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34th Malaysian Association of Clinical Biochemists Annual Conference in collaboration with 2nd Malaysian Biomedical Science Association Symposium 2024

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ABSTRACT

The Malaysian Association of Clinical Biochemists (MACB) and the Malaysian Biomedical Science Association (MyBiomed) successfully organized the 34th MACB Conference in conjunction with the 2nd MyBiomed Symposium 2024. Under the theme 'Towards Holistic Integration and Sustainability in Medical Laboratories, the event brought together experts from various disciplines to explore advancements and sustainability in laboratory medicine and biomedical science. The conference featured a comprehensive program including plenary lectures, industrial talks, oral and poster presentations, and trade exhibitions. It provided a platform for the exchange of scientific knowledge and insights into emerging technologies and practices. A diverse range of professionals participated, including local and international chemical pathologists, clinical biochemists, medical laboratory scientists, researchers, and postgraduate students. Key topics covered included natural product research, herbs and metabolic disease, molecular diagnostics, pharmacology and toxicology, endocrinology, reference intervals, value-based laboratory medicine, newborn screening, the role of digital technologies in medical labs, and strategies for developing green & sustainable medical laboratories. The discussions were aligned with the conference's goal of fostering integration and sustainable practices in the medical laboratory sector, reflecting the latest trends and future directions of the field. The event achieved its aim of providing participants with valuable updates on scientific and technological innovations while fostering collaboration and dialogue among laboratory professionals at a global scale.

Keywords: *Biomedical sciences; clinical biochemist; research; laboratories and technology*

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ABSTRACTS

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The temporal impact of aluminium on memory function and amyloid-β accumulation in rats

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Abstract

Aluminium is a non-essential element that poses a potential risk for neurodegenerative diseases, including Alzheimer's disease (AD). Despite the known association between aluminium exposure and neurotoxicity, the temporal dynamics of aluminium-induced cognitive deficits and Amyloid-β (Aβ) accumulation in the context of AD remain limited. This preliminary study aims to investigate the impact of aluminium exposure on memory in an AD rat model at two-time points: 14 days (T14) and 42 days (T42), using 200 mg/kg/day of aluminium chloride (AlCl3) administered via oral gavage. The memory function was assessed via a 2-Novel Object Recognition (2-NOR) test. Post-exposure, brain tissues were analysed for aluminium concentration using inductively coupled plasma mass spectrometry (ICP-MS) and Aβ levels using an enzyme-linked immunosorbent assay (ELISA). Results indicated that aluminium exposure significantly impaired memory in the AD group compared to controls ($p<0.001$). Aluminium concentrations were notably higher in the AD groups at both T14 ($p<0.01$) and T42 ($p<0.01$), with a significant increase observed from T14 to T42 ($p<0.05$). Moreover, a strong negative correlation between aluminium and soluble Aβ concentrations was identified ($p<0.05$). Aluminium exposure may exacerbate cognitive deficits by inducing oxidative stress or inflammatory responses, potentially disrupting soluble Aβ clearance. This disruption can lead to the conversion of soluble Aβ into its insoluble form, contributing to Aβ accumulation in AD. Understanding these temporal dynamics is crucial for identifying critical periods of aluminium exposure impact. Further proteomic and transcriptomic studies are essential to elucidate the mechanistic pathways involved and to identify key pathways and potential biomarkers of aluminium-induced neurotoxicity.

Keywords: *Aluminium; Alzheimer's disease; amyloid-β; memory impairment and neurodegenerative model*

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External quality assurance programme for drug testing in the MOH laboratories: Performance for 2023

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Abstract

Drug testing started in Malaysia in 1970 at the Institute of Medical Research (IMR) and then in pathology laboratories in 1980. The National Drug Quality Control Programme (NDQCP) was introduced as an external quality programme for drug testing by IMR and stopped in 2018. NDQCP was reactivated by Hospital Kuala Lumpur (HKL) in 2019. Urine samples were distributed to 60 hospitals, consisting of four samples per year for opiates and cannabis and two samples per year for amphetamine-type stimulants (ATS). The tests included opiates (morphine and codeine), cannabis (THC) and ATS (amphetamine, methamphetamine, MDA, MDMA). Techniques for analysis were immunoassay by test strips or chemistry analyser for screening, thin layer chromatography (TLC) for confirmation of opiates and cannabis and gas chromatography-mass spectrometry (GCMS) for confirmation of ATS. Samples were prepared using Certified Reference Material by a certified provider and distributed by a local company to the hospitals. The results were analysed by HKL. showed that chemistry analysers were more accurate than test strips for screening tests. Many hospitals did not give a correct interpretation for TLC, especially near the cut-off values. ATS confirmations done by GCMS at seven hospitals showed good results except for one laboratory. The TLC method needs to be verified, and personnel performing the tests need to be trained and competent. The GCMS method should be performed using quantitative methods, and the performance of those hospitals should be monitored by HKL. As a way forward, drug testing services need to be centralised.

Keywords: *Drug testing; GCMS; opiates; THC and TLC*

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Proteomic and cellular integrity analysis in response to lysosomal dysfunction in human brain endothelial cells

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Abstract

Lysosomes are crucial organelles for cellular breakdown and homeostasis maintenance. Its failure has been associated with amyloid deposition and blood-brain barrier (BBB) dysfunction in Alzheimer's disease (AD) development. The pathological events are mediated by proteins influencing cell biochemical reactions, thereby determining the phenotype or disease progression. This study developed a lysosomal dysfunction model to investigate changes in protein expression and permeability of brain endothelial cells. Human brain endothelial cells (HBEC-5i) were treated with a lysosomotropic agent, chloroquine, for 24 hours, and the treatment concentrations were determined by MTT assay: IC_{10} (17.5 µM), IC_{25} (70.5 µM), and IC_{50} (125 µM). Inhibition of the lysosomal function led to vacuole accumulation in cells, and capillary-based immunoassay showed a significant increase in autophagy marker, LC3-II, at IC_{25} (p<0.05). Furthermore, intracellular Aβ42 measured by ELISA also recorded a significant increase at IC_{25} (p<0.05). Therefore, the optimal concentration of IC_{25} was employed in the subsequent experiments. Liquid chromatography-mass spectrometry (LC-MS/MS) identified 27 significantly expressed proteins (p<0.05), with decreased expression of lysosomal proteases *CTSD* (-3.4) and *CTSB* (-3.8). Conversely, *SQSTM1* (+2.4) and *EEA1* (+1.7) increased in expression. Gene ontology revealed enrichment in the transcription and apoptosis process, while protein-protein interaction depicted three protein clusters involved in translation, DNA modification and lysosomal degradation process. Additionally, a significant increase in cell monolayer integrity (p<0.05) was observed using transendothelium electrical resistance (TEER). These findings demonstrated that lysosomal dysfunction alters cellular proteins associated with lysosomal degradative networks and impairs the barrier function of the brain endothelium, which may contribute to the onset of neurodegenerative diseases.

Keywords: *Amyloid angiopathy; Alzheimer's disease; autophagic vacuoles; lysosome dysfunction and proteomic*

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Investigation of wound healing properties of coastal medicinal plant *Vitex rotundifolia* fruits extracts and fractions

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Abstract

The fruits of the coastal plant *Vitex rotundifolia*, also known as Fructus viticis, have long been used to treat a variety of diseases, but little research has been conducted on their wound-healing properties. In this study, we investigated the wound healing properties of *V. rotundifolia* fruit methanolic crude extract (VRFME) and its fractions in terms of antioxidants, total phenolic content (TPC), anti-collagenase, and anti-elastase activity, as well as their ability to heal full-thickness wounds in zebrafish models. The antioxidants in VRFME and their fractions were measured using a DPPH scavenging assay at various concentrations (0-0.5 mg/mL). Meanwhile, gelatine digestion and spectrophotometric assays were used to determine anti-collagenase and anti-elastase activity at concentrations ranging from 0 to 1 mg/mL. In an animal study, formulated VRFME using vaseline (50% w/w) was applied to a full-thickness wound in a zebrafish model, and gross examination was performed daily for 14 days. At the end of the experiment, the tissue sample was histopathologically analysed. VRFME had the highest bioactivities, including a potent antioxidant (IC_{50}: 0.027 mg/mL), high TPC (16.8±0.5 mg GAE/g), anti-collagenase (24.7±1.2%) and anti-elastase (50.4±1.8%). In addition, *in vivo* study found that 50% of the formulated VRME significantly ($p<0.05$) improved wound healing closure from day 0 to day 14 compared to the control group. Histopathology analysis confirmed the healing process associated with better tissue regeneration and collagen deposition. In conclusion, *V. rotundifolia* fruits have the potential to be further utilised and developed into pharmaceutical and nutraceutical products, particularly for dermal-associated diseases.

Keywords: *Coastal plants; collagen; Danio rerio; Fructus viticis and wound healing*

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Progressive cognitive decline and biomarker shift in an Alzheimer's disease model rats

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Abstract

Alzheimer's disease (AD) is characterised by progressive cognitive decline and neurobiological changes, including the accumulation of amyloid plaques and alterations in neurotrophic supports. However, the precise impact of aluminium exposure on AD biomarkers and its progression remain poorly understood. This study seeks to elucidate the neurobiological changes associated with AD progression following aluminium exposure. To achieve this, thirty rats were randomly assigned to three aluminium exposure durations (200 mg/kg/day of aluminium chloride via oral gavage): 14 days (D14), 28 days (D28), and 42 days (D42). Memory function was assessed using the 2-Novel Object Recognition (NOR) test. Following exposure, the rats' hippocampi were isolated and analysed for Aβ, BDNF, proBDNF, and AChE activity levels. Results showed that aluminium exposure significantly impaired memory in the AD group compared to controls ($p< 0.001$). Aβ levels were significantly elevated in the AD group at D14 ($p < 0.01$). BDNF levels decreased significantly at D42 ($p< 0.001$), while proBDNF levels increased at both D14 ($p< 0.05$), and D42 ($p< 0.01$). A moderate negative correlation between proBDNF and mature BDNF levels was observed ($p< 0.05$). AChE activity increased at D14 ($p < 0.05$), but decreased at D42 ($p< 0.01$). The negative correlation between proBDNF and mature BDNF levels suggests dysregulated pro-BDNF processing, potentially exacerbating synaptic dysfunction and cognitive decline in AD. Furthermore, the decline in AChE levels may exacerbate this condition, highlighting the intricate interplay between neurotrophic factors and cholinergic neurotransmission in AD pathophysiology. Further research is warranted to elucidate the underlying mechanisms.

Keywords: *Alzheimer's disease; brain-derived neurotrophic factor; memory impairment; proBDNF and synaptic pathway*

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Tissue rigidity in pre-malignant and malignant lung squamous cell carcinoma stages *in vivo*

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Abstract

Lung squamous cell carcinoma (LSCC), a subtype of non-small cell lung cancer, has resulted in numerous deaths globally. One of the emerging hallmarks of cancer is increased tissue rigidity, which is crucial for cancer growth. This increased rigidity is linked to treatment resistance, poor prognosis, and lower survival rates in cancer patients. Despite its importance, the characterisation of tissue rigidity in the dual stages of lung squamous cell carcinoma (SCC) carcinogenesis remains undefined. Therefore, this study focused on investigating tissue rigidity in both the pre-malignant and malignant stages of lung SCC in vivo. BALB/c mice were randomly assigned to 4 groups (n=8 mice per group): pre-malignant (PM) and malignant (M) groups, along with vehicle control (VC) groups for both PM and M. N-nitroso-tris-chloroethylurea (NTCU) was used to induce PM and M lung SCC in the respective groups for 15 and 30 weeks. Significant increases in both collagen content and tenascin-C protein expression were observed in the M group ($p<0.05$) compared to the other groups. Additionally, collagen rigidity analysis indicated a notable increase in the M group ($p<0.05$) compared to the others. In conclusion, this study suggests that tissue rigidity increases as carcinogenesis progresses from the pre-malignant to the malignant stage. This finding supports the potential of targeting tissue rigidity as a novel mechano-therapeutic approach for lung SCC.

Keywords: *Lung cancer; tissue rigidity; squamous cell carcinoma; pre-malignant and malignant*

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Unveiling the pandemic's footprint: temporal shifts in creatinine levels before, during, and after the COVID-19 pandemic

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Abstract

The COVID-19 pandemic has raised questions about its impact on biochemical markers through prolonged exposure and vaccination. Temporal analysis of biomarkers, such as creatinine, equips laboratories to understand trends and fluctuations in response to health crises. Thus, the examination of biomarker reference intervals is critical to ensure that the intervals reflect the current health status of the intended population. Using laboratory data from 2019 to 2023, the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) method is employed to estimate reference intervals for creatinine. The Differential Evolution (DE) algorithm, optimised DBSCAN hyperparameters to cluster creatinine data and effectively manage outliers. The quality and stability of the clustering were rigorously evaluated using internal, external, and error metrics. Temporal trends were then visualised with bar charts, and year-to-year differences were examined using significance tests to assess practical significance, ensuring the precision and reliability of our findings. The performance metrics validated the DE-optimized DBSCAN algorithm's effectiveness in clustering creatinine data, revealing significant year-to-year variations from 2019 to 2023. Statistical tests indicated significant differences ($p < 0.01$) in creatinine levels between years, with most comparisons showing small effect sizes (Cohen's $d < 0.5$). However, the 2021 to 2023 difference was more substantial, with a medium effect size (Cohen's $d = 0.545$). Our study reveals significant temporal variations in creatinine intervals, with notable increases during the pandemic. Continuous monitoring of biomarker levels is crucial for understanding post-pandemic health changes, and the indirect method using existing laboratory data offers a convenient approach for ongoing surveillance.

Keywords: *COVID-19; creatinine; indirect reference interval; temporal analysis and unsupervised machine learning*

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Vitamin D status and related sociodemographic factors in Malaysia: Secondary data from the Pantai Premier Pathology Laboratory

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Abstract

Vitamin D is a fat-soluble vitamin important for bone metabolism and maintaining optimal mineral levels in the body. The human body cannot produce vitamin D on its own; therefore, it can be obtained directly from sunlight or food sources. Despite Malaysia receiving ample sunlight throughout the year, it is necessary to identify whether there is a vitamin D deficiency based on sociodemographic factors among the population in Malaysia. Secondary data comprising 62,388 records was received from private hospitals across Malaysia, spanning from 2017 to 2021. This data includes sociodemographic information about participants and their vitamin D levels (nmol/L), and it was analysed using the Complex Samples module of SPSS 29 software to represent the population of Malaysia. Overall, this secondary data represents approximately 3.02 million people, with the highest number of participants in 2021 (45.2%) compared to 2017 (3.0%). The high rate of vitamin D screenings in 2021 may be due to more individuals seeking health check-ups because of the ongoing pandemic. In terms of vitamin D levels, 63.8% (equivalent to 19.3 million people in Malaysia) were found to have a vitamin D deficiency, compared to 34.7% who had normal vitamin D levels. The Malaysian population remains exposed to the risk of vitamin D deficiency despite living in a tropical country and having access to vitamin D-rich food sources. This can lead to bone health issues such as osteoporosis and rickets. Therefore, further research is necessary to identify the main causes of vitamin D deficiency and appropriate preventive measures.

Keywords: *Deficiency; population; secondary data; sociodemographic factors and vitamin D*

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Investigating the chemokine gene expression profiles involved in neutrophil and macrophage recruitment in an acute inflammation model induced by red seaweed extract Lambda-carrageenan

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Abstract

Activation of Toll-like receptor 4 (TLR4) ligands, including λ-carrageenan, a red seaweed extract, leads to an upregulation of pro-inflammatory molecules such as chemokines. These molecules, secreted by TLR4-expressing cells, promote immune cell infiltration, inflammation, and the sensitisation of primary afferent nociceptors, resulting in pain. However, the specific expression of chemokines upon carrageenan activation remains incompletely understood. This study aims to elucidate the chemokine and pro-inflammatory gene expression profiles following TLR4 activation using λ-carrageenan-induced acute inflammatory pain in rat paw tissues. Intraplantar subcutaneous injection of 2% (v/v) λ-carrageenan (100 µl) or saline control into the hind paw of Sprague Dawley rats significantly altered hind-limb weight bearing and increased paw volume, indicative of hyperalgesia and inflammation. Plantar skin samples were collected 2 hours post-injection for mRNA extraction. Gene expression analysis of 35 genes associated with chemokines, neutrophil markers, and the NF-κB signalling pathway was performed using Taqman Low Density Arrays. As anticipated, carrageenan significantly upregulated the expression of several chemokines: CCL4, CXCL2, CXCL1, and CCL3, with a fold change exceeding 50 compared to the control paw. Additionally, carrageenan differentially affected the expression of neutrophil markers and NF-κB pathway genes. These findings suggest that future research on anti-inflammatory agents should target specific chemokines that regulate the infiltration of neutrophils and monocytes/macrophages, as these processes are critical in the development of oedema and pain.

Keywords*: Acute inflammation; chemokines; TLR4; seaweed and pain*

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Comparison of polyphenolic compounds of three different types of roselle extracts and toxicity assessment of roselle extract via acute and subacute toxicity studies

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Abstract

Roselle has been reported to contain polyphenolic compounds that exert antioxidative, antihyperlipidemic, anticancer, and anti-inflammatory effects. However, polyphenolic compounds in roselle calyx extract are highly influenced by extraction methods. Though roselle has been extensively studied, there is limited knowledge about roselle's toxic profile. Thus, this study aimed to quantify the polyphenolic compounds in three different types of extract and determine the toxic effects via acute and subacute toxicity studies. The polyphenolic compounds of aqueous (AR), ethanolic aqueous (EAR), polyphenol rich extract (HPE) was analysed by high pressure liquid chromatography. The extracts that contain highest polyphenolic compounds proceeded with toxicity studies. 28 male Sprague-Dawley rats were used for both toxicity studies. The rats were administered a single dose of 500, 1000, and 2000 mg/kg of extract for acute toxicity study and kept monitored for 14 days. For subacute toxicity study, the dose of 125, 250 and 500 mg/kg were administered daily for 28 days. Blood samples were collected for biochemical test and organs were harvested for histology study. HPE was found to have highest chlorogenic acid. However, EAR has higher caffeic acid, rutin, delphinidin-3-sambubioside and cyanidin-3-sambubioside content compared to other extracts. An acute toxicity study revealed that LD50 of aqueous ethanolic extract is more than 2000 mg/kg. For the subacute toxicity study, the liver, lipid, and kidney profiles were in the normal range and the histology study showed normal tissue structure. Therefore, this study revealed that EAR contains the highest polyphenolic compounds and has no toxic effects. Therefore, EAR can be developed as a nutraceutical for alleviating diseases.

Keywords*: roselle; polyphenol; nutraceutical; extraction and toxic profile*

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Pooled evidence on the effect of exercise towards cognitive function and physical health through biochemical changes: A scoping review

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Abstract

Physical exercise, which includes resistance and aerobic training, provides numerous benefits, including improved physical health, cognitive function, and disease prevention. Physical exercise induces numerous biochemical changes that counteract the deterioration of cognitive function and physical strength associated with ageing. Moreover, exercise timing or chrono-exercise also studies how the body's internal clock, known as the circadian rhythm, responds to exercise and improves the metabolic processes. Our scoping review aims to investigate how various forms of exercise affect the biochemical processes. The final analysis included the aerobic, resistance and combination of exercise studies. The result shows that exercise affects many aspects, such as physical and muscle performance, energy production, cardiovascular and metabolic health and cognitive and mental health. It involves various biochemical processes such as gene regulation, alters hormone and growth factor secretion, and affects enzyme and protein activity. Additionally, exercise is crucial in metabolism, maintaining homeostasis, and enhancing respiratory function. However, the explanation of the interaction of biochemical or molecular processes remains unclear. Also, the long-term effects of exercise interventions on specific health outcomes remain insufficiently explored. Our findings elucidate the comprehensive benefits of both types of exercise despite having different protocols on various aspects of physical health to optimise the interventions for enhancing cognitive function and physical performance.

Keywords: *exercise; rodent; scoping review; timing*

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EVNol Suprabio™ protects testicular steroidogenesis via reproductive hormone regulation in bisphenol F-induced Sprague Dawley rats

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Abstract

Bisphenol F (BPF) is one of the endocrine disruptors which causes reproductive hormonal alterations. EVNol SupraBioTM (EVNol) is a tocotrienol-rich supplement that is widely known for its anti-inflammatory benefits. However, it is unclear how EVNol affects alterations in testicular steroidogenesis induced by BPF via inflammation. Present study was conducted to explore the effects of EVNol on hormonal regulation in testis induced by BPF by evaluating reproductive hormone analysis (plasma), inflammatory and antioxidant status of Leydig cells. Forty male Sprague-Dawley rats (weighed 220-250g) were randomly assigned to five groups: control group (1mg/kg corn oil), EV100 (100mg/kg EVNol), BPF (10 mg/kg BPF), BE50 (50 mg/kg EVNol + BPF) and BE100 (EV100+ BPF). Treatments were administered daily via oral gavage for 35 days, with (BE) rats being given EVNol 30 minutes before administration of BPF. Current results revealed that EVNol reduced inflammation as level of arachidonic acid (AA) significantly increased in EV100 and BE100 groups whereas PGE-2 significantly decreased in EV100 group compared to BPF group. Expression of COX-2 significantly reduced in EV100 and BE100 groups compared to BPF group. A significant increase of GSH in EV100 group indicated enhanced antioxidant capacity compared to BPF group. Cholesterol levels significantly elevated in EV100 group compared to BPF group. Hormone analysis showed significantly increased pregnenolone and decreased estradiol levels in EV100 and BE100 groups compared to BPF groups. Levels of testosterone in EV100 group were significantly higher compared to BPF group. Overall, 100 mg/kg of EVNol protects testicular steroidogenesis in BPF-induced rats by alleviating inflammation.

Keywords*: Bisphenol F; BPA analogue; EVNol SupraBio™; testicular steroidogenesis and tocotrienol*

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Epidemiological surveillance of upper respiratory tract infection in a private tertiary care hospital: A preliminary study

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Abstract

Significant advancements in Polymerase Chain Reaction (PCR) technology have revolutionised the rapid detection of multiple pathogens within a short timeframe. This breakthrough has notably expedited doctors' ability to diagnose patients with greater precision and ultimately improve patient care. A study encompassing 11,717 cases associated with upper respiratory tract conditions, spanning from 2020 until 2023, was curated from a comprehensive database housing a rapid pathogen detection system using a syndromic surveillance tool. Twenty-three pathogens relevant to upper respiratory tract diseases were screened. Notably, cases positive for Coronaviruses such as Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43 and SARS-CoV-2 were excluded, given the pandemic status of SARS-CoV-2 in that period of time. The most prevalent infections observed were Human rhinovirus/enterovirus (HRV/ENT), accounting for 36.78% of the respiratory infection cases ($p \leq 0.05$), while the least prevalent infections were Influenza A subtype H1-09, constituting merely 0.02% of cases ($p \leq 0.05$). This study underscores the potential for future longitudinal research endeavours, suggesting the inclusion of additional factors such as age, specific diagnoses, and an expanded sample size. Such investigations hold promise for further elucidating the complexities of upper respiratory tract diseases and refining diagnostic methodologies.

Keywords: *Epidemiology; real-time; infectious diseases; rapid test and molecular*

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Pseudohyponatremia in hypertriglyceridemia patient: How to manage the analytical interference

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Abstract

Most central clinical laboratories employ testing for sodium using indirect ion selective electrode (ISE) in automated analysers. Pseudohyponatremia is well known to be associated with hypertriglyceridemia when analysed with indirect ISE. We present a case of a 50-year-old gentleman with type 2 diabetes mellitus, primary hypothyroidism and severe hypertriglyceridemia.A plasma sample received appeared milky, and the Lipemia index showed 4+, equivalent to approximately >500 mg/dL Intralipid. Triglycerides level was 53.6 mmol/L, total cholesterol 14.9 mmol/L and HDL cholesterol 0.46 mmol/L. We noted his sodium level was 125 mmol/L (indirect ISE). The milky plasma was then subjected to high-speed microcentrifugation with a configuration setting of 14000 rpm for 10 minutes. The creamy supernatant was removed, and the clear infranatant was then re-analysed. The infranatant sample yielded sodium of 133 mmol/L, and the result was compared with the direct ISE sample, where the sodium level was 136 mmol/L. Upon reviewing his previous blood results, the baseline indirect ISE sodium levels were 138 mmol/L and 132 mmol/L when triglycerides levels were 8.0 mmol/L and 18.6 mmol/L, respectively. Chemical methods using polar solvents and the analytical method of ultracentrifugation (Clinical & Laboratory Standards Institute recommendation) are used to remove lipemia in serum/plasma samples. However, due to the cost and limited availability of these two methods, high-speed microcentrifugation can be employed to tackle the issue of pseudohyponatremia in samples with high triglyceride for routine clinical biochemistry laboratory.

Keywords: *High-speed microcentrifuge; hypertriglyceridemia; indirect ion selective electrode and pseudohyponatremia*

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Evaluation of the iCHEM velocity urine chemistry analyzer in Hospital Al-Sultan Abdullah, UITM Puncak Alam

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Abstract

The placement of a new urine chemistry analyzer, Beckman Coulter iChem Velocity system necessitates evaluation study to be carried out. The study aims to evaluate the precision, specificity, sensitivity, and concordance of the new analyzer. Precision was carried out according to Clinical Laboratory Standard Institute (CLSI) EP15-A2 using internal quality controls (CA, CB, and CC IQC). The acceptance criteria for precision study were established as 90% of negative samples producing negative results distributed within 2 adjacent concentration ranges. The specificity and sensitivity were calculated from the known positive and negative cases. The concordance of results with referenced urine chemistry analyzer, Roche Cobas u411 were evaluated. The acceptance criteria for the concordance study were set as 100% for ± 1 grade. Within-run and between-run imprecision showed 100% agreement except glucose on CA IQC (80%) and ketone on CB IQC (60%); glucose on CA IQC (56%), ketone on CB IQC (60%), and urobilinogen on CB IQC (68%) respectively. The specificity ranged between 74% and 100% with the blood parameter showing the lowest specificity (74%). The sensitivity ranged from 66% to 100% with protein parameter has the lowest sensitivity (66%), while the sensitivity for bilirubin and urobilinogen were not calculated due to insufficient positive samples. The iChem VELOCITY had 100% agreement within ± 1 grade except pH (92%), SG (90%), and blood (80%). iChem VELOCITY showed an overall good performance, having met the defined acceptance criteria and demonstrated high percentage of analyte agreement with the other commercially available urine chemistry instrument.

Keywords: *Method evaluation; correlation study; semiquantitative and concordance study*

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HbA1c reportability of heterozygous variant samples study subjected to P3 peak cutoff criteria between Variant II Turbo and Bio-Rad D-10

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Abstract

Glycated hemoglobin (HbA1c) has become the biomarker for diagnosis and measurement of glycemic control. High-Performance Liquid Chromatography (HPLC) is a gold standard method to measure HbA1c, seemingly Bio-Rad D-10 and Variant II Turbo are the applications of HPLC. This study is conducted to evaluate the difference in HbA1c results for unreportable patient samples of heterozygous variant with P3 >5% from VIIT in Hospital Serdang with D-10 in Hospital Putrajaya. Can these patient results be directly reported from VIIT and equivalent to the reportable results with D-10? Total of 55 unreportable patient samples of heterozygous variant with P3>5% from VIIT are selected for this study (August- October 2021). After being tested at VIIT, samples are measured at D-10. From the total of 55 unreportable heterozygous patient samples in VIIT, all of it can be reportable through D-10 with only 9 samples needed to include caution/ comment as exceeded P3>10%. Passing-Bablok regression analysis entails a linear relationship between VIIIT and D-10. Bland-Altman Plot shows VIIT correlates at a higher bias than D-10 which operated at a bias of 3.25% with a bias unit of 0.2113. VIIT and D-10 show a good linear relationship and correlate well with acceptable regression outcome. Based on the data statistics, it is concluded that HbA1c results for normal and heterozygous patients on VIIT with P3>5% and <10% are reportable, with a comment at the highest bias of 3.25%.

Keywords*: HbA1c; Bio-Rad D-10; Variant II Turbo and HPLC*

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The impact of automated verification on the workflow of routine complete blood count (CBC)

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Abstract

Complete blood count (CBC) analysis is one of the most common routine tests performed on patients in hospitals. However, the manual validation on CBC analysis is laborious and time-consuming. The aim of this study was to evaluate the impact of introducing automated verification steps in routine CBC analysis by assessing the turnaround time (TAT) and overall workload. The data used were CBC samples collected retrospectively from October 2023 to December 2023 in the routine hematology unit of laboratory medicine department, University Malaya Medical Centre. Inclusion criteria were based on customized rules created for fully automated test validation for routine samples using Sysmex® XN9000 series middleware (Sysmex Corp., Kobe, Japan) as well as the laboratory information system (LIS). In these customized rules, analytical measurement ranges (AMR), delta check, critical values and all analytical flags from the analyser were used as part of the algorithm in designing the auto-verification step. The duration of the complete blood count (CBC) analysis ranges from acceptance by the laboratory to verification of the results. A t-test for hypothesis testing was applied to determine the changes in manual and automated validation to determine their significance. The change in the data is considered significant if the P value <0.05 at a 95% confidence interval. A total of 49,819 CBC profiles were analyzed. The hospital quality system targets a CBC TAT of less than 90 minutes. The study indicates a significant difference in turnaround time between auto verification (mean = 6.17 minutes) and manual validation (mean = 38.26 minutes) for CBC profiles, with a large effect size (d = 2.38), suggesting a substantial impact on efficiency. The p-value (<.001) suggests strong evidence against the null hypothesis, supporting the statistical significance of this difference. The delivery time for routine CBC test was considerably shortened by the automatic verification method. The reduction of the number of samples needed for manual slide review, provides the laboratory technicians or specialists, more time to assess for any haematological abnormalities. This enabled more effective detection of hematologic abnormalities during slide review. Also, ensures the achievement of good-quality reports. The auto-verification rule has also improved the overall workload on routine haematology and provides a timely assessment that improves overall patient management.

Keywords: *Auto-verification; manual validation; complete blood count; workload; turnaround time*

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Performance verification of the high sensitivity Troponin I algorithms to rule-out NSTEMI in the Emergency Department of University Malaya Medical Center

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Abstract

Rapid diagnosis of acute myocardial infarction (AMI) is crucial to initiate timely and appropriate management as well as identifying low risk patients who can be safely discharged from ED relatively quickly. The introduction of high sensitivity assays allows for reliable quantification of cardiac troponin at lower concentrations. International Federation Clinical Chemistry (IFCC) Taskforce stated that "Relative change value (RCV)"or "delta change value" in serial measurements of hs-Troponins is more accurate for AMI diagnosis than a single value, as solitary elevation of hs-Troponins can also be due to other causes of chronic cardiac or non-cardiac diseases. European Society of Cardiology (ESC) guidelines recommend the use of assay dependent hs-Troponins in algorithms based on specifically timed serial samplings for early AMI rule-out decisions. The 0-1, 0-2 and 0-3-hour algorithms have been established and validated for their safety and method specific hs-cardiac troponins' RCV or delta value between measurements were used as the cut-off reference to rule out AMI. The hs-Trop I method used was the Siemens TNIH reagent on the Atellica platform. A total of 233 patients presenting to UMMC ED and investigated for AMI were recruited. Blood sampling times were based on the corresponding algorithms with baseline hs-Troponin I taken at presentation (0-hour) and the subsequent ones at 1, 2 and 3 hours post presentation, respectively, based on the timing determined by each algorithm. The number of patients was N=45 for the 0/1H, N= 44 for the 0/2H and N=144 for the 0/3H rule-out algorithms. RCV/delta values used were those established and validated by Boeddinghaus and Chapman for Siemens platforms available in the 2020 NICE guidelines. The 0/1H algorithm (N=45) showed 100% rule-out sensitivity and NPV with 87.5% rule-in specificity and 73% PPV. The 0/2H algorithm (N=44) showed 100% rule-out sensitivity and NPV with 80% rule-in specificity and 56% PPV. The 0/3H algorithm (N=114) showed 97% rule-out sensitivity, 99% NPV with 48% rule-in specificity and 37% PPV. Our study has verified that all the 3 rule-out algorithms produced very good NSTEMI rule-out sensitivity (>90%) based on the ESC established and validated RCV/delta value using the Siemens TNIH reagent on the Siemens Atellica platform. The algorithms however, did not produce high specificity to rule-in NSTEMI, which is consistent with those established in the ESC validated studies. These algorithms should therefore, be utilized strictly for the purpose of ruling out NSTEMI in the ED setting and not for diagnosing NSTEMI. The trend in UMMC Emergency Department showed that hs-TropI was mostly sampled using the 0/3H algorithm, reflecting the difficulty in keeping with the 1 or 2 hours post-presentation sampling in a tertiary hospital's emergency setting where a large variety and severity of cases were attended to.

Keywords: *High sensitivity Troponin I; serial measurements; non-ST elevation myocardial infarction; rule-out algorithms and relative change value*

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Potential anticancer peptides from serine protease precursor of *L. rhinocerus* TM02®: An *in silico* approach

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Abstract

The development of new anticancer agents was imperative due to existing treatments' limitations and side effects. Mushroom-derived proteins garnered attention for their anticancer potential, particularly low-carbohydrate proteins. Peptides, generated from protein digestion, exhibited diverse bioactivities, including anticancer effects. This study aimed to design potential anticancer peptides (ACPs) from the serine protease precursor of *Lignosus rhinocerus* TM02® (tiger milk mushroom) using an *in silico* approach. Specifically, we targeted the cytotoxic protein fraction F5, previously identified in *L. rhinocerus*. F5 demonstrated selective cytotoxicity against a human breast adenocarcinoma cell line (MCF7) and induced apoptosis via crosstalk between the extrinsic and intrinsic apoptotic pathways, which may have important implications for the development of new therapeutic strategies for the treatment of cancer. F5 contains three predicted non-synonymous single nucleotide polymorphisms (T > C) and an alternative 5' splice site. In this study, mutations were introduced, and their anticancer activity was predicted via computational approaches. Through binding analysis with antiapoptotic proteins (BCL-2, Mcl-1, Bcl-XL), three potential anticancer peptides (TM-SP_1, TM-SP_2, and TM-SP_3) were identified, demonstrating good stability and suitability as anticancer peptides. This study represented the first exploration of potential ACPs derived from *L. rhinocerus*, utilising a combination of bioinformatics and biotechnology approaches. The identified novel ACPs pave the way for further exploration of its therapeutic potential in cancer treatment.

Keywords: *Anticancer peptides; Lignosus rhinocerus; serine protease and in silico design*

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Unveiling a subtle variant of Conn's Syndrome - A case report

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Abstract

The classical signs of primary hyperaldosteronism (PA) are hypertension and hypokalemia which occurs in 60% to 90% of patients. However, rarely PA presents with normal blood pressure. A 43-year-old woman presented to our hospital with bilateral lower limb weakness. Her previous history suggested a fluctuating serum potassium between 1.5 and 2.3mmol/l. She had no other past medical history, unremarkable physical examination with normal blood pressure. The initial serum potassium at our center was 3.0mmol/L. Plasma aldosterone was 1779pmol/L while plasma renin activity was 7.0mU/L with a raised aldosterone renin ratio (ARR) of 231pmol/mU. A CT scan showed a well-defined nodule measuring 2cm x 1.6cm x 1.8cm at the right adrenal gland. Adrenal vein sampling (AVS) suggested lateralization to the right adrenal adenoma. The understanding of primary aldosteronism (PA) has indeed undergone refinement, particularly regarding its association with hypertension. The traditional view linking PA predominantly with hypertension has been challenged by emerging evidence suggesting that normotensive PA exists. This recognition underscores the importance of considering a broader spectrum of presentations during screening and diagnosis, ensuring that patients receive appropriate care regardless of their blood pressure status. As research continues to shed light on these nuances, it will be interesting to see how clinical practice guidelines adapt to accommodate these insights.

Keywords: *Adrenal adenoma; aldosterone renin ratio; hypertension; hypokalemia and primary hyperaldosteronism*

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The vascular effects of *Lignosus rhinocerus* TM02® and xLr™ supplementation on obesity-induced endothelial dysfunction

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Abstract

Obesity-induced endothelial dysfunction is a significant risk factor for the development of arteriosclerosis. *Lignosus rhinocerus* (Tiger Milk mushroom, TMM) has been found to possess various bioactivities. Historically, TMM has been consumed as traditional medicine by various communities in Southeast Asia and China. However, its role in alleviating endothelial dysfunction remains to be elucidated. This study aims to investigate the effects of TMM supplementation, using uniquely cultivated *L. rhinocerus* TM02® sclerotia powder (TM02®) or its patented cold-water extract (xLr™), on metabolic parameters and vascular functions in an obese mice model. Obese C57BL/6J mice fed with high-fat diet were supplemented with TM02® (500 mg/kg), xLr™ (50 mg/kg) or aminoguanidine (200 mg/kg) orally for 4 weeks. Body weight and fasting blood glucose levels were monitored weekly, with an oral glucose tolerance test (OGTT) performed on the final week of treatment. Vascular reactivity in the aorta was measured using a wire myograph at the treatment endpoint. Our results showed that untreated obese mice had increased body weight, elevated fasting blood glucose and impaired OGTT. Both TM02® and xLr™ supplementation revealed significant reduction in fasting blood glucose levels and improved OGTT results compared with control, without affecting body weight. Furthermore, supplementation with TM02® significantly alleviated endothelial dysfunction in obese mice. Our findings demonstrate that both TM02® and xLr™ supplementation can potentially improve glucose intolerance and vascular function in obesity. This study delineates the prospective of using TM02® and xLr™ as an adjunct, natural supplement to preserve endothelial function and manage cardiovascular complications associated with obesity.

Keywords*: Cold-water extract xLr™; endothelial dysfunction; glucose intolerance; Lignosus rhinocerus TM02® and obesity*

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Analysing trends: a comprehensive audit of common patterns in CSF protein electrophoresis reports

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Abstract

Cerebrospinal fluid (CSF) protein isoelectric focusing is an important tool in the evaluation of neurological disorders and can provide valuable insights into the underlying pathophysiology of these conditions. The aim of this study is to assess the most common type of CSF protein electrophoresis reported in UMMC and the medical conditions commonly associated CSF migration pattern and electrophoresis profiles. A total of 268 samples were analysed during the period of 2016-2021. Patients' demographic (age, gender and race), clinical history and CSF protein electrophoresis results were retrieved from Laboratory Information System (LIS). The patterns were divided into five groups according to the CSF isoelectric focusing, i.e., type 1 to type 5. Type 1 (n=194) is the most common finding followed by type 4 (n=35), type 2 (n=29), type 3 (n=9), and type 5 (n=1). The various medical conditions associated with positive (Type 2,3,4 and 5) CSF oligoclonal bands (OCB) are transverse myelitis, neuromyelitis optica spectrum disorders, optic neuritis and others. Type 2 has been mostly found in multiple sclerosis (MS) patients (n=13), however MS was reported in other types also; type 1 (n=5), Type 4 (n=1) and Type 5 (n=1). Only one patient had Type 5 pattern, who has been diagnosed with MS. CSF protein electrophoresis analysis carried out a great value in identifying OCB, which can aid in the diagnosis of MS and other inflammatory and non-inflammatory diseases of the CNS. However, this test cannot be used alone to diagnose any specific disease.

Keywords: *CSF protein electrophoresis; CSF type; multiple sclerosis; oligoclonal bands and positive CSF OCB*

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Immune-mediated diabetes positivity among diabetes mellitus patients in Malaysia

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Abstract

Immune Mediated Diabetes (IDM) accounts for 5–10% of Diabetes Mellitus (DM). Circulating autoantibodies namely glutamate decarboxylase (GADA), islet antigen-2 (IA2) and islet cell cytoplasmic (ICA) directed to the pancreatic islets lead to progressive pancreatic beta-cell failure. There is limited data reported on the positivity of IDM in DM patients in Malaysia. Identifying the positivity of autoantibodies enables proper classification of DM and is clinically relevant for appropriate therapy. This study aims to determine the frequency of IDM positivity and their association with demographic characteristics. All requests for IDM performed between 1 January 2023 to 31 December 2023 (n=2974), were screened and analysed. Results are considered positive if at least one autoantibody shows positivity. The cutoff point was according to manufacturer's recommendation (Snibe Maglumi 2000), 17 IU/ml for GADA, and 28 IU/ml for ICA and IA2. Statistical analysis was performed with SPSS Version 25.0., descriptive statistics for IDM positivity analysis and Pearson's chi-square to determine association between categorical variables. 533/2974 (17.9%) was positive for IDM; of these, 350 (65.7%) had multiple positivity, single positivity, 183 (34.3%). GADA was found to be the most prevalent, 500 (93.8%), followed by ICA, 340 (63.8%) and IA2, 116 (21.8%). Chi-square test of independence showed significant association between age and IDM positivity ($p<.001$), while there was no significant association between gender and race with IDM positivity (all $p> .001$) GADA is the most significant autoantibodies in ruling out the IDM. It has the highest frequency in both single and multiple positivity. IDM was found to be associated with age and no significant association was found between gender and race with IDM positivity.

Keywords: *Autoantibodies; demographic characteristics; diabetes mellitus; IDM positivity and immune mediated diabetes*

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Performance of immunotyping and immunofixation in identifying and characterising monoclonal immunoglobulin: A comparison of methods

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Abstract

Immunotyping (IT) and immunofixation (IF) are methods used for confirming and characterising monoclonal immunoglobulin, performed following serum protein electrophoresis. Our institution was in a transition from gel-based to capillary-based electrophoresis; this study compared the two methods' performance in the local setting. IT and IF of 92 samples selected over four consecutive months were blindly interpreted by four interpreters in comparison with a three-test panel (agarose gel electrophoresis, IF, and serum free light chains). IF gels performed for routine reporting and IT analysed within the sample stability period were used for interpretation. The two methods isotyping agreement was assessed using 62 IF-confirmed monoclonal immunoglobulin samples. Additional evaluations include the impact of continual training on IT performance. IF was significantly more sensitive than IT (95.4% and 84.6%, respectively; 95%CI difference between sensitivities: 1.1%-18.9%) in monoclonal immunoglobulin detection, but not between specificities (85.2% and 77.8%, respectively; 95%CI difference between specificities: -9.7%-24.2%). The two methods' isotyping agreement was 69.4%. IT performed sub-optimal to IF in samples with monoclonal immunoglobulin ≤3.0g/L, monoclonal IgM, monoclonal light chain, monoclonal immunoglobulin migrating outside the gamma region, and multiple monoclonal bands, particularly if the additional band(s) exhibited the first and fourth features mentioned earlier. Both methods encountered difficulties with correct interpretation of oligoclonal bands. Continuous training significantly improved IT sensitivity ($p<0.05$), but not specificity ($p=0.46$). IF is needed as a supplementary test to IT in challenging instances to enhance monoclonal Ig detection and isotyping. IT sensitivity may improve with further training and expertise.

Keywords: *immunofixation; immunosubtraction; immunotyping; monoclonal gammopathy and monoclonal immunoglobulin*

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Detection of creatine and guanidinoacetate: procedure transfer from HPLC-MS/MS to UPLC-MS/MS

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Abstract

The diagnostic development for the detection of creatine (Cr) and guanidinoacetate (GAA) was started at the Institute for Medical Research in 2017 using Tandem Mass Spectrometry, Micromass Quattro Macro, combined with Waters 2795 Alliance HPLC (HPLC-MS/MS). Due to aging instrumentation, we aim to optimize MS/MS conditions and validate the performance using the Xevo-TQ-S micro–triple quadropole Mass Spectrometry-Acquity UPLC Class (UPLC-MS/MS). Infusion solutions were prepared by mixing a standard solution containing 1000 µmol/L (Cr), 250 µmol/L (GAA), 350 µmol/L internal standard Cr (d3-Cr), and 200 µmol/L internal standard GAA (d2-GAA). Twenty-five microliters of urine and plasma samples were extracted using methanol and acetonitrile and derivatized with mixture of acetyl chloride and butanol. The volume injection is reduced to 10 µL, and solvent consumption is diminished. Resolution is improved in the UPLC-chromatogram; peaks are narrower and have a much shorter run time of 0.18 minutes (Cr and d3-Cr) and 0.19 minutes (GAA and d2-GAA). Peak intensity has improved two times. The validation method was improved for precision studies (less than 5%), detection and quantitation limits were reduced, and accuracy by bias and recovery were found to be satisfactory and almost the same. A method comparison was performed with the patient's samples, showing similar results between the HPLC-MS/MS and UPLC-MS/MS. We successfully optimized and validated the UPLC–MS/MS method that gives better performance. Moreover, it allows us to reduce the required amount of sample and obtain a higher sensitivity with a much shorter run-time.

Keywords*: Creatine; guanidinoacetate; HPLC-MS/MS; UPLC-MS/MS and method development*

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A comparison study between bilirubinometer Olidef AG and Abbott Architect c8000

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Abstract

In Malaysia, newborns with jaundice are primarily monitored in public and private primary care clinics to facilitate timely treatment and prevent severe neonatal hyperbilirubinemia. Numerous point-of-care testing (POCT) devices are available in the market for determining bilirubin levels, either transcutaneously or using a capillary sample. This study aims to compare the results of the Olidef AG POCT device (photometric principle) with the Abbott Architect c8000 (diazonium salt reaction) and to evaluate the bias at medical decision limits. Forty patients' serum samples were collected and categorized based on the medical decision limits (less than 85 µmol/L, 85-290 µmol/L, and more than 290 µmol/L). The samples were measured using both Olidef AG and Abbott Architect c8000. Regression analysis was conducted, and bias was determined using the linear regression equation. Pearson correlation analysis revealed a strong correlation between the results from both analyzers (r=0.998) with an R-squared value of 0.997. Total error (TE) reveal satisfactory performance, with TE were below the defined optimal total allowable error. Olidef AG POCT device demonstrated comparable results in measuring total serum bilirubin at medical decision levels. However, further evaluation with capillary samples is warranted, as these are the primary samples used in primary care clinics.

Keywords: *Bilirubinometer; bilirubin measurement; neonatal hyperbilirubinemia; neonatal jaundice; POCT device comparison study*

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Factitiously low total creatine kinase activity in severe rhabdomyolysis: A case report

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Abstract

Factitiously low total CK activity can occur in severe rhabdomyolysis, potentially causing misdiagnosis and inappropriate patient management. To emphasize the importance of laboratory vigilance in reporting total CK activity in rhabdomyolysis. A 60-year-old female with underlying diabetes mellitus, was diagnosed with septic shock secondary to a nosocomial infection suspected of rhabdomyolysis. However, the total CK activity was unexpectedly low (<7 U/L N: 26-192 U/L). Post-dilution procedures showed markedly elevated total CK activity (>22,000 U/L (1:11)). Other laboratory investigations showed features of acute kidney injury and liver impairment. Unfortunately, the patient succumbed despite optimized medical treatment due to multi-organ failures. Measurement of CK is considered as a diagnostic test for rhabdomyolysis. Most of the laboratory nowadays measures total CK activity using enzymatic coupled with spectrophotometry method. However, substrate depletion can occur in severe rhabdomyolysis in which creatine phosphate get consumed by a very high concentration of CK in sample before the kinetic measurement is initiated, leading to factitiously low total CK activity. Sample dilution can be done to obtain the accurate total CK activity, thus avoiding error in result reporting. Factitiously low total CK activity can occur in severe rhabdomyolysis due to analytical interference. Clinical correlation is paramount in interpreting laboratory results to safeguard patient management.

Keywords: *Analytical interference; clinical chemistry; creatine kinase and laboratory medicine*

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Unveiling the genomic signatures of periodontitis: A bibliometric analysis of emerging biomarkers

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Abstract

Periodontitis, a widespread inflammatory illness that affects tooth-supporting tissues, has long been the focus of intense research because of its complicated aetiology and major public health implications. The VOSviewer tool was used to locate research hotspots, assess the intellectual structure of genetic biomarkers in periodontitis, and identify trends, key articles, and future directions. The study analyzed 983 publications from 57,363 retrieved papers on periodontitis genomic from 2000-2023, revealing a significant increase since 2008, using Microsoft Excel and VOSviewer for data visualization. We discover worldwide, researchers from India ranked first in productivity with 191 published documents, followed by United State (170), China (95), United Kingdom (78) and Brazil (66). Sorsa Timo A. from Sweden is the author with the most contributions. The research hotspots of this field, according to keyword analysis, are mainly concentrated on dentistry, medicine, biochemistry, genetics, molecular biology, immunology and microbiology. The Journal of Clinical Periodontology published the highest number of articles (41) that received the highest citations. "Periodontitis", followed by "biomarkers" and "genomic", were the most common keywords used by the authors in the paper related to uncover emerging themes and relevant genetic biomarkers. The Karolinska Institutet, Sweden was the most prominent with 55 articles, followed by University of Helsingin, Yliopisto Finland (45 articles), Helsinki University Hospital (45 articles), Universidade de São Paulo, Brazil (22 articles), and University of Michigan, Ann Arbor, United States (20 articles). Our study reveals genomic indicators like immune response gene changes, inflammation, and microbial colonization, and explores historical research patterns, offering insights for future biomarker discoveries and personalized therapeutic methods. This bibliometric study not only reveals the major genetic traits implicated in periodontitis, but it also provides a valuable resource for scholars traversing the vast landscape of periodontal research.

Keywords: *Bibliometric analysis; biomarkers; periodontitis and VOSviewer*

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Effects of aged tualang honey containing 5-hydroxymethylfurfural on fertility and teratogenicity in female Sprague Dawley rats

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Abstract

Aged tualang honey (ATH) is popular in Malaysia, especially among the older populace, for its perceived nutritional benefits. However, safety concerns arise due to the elevated levels of 5-hydroxymethylfurfural (HMF) present in ATH. This study evaluated the effects of ATH containing HMF on fertility, progression of pregnancy, and embryo-foetal development in 32 female Sprague-Dawley rats. Female rats were orally administered with i) distilled water (control); ii) ATH (4-year-old storage) (200 mg/kg); iii) purified HMF (154.9 mg/kg) corresponding to actual HMF level in ATH; or iv) combination of fresh honey (FTH) (200 mg/kg) + purified HMF (154.9 mg/kg). Treatment commenced from the pre-mating period and continued until day 19 of pregnancy. Various reproductive parameters were assessed throughout the study until the day of necropsy (day 20th of pregnancy). Results indicated no significant differences in general health, behaviour, and maternal body weights across the groups. The oestrous cycle length remained statistically unaffected, despite a few rats showing irregular cycles. Reproductive parameters, including mating and pregnancy indices, numbers of corpora lutea and implantation sites, pre- and post-implantation losses, and reproductive organ weights, showed no significant changes. Foetal outcomes such as the number of live or dead foetuses, sex ratio, and body weight were also statistically unaffected by the treatment. Importantly, no signs of teratogenicity were observed in the foetuses. These findings suggest that ATH containing HMF did not exhibit significant toxicity on fertility or teratogenicity but slightly impacted the oestrous cycle and caused low foetal weight.

Keywords*: Aged tualang honey; 5-hydroxymethylfurfural; fertility; Sprague-Dawley rats and teratogenicity*

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Addressing reagent inter-lot variation in amphetamine-type stimulants testing

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Abstract

Amphetamine Type Stimulants (ATS) are synthetic drugs listed in the Dangerous Drugs Act 1952. Screening for ATS is typically done via immunoassay with a 1000 ng/ml cut-off. In order to maintain accuracy, Drug Lab Hospital Kuala Lumpur regularly verifies new reagent lots to ensure consistent results. In the lot-to-lot study, five urine samples at 750 ng/ml and two at 1250 ng/ml were tested with both the current and candidate reagents on the Cobas 6000 analyzer. After detecting discrepancies, 40 urine samples ranging from 200 ng/ml to 8000 ng/ml were further tested, and a linear plot was created. The bias exceeded 20% of the Analytical Performance Specification (APS), triggering rejection. The linear plot showed an R^2 value of 0.9517, and at the 1000 ng/ml cut- off, the bias was 21.02%. Results were nearly 20% lower with the candidate reagent, prompting a detailed review. Consequently, the cut-off for the candidate reagent was adjusted to 800 ng/ml for accuracy. A new cut-off for ATS screening was established due to differences between the current and candidate reagents. Reagent lot verification is essential in drug abuse testing to avoid false positives or negatives. The lot differences were also evidenced in Noklus template as long-term monitoring of new reagent lot change. Setting cut-off values for the in-use reagent is crucial, as they may need adjustment if variations are detected.

Keywords*: Amphetamine Type Stimulants (ATS); immunoassa and noklus*

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A case report: Clinical and biochemically diagnosed citrullinemia type 1

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Abstract

Citrullinemia type 1 is an inherited disorder that often presents as a hyperammonemic encephalopathy in the neonatal period, or it can also develop in the late-infantile period and in adults. It is caused by a deficiency of the enzyme argininosuccinate synthase, which converts citrulline and aspartate into argininosuccinate. Complete defects in this enzyme result in markedly elevated levels of citrulline, whereas partial defects result in milder clinical phenotypes. A 17-month-old boy (previously born prematurely at 32 weeks, from a consanguineous marriage), was referred to UMMC for hyperammonemia and lethargy. He had multiple unidentified metabolic crises preceded by various episodes of viral infection since the age of two months. As a consequence, he developed severe hepatitis and coagulopathy. Laboratory investigations showed severe coagulopathy (INR 5-10) with deranged aminotransferases (AST 5675 U/L, ALT 2819 U/L) and hyperammoniemia (268 umol/L). Plasma citrulline was markedly elevated (1786 umol/L) with presence of orotic acid and uracil in the urine organic acid analysis. Provisional diagnosis of citrullinemia type 1 was made. Multigene panel testing revealed a carrier for heterozygous ARSA pathogenic variant (maternal inherited), which is associated with autosomal recessive metachromatic leukodystrophy. Whole genome sequencing revealed ASS1 homozygous variant - Variant of Uncertain Significance (VUS). This patient is likely to have Citrullinemia type 1 based on the clinical presentation and biochemical profile even though unable to confirm molecularly. Patient had responded well to protein restriction and oral ammonia scavenger, with normalization of subsequence aminotransferases and serum ammonia.

Keywords: *Argininosuccinate; ASS1 gene; citrullinemia; hyperammonemia and inherited*

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The effect of lysosomal dysfunction on proteomic expression in human brain endothelial cells (HBEC-5i)

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Abstract

Lysosomes are acidic organelles essential for cellular homeostasis, degrading cellular components such as nucleic acids, lipids, and proteins. Lysosomal dysfunction can result in the accumulation of undegraded proteins, disrupting cellular functions and contributing to diseases such as Alzheimer's. Despite the vascular system's involvement in Alzheimer's etiology, the proteomic changes linked to lysosomal dysfunction are not well understood. This study investigated the effect of lysosomal dysfunction on the proteomic expression of human brain endothelial cells (HBEC-5i) using the lysosome inhibitor chloroquine. Proteomic changes were analyzed at chloroquine concentrations of IC_{10} (17.5 µM), IC_{25} (70.5 µM), and IC_{50} (125 µM) using LCMS-MS and Perseus bioinformatics software. The DAVID bioinformatics tool identified biological and molecular processes, while STRING software determined cell protein-protein interactions. The findings of the study show more significant changes in protein expression with increasing concentration, and the main proteins identified as involved in lysosomal dysfunction are Y-box binding protein 1 (YBX1), Sequestosome-1 (SQSTM1), Cathepsin B (CTSB), and Cathepsin D (CTSD). Protein-protein interactions (PPIs) between SQSTM1, CTSB, and CTSD were identified at IC_{25} and IC_{50} concentrations. Cellular responses to chloroquine include disruptions in cellular components such as the cytoplasm and extracellular exosomes, changes in molecular functions such as RNA binding, and activation of apoptosis-related and translational processes. Additionally, proteins associated with Alzheimer's, such as Cellular tumor antigen p53 (TP53), CTSB, SQSTM1, YBX1, and Kinesin heavy chain-1 (KIF5B), showed altered expression. These findings provide insight into the molecular mechanisms of lysosomal dysfunction and its role in disease progression, highlighting potential therapeutic targets.

Keywords*: Alzheimer's; chloroquine; endothelial cells; lysosome and protein*

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Morphological features evaluation using differential image pre-processing methods for classification of mice myeloid colony forming unit

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Abstract

Hematopoietic stem/progenitor cells (HSPCs) are multipotent stem cells crucial in maintaining the hematopoiesis and are increasingly used in medical treatments. Colony-forming unit (CFU) assay is an in vitro method assessing HSPCs proliferation and differentiation potency through microscopic morphological observation of formed CFUs. But the assay is time-consuming, requires trained labour and prone to produce errors in data analysis. Automated bioimage methods can overcome these limitations but requires input of numerous, high-quality qualified images. This study aims to develop an automated bioimage system through morphological feature characterization for classification of myeloid progenitors comprise of three CFUs (CFU-GM, CFU-G, CFU-M). CFUs images obtained without a pre-processing method and with differential pre-processing methods (imadjust, adapthisteq, histeq) were used for morphological feature extraction and enhancement of qualified images. Results showed that the imadjust method yielded the highest percentage of qualified images (45-65%), although not significantly different from unprocessed images. Analysis at 4x magnification shows that images without pre-processing, and those with imadjust and histeq, recorded eight out of eleven significant features distinguishing the three CFUs, while adapthisteq recorded six. At 10x magnification, five out of eleven features can distinguish all myeloid CFU (without pre-processing, imadjust), whereas histeq and adapthisteq methods can only distinguish one to two types of CFU. In conclusion, morphological features have the potential for development of myeloid CFU classification system. However, the three pre-processing methods in this study did not significantly improve the quality of images for morphological characterization compared to those without the pre-processing.

Keywords*: Machine learning; pre-processing methods; morphological features; hematopoietic stem/progenitor cell and colony-forming unit*

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EVNOL SUPRABIO™ attenuates prostate gland damaged in Sprague Dawley rats induced by bisphenol F

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Abstract

Bisphenol F (BPF) is an endocrine-disrupting chemical known to disturb the male reproductive hormones, leading to Benign Prostatic Hyperplasia (BPH). EVNol SupraBio™,a palm oil-derived vitamin E with enhanced oral adsorption, has shown potential in protecting male reproductive system. However, its specific impact on prostate gland damage remains unstudied. This research aimed to assess the protective effect of EVNol SupraBio™ on BPF-induced prostate gland damage. 40 male Sprague Dawley rats were randomly divided into five groups: control (1 mg/kg corn oil), EV (100 mg/kg EVNol SuproBio™), BPF (10 mg/kg), BE50 (50 mg/kg EV and 10 mg/kg BPF), and BE100 (100 mg/kg EV and 10 mg/kg BPF). EV was administered 30 minutes before BPF for 35 days. Results showed that testosterone levels in the BE50 and BE100 groups were significantly higher than in the BPF group ($p<0.05$). The BE100 group had significantly lower estradiol levels compared to the BPF group ($p<0.05$), while no significant differences were observed in LH levels. Prostate tissue thickness and expression of Estrogen Receptor α (ERα) significantly ($p<0.05$) decrease in BE50 and BE100 compared to the BPF group. For ERβ, BE50 decrease significantly compared to BPF group ($p<0.05$). EVNol SupraBio™ potentially regulates testosterone production and reduce estrogen synthesis. Additionally, EVNol Suprabio™ also potentially regulates the ERα and ERβ thereby reducing epithelial cell proliferation in the prostate of Sprague-Dawley rats induced by BPF. These findings suggest that EVNol SupraBio™ could mitigate the adverse effects of BPF on the prostate gland.

Keywords: *Bisphenol F; benign prostatic hyperplasia; vitamin E; male reproductive hormones and estrogen receptor*

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Benzodiazepine analysis by an LC-MS/MS at Drug & Toxicology Laboratory Hospital Kuala Lumpur illustrates usage patterns of suspected drug users in Malaysia

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Abstract

Benzodiazepines are commonly prescribed, particularly for anxiety and insomnia. However, chronic use can lead to addiction. In 2023, 5009 medicolegal urine samples were analysed for the presence of benzodiazepines. There are 18 different benzodiazepines and metabolites that can be detected by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in the Drug & Toxicology Laboratory. Notably, only nimetazepam and flunitrazepam are listed in Malaysia's Dangerous Drugs Act (DDA) 1952. Nimetazepam is more typically found in illicit Erimin-5 tablets compared to flunitrazepam, yet there is a growing trend of using other benzodiazepines that are not controlled under the DDA 1952. We assessed the prevalence of individual benzodiazepines in urine samples from suspected drug users by using LC-MS/MS. A concentration of 100ng/mL was used as the cut-off for positive reporting of benzodiazepines. Out of the samples analysed, 1584 tested positive for benzodiazepines. The most frequently detected were nimetazepam metabolites, 7-aminonimetazepam (68%) and 7-aminonitrazepam (51%). Other benzodiazepines identified included nitrazepam (5%), oxazepam (5%), temazepam (3%), nordiazepam (2%), and 7-aminoclonazepam (2%). Only 24 samples were positive for nimetazepam, and none for flunitrazepam. In conclusion, the trend of the most common benzodiazepines that can be detected in suspected drug users' urine samples has been successfully identified. As there is still a shortage of data and information regarding benzodiazepine products in the market, ranging from licit products to illicit drugs, this information on other benzodiazepines and metabolites found, could be proposed to be regulated as it is not yet enlisted in the DDA 1952.

Keywords: *LC-MS/MS; benzodiazepines; Dangerous Drugs Act 1952; drug of abuse and Erimin-5*

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Evaluation of cytotoxic effect of hydroquinone on human brain endothelial cells, HBEC-5i

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Abstract

Hydroquinone (HQ) is a major benzene metabolite that can be closely found in daily life such as in skin-lightening agents, tar in cigarette smoke, oil refinery factory, and petroleum industry. It is associated with adverse effects to the individual who works in the oil and gas industry. Overexposure to HQ has been proven to induce the NF- κB pathway causing inflammation reaction. Recently, there has been a rising concern about the association of HQ with disruption of blood-brain barrier (BBB) permeability due to inflammation which can lead to cerebral oedema and sepsis-related encephalopathy. However, the effect of HQ on human brain endothelial cells (HBEC) remains elusive. To address the effect of HQ in inducing cytotoxicity, a study on HQ targeting HBEC-5i was conducted. HBEC-5i was cultured in Dulbecco's Modified Eagle Medium/Nutrient F-12 Ham media with 10% foetal bovine serum, and 1% Penicillin-Streptomycin in 37°C, 5% CO2. HBEC-5i were exposed to HQ and lipopolysaccharide for 18H in triplicate. Cytotoxicity assay was conducted with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to determine the inhibitory concentration (IC) at IC_{50} for HQ. Lipopolysaccharide (LPS) was used as positive control. The IC_{50} for HQ was 120 µM meanwhile LPS was recorded at 0.1 µM. The morphological changes observed identified cell shrinkage and loss of cell volume that was prominent at IC_{50} in HQ that also present in the positive control group which may indicate apoptosis. This study showed the cytotoxic effect of HQ on HBECs could lead to cell death potentially can impair BBB function.

***Keywords**: Apoptosis; benzene; blood vessel; blood brain barrier and inflammation*

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The effects of S-allyl cysteine (Sac) on vascular reactivity in ovariectomised rats

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Abstract

Cardiovascular diseases (CVDs) are a major global health issue, particularly among menopausal women who experience hormonal fluctuations and increased oxidative stress. Estrogen, a key hormone in females, provides cardioprotection by upregulating cystathionine γ-lyase (CSE) expression and enhancing hydrogen sulfide (H_2S) production. S-allyl cysteine (SAC), found abundantly in aged garlic extract, has been shown to protect against vascular dysfunction through antioxidative mechanisms. Thus, this study aims to investigate the vasodilatory effects of SAC in ovariectomized rats (OVX), a model for estrogen deficiency in menopausal women. Female Wistar rats (170-220 g, N=32) underwent either ovariectomy or sham operations and were given a 21-day recovery period before being sacrificed. The rats' aortic rings were isolated and suspended in a tissue bath and placed between 2 tungsten wires, connected to an isometric force transducer. Pre-constriction with phenylephrine (PE, 10^{-6} M) was followed by SAC incubation and a cumulative dose-response curve using acetylcholine (ACh, 10^{-9} M to 10^{-4} M). Statistical analysis was performed using one-way and two-way ANOVA with significance set at $p<0.05$. Results indicated a significant increase in aortic relaxation after SAC incubation. SAC also led to a higher percentage of KCl-induced contraction, suggesting activation of potassium ion channels in the endothelium. This was supported by the observation that DL-propargylglycine (PAG), a CSE inhibitor, blocked SAC's vasodilatory effect. The study concludes that SAC modulates vascular function and blood pressure regulation, likely through the CSE/H_2S signaling pathway. These findings highlight SAC can be a natural supplementation that aids in cardioprotection for menopausal women.

***Keywords**: S-allyl cysteine; ovariectomized; hydrogen sulfide and CSE/H_2S signaling pathway*

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Trimester-specific reference intervals for thyroid function tests in pregnant Malaysian women

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Abstract

Thyroid dysfunction during pregnancy can adversely affect both maternal health and fetal development. International guidelines recommend using population-based trimester- and assay-specific reference interval (RI) for thyroid testing in pregnancy. This study aimed to establish a trimester-specific RI for thyroid function tests (TFT) in Malaysian pregnant women. Serum free thyroxine (fT4) and thyroid stimulating hormone (TSH) levels were measured in 1331 pregnant women receiving antenatal care in a large tertiary hospital in Selangor. Patients with medical conditions and positive thyroid auto-antibodies were excluded. fT4 and TSH were measured using the immunoassay analyzer, Siemens Atellica. Non-parametric analysis (2.5-97.5th percentile) was performed to determine the trimester-specific RI after removal of outliers. The TSH RI were 0.049-2.746, 0.148-3.118 and 0.181-2.955 mIU/L for the first, second and third trimester, respectively. Meanwhile, the fT4 reference intervals were 11.47-18.93, 10.22-17.18 and 9.20-16.35 pmol/L for the first, second and third trimester, respectively. From the first trimester to the third trimester, the median TSH levels showed an increasing trend, while the median fT4 levels showed a decreasing trend with each trimester. Establishing population-based trimester-specific RI is essential for accurate assessment of thyroid function in pregnant women. The RI established in our study will aid clinical decision making in the diagnosis and management of thyroid dysfunction in pregnancy in the local population.

Keywords: *Malaysian; pregnancy; reference interval; thyroid and trimester*

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The effects of surfactants in increasing solubility of hexane extract *Swietenia macrophylla*

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Abstract

Swietenia macrophylla, commonly known as mahogany, is a tropical and subtropical plant valued for its high-quality wood and medicinal properties. Its seeds are rich in bioactive compounds with anti-inflammatory, antimutagenic, and antitumor effects. However, the hexane extract from this plant is poorly soluble in water, necessitating a solvent to enhance its solubility and allow non-polar components to impact biological activity evaluations effectively. This study aimed to identify an effective surfactant to improve the solubility of *S. macrophylla* hexane extract. Two surfactants, sodium dodecyl sulfate (SDS) and Tween 20, were tested for solubility enhancement. Following solubility tests, MTT assays were performed using HepG2 cells to determine the extract's cytotoxicity in various solvents, including distilled water, dimethyl sulfoxide (DMSO), and 0.5% SDS, with a 24-hour incubation period. The solubility tests revealed that 0.5% SDS effectively dissolved the hexane extract. MTT assay results showed the lowest IC_{50} value, 14 µg/mL, for the hexane extract in 0.5% SDS, indicating strong cytotoxicity against HepG2 cells. In contrast, the IC_{50} value for the extract in DMSO was significantly higher at 146 µg/mL, and no detectable IC_{50} value was observed in distilled water. In conclusion, SDS proves to be a suitable surfactant for dissolving the hexane extract of *S. macrophylla*, enabling it to exhibit a potent cytotoxic effect on HepG2 cells.

Keywords: *Cytotoxicity; natural product, SDS; solubility and Tween 20*

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The effect of *Hibiscus sabdariffa* aqueous extract on biological markers of bone formation and reabsorption in the ovariectomised osteoporosis rat model

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Abstract

Women who have menopause will experience a decrease in estrogen levels often associated with osteoporosis problems. Hibiscus sabdariffa extract (HSE) has proven to be a powerful anti-inflammatory agent in a variety of models, but studies on its effects on post-menopausal osteoporosis problems are still limited. The aim of this study was to study the effects of HSE on the biological markers of formation and reabsorption as well as the strength of the femur bone in a model of osteoporosis mice with ovariectomy. A total of 32 female Sprague-Dawley mice with an estimated weight between 250-300g were randomly divided into four groups (n=8): (1) the SHAM group, (2) the ovariectomy control group, (3) the ovariectomy-HSE group (OVX-HSA) and (4) the ovariectomy-ZLD group. (OVX-ZLD). All mice underwent a bilateral ovariectomy (OVX) procedure under KTX anaesthesia. (im). For the SHAM group, the same surgical procedure was performed without removing the rat's ovaries. The mice were left for six weeks for a period of recovery from the ovariectomy surgical process and to form a menopausal model with osteoporosis. For the SHAM and OVX groups, the solution was given orally daily for four weeks. For the OVX-HSE group, HSE (100mg/kg) was given orally daily for four weeks while for the OVX-ZLD group, ZLD (0.1 mg/kg), was given (ip) twice a week. After four weeks, blood plasma and femur bone were taken to measure bone biomarker osteocalcin for bone formation and CTX-1 for bone resorption while testing bone strength for three things: pressure, tension, and maximum load. The results of the study showed no significant changes ($p>0.05$) in both bone biological markers. However, there is a trend that can be seen in the OVX-HSE and OVX-ZLD groups where the study results showed a slight increase in osteocalcin and a decrease in CTX-1 compared to the other groups. Mechanical strength also shows no significant difference ($p>0.05$). However, a trend can be seen where the OVX-HSE group and the OWX-ZLD group show improvements in all three parameters tested including the Young's modulus value. In conclusion, HSE may have an early effect on bone biomarker and bone strength, although insignificant in this study. However, further research is needed to obtain a more accurate assessment of the potential of this HSE therapy for bones.

Keywords: *Hibiscus sabdariffa; menopausal osteoporosis; bone marker and bone strength*

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Beyond borders: Challenges in diagnosing extrapulmonary TB

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Abstract

Tuberculosis remains a significant public health challenge globally, particularly in regions with high prevalence like Malaysia. Diagnosing tuberculosis is often complicated by atypical presentations and difficulties in obtaining definitive diagnostic samples. We present a case of extrapulmonary tuberculosis (EPTB) manifestations, highlighting diagnostic challenges. A 25-year-old foreign worker presented with a productive cough, fever, pleuritic chest pain, progressive dyspnoea for one month and had a history of tuberculosis sick contact. Physical examination showed reduced air entry over the left lower lung. Radiology revealed left lower zone consolidation with a blunted costophrenic angle. He was treated for left hydropneumothorax secondary to lung empyema and investigated for tuberculosis. Initial microbiological investigations for sputum were negative for tuberculosis. Multiple ultrasound-guided pleural taps were attempted before pleural fluid was successfully drained for further analysis. The pleural fluid analysis showed exudative effusion with high adenosine deaminase (ADA) levels, leading to empiric anti-TB. Pleural fluid ADA is useful in diagnosing tuberculous pleural effusion (TPE) at cutoff values of 29.6 U/L which gives 97.6% sensitivity and 90.4% specificity. In this case, confirmation of the diagnosis of TPE through a positive pleural fluid Mycobacterium tuberculosis culture required eight weeks. Moreover, the difficulty in obtaining the sample also had delayed the result of high pleural fluid ADA levels to diagnose TPE. Serum ADA is another potential alternative marker with cutoff of >21.2 IU/L, poses 94% sensitivity and 83% specificity to diagnose EPTB. Pleural fluid or serum ADA, alongside clinical evaluation, could expedite EPTB diagnosis and treatment, especially in tuberculosis endemic regions.

***Keywords**: Adenosine Deaminase (ADA); extrapulmonary tuberculosis (EPTB) and tuberculous pleural effusions (TPE)*

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Assessment of acute toxicity and cartilage development in zebrafish embryos and larvae exposed to disodium phenytoin

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Abstract

Phenytoin is an anti-seizure drug prescribed orally to treat epilepsy. Studies have shown, however, that phenytoin exerts adverse effects on cartilage development. Here, we introduce a new compound, disodium phenytoin (5,5-diphenylhydantoin disodium), a water-soluble salt synthesized from the reaction of phenytoin (5,5-diphenylhydantoin) and sodium hydroxide. Disodium phenytoin has a lower melting point and better solubility in water compared to phenytoin and could potentially be used therapeutically. Biosafety of disodium phenytoin was screened in zebrafish embryos by performing Fish Embryo Toxicity (FET) test adapted from the OECD Guidelines Test no. 236, while cartilage development was assessed by whole mount Alcian blue staining at 5 days post fertilization (dpf). Zebrafish embryos in 24-well plates (20 embryos per well) were exposed to disodium phenytoin solution from 4 hours post fertilization for 96 hours and monitored every 24 hours. At the end of the exposure period, LC50 (50% lethal concentration) was undetermined because the highest concentration of disodium phenytoin used did not result in 100% mortality. Additionally the highest concentrations yielded crystallized solids in solution. We concluded that disodium phenytoin within the tested range was not lethal in zebrafish embryos and that lower concentrations should be used to avoid crystallization. Subsequently, 3 – 5 dpf larvae were exposed to disodium phenytoin at 50µM and 75µM to ascertain the effect of exposure on craniofacial cartilage development. Disodium phenytoin did not visibly cause cartilage impairment in either concentration of disodium phenytoin, exhibiting no difference to the controls that were exposed to distilled water alone.

Keywords*: Acute toxicity; cartilage development; disodium phenytoin; phenytoin and zebrafish*

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Elucidating the effects of *Hibiscus sabdariffa* aqueous extract on oxidative stress of gingival tissue in induced periodontitis of ovariectomised rats

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Abstract

Periodontitis increases the production of free radicals, resulting in oxidative stress. This condition leads to chronic inflammation and gingival tissue damage. Natural plant extracts have been reported to provide significant benefits in various diseases due to their antioxidant properties. The aim of this study was to examine the effects of an aqueous extract of roselle (*Hibiscus sabdariffa*, HSE) on oxidative stress in the gingival tissue of ovariectomized rats with experimental periodontitis. Thirty-two Sprague Dawley rats (200g–350g) were randomly divided into four groups (n=8): a control group (SHAM), ovariectomy + periodontitis group (OVX+EP), ovariectomy + periodontitis with zoledronate treatment group (OVX+EP+ZLD) and ovariectomy +periodontitis with roselle treatment group (OVX+EP+HSE). ZLD (0.1 mg/kg) and HSE (100 mg/kg) were administered for a 28-day treatment period, while the SHAM and OV+EP groups were left untreated. Biochemical analysis, including levels of oxidative damage markers and antioxidants was conducted. The results showed a significant decrease ($p<0.05$) in the oxidative damage marker for protein carbonyl in the OVX+EP+HSE group compared to the OVX + EP group. There was a low level of malondialdehyde (MDA) in OVX+EP+HSE compared to OV+EP, but no significant difference observed. Meanwhile, there was a trend of improvement in antioxidant status for superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) However no significant difference observed between the OVX+EP+HSE and the OVX+EP group. Therefore, these findings suggest that HSE treatment has the potential to reduce oxidative stress in periodontitis conditions.

Keywords: *Periodontitis; oxidative stress; ovariectomized rats and roselle*

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Comparison of urine sample analyze on Hydragel HR and Hydragel Protein (E)

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Abstract

Protein electrophoresis is a well-established technique routinely used in clinical laboratories for screening of serum and other biological fluids for protein abnormalities. In HKL, protein electrophoresis analysis for serum and urine samples has long been performed using Hydragel 30 Protein E. It is more advisable to use high resolution techniques which is Hydragel HR when working with urine samples. To analyze urine protein electrophoresis, evaluation was conducted because of migration from Hydragel Protein (E) to Hydragel HR. The assessment is also essential to meet MS ISO 15189 accreditation requirements. Our objectives are to evaluate the urine samples analyzed on Hydragel HR and Hydragel 30 Protein (E). In addition, we want to evaluate the correlation between two Sebia Hydrasys equipment available in our lab. Quantitative and qualitative results were reported. In a precision study of Hydragel HR, the results were within the manufacturer's claim. The urine protein electrophoresis on Hydragel HR correlates well between the two Sebia Hydrasys equipment. The results also correlate well between urine analysis using Hydragel HR and Hydragel 30 Protein (E). The Hydragel HR is more sensitive and requires an additional procedure before being used for analysis. Although the results are comparable between the two types of gels, the analysis was done qualitatively (positive and negative) in this study. Quantitative results (g/L) require further evaluation using semiquantitative results from densitometry scanning of gels. The urine can be compared and analysed using Hydragel HR and Hydragel 30 qualitatively and quantitatively.

Keywords: *Bence Jones protein; hydragel urine protein electrophoresis; monoclonal free light chains and multiple myeloma*

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Effects of oral pterostilbene administration towards the liver of UVB-induced skin photoaging BALB/c mouse

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Abstract

Ultraviolet B (UVB) radiation emitted by the sun causes detrimental effects on humans by inducing oxidative stress. Pterostilbene has shown its effectiveness as a photoprotective agent in both *in vitro* and *in vivo* studies on the skin. However, only a few studies have examined the photoprotective effects of pterostilbene against UVB effects on non-skin tissues. This research aimed to investigate the effects of oral pterostilbene towards the liver of mice in a UVB-induced skin photoaging BALB/c mouse model. BALB/c mice were divided into four groups: negative control (UVB (-)), vehicle control (UVB (+)), low-dose treatment (30 mg/kg pterostilbene, PTE 30), and high-dose treatment (60 mg/kg pterostilbene, PTE 60), with each group consisting of 6 mice (n=6). The antioxidant status, indicated by the level of non-enzymatic antioxidant glutathione (GSH), the activity of enzymatic antioxidants, superoxide dismutase (SOD) and catalase (CAT), was assessed. Statistical analysis revealed no significant differences in GSH level, SOD activity, and CAT activity among the four mice groups ($p>0.05$). Despite the lack of statistically significant differences in antioxidant status between mice groups with or without pterostilbene treatment, the decreasing trend observed in the antioxidant parameters of the vehicle group suggests a possible systemic effect after intensive UVB exposure, whereas the increasing trend observed in the high-dose treatment group indicates the potential of oral pterostilbene as a complementary photoprotective agent against UVB-induced oxidative damage. Further investigations are warranted to better understand the underlying relationship and evaluate the systemic effects of the test compound.

Keywords*: Liver, oxidative stress; photoprotection; pterostilbene and ultraviolet B*

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Cytotoxic and genotoxic effects of FLDP-8 compound curcuminoid analogue on HCT 116 human colon cancer cell

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Abstract

Colorectal cancer (CRC) is a leading cause of cancer deaths globally. Conventional treatments like surgery and chemotherapy often cause long-term side effects, prompting research into natural products for safer anticancer agents. Therefore, research has focused on the analogue of Curcumin from Curcuma longa (turmeric), namely FLDP-8, against HCT 116 colon cancer cells. In this study, the cytotoxic effects of the compound were determined using the MTT assay, while cell death mode was assessed using flow cytometry techniques based on Annexin V-FITC staining and PI/RNase. Alkaline comet assay was performed to determine genotoxic effects. Based on the study results, the MTT assay recorded a significant difference ($p<0.05$) between the negative control and HCT 116 cells treated with FLDP-8 and positive controls, Curcumin, and Menadione. The IC50 values for each treatment were as follows: FLDP-8 compound (2.45±0.70) µg/mL, Curcumin (24±11.30 µg/mL), and Menadione (2.35±0.52 µg/mL). Flow cytometry analysis for cell death mode found that most treated cells underwent late apoptosis with significant differences compared to the negative control ($p<0.05$), namely 77.68 ± 0.78% (FLDP-8), 98.33 ± 0.17% (Curcumin). Alkaline comet assay showed significant tail intensity ($p<0.05$) in FLDP-8 and Curcumin compound treatments compared to the negative control and simple DNA damage scale (tail intensity 20-40%) at each treatment hour. In conclusion, compound FLDP-8, a Curcuminoid analogue has enhanced the anticancer effectiveness on HCT 116 cells through apoptosis and DNA damage.

***Keywords**: Colorectal cancer; curcumin analogues; cytotoxicity; apoptosis and genotoxicity*

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Implementation of outreach system to external facilities: Effect on turnaround time and laboratory errors

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Abstract

The Chemical Pathology Unit at Department of Pathology, Hospital Sultan Idris Shah, Serdang (HSIS) received a high volume of requests, mainly immunoassay tests from 10 external facilities. Despite Hospital Sultan Idris Shah, Serdang as a Total Hospital Information System (THIS), all requests from external facilities are sent via request form. Errors in transcription related to external samples, including incorrect patient identification, incorrect entry of test orders, and location entries, have been identified as contributing factors to delays in both laboratory Turnaround Time (LTAT) and external patient management. To evaluate the effectiveness of LIS Outreach System whereby the ordering test and accessing of reports can be performed in external facilities. The LIS Outreach System was implemented in the Chemical Pathology Unit in February 2024. The system was linked to all 10 Health Clinics and 1 Hospital under the Hospital Sultan Idris Shah, Serdang supervision. Access and passwords were given to all users. All patient's information and test requests were entered into the system on their site and samples were sent together with a sent list to the Chemical Pathology Unit, HSIS. Samples were acknowledged upon receipt in the system and will be analysed on an interfacing analyser. Validated results will be automatically transmitted into the system. Results and other reports such as rejection reports can be viewed on the system at their site. The LTAT data was collected retrospectively, with data from January 2024 collected as pre-implementation and data from March 2024 as post-implementation. Data was analysed starting from the time samples were received at the Receiving Counter until reporting into the system. The rejection data from the year 2023 and March 2024 was collected. The result showed an 82% improvement on LTAT and 95% reduction of rejection due to transcription errors. The implementation of LIS Outreach

Keywords: *LTAT; outreach system; laboratory errors; external facilities and interfacing*

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Measurement of curvature parameters in adult zebrafish using computed tomography

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Abstract

Scoliosis is a common orthopaedic condition in humans. Mild cases can impact the range of motion of the spinal joints, while severe cases can cause various peripheral nerve disorders. In rare cases, scoliosis can even lead to serious cardiopulmonary problems that endanger life. To explore the causes of scoliosis in humans, the zebrafish (*Danio rerio*) scoliosis model is widely accepted due to the mechanical force of the zebrafish's spine is very similar to humans. However, the model has yet to be fully characterised. Unlike humans, zebrafish vertebrae numbers differ between animals. Currently, no studies have examined the effect of vertebrae number in relation to scoliosis curvature in the zebrafish model. To establish the thoracic kyphotic curve (TKC), maximal kyphotic curve (MKC), spine length, and vertebrae number parameters, eight adult zebrafish with visible scoliosis was imaged from maxillary tip to the last vertebrae using a micro-CT. Images was analysed using DragonFly software. All scoliotic zebrafish demonstrated curvature in the precaudal, caudal, and caudal fin region of the zebrafish spine with a marked difference in TKC (22°-66°) and MKC (35°-66°). These zebrafish also had a higher number of vertebrae compared to the controls. In conclusion, the adult zebrafish scoliosis model provides measurable parameters that can be used in exploring scoliosis development and testing potential treatments.

Keywords: *Computed tomography; scoliosis; vertebrae and zebrafish*

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Time-kill assay of n-(2-bromoethyl)-7-chloroquinolin-4-amine (ACP4A) with linezolid and vancomycin antibiotics against MRSA ATCC 33591

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of gram-positive bacterium that becomes resistant towards multiple antibiotics leading to the failure of MRSA infection treatment. A new series of synthetic quinoline derivatives, N-(2-Bromoethyl)-7-Chloroquinolin-4-Amine (ACP4A) has proven to have a promising antibacterial activity against *Staphylococcus aureus* based on the previous study on the antibacterial activity against MRSA. Hence, this study aimed to evaluate the antibacterial activity of ACP4A and the Time-Kill Assay (TKA) of ACP4A against MRSA ATCC 33591. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using micro broth dilution and streak plate method. The TKA was determined using micro broth dilution throughout 24 hours of treatment to understand the interactions between microbial strains and antimicrobial agents. The MIC and MBC of ACP4A against MRSA ATCC 33591 are 25 µg/ml and 50 µg/ml respectively, showing bacteriostatic action of ACP4A. In the study, ACP4A showed greater antibacterial activity against MRSA compared to linezolid, that has a higher MIC value of 31.25 µg/ml with bacteriostatic action. It is also proven that vancomycin is the most effective antibacterial activity with the lowest MIC value of 1.95 µg/ml with bacteriostatic action. TKA analysis of ACP4A 1xMIC shows bacteriostatic action as there is decrease in number of colonies <3 *Log*10 CFU/ml. On the other hand, vancomycin shows bactericidal action as there is constant decrease in number of colonies ≥3 *Log*10 CFU/ml. The statistical differences for MIC and MBC between the groups were assessed by a parametric test which is chi-square while for TKA is mixed ANOVA. In conclusion, ACP4A has the potential to be developed as an alternative treatment against MRSA ATCC 33591 with bacteriostatic action and helps to slow the development of bacterial antibiotic resistance.

Keywords: *Methicillin-resistant Staphylococcus aureus; n-(2-bromoethyl)-7-chloroquinolin-4-amine; minimum inhibitory concentration; minimum bactericidal concentration and time-kill assay*

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The effect of aluminium exposure on the deterioration of memory function of rats through oxidative stress in the striatum

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Abstract

Aluminium (Al) exposure is linked to alterations in glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) levels. This study examined the impact of aluminium on MDA, SOD, and GSH levels in the striatum of rats, a region crucial for memory processing. Twenty rats were randomly divided into two groups and subjected to oral gavage with aluminium ($AlCl_3$) at 200 mg/kg/day: 14 days (T14) and 28 days (T28). The 2-Novel Object Recognition (2-NOR) test was used to assess the memory capacity of rats. The rats' striatum tissue was isolated and homogenized after termination to measure MDA, SOD, and GSH levels. Results indicated that MDA levels in T14 and T28 were significantly higher than IN controls (C14, C28, respectively) ($p<0.05$) and were higher at T14 than at T28 ($p<0.05$). SOD levels were lower in T14 than in C14 but higher in T28 than in C28 ($p<0.05$), with significant differences between T14 and T28 ($p<0.05$). GSH levels in T14 and T28 were significantly lower than in controls (C14, C28) ($p<0.05$). The 2-NOR test revealed that memory function in T14 and T28 was significantly lower than in controls (C14, C28) ($p<0.05$), with no significant difference observed between T14 and T28. These findings suggest that oxidative stress, resulting from an imbalance between antioxidants and oxidants, contributes to neurotoxicity and cognitive decline. In conclusion, 14 and 28 days of aluminium exposure led to the deterioration of memory function in rats, mediated by oxidative stress in the striatum, a key brain region involved in memory processing.

Keywords: *Neurodegenerations; memory impairment; oxidative stress; aluminium and striatum*

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A clinical audit on the appropriateness of thyroid antibodies test

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Abstract

Thyroid disorders are prevalent, impacting millions globally. An accurate assessment of thyroid function is vital for guiding treatment. This study aimed to improve thyroid antibodies test requests based on the Malaysian CPG on Management of Thyroid Disorders 2019 and other relevant guidelines. This retrospective audit from June 1 to June 30, 2023, in Chemical Pathology, Hospital Kuala Lumpur involved extracting data on thyroid antibodies [anti-thyroglobulin (anti-TG), anti-thyroid peroxidase (anti-TPO), and anti-thyroid stimulating hormone receptor (anti-TSHR)] and thyroglobulin from the electronic laboratory information system. Request forms were collected to determine test indications. A total of 2258 tests were requested. Among these, anti-TSHR had the highest count at 977, followed by anti-TG (507), thyroglobulin (397), and anti-TPO with 377 requests. The main reasons for ordering anti-TSHR were hyperthyroidism, suspected Graves' disease, and pregnancy-related hyperthyroidism. Anti-TPO tests were mostly requested for hyperthyroidism, hypothyroidism, and suspected Graves' disease. Anti-Tg and Tg are mainly ordered for thyroid cancer and hyperthyroidism. Anti-TPO was found to have a higher percentage of non-indicated tests (55.2%), followed by anti-TG at 29.4%, anti-TSHR at 10.5% and thyroglobulin at 10.3%. The high percentage of non-indicated anti-TPO tests may result from a lack of adherence to clinical guidelines or a misunderstanding of the test's appropriate use. Unnecessary testing can lead to increased healthcare costs and patient anxiety without providing additional clinical benefit. Adherence to guidelines is an effective intervention to reduce non-indicated testing and overutilization of laboratory costs.

Keywords*: Thyroid antibodies; thyroid disorder; anti-TSHR; anti-TPO; anti-Tg*

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Role of heat shock protein 70 in extracellular vesicles derived from colorectal cancer

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Abstract

Heat Shock Protein 70 (HSP-70) is a stress-responsive molecular chaperone found in tumor tissues including colorectal cancer (CRC), which is the third most common cancer worldwide. HSP-70 has been found in extracellular vesicles (EVs) and studies have shown that it is associated with CRC biomarkers that function in cell proliferation and migration. This study was performed to investigate the role of HSP-70 in EVs derived from CRC. In silico analysis confirmed the presence of HSP-70 expression in the tissues of CRC patients with higher levels indicating a decreased survival probability. High expression of HSP-70 can affect the pathologic stage ($p=0.01317$), neoplasm status of patients ($p=0.04062$), lymphatic invasion, ($p=0.04527$) and perineural invasion ($p=0.03531$) significantly. HSP-70 expression of CRC patient samples (mean ± standard error of the mean = 118.10 ± 29.34) was higher than EV samples of non-CRC patients (mean ± standard error of the mean = 71.71 ± 19.70). HT-29 confirmed to have high HSP-70 expression was selected as a cell line model and HSP-70 inhibitor treatment (VER-155008) was performed to confirm the decrease in HSP-70 expression. Two EVs groups namely VER-155008 treatment group (TVER EV) and untreated group (UT EV) were isolated and purified. Flow cytometry analysis confirmed that the decrease in HSP-70 expression for the VER group was significant ($p=0.0055$). The cells migration assay showed that the VER group significantly decreased wound closure percentage at 24 hours ($p=0.039$) and 48 hours ($p=0.016$). The CCK-8 proliferation test was conducted showing that the VER group could lower the percentage of the cells viability significantly ($p<0.0001$) compared to the UT group. In conclusion, the expression of HSP-70 in the patients' tissues can be correlated with survival probabilities. HSP-70 inhibition can significantly reduce the migration and proliferation ability of CRC cells. Therefore, this study suggests that the expression of HSP-70 in EVs can influence the activity and function of CRC cells.

Keywords*: Colorectal cancer; heat shock protein 70; extracellular vesicles; HT-29 and migration*

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Low electromagnetic field exposure as wound healing accelerator: A potential setback

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Abstract

Recent studies have shown that exposure to electromagnetic fields (EMF) may have a positive effect on the wound healing process. Therefore, this study was conducted to investigate the effectiveness and potential side effects of EMF exposures on the open wound healing process in rats. Four groups of 24 male Sprague Dawley rats (350 – 450g) were used: a negative control (normal saline), positive control (povidone iodin), treatment with EMF 0.8 mT and 1.2 mT strengths. Macroscopic observation revealed that EMF exposure-treated wounds at 0.8 mT and 1.2 mT strengths exhibited the quickest healing process, initiated as early as 24 hours after wound induction. Scab formation was slightly delayed on day-7 as compared to the negative control group. Wound contraction increased over days for the 0.8 mT EMF exposure-treated wounds (5.00 ± 0.51 %, 9.69 ± 1.07%, 25.00 ± 5.10%, 75.00 ± 4.05% and 96.87 ± 3.12%) and for 1.2 mT EMF-exposure treated wounds (5.63 ± 0.63%, 6.88 ± 0.63%, 31.25 ± 6.25%, 75.63 ± 1.88% and 93.75 ± 6.25%). While all results shown positive potentials as wound healing accelerator, our histological observations on lungs and kidneys tissues revealed cellular damages, leading to increased permeability of capillaries and cell walls with increased EMF strength and exposure duration. In conclusion, treatment using EMF exposure at 0.8 mT and 1.2 mT frequencies showed potential to accelerate early-stage wound contraction but also had adverse impact at the lung and kidney soft tissues; suggesting a thorough investigation is required in future studies.

Keywords*: Electromagnetic fields; wound healing; lung; kidney and histology*

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Texture features evaluation using differential image pre-processing methods for classification of mice myeloid colony forming unit

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Abstract

Hematopoietic stem/progenitor cells are vital in regulating the hematopoietic system due to their ability to differentiate into various types of mature blood cells. Colony Forming Unit (CFU) assay is an in-vitro method to identify and enumerate hematopoietic progenitor cells via formation of CFUs. However, this method is time-consuming, prone to produce errors and requires trained expertise. To overcome the limitations, this study aims to develop an automated bio-imaging system for CFUs analysis of which requires input with high numbers of qualified images. CFUs images of myeloid progenitors consisting of CFU-granulocyte (CFU-G), CFU-monocyte (CFU-M) and CFU-granulocyte-monocyte (CFU-GM) obtained through without pre-processing and with differential pre-processing methods (imadjust, adapthisteq, and histeq) were used to characterize 14 texture features and enhancement of qualified images. Pre-processing methods were carried out using MATLAB software version 2023. 14 texture features were extracted from CFU-G, CFU-M, and CFU-GM images at 4x and 10x microscope magnification using the Gray-Level Co-occurrence Matrix (GLCM). Almost all texture features showed significant differences ($p<0.05$) when compared at 2 levels of microscopic magnification. However, no significant differences ($p>0.05$) of texture features were noted when CFUs images were compared across groups of with and without pre-processing methods. Overall, the use of pre-processing methods does not improve the number and quality of the images for textural features analysis of myeloid CFUs. The use of images without pre-processing is sufficient for classification of three types of myeloid CFUs.

Keywords *Machine learning; pre-processing methods; texture features; hematopoietic stem/progenitor cell and myeloid colony-forming unit*

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Cytoprotective effect of *Clinacanthus nutans* on H202-induced oxidative stress on 4T1 cell

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Abstract

Triple negative breast cancer (TNBC) is an aggressive type of breast cancer with poor prognosis. Current treatments for TNBC are debatable in terms of improving patients' quality of life due to the lack of hormone receptors. *Clinachantus nutans* leaf extracts are popular in South Asia for treating various diseases, showing non-cytotoxic effects on normal cells and cytotoxic effects on cancer cells. This study aimed to determine the cytoprotective effects of *C. nutans* extract on 4T1 cells affected by oxidative stress induced by hydrogen peroxide (H2O2). The sulforhodamine B (SRB) assay determined the cytotoxic effects of *C. nutans* on 4T1 cells, showing an IC50 of 0.67 mg/ml. Cell viability percentages ranged from 102.23% at 0.063 mg/ml to 40.52% at 1.000 mg/ml. For H2O2, the IC50 was 0.063 mM. Significant decreases in cell viability were observed from 0.125 mM to 2.00 mM. Pre-treatment with *C. nutans* before 2 mM H2O2 exposure resulted in 75.27% cell viability, significantly higher than the ative control. Pre-treatment with NAC showed 59.53% cell viability. The results indicate that *C. nutans* extract has potential cytoprotective effects against oxidative stress in 4T1 cells. The significant difference in cell viability between *C. nutans* and NAC pre-treatments highlights the efficacy of *C. nutans. C. nutans* extract demonstrates potential cytoprotective effects, but further studies are necessary. Future research should include antioxidant activity assays and measurements of oxidative stress markers like malondialdehyde (MDA) levels before and after treatment.

Keywords: *Clinacanthus nutans; natural product; triple negative breast cancer; 4T1 and oxidative stress*

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Inhibitory effects of daidzein on 5α-reductase and prostate histomorphometry in testosterone-induced benign prostatic hyperplasia in Sprague Dawley rats

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Abstract

Benign prostatic hyperplasia (BPH) is a common prostate disorder in ageing males, characterised by abnormal prostatic cell growth. Current pharmaceutical treatments often have side effects, highlighting the need for alternative options such as natural compounds. Daidzein, an isoflavone, mostly found in soybeans, has various potential health benefits, including anticancer, anti- inflammatory, and neuroprotective effects. Studies have shown that high doses of daidzein can inhibit cell growth in BPH cells. This study aimed to assess the effect of daidzein on 5α-reductase activity and prostate histoarchitecture in BPH-induced rats. The male Sprague Dawley rats were randomly assigned to six groups (n=6): Sham, BPH-induced, and three BPH-induced treatment groups (finasteride (1mg/kg) as a positive control, and daidzein (10 and 100 mg/kg) intragastrically for 30 days, respectively). BPH was induced by daily injection of testosterone propionate (3mg/kg, s.c). The study investigated the serum dihydrotestosterone (DHT) levels, prostatic index, and structural changes in the prostate. The results showed that daidzein treatment significantly reduced prostatic weight, prostatic index, and serum DHT levels in BPH-induced rats ($p<0.05$). Furthermore, the histological analysis of the prostate showed marked improvements in the thickness of epithelial cells, fewer epithelial involutions of glandular tissues and stromal improvement with a significantly lower histo-score ($p<0.05$). The study concluded that daidzein treatment mitigated the development of BPH. In summary, daidzein shows promise as a natural treatment option for BPH, with potential benefits and without the side effects associated with pharmaceutical treatments.

Keywords*: Benign prostatic hyperplasia; dihydrotestosterone; 5α-reductase; histomorphology and daidzein*

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Silent intruders: Zoonotic malaria amongst the asymptomatic and submicroscopic in Malaysia

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Abstract

Despite successfully eliminating human-to-human malaria transmission, Malaysia continues to face a significant challenge from zoonotic malaria infections. Furthermore, submicroscopic infections have become a crucial barrier to reducing the number of malaria cases, particularly in areas characterized by low transmission rates. This study aims to unveil a groundbreaking discovery: the presence of zoonotic malaria infections within asymptomatic carriers of submicroscopic infections. A community-based cross-sectional study was conducted in July 2020 in 23 localities across four states (Sabah, Perak, Kelantan, and Johor) to determine the prevalence of asymptomatic submicroscopic malaria. Blood samples were obtained from asymptomatic villagers with no history of malaria for the past 4 weeks of blood collection. Giemsa-stained blood slides from 3,322 samples were examined by expert microscopists. Upon examination, no parasites were detected. However, it was found that the prevalence of submicroscopic malaria was 1.86% (62/3,3322) by PCR and the species distribution was 0.75% *Plasmodium malariae*, 0.54% *P. vivax,* 0.45% *P. knowlesi*, 0.03% *P. falciparum*, 0.03% *P. cynomolgi* and 0.06% mixed species. Submicroscopic zoonotic malaria, while less common and less studied compared to human-to-human transmitted malaria, is an important and emerging area of concern, particularly in regions where zoonotic transmission cycles exist and it implies that these infections are not detectable by standard microscopy but can be identified using more sensitive molecular techniques such as PCR. Malaria elimination needs a concentration of activities towards identification of low-level residual transmission and intensification of efforts to eliminate the last few infections.

Keywords: *Elimination; malaria; reintroduction and submicroscopic*

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Oxidative stress-induced endothelial dysfunction in REM sleep-deprived animal model

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Abstract

Oxidative stress is associated with REM sleep deprivation (REMsd), which can lead to endothelial dysfunction, an indicator and predictor of cardiovascular disease (CVD). This study aimed to investigate the effects of REMsd on the endothelium in a REM sleep-deprived rat model. Twenty-eight male Sprague-Dawley (SD) rats were randomly divided into four groups (n=7): free-moving control rats (FMC), 72-h REM sleep-deprived rats (72-h REMsd), tank control rats (TC), and 72-h sleep recovered rats after 72-h of REM sleep deprivation (SR). Rats were deprived of REM sleep using the inverted flowerpot technique. Food consumption (Fc), body weight gain (BWg) and systolic blood pressure (SBP) were monitored. The descending thoracic aorta was isolated for oxidative stress markers measurement, *in vitro* functional vessel study, endothelial nitric oxide synthase (eNOS) protein expression by western blotting and histopathology examination. The results showed that 72-h REMsd decreased BWg significantly despite a significant increase in Fc, increased SBP, increased oxidative stress, impaired endothelial function, and caused endothelial damage. Antioxidant markers, including superoxide dismutase (SOD) activity, total antioxidant capacity (TAC), catalase (CAT), and glutathione (GSH) were significantly decreased while malondialdehyde (MDA) levels were significantly increased in 72-h REMsd rats. REMsd impaired vasorelaxation and caused hypercontractility. In the 72-h REMsd rats, levels of eNOS were significantly decreased and histopathology of endothelium revealed features of endothelial damage. The current work shows that REMsd is associated with endothelial dysfunction and damage, which is thought to be related to increased oxidative stress. Oxidative stress-induced lipid peroxidation is responsible for the damage, evidenced by increased MDA levels. Sleep recovery may revert the detrimental effects of REMsd.

Keywords: *Endothelial damage; endothelial dysfunction; oxidative stress and REM Sleep deprivation*

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Hibiscus sabdariffa linn. (Roselle) polyphenol extract (HPE) attenuates aorta redox imbalance in a diabetic rat model

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Abstract

Hyperglycemia and redox imbalance in diabetes mellitus cause oxidative stress of the endothelial cells which leads to vascular dysfunction. Polyphenol-rich extract of *Hibiscus sabdariffa* Linn. (HPE) is a potent antioxidant agent attributable to its polyphenolic compounds which has prevented aortic damage progression in experimental animals. Therefore, this study aimed to investigate the protective mechanism given by HPE. Methods Forty male Sprague-Dawley rats were divided into five groups; non-diabetes mellitus (NDM), diabetes mellitus without treatment (DM), diabetes mellitus treated with 100 mg/kg (DMRL) and 200 mg/kg HPE (DMRH) whilst diabetes treated with 150 mg/kg metformin (DMM) as positive control. The diabetic rats were induced by using a single injection of streptozotocin (55 mg/kg) intraperitoneally. After four weeks, HPE and metformin treatment were given daily for 4 weeks duration. At the end of the experiment, blood and the aorta were harvested for further analyses. The results demonstrated that HPE supplementation improved blood glucose level, reduced total cholesterol, triglyceride, and LDL level, however increased HDL level. HPE supplementation also improved the systolic, diastolic, and arterial blood pressure. Interestingly, HPE improved redox imbalance by increasing glutathione (GSH) level and superoxide dismutase (SOD) activity as well as reducing malondialdehyde (MDA) and advanced oxidation protein product (AOPP) level of aorta. Conclusion The findings of this study suggested that HPE was able to improve blood pressure and lipid profile as well as attenuated redox imbalance of the vascular in diabetic conditions.

Keywords: *antioxidants; cardiovascular disease; hyperglycemia and vascular dysfunction*

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The effects of *Hibiscus sabdariffa* linn. aqueous extract on oxidative stress and morphological changes on femur in ovariectomized-induced osteoporotic rats

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Abstract

Estrogen deficiency in postmenopausal women is often associated with osteoporosis. *Hibiscus sabdariffa Linn.* (roselle) is rich in anthocyanin, which has antioxidant properties, but its potential in treating osteoporosis caused by menopause remains unclear. This study aimed to investigate the effects of roselle aqueous extract (HSE) on oxidative stress and morphological changes of femur in ovariectomized-induced osteoporotic rats. Twenty-four female Sprague Dawley rats (250-300 g) were divided into four groups: Sham, Ovariectomy (OVX), Ovariectomy+HSE (OVX+HSE) and Ovariectomy+Zoledronate (OVX+ZLD). Bilateral ovariectomy was performed under KTX anaesthesia. The rats were left for 6 weeks to develop osteoporosis followed by treatment for 4 weeks. The OVX+HSE group was given HSE (100 mg/kg orally), while the OVX+ZLD group was given zoledronate (0.1 mg/kg intraperitoneally). Sham and OVX groups were given distilled water. The rats were sacrificed, and femurs were taken for biochemical and histological tests. Biochemical tests include the malondialdehyde (MDA) level, superoxide dismutase (SOD) activity and catalase (CAT) activity. The study found a significant decrease in the MDA level for the OVX+HSE group compared to the OVX group. However, no significant differences were observed for SOD and CAT activities between these groups. Histological observation showed an increase in trabecular bone thickness and a decrease in the number of adipocytes in the OVX+HSE group compared to the OVX group. Therefore, HSE has the potential to prevent oxidative stress and morphological changes of the femur in ovariectomized-induced osteoporotic rats.

Keywords: *Femur; postmenopausal; osteoporosis; oxidative stress and roselle*

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Therapeutic effects and risks of low electromagnet field (EMF) exposure on diabetic wound healing

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Abstract

Research has proposed alternative treatments to enhance diabetic wound healing such as low electromagnetic field (EMF) exposure, which can enhance the treatment within specific frequencies and exposure durations. This study was conducted on induced diabetic rats to investigate the effects of EMF exposure of 0.8mT and 1.2mT on open wounds. Four groups of induced diabetic rats: a positive control (iodine), negative control (normal saline), and two treatment groups of 0.8mT EMF and 1.2mT exposure respectively. Each rat was given streptozotocin via intraperitoneal injection to induce diabetes and two open wounds, measuring a diameter of 8mm width and 2mm depth. Iodine and normal saline were administered over 21 days, while for five consecutive days, 30 minute EMF exposure of 0.8mT and 1.2mT were conducted. The 0.8mT treatment group's macroscopic observations revealed progressive wound healing of (48.75±1.11)% at day 14 then to highest percentage at (60.31±0.20)% on day 21. Nevertheless, adverse effects on other organs were also observed with both frequencies. The 0.8mT treatment group experienced fewer side effects, including bronchial wall thickening, kidney podocytes swelling, and signs of necrotic brain cells as compared to the control group. In conclusion, 0.8mT EMF exposure accelerates diabetic wound healing particularly proliferation and remodelling phases by 60% compared to untreated diabetic rats, at the expense other organs may be impacted alongside affecting structure alterations to its functionality. More studies were needed before EMF exposure can be employed to treat diabetic wound healing in the future.

Keywords*: Delayed wound healing; diabetes; duration; frequency and low EMF*

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Elucidating angiogenesis mechanism of stem cells from deciduous teeth incorporated with chitosan hydrogel via MAPK/ERK pathway

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Abstract

The combination of stem cells from human exfoliated deciduous teeth (SHEDs) with chitosan hydrogel holds significant potential for advancements in regenerative medicine, particularly for dental issues like periodontal disease and dental trauma. Eventhough Root Canal Treatment (RCT) is commonly used, it has limitations such as an increased risk of tooth fracture and reinfection. SHEDs offer a promising alternative by fostering pulp regeneration through multiple mechanisms, and chitosan hydrogel's unique properties, including biocompatibility and controlled release of bioactive molecules, enhance the efficacy of SHEDs. This study investigated the proliferation activity, angiogenesis activity, and CD31 mRNA expression levels of SHEDs, contributing to advanced regenerative therapies. The optimal chitosan hydrogel formulation, prepared with a β-glycerophosphate concentration of 50mg mixed with 130 mg chitosan powder, was selected for its superior porosity and pore sizes, confirmed through Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray (EDX) analyses. Five experimental groups were as follows: Cells only, Cells + Hydrogel, Cells + Hydrogel + VEGF, Cells + Hydrogel + ERK inhibitor, and Cells + Hydrogel + VEGF + ERK inhibitor. BrdU assays showed time-dependent increases in SHEDs proliferation across all groups, with the hydrogel + VEGF group having the highest proliferation rate and the hydrogel + inhibitor group effectively inhibiting proliferation. Angiogenesis assays indicated increased angiogenesis activity, with the hydrogel + VEGF group showing the highest activity and the hydrogel + inhibitor group capable of suppressing it. These findings demonstrate the potential of SHEDs and chitosan hydrogel combinations in promoting tissue regeneration and therapeutic interventions, particularly in dental applications.

Keywords*: Angiogenesis; chitosan hydrogel; proliferation; regenerative medicine and SHEDs*

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Mitigation of diabetic cardiomyopathy progression through roselle polyphenol-rich extract by modulating PKC-MAPK pathway

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Abstract

Roselle polyphenol-rich extract (HPE) supplemented at early development of diabetes mellitus prevented diabetic cardiomyopathy (DCM) in experimental rats. However, it is not known whether HPE could limit or treat progressed DCM. This study aimed to investigate the protective mechanism of HPE in limiting the progression of DCM in diabetic rats. Male Sprague-Dawley rats were divided into four groups: untreated diabetic (DM), HPE supplementation (DMR; 100 mg/kg), and metformin-treated (DMM; 150 mg/kg). Type 1 diabetes was induced with streptozotocin. After four weeks of untreated diabetes, supplementation was administered for an additional four weeks. At the end of the experiments, all rats were sacrificed, and the hearts were extracted for cardiac structural and functional assessment in addition to biochemical and protein study of the parameters related to oxidative stress, myocardial inflammation, and cardiomyocyte apoptosis markers. Findings showed diabetes led to systemic issues like hyperglycaemia and dyslipidaemia and a significant decline in cardiac function, affecting both diastolic and systolic functions. Interestingly, HPE supplementation suppressed the activation of PKC-α, PKC-βII and MAPK as observed in the reduced phosphorylation of JNK, ERK1/2, and p38 MAPK. HPE inhibited NADPH oxidase activation, reduced oxidative stress, and increased antioxidant status. Reductions in cardiac pro-inflammatory (IL-1β and TNF-α) and apoptotic (cytochrome C and caspase-3) protein expression were also observed in HPE-supplemented rats. In addition, impediment of cardiac remodelling was evidenced by significant reductions in myocardial fibrosis and cardiomyocyte hypertrophy, consequently preventing deterioration of cardiac systolic and diastolic dysfunction. In summary, HPE mitigated DCM progression by targeting the PKC-MAPK pathway.

Keywords*: Cardiac function; diabetes mellitus; Hibiscus sabdariffa; oxidative stress and remodelling*

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A case report: persistently elevated thyroid stimulating hormone

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Abstract

This is a case of WS, a 19-year-old male diagnosed with congenital hypothyroidism and was managed with a daily dose of Levothyroxine. Despite receiving maximal dosage, WS exhibited persistent discordance of thyroid function tests (TFT) characterised by elevated thyroid stimulating hormone (TSH) and normal free-thyroxine (fT4) levels, prompting suspicion of possible antibody interference. Suspecting antibody interference in immunoassay, the primary team had sent TFT to a private laboratory for results comparison. However, the results were inconclusive. The team has explored alternative explanations, including non-adherence to Levothyroxine. The observation of overt hypothyroidism in a subsequent TFT prompted further investigation of patient's compliance and also dose adjustments by changing to a weekly dosage regimen. Despite escalated Levothyroxine doses, WS continued to have weight gain and subclinical hypothyroidism biochemically. A thyroxine absorption test was subsequently conducted to differentiate between non-adherence and thyroxine malabsorption. The results of thyroxine absorption test revealed >100% increase in fT4 levels at the fourth hour, indicating adequate Levothyroxine absorption, suggesting non-compliance as the cause of persistent discordance TFT in this patient. The thyroxine absorption test emerges as a valuable tool in distinguishing between non-adherence and malabsorption in hypothyroid treatment. In this patient's case, it facilitated the identification of non-compliance, preventing the need for further invasive investigations related to Levothyroxine malabsorption. These findings highlight the importance of patient adherence to treatment given in achieving therapeutic success in hypothyroid management.

Keywords: *Compliance; free thyroxine; interference; levothyroxine and thyroxine absorption test*

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Triple action of M1 peptides: suppression of growth, angiogenesis inhibition, and apoptosis induction in metastatic breast cancer

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Abstract

Breast cancer is a significant global health concern, leading to high mortality rates and affecting millions of individuals worldwide. However, the development of resistance by cancer cells remains a challenge, and conventional chemotherapy agents often result in relapse and metastasis due to their non-selective nature. Our developed biotherapeutic peptide (M1) showed anticancer properties targeting colon, lung and prostate cancers However, their anticancer activity on breast cancer cells is still unknown. The M1 displayed low toxicity and haemolytic activities. The in-vitro validation of antiproliferation activity of these M1 peptides on different metastatic cancer cells was studied. The interaction of M1 peptides with different anti-apoptotic Bcl-2 family proteins was investigated to elucidate its potential to induce apoptotic cell death. The selective antiproliferation activity of M1 was observed by targeting only metastatic cancer cells without interrupting normal cells' viability. In comparison to apoptotic-inducer drugs (venetoclax) in protein-peptide studies, M1 showed greatest binding efficacy towards Bcl-2, Bcl-xL and Mcl-1 thus inhibiting their anti-apoptotic functions. Moreover, the M1 peptide has shown strong affinity towards the VEGFR2 binding site and thus blocking the VEGF-VEGFR interaction. From our preliminary in-vitro studies, these peptides reduced proliferation and expression of metastasis-associated proteins (Slug and Snail) in both highly metastatic MDA-MB-231 and PC-3M cells. Future studies should continue to investigate their therapeutic potentials and underlying mechanisms of anticancer actions.

Keywords*: Angiogenesis; apoptosis; breast cancer; metastasis and peptide*

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Successful reversal of paracetamol-induced hepatotoxicity with acetylcysteine: A case report

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Abstract

Paracetamol overdose in Malaysia, often linked to parasuicide attempts. Despite the effectiveness of acetylcysteine as an antidote, hepatotoxicity and fatalities can still occur. We present a case of paracetamol-induced hepatotoxicity, successfully managed with timely acetylcysteine administration. A 26-year-old Indian male previously healthy presented to emergency department following alleged paracetamol ingestion the previous day, driven by suicidal ideation after a personal rejection. The patient was tachycardic but other vital signs were stable. Laboratory tests indicated hepatotoxicity (AST up to 1461 U/L, ALT up to 2439 U/L, bilirubin highest of 65 µmol/L), coagulopathy (APTT prolonged (109.5 s), PT prolonged (95.2 s), INR highest of 7.17) and serum paracetamol level of 1070 µmol/L. Renal profiles and calcium levels were normal. Diagnosis was confirmed using the King's College Criteria for paracetamol toxicity. As the paracetamol level was above the Rumack-Matthew nomogram treatment line, the patient was started on a 5-day course of intravenous N-acetylcysteine and diagnosed as adjustment disorder with anxiety distress. Before discharge, AST and ALT levels decreased markedly, and coagulation parameters normalized, indicating a favorable response to acetylcysteine therapy. The patient was discharged in stable condition. This case highlights the crucial role of laboratory diagnostics and the Rumack-Matthew nomogram in managing paracetamol-induced hepatotoxicity. While the nomogram is invaluable in acute overdose settings, it is less reliable for staggered or chronic overdoses and not applicable to extended-release paracetamol toxicity. Despite these limitations, prompt and accurate laboratory assessment, as demonstrated in this case, is essential for effective treatment and recovery in acute paracetamol overdose.

Keywords: *Coagulopathy; hepatotoxicity; n-acetylcysteine; paracetamol and Rumack-Matthew nomogram*

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Knowledge, attitude and practices (KAP) towards the prevention of thalassemia among unmarried adults in Selangor, Malaysia: A cross-sectional study

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Abstract

Thalassemia is an inherited blood disorder that can cause anemia, posing a major public health problem worldwide, including in Malaysia. Unmarried adults are the main population group for thalassemia screening because they are at risk of having thalassaemic children if they are carriers. Therefore, assessing their level of knowledge, attitude, and practice (KAP) is important. An online cross-sectional study was conducted among 177 unmarried adults in Selangor, Malaysia. Nine districts in Selangor were selected as study locations, namely Gombak, Petaling, Kuala Selangor, Hulu Langat, Hulu Selangor, Klang, Sabak Bernam, and Kuala Langat. The level of KPA against thalassemia prevention was measured through a questionnaire with a Cronbach's Alpha value of 0.86, involving sociodemographic information, knowledge about thalassemia, attitudes, and practices towards thalassemia prevention. Mann Whitney U test, ANOVA, Kruskal Wallis H, and Pearson correlation were used to analyse the data. The study showed that unmarried adults have a high level of KAP. Additionally, there was a significant difference in the level of knowledge with age ($p=0.01$) and learning background ($p=0.04$), as well as in the level of attitude and practice with family income ($p=0.02$). Furthermore, the results of the study also showed a significant correlation between (knowledge-practice) ($r=0.349$, $p=<0.001$) and (practice-attitude) ($r=0.423$, $p=<0.001$). In conclusion, the Malaysian Ministry of Health and the Ministry of Education should raise awareness about thalassemia and its prevention to decrease its prevalence in Malaysia.

Keywords: *KAP; Malaysia; screening; thalassemia and unmarried adults*

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Knowledge, attitude and preventive practice towards sexually transmitted diseases among Malaysian undergraduate students in public universities: A cross-sectional study

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Abstract

The increasing trend of sexually transmitted diseases (STDs) among young adults between 18 and 25 years old is an alarming issue, as they are still unable to distinguish between risk and reward. Assessing their knowledge, attitude and practice (KAP) is important in controlling the number of rising cases of STDs. An online cross-sectional study has been conducted among 575 undergraduate students from five public universities across Malaysia. The level of KAP towards STDs has been measured through a questionnaire involving sociodemographic information, knowledge of STDs, attitudes towards STDs and preventive practice towards STDs with the Cronbach's alpha value for the questionnaire being 0.722. Descriptive analysis, the Mann-Whitney test and Fisher's exact test have been conducted. Results showed that 67.0% of students have poor knowledge of STDs, 75.5% of students have good attitudes towards STDs and 96.7% of students have never had sexual intercourse. There was a significant difference in the level of knowledge ($Z = -3.285$, $p = 0.001$) and attitudes ($Z = -5.323$, $p = 0.000$), while there was no significant difference in the level of preventive practices ($Z = -0.008$, $p = 0.994$) towards STDs between health science or science students and non-health science or non-science students. There was no association between the level of knowledge ($p = 1.000$) and attitude ($p = 0.437$) towards preventive practices against STDs. In conclusion, the public university undergraduate students who participated in this study have a poor level of knowledge but a good level of attitude and preventive practice towards STDs.

Keywords*: Attitude; knowledge; practice; sexually transmitted diseases (STDs) and students*

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CSF oligoclonal bands: A sensitive tool for CNS metastases in multiple myeloma – a case series

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Abstract

Cerebrospinal fluid (CSF) oligoclonal bands (OBs) play a crucial role in supporting the diagnosis of multiple sclerosis and other neuroinflammatory disorders. We report two cases of known IgG kappa multiple myeloma (MM) patients with a distinctive ladder pattern of monoclonal bands in CSF OBs. Case A presented with bilateral upper limb weakness and numbness. Spine magnetic resonance imaging (MRI) revealed multiple masses with intraspinal extension, causing compression from C6 to T3. Decompression surgery and biopsy were performed. Case B is a patient who experienced double vision. Brain MRI were inconclusive, leading to a clinical diagnosis of bilateral abducens cranial nerve palsy. Lumbar punctures were conducted in both patients, revealing dense, closely clustered monoclonal bands with a ladder pattern in CSF OBs. In Case A, CSF cytology indicated monocytes and occasional lympho-plasmacytoid cells, with limited further characterization. Histopathological examination of the mass confirmed > 90% atypical tumour cells consistent with underlying MM. Case B's additional CSF investigations (cytology, flow cytometry, and biochemistry) showed no abnormal findings. Neurological symptoms in these patients may arise from primary neuroinflammatory disorders of MM progression. While Case A benefited from straightforward radiological and laboratory investigations pointing to central nervous system (CNS) involvement, Case B's negative findings emphasized the critical role of CSF OBs in diagnosing CNS metastases of MM. CSF OBs serve as a valuable diagnostic tool, especially when other investigations yield inconclusive results. Understanding their significance can guide accurate diagnoses and inform treatment decisions.

Keywords*: Cerebrospinal fluids; CNS metastasis; multiple myeloma; neuroinflammatory disorders and oligoclonal bands*

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Navigating HbA1a challenges in HbA1c reporting: A clinical dilemma

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Abstract

Haemoglobin A1c (HbA1c) serves as a valuable tool for diagnosing and monitoring glycaemic control in diabetes mellitus. HbA1a, a non-enzymatic glycation product of either fructose-1,6-diphosphate or glucose-6-phosphate with haemoglobin, should ideally be lower than HbA1c. We present a case of elevated HbA1c with a higher HbA1a value. The patient, an elderly woman with underlying diabetes mellitus and dyslipidaemia, was admitted for urosepsis complicated by hospital-acquired pneumonia. High-performance liquid chromatography (HPLC) analysis revealed an HbA1a value of 11.1%, significantly exceeding the HbA1c level (8.2%). Despite serial dilutions, HbA1a proportions remained persistently elevated (>10%). Previous HbA1c analysis done at another hospital using the capillary electrophoresis (CE) method reported 8.3% with no abnormal haemoglobin peak. Fasting blood sugar (FBS) was elevated at 13.1 mmol/L (normal interval: 3.9 – 6.0 mmol/L). Normal haemoglobin analysis by CE method excludes the common haemoglobin variants that may co-elute in the HbA1a region. The discordance between elevated HbA1c and higher FBS suggests that HbA1a complicates the accurate HbA1c reporting. Although HbA1a is not part of the acceptance criteria for HbA1c reporting, its presence can confound results. This could substantially impact the interpretation of normal HbA1c in poorly controlled diabetic patients. Compared to the CE method, the HPLC method is able to identify HbA1a peak. We propose that the excess blood glucose preferentially glycates with haemoglobin to form HbA1a rather than HbA1c. Thus, relying solely on HbA1c may not reliably indicate glycaemic control in such cases. Clinicians should integrate other glucose monitoring modalities and clinical findings for a comprehensive assessment.

Keywords*: Diabetes mellitus; glycaemic control; HbA1a and high-performance liquid chromatography*

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Reprogramming of stem cell into synthetic mRNAs transcription factor

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Abstract

Diabetes Mellitus (DM) is a prevalent chronic metabolic disorder impacting 422 million individuals globally, with an annual death toll of 1.6 million. Autoimmune β-cells destruction and insulin resistance is the underlying cause of diabetes. This study focuses on differentiating pluripotent stem cells into pancreatic β-cells using synthetic mRNA transcription factors as treatment instead of donor islet cell transplantation. The primary objective is to differentiate human mesenchymal stem cells into functional β-cells by modulating key transcription factors: PDX1, PAX4, NGN3, MafA, and GLIS3, vital in natural pancreatic β-cells differentiation. This study involves three distinct phases: (1) synthesis of synthetic mRNA, (2) *in vitro* investigations, and (3) *in vivo* transplantation. The successful transfection assessment encompasses gene expression analysis, cell viability, apoptosis assay, immunohistochemistry staining, and glucose-stimulated insulin assay. Results reveal increased viability of terminally differentiated cells (>80% vs. 72.71%control), affirming the safety of mRNA-mediated differentiation. RT-PCR demonstrates amplified insulin mRNA expression in PNMPG-differentiated β-cells ($P<0.05$), emphasizing effective insulin induction. *In vivo* analysis examines weekly glucose readings and oral glucose tolerance test (OGTT) data to evaluate differentiated cell impact, revealing encouraging glucose regulation trends. Moreover, C-peptide, insulin receptor and insulin level exhibit significant differences in the differentiated cells compared to control groups ($p<0.05$). In conclusion, synthetic mRNA transcription factors drive huMSC differentiation into β-cells, elevating cell viability and β-cell marker expression. This innovative approach holds potential as an alternative for the treatment of Diabetes Mellitus.

Keywords*: β-cells; cell differentiation; diabetes mellitus; mesenchymal stem cell and synthetic mRNA*

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Wound healing breakthroughs: A scoping review of low electromagnetic field (EMF) therapy's immunomodulatory potential

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Abstract

The study aims to explore the immunomodulatory potential of low electromagnetic field (EMF) exposure therapy in wound healing, particularly on the immune cell response and underlying mechanisms. Scoping review research method was carried out to study reports of immune cell responses to low-frequency EMF therapy in existing research published between 2018 and 2023 on three electronic academic databases. A total of 19 articles were selected out of 3,567 articles. Analyses conducted found that exposure to low-frequency EMF can promote faster wound healing through several mechanisms including MMP-9 and MMP-2 matrix activation, influence on MSC stem cell migration, increased early expression of pro-inflammatory cytokines, influences on macrophage cell activity and migrations, incentives for the transition of the wound-healing phase from inflammatory to proliferation phase, promotions against fibroblast-like synoviocytes (FLS) migration, enhanced antioxidant defence systems, control of oxidative pressure and neurotrophic factors, effects on T-helper cells, and acceleration of tissue regeneration. The frequency of 50 Hz is identified as the most optimal and safe for therapeutic purposes, with a magnetic field density of 1 mT as the safest and can give a significant immunomodulatory effect. An eight-hour exposure period is an appropriate and optimal period to provide therapeutic effects without the risk of unwanted side effects. This period may involve staged or scheduled exposure sessions, rather than continuously. The conclusion finds that low-frequency EMF therapy has shown positive effects in improving wound healing and modulating immune response through several immunomodulatory response pathways and mechanisms, suitable as a non-invasive approach to wound healing.

Keywords: *EMF; healing; immunomodulatory effects; low frequency and wound*

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The effects of roselle aqueous extract on the RANKL and OPG protein expressions in the gingiva of ovariectomy-induced periodontitis rats

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Abstract

Periodontitis is a gum disease, presented by bacterial plaque and destruction of the alveolar bone. Periodontitis development is higher in osteoporotic patients. RANKL and OPG proteins play a crucial role in bone homeostasis. Roselle calyces have been shown to exhibit multiple health benefits. The objective of this study is to investigate the effects of roselle on the RANKL and OPG protein expressions in ovariectomy-induced periodontitis rats. Bioactive compound determination for roselle was performed using high-performance liquid chromatography. A total of 32 Sprague-Dawley rats were divided into four groups: zoledronate (ZLD), roselle (HSE), negative control (OVP) and Sham. Ovariectomy was performed on HSE, ZLD and OVP groups to induce osteoporosis. After six weeks, HSE, ZLD and OVP groups were induced with periodontitis using orthodontic ligature wire. Treatments were then given for four weeks. Zoledronate (0.1mg/kg) was given twice a week via intraperitoneal injection. Roselle aqueous extract (100 mg/kg) was given daily via oral gavage. The Gingiva of the rats were isolated. RANKL and OPG protein were measured using western blotting. Anthocyanin, phenolic acids and flavonol were detected in roselle aqueous extract. The result showed no significant difference in RANKL/OPG ratios for HSE (1.327 ± 0.6552), ZLD (1.041 ± 0.5135), OVP (2.041 ± 0.6001) and Sham (0.8017 ± 0.2267) with $p>0.05$. The OVP group had the highest RANKL/OPG ratio, indicating high bone resorption. The HSE group showed a reduction in RANKL and an increase in OPG, indicating a reduction in bone resorption. The potential of roselle as a therapeutic agent to prevent periodontitis and treat osteoporosis needs to be studied more in-depth.

Keywords: *OPG; osteoporosis; periodontitis; RANKL and roselle*

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The effect of carvacryl-2-oxoethylgallate on cardiac fibrosis and hypertrophy in doxorubicin-induced cardiotoxicity model

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Abstract

Doxorubicin is a commonly used anthracycline in chemotherapy, but its cardiotoxic effects limit its effectiveness. Carvacrol, a phenolic monoterpenoid with antioxidant and cardioprotective properties, has not been studied for its ability to prevent doxorubicin-induced cardiotoxicity. This research aims to investigate how a synthesized carvacrol derivative, carvacryl-2-oxoethylgallate, impacts the histological changes from doxorubicin-induced cardiotoxicity. A total of 24 male Sprague-Dawley rats (200 ~ 250 g) were randomly divided into four groups: control, cardiotoxicity (DOX), and dose therapy 1 (DOX+CAR 25 mg/kg), dose therapy 2 (DOX+CAR 50 mg/kg), where each group consists of eight rats. The treatment group was given 25 mg/kg and 50 mg/kg of carvacrol-2-oxoethylgallate for 14 consecutive days (p.o). The DOX and CAR+DOX groups were given doxorubicin (15 mg/kg, i.p) on day 15 to induce cardiotoxicity, while the control group was given DMSO 0.5% (i.p). All rats were allowed to recover for 3 days before being sacrificed on day-19 and the hearts were excised. Histological observations showed significantly higher ($p<0.05$) hypertrophy and fibrosis quantification in the cardiotoxicity group compared to the control group. Pre-treatment with carvacryl-2-oxoethylgallate prevented structural changes in both CAR+DOX groups, demonstrating cardioprotective effects. The gene expression ANP and BNP differed significantly ($p<0.05$) between the DOX+CAR groups, while Smad2 and Smad3 showed no significant differences. However, Smad3 tended to have the highest expression pattern in the DOX group. In addition, TGF-β1 protein expression was significantly different ($p<0.05$) among the groups. These findings show that supplementation with carvacryl-2oxoethylgallate could potentially prevent cardiotoxicity, especially in preserving the heart structure.

Keywords: *Carvacryl-2-oxoethylgallate; cardiotoxicity; doxorubicin; fibrosis and hypertrophy*

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Protective effect of zerumbone on vital organs of aluminium chloride induced Alzheimer rat

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Abstract

Zingiber zerumbet from the Zingiberaceae family is a plant that has many nutrients and is believed to have antioxidant, anti-inflammatory, anticancer and antibacterial effects. In this study, the protective effect of Zerumbone on the vital organs of male Wistar rats induced with aluminium chloride as an Alzheimer's model was carried out. A total of 36 male Wistar rats were divided into 6 groups, namely the negative control group (distilled water), the positive control group NAC (N-acetylcysteine 20 mg/kg), the $AlCl_3$ group (aluminium chloride 25 mg/kg), the ZER 10 group (Zerumbone 10 mg/ kg), ZER 20 (Zerumbone 20 mg/kg) and ZER 30 (Zerumbone 30 mg/kg). All groups of rats received their respective treatments for 42 days. $AlCl_3$ treatment was only given after 14 days to all groups except the negative control group. This is a neuroprotective Alzheimer's rat model. Rats were subjected to behavioral tests on day 14, 28 and 42 and killed on day 43. Samples of important organs such as kidneys, liver, lungs and heart are taken for the purpose of biochemical and histology analysis. The results of this study show that the total protein level for the Zerumbone group shows an increasing pattern according to the concentration of Zerumbone for all important organs. Total protein levels for the lung organs of the ZER 30 group and the heart organs of the ZER 20 and ZER 30 group showed significantly higher levels ($p<0.05$) compared to the negative group. For the levels of SOD and GSH in all four organs of the rats, there was no significant level ($p>0.05$) compared to the negative group. For the MDA level of the kidney organ, it shows a pattern of decreasing MDA level according to the concentration of Zerumbone, which is for the ZER 20 and ZER 30 group, which are significantly lower ($p<0.05$) compared to the negative group. However, MDA levels for liver, lung and heart organs are not significant ($p>0.05$) compared to the negative group. Analysis of the behavior of rats induced by aluminium chloride showed an effect on the change in the memory level of rats but not significantly ($p>0.05$). Histology analysis of the kidney and liver organs of all study groups showed a normal structure except for slight structural changes in the $AlCl_3$ group. In conclusion, this study suggests that Zerumbone can have a protective effect on the vital organs of aluminium chloride-induced Alzheimer's rats.

***Keywords**: Alzheimer; antioxidant; dementia; Zerumbone and Zingiber zerumbet*

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Determination of the presence of parasites and bacteria, and the effects of fecal contamination in shellfish from Pasir Penambang public market, Kuala Selangor

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Abstract

Mollusk or shellfish such as *Cerithidea obtusa*, *Meretrix meretrix* and *Anadara granosa* are among the shellfish that Malaysians often eat whether cooked or raw. At the same time, mollusca is also an intermediate host to some parasites and bacteria where parasites and bacteria are among the causes of increased health problems in humans such as vibrio infection, parasitic infections and food poisoning. Therefore, this study was carried out to determine the presence of parasites and bacteria as well as the effects of fecal contamination in this mollusca. A total of three species of mollusca, namely *Cerithidea obtusa*, *Meretrix meretrix* and *Anadara granosa* were purchased from the Pasir Penambang Public Market, Kuala Selangor and were homogenized to see the presence of parasites as well as the spreading method was conducted for the detection of the number of bacterial colonies and bacterial growth. From the studies conducted, there was no parasite found. However, there were several protists found from the mollusca which also might give a bad effect to humans. In addition, bacterial growth can also be seen from each mollusca plate in order. This shows that good handling of foods such as mollusca is very important and should be taken into account to avoid adverse effects such as food poisoning when consumed.

Keywords: *Bacteria; fecal contamination; mollusca and parasites*

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In vitro antibacterial activity of Raed Sabun Mandian Semambu® body wash product against skin pathogen bacteria

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Abstract

Antibacterial body washes aim to slow or prevent bacterial growth on the skin, thereby preventing infections. Common bacterial strains associated with skin infections include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. Antibacterial body washes containing natural ingredients are preferred for their ability to prevent skin infections without causing adverse effects like redness and irritation. Raed Sabun Mandian Semambu® is formulated with neem, frankincense, anise, clove, and chamomile essential oils, along with pro-vitamin B5, and has been tested for antibacterial effects against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. The inhibition zone diameter was determined using the well diffusion method. The minimum inhibitory concentration (MIC) was assessed using the broth microdilution method, while the minimum bactericidal concentration (MBC) was evaluated using the plate streak technique. The time-kill assay was conducted at 0.5x MIC and 1x MIC for *S. aureus* and *S. epidermidis*, and at 0.5x MIC, 1x MIC, and 2x MIC for *S. pyogenes*, with incubation at 0, 2, 4, 6, 8, 10, and 24 hours. Results show inhibition zone diameters at product concentrations of 25%, 50%, 75%, and 100%. The MIC values indicate that the product inhibits the growth of all tested bacteria. The MBC/MIC ratio demonstrates a bactericidal effect on all bacterial strains. The time-kill assay revealed a bactericidal effect at 1x MIC for *S. aureus* and *S. epidermidis*, and at 2x MIC for *S. pyogenes*. In conclusion, Raed Sabun Mandian Semambu® exhibits a bactericidal effect on all tested bacteria.

Keywords: *Antibacterial body wash; bactericidal effect; natural ingredients; neem extract and skin infection*

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Assessment of the effectiveness of mosquito larvae trapping device enriched with pyriproxyfen as dengue vector control in Taman Prima Saujana Kajang

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Abstract

Dengue fever, caused by the dengue virus (DENV) of the Flavivirus genus within the Flaviviridae family, remains a significant global public health concern, including Malaysia. Dengue outbreaks are typically initiated by the bites of infective *Aedes aegypti* and *Aedes albopictus.* Various program measures have been implemented to control dengue outbreaks, yet reported cases of dengue fever continue to rise annually. Thus, this study aimed to assess the effectiveness of the Mosquito Larvae Trapping Device (MLTD) intervention, supplemented with pyriproxyfen, in mitigating *Aedes* spp. populations. The study was conducted in Taman Prima Saujana Kajang as treatment location and Taman Kantan Permai Kajang as the control location over a 20-week period, segmented into pre-treatment, treatment, and post-treatment phases. During the first four weeks of the pre-treatment and the last four weeks of the post-treatment phases, three traditional ovitraps were placed at the study sites, while during the treatment phase, two ovitraps along with one MLTD were placed at each sampling point in the treatment area. *Aedes* spp. mosquito populations, Ovitrap Positive Index (OPI), and Average Egg Index (AEI) were considered in this study. *Ae. albopictus* was found to dominate outdoor residential areas compared to *Ae. aegypti.* The OPI values indicated that the study sites were at high risk of becoming dengue fever outbreak areas, while the AEI values showed a difference compared to the control site. There was a significant difference ($p < 0.05$) in the number of eggs at the treatment location between the pre-treatment and post-treatment phases. In conclusion, MLTD demonstrated good effectiveness in reducing *Aedes* spp. mosquito populations in Taman Prima Saujana Kajang.

Keywords: *Aedes; ovitraps; pyriproxyfen and vector control*

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The changes in expression of GABA (A) receptor α2 subunit in the CA1 and CA3 hippocampal of the lithium-pilocarpine status epilepticus rat model

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Abstract

Studies on the distribution of GABAA receptor (GABAAR) subtypes have shown that α2-containing receptors represent approximately 15 - 20% of all GABAARs. This receptor subtype is believed to play a significant role in mediating anxiolytic and antidepressant-like properties. Consequently, α2-containing GABAARs have emerged as potential targets for the treatment of anxiety and depression. The localisation and expression of the GABAR α2 subunit in human and animal models have been studied extensively, particularly in anxiety. This study assessed the expression and localisation of α2 subunit in specific hippocampal subregions, such as CA1 and CA3, crucial for developing targeted therapies for anxiety. In this work, pilot studies of Sprague-Dawley rats (250 - 300g) were randomly allocated into two groups (Control; Epilepsy) in which models of status epilepticus with high morbidity and low mortality rate were developed. Epilepsy was induced via systemic administration of isomorphic lithium-pilocarpine. After 21 days, the rats were euthanised, and their brains were harvested and fixed. Coronal brain sections were prepared and subjected to immunohistochemical staining. Fluorescence immunohistochemistry was conducted to assess the α2 fluorescence intensity in the hippocampal area, especially CA1 and CA3 subregions. Our findings revealed downregulation of α2 expression in the CA1 and CA3 subregions between the control and epilepsy models ($p < 0.05$ and < 0.01, respectively). This indicated a pronounced expression of α2 in both subregions in epileptic conditions, suggesting potential therapeutic in anxiolytic.

Keywords*: GABA (A) receptors; α2 subunit and status epilepticus*

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The evaluation of effectiveness of various mosquito trapping methods for the disease-carrying vectors

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Abstract

The Human Landing Catch (HLC) method is the most effective method in catching mosquitoes for entomological research up until it has been recognized as the standard goal according to past researchers. This study was conducted in Taman Rimba Komuniti, Kota Damansara for 16 weeks using a 4x4 Latin Square research design four methods of trapping mosquitoes are used, including the HLC method, the CDC light trap with three different sources, which uses dry ice, yeast and sugar as well as hydrochloric acid (HCl) and calcium carbonate ($CaCO_3$). Optimization tests for yeast and sugar as well as hydrochloric acid and calcium carbonate were carried out first in the laboratory. The results of the analysis found that there is a significant difference ($p<0.05$) between all of the methods used. However, there is no significant difference to the three CDC light traps with different carbon dioxide sources (dry ice; yeast and sugar; hydrochloric acid and calcium carbonate) because of the $p=0.077$, which is not less than 0.05 between the three sources of carbon dioxide used. As a summary of the study, the HLC method is still the most effective method because the number of mosquitoes caught is far more compared to the other traps, which is 962 out of 1227 mosquitoes. However, the CDC light trap method using dry ice got the highest number of mosquitoes (180) compared to the CDC light trap using yeast and sugar (48) and the CDC light trap using hydrochloric acid and calcium carbonate (37).

Keywords: *CDC light trap; dry ice; human landing catch; hydrochloric acid and yeast*

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Evaluation of anticancer activity of agarwood ethanol extract from *Aquilaria malaccensis* on acute lymphoblastic leukemia Jurkat E6-1 cells

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Abstract

Acute lymphoblastic leukemia (ALL) is a type of leukemia that occurs in children. Various forms of treatment have been performed to treat leukemia patients such as chemotherapy, radiation therapy and immunotherapy. This prompted researchers to look for alternatives to existing treatments by testing a variety of natural compounds or products to find effective anti-cancer agents with fewer side effects on patients. Therefore, this study focused on the potential of the ethanol extract of naturally formed agarwood from *Aquilaria malaccensis* against acute lymphoblastic leukemia cells in children, Jurkat E6-1. In this study, the cytotoxic effects of extracts were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, while the cell death mode was assessed by flow cytometry technique based on Annexin V-FITC (fluorescein isothiocyanate) coloring. The results of the study found that MTT assay recorded a significant difference ($p<0.05$) between the negative control and Jurkat E6-1 cells treated with agarwood ethanol extract and menadione (positive control). The IC_{50} value for the treatment of agarwood ethanol extract with Jurkat E6-1 was 140.00 $\pm$ 3.18 µg/ml, whereas for menadione treatment, it was 9.50 $\pm$ 0.29 µM. The flow cytometric analysis for the mortality mode of cells found that 36.87% of the cells treated with agarwood ethanol extract and 40.1% of cells treated with menadione suffered late apoptosis. In conclusion, agarwood ethanol extracts have shown anti-leukemia effects on Jurkat E6-1 cells with cytotoxic inhibition through late apoptosis. Nevertheless, more thorough research needs to be carried out to prove the effectiveness of this agarwood ethanol extract as a chemotherapeutic agent.

Keywords: *Acute lymphoblastic leukemia; agarwood; anticancer activity; apoptosis and ethanol extract*

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Elucidating the repellent effects of *Coleus amboinicus* lour. Essential oil against *Aedes aegypti* linn. (Diptera: *culicidae*)

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Abstract

Increasing dengue cases in Malaysia posed a significant public health challenge. Despite widespread use of synthetic repellents, their efficacy is diminishing due to increasing mosquito resistance and potential health risks associated with prolonged chemical exposure. Additionally, the environmental impact of synthetic repellents raises concerns, highlighting the urgent need for sustainable and effective vector control strategies. Thus, natural products such as essential oils have garnered attention as natural and environmentally friendly alternatives to synthetic repellents. Hence, this study aimed to elucidate the effectiveness of *Coleus amboinicus* essential oil (CAEO) against *Ae. aegypti* in Malaysia by determining the effective doses (ED50, ED90) and complete protection time (CPT) of CAEO. The findings reveal a positive correlation between increasing CAEO concentrations and repellency, with ED50 and ED90 values identified at 6.14% and 24.74%, respectively. Notably, CAEO maintained at least an 80% repellency rate over a 4-hours period, underscoring its potential as an effective repellent. While DEET achieved 100% repellency for the same duration, CAEO emerged as a viable alternative, showing no significant difference in repellency over the duration of 4-hours of exposure. Besides, further analysis using GC-MS on the CAEO had identified major components such as carbamic acid (30.75%), caryophyllene (6.52%), ρ-cymene (6.40%), caryophyllene oxide (4.83%), humulene (4.64%) and γ-terpinene (4.45%), where some of these compounds have known insecticidal and mosquito repellent properties. Thus, this study underscores the potential application of CAEO as a natural repellent against *Aedes* mosquitoes, offering an effective and sustainable option in replacement of synthetic repellents like DEET.

Keywords: *Aedes aegypti; Coleus amboinicus; essential oil and repellent activity*

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Unravelling the cytotoxicity and mode of cell death of di- and triphenyltin(iv) alkylbenzyldithiocarbamate compounds against human lung carcinoma cell line (A549)

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Abstract

Lung cancer is the leading cause of cancer-related mortality worldwide, and cisplatin remains a core chemotherapy despite its limitations. Due to their impressive anti-proliferative properties and stability, organotin(IV) dithiocarbamate compounds have been explored as potential anti-cancer agents. In this study, we evaluated the cytotoxicity via MTT assay, morphological changes, and mode of cell death via Annexin V-FITC/PI of two newly synthesized organotin compounds, diphenyltin(IV) *N*-methyl-*N*- benzyldithiocarbamates compound (compound 1) and triphenyltin(IV) *N*-ethyl-*N*-benzyldithiocarbamates compound (compound 2), in human lung carcinoma cell lines (A549). Our results demonstrated that these compounds exhibit potent antiproliferative activity, with compound 2 being more toxic (IC_{50} = 0.52 ± 0.06 µM) than compound 1 (IC_{50} = 1.86 ± 0.15 µM). Morphological observation was conducted with IC_{50} values at 24 hours. The result demonstrated that the changes have the characteristics of apoptosis, such as cell shrinkage and membrane blebbing. In addition, apoptosis characteristics such as cell shrinkage and membrane blebbing have been observed. Moreover, both compounds induce apoptotic cell death, with compound 2 resulting in approximately 91% compared to 54% for compound 1. Our findings suggest that organotin(IV) dithiocarbamates may offer a promising alternative to cisplatin in treating lung cancer. These findings indicate that organotin(IV) dithiocarbamate compounds have potential as novel anti-cancer agents, and further research is warranted to elucidate their specific mechanisms of action in A549 cells.

Keywords: *A549 cells line; anticancer; cytotoxicity; dithiocarbamate and organotin,*

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Preliminary investigation on the immunomodulatory effects of sang huang (*Phellinus linteus* ligno-sh02) extract (xSH™) on macrophages

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Abstract

Extracts from the medicinal mushroom *Phellinus linteus* have been used in traditional medicine against various diseases and claimed to possess immunomodulatory properties with the likelihood of compound interaction with immune cells such as macrophages. Various results have been reported in previous studies, with some suggesting its anti-inflammatory activities and others proposing its immunomodulatory effects. Hence, the current study was carried out to further investigate the immunomodulatory properties of *P. linteus* extract (xSH™) on macrophages. MTT assay was performed on RAW264.7 and phorbol-12-myristate 13-acetate (PMA)-differentiated THP-1 cells treated with 50 to 500 µg/ml of xSH™. xSH™ did not exhibit cytotoxic effects at concentrations of up to 350 µg/ml, thus concentrations of 50, 150, 300 µg/ml were chosen for further investigatory works. Macrophages were stimulated with lipopolysaccharide and treated with 50, 150, 300 µg/ml of xSH™ for 24 hours. Subsequent assays for the measurement of radical scavenging activities, nitric oxide (NO) production, reactive oxygen species (ROS) generation, and cytokine expression were performed. xSH™ did not display DPPH-scavenging activities, and showed no significant modulation of NO and ROS production. However, gene expression analysis revealed significant enhancement of inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor-necrosis factor (TNF)-α in macrophages. Thus, despite the absence of radical-scavenging activities, the pro-inflammatory properties of xSH™ suggests its potential as an immune enhancer for use in conditions such as immuno-deficiency disorders and chemotherapy. Nevertheless, further studies on the underlying mechanisms behind the lack of radical scavenging properties but substantial cytokine-enhancing effects should be performed.

Keywords*: Immunomodulatory; medicinal mushroom; macrophages and Phellinus linteus*

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Assessment of antibacterial activity of *Moringa oleifera* leaf extract (MOLE) against *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents a substantial nationwide health risk due to its resistance to conventional antibiotics. Researchers are currently investigating alternatives to address this pressing issue. One such approach involves the usage of herbal medicine. Among the prospective herbs, *Moringa oleifera* leaf extract (MOLE) has demonstrated potential in combating bacterial infections. This study explores the antibacterial effects of ethanolic leaf extracts of *M. oleifera* against methicillin-resistant and -susceptible *S. aureus* (MRSA and MSSA). The antibacterial activity of ethanolic extract of MOLE was evaluated using the Kirby-Bauer disc diffusion method. Five different concentrations of MOLE ranging between 50 to 800 mg/mL were tested against MRSA and MSSA. The phytochemical composition of MOLE was analysed using liquid chromatography-mass spectrometry (LC-MS) MOLE exhibited significant dose-dependent antibacterial activity against both MRSA and MSSA strains, with higher concentrations demonstrating greater efficacy. MOLE displayed antibacterial activity comparable to the positive control for MRSA at a concentration of 800 mg/mL. LC-MS analysis revealed a diverse array of bioactive compounds in MOLE including flavonoids, alkaloids, phenolics, and glucosinolates, which are known for their antibacterial properties. Data from this study strongly suggest that MOLE exhibits *in vitro* antibacterial activity against both MSSA and MRSA. The identification of bioactive compounds through LC-MS underscores its potential as a natural antimicrobial agent. Future studies should focus on elucidating the mechanisms underlying MOLE's antibacterial effects and exploring its therapeutic potential.

Keywords: *Moringa oleifera; antibacterial; methicillin-resistant S. aureus and methicillin-susceptible S. aureus*

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Epigenetic changes modulated by inhibitor of Kappa B Kinase Alpha (IKKα) in a pancreatic ductal adenocarcinoma (PDAC) cell line

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, with epigenetic modifications such as DNA methylation significantly contributing to tumour heterogeneity, metastasis, and disease progression. Recent studies have also suggested that inhibitor of kappa B kinase alpha (IKKα) plays a central role in PDAC initiation and progression *via* NF-κB-mediated gene expression. However, the role of IKKα and its epigenetic changes in PDAC are not fully understood. In this study, we aimed to determine the epigenetic changes associated with IKKα and to identify gene pathways that are regulated by IKKα in a PDAC cell line. Findings revealed genomic regions that were hypermethylated in the IKKα knockout cells relative to the parental cell lines were significantly enriched for genes involved in *ERBB2*-related signaling pathways, such as the PTK6-ERK/MAPK, and PI3K events. qRT-PCR confirmed the downregulation of *ERBB2* expression in the IKKα knockout cell line. Notably, the cell line exhibited a reduced cell proliferation rate when compared to the wildtype, but did not exhibit a significant difference in cell migration. Subset genes such as *NRG1, NRG3, ERBB4*, and *EGFR* were also identified to be regulated by IKKα. In contrast, no cellular pathways were significantly enriched in the hypomethylated regions after adjusting for multiple testing. Our data implies that perturbations to IKKα expression result in epigenetic alterations in a PDAC cell line *via* NF-κB dependent and/or independent pathways. These distinct modulations involving IKKα highlights its potential as a PDAC biomarker for future treatment strategies.

Keywords*: DNA methylation; epigenetics; IKKα; NF-κB and PDAC*

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Protective potential of exosome-formulated roselle extract in myocardial injury-associated hypercholesterolemia rats

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Abstract

Despite exosomes being proven to be excellent drug-delivering agents, there is a lack of studies exploring the exosome-based approach to enhancing the roselle's health-promoting properties. Therefore, this novel study aimed at the potential of exosome-formulated roselle extract (Aehs-Exo) in myocardial injury (MI) associated hypercholesterolemic (HC) rats. A total of 18 Sprague-Dawley rats (250-300g) were randomly allotted into three groups (Control; HC-MI; Aehs-Exo). Rats were either fed with a 4% high-cholesterol diet (HCD) or standard chow for six weeks, then another four weeks of Aehs-Exo (100mg/kg, p.o) or vehicle with diet maintained accordingly. Rats were subjected to MI by subcutaneous injection of isoprenaline-hydrochloride (85mg/kg) for the last two consecutive days prior to euthanisation. HC-MI subjected rats exhibited significantly increased BMI, total cholesterol (TC), HDL, LDL, TC/HDL ratio, and cardiac troponin-T, validating the model. Aehs-Exo supplementation greatly restored the lipid profile, liver (ALP, AST, ALT) and kidney (urea, creatinine, and uric acid) function test. In parallel, significant amelioration of oxidative stress (MDA) and antioxidant (SOD, GSH) parameter were also seen. Cardiac histological observation revealed prominent perivascular fibrosis and necrosis with inflammatory cells in untreated HC-MI rats. Liver histological observations confirm the presence of steatohepatitis and ballooning degeneration, suggestive of non-alcoholic fatty liver disease development. Meanwhile, prominent degeneration of glomeruli and fibrosis is seen in renal histology. Interestingly, these histological changes were markedly improved by Aehs-Exo treatment. In conclusion, this study provides novel information on the therapeutic potential of exosome-encapsulated roselle treatment, which could be useful in treating HC-associated MI in clinical settings.

Keywords: *Exosome; roselle; hypercholesterolemia and non-alcoholic fatty liver disease*

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Photoprotective bioactivity of *Pseudokirchneriella subcapitata* extracts on ultraviolet light-irradiated human keratinocytes

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Abstract

Ultraviolet B (UVB) exposure contributes to photo-aging, which is associated with compromised keratinocyte viability and function. An emerging source of powerful phytoproducts in microalgae may harbour photoprotective properties, which remain to be elucidated. We aimed to evaluate the effect of the methanol extract (PSME) and aqueous extract (PSAE) of the green microalga *Pseudokirchneriella subcapitata* on the viability, migration, and proliferation of a UV-irradiated human keratinocyte cell line, HaCaT. Our study found that PSME treatment resulted in 20% cytotoxicity, however, 0.1 mg/mL PSAE exposure increased cell proliferation by 70%, and decreased gap width by 39% in the scratch assay after UV irradiation. The low cell viability in PSME as compared to PSAE may be due to the decreased activity of glutathione-related enzymes in the methanol intoxication of cells. The increase in cell proliferation and gap closure may be due to the presence of mycosporine-like amino acids (MAAs) that provide photoprotection capabilities. In conclusion, PSAE significantly increased cell viability, migration, and proliferation of UV-irradiated human keratinocyte cells, highlighting the potential of *P. subcapitata* microalgae in UV photoprotection.

Keywords: *Cell proliferation; cell viability; HaCaT cell line; microalgae and UV radiation*

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Unravelling the behavioural changes following *Cosmos caudatus* aqueous extract supplementation on $ALCL_3$-induced rats

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Abstract

Cosmos caudatus (C.C.), also known as 'ulam raja,' is a local plant recognised for its antioxidant properties and potential therapeutic effects on diseases associated with free radicals, including neurodegenerative disorders (You et al. 2021). This study aims to evaluate the impact of C.C. supplementation on $AlCl_3$-induced cognitive impairment in rats. Male Wistar rats were assigned to either a control group or a C.C. supplementation group. The experimental design included a series of neurobehavioral tests: the novel object recognition test, the open field test, and the Y-maze test, to assess the neuroprotective efficacy against neurotoxicity. Administration of C.C. (100 mg/kg, orally) for 21 days significantly mitigated $AlCl_3$-induced cognitive deficits, as evidenced by improved performance in behavioral tasks. Specifically, there was a significant increase in the percentage of spontaneous alternation in the Y-maze task ($p<0.05$). Additionally, the C.C. group showed notable improvements in anxiety and locomotor activity in the open field test ($p<0.05$) compared to the control group. These findings suggest that C.C. possesses neuroprotective properties and holds promise as a potential therapeutic agent for treating cognitive impairments.

Keywords: *Cognition; Cosmos caudatus and neurobehavioral*

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A comparative study of plasma and dried blood spot acylcarnitines in fatty acid oxidation disorders and organic acidurias

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Abstract

Analysis of blood acylcarnitines is used in the investigation of fatty acid oxidation disorders (FAOD) and organic acidurias. Dried blood spot (DBS) sample is regarded as the preferred sample for newborn screening programmes due to its certain characteristics. However, there is limited data on the correlation of acylcarnitines in plasma and DBS. This study aims to compare individual acylcarnitines as well as the profile between plasma and DBS in FAOD and organic acidurias. 55 paired plasma and DBS samples of known FAOD and organic acidurias were studied. Individual acylcarnitines from both matrices were analysed as their butyl esters by electrospray ionisation tandem mass spectrometry (ESI- MS/MS) in multiple reaction monitoring (MRM) mode. The similarities and differences in acylcarnitines concentrations and profile interpretations between plasma and DBS for individual diseases were described. Free carnitine elevations in patients with CPTl a deficiency were two times higher in DBS than plasma. Long-chain acylcarnitines elevations in patients with CACT and CPT2 deficiency were more strikingly demonstrated in plasma than in DBS. Long-chain hydroxylacylcarnitines elevations in MTP/LCHAD deficiency were equally detected on both matrices. Elevations of the main acylcarnitines were seen in both matrices of patients with propionic aciduria, glutaric aciduria type 1 and isovaleric aciduria. In methylmalonic aciduria, 3/19 (15.8%) patients had DBS C3-acylcarnitine within the reference range. DBS is better at detecting CPTla deficiency, while plasma is better at detecting CPT2/CACT deficiency. Both plasma and DBS are equally useful in the investigation of suspected LCHAD/MTP deficiency and common organic acidurias.

Keywords: *Acylcarnitine profile; dried blood spot; fatty acid oxidation disorders; organic acidurias and tandem mass spectrometry*

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Low anti-glutamic acid decarboxylase-65 and insulin autoantibodies in Type 1 Diabetes Mellitus patients of Eastern Nepal

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Abstract

There is limited data available on T1DM in the Nepalese population, and there are negligible reports on the antibody status in the T1DM of the Nepalese population. This study aimed to estimate the autoantibodies, namely glutamic acid decarboxylase-65 (GADA) and Insulin autoantibody (IAA) positivity and assess the serum vitamin D levels in Type 1 DM patients of eastern Nepal. This study was conducted among 54 patients diagnosed/suspected of T1DM attending the tertiary centre of eastern Nepal for a period of one year. Convenient sampling was used to recruit the patients. Serum GADA and IAA were estimated using a chemiluminescence immunoassay (CLIA). Quantitative variables were expressed as a mean and standard deviation. Fifty-four patients (36 Female and 18 male) were enrolled. The mean age of the patients was 22.44 ± 9.69 years. GADA positivity was present in 18.5% of the patients, and IAA was positive in 24.07% of the patients. The mean Vitamin D level was 22.53 ± 6.78 ng/ml in T1DM patients. Low vitamin D was associated with antibody (GADA and IAA) positivity in T1DM patients. Our study findings depict the prevalence of autoantibodies in T1DM patients of eastern Nepal (GADA= 18.5%; IAA= 24.07%), though lower than the studies done in other countries. The majority of the patients (63%) were vitamin D deficient. Hence, regular screening of the possible autoimmune disease in T1DM should be done for better patient care.

Keywords: *Autoantibodies; autoimmunity; GADA; IAA and Type 1 diabetes*

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