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaakob Latif, Bandar Tun Razak, Cheras 56000, Kuala Lumpur, Malaysia.
3Drug and Herbal Research Centre, Faculty of Pharmacy, Jalan Raja Muda Abdul Aziz, 53000 Kuala Lumpur, Malaysia.

*Correspondence:*
Department of Anatomy, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaakob Latif, Bandar Tun Razak, Cheras 56000 Kuala Lumpur, Malaysia.
Email: fairusahmad@ukm.edu.my
DOI [https://doi.org/10.28916/lsmb.6.1.2022.108](https://doi.org/10.28916/lsmb.6.1.2022.108)

Abstract

*Piper sarmentosum* Roxb. has an anti-obesity effect by increasing adiponectin and inhibiting the 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), hexose-6-phosphate dehydrogenase enzyme (H6PD), peroxisome proliferator-activated receptor gamma (PPARγ) and leptin. This study aimed to identify the effects of the fraction of *P. sarmentosum* leaves (FrPS) and glycyrrihizic acid (GCA) on the adipogenesis of 3T3L1 preadipocytes. The 3T3L1 preadipocytes were cultured with the induction medium (day 1), a differential medium I (day 3), a differential medium II (day 5) and a culture medium (day 7 to day 15). The treated groups were cultured without the treatment from day 3 to day 8 and then the cultured adipocytes were treated with the doses 6.0 mg/ml of FrPS and 1.92 mg/ml of GCA from day 9 to day 15 of the culture. The treated groups were cultured without the treatment from day 3 to day 8 and then the cultured adipocytes were treated with the doses 6.0 mg/ml of FrPS and 1.92 mg/ml of GCA from day 9 to day 15 of the culture. The immunocytochemistry of the cultured adipocytes was to determine the expression of the 11βHSD1, H6PD, PPARγ, adiponectin and leptin proteins. The culture at day 15 was preserved with 4 % paraformaldehyde and 0.25 % TritonX100 solutions. The adipocytes were then incubated with the peroxidase inhibitor solution, primary antibodies, polymer anti-rabbit with HRP and chromogen substrate solutions. The cells were stained with Mayer haematoxylin and the intensity of the staining was measured. The results suggest that the FrPS was unable to increase the reductase enzyme of the 11βHSD1 due to the insignificant value of the H6PD protein expression (p >0.05). As a result, the dehydrogenase activity of the 11βHSD1 protein expression increased significantly (p <0.05). In addition, there was no significant difference in the adiponectin and leptin protein expressions after treatment with FrPS (p >0.05). This was because of the inhibition of PPARγ protein expression by FrPS (p <0.05). Therefore, the fraction of the *Piper sarmentosum* leaves (FrPS) has the potential to be utilized as an anti-obesity agent in obesity.

**Keywords:** *Piper sarmentosum*; obesity; protein expression; 3T3 L1 preadipocytes and culture
Evaluation of OmpK36 genotyping on *Klebsiella pneumoniae* ESBL and non-ESBL clinical isolates

Hazmin Hazman¹, Sze Ni Tee ¹, Nurul Asyikin Abdul Rahman¹,³, Nur Afiza Aziz⁴, Mohd Nasir Mohd Desa¹, Siti Norbaya Masri² and Nurshahira Sulaiman¹,*

¹Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
³School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA Negeri Sembilan, Kuala Pilah Branch, 72000 Kuala Pilah, Negeri Sembilan, Malaysia.
⁴Microbiology Unit, Department of Pathology, Hospital Sultanah Aminah, Jalan Persiaran Sultan Abu Bakar, 80100 Johor Bahru, Johor, Malaysia.

*Correspondence:
Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: nurshahira@upm.edu.my
DOI: https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

*Klebsiella pneumoniae* is a Gram-negative bacterium which is commonly associated with many life-threatening community-acquired and nosocomial infections such as urinary tract infections, pneumonia and bacteraemia. Treatment of *K. pneumoniae* infections is severely limited due to its ability to produce extended-spectrum β-lactamase (ESBL), which makes it resistant to almost all β-lactamase antibiotics. Detection of the OmpK36 porin variant, which is closely associated with antibiotic resistance, may be an indicator for understanding the risk of infection and the molecular epidemiology of these isolates. The study aims to determine the association between the OmpK36 genotype and the antimicrobial resistance profile of *K. pneumoniae* clinical isolates. A total of 212 *K. pneumoniae* isolates were collected from Hospital Sultanah Aminah Johor Bahru (HSAJB) and Hospital Pengajar Universiti Putra Malaysia (HPUPM). An antibiotic diffusion test was performed to identify the isolates with ESBL-producing phenotype and to determine the antimicrobial resistance profile. The DNA of *K. pneumoniae* were extracted and PCR-based ompK36 typing was carried out to classify the isolates into Group A, B, C and D. Statistical analyses were performed using the Pearson chi-square test. In this study, four OmpK36 types (groups A, B, C, and D) were identified in 36, 24, 79, and 44 isolates, respectively; 29 isolates were untypeable. Our results showed that the OmpK36 Group C isolates were more associated with ESBL phenotype and bloodstream infections than OmpK36 non-C group isolates. This study suggests that OmpK36 genotyping may serve as an important epidemiologic tool associated with antimicrobial resistance in bloodstream isolates.

**Keywords:** OmpK36 genotyping; PCR; Klebsiella pneumoniae and antimicrobial resistance
Ethanolic extract of \textit{Centella asiatica} (EECA) increases the transdifferentiated neural stem cell (NSC)-derived neurospheres from amniotic fluid stem cells

Siti Sarah Mustaffa Al Bakri$^1$, Kah Wen Ding$^1$, Winnie Khor$^1$ and Norshariza Nordin$^{1,2,*}$

$^1$Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
$^2$Genetics and Regenerative Medicine (ReGEN) Research Group, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: shariza@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The incidence of neurodegenerative diseases (NDs) across the globe is alarming. Transplantation of neural stem cells (NSCs) is one of the emerging treatments used to treat NDs. However, the inaccessibility of the brain to isolate endogenous NSCs has limited its application highlighting the essential of exogenous source NSCs. Amniotic fluid stem cells (AFSCs) are broad multipotent stem cells which have shown neurogenic properties and are able to transdifferentiate into NSCs. Unfortunately, the efficiency of transdifferentiation is still insufficient to generate enough NSCs for the neurotransplantation procedure, therefore an enhancer is required. \textit{Centella asiatica} (CA) has proven to have a neuroenhancement effect and could be a potential inducer to enhance the transdifferentiation of AFSCs to NSCs. Thus, this study aims to investigate the enhancement effect of the ethanolic extract of \textit{Centella Asiatica} (EECA) in promoting transdifferentiated NSCs through the formation of good-quality neurospheres. Rat Full-term amniotic fluid stem cells (R3) were cultured in Embryonic Stem Cell Medium prior to transdifferentiation to NSCs by monolayer adherent culture for two days in the NSC medium with EECA at the concentration of 1µg/ml and 10 µg/ml and 50µM dbCAMP (positive control) in addition to the untreated group (negative control). Then, the R3-derived NSCs were subjected to neurosphere formation assay by culturing it in the neurobasal/B27 on a 100mm uncoated bacteriological Petri dish for three days and evaluated on the number and size (diameter, µm) using ImageJ software. RNA was extracted from R3-derived NSCs of all groups and relative expression of NSC-specific marker (Sox1) normalised against a housekeeping gene (GAPDH), was determined by RT-qPCR. The R3-derived NSCs have successfully formed neurospheres expressing the NSC-specific markers (Nestin, Sox1) with the group treated with EECA showing a higher percentage of good quality neurosphere (50-100µm, diameter) compared to the controls group. The findings of this study prove that EECA is able to enhance the transdifferentiation of AFSC to NSC and promote the production of good-quality neurospheres.

\textbf{Keywords:} Neural stem cells; amniotic fluid stem cells; neurospheres and \textit{Centella asiatica}
Role of BPA in inducing obesity by in vivo study

Al-Salihi Ahmed Rashid Abdulhameed1, Vuanghao Lim2, Hasnah Bahari1, Boon Yin Khoo3, Muhammad Nazrul Hakim Abdullah4, Jun Jie Tan2 and Yoke Keong Yong1,*

1Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
2Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Penang, Malaysia.
3Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Penang, Malaysia.
4Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Correspondence:
Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: yoke.keong@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Bisphenol A (BPA) is an endocrine-disrupting agent used in the production of polycarbonate plastics and epoxy resins. There is a strong correlation between BPA exposure and several chronic metabolic disorders including atherosclerosis, heart disease, obesity, diabetes, and hypertension. This study aimed to determine the chronic exposure effect of the BPA on body weight and lipid profile in a rat model. Thirty-two Sprague Dawley rats were divided into four groups (n=6), (G1) normal diet (ND)+vehicle, (G2) high-fat diet (HFD)+vehicle, (G3) ND+25mg/kg BPA and (G4) HFD+25mg/kg BPA administered orally for 20 weeks. The body weight and food consumption of the rats were recorded weekly. At the end of week 20, the animals were sacrificed, and serum and adipose tissues, including white adipose tissue (RPwat, visceral, and gonadal) were collected for lipid profile, fasting blood sugar and weight. In addition, body length and body circumference measurement. G4 showed significant (P<0.05) higher calorie intake as compared to the control (G1). However, body weight in all groups increased by more than 10% compared to the G1, suggesting that chronic exposure to BPA may lead to obesity. Lipid profile revealed T.C and H.D.L in all groups were higher than control, while only G4 showed a significant increase in T.G level as compared to G1. Meanwhile, we found non-significant results in the LDL, F.B.S, and body length in all groups compared to G1. Lastly, the body circumference and total weight of white adipose tissue (RPwat, visceral, and gonadal) collected in the G2 and G4 showed a significant increase in weight compared to G1. The current finding demonstrated that BPA has a significant influence on rat body weight gain and the occurrence of obesity. Further investigation in terms of the mechanism of action is warranted.

Keywords: BPA; white adipose tissue; obesity and in vivo study
Optimisation of in-house growth medium on the proliferation of primary human skeletal myoblast and Duchenne Muscular Dystrophy skeletal myoblast: A comparative assessment

Chung Liang Lam, Nadiah Sulaiman and Muhammad Dain Yazid*

Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaa’cob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia.

*Correspondence:
12th Floor, Clinical Block, Faculty of Medicine, Hospital Cancelor Tunku Muhkriz (HUKM), Jalan Yaa’cob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia.
Email: dain@ukm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Duchenne Muscular Dystrophy (DMD) is an X-linked progressive muscle-wasting disease arising from the total absence of dystrophin. To date, there is still no cure as the obstacle to this lies in grey areas in understanding the disease at a subcellular level. As primary cells best reflect their physiological state in vivo, they are vital to research focused on generating the most biologically relevant and accurate data. However, establishing a stable primary cell culture has been a proven challenge considering the need to optimise the perfect supplement cocktail comprised of cell-specific nutrients and growth factors. In this study, we aim to determine a cost-effective in-house growth medium as an alternative to commercially available complete growth medium for the proliferation of both primary human skeletal myoblast (HSkM) and DMD skeletal myoblast (DMD-SkM). HSkM and DMD-SkM were grown in Dulbecco’s Modified Eagle Medium (DMEM) with varying concentrations of fetal bovine serum and L-glutamine and in a commercially available growth medium. Comparatively, both myoblasts were assessed based on their morphology, proliferative capabilities, and the characterisation of muscle-specific markers. We find that both skeletal myoblasts grown in DMEM were morphologically very elongated and had a lower population doubling time as compared to the commercially available growth medium. Our results suggest that the in-house growth medium is unsuitable for optimal growth and proliferation of primary skeletal myoblasts. This may be overcome with the addition of more skeletal muscle-specific growth factors, however, will not be a cost-effective approach as more optimisation is required.

Keywords: Skeletal muscle myoblasts; growth medium; optimisation and primary cells
QSAR modelling and molecular docking of benzimidazole analogues as an anticancer agent

Raadhika Arunasalam¹, Kok Wai Lam² and Hasiah Ab. Hamid¹,*

¹Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
²Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
*Correspondence: Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: hasiah@upm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Cancer contributed to 18.1 million cases and almost 10 million deaths in 2020 and will keep increasing to 16.4 million by 2040. Due to the adverse effects of existing chemotherapy drugs, there is a need for a new chemotherapeutic compound which can act as an effective anticancer agent. Caspase-8 plays an important role in the apoptosis mechanism and is one of the cancer hallmarks. Benzimidazole is widely known due to its varying medicinal properties yet benzimidazole is a relatively new candidate as an anticancer agent. This study aims to develop a validated QSAR model, predict the characteristics for higher biological activity and analyse possible interactions of synthesised compounds against caspase-8. A validated QSAR model is created using hundred thirty-nine (139) benzimidazole compounds by using the genetic approximation function (GFA) method. 10 QSAR models were computed, and external validation of chosen parameters was established. Finally, 3 QSAR models were chosen as the best models using two software which are AutoDock and Achilles Blind Docking Server. The statistical equation of the QSAR model revealed that benzimidazole compounds containing a subpopulation of amine groups, a high number of hydrogen donors and exhibit superconductivity inclines towards higher biological activity in the MCF7 cancer cell line. The molecular docking data also showed that all 5 synthesised benzimidazole analogues can bind against caspase-8. Based on the QSAR model, the amine subpopulation contributes the highest towards benzimidazole analogues’ biological activity. Amines are weak bases which act as hydrogen donors and have electronegative elements allowing hydrogen bonding. Hydrogen bonds contribute to the bioavailability of the drug as it affects diffusion, stability, recognition, and interaction. In addition, the higher binding energy is also highly contributed by the number of hydrogen bonds created during docking. Findings of QSAR analysis and molecular docking demonstrate the potential of benzimidazole analogues as anticancer agents.

Keywords: Benzimidazole; QSAR analysis; molecular docking and anticancer agent
Antimicrobial and wound healing effects of postbiotics derived from *Lactiplantibacillus plantarum* LAB1 and LAB 12, and *Pediococcus pentosaceus* LAB3 and LAB6, *in vitro*

As-Zainill Faris Zain Khairull, Danial Akmal Ahmad Makmom, Ila Sazleen Amlan, Mohamad Lokman Abdul Ghani, Kalavathy Ramasamy and Siong Meng Lim*

Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.

*Correspondence:
Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.
Email: lim219@uitm.edu.my
DOI https://doi.org/10.28916/ljsmb.6.1.2022.108

Abstract

Skin acne is closely associated with cellular wounds, which may lead to bacterial infections if left untreated. Although current treatments against acne are available in the form of topical retinoids, benzoyl peroxide, and oral and topical antibiotics; the efficacy of these treatments is often compromised by antibiotic resistance and side effects. This raises the need for safer and more effective alternatives. Postbiotics, which are by-products from the fermentation of probiotics that provide additional bioactivity to the host, appear to be a viable option. The present study aimed at evaluating the potential antimicrobial and wound healing effects of postbiotics derived from lactic acid bacteria (LAB) which included *Lactiplantibacillus plantarum* LAB1 and LAB 12, and *Pediococcus pentosaceus* LAB3 and LAB6 *in vitro*. For this purpose, the disk diffusion assay was performed for the assessment of the antimicrobial activity of the LAB-derived postbiotics against *Staphylococcus aureus* (ATCC433001 and 6538) and *Propionibacterium acnes* (ATCC6919). The resultant zones of inhibition were measured. As for wound healing properties, the Sulforhodamine B (SRB) assay was first performed to determine the highest subtoxic concentration of the LAB-derived postbiotics. This was followed by the scratch assay, whereby a scratch was created on the monolayer normal human dermal fibroblasts (NHDF) by using a pipette tip, after which LAB-derived postbiotics were added. The wound closure was analysed qualitatively and quantitatively at 0 and 24th hours. All tested LAB-derived postbiotics did not form inhibition zones against *S. aureus* and *P. acnes* but significantly (p<0.05) induced wound closure. LAB1-derived postbiotics exhibited the greatest wound healing effects (58%) on the scratched monolayer NHDF, followed by LAB3- (54%), LAB6-(36%) and LAB12-derived postbiotics (29%). The present findings warrant the determination of the composition of the LAB-derived postbiotics and validation of their wound healing properties using *in vivo* models in future studies.

**Keywords:** Acne; lactic acid bacteria; postbiotics; wound healing and antimicrobial
Genetic polymorphism of the serotonin transporter (SERT) in variable stress intolerance response

Jastina Dee Anna Jack¹, Nur Izzati Umar Zaman² and Lay Kek Teh¹,²,*

¹Faculty of Pharmacy, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.
²Integrative Pharmacogenomics Institute, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.

*Correspondence: Integrative Pharmacogenomics Institute, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300, Selangor Darul Ehsan, Malaysia.
Email: tehlaykek@uitm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The COVID-19 pandemic has negatively affected both the physical and mental health of people worldwide. Consequently, a spike in psychological distress such as stress, anxiety, and depression has been reported during the pandemic. An individual’s response to tolerating stress is different; one may be resilient, and one is vulnerable in the face of trauma leading to the development of severe mental illnesses such as depression. We can predict a person’s susceptibility to stress and risk of developing a disease or disorder through genetic testing if the specific genetic marker has been known. The serotonin transporter (SERT) is encoded by SLC6A4, and its gene polymorphism is referred to as the 5HTTLPR, giving rise to short (S) and long (L) alleles. In previous studies, the carriers of the S allele were reported to have a higher propensity to stress-induced depression than those of the L allele carriers. In the present study, a validated PCR-based method was used for 5HTTLPR genotyping. The subjects’ DASS-21 data was used to identify their severity level of depression and we further divided the randomly selected subjects into normal versus depressed groups. The association of 5HTTLPR with the occurrence of depression was analysed using the Chi-square test. As a result, the frequency of L/L, L/S and S/S genotypes in the depressed group was 25%, 50%, and 25%, respectively. However, these frequencies were 23.5%, 58.8% and 17.6%, respectively in the normal group. The distribution of the different 5HTTLPR genotypes (L/L, L/S and S/S) did not show any significant differences between depressed and normal subjects, χ²(2) = 0.335, p = 0.85. Due to the small sample size (N=33), there was no significant relationship between depression and the allele distribution among the groups in this study. A larger sample should be recruited alongside environmental factors to understand the role of different genes on depression in the future.

Keywords: Serotonin transporter (SERT); genetic polymorphism; variable stress intolerance response and university students
Potential African herbs used in the management of hypertension

Nur Yuhaniz Mohd Fauzi and Choo Chee Yan*

Department of Pharmaceutical Pharmacology & Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

*Correspondence:
Department of Pharmaceutical Pharmacology & Chemistry, Faculty of Pharmacy, Universiti Teknologi Mara (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.
Email: choo715@uitm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Hypertension is a worldwide health concern and can lead to severe health risks mainly associated with cardiovascular diseases. Current antihypertensive drugs in the market are constrained by side effects. This review aims to identify alternative medicinal plants and herbs used in hypertension management and to review their effectiveness and mechanism of action as well as the traditional uses of each plant. Databases used to search for information were PubMed, ScienceDirect, Scopus, and Google Scholar reporting from January 2015 until June 2022. The search terms used were “hypertension”, “antihypertensive”, “medicinal plants”, “traditional herbs”, “extracts”, “reduce blood pressure” and “African herbs”. A total of twenty plants were identified showing blood pressure reduction or prevention of blood pressure increases and were thematically categorised under four mechanisms which are 1) antioxidant properties, 2) inhibition of ACE activity, 3) lipid-lowering effects, and 4) blockade of calcium channels. Some herbs displayed traditional uses more than just being used in hypertension management. More effort is needed to enrich these plant extracts with the recommended bioactive to optimise the effective dose and confirm that the effect is reproducible by standardisation of these bioactive for production or clinical trials as well as to conduct well-conducted safety and toxicity studies for these extracts.

Keywords: Hypertension; antihypertensive; alternative treatment and complementary therapy
Neuroprotective activity of astaxanthin: Distribution comparison between astaxanthin in an oil solution and macroemulsion

Nur Fatihah Khairunisah Abdul Fatah1, Mazzura Wan Chik1, Meor Mohd Redzuan Meor Mohd Affandi2 and Gurmeet Kaur Surindar Singh1,*

1Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.
2Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor Branch, 42300 Bandar Puncak Alam, Selangor, Malaysia.

*Correspondence:
Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.
Email: gurmeet9952@uitm.edu.my
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Abstract

The astaxanthin (AST) formulation with smaller droplet sizes has been developed to address its bioavailability and distribution problems. However, its distribution in the brain has yet to be explored. Furthermore, the spleen has also been found to contribute to neuroprotective activity by regulating the immunological response and clearance of beta-amyloid in the brain. The role of the brain-spleen axis in dementia, however, remains to be fully elucidated. This study aimed to prove that different types of AST formulation, namely oil solution and macroemulsion, can be distributed in rats’ brains and spleens. Besides, this study also intends to assess whether AST concentration in the brain and spleen would increase by an increment in the dose given to the rats. Thirty male Sprague Dawley rats were divided into five groups (n = 6): control (pure palm olein), oil solution (320 mg/kg), 640 mg/kg), and macroemulsion (320 mg/kg, 640 mg/kg). The treatments were orally fed for twenty-eight days, followed by the isolation of the rats' spleen and brain regions, namely, hippocampus, cerebellum, and cortex for tissue distribution studies using high-performance liquid chromatography (HPLC). AST was detected in the spleen but at a low concentration, whereas AST in the brain and macroemulsion AST were unable to be detected. Additionally, a higher AST concentration was observed at a greater strength. In short, we did not manage to prove the AST distribution in the brain, particularly when macroemulsion was used. However, we observed an increase in AST concentration in the spleen when greater strength was given.

Keywords: Dementia; astaxanthin; droplet size; distribution and brain
A cross-sectional study of the prevalence of readmission among substance use disorder patients and the factors associated with it

Loshanah Selvaraj¹, Jaya Kumar²*, Mohd Fadzli Mohamad Isa³, Rashidi Mohamed Pakri Mohamed⁴ and Isa Naina Mohamed⁵

¹Department of Physiology, Faculty of Medicine, National University of Malaysia, 56000 Kuala Lumpur, Malaysia.
²Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
³Department of Psychiatry and Mental Health, Hospital Kuala Lumpur, 50586 Kuala Lumpur, Malaysia.
⁴Department of Family Medicine, Faculty of Medicine, National University of Malaysia, 56000 Kuala Lumpur, Malaysia.
⁵Department of Pharmacology, Faculty of Medicine, National University of Malaysia, 56000 Kuala Lumpur, Malaysia.

*Correspondence:
Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Email: jayakumar@ustm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

This paper identifies the prevalence of readmission among substance use disorder patients and the factors associated with it. Substance use disorder (SUD) is the persistent use of drugs (including alcohol) despite substantial harm and adverse consequences. The Malaysian government has stated that drug abuse is not only a social problem, but also a threat to national security considering its widespread negative social, political, and economic consequences. Management of chronic drug users is challenging due to recurring relapses and hospital readmissions. Existing literature has correlated numerous factors to relapse or readmission such as sociodemographic, clinical and patient history with previous admissions as well as types of admission. Even so, non-severe symptoms, such as changes in heart rate, irritable behaviour, tremor, nausea, sweating, changes in appetite and weight, and withdrawal symptoms (including craving), which are commonly recorded in the clinical setting may serve as a clinical marker that could indicate a patient’s likelihood for future readmission. Results from this study will provide novel information on the relationship between sociodemographic and clinical (withdrawal symptoms, cravings, non-severe symptoms) that lead to readmission among substance use disorder patients. Furthermore, results from this study will allow the clinician to better address the specific needs of the population at risk and to prevent costly inpatient treatment.

Keywords: Substance use disorder; readmission and relapse
In vivo toxicity study on the effect of aqueous propolis extract derived from *Geniotrigona thoracica* sp in mice: A pilot study

Nurul Alina Muhamad Suhaini, Mohd Faeiz Pauzi*, Siti Norazlina Juhari and Noor Azlina Abu Bakar

Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, 20400 Kuala Terengganu, Malaysia

*Correspondence:
Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, 20400 Kuala Terengganu, Terengganu, Malaysia.
Email: faeizpauzi@unisza.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

*Geniotrigona thoracica* sp is a stingless bee species of the *Trigona* family. Propolis is a wax-cum-resin substance that is produced by bees and has been utilised as traditional folk medicine in treating inflammatory pain. It is compulsory to conduct a nonclinical toxicity study because of its curative effects on human health. This study aims to evaluate the adverse effects of the aqueous extract of propolis following oral administration in mice by mean body weight, relative organ weight and kidney histopathology. Aqueous extract of propolis was administered orally at the three doses of 1000, 2000, and 4000 mg/kg body weight for 14 days. Body weight was taken initially and periodically once a week for the next 14 days. At the end of the study, the mice were sacrificed, and the kidney sections were collected for histopathological analysis. The mean body weight of the mice showed no significant differences among all groups (p>0.05) suggesting that oral administration of aqueous propolis extract on mice did not influence the body weight. The treated mice did not show any significant difference based on relative organ weight compared to their respective control groups (p>0.05) which might suggest that propolis at the three doses regime did not have a toxicity effect on the mice's kidneys. The histopathologic severity grades revealed mild to moderate microscopic lesions such as vascular congestion and thrombosis, which are indications of toxicity in this animal model. It explained that the tested material is toxic when excreted to the kidneys, producing mild to moderate histological lesions. Further biochemical investigations on serum levels in mice will be necessary for its visible results and an overall interpretation.

Keywords: *Geniotrigona thoracica* sp; aqueous propolis extract; kidney; toxicity and histopathological
Minocycline reduced tumour necrotic factor-alpha (TNF-\(\alpha\)) in lipopolysaccharide (LPS)-induced neuroinflammation rat model

Entesar Yaseen Abd Qaid¹, Rahimah Zakaria², Zuraidah Abdullah¹ and Idris Long¹,*

¹Biomedicine Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
²Physiology Department, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

*Correspondence: Biomedicine Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
Email: idriskk@usm.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Lipopolysaccharide (LPS) is used to induce neuroinflammation. The effects of minocycline on tumour necrotic factor-alpha (TNF-\(\alpha\)) activation-induced LPS are not clearly understood. This study aims to elucidate the effects of LPS and minocycline on TNF-\(\alpha\) in comparison to a clinically approved drug named memantine. A total of fifty male SD rats were divided into: (i) control, (ii) LPS, (iii) LPS-treated with minocycline 25 mg/kg, (iv) LPS-treated with minocycline 50 mg/kg, and (v) LPS-treated with memantine 10 mg/kg. Minocycline and memantine treatments were administered intraperitoneally once daily for 2 weeks and LPS was injected once on day 5. Immunohistochemistry and western blot for TNF-\(\alpha\) protein were performed to measure its expression and level in the hippocampus and cortex. Immunohistochemistry and western blotting showed that LPS significantly increased TNF-\(\alpha\) expression and density (\(p>0.05\)). Minocycline treatment, dependent on dose, reduced TNF-\(\alpha\) expression and density (\(p>0.05\)) comparable to the memantine effect. Dependent on the dose, minocycline reduced TNF-\(\alpha\) expression in the LPS rat model of neuroinflammation in comparison to memantine. Thus, minocycline has potential preventive-therapeutic effects in neuroinflammatory diseases such as Alzheimer’s disease.

Keywords: Tumour necrotic factor-alpha; lipopolysaccharide; minocycline and memantine
Elements of effective anatomy teaching from the perspective of medical students

Saiful Bahri Talip1,2,*, Muhamad Saiful Bahri Yusoff2, Zul Izhar Mohd Ismail3 and Siti Nurma Hanim Hadie3

1Basic Medical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
2Department of Medical Education, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
3Department of Anatomy, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

*Correspondence:
Basic Medical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
Email: tsbahri@unimas.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Previous research shows that effective anatomy teaching depends on several factors including the teaching approaches and lecturers' behaviour. However, evidence is scarce on effective teaching in anatomical sciences education. Therefore, this study aims to identify the elements of effective anatomy teaching from the perspective of medical students. A qualitative study was conducted by using a focus group discussion involving 52 medical students from one public medical school in Malaysia. The interview data were transcribed, converted into electronic format, and analysed using thematic analysis via the ATLAS.ti software. The analysis produced three themes, namely: (1) lecturer's attributes and behaviour; (2) teaching approaches and strategies; and (3) teaching support. Each theme overlies several sub-themes that reflect a considerable number of effective teaching elements. In general, there are thirteen effective teaching elements generated, namely, (1) experience teacher; (2) self-confidence; (3) passionate teaching (4) teaching effort; (5) teachers’ character; (6) integrating different approach; (7) group activity task; (8) good lecturing strategies and techniques; (9) teaching and learning through visual modalities; (10) intraclass activity; (11) teaching tools and facilities; and (12) supplementary teaching session; and (13) online related learning. The findings of this study serve as a foundation for producing effective teaching guidelines that can enhance anatomy teaching in the future.

Keywords: Elements of effective teaching; focus group discussion; thematic analysis; anatomy teaching and teacher behaviour
An insight towards establishing a faster metabolic syndrome animal model

Mohamad Fairuz Yahaya¹*, Nellysha Namela M.A. Kadar², Fairsus Ahmad¹ and Teoh Seong Lin¹

¹Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia.
²Department of Biomedical Sciences and Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.

*Correspondence:
Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia.
Email: mfairuzy@ukm.edu.my
DOI https://doi.org/10.28916/lismb.6.1.2022.108

Abstract

One of the major hurdles in creating and establishing a reliable metabolic syndrome animal model is the length of time taken for each of its components to manifest. Metabolic syndrome (MetS) is a cluster of conditions that include central obesity, insulin resistance, dyslipidaemia, and high blood pressure. Due to the multitude of disorders, researchers in this field not only face a challenge in creating an established model but also a more rapid yet reliable one. There have been numerous rodent models available but many would take as long as 16-weeks to create one that has at least three out of five criteria to be confirmed as a MetS-induced model. Consequently, we carried out the experiments to find out if the same diet that induced the syndrome at 16-weeks on a rat can induce a much earlier disorder at 8-weeks. Using a similar method of force-feeding with a high carbohydrate and high fructose (HCHF) diet, we tested this on 2 cohorts of Wistar rats at a different time point against a control that received a normal diet ad libitum. Prior to sacrifice, their abdominal circumference, weight, fasting blood glucose (FBS), lipid profile and blood pressure were measured. In the first cohort, only the systolic blood pressure from the MetS-induced group showed a significant increase (p<0.05) compared to the control. However, in the second cohort, we were able to induce a significant (p<0.05) increase in FBS, diastolic BP, and triglycerides; and reduce the high-density lipoprotein. These conflicting results showed that the induction of MetS through a special diet is possible after a short duration of feeding but the result needs to be treated with caution. More experiments are needed to verify this but until then it is advisable to induce the condition using a longer duration of feeding with the special diet.

Keywords: Metabolic syndrome; high carbohydrate high fructose diet and animal model
Knowledge level on main factors of hand, foot, and mouth disease (HFMD) among parents in Kuala Lumpur

Nurul Ashilah Masro1, NurAqilah Azman1, Nur Aliah Yasmin Mohd Lokman1, Siti Nur Balqis Mohamad Sabri1, Muhammad Shahrizat Ruslan1, Muhamad Zakuan Abdullah1,* and Nur Shahirah Nasir2

1Department of Medical Science & Technology, Faculty of Health Sciences, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.
2Faculty of General Study and Foundation, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.

*Correspondence:
Department of Medical Science & Technology, Faculty of Health Sciences, University College of MAIWP International, 68100 Batu Caves, Kuala Lumpur, Malaysia.
Email: m.zakuan@ucmi.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Hand, foot, and mouth disease (HFMD) affect millions of children across Asia each year. Previously, HFMD was the last outbreak in Malaysia in 1997. HFMD cases emerged again and significantly increased all over Malaysia until today. Recently, outbreaks of two enteroviruses were recorded in Selangor followed by Kuala Lumpur which occur mostly in nurseries, child care centres, and private homes. Good knowledge of the parent is one of the factors that potentially contributed to the volume of cases. Therefore, this study aimed to identify the knowledge level of HFMD among parents who have children under 6 years old. A cross-sectional questionnaire was conducted on 26 parents from low-cost residential areas who were selected as respondents in Cheras. The study showed that 100% of the respondents have a high level of knowledge of HFMD. No significant difference in knowledge was found between males and females. Meanwhile, working and non-working respondents do not also influence their knowledge. Throughout the study, most of the respondents (29%) know poor hygiene is the main factor of the HFMD outbreak followed by coughing and sneezing (24%), crowded settings (15%), sharing toys (15%), close social interaction (13%) and hot weather (4%). The form of town halls, webinars, and briefings on disinfecting processes influence the spread of knowledge and awareness in the community. Further information regarding HFMD can be easily accessed from social networks, friends, and family members who have been infected. In conclusion, increased knowledge and understanding among residents would help to decrease the cases of HFMD in their population. Therefore, proper and continuous public health education should be done towards eradicating infectious diseases from re-emerging.

Keywords: HFMD; enterovirus and nursery
A study on the prothrombin time, activated partial thromboplastin time and coagulase tests of expired frozen plasma

Mohamed Akmal Mohamed Ali1,2 and Hamidah Abu Bakar1,3,*

1Faculty of Health Sciences, Universiti Selangor (UNISEL), Shah Alam Campus, Jalan Zikron A7/A, Section 7, 40000 Shah Alam, Selangor Darul Ehsan, Malaysia.
2Department of Transfusion and Pathology, Hospital Kota Tinggi, 81900 Kota Tinggi, Johor, Malaysia.
3Faculty of Pharmacy, University College MAIWP International, No.3, Jalan 31/10A, Kawasan Perindustrian IKS, Taman Batu Muda, 68100 Batu Caves, Kuala Lumpur, Malaysia.

*Correspondence:
Faculty of Pharmacy, University College MAIWP International, No.3, Jalan 31/10A, Kawasan Perindustrian IKS, Taman Batu Muda, 68100 Batu Caves, Kuala Lumpur, Malaysia.
Email: dr.hamidah@ucmi.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The expired plasma has been used in some clinical settings due to discrepancies in the shelf life of the frozen plasma. In general, many of these blood products have been discarded based on the expiry date as it is assumed that they might contain degraded active coagulation factors. This might lead to unnecessary sample rejection, hence resulting in an increase in health practitioners’ workload, time and material wastage. Thus, the present research aimed to evaluate the activity of expired frozen plasma (EFP) levels by measuring the prothrombin time (PT), activated partial thromboplastin time (APTT) and coagulase tests. Thirty blood plasma were collected from the Centre of Collection in Hospital Sultanah Aminah (HSAJB). PT, APTT and coagulase tests were then conducted on different days (days 1, 6, 11, 16, 21, 26, 31). The coagulase test showed that there was no difference between expired and non-expired samples. However, the PT and APTT tests demonstrated that the coagulation activities of the expired samples were significantly lower (P<0.001) than the non-expired samples. The present data indicate that non-expired blood plasma must be used in coagulation factor diagnosis. Increasing the sample size and performing different specific tests might be helpful to enhance the accuracy of the observation.

Keywords: Expired frozen plasma; prothrombin time test; activated partial thromboplastin time test and coagulase test
Tele exercise for the patients post stroke: A systematic review

Ardalan Shariat*

Department of Digital Health, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

*Correspondence: Department of Digital Health, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Email: ardalansh2002@gmail.com
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Patients post stroke face with different kinds of disability. Once they finish their physiotherapy phase, they have to do different kinds of exercise training. So, they need to go to the occupational therapists which is costly in time and money. So, the main aim of this systematic review is to focus on tele exercise as a kind of rehabilitation for the patients post stroke once they have finished their physiotherapy phase and need to do the regular exercise training. Multiple databases including Scopus, Web of Sciences, PubMed and Google Scholar were searched for the published papers in English till 2022. The main keywords for this search were: tele health, tele medicine, tele exercise, post stroke patients, rehabilitation, occupational therapy and exercise therapy. Selected papers were inserted in Mendeley for further evaluation based on their abstract. Among 830 selected papers, finally 240 papers were checked based on their abstracts. A total of 11 trials were in line with our eligibility criteria based on their methodology. Following the analyses of those 11 papers, it was shown that, using tele exercise for the rehabilitation of the patients post stroke, could be a useful method with lower price in comparison with face-to-face occupational therapy in the clinics. It appears that giving online consultation with clear explanations and also doing exercise training using online platforms, are the cost effective and useful methods in comparison with face-to-face consultation and doing physical exercises. So, as a home message, it is suggested for occupational therapists to use tele exercise for therapeutic purposes for the patients post stroke who have finished their physiotherapy phase and need to do the exercise training.

Keywords: Tele exercise; tele health; stroke and rehabilitation
Effect of thymoquinone nanoparticles on osteosarcoma three-dimensional cell viability

Halimatun Saadiah Abdul Wahab¹, Aisha Mohd Din²*, Gabriele Ruth Anisah Froemming³ and Hairil-Rashmizal Abdul Razak⁴

¹Faculty of Health Sciences, Universiti Teknologi MARA, Jln Pesona, 42300 Puncak Alam, Selangor, Malaysia.
²Department of Basic Sciences, Faculty of Health Sciences, Universiti Teknologi MARA, Jln Pesona, 42300 Puncak Alam, Selangor, Malaysia.
³Faculty of Medicine and Allied Health Sciences, University Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
⁴South Cloisters, University of Exeter, St Luke’s Campus, Heavitree Road, Exeter, EX1 2LU, United Kingdom.

*Correspondence: Department of Basic Sciences, Faculty of Health Sciences, Universiti Teknologi MARA, Jln Pesona, 42300 Puncak Alam, Selangor, Malaysia. Email: aishamohddin@uitm.edu.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Osteosarcoma is an aggressive malignancy of the bone in adolescents. Despite standard chemotherapy having improved the long-term survival rate, the outcome for patients with recurrent and metastatic osteosarcoma remains dramatically poor. Development of the osteosarcoma three-dimensional (3D) system is important when finding novel therapies as it mimics the tumour microenvironment in vitro. Thymoquinone (TQ) has been reported to exhibit antiproliferative effects on the colon, breast, ovary, lungs, and osteosarcoma. Incorporating TQ into nanoparticles can sustain and prolong drug delivery. Therefore, this study aims to look at the cell viability of human foetal osteoblast (hFOB), osteosarcoma (MG-63), and co-culture (hFOB and MG-63) spheroids. The 3D culture was performed using the spotting method on a 96-well collagen scaffold plate with a seeding density of 45,000 cells/spot and 25,000 cells/spot for hFOB and MG-63 respectively. After day three, the spheroids were formed. The spheroids were then treated with varying TNP concentrations, between 2000-8000 ug/mL for 48h. The size of the spheroids was measured using Image J software. After 48h, an MTS assay was performed. The cell viability results showed the IC₅₀ concentration of TNP when treated on MG-63 spheroids was at 5000 ug/mL with no significant reduction in hFOB cell viability. The MG-63 spheroids were also seen to be significantly reduced in size but not in hFOB spheroids. This result suggests that TNP selectively kills MG-63 tissue with very minimal effect on normal bone tissue.

Keywords: Nanoparticles; thymoquinone; 3D culture; osteosarcoma and human foetal osteoblast cells
Antioxidant properties of agarwood, mango shoots, bamboo starfruits, nutmeg shells, and noni fruit extract

Mohd Kamal Nik Hasan1,*, Muhammad Danial Che Ramli2, Zaridah Mad Zaki1, Abd Rashid Li1, Azman Mohamed1, Muhammad Khair Mohd Ayob1, Mohd Syahmi Mohd Azha1, Andik Nurul Shafika Hasni2, Zurain Syahira Zalman Zain2 and Nurul Rahmah Anitasari Ariyadi Jemiran2

1Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
2Department of Faculty of Health and Life Sciences, Management and Sciences University, 40100 Shah Alam, Malaysia.

*Correspondence: Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
Email: mohdkamal@frim.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Agarwood leaves, Mango shoots, Bamboo starfruit, Nutmeg shells, and Noni fruit are among the herbs that are part of medicinal herbs in Malaysia. These herbs are famous for their smell, use, and medicinal purposes. The purpose of this study is to determine the antioxidant properties of these herbal plants. The raw materials were cut, dried, and ground. The ground herb was boiled at 100°C for 1 hour. Then, the boiled water was evaporated using a rotavap until it became a powder extract. The antioxidant activity of this extract powder was measured for DPPH, FRAP, TPC, and MDA tests. The results of the study show that Agarwood leaves, Mango shoots, Bamboo starfruit, Nutmeg shells, and Noni fruit have different antioxidant properties. It can be concluded that the best herbal plant with the highest antioxidant activity is the Agarwood leaf followed by Mango shoots, Bamboo starfruit, Noni fruit, and finally Nutmeg shells.

Keywords: Agarwood; mango shoots; bamboo starfruits; nutmeg shells; noni fruits; antioxidant
Oral acute toxicity of aqueous extract of *Elephantopus scaber* Linn. (Tutup bumi) in Sprague-Dawley rats

Mohd Syahmi Mohd Azha¹,*, Mohd Kamal Nik Hasan¹, Azman Mohamed¹, Zaridah Mad Zaki¹, Abd Rashid Li¹, Muhammad Khair Mohd Ayob¹ and Nur Farina Mohd Rizal²

¹Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
²Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

*Correspondence:
Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
Email: msyahmi@frim.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

Plants have been used since the beginning of time to treat various diseases. One of these plants is *Elephantopus scaber* Linn. (*E. scaber*). This study evaluated the acute toxicity effect of aqueous extract of *E. scaber* using female albino Sprague-Dawley rats. The extract was administered orally to each female rat at a single dose of 2000 mg/kg on the first day. The rats were observed for 14 days. The acute toxicity of oral administration of aqueous extracts of *E. scaber* on the rats within 14 days did not show any toxicity effects at the dose of 2000 mg/kg. All the test systems survived the study period of 14 days. The results showed that the *E. scaber* is non-toxic at the tested dose.

Keywords: *Elephantopus scaber* Linn.; acute toxicity; aqueous; Sprague-Dawley and oral
Acute toxicity study of aqueous leaf extract of *Senna alata* (Gelenggang) by oral administration in Sprague Dawley rats

Azman Mohamed¹,*, Mohd Kamal Nik Hassan¹, Zaridah Mad Zaki¹, Abd Rashid Li¹, Muhammad Khair Mohd Ayob¹, Mohd Syahmi Mohd Azha¹ and Nur Farina Mohd Rizal²

¹Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
²Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

*Correspondence:* Department of Natural Products, Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.
Email: azmanm@frim.gov.my
DOI https://doi.org/10.28916/lsmb.6.1.2022.108

Abstract

The main source of therapeutic chemicals in medicinal plants can be used in the development of new therapeutic compounds along with their wide biological applications. *Senna alata* (*S. alata*) is one of the medicinal plants that has been the subject of scientific investigation. This study evaluated the toxicity of *S. alata* aqueous leaf extract using albino Sprague-Dawley rats. In this acute toxicity test, the aqueous leaf extract of *S. alata* was administered orally to each male and female rat. Sprague-Dawley rat weighing from 100g to 200g. Sprague-Dawley rats were administered orally once on Day 0 with aqueous leaf extract of *S. alata* at a dose of 2000 mg/kg. The rat was observed for 14 days. Observations were made and a necropsy on each rat was carried out on Day 14. Oral administration of an aqueous extract of *S. alata* leaves in albino rats after 14 days did not cause any death at a dose of 2000 mg/kg. All rats survived the 14-day study period. No signs of intoxication were found on every internal and external body of the rat. The results showed that the dried leaves of *S. alata* were not toxic at the tested doses.

**Keywords:** *Senna alata*; aqueous extract; Sprague Dawley; acute toxicity and oral administration
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