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ORAL PRESENTATION

OA1

Structure-Based Drug Discovery of Curcuminoid Analogues as Potential Antimycobacterial Drug Candidates for *Mycobacterium Tuberculosis* CYP121 Drug Target

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ABSTRACT

Introduction: Tuberculosis (TB) remains to be a serious global health issue due to the emergence of multidrug-resistant tuberculosis (MDR-TB) and total drug-resistant tuberculosis (TDR-TB). This raises the importance to develop novel drug candidates to combat the drug-resistant TB variants. CYP121 of *Mycobacterium tuberculosis* (*M. tb*) is considered a crucial target for the development of therapeutic agents to treat TB. This study focuses on uncovering new *M. tb* CYP121 inhibitors by performing ensemble docking using curcuminoid analogs based on the structure-based drug discovery principle. **Methods:** In this work, molecular dynamics simulation (MDS) was carried out on two different systems, i.e., CYP121 without ligand and CYP121 in complex with a potent azole inhibitor 69M using the AMBER16 suite of program. Then, all the generated trajectories were clustered into 70 ensemble conformations using an agglomerative hierarchical algorithm. Virtual screening of 328 curcuminoid compounds against 70 ensemble conformations of CYP121 was performed using EasyDock Vina software. **Results:** The best consensus of CYP121 inhibitors were selected based on the highest binding affinity value than its original ligand (-9.6 kcal/mol) and the interaction with CYP121 cofactor HEM which is substantial for catalytic activity of CYP121. We found out that the top ten curcuminoid consensus were mainly from sulfonamide and pyrazoline group of compounds which illustrated steady binding affinity values and great interactions with cofactor and surrounding residues at the CYP121 active site. The selected curcuminoid compounds were then screened for ADMET prediction. **Conclusion:** The best curcuminoid compounds are anticipated to introduce to MDS to study the behavior of CYP121 at both structural and residual levels and then synthesize to evaluate further potential biological activities. This study could serve as a preliminary study towards developing promising CYP121 inhibitors.

Keywords: Tuberculosis, CYP121, Drug Resistance, Ensemble Docking, Curcuminoid Compounds

OA2

Indole-2-carboxamides as New Anti-Mycobacterial Agents: Design, Synthesis, Biological Evaluation and Molecular Modeling Against mmpL3

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ABSTRACT

Introduction: Tuberculosis (TB), an airborne disease caused by *Mycobacterium tuberculosis*, has infected millions of people and been responsible for their deaths. **Methods:** Toward the anti-TB endeavor, the synthesis of total twenty-four indole-2-carboxamide derivatives as potent anti-TB agents have been carried out using CDI-mediated amidation. **Results:** The biological evaluation against H37Rv revealed compounds 5d, 5e and 5u with MICs in the range of 3.125-12.5 µg/mL using MABA assay. Further, compound 5u was tested against RAW 264.7 cell by MTT assay and showed 32% growth inhibitions. The structure activity relationship of the indole-2-carboxamides has been established for antimycobacterial activity. The physicochemical properties and ADMET parameters of the 5d, 5e and 5u using pKCSM and SwissADME revealed their suitability as promising drug candidates. Molecular docking studies using AutoDock Vina revealed binding of 5u with the catalytic site of mmpL3 (PDB ID: 6AJG). The MD simulations of the most active compound 5u using GROMACS 2020.1 revealed its stability at the protein active site. **Conclusions:** Further optimization of indole-2-carboxamies may reveal the potentiation of identified anti-mycobacterial drug candidates.

Keywords: Indole-2-carboxamides, Anti-TB Agents, mmpL3, ADMET Assay, Molecular Modelling

OA4

Plant-Derived Extracellular Vesicles: Investigating Potential Inhibitor of Cyclin A of Colorectal Cancer Through *In Silico* Molecular Docking Approach

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ABSTRACT

Introduction: Colorectal cancer (CRC) ranks as the second deadliest cancer globally, with limitations in chemotherapy effectiveness due to complications and the emergence of resistance and recurrence. Plant-derived extracellular exosomes and vesicles (PDEV) serve as an innovative drug delivery system, offering targeted transport of bioactive compounds, immunomodulation, and potential resistance alleviation while containing secondary metabolites with unexplored anti-CRC potential. This study employs *in silico* docking, including protein/nucleotide exploration, molecular docking, and binding energy calculations, to screen PDEVs metabolites for an anti-CRC inhibitor targeting Cyclin A (PDBID:6GUE) through ligand- and structure-based approaches. **Methods:** A comprehensive literature search in Scopus, Web of Science, and PubMed used "Plant-derived Extracellular vesicles or nanovesicles or exosomes and secondary metabolites" to identify PDEV secondary metabolites. QSAR and ADMET analyses determined PIC50 values and compound behavior. Selected compounds underwent molecular docking using Cb-doc (http://clab.labshare.cn/cb-dock/php/blinddock.php) to assess binding interactions with the target protein. **Results:** This study screened 59 citations, yielding 26 research articles. Only three mentioned compounds are found in PDEV. Sixteen compounds with PIC_{50} values below 10 were identified, but ADMET analysis indicated potential toxicity for some, leading to the selection of 8 compounds for molecular docking. Among them, ferulic acid (-7.3) was chosen due to its non-toxic profile according to ADMET and a PIC50 of 4.13, lower than other compounds. The drug-likeness assessment also adhered to Lipinski's Rule of Five. Molecular docking demonstrated ferulic acid's interaction with specific amino acids in the target protein, mirroring co-crystal findings. Consequently, ferulic acid emerged as a lead compound, exhibiting strong binding, favorable pharmacokinetics, and drug-like characteristics against 6GUE. **Conclusion:** The compelling findings underscore ferulic acid's potential as a promising candidate for further development as an anti-CRC agent, warranting rigorous *in vitro* and *in vivo* investigations.

Keywords: Extracellular vesicles, Exosomes, Nanovesicles, Cancer

OB1

TRF Adjuvant Improves Dendritic Cell Vaccine Efficacy in Mouse Model of Breast Cancer

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ABSTRACT

Introduction: Dendritic cells (DCs) are immune cells that can present antigens to T-cells and stimulate immune responses. Tocotrienol-rich fraction (TRF) from palm oil has been reported to have anti-cancer and immune-enhancing effects. **Methods:** In this study, TRF was used as an adjuvant to enhance the effectiveness of DC vaccines in treating mouse mammary cancer. **Results:** The results of the study revealed that early treatment significantly improved the prognosis of the mice with cancer. They also found that the tumours in the animals that were treated at the G1 stage had lower volume compared to those in the other groups. Interferon-gamma (IFN-γ) and interleukin-12 (IL-12) productions showed the highest level in the group exposed to the earliest vaccine therapy and combination with TRF. Similar pattern for tumour inhibition was observed in other groups. Furthermore, PD-1 and PD-L1 were found to be significantly down-regulated in the early treatment groups, compared to the delay treatment groups. The higher interactions of cell surface proteins (PD1 and PD-L1) elevate the progression of tumours in the tumour microenvironment. Therefore, early treatment inhibited the interaction of cell surface proteins in the tumour microenvironment. **Conclusion:** In conclusion, TRF can be used as an adjuvant to enhance tumour-specific immune response induced by DC-based vaccines in a syngeneic mouse model of breast cancer. Earlier treatment modality exposure to the mouse model warranted the best inhibition in tumour-bearing mice and increased higher anti-tumour immune response. Hence, DC-based vaccines together with TRF as an adjuvant may be clinically useful as a new immunotherapeutic approach towards cancers.

Keywords: TRF, Adjuvant, Dendritic Cell Vaccine, Breast Cancer, Immunotherapy

OB2

Cyclodextrin Inclusion Complex of Tetrahydrocurcumin (THC) Augments Solubility And *In Vitro* Anticancer Activity Against Colorectal Cancer

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ABSTRACT

Introduction: Tetrahydrocurcumin (THC), a hydrophobic polyphenolic bioactive substance extracted from turmeric, has been established as a natural anticancer agent. Unfortunately, its sparing solubility (approximately 1.3%) in water and its reduced systemic bioavailability has limited its efficacy. This study explores the use of an organic-based drug delivery approach via encapsulation to circumvent the pitfalls of THC's poor solubility and potentially improve its chemotherapeutic properties. **Methods:** An inclusion complex of THC with β-cyclodextrin (β-CD) at a molar ratio of 2:1 was formed and characterized using UV-vis spectroscopy, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The inclusion complex's solubility assessment and drug release study were evaluated and compared with pure THC. The anticancer effects of the inclusion complex on colorectal cancer cells (SW480 and HCT116 cells) were investigated by MTT assays, migration assays, Transwell invasion assays, Annexin-V/PI staining assays, and poly adenosine diphosphate-ribose polymerase (PARP) cleavage assays. **Results:** The inclusion complex displayed higher aqueous dispersion (65-fold) and its physiochemical characterization confirmed the successful formation of a β-CD inclusion complex encompassing a hydrophobic cavity. Through the presence of an inclusion complex, cell viability was potentially reduced with an SI value >10 while the apoptosis rate was increased ($p < 0.05$) in vitro. Additionally, the complexation further enhanced both anti-migration and anti-invasion capabilities in comparison to pure THC. Both formulations were consistent in terms of caspase 3 activation. **Conclusion:** These findings provide evidence of the potential use of this formulation in rendering THC and conceptually other hydrophobic agents with an improved chemotherapeutic efficacy against various malignancies.

Keywords: Tetrahydrocurcumin, Cyclodextrin, Inclusion Complex, Solubility, Colorectal Cancer

OB3

Erlotinib Loaded Lipidic Nanocarriers for Loco-Regional Therapy in Management of Oral Cancer: In-vitro and In-vivo Evaluation

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ABSTRACT

Introduction: As per the Globocan report of 2018, head and neck cancer ranks 2nd in terms of incidence in India. The carcinoma starts in squamous cells and leads to development of oral squamous cell carcinoma (OSCC) in more than 80% of oral cancers. Conventional treatment strategies have several side effects mandating the need for novel drug delivery. Recently, local site-specific delivery in oral cavity has been explored to treat cancerous lesions and those detected at early stages. This helps in reducing dose required and decreasing side effects without compromising therapeutic efficacy. The current research evaluates the feasibility of Erlotinib (ERB) loaded lipidic nanocarrier (ELNC) gel for treatment of chemically induced oral cancer in a rat model. **Method:** ELNC was prepared using hot homogenization technique with size reduction by high-pressure homogenization. The ELNC was characterized for PS, PDI, %EE, ZP and assessed for in-vitro cytotoxicity in KB-3-1 cell line. The nanocarriers were freeze-dried using mannitol as cryoprotectant and characterized further using DSC and XRD. Furthermore, the developed nanocarriers were loaded in carbopol gel and administered locally at the site of oral cancer in rat to evaluate in-vivo efficacy and cytokine levels. **Results:** ELNC sizes were in the range between 350nm to 380nm, PDI less than 0.35 and %EE up to 75%. The particles were stable with ZP up to -25mV. Freeze dried ELNC characterized by DSC and XRD revealed drug present in amorphous form inside LNC. Cytotoxicity studies showed potent anti-cancer effects with IC50 values of plain ERB (558.94 ±103.6nM) and ELNC (686.25 ± 44.8 nM). **Conclusion:** Pharmacological efficacy studies revealed, nanoformulation could decrease tumor size as compared to plain drug. Furthermore, decrease in cytokine levels, IL-6, IL-1β and TNF-α were observed with ELNC as compared to plain indicating decreased inflammatory conditions at the site of tumor with ERB loaded nanocarrier.

Keywords: Erlotinib, Oral Cancer, Lipidic Nanocarrier, Cytokine, High Pressure Homogenization

OB4

The Effects of Polyethyleneimine (PEI) Combined with Cisplatin on Cytotoxicity, Colony Formation, and Apoptosis Mechanisms Against Triple-Negative Breast Cancer Cell Lines

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ABSTRACT

Introduction: Triple-negative breast cancer (TNBC) is a distinctive subtype characterized by a lack of three important receptors and molecular pathways. Conventional chemotherapy options like cisplatin is ineffective against TNBC. Recent studies have focused on the potential use of polyethyleneimine (PEI) as a co-delivery system for anticancer drugs to improve therapeutic efficacy. Acting as a transfection agent, PEI aids nuclear DNA binding and avoids endosomal barrier. PEI/cisplatin combination represents a promising strategy to improve cisplatin's efficacy in treating TNBC. **Methods:** 7 dfferent types of PEI were used in this research. Water Tetrazolium (WST)-1 assay, colony-forming assay, and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) were performed to determine cell viability and apoptosis mechanisms. **Results:** All PEI/cisplatin combinations increased cytotoxicity and reduced colony-forming ability. PEI with molecular weights of 600 and 750 kDa were excluded from colony-forming assay and RT-qPCR analysis as excessively high molecular weights caused irregular cell death. CASP3 and TP53 gene expressions were upregulated, meanwhile BCL2 was downregulated following treatments. Low molecular weights PEI (0.8, 1.3, 2.5, and 5 kDa) at concentrations of 5 and 10 ppm showed potential when combined with cisplatin. However, the results were not significant, likely due to low concentrations used. PEI with higher molecular weights (25 kDa) showed lowest cell survival rate, a 15.7% decrease at 25:5 ppm PEI/cisplatin, and zero colony counts. Overexpresion of BCL2 by 59.4-fold was observed at a concentration of 10:5 ppm PEI/cisplatin compared to cisplatin alone. **Conclusion:** The PEI/cisplatin combination improves in vitro efficacy against TNBC. PEI demonstrated unique properties in inducing cell death through variation in molecular weights, concentration, and structural characteristics. PEI with higher molecular weights exhibited cytotoxicity limitations that might lead to non-apoptotic cell death mechanisms. Further investigations including advanced nanoparticle formulation, assessing PEI toxicity and examining its effects on molecular regulation are necessitated.

Keywords: Triple Negative Breast Cancer, Cisplatin, Cationic Polymer, Polyethyleneimine, Drug Delivery

OC1

Alkylated Indole Hybrids: Synthesis and RTU Formulation Development for Treatment of Cancer

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ABSTRACT

Introduction: Indole heterocyclic compounds have shown diverse biological activities, making them privileged scaffold for drug development. In this study, we focused on design, synthesis and formulation of hybrid indole compounds containing 2-chloro-N-(2-chloroethyl)-N-methylethanamine as promising anti-cancer agents. **Methods:** In-silico study was conducted, wherein a library of 1500 indole compounds were designed, screened based on electrostatic properties and shape similarities using TorchLite 10.5.0. The eXtened Electron Distribution (XED) pattern was studied and compared with reference listed drugs (RLDs). The binding affinity of designed ligands to DNA target (PDB ID: 1AXL), was studied using Glide program. Multivariate analysis, ECFP-6 fingerprints and scatter plots were used which demonstrated closeness of designed compounds with RLDs. Alkylated indole derivatives were synthesized from substituted indoles with oxalyl chloride and replacement of chloride using diethanolamine, with further chlorination using thionyl chloride. The compounds were effectively characterized using physicochemical properties and spectral analysis. In vitro MTT assay was performed to evaluate anti-cancer potential of synthesized compounds. Stable and cost-effective Ready To Use (RTU) formulation of AGSPBM1006 indole alkylating agent was developed using 95% dehydrated alcohol as vehicle. The stability testing (accelerated condition: 40°C/75%RH) and assay of related substances present in formulation were studied using validated RP-HPLC method. **Results:** In the in-silico study, designed compound AGSPBM1006 showed lowest binding energy (-9.130) when compared to Bendamustine RLD (-6.232) and is capable of binding with minor grove in the active site. The compound demonstrated comparable IC50 values (2.20 and 3.97) to RLD bendamustine (2.31 and 4.35) against HEPG2 and MCF-7 cell lines respectively. Furthermore, stable RTU formulation of AGSPBM1006 was formulated using QbD approach with critical process parameters optimized through DoE. **Conclusion:** The alkylated Indole hybrid compound AGSPBM1006 identified through rigorous in-silico studies with promising anti-cancer potential can serve as a lead for further investigations for development of novel anti-cancer agents.

Keywords: Indole derivatives, Anticancer, In-silico studies, Alkylating agents, RTU injection

OC2

In silico Discovery of RIOK3 Inhibitors Against Pancreatic Ductal Adenocarcinoma via Molecular Docking, Molecular Dynamics Simulations, and ADMET Prediction

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ABSTRACT

Introduction: The highly metastatic pancreatic ductal adenocarcinoma (PDAC) is an exceptionally aggressive malignant disease. It has been demonstrated that PDAC invasion and metastasis has shown a strong correlation with RIO Kinase 3 enzyme (RIOK3) activity. RIOK3 promotes the invasion and metastasis of PDAC cells by stabilizing Focal Adhesion Kinase (FAK) protein expression and increasing its phosphorylation. Thus, targeting RIOK3 offers a novel treatment approach. In the field of protein prediction, ab initio techniques have commonly relied solely on physiochemical interactions for potential determination. The revolutionary AlphaFold2, a neural network-based model created by Deep Mind, on the other hand, has ushered in a new age in protein structure prediction. In this study, drug repurposing approach will be used to identify therapeutic inhibitor against RIOK3. **Method:** The structure of the RIOK3 was predicted via I-TASSER server and Alphafold 2. The accuracy of these predictions was evaluated using Saves Server. Subsequent docking studies were conducted with FDA-approved drugs using AutoDock Vina. The pharmacokinetic and pharmacodynamic properties were examined using SwissADME. Molecular dynamics simulation and in vitro experiments were used for validation. MTT assays were performed to assess the effect of the RIOK3 inhibitors on cell growth and survival. **Result:** Our study results identified the top five molecules as possible RIOK3 inhibitors, with binding energies ranging from -11.8 to -10.8 kcal/mol. **Conclusion:** The in silico and in vitro experiments conducted on these compounds support their potential as key anticancer candidates. The present research provides valuable structural insights that can contribute to further comprehend PDAC therapy strategies by targeting RIOK3.

Keywords: Pancreatic Ductal Adenocarcinoma, AlphaFold2, Ab Initio, RIOK3, AutoDock Vina

OC3

Anticancer Activity of *Annona muricata* Leaf Extract and Fractions Againts MCF-7 cells

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ABSTRACT

Introduction: Breast cancer is the most prevalent cancer among women globally, with a growing annual incidence rate. Soursop, scientifically known as *Annona muricata L* (*A. muricata*), is a traditional medicinal plant renowned for its potential as an anticancer remedy. This study aimed to clarify the cytotoxic effect and mechanism of *A. muricata* leaf extract and fractions. However, more studies are still warranted. **Methods:** In this research, we prepared ethanol extract and three solvents fractions (ethyl acetate, n-hexane, and water) from *A. muricata* leaves. The anti-proliferative and cytotoxic effects of these extract and fractions were evaluated on MCF7 breast cancer cells and CV1 normal kidney cells. Observation of cell morphology was performed by staining using mixture of propidium iodide and 4β,6-diamidino-2-phenylindole, indicating an ongoing process of apoptotic cell death in MCF7 cells. To elucidate the apoptotic cell death mechanism, we assessed the mRNA expression of key components in the caspase cascade, including caspase-9, caspase-3, PARP-1, and the anti-apoptotic protein Bcl-2. **Results:** The ethanol extract, ethyl acetate, n-hexane, and water fractions derived from *A. muricata* leaves exhibited IC_{50} values of 5.3, 2.86, 3.08, and 48.31 µg/mL, respectively, against MCF7 cells, while showing no toxicity in CV1 cells. Exposure to *A. muricata* leaf ethanol extract and the ethyl acetate fraction induced distinct morphological changes in MCF7 cells within 6 hours. These changes included membrane and nuclear alterations indicative of apoptosis. The mechanism underlying this potent cytotoxic activity in MCF7 cells was linked to a decrease in the expression of Bcl-2 mRNA, alongside an increase in caspase-9 and caspase-3 mRNA expressions. **Conclusion:** The leaves of the *A. muricata* medicinal plant contain compounds that, upon extraction, exerted highly effective anticancer activity against MCF7 breast cancer cells by inducing apoptotic cell death.

Keywords: Soursop, *Annona muricata* L., MCF7 Breast Cancer Cell, Cytotoxicity, Caspase-Cascade

OC4

New Dimers of Dipyridothiazines with Anticancer Activities

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ABSTRACT

Introduction: Phenothiazines are classes of heterocyclic compounds with wide spectrum of biological properties especially in the mental disorders. Recent reports have shown promising anticancer, antiplasmid, antibacterial, anti-inflammatory and immunosuppressive activities of both classical and new derivatives of phenothiazines. Previously synthesized library of dipyridothiazines have demonstrated interesting antiproliferative, anticancer, antioxidant and immunosuppressive activities. The current project aims to obtain new derivatives of dipyridothiazines, which are expected to possess promising anticancer activity. **Methods:** New twelve dimer of dipyridothiazines were synthesized effective reactions of involving dipyridothiazines with selected linkers, in the presence of sodium hydride, and dimethylformamide (DMF). The structures of the new compounds were determine using NMR and 2D NMR spectroscopy (COSY, ROESY, HSQC, HMBC) as well as mass spectrometry (HR MS). All compounds were then subjected to biologically evaluation for their anticancer activity against colon (SW480) and breast cancer cell lines (MCF7). **Results:** Promising results were obtained, which encourage further research to explore the mechanism of anticancer activity and cytotoxicity. **Conclusion:** The research study underscores the potential importance of new dimers from dipirydothiazines in the search for targeted anti-cancer agents.

Keywords: Dipyridothiazines, Structural Analysis, Anticancer Action

OD1

Uncovering the Potential of Androgen Receptor as A Therapeutic Biomarker for Triple Negative Breast Cancers

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ABSTRACT

Introduction: Triple Negative Breast Cancer (TNBC) is a dreadful molecular subtype due to its aggressiveness and heterogeneity. The presence of Androgen Receptor (AR) signaling plays a pivotal role in tumor progression and metastasis. The anti-androgens are exhibiting better responses in treating prostate cancers. Luminal-Androgen Receptor (LAR+TNBC) is one of the TNBC subtypes in which AR is present. In this study, it has been envisaged that targeting AR with AR antagonists for the treatment of TNBCs would be a better therapeutic regimen. We also identified the incidence of Androgen Receptor positivity in TNBC patients of Western India. **Methods:** The effectiveness of AR antagonists (Bicalutamide & Enzalutamide) were tested using in-vitro studies. Assessment of cell viability was determined by MTT assay in Human Breast cancer cell lines MDA-MB-453 (LAR^+TNBC), MDA-MB-231 (TNBC), and MCF-7 (ER^+PR^+). The genotypic and phenotypic expressions of AR were analyzed via qRT-PCR and western blotting, respectively. Fifty TNBC patients were enrolled at The Gujarat Cancer and Research Institute, Ahmedabad, and AR expression was determined by Immunohistochemistry. **Results:** Based on the cell IC-50 values, both AR antagonists showed significant response in both TNBC cell lines but not significant in MCF-7. Moreover, Enzalutamide showed a better response in both TNBC cell lines than Bicalutamide. In the case of the study in TNBC patients, AR positivity was 18%, and, the incidence was correlated with clinicopathological prognosticators and disease status. **Conclusion:** The incidence of AR positivity in TNBC patients is about 20% and the preliminary in vitro studies showed that both AR antagonists can be explored as a therapeutic regimen for TNBCs. However, further, the role of AR signaling and its underlying mechanisms will be explored for a better understanding of AR as an independent biomarker for TNBCs.

Keywords: Androgen Receptor, Triple-Negative Breast Cancer, Bicalutamide, Enzalutamide, AR Antagonists, Dihydrotestosterone

OD2

Factors Related to Hematotoxicity, Hepatotoxicity, and Nephrotoxicity in Acute Lymphoblastic Leukemia during the Induction Phase at Dr. Hasan Sadikin General Hospital, Bandung, from 2020 to 2023

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) in children makes up approximately 70% of all cancer cases in Indonesia. The primary treatment for childhood ALL involves chemotherapy with more complex regimens that significantly contribute to adverse drug effects (ADRs). This study aims to evaluate the severity of ADRs and determine the association between patient characteristics and drug toxicity during the induction phase. **Methods:** An observational cohort study was conducted using Indonesian Pediatric Cancer Registry (IP-CAR) by including children diagnosed with ALL. Data were collected retrospectively from 2020 to early 2023. Hematotoxicity, hepatotoxicity, and nephrotoxicity were defined based on Common Terminology Criteria for Adverse Events (CTCAE v.5). Uni and multivariate statistical analysis was conducted to estimate the association between factors and the outcomes. **Results:** A total of 85 subjects were included. The most frequently observed ADRs were decreased neutrophil counts (67.95%), decreased platelet counts (59.52%), and anemia (30.59%). Significant differences were found between sex and ADR anemia, age and decreased neutrophil count, risk stratification and decreased neutrophil count, as well as BMI and increased alanine transaminase (ALT) serum levels (p=0.049; p=0.004; p = 0.037; p = 0.022, respectively). The results of the multivariate analysis indicate that higher age is linked to a protective effect against reduced neutrophil count (OR = 0.85; 95% CI 0.75–0.96; p = 0.011) and decreased platelet count (OR = 0.89; 95% CI 0.81-0.99; p = 0.049). Moreover, an increase in BMI also demonstrates a protective association with elevated serum ALT levels (OR = 0.43; 95% CI 0.2-0.92; p = 0.029). **Conclusion:** The occurrence of drug toxicity during the initiation period is associated with younger age and lower Body mass index (BMI) in pediatric ALL patients.

Keywords: Acute lymphoblastic leukemia, Children, Adverse drug reaction, Chemotherapy, Toxicity

OD3

Insufficient Induction of Cytotoxic Agents Affects the Expression of the TGF-β-related Gene in Breast Cancer Cell Lines

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ABSTRACT

Introduction: Insufficient intracellular concentration of cytotoxic agents alters drug sensitivity in breast cancer. Transforming growth factor-β signalling pathway affects transcriptional activations of target genes that regulate epithelial-mesenchymal transition (EMT) and drug sensitivity. This experiment investigates the effect of insufficient induction of cytotoxic agents on regulating TGF-β-related gene expressions in breast cancer cell lines. **Methods:** The cytotoxicity assay was performed before and after two cycles of induction with doxorubicin and cisplatin in MDA-MB-231 and MCF-7 cells. Expression levels of *TGIF1, SNA1*, and *PMEPA1* were quantified in both the parental cells and cells subjected to two cycles of induction. **Results:** A significant increase in the IC_{50} values for both doxorubicin- and cisplatin-inductions were observed in MDA-MB-231 cells, but not MCF-7 cells. In MDA-MB-231 cells, TGIF1 and SNA1 expression levels were upregulated following induction with both doxorubicin and cisplatin. In contrast, in MCF-7 cells, this upregulation was only evident after doxorubicin induction. Interestingly, *PMEPA1* expression level decreased after induction with both doxorubicin and cisplatin in MCF-7 cells. However, this effect was only observed with cisplatin induction in MDA-MB-231 cells. **Conclusion:** Insufficient concentration of cytotoxic agents was found to regulate the expression levels of genes related to TGF-β and EMT regulators, including *TGIF1, SNA1*, and *PMEPA1* in breast cancer cells. *PMEPA1* exhibited an opposite regulation manner compared to *TGIF1* and *SNA1* in distinct cell types and in response to various cytotoxicity agents. This suggests the dynamic interplay between these genes in different cellular contexts and with different cytotoxicity agents.

Keywords: Insufficient Induction, Doxorubicin, Cisplatin, Breast Cancer, TGF-β Related Genes

OE1

Exploring Nature's Toolbox: Isolation of Plant-Based Extracellular Vesicles

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ABSTRACT

Introduction: Extracellular vesicles (EVs), a diverse group of membrane-enclosed nanoparticles, are important in cellular communication, shuttling proteins and RNA between prokaryotic and eukaryotic cells. These naturally derived vesicles offer distinct advantages as drug carriers over synthetic counterparts. While exploration of plant-derived EVs have only gained recent attention, their potential in biomedical applications is significant. The initial hurdle lies in successful isolation, a critical first step explored in the present study. **Methods:** The present study employed fruit juice for EV purification through an optimized hybrid method of modified centrifugation-ultracentrifugation and use of size exclusion column purification. The physical attributes of EV (size, shape, and purity) were further characterized using transmission electron microscopy (TEM), zetasizer, and nanoparticle tracking analysis (NTA) techniques. **Results:** The results demonstrated successful isolation of plant EVs by integrating differential centrifugation and size exclusion chromatography. TEM imagery revealed unique concave structure and bilayer composition, distinguishing exosomes (25-100 nm) from microvesicles (>300 nm). Zetasizer analysis confirmed the presence of particles beyond 300 nm, with zeta potentials measuring -17 mV. NTA quantified EV concentration at 4.04 x 10^9 particles/ml. **Conclusion:** The scarcity of comprehensive investigations centered around isolation of plant-derived EVs underscores the shortage of references and standardized protocols. The present study proposed an optimized isolation method combining optimised ultracentrifugation and additional purification step. The outcome was the attainment of pristine cup-shaped vesicles with higher concentration and smaller size, thus surmounting initial limitations. This study potentially contributes to the foundational understanding of plant-derived EVs and offers insight into their potential utility as effective drug carriers.

Keywords: Extracellular Vesicles, Plant-derived EV, Ultracentrifugation, Size exclusion chromatography, Exosomes

OE2

Design, Optimise, Standardise and Validate In Vitro Human Blood-Brain Barrier Models

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ABSTRACT

Introduction: The brain endothelial cells, pericytes, and astrocytes communicate with each other to regulate the properties of blood-brain barrier (BBB). They share a common basement membrane to mimic the anatomical, functional, and microenvironmental situation in vivo. **Methods:** Construction of monoculture, coculture, and triculture models were performed using human microvascular endothelial cells (hCMEC/D3), human brain vascular pericytes (HBVP), and normal human astrocytes (NHA). Human extracellular matrix (ECM) proteins combinations were optimised using collagen-IV (10 µg/ml), fibronectin (5 µg/ml), laminin (5 µg/ml), agrin (1 µg/ml), and perlecan (10 µg/ml) at the ratios (100:100:100:100:100; 50:20:20:5:5; 56:18:18:4:4; 62:16:16:3:3, v/v, %). Transepithelial/ transendothelial electrical resistance (TEER), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were subsequently analysed. **Results**: Using 1 µm-translucent-PET in the thin-layer protocol, only at a ratio 56:18:18:4:4 is statistically significant ($P<0.0016$), TEER was 30±0.94 ohm*cm^2. The hCMEC/D3 seeding density at 6x10^4 cells/ml generated the highest TEER value at day 6. The hCMEC/D3 monoculture model showed a peak TEER of 36±0.50 ohm*cm^2 on day 6. TEM of endothelial cells showed close apposition to each other, with electron-dense areas at points of contact between adjacent cells, likely indicating the presence of adherent and tight junction complexes. SEM of hCMEC/D3 cells showed a confluent cell monolayer composed of closely apposed cells. HBVP seeding density at 4x10^4 cells/cm^2 in hCMEC/D3-HBVP coculture models produced the highest TEER (41±0.97 ohm*cm^2) at day 6 ($p<0.0001$). NHA seeding density at 4x10^4 cells/cm^2 in hCMEC/D3-HBVP-NHA triculture-models produced the highest TEER (103±0.97 ohm*cm^2) at day 6 ($p<0.0001$). The hCMEC/D3-HBVP-NHA triculture-model produced the highest statistically significant TEER ($p<0.0001$) 103±0.97 ohm*cm^2 compared to the hCMEC/D3-HBVP coculture-model 41±0.49 ohm*cm^2 and the hCMEC/D3 monoculture-model 35±0.97 ohm*cm^2. **Conclusion:** The designed, optimised, standardised, and validated in vitro human BBB models were successfully constructed to achieve intact, structural, and functional BBB barrier formation.

Keywords: In vitro Human BBB Model, Human ECM, Transwell Model, Optimisation, Validation

OE3

Identification and Comparison of Phytoconstituents of Oil, Leaf and Rhizomes of *Elettariopsis curtisii* via GCMS and *In Vitro* Antioxidant Extracts Analysis

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ABSTRACT

Introduction: *Elettariopsis curtisii* is a medicinal plant that belongs to the family Zingiberaceae and is mostly found in Peninsular Malaysia, Thailand, and Borneo. *E. curtisii* grows from rhizomes on humid and wet ground in the shade. It is also called Pokok Kesing or Pokok Pepijat because it has a pungent smell that is like a stinking bug. It shows many pharmacological activities. Previous studies reported the phytoconstituents of rhizome oil only, with very little information available about the metabolites present in other plant parts of *E. curtisii*. Thus, the aim of the study was to analyse and identify the metabolites present in oil, leaf, and rhizomes, and consequently, in vitro antioxidant activity analysis via the DPPH method. **Method:** The extraction of volatile oils from rhizomes and leaves was carried out via hydro distillation using Clevenger apparatus and simple cold maceration using methanol as a solvent. All samples were analysed by GCMS, and phytoconstituents were identified based on the comparison in the NIST08 library. The antioxidant evaluation of rhizomes and leaf extracts was carried out via a DPPH assay. **Results:** There were 12 (rhizome oil), 4 (leaf oil), 46 (methanol rhizome extract), and 9 (methanol leaf extract) compounds detected via GCMS analysis. The most abundant phytoconstituents were aldehydes, with (E)-2-decenal (68.39%) in rhizome oil and (E)-2-octenal in leaf oil, and methanol rhizome extract and methanol leaf extract with values of 41.11%, 40.68%, and 54.14%, respectively. Antioxidant results show that the methanol leaf extract is found to be more potent than the methanol extract of the rhizome. **Conclusion:** We concluded that phytoconstituents identified in the methanolic leaf extract of *E. curtisii* could be a potential source for antioxidant activity. Further isolation and identification of potent, pure compounds can be made from the methanolic leaf extract of *E. curtisii*.

Keywords: *Elettariopsis curtisii*, GCMS Analysis, Antioxidant Activity

OE4

Comparison of Green Technology Supercritical Fluid Extraction with Conventional Extraction Technique for Betulinic Acid, A Potential Anticancer Agent, from *Dillenia indica* Linn. Bark using Experimental Design

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ABSTRACT

Introduction: Betulinic acid has promising anticancer activity and present in appreciable quantity in *Dillenia indica* Linn bark commonly known as 'Karmal'. For the separation of betulinic acid from the bark of *Dillenia indica* Linn, supercritical fluid extraction was examined and compared to conventional extraction methods. The present investigation's goal was to isolate betulinic acid in pure form and compare ancient and novel extraction procedure. **Methods:** Soxhlet extraction followed by separation with column chromatography was applied and yield obtained was compared with super critical fluid extraction. The optimum extraction conditions for betulinic acid were also investigated by supercritical fluid extraction (SFE) using response surface methodology based on 3 factor 3 level Box-Behnken experimental design. Experiment design for SFE was performed to evaluate the combination effect of three independent variables like co-solvent concentration, temperature (35-60°C) and pressure (100-200 bar). Betulinic acid obtained was quantified using developed High Performance Thin Layer Chromatography method. **Results:** Analysis of variance showed that the "p-value" of SFE 0.0141 which indicate that models were statistically significant ($p<0.05$). and "coefficient of determination" (R^2 value) is 0.94 which indicate that the model showed the goodness of fit. The optimum conditions for the efficient super critical fluid extraction of betulinic acid were co-solvent concentration 10%, extraction temperature 50°C and extraction pressure 200bar. **Conclusion:** Soxhlet extraction technique followed by column chromatography is advantageous because SFE require specialized equipment while the conventional method required more amount of chemicals and reagents. Application of chemo metric tools in optimization of methods is that it reduces the number of experiments, reagent consumption and tedious laboratory work. According to the results of the SFE experimental design, the co-solvent concentration and extraction pressure have the largest influence and improve the percentage yield, while the extraction temperature has a negative impact and decreases the total yield.

Keywords: Betulinic Acid, *Dillenia indica* Bark, Green Technology, Experimental Design

OG1

Anticancer Potential of Angucyclone Polyketides from *Streptomyces carlesensis* Strain DSD011 against Human Lung, Colorectal, Breast, and Ovarian Cancer Cell Lines

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ABSTRACT

Introduction: Cancer morbidity and mortality have been increasing at an alarming rate, urging drug discovery programs to discover potential anticancer compounds from natural products. The Philippine marine sediments harbors bacteria that produce metabolites with potent bioactivities, especially those that belong to the genus *Streptomyce*s. This study aimed to determine the antiproliferative activity of *Streptomyces carlesensis* DSD011T isolated from the marine sediments of Islas de Gigantes, Iloilo, Philippines against four cancer cell lines. **Method:** *Streptomyces carlesensis* DSD011T extract was purified by HPLC to yield semi-pure and pure compounds, which were characterized by spectroscopic analysis (HRMS and NMR). These compounds were tested using MTT assay against cells of human colorectal cancer (HCT-116), human ovarian cancer (A2780), human breast cancer (MCF-7), and human lung cancer (A549) at final concentrations of 100 and 10 µg/mL. The positive controls used were 5-fluorouracil, cisplatin, tamoxifen, and doxorubicin hydrochloride for HCT-116, A2780, MCF-7, and A549, respectively. **Results:** Results showed that several fractions demonstrated cancer cell growth inhibitory activity. Specifically, fraction DSD011G-6I2H41 exerted the most notable antiproliferative activity at both testing concentrations. At 100 µg/mL, the fraction showed 85%, 92%, 83%, and 78% growth inhibition against HCT-116, A2780, MCF-7, and A549, respectively. Conversely, the fraction demonstrated 84%, 97%, 92%, and 69% growth inhibitory activity at 10 µg/mL against HCT-116, A2780, MCF-7, and A549, respectively. The MS and NMR analysis indicate that these fractions contain angucycline polyketide compounds, known to have anticancer activities. Additionally, unpaired t-test with Welch correction showed that there is no significant difference ($p \geq 0.05$) between the bioactivities exhibited by fraction DSD011G6I2H41 at concentrations 100 and 10 µg/mL, indicating that the tested fraction has high antiproliferative activity even at a low testing concentration. **Conclusion:** *Streptomyces carlesensis* DSD011T isolated from the marine sediments of Islas de Gigantes is a promising candidate for anticancer drug discovery.

Keywords: Anticancer, Drug Discovery, Marine Sediment, *Streptomyces carlesensis*

OF1

Effects of Nipa (*Nypa fruticans Wurmb.*) Vinegar on Biochemical Parameters of Type 2 Diabetes Rat Model

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ABSTRACT

Introduction: Nipa palm vinegar had shown promising blood glucose lowering effects in type 1 diabetes rat model. Thus, this study is designed to further explore the possible antihyperglycemic effect of nipa palm vinegar in type 2 diabetes mellitus. **Methods:** Nipa palm vinegar's aqueous extract was prepared using liquid-liquid extraction. Type 2 diabetes model was induced in *Sprague Dawley* rats using a high-fat diet and low-dose streptozotocin (30 mg/kg body weight). The diabetic rats were treated with three doses of aqueous extract (250, 500, and 1000 mg/kg body weight) for 28 days. Analysis of glucose, insulin, incretin hormones (GLP-1 and GIP), liver enzymes (AST and ALT), DPP4, and lipid profiles (TC, HDL, TG, and LDL) were carried out. **Results:** Single administration of the extract significantly reduced blood glucose levels respectively at the dose of 250 mg/kg, 500 mg/kg, and 1000 mg/kg as compared to the negative control at $p<0.05$. After 28 days of treatment, there were significant differences between the 1000 mg/kg dose and the diabetic group at $p<0.05$ in decreasing the blood glucose and cholesterol levels. A significant ($p<0.05$) increase in the insulin level was observed in the groups treated with NPV at the doses of 1000 mg/kg and 250 mg/kg. In addition, the dose of 250 mg/kg lowered lipid content (TG and LDL) and the level of ALT enzyme in the liver. **Conclusion:** The overall study result has demonstrated the potential of nipa vinegar aqueous extract in normalizing biochemical parameters related to type 2 diabetes mellitus.

Keywords: Nipa Palm Vinegar, Type 2 Diabetes, Streptozotocin, Biochemical Parameters

OF2

Dexamethasone Reverses *Aspergillus fumigatus*-Induced Severe Asthma by Reprogramming Pulmonary Metabolism

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ABSTRACT

Introduction: In difficult-to-treat conditions such as cancer and autoimmune diseases, several drugs have demonstrated that reprogramming cellular metabolism translated to anti-inflammatory effects. Corticosteroids are mainstay therapeutic agents for patients with the predominant *Aspergillus fumigatus* (Af)-induced severe eosinophilic asthma subtype. Yet, its immunometabolic modulatory mechanism in severe asthma remains unknown. Unravelling this relationship would unearth novel targets that could support candidate drug development efforts aimed at advancing the management of severe asthma. **Methods:** Mice were exposed to repeated intratracheal administrations of Af aeroallergen before therapeutic intervention with dexamethasone (1 mg/kg). Extent of airway inflammation was determined through total airway cell counts, histological analysis, and levels of pro-inflammatory cytokines. Real-time shifts in metabolism were measured using Seahorse bioanalyzers in lung single cells and primary eosinophils. Expression of rate-limiting metabolic enzymes were studied via Western blotting and immunofluorescence. Statistical significance was determined by applying one-way ANOVA followed by a Dunnett's test. **Results:** Dexamethasone markedly reduced Af-induced airway inflammation as demonstrated by a significant reduction in airway eosinophils ($p<0.0001$), extent of peribronchial infiltration ($p=0.0013$), and levels of pro-inflammatory cytokines interleukin (IL)-4 ($p=0.0043$), IL-13 ($p=0.0473$), and eotaxin-1 ($p=0.0014$). This was accompanied by a suppression in glycolytic activity and key glycolytic enzymes, such as the terminal glycolytic enzyme lactate dehydrogenase, in both lung single cells and activated ($CD69^+CD80^+$) primary eosinophils. Dexamethasone's anti-inflammatory effects were further accompanied by an overall reduction in glutaminolysis, shown by a reduction in glutaminase ($p=0.001$) and arginase 2 ($p=0.0049$), and fatty acid synthesis, which was demonstrated by a reduction in the palmitate-producing fatty acid synthase enzyme ($p=0.023$). Notably, the reduction in airway eosinophils following dexamethasone treatment was accompanied by an upregulation in eosinophil mitochondrial proton leak ($p=0.0037$). **Conclusion:** Targeting dysregulations in lung glycolysis, glutaminolysis, and fatty acid synthesis, or inducing eosinophil mitochondrial proton leak could be emerging anti-inflammatory strategies against severe asthma.

Keywords: Eosinophil, Metabolic Reprogramming, Inflammation, Steroid, Lung

OF3

Nimbolide Alleviates Insulin Resistance through Increased Glucose Uptake and Activation of Glucose Transporter 4 (GLUT4) in L6 Myoblasts

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ABSTRACT

Introduction: *Azadirachta indica* (Neem) is a deciduous plant that is native to countries in South Asia including Malaysia. This plant has been widely reported for its pharmacological properties in treating diabetes, neurological disorders, and stomach ulcers. Our previous study showed that the crude extract of *A. indica* was able to prevent the formation of advanced glycation end products (AGEs) via the modulation of aldose reductase (ALR2) and glyoxalase 1 (GLO1) enzymes where nimbolide, a limonoid triterpene, was identified as a major compound through liquid chromatography-mass spectrometry (LC-MS). This study aimed to determine the antidiabetic potential of nimbolide through its glucose uptake ability in L6 myoblasts using glucose transporter 4 (GLUT4). **Methods:** Differentiated L6 myoblasts were treated for 24 hours with 1 mM metformin (positive control) and nimbolide (1, 5 and 10 µM) before performing the glucose uptake assay using the Promega Glucose Uptake-Glo™ Assay Kit and an ELISA test to determine the protein expression of GLUT4 in these cells. The rate of glucose uptake was calculated upon reading the luminescence signals from the glucose uptake assay whereas the GLUT4 concentration was extrapolated from the ELISA standard curve. **Results:** Nimbolide was able to promote glucose uptake in L6 myoblasts in a concentration-dependent manner. This was confirmed with the rate of glucose uptake where cells treated with 10 µM nimbolide was the highest. The ELISA analysis showed that nimbolide was able to stimulate GLUT4 in a concentration-dependent manner, suggesting that the increase in the rate of glucose uptake is associated to the increased protein expression of GLUT4 in the cells. **Conclusion:** Nimbolide showed promising results of promoting glucose uptake in L6 myoblasts through GLUT4, suggesting its potential as a therapeutic compound in treating insulin resistance and diabetes.

Keywords: Nimbolide, *Azadirachta indica*, Glucose Uptake, Glucose Transporter 4, Insulin Resistance

OF4

In-vitro Alpha-Amylase Inhibitory Activity of Selected Medicinal Plants Used by the Sundanese Community for Managing Diabetes

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a complex, multifactorial disease which demands multiple therapeutic approaches. It is characterised by high blood glucose levels resulting from insufficient insulin production or impaired insulin functionality, hindering glucose uptake by cells. Ethnomedicine and ethnobotany research has indicated that, prior to the discovery of insulin and modern blood glucose lowering medications, traditional herbal remedies were used for diabetes management. The present study aims to evaluate the alpha-amylase inhibitory activity of selected medicinal plants traditionally used to manage diabetes within the Sundanese community in West Java, Indonesia. **Methods:** Dried leaves of *Moringa oleifera* L., *Physalis angulata* L., and *Annona muricata* L. were individually extracted using 70% ethanol. The α-amylase inhibition assay was performed using human α-amylase inhibitor screening kit. Total phenolic and flavonoid content analyses were determined spectrophotometrically using Folin-Ciocalteu's reagent and aluminium chloride ($AlCl_3$), respectively. **Results:** The study revealed that the highest inhibition of α-amylase activity (19.3%) was achieved by the *A. muricata* leaves extract at a dose of 500 µg/ml. The analysis of total phenolic and flavonoid contents in *A. muricata* leaves extract were 18.97±0.006 mg gallic acid equivalent (GAE)/g and 53.06±0.002 mg quercetin equivalent (QE)/g, respectively. **Conclusion:** These findings provide promising evidence for further investigations into the antidiabetic potential of *A. muricata* leaves.

Keywords: Diabetes Mellitus, Ethnomedicine, *Anona muricata*

OF5

Investigation of Serum Cartilage Oligomeric Matrix Protein Levels and WOMAC Index in Patients with Knee Osteoarthritis in Bandung, Indonesia

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ABSTRACT

Introduction: Osteoarthritis (OA) is often associated with fractures, which can potentially be predicted through the evaluation of serum calcium levels or specific biomarkers like cartilage oligomeric matrix protein (COMP). The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) serves as a reliable tool for assessing the symptoms and physical disability experienced by patients with hip or knee OA. This study aims to investigate association of serum calcium levels, serum cartilage oligomeric matrix protein levels, and WOMAC scores in patients with knee OA. **Methods:** The study recruited patients from the orthopedic clinic of a private hospital in Bandung, Indonesia. There were 18 participants each among normal controls and patients with knee OA who met the inclusion criteria. Serum calcium levels were determined by colorimetry method, serum COMP levels were measured through enzyme-linked immunosorbent assay (ELISA), and WOMAC scores were assessed through a structured questionnaire. **Results:** The study found that the serum calcium level among the patients with knee OA was 113.36 ± 81.04 mg/mL, the serum COMP level was 773.02 ± 343.48 ng/mL, and the WOMAC score was 32.89 ± 0.34. Meanwhile, the serum calcium level and the serum COMP level for normal controls were 210.50 ± 49.26 mg/mL and 534.92 ± 315.55 ng/mL, respectively. **Conclusion:** In the patients with knee OA, the serum COMP level was higher compared to normal controls, indicating increased disease severity. Conversely, the serum calcium level in patients with knee OA was lower compared to normal controls.

Keywords: Health Monitoring, Osteoarthritis, Colorimetry Method, ELISA

OF6

Simultaneous Quantification of Whole Blood Hydroxychloroquine and Desethylhydroxychloroquine for Lupus Erythematosus Patients

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ABSTRACT

Introduction: Hydroxychloroquine is the first-line drug for lupus erythematosus. Quantifying whole blood hydroxychloroquine (WBHCQ) and its metabolite, desethylhydroxychloroquine (DHCQ), provides insight into its pharmacokinetic and pharmacodynamic, for treatment optimization. Methods to detect WBHCQ were previously described with variable detection and quantification limits. However, methods for DHCQ quantification were rarely described. We aimed to develop and validate a method for the detection and quantitation of HCQ and DHCQ for the monitoring of treatment response in lupus patients. **Methods:** A simple and sensitive, high-performance liquid chromatography (HPLC) with fluorescence detection method for the simultaneous detection and quantification of HCQ and DHCQ, in human blood was refined from previously published methods and validated. The blood sample was prepared by precipitating proteins with 2-fold methanol after the addition of internal standard chloroquine (CQ) and separated on an ACER Excel UHPLC C18 column (150 x 4.6 mm with 5 m particle size) as a stationary phase with a mobile phase consisting of 70% acetonitrile, 30% 20 mM sodium monophosphate buffer, and 0.25% v/v triethylamine (pH 8.0). Fluorescence detection was used to detect the analytes at excitation and emission wavelengths of 337 and 405 nm, respectively. **Results:** The method was linear for both analytes over the 3 - 3000 ng/mL range with r2 >0.999, and the chromatographic run time was 10 minutes. The intra- and inter-day precision values with the %RSD ranged from 1.56% to 14.73%. The method showed a good sensitivity with a LOD and LOQ of 12.41 ng/mL and 37.5 ng/mL for HCQ, and 11.05 ng/mL and 33.5 ng/mL for DHCQ, respectively. Our method has shown similar sensitivity to other published methods. **Conclusion:** This improvised method has successfully detected and quantified both HCQ and DHCQ simultaneously with high sensitivity. This method can be adapted for therapeutic drug monitoring (TDM) for patients on HCQ.

Keywords: Cutaneous Lupus Erythematosus, Hydroxychloroquine, Whole Blood Hydroxychloroquine Concentration, Desethylhydroxychloroquine

OG2

Elucidating the Mechanisms of Combination Therapy Using Palm Vitamin E And Commercial Anti Leukemic Drug (Cytarabine) In Cell-Based Models of Acute Myeloid Leukaemia

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ABSTRACT

Introduction: Acute myeloid leukaemia (AML) is a malignant disease of the bone marrow. The main treatment for most AML is chemotherapy, targeted therapy drug and stem cell transplant. Tocotrienol were proven to demonstrate high antioxidant performance in Chronic Myeloid Leukaemia. **Methods:** Water soluble tetrazolium (WST) assay, Caspase activities, Real Time Polymerase Chain Reaction (RT PCR), and Next Generation Sequencing (NGS) were assessed for three AML cell lines with single isomers of palm tocotrienol (δT3 and γT3) and the mix fraction called tocotrienol-rich fraction (TRF) and AML drug, cytarabine. **Results:** TRF is highly effective in inhibiting cell growth of THP 1, HL 60 and Kasumi 6 at different cell percentage ($p<0.05$). The best inhibition was found in Kasumi 6 with TRF treatment followed by HL 60 and THP 1. For combination study, the best inhibition was also found in Kasumi 6 with combination treatment ($p<0.05$). All caspases' activities in Kasumi 6, THP 1 dan HL 60 are significantly increased in 72 hours of incubation. The expression of MIG-6 gene, a tumour suppressor gene was upregulated while the expression of API-5, an apoptosis inhibitor gene was down-regulated in all three AML cell lines treated with the various forms of T3 with or without cytarabine. In NGS analysis, the highest fragments per kilobase of exon per million mapped fragments (FPKM) value was observed in the HL-60 cells treated with the combination of cytarabine and TRF, followed by HL-60 treated with cytarabine alone. Higher FPKM value observed in the combination group may indicate that there were more genes and interactions involved. NGS study also showed many key genes essential for cell viability were differentially regulated. **Conclusion:** All tocotrienol isoforms demonstrated potent anti-proliferative effects on the three AML human cell lines tested in this study, which was better or comparable to that observed with cytarabine.

Keywords: Acute Myeloid Leukaemia, Palm Tocotrienol, Tocotrienol Rich Fraction, Next Generation Sequencing, Gene Ontology

OG3

Design, Synthesis, and *In-Vitro* Biological Evaluation of Novel THQ Derivatives as Anticancer Agents

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ABSTRACT

Introduction: The lack of effective treatments and drug resistance provides significant challenges in the treatment of cancer. PI3K/AKT/mTOR pathway was chosen in the current study as a key alternative target for the mitigation of cancer which can resolve the issue of futile treatment and drug resistance. **Methods:** The mTOR inhibitors were designed based on pharmacophore-based virtual screening findings and the contour map analysis of field-based and atom-based 3D-QSAR models. By combining the virtual hits with the compounds from the preclinical and clinical studies, the common active aspects for activity against mTOR (C1 and C2) were identified leading to a knowledge-based selection of tetrahydroquinoline (THQ) scaffold. Further, these novel tetrahydroquinoline (THQ) derivatives were designed and synthesized. Spectral characterization of these compounds was carried out with the aid of ^{1}H NMR, ^{13}C NMR, and D_2O exchange, confirming the formation of the desired compounds. Moreover, these optimized THQ derivatives were evaluated against the various panel of cell lines viz. colon cancer (HT-29), breast cancer (MCF-7), and lung cancer (A-549). **Results:** Among all the synthesized compounds, compound UC-BzCl-01 showed promising anticancer activity against the panel of these cancer cell lines. Further, FACS analysis of these THQ derivatives was carried out where UC-BzCl-01 demonstrated apoptotic characteristics. **Conclusion:** Based on the results of the study, UC-BzCl-01 may be explored further as a possible mTOR inhibitor and potential anticancer agent.

Keywords: Cancer, Atom Based-QSAR, mTOR, Tetrahydroquinoline, Apoptosis

OG4

Synthesis of Xanthone Derivatives and Jacareubin Derivatives as Potential Therapeutic Agents via *In-Silico* Approach

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ABSTRACT

Introduction: Cancer is distinguished by the uncontrolled proliferation of cells, resulting in the formation of solid tumors and the potential for metastasis. Predominant cancers in males encompass lung, prostate, and colorectal cancer. In contrast, the most prevalent cancer types among females include breast, lung, and cervical cancer. **Methods:** The Grover, Shah, and Shah (GSS) reaction method was employed for a one-pot synthesis of xanthonoids. The synthesis involves phenol-benzoic acid condensation and direct cyclization of the benzophenone intermediate. The resulting xanthonoid products demonstrated significant binding affinities to receptors associated with inhibiting various cancers such as breast, prostate, cervical, and lung cancers. Docking analysis using specific receptors (3EQM, 7TAI, 4XR8, and 6MNX) revealed interactions between the synthesized xanthonoids and the receptors. Additionally, compounds (1-20) were observed to interact with the 4G3D receptor, known for its broad inhibitory effect against various cancer types and its ability to enhance the body's defensive mechanisms. **Results:** The synthesis of xanthone derivatives (compounds 1-20) was accomplished. Sequel to the molecular docking analysis, compound 14 displayed substantial binding affinities for three distinct receptors: 3EQM, 7TAI, and 4XR8, with values of -9.8 kcal/mol, -11.6 kcal/mol, and -11.0 kcal/mol respectively. This compound demonstrated a specific potential for inhibiting estrogen-dependent breast cancer, prostate cancer, and cervical cancer. Moving on to Compound 16, it exhibited the highest binding affinity with a binding energy of -11.1 kcal/mol for the inhibition of non-small cell lung cancer (NSCLC). Compound 12 displayed the best-fit binding affinity at -9.2 kcal/mol with the crystal structure of human NF-kappaB inducing kinase, NIK (4G3D). **Conclusion:** A recent study discovered that compounds 12, 14, and 16 show significant efficacy against selective receptors, suggesting their potential for inhibiting human cancers. However, the specific interactions between these compounds and their respective receptors are not yet fully understood and depend on experimental methodologies.

Keywords: Molecular Docking, Receptor, Therapeutic Agents, Toxicity, Xanthone

OG5

The Effect of Wnt Signaling Activation on *AXIN2* level and Spheroid Formation in TMEPAI Knockdown Colon Cancer Cell lines

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ABSTRACT

Introduction: TMEPAI is a novel oncogenic protein constitutively and highly expressed in cancers. TMEPAI is associated with poor prognosis, whereas the mechanism of how TMEPAI is involved in tumorigenesis is not entirely understood. TMEPAI is known as a TGF-β related molecule, and our previous finding showed that TMEPAI knockout in triple-negative breast cancer cell lines promoted β-catenin nuclear accumulation and upregulated *AXIN2* levels. Here, we investigate the effect of Wnt signaling activation on *AXIN2* level and spheroid formation in TMEPAI knockdown cells in colon cancer. **Methods:** Caco-2 and DLD-1 cells were used in this experiment as colon cancer models. Wnt3A conditioned medium activates Wnt signaling. The *AXIN2* level was quantified using qRT-PCR methods, and in vitro tumorigenesis was performed using spheroid formation assay. **Results:** In activated Wnt signaling, the *AXIN2* level was not significantly induced in Caco-2 cells and up-regulated in DLD-1 cells. TMEPAI knockdown in Caco-2 cells increased sphere sizes compared to the control, and Wnt3A treatment further increased the size and number of spheres. TMEPAI knockdown reduced the size and number of spheres compared to control in DLD-1 cells, and Wnt3A treatment further reduced sphere size and number. According to the Sanger database, Caco-2 and DLD-1 cells have constitutively activated Wnt signaling by APC mutation, while only Caco-2 cells have additional *SMAD4* and *CTNNB1* mutation. **Conclusion:** This result shows different effects of TMEPAI knockdown on *AXIN2* level and spheroid formation by different activated signaling and mutation in colon cancer cell lines.

Keywords: TMEPAI, Signaling Activation, Wnt Signaling, Tumorigenesis

OG6

The Antinociceptive Effects of 5-HT_3 Receptor Antagonist in Chemotherapy-Induced Peripheral Neuropathy (CIPN) in a Rat Model

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ABSTRACT

Introduction: Chemotherapy-Induced Peripheral Neuropathy (CIPN) is the second most common dose-limiting adverse effect that significantly impacts the quality of life of cancer patients and survivors. Effective treatments for CIPN have so far not been well-defined. Previous studies have shown that various neuropathic pain could be blocked by the 5-HT_3 receptor antagonist. Considering that 5-HT_3 receptor antagonists are already clinically used to treat chemotherapy-induced nausea and vomiting in cancer patients, it is worthwhile to explore 5-HT_3 receptor and its antagonist in CIPN. The present study aims to examine the role of 5-HT_3 receptor and its antagonists on CIPN in rats as animal model. **Method:** The effects of 5-HT_3 receptor (palonosetron and ondansetron) on CIPN were examined via mechanical allodynia test using the Von Frey filament method and cold allodynia test using acetone drop. The rats were induced with cisplatin (4mg/kg) weekly up to 3 cycles followed by palonosetron (3.1mg/kg) or ondansetron (148.48mg/kg) treatment orally for 1 week. The nociceptive behaviors were evaluated on the 7th day of the 1-week treatment. The involvement of 5-HT_{3R} was further validated by administrating mCPBG, a 5-HT_{3R} agonist prior to the administration of palonosetron. **Results:** In mechanical allodynia test, our results indicated that both palonosetron and ondansetron significantly reduced cisplatin-induced pain but not for cold allodynia. Palonosetron effectively counteracted mCPBG action which showed that the mechanical allodynia reduction was modulated via 5-HT_3 receptor. **Conclusion:** Blockade of 5-HT_3 receptor by its antagonist induces an antinociceptive effects on CIPN and suggests that the drugs especially palonosetron may have potential clinical utility for the management of CIPN.

Keywords: Chemotherapy-Induced Peripheral Neuropathy (CIPN); 5-HT_3 Receptor; Palonosetron; Ondansetron

OG7

Elucidating Interaction of Gold Nanoparticles on Expression of Tumor Necrosis Factor Receptor 2 (TNFR2) Positive Cells in Human Peripheral Blood Lymphocytes of Rheumatoid Arthritis Patients

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation in the joints. Regulatory T (Treg) cells are very important in keeping the immune system in balance. Previous research revealed that TNFR2+ Treg cells are downregulated particularly during RA inflammation. To tackle this challenge, gold nanoparticles (GNPs) may influence immune responses to enhance the production of TNFR2+ Treg cells. The aim of this study is to elucidate the interaction between GNPs and TNFR2+ Treg cells. **Methods:** The PBMC from RA patients were cultured into different media supplied with lipopolysaccharide (LPS), GNPs, etanercept and tumor necrosis factor-alpha (TNF-α) for two days at 37°C in 5% CO_2. The lymphocytes were harvested and stained with Treg markers: CD4, CD25, CD127, Foxp3, TNFR1 and TNFR2. The phenotyping of the cells was evaluated using flow cytometer and analysis was performed by FlowJo software. **Results:** The proliferation of TNFR2+ Treg cells induced with GNPs is comparable to LPS, etanercept and in TNF-α. Our findings indicate that GNPs have the ability to enhance the proliferation of TNFR2+Treg cells. **Conclusion:** This study offers valuable insights to the understanding of the immunomodulatory impacts of GNPs on TNFR2+ Treg cells and emphasize the possibility of their applications in the field of immunotherapy and the management in RA disease.

Keywords: TNFR2, Rheumatoid Arthritis, Nanoparticles, Autoimmune

OH1

Prognostic Models Predicting Acute Ischemic Stroke Outcomes: A Population-Based Study

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ABSTRACT

Introduction: The outcomes after acute ischemic stroke (AIS) management remain variable. This study aimed to develop and validate a prognostic model that predicts 90-day outcomes following a first-ever acute ischemic stroke. **Methods:** We included 899 adult patients (aged 18 years and above) with first-ever acute ischemic stroke enrolled in the Malaysian National Stroke Registry (NSR) from 2010 to 2020. The outcomes measured were all-cause mortality and functional disability measured using the modified Rankin score (mRS) (≥3). A multivariable logistic regression was utilized for the prognostic modeling using 75:25 (development: validation). **Results:** The mean ± SD age of the patients was 60.1 ± 10.8 years, with the majority males (60.8%). The final model predicting mortality and disability included common predictors such as Glasgow coma scale (moderate-severe, GCS ≤8) [adjusted odds ratio, OR 2.66, 95% confidence interval, CI (1.31-5.40); 1.56 (1.00-2.45)], diabetes [2.42 (1.41-4.16); 1.60 (1.60-2.20)], and non-adherence to antiplatelet within 48 hrs. [2.30 (1.26-4.20); 1.99 (1.28-3.09)], to lipid-lowering therapy (2.09 (1.10-4.00); 1.77 (1.26-2.48)], to stroke education [39.61 (21.92-71.57); 11.46 (6.84-19.21)] and to rehabilitation [10.75 (6.00-19.25; 2.19 (1.59-3.09)], respectively. Other predictors of mortality were age ≥ 60 years, non-adherence to dysphagia screening, and antiplatelet upon discharge, while another predictor of disability was the female gender. The final models achieved acceptable validation performance using discrimination and calibration - mortality [AUROC=0.94; HL p=0.630] and disability [AUROC=0.78; HL p=0.967], respectively. **Conclusion:** A validated prognostic model that predicts 90-day mortality and functional disability following the management of the first-ever acute ischemic stroke in Malaysia was developed. The model demonstrated acceptable validation performance. In addition, the model scores could serve as a template for integration into an easy-to-use web-based risk calculator for clinicians, patients and other stakeholders.

Keywords: Acute ischemic stroke, Prognostic model, Mortality, Disability, Modified Rankin scale

OH2

Predictive Factors for Length of Stay (LOS) Among COVID-19 Patient in Hospital Bandung City, Indonesia

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ABSTRACT

Introduction: As of September 3, 2023, the World Health Organization (WHO) has reported a staggering 770,563,467 confirmed cases of COVID-19, with 6,957,216 unfortunate fatalities, all attributed to the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The management of COVID-19 frequently necessitates extended hospital stays, which not only strain healthcare resources but also impose a significant economic burden. Thus, it is important to comprehensively investigate the factors that predict the length of hospitalization in COVID-19 cases. **Methods:** This study employed a cross-sectional observational design, utilizing secondary data sourced from the medical records of a provincial hospital in Indonesia. Demographic characteristics, comorbidities, and hospital length of stay data were collected through purposive sampling techniques. The collected data were analyzed using chi-square statistical test. **Results:** Most COVID-19 patients were male, constituting 55.09% of the total cases. The highest proportion of cases fell within the age groups of 30-39 years and 60-69 years, accounting for 24.24% each. The majority of patients presented with comorbidities, with a prevalence of 72.27%. The prevalent comorbid conditions included hypertension, diabetes, asthma, and pneumonia. In 2020, the highest recorded total length of stay (LOS) exceeded 20 days, with an average LOS (AvLOS) of 28.818 days. Significant predictive factors associated with LOS were identified, including age (p-value: 0.001), gender (p-value: 0.0012), and comorbid status (p-value: 0.004). **Conclusion:** Several patient characteristics serve as valuable predictors for hospital LOS among COVID-19 patients. This information can prove instrumental in the development of triage systems and interventions aimed at reducing LOS in these cases.

Keywords: LOS, COVID-19, Predictive Factors

OH3

Treatment with Red Yeast Rice Improves Endothelial Dysfunction in Spontaneously Hypertensive Rats

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ABSTRACT

Introduction: Hypertension is closely associated with endothelial dysfunction, a condition caused by an imbalance between nitric oxide (NO) and reactive oxygen species (ROS), leading to impaired endothelium-dependent vasorelaxation due to reduced NO-cyclic GMP (cGMP) signaling. Red yeast rice (RYR) is a traditional folk medicine produced from the fermentation of rice with *Monascus purpereus* mould. RYR is reported to have anti-hypertensive properties but its effect in improving endothelial dysfunction is yet to be fully elucidated. Thus, the present study aimed to investigate if treatment with RYR improves endothelial dysfunction in Spontaneously Hypertensive Rats (SHR). **Methods:** Male SHR of age 10-12 weeks were administered with RYR (100 mg/kg/day). Male Wistar-Kyoto (WKY) rats of same age were used as normotensive controls. Drug administration was performed for 12 weeks through oral gavage. Systolic blood pressure was measured by tail-cuff method. Vascular reactivity was determined using isolated aortic rings in organ bath. The levels of vascular NO and ROS were measured using dihydroethidium (DHE) and difluorofluorescein acetate (DAF-FM) fluorescence assay respectively, while vascular tetrahydrobiopterin (BH_4) and cGMP levels were determined using commercial assay kits. **Results:** Treatment with RYR reduced elevated systolic blood pressure and enhanced endothelium-dependent vasorelaxation in isolated aortic rings of treated SHR. Furthermore, the level of vascular ROS was decreased and the levels of NO, BH_4 and cGMP in the aorta were significantly increased. **Conclusion:** The present study demonstrated that treatment with RYR for 12 weeks improved endothelial dysfunction partly via reduction of oxidative stress, leading to the decreased eNOS uncoupling and enhanced NO-cGMP signaling.

Keywords: Red Yeast Rice, Oxidative Stress, Nitric Oxide, Endothelial Dysfunction, Hypertension

OH4

An *In Vitro* and *In Silico* Study of The Antihyperlipidemic Effect of (MY-A and MY-B)-4-Quinobenzothiazini Butane-Sulfonic Acids

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ABSTRACT

Introduction: Hyperlipidemia, a medical disorder characterized by elevated lipid levels in the blood, is often associated with an increased risk of heart diseases and other cardiovascular ailments. The current line of antihyperlipidemic drugs may have certain undesirable side effects and offer only partial success in treating hyperlipidemia. Hence, the current study aims to investigate the antihyperlipidemic activity of MY-A and MY-B compounds to act as a complement to the current gamut of antihyperlipidemic drugs. **Methods:** L6 myoblasts were used to determine the cytotoxicity of MY-A and MY-B where the cells were cultured and treated with these compounds at a concentration range of 0.01-2.58 µmol/mL for a period of 24 hours. The cell viability was then evaluated using the MTT cytotoxicity assay. Consequently, in vitro antihyperlipidemic studies were performed to assess the inhibitory effect of varying concentrations of MY-A and MY-B (0.01-2.58 µmol/mL) on pancreatic lipase, cholesterol esterase and HMG CoA reductase enzymes. Lastly, an *in silico* study was performed using AutoDock Vina 1.2.0 to determine the binding strength of MY-A and MY-B with these enzymes. Results: MY-B showed negligible signs of toxicity against L6 cells with an IC_{50} value of 43.27 ± 0.35 µmol/mL. The *in vitro* antihyperlipidemic studies showed that MY-A and MY-B effectively inhibited pancreatic lipase and cholesterol esterase in a concentration-dependent manner. The Lineweaver-Burk plot analysis showed the MY-B displayed the highest level of HMG CoA reductase inhibitory efficacy, at approximately 68%. The *in silico* study corroborated these findings by revealing a strong binding energy of MY-A and MY-B against the three enzymes among which, MY-B showed a significantly stronger affinity to these enzymes as compared to MY-A. Conclusion: MY-A and MY-B showed great potential as antihyperlipidemic agents which could provide greater therapeutic benefits in improving cholesterol metabolism and thus, relieving hyperlipidemia.

Keywords: 4-Quinobenzothiazini Butane-Sulfonic Acids, Antihyperlipidemic, Pancreatic Lipase, Cholesterol Esterase, HMG Coa Reductase

OH6

Effects of Palm Carotene Mixture on Static Bone Histomorphometry of Bovine Bone Scaffold Co-cultured with Osteoblasts and Osteoclasts

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ABSTRACT

Introduction: Palm carotene exists naturally in complex mixture consisting of alpha-, beta-, and gamma-isomers. The effects of beta-carotene on bone have been previously reported, but the effects of other isomer either individually or in combination have not been investigated. This study aimed to investigate the effects of palm carotene mixture on static bone histomorphometry using bovine bone scaffold co-cultured with osteoblasts and osteoclasts, serving as an in vitro model that mimics endogenous bone microenvironment. **Methods:** The decellularised and demineralised bovine bone scaffold were randomised into five experimental groups: (a) native bone, (b) osteoporotic bone, (c) osteoporotic bone co-cultured with osteoblast-osteoclast, (d) osteoporotic bone co-cultured with osteoblast-osteoclast and treated with 12.5 µg/mL palm carotene mixture, and (e) osteoporotic bone co-cultured with osteoblast-osteoclast and treated with 10 nM alendronate. After 21 days of treatment, bone scaffolds were decalcified and stained with haematoxylin and eosin to assess the static bone parameters. **Results:** Bone scaffolds subjected with decellularisation and demineralisation had lower osteoblast number, osteoclast number, and osteoid surface as compared to native bone. The seeding of osteoblasts and osteoclasts increased osteoclast number in the bone scaffolds as compared to those without bone cells. Treatment of palm carotene mixture increased osteoblast number and osteoid volume as compared to the non-treated bone scaffolds. Bone scaffolds treated of alendronate showed raised osteoid volume as compared to the non-treated bone scaffolds. **Conclusion:** Palm carotene mixture increases osteoblast number and osteoid volume, suggesting its potential bone-protective effects.

Keywords: Bone scaffold, Carotene, Vitamin A, Osteoporosis

OH7

Risk Analysis of Lead and Cadmium Contamination in Staple Foods in Jakarta and Bandung, Indonesia

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ABSTRACT

Introduction: Rice is a staple food in Indonesia. There is even a saying in Indonesian society, "you do not say you have eaten before taking rice". Official data from the Food and Drug Monitoring Agency of the Republic of Indonesia suggests an average daily rice consumption of 200 grams/ person, while the Food Security Agency reports a higher figure of 320 grams/ person/ day. Previous research highlighted high levels of heavy metals contamination, particularly lead (Pb) and cadmium (Cd) in rice. According to WHO in 2011, Pb lacks a defined safety threshold, with a Point of Departure (POD) set at 0.6 µg/kg bw/day, capable of causing a 1 IQ point reduction in children while 1.2 µg/kg bw/day linked to a 1 mmHg increase in blood pressure in adults. Contrarily, Cd is considered to have a safety threshold with Provisional Tolerable Monthly Intake (PTMI) set at 25 µg/kg bw/month. This study aims to provide essential risk analysis data related to Pb and Cd exposure through rice consumption. **Methods:** Rice samples sourced from various traditional markets in Jakarta and Bandung underwent analysis for Pb and Cd levels using the Atomic Absorption Spectrophotometry (Graphite Furnace) technique. This data obtained was subsequently used in the calculation of Excess Cancer Risk (ECR), which is linked to the daily rice intake of the Indonesian population. **Results:** The findings of this study reveal that the levels of Pb and Cd in the analyzed rice samples fall within safe thresholds, as determined by the Risk Analysis based on both the measured metal levels and the daily rice consumption per kilogram of body weight among the Indonesian populace. **Conclusion:** Risk analysis related to exposure of Indonesian communities to Pb and Cd through rice consumption is categorized as within safe limits.

Keywords: Risk Analysis, Lead, Cadmium, Staple Food

OI1

Assessing The Influence of DOTAP: Lipid Ratio on Lipid Nanoparticles Serving as Genetic Material Delivery System

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ABSTRACT

Introduction: Lipid Nanoparticles (LNPs) represent a lipid-based delivery system, consisting of both solid and liquid lipids. This unique combination allows LNPs a greater drug loading capacity, making them an ideal carrier for both hydrophilic and hydrophobic drugs. Thus, LNPs are widely used as a delivery system to transport genetic material into the nucleus. Among the diverse array of lipids, cationic lipids play an important role in influencing drug biodistribution and efficacy. One such cationic lipid is DOTAP (1,2-Dioleoyl-3-trimethylammonium propane). This study aims to investigate the effect of varying DOTAP ratios in comparison to other lipid components within LNP formulations. **Method:** LNPs was prepared using emulsification-ultrasonication method. Four different formulations of LNPs were prepared, each featuring a unique DOTAP: Lipids ratio denoted as F1 (0.00), F2 (0.04), F3 (0.08) and F4 (0.15). The physicochemical characteristics of the LNPs, including particle size, polydispersity index and zeta potential measurements were assessed. Furthermore, cell viability test and cellular uptake assay were conducted using Hepa1-6 cells. **Results:** LNP particle size increased with increasing DOTAP concentration across the formulations. Specifically, the particle sizes measured were 73.2±2.1, 118.4±6.4, 131.1±9.7, and 158.3±2.2 nm, respectively. The polydispersity index value also exhibited a corresponding increase, measuring 0.294±0.043, 0.244±0.023, 0.237±0.017, and 0.299±0.019. Being a positively charged lipid, an increase in concentration of DOTAP causes the LNPs to become more positively charged, resulting in surface charges of -3.24±0.80, 5.24±1.20, 7.44±0.65, and 8.65±0.22 mV. Cell viability test and cellular uptake assay were performed using F2 formulation. The IC50 value for cell viability was determined to be 218.30 µg/mL. It was observed that cellular uptake of LNP F2 predominantly occurred through Clathrin-Mediated Endocytosis (CME). **Conclusion:** The present findings demonstrate the importance of carefully considering the concentration of cationic lipid bases in LNP formulations, as they would affect both particle size and LNP surface charge.

Keywords: Cationic Lipid, DOTAP, Genetic Material, Lipid Nanoparticle, Physicochemical Propertie

OI2

Nanoformulation of A Standardised *Andrographis paniculata* (Burm.) Nees Aqueous Extract Improves Pharmacokinetics Profile

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ABSTRACT

Introduction: The therapeutic efficacy of orally administered natural products is often limited by poor oral bioavailability, which may be due to poor water solubility, limited intestinal absorption, and being substrates of the efflux pump and cytochrome P450. *Andrographis paniculata* (AP) is widely used traditionally with proven prophylactic potential in mouse asthma models. However, the major active phytoconstituents exhibit poor oral bioavailability. This study aimed to formulate and determine the pharmacokinetic profile of a standardised lecithin phospholipid-based nanoformulation of AP aqueous extract (FAPAE) in mice. **Methods:** The optimisation of the nanoformulation was done at various ratios of compound:lecithin (1:1, 1:2, and 1:3) using a thin-film hydration technique. FAPAE was characterised using Zeta nanosizer, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), and encapsulation efficiency (EE) was determined. A single oral dose of 200 mg/kg was administered to female Balb/c mice (6 mice per group). At predetermined intervals of 5, 10, 15, 30, 60, 120, and 180 minutes, animals were anesthetised, and terminal blood samples were collected. The samples were processed using liquid-liquid extraction methods. PKsolver software was used to analyse the pharmacokinetic parameters. **Results:** The optimised FAPAE (1:3) exhibited a vesicular size of 108.60 ± 7.91, a polydispersible index of 0.25 ± 0.01, and a zeta potential of -48.90 ± 4.51 mV. TEM and SEM reveal a spherical-shaped particle, with an EE of 68.15%, 75.00%, and 71.96% for AGP, NAG, and DDAG, respectively. The *in vivo* pharmacokinetics study revealed an increase in the AUC_{0-180} from 67.63 to 103.45, 297.44 to 365.02, and 125.54 to 163.88, while the absorption rate constant increased from 1.26 to 2.56, 4.03 to 5.09, and 1.44 to 2.19 µM/mL for AGP, NAG, and DDAG, respectively, in FAPAE compared to APAE. **Conclusion:** FAPAE shows promise as a nanoformulation for enhancing the absorption and oral bioavailability of the active compounds found in APAE.

Keywords: *Andrographis paniculata*, Nanoformulation, Pharmacokinetics, AUC, Andrographolide

OI3

Calcium Nanoliposomes: Potential Insulin Release Stimulator *In Vivo*

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ABSTRACT

Introduction: Glucose metabolism triggers ATP production, leading to closure of K-ATP channels, which in turn causes depolarization and the opening of calcium channels. The influx of intracellular calcium concentrations subsequently stimulates insulin secretion. Structural similarity of liposomes and cell membranes render them effective at facilitating the entry of concentrated calcium into pancreatic cells. This study aims to formulate calcium-loaded nanoliposomes for stimulating insulin release in pancreatic cells. **Method:** Calcium nanoliposomes were prepared using thin-layer hydration method. Characterization included observation of morphology, as well as assessment of particle diameter, polydispersity index, zeta potential, pH, and entrapment efficiency. In vivo experimentation was conducted using a glucose load model in a group of male white mice weighing between 30 to 40 grams and aged 40 to 50 days. Each group received a 50% glucose load. Blood glucose levels were measured at 30, 60, 90, 120, and 150-minute intervals using a glucometer. Blood samples (1-2 microliters) were collected from the tail vein. The experimental groups were: Group 1 received calcium nanoliposome, Group 2 received empty nanoliposomes, Group 3 received glibenclamide, and Group 4 received distilled water. **Results:** Glucose tolerance measurements showed significant differences among the groups. The group receiving calcium nanoliposomes showed significant reduction in blood glucose levels compared to the control group. Specifically, in Group 1, blood glucose levels were maintained at 186 mg/dL after 30 minutes of glucose loading, followed by significant reductions at 60, 90, 120, and 150 minutes, with values of 142, 128, 113, and 93 mg/dL, respectively. Group 2 displayed respective blood glucose levels of 277, 240, 205, 155, and 112 mg/dL at the same time points. Contrarily, Group 3 exhibited blood glucose levels of 135, 106, 87, 81, and 71 mg/dL, respectively. **Conclusion:** These results suggest the potential of calcium nanoliposomes as an insulin release stimulant.

Keywords: Calcium, Nanoliposome, Insulin

OI4

Synthesis, Characterization, Stability of NanoGraphene Oxide Functionalized with Pluronic (NanoGO-PF), and Its Biocompatibility Study in Zebrafish Embryos for Delivery of Hydrophobic Compound

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ABSTRACT

Introduction: Graphene oxide (GO) has yielded new promising potentials as a nanoplatform for various applications, especially in pharmaceutical science. However, the major hurdles of GO in clinical settings are biocompatibility and stability as reported in the literature. The present study investigated the effects of GO functionalization with Pluronic (PF) on the biocompatibility and stability of GO, as well as its capability as a nanocarrier for hydrophobic compound. **Methods:** The GO, NanoGO and PF loaded graphene oxide (NanoGO-PF) were characterized by dynamic light scattering, UV-Vis spectroscopy, Raman spectroscopy, FT-IR, XRD, scanning electron microscopy coupled with EDX, and transmission electron microscopy (TEM). The storage stability of NanoGO-PF and in various media, were evaluated for size, size distribution and zeta potential. Toxicity profile of NanoGO-PF (0-100 µg/mL) on zebrafish embryonic model was recorded for 96 hours post-fertilization. Lastly, the ability of NanoGO-PF to load curcumin (CUR) was assessed. **Results:** NanoGO has a significant smaller hydrodynamic size, compared to GO (~119 nm), but increase in size to ~230 nm was observed following functionalization with PF. The attachment of PF onto NanoGO was found to be ≥50% and was further confirmed with UV-vis and FT-IR analyses. NanoGO-PF was found to be stable in storage and in several biological media. NanoGO-PF exhibited improved biocompatibility in zebrafish embryos with the ability to load CUR at different respective ratios. **Conclusion:** Findings from the present study provide pivotal data on the advancement of a compatible and stable GO nanocarriers.

Keywords: Graphene Oxide, Pluronic, Toxicity, Zebrafish, Stability

OI5

Customizing Cationic Lipid Nanoparticles for Promising Gene Therapy Applications

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ABSTRACT

Introduction: Gene therapy is a medical approach aimed at treating or preventing diseases by addressing fundamental genetic irregularities. Unlike conventional approaches reliant on drugs or surgical interventions, gene therapy employs innovative techniques to rectify genetic anomalies, including gene mutations. This therapeutic approach holds great promise, particularly in its capacity to precisely target the nucleus through the utilization of cationic lipid nanoparticle (cLNP) delivery systems. **Methods:** In this study, we formulated cLNPs using an emulsification methodology. The attributes of cLNPs, including size, polydispersity index, zeta potential, and morphological structure, were assessed using transmission electron microscopy. Entrapment efficiency was also being performed. For cLNP-mediated transfection, incorporation of cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) enabled electrostatic interactions with nucleic acids. To gauge the penetration ability of cLNPs into TM4 cells and their subsequent nucleus targeting, enhanced green fluorescent protein (EGFP) was used as a marker protein. EGFP served as confirmation for cLNP's efficacy in delivering the pEGFP gene into the nucleus, where the expression of EGFP protein is visually observed via confocal microscopy. **Results:** With a particle size of 146.17±33.47 nm and a positive particle charge of +0.43 mV, the cLNPs showed remarkable ability to penetrate TM4 cells. The polydispersity index showed exceptional size uniformity, with a value of 0.313±0.022 and entrapment efficiency of 90.03±0.055%. The TEM results showed the spherical morphology of these cLNPs, confirming their robust structural integrity. The interaction between materials forming the cLNPs was further evident through the difference in Tm, as observed in the DSC results. The cLNP were also able to internalise the nucleus, as indicated by the expression of EGFP resulting from the successful delivery of pEGFP by the cLNP into the cells. **Conclusion:** The characterization data of these cLNPs shows their potential as an efficacious gene therapy delivery system for addressing complex diseases within cellular contexts.

Keywords: Cationic Lipid Nanoparticle (cLNP), Enhanced Green Fluorescent Protein (EGFP), Gene Therapy, Nucleus, TM4 Cells

OI6

Cellular Uptake of Liposome from *Mycobacterium smegmatis* in Human Peripheral Blood Monocytes

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ABSTRACT

Introduction: Liposomes are a type of lipid-based nanoparticle formed by the self-assembly of phospholipid molecules in an aqueous medium. Liposomes have unique properties such as biodegradable, biocompatible, reduced toxicity, strong immunogenicity, and good target availability. Nowadays there has been attention is focused on liposomes derived from bacterial lipids as potential carriers for targeted delivery in biomedical research for their adjuvant effects on moDCs. Thus, we aim to investigate the cellular uptake of liposomes derived from Mycobacterium smegmatis on moDCs. **Methods:** Liposomes were synthesized from total lipids of *M. smegmatis* and characterized by field emission scanning electron microscope (FESEM). MoDCs were sorted from human PBMC. The immature moDCs were treated with LPS, liposomes and inhibitor to form mature moDCs. The uptake of moDCs was observed and analyzed with FACS analysis, FESEM and confocal microscopy. **Results:** FESEM images of liposomes showing the spherical structures with average size between 20nm-80nm that can be classified as small unilamellar vesicles (SUV). For the cell surface marker, the MFI of CD80 and CD86 shows no significant difference compared to control group. FESEM and confocal microscopy images have shown the uptake of liposomes and it was internalized by moDCs. **Conclusion:** We successfully synthesized natural liposomes derived from total lipid of *M. smegmatis* before introducing with moDCs. Therefore, liposomes derived from total lipid of *M. smegmatis* were taken up and internalized by moDCs with the purpose of initiating and modulating immune responses.

Keywords: Liposomes, Inhibitors, moDCs, Cellular Uptake

OI7

Understanding The Effect of Introducing Biopolymer at Two Distinct Phases During Homogenization in A Double Solvent Evaporation Technique: A Study Using Simvastatin as A Model Drug for Bone Tissue Regeneration

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ABSTRACT

Introduction: Simvastatin (SIM), a cholesterol-lowering medication, demonstrated potential osteogenic effects, rendering it an attractive choice for bone scaffolds. This research aims to study the encapsulation efficiency, yields, cumulative release, and mechanical properties of porous SIM microparticles (SIM/PMP). Three biopolymers were used in this study, namely chitosan, pectin, and pluronic F127. **Methods:** SIM-loaded-PLGA microparticles with 0.05% and 1% tripolyphosphate -chitosan (TPP-Chi), 0.4% and 1.0% pectin (Pec), and 0.13% and 0.5% of pluronic F127 (F127) were fabricated using double emulsion solvent evaporation. All the biopolymers were added at two distinct positions, either in the internal phase (MM1) or aqueous phase (MM2). All microparticles underwent a 48-hour lyophilization process. The percentage of yields, encapsulation efficiency and SIM cumulative release were further analysed. **Results:** A two-way ANOVA analysis indicated a significant different in the percentage of yield between different group of biopolymers in MM1 and MM2 with $F(6,28) = 4.712$, $p = 0.002$, and partial Eta Square (χ^2) = 0.502. A post-hoc analysis was conducted to examine the multiple comparisons of encapsulation entrapment across all groups. Statistically significant difference in SIM release was observed between 0.5% TPP-Chi and 1.0% TPP-Chi at $F(4.263, 10.65) = [11.34]$, $p < 0.001$, and partial Eta Square $\chi^2 = 0.819$, over the 21 days of SIM cumulative release. **Conclusion:** The release profile of SIM/PMP, when compared to SIM/PMP with additional biopolymers using TPP-chi, Pec and F127, demonstrated increased encapsulation efficiency and prolonged release. Notably, formulations such as TPP-Chi 1.0% SIM/PMP (MM2), Pec 1.0% SIM/PMP (MM1), and F127 0.13% SIM/PMP (MM1) exhibited a slower and extended SIM release compared to other formulations. Among all these formulations, F127 0.13% SIM/PMP (MM1) displayed the most favorable mechanical properties.

Keywords: Simvastatin, Chitosan, Pectin, Pluronic Acid, Tissue Engineering

OJ1

Dependence Potential of Mitragynine (Kratom): Behavioural Pharmacology in Rodents

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ABSTRACT

Introduction: Kratom (*Mitragyna speciosa* Korth), has been widely explored for its potential therapeutic value as an opioid substitute. However, studies on the physiological and psychological dependence-producing properties of its main psychoactive compound, mitragynine (MG), remains limited. This study examined the dependence-producing effects of MG using operant-scheduled behaviour experiments in rats. The potential therapeutic effect of MG was investigated by comparison to buprenorphine in morphine-dependent rats. The pentylenetetrazol (PTZ) discrimination assay was used to investigate the generalisation effects of withdrawal from MG to anxiogenic PTZ stimulus. **Methods:** The rats received chronic administration of both MG and morphine to assess the development of physiological dependence. This involved monitoring the cessation of drug treatment and observing antagonist-precipitated withdrawal responses. Subsequently, the study examined the effects of MG substitution on naloxone-precipitated morphine withdrawal effects. Concurrently, another group of rats underwent chronic treatment with either MG or morphine, followed by naloxone-induced withdrawal. These rats underwent PTZ discrimination assays at 2-, 8- and 24-hour intervals post the last MG or morphine dose to assess PTZ generalization responses. **Results:** Unlike morphine, MG-treated rats showed no suppression of response rates following cessation of MG treatment. However, withdrawal effects were evident following naloxone precipitation. Higher MG doses (10 and 30 mg/kg) attenuated the naloxone-precipitated morphine withdrawal effects, while smaller doses of buprenorphine (0.3 and 1.0 mg/kg) achieved a similar outcome. In contrast to morphine which produced a time-dependent generalisation to the PTZ stimulus, naloxone did not induce withdrawal effects in MG-treated rats, as they consistently selected the vehicle lever across three withdrawal time points. **Conclusion:** This study suggests that MG induces less severe physiological and psychological dependence compared to morphine, while demonstrating potential in alleviating the physical symptoms associated with morphine withdrawal. These findings align with the desired characteristics of novel pharmacotherapeutic interventions for managing opioid use disorder (OUD).

Keywords: Mitragynine, Kratom, Opioid, Dependence, Rats

OJ2

Application of Median Nerve Electrical Stimulation to Restore Neuronal Function and Promote Myelin Regeneration after Stroke in Rats

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ABSTRACT

Introduction: Upper limb motor dysfunction is one of the common complications of stroke patients. This symptom is associated with disruption of normal neuronal function. Restoration of the upper limb motor dysfunction following stroke remains a major challenge for rehabilitation medicine. Clinically, we had demonstrated that median nerve electrical stimulation (MNES) could significantly improve the upper limb motor function in stroke patients, but the underlying mechanism is still unclear. Myelin regeneration plays an important role in the restoration of nerve function in stroke. In this study, we aimed to investigate the effects of MNES on myelin regeneration in a rat stroke model. **Methods:** Adult male Sprague-Dawley rats (n=24) were divided into three groups, i.e., sham group, left middle cerebral artery occlusion stroke model (MCAO group), and stroke model receiving MNES treatment (MNES group). The MNES group received MNES intervention (once per day for 7 times) on the injured side forelimb at 3 days after MCAO. The neural functional recovery was evaluated by neurological severity score (NSS), Rotarod (RR), and foot fault test (FFT). The expression of myelin basic protein (MBP) was detected by Western blotting. The myelin thickness and number of medullated fibers in the penumbra area were observed by transmission electron microscopy (TEM). **Results:** The MNES group had significantly improved the performance of NSS, RR, and FFT scores compared to the MCAO group ($p<0.01$). Also, the expression level of MBP protein in the MNES group was increased ($p<0.05$). Under TEM, compared to the MCAO group, the MNES group had increased myelin thickness ($p<0.01$) and number of medullated fibers ($p<0.05$), the shape of nerve fibers was regular, and the lamellar structure was dense. **Conclusion:** The MNES treatment could help in restoring neuronal function by promoting the expression of MBP, myelin regeneration, and improving the microstructure of myelin after stroke.

Keywords: Stroke, Median nerve electrical stimulation, Neuronal function recovery, Myelin regeneration

OJ3

Efficient Synthesis of (-)-Swainsonine Using Inexpensive and Readily Accessible Ascorbic Acid as a Starting Material

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ABSTRACT

Introduction: Swainsonine, an α-mannosidase II inhibitor, demonstrated capability to inhibit glycosylation pathways during pre-clinical investigations. However, this compound fails in clinical trial due to severe side effects including pulmonary oedema and neurological disorders, primarily arise from simultaneous inhibition of both lysosomal α-mannosidase II and Golgi α-mannosidase. To address these challenges, swainsonine analogues exhibiting greater selectivity towards Golgi α-mannosidase, as oppose to lysosomal α-mannosidase, are needed. To create highly selective analogs, it is imperative to begin with the parent compound swainsonine prior substitution. As it is costly to purchase swainsonine directly, we tested and developing a synthesis scheme for the preparation of swainsonine using D-isoascorbic acid as the chiral starting material. **Methods:** The medicinal chemistry synthesis pathway for swainsonine involves a series of protection and deprotection steps. Key transformations in the process include Wittig olefination, Huisgen 1,3-dipolar cycloaddition and hydroboration-oxidation. Purification of the products was carried out using the classical column chromatography Si-gel G60 (230-400 mesh, Merck). The 1H and 13C NMR spectra was registered in $CDCl_3$ with Joel Resonance ECZ400S 400 MHz (1H) and 100 MHz (13C), using TMS as the internal standard. High-resolution mass spectra (HRMS) were obtained on an Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight Liquid Chromatography/ Mass Spectrometry (Q-TOF LC/MS) system. Specific optical rotation was determined using Anton Paar MCP 500 polarimeter. **Results:** The parental compound (-)-swainsonine was successfully synthesized from D-isoascorbic acid through a 13-step process. Following published protocols, lactol-aldehyde tautomerism issue was encountered, leading to failure in obtaining precursor 8. We subsequently explored an alternative route and improved the access towards precursor 8 with few additional steps to prevent intramolecular cyclisation. **Conclusion:** In summary, we have succeeded in producing swainsonine skeleton from the inexpensive and readily available D-isoascorbic acid. While extending the number of steps involved, present modification led to improved accessibility to olefinic alcohol 8.

Keywords: Swainsonine, Glycosylation, Golgi α-mannosidase, Lactol-Aldehyde Tautomerism

OJ4

An Ensemble Docking Strategy for The Discovery of Novel Dengue Virus Inhibitors RNA-Dependent RNA Polymerase

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ABSTRACT

Introduction: Dengue fever is a significant health concern in tropical regions, transmitted by infected mosquitoes. Developing effective antiviral drugs is crucial to combat the disease. The NS5 RNA-dependent RNA polymerase (RdRp) is a potential therapeutic target due to its role in viral replication. This study aims to explore NS5 RdRp as a target for antiviral agents against dengue virus (using an ensemble docking strategy). **Methods:** Molecular dynamics (MD) simulations were conducted to study the behaviour and dynamics of NS5 RdRp apoprotein (PDB code: 2J7U) and the NS5 RdRp complex (PDB code: 2J7W) over 150 nanoseconds (ns). An ensemble docking strategy screened a compound library for potential RdRp inhibitors. **Results:** The root-mean-square deviation (RMSD) analysis revealed stable conformations for both the apoprotein and complex. Ligand binding reduced the complex's flexibility. The observed significant flexibility in the Met342-Arg352 region of the apoprotein, as revealed by RMSF analysis, implies its potential involvement in dynamic structural changes, possibly linked to specific functional activities. The relatively stable dynamics observed in all the modeled systems, with fluctuations ranging from 2.0 to 5.9 E, suggest that these protein structures are well equilibrated and remain structurally robust without undergoing substantial conformational alterations throughout the simulation. Dynamic cross-correlation matrix (DCCM) analysis duemonstrated shifts in correlation, indicating changes in collective motions. MD trajectories clustered into 225 protein structures, screened with 328 curcuminoids. Ligands 90, 175, and 1CJS displayed high affinity compared to GTP, with ligand 90 showing stable binding interactions. Binding affinity evaluated using MM-PBSA indicated that ligand 90 had a high affinity of -127.6 kcal/mol, suggesting it as a potent RdRp inhibitor for DENV. **Conclusion:** Compound 90 is a potent RdRp inhibitor for DENV. Our analysis offers valuable insights into NS5 RdRp behavior and ligand interactions, aiding antiviral strategy development against dengue virus.

Keywords: Rdrp, Dengue Virus, Ensemble Docking, Molecular Dynamics Simulations, Virtual Screening

OJ5

RNA Sequencing Reveals Transcriptomic Changes in HEK293 Cells Following Introduction of rs16851030 DNA Variant

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ABSTRACT

Introduction: Rs16851030, a single-nucleotide variant located in the 3'-untranslated region of the *ADORA1* gene, has been proposed as a potential marker of caffeine sensitivity in preterm apnoea, aspirin-induced asthma, and the development of acute chest syndrome. However, its functional significance is still unconfirmed. This study aimed to elucidate the functional impact of rs16851030 by using CRISPR/Cas9 to induce physiological changes associated with the DNA variant. **Methods:** The rs16851030 variant was introduced into HEK293 cells through homology-directed repair induced by a combination of a sgRNA, a plasmid-encoded CRISPR enzyme, and a single-stranded oligodeoxynucleotide donor template. Edited cells were then fluorescence-enriched, sorted, isolated, and grown into single-cell clones. The single-base edit was confirmed by Sanger sequencing. Finally, RNA sequencing was performed to elucidate the pathways affected by rs16851030. **Results:** rs16851030-mutant cells were found to be more susceptible to the adverse consequences of hypoxia. Following 24 h of exposure to hypoxia, both CRISPR-edited clones 1 and 2 exhibited lower levels of viability than the wild-type cells (75.45% and 74.47% vs 96.34%). **Conclusion:** Our study therefore provides valuable information about key pathways associated with rs16851030 DNA variant. The molecular mechanisms that underpinned the increased vulnerability to hypoxia should be explored in the future by investigating the transcriptomic changes caused by rs16851030 in hypoxic condition.

Keywords: rs16851030, CRISPR/Cas9, Hypoxia, Caffeine, Preterm Apnoea

OJ6

Characterization, Cytotoxicity Assessment, and In Vivo Evaluation of Chitosan/Alginate Polymeric Nanoparticle-Loaded with α-Mangostin Against Breast Cancer

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ABSTRACT

Introduction: α-Mangostin (Amg), a compound isolated from the rind of the mangosteen (*Garcinia mangostana* L.), exhibits promising anticancer properties. However, its efficacy has been constrained by low solubility and selectivity towards cancer cells. To address this limitation, researchers have developed chitosan/alginate polymeric nanoparticles (NANO-AMCAL) to increase the efficiency of Amg. Initial in vitro research demonstrated the remarkable efficacy of NANO-AMCAL against breast cancer cells. The present study aims to assess NANO-AMCAL's potential for breast cancer treatment in Wistar rats (*Rattus norvegicus*) and determine the optimal dosage. **Methods:** Seven treatment groups, comprising normal (without DMBA induction), control, tamoxifen, Amg Pure (P.Amg), NANO-AMCAL 5 mg, NANO-AMCAL 10 mg, and NANO-AMCAL 20 mg, were established. The rats were subjected to subcutaneous administration of 7,12-dimethylbenz(a)anthracene (DMBA), a carcinogenic substance. Their body weight and tumor volume were measured every three days throughout the treatment period. On day 14, surgical procedures were conducted. Histopathological examinations were carried out on breast and lung tissues to assess the treatment's effectiveness. **Results:** The results showed that NANO-AMCAL significantly enhanced the anticancer activity of Amg in treating breast cancer in Wistar rats. NANO-AMCAL containing 0.33 mg of Amg had a healing effect three times better than 20 mg pure Amg and was comparable to tamoxifen. The effective dose of NANO-AMCAL for anti-breast cancer treatment in Wistar rats was found to be 20 mg, which exhibited a good healing response, and the tumor volume continued to decrease up to 17.43% on the 14th day. Furthermore, histopathological tests showed tissue repair and no metastases. **Conclusion:** These results imply that NANO-AMCAL could be a promising therapeutic choice for the treatment of breast cancer.

Keywords: α-Mangostin, Nanoparticles, DMBA, *In Vivo*, Breast Cancer

OJ7

Assessment of Anti-Inflammatory Potential of Dayak Onion (*Eleutherine bulbosa* (Mill.) Urb.) Extract in Comparison to Celecoxib

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ABSTRACT

Introduction: *Eleutherine bulbosa* (Mill.) Urb., commonly known as Dayak onion, has a rich history in traditional medicine due to its diverse health benefits, including anti-inflammatory, anticancer, and immunostimulant activities. This study assessed the in vitro anti-inflammatory potential of the ethyl acetate fraction derived from Dayak onion bulb ethanol extract (DOF) in comparison to the nonsteroidal anti-inflammatory drug (NSAID) celecoxib (Cxb). **Methods:** DOF was procured via maceration extraction using ethanol as the primary solvent, followed by a subsequent liquid-liquid extraction using ethyl acetate. The anti-inflammatory activity of DOF was evaluated by measuring its ability to inhibit the production of the proinflammatory mediator nitric oxide (NO) in lipopolysaccharide-induced RAW 264.7 macrophages. **Results:** DOF, at concentrations up to 500 µg/mL, resulted a cell viability of 117.77% in RAW 264.7 macrophages when compared to the control. These data indicate its safety within this concentration range. In contrast, treatment of celecoxib at concentrations of 2 and 10 µg/mL resulted in cell viabilities of 128.99 and 134.04%, respectively when compared to the control. At a higher concentration of 50 µg/mL, however, it resulted in a reduced cell viability of 62.81%. These findings indicate that celecoxib at concentrations of 10 µg/mL or lower can be considered safe for use, whereas at higher concentrations, such as 50 µg/mL or above, it manifests cytotoxic effects on RAW 264.7 macrophages. DOF, at concentrations of 10, 20, 40, and 100 µg/mL provided successive inhibition of NO production in lipopolysaccharide-induced RAW 264.7 macrophage cells at 6.07, 9.52, 15.64, and 24.97%, respectively. Cxb at concentrations of 5, 10, and 20 µg/mL resulted in successive NO inhibitions at 8.89, 18.19, and 28.68%. **Conclusion:** DOF exhibits promising anti-inflammatory properties that warrant further investigation for potential therapeutic applications due to its non-toxic nature and ability to inhibit proinflammatory mediators.

Keywords: Dayak Onion (*Eleutherine bulbosa* (Mill.) Urb.) Extract, Anti-inflammatory Assessment, Celecoxib, Nitric Oxide Inhibition, RAW 264.7 Macrophages

POSTER PRESENTATION

PA1

Active Targeting Docetaxel-loaded Nanocapsules Primarily Composed of Polycaprolactone, Chitosan-Folate, and TPGS for Lung Cancer Treatment

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ABSTRACT

Introduction: Docetaxel, approved for the second-line treatment of advanced non-small cell lung cancer, is associated with several issues, including low tumor selectivity. Encapsulating Docetaxel into folate-functionalized nanocapsules helps to actively target folate receptors (FR) commonly overexpressed on lung tumor surfaces. **Methods:** Docetaxel and sorbitan monostearate are dissolved in ethanol followed by the addition of this ethanolic solution into polycaprolactone dissolved in acetone to form the organic phase. The organic phase is slowly added into the sonicated aqueous phase (TPGS dissolved in distilled water) to form nanoemulsion. The organic solvents are removed using a rotatory evaporator to induce nanoprecipitation. Finally, the chitosan-folate solution is slowly titrated into the sonicated concentrated emulsion followed by centrifugation to collect the nanocapsules. **Results:** Prior to the addition of chitosan-folate, the nanocapsules formed have a negative zeta potential (-18.7 ± 1.3 mV) contributed by the PEGylated surface. The positive-charged chitosan-folate interacts with the negative-charged surface to form the outer coating, a similar concept to ionotropic gelation. The nanocapsules have a median size of around 200 nm suitable for systemic circulation and negative zeta potential (-8.5 ± 0.5 mV), accorded by a novel combination of TPGS and chitosan-folate to mitigate renal elimination linked to positive-charged nanoparticles. The nanocapsule boasts a high encapsulation efficiency of close to 99%. Characterization using a transmission electron microscope reveals that the nanocapsules have a globular structure suitable for parenteral administration. **Conclusion:** The characteristic of nanocapsules produced seems promising to tackle drug delivery issues discussed in various literature. The targeting effectiveness will be determined through in-vitro cytotoxicity using lung tumor cells (FR-positive H1299 and FR-negative A549) and normal lung BEAS-2B. Depending on the in-vitro results, the effectiveness will be validated further through in-vivo pharmacokinetic, biodistribution, and toxicity studies.

Keywords: Non-Small Cell Lung Cancer, Drug Delivery, Docetaxel, Polymeric Nanoparticles, Folate Targeting

PA2

Design and Development of Self-Propelling, Magnetic, Protein Conjugated Drug Delivery System

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ABSTRACT

Introduction: Biocompatible, self-propelling and magnetic nanobots with high efficiency for drug loading and tumor targeting emerge as a promising strategy to address serious issues of currently used anticancer drugs. Here, we report plasma protein conjugated magnetic nanobots loaded with doxorubicin as a dynamic and targeting drug delivery platform. **Methods:** Novel magnetic nanobots have been fabricated by conjugating plasma proteins loaded by DOX with Fe_3O_4 nanoparticles. Propulsion and magnetic behaviour, Drug release kinetics and cytotoxicity analysis were studied. **Results:** Chemically conjugated plasma proteins create biocompatible surface which provides large chemical space for drug loading/conjugation and accessibility to multiple bio-macro molecules for tumor targeting e.g., albumin, transferrin etc. Moreover, self-propulsion and magnetic navigation born due to Fe_3O_4 nanoparticles enable this nano-system with dynamic functionality. Fe_3O_4 based nanobots were found to autonomously propel in biologically relevant media such as human blood serum and PBS. Propelling velocity, time and total distance travelled by nanobots were found to be increased with increasing H_2O_2 concentration. The designed nanobots showed pH responsive drug release, representing its selective activation and controlled drug release in tumor microenvironment. Cytotoxicity study confirmed the potential of nanobots to kill cancer cells more effectively than free DOX. **Conclusion:** This research work presenting a novel platform for nanobots that demonstrates multiple functions such as self-propulsion; tumor targeting and pH responsive drug release can be applied for delivery of highly toxic anticancer drugs and other cargo.

Keywords: Self-propelling, Drug Delivery System, Anticancer, Magnetic, Nanobots

PA3

Nanotheranostics Utilizing 5-Fluorouracil in Cancer Management: An In-Depth Analysis of Efficacy, Safety, and Diagnostic Applications

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ABSTRACT

Introduction: The utilisation of nanocarriers for drug delivery in cancer treatment is further enhanced by incorporating diagnostic functionality. These dual-purpose nanotheranostic agents (NTAs), serve as a single platform capable of both treatment and real-time monitoring of cancer progression simultaneously. The wide range of materials utilised in constructing NTAs may lead to significant variations in their properties. Therefore, a systematic review was conducted to consolidate current NTAs incorporating 5-fluorouracil (5FU) and elucidate their differences in toxicity, efficacy, and imaging performance. **Methods:** Medline and Embase databases were searched up to 18th March 2022 to include articles with keywords of "cancer," "theranostics," "nanoparticle," "in vivo" and "fluorouracil" in combination. Publications were screened if they met the following criteria: original research involving 5FU, utilising an animal cancer model, and reported outcomes related to efficacy, toxicity, or diagnostics. **Results:** Nine studies were included in the analysis, with 44.4% developing NTAs using inorganic materials, mainly gold nanoparticles. Another 33.3% developed NTA using the hybrid of organic and inorganic materials while two studies used organic material only to achieve nanotheranostic properties. The 5FU-NTAs were categorised based on their functions: active targeting only (50.0%), thermal ablation only (33.3%) and a combination of both (16.67%). Irrespective of the materials used, all functionalised NTAs consistently outperformed the non-functionalised nanoparticles, evidenced by a tumour volume reduction exceeding 40% compared to the control. All NTAs did not result in significant toxicity based on the body weight change. For imaging, the NTAs tagged with targeting moiety achieved maximum tumour accumulation faster (within 6 hours). **Conclusion:** The functionalised NTAs hold promises for all-in-one management of advanced cancer. To further improve the quality of current preclinical practice, this review proposed a checklist of parameters (PICANT) to recommend researchers for nanoparticle testing in animal cancer studies.

Keywords: Nanoparticles, Cancer, Thermal Ablation, Tumour-Targeting, *In Vivo*

PA4

Anticancer Potential of Andaliman (*Zanthoxylum acanthopodium* DC.) Fruit Ethanol Extract

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ABSTRACT

Introduction: Andaliman (*Zanthoxylum acanthopodium* DC.) is a shrub renowned for its fruit, a widely used spice in traditional cuisine. The 70% ethanol extract of Andaliman fruit is enriched with alkaloids, polyphenols, terpenoids and flavonoids which are recognized for potential anticancer activity. This research aims to study anticancer potential of Andaliman Fruit Ethanol Extract (EEBA) by evaluating its cytotoxic effects on MCF-7 breast cancer and HEK 293-A human embryonic kidney cell lines. **Methods:** EEBA was prepared from the fruits using 70% ethanol. Phytochemical screening on the extract was conducted by using Harborne method. Cytotoxicity assessments were conducted using the WST-1 method on both HEK 293-A and MCF-7 cell lines. Measurements were taken at 450 nm. Annexin V-PI staining was used to analyse cell death via apoptosis. Non-linear regression analysis was performed using GraphPad Prism and Microsoft Excel 2016. **Results:** Phytochemical screening of EEBA revealed the presence of alkaloids, polyphenols, flavonoids, monoterpenes, and steroids. The IC50 values for EEBA were determined to be 67.42 µg/mL for HEK 293-A cells and 100.2 µg/mL for MCF-7 cells. To assess selectivity, the toxicity of the extract on MCF-7 cells was compared to normal HEK 293-A cells, resulting in a selectivity value (IS) of 0.67. A high selectivity index is typically considered when IS > 3. Hence, the extract was considered to have low selectivity against MCF-7 cell line. Annexin V-P1 results revealed the early stage of apoptosis, as well as necrosis. **Conclusion:** EEBA showed potential cytotoxicity against both HEK 293-A and MCF-7 cell line, with IC50 values of 67.42 µg/mL and 100.2 µg/mL, respectively.

Keywords: Andaliman, *Zanthoxylum acanthopodium* DC., Cytotoxicity, MCF-7 Cell

PA5

The Role of Peroxisome Proliferator-Activated Receptor-β/δ Antagonist in Melanogenesis

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ABSTRACT

Introduction: Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) is a ligand-activated transcription factor that belongs to the superfamily of nuclear hormone receptors. Antagonists targeting PPARβ/δ have been known to play an important role in various cellular responses including cell proliferation, differentiation, regulation of inflammation, and the maintenance of energy homeostasis. Notably, PPARβ/δ is expressed in both mouse and human melanocytes. The role of PPARβ/δ in melanogenesis remains unexplored. **Methods:** The B16F10 murine melanoma cell line derived from C57BL/6J mouse was exposed to a novel PPARβ/δ antagonist. The melanin secretion and the expression of Microphthalmia-associated transcription factor (Mitf) were subsequently assessed. **Results:** The exposure of B16F10 cells to the PPARβ/δ antagonist led to a significant reduction in melanin secretion by these cells when stimulated by alpha-melanocyte stimulating hormone. Importantly, this reduction in melanin secretion did not induce toxicity in the cells. A concomitant decrease in Mitf expression within mouse melanoma B16/F10 cells after the treatment was also noticed. **Conclusion:** Our results suggest that PPARβ/δ might play a role in regulating melanogenesis.

Keywords: Peroxisome Proliferator-Activated Receptor-β/δ, Antagonists, Melanogenesis

PA6

A Broad-Spectrum Antiviral Targeting RNA Viruses

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ABSTRACT

Introduction: Dengue virus (DENV), a mosquito-borne flavivirus, continues to be a major public health threat in many countries and there are no antiviral therapeutics available. Limited protective efficacy across four dengue serotypes of the current available DENGVAXIA and QDENGA vaccine prompt the need to search for alternative. **Methods:** In this work, we discovered a sulfonyl anthranilic acid (SAA) derivative of the 2,1-benzothiazine 2,2-dioxide core that was previously used to develop DENV NS5 polymerase inhibitors. Dose-response inhibition experiment of SAA against DENV was performed to determine the EC_{50} and CC_{50} values. Time-of-drug-addition assay (TODA) was carried out to investigate the mechanism of action of the most potent compound - FlaR18, followed by quantification of viral RNA level and viral protein production. The efficacy of FlaR18 is also evaluated in different cell lines. Thermal proteome profiling (TPP) was performed to investigate the binding target of FlaR18. **Results:** Of the 38 SAA derivatives, several exhibited potent anti-DENV-2 activity in the cell-based inhibition assay, but surprisingly did not inhibit DENV NS5 polymerase activity. Notably, compound FlaR18 showed EC50 values in the range of 0.3 to 0.6 μM against the four dengue serotypes (DENV-1-4) and different RNA viruses. Time of addition assay revealed that analogue FlaR18 is a post-entry replication inhibitor that appears to be specific for cells of primate origin, implicating a host target. We have taken a high throughput proteomic approach, Cellular Thermal Shift Assay coupled to Mass Spec (MS-CETSA), to identify potential host targets that are currently being validated in gene knock out assays to elucidate the mechanism of action for compound FlaR18. **Conclusion:** Compound FlaR18 could serve as a lead for more potent inhibitors against the target since it also shows similar antiviral efficacy against other RNA viruses that have been tested.

Keywords: Dengue Virus, Host-Directed Antiviral, Cell-Based Infection Assay, Mass-Spectrometry

PA7

Exploring the Therapeutic Potential: *In Vitro* Assessment of the Dietary Supplement L-Citrulline's Antiglycation and Antioxidant Properties

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a worldwide health issue characterized by hyperglycemia due to insulin resistance, which causes protein glycation and oxidative stress. Protein glycation and oxidative stress play critical roles in the pathogenesis of DM by contributing to impaired insulin sensitivity and pancreatic beta-cell dysfunction. The rising prevalence of diabetes mellitus has spurred a search for a new, affordable, and effective medication. The primary goal of this study is to investigate the anti-glycation and antioxidant properties of the dietary supplement L-Citrulline. **Methods:** The researcher employed a two-reaction model system to study the dietary supplement L-Citrulline's impact on advanced glycation end product (AGE) formation. This involved in vitro assays, including the BSA-Glucose and BSA-MGO assays. Additionally, the supplement's antioxidant abilities were assessed by measuring its metal ion binding capacity through absorbance reading and its reactive oxygen species scavenging potential using differentiated C2C12 myoblasts in a fluorescence measurement. **Results:** In antiglycation assays, at 100 ppm, dietary supplement L-Citrulline inhibited AGEs by 52.19 ± 0.39% (BSA-Glucose) and 49.64 ± 0.27% (BSA-MGO) when compared to the control. It also chelated Fe2+ ions, reducing activity by 68.58 ± 0.45% at 100 ppm when compared to the control. In the reactive oxygen species assay with Glucolipotoxicity (GLT) media, reactive species levels increased significantly by 173.48 ± 9.37% compared to the control, but adding 10 mM, dietary supplement L-Citrulline reduced this increase significantly to 98.42 ± 5.04%. These findings suggest that dietary L-Citrulline has therapeutic potential in eliminating reactive oxygen species (ROS) in skeletal muscle cells as a dietary supplement. **Conclusion:** The study findings indicate that L-Citrulline, a dietary supplement, effectively inhibits glycation at various concentrations and demonstrates significant antioxidant efficacy. These results suggest its potential as a treatment for diabetes mellitus.

Keywords: Type 2 Diabetes Mellitus, Hyperglycemia, In vitro, Dietary Supplement L-Citrulline

PA8

α-Amylase and α-Glucosidase Inhibitory Potential of the Different Solvent Extracts from the Air-Dried Leaves of *Crescentia cujete* Linn.

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ABSTRACT

Introduction: Diabetes mellitus is characterized by high blood sugar levels resulting from defects in insulin secretion, insulin action, or both, in the absence of treatment. Approximately 90% of people with diabetes are classified as having type 2 diabetes mellitus (T2DM), 8% have type 1 diabetes mellitus (T1DM), and 2% have a rare type of diabetes. Crescentia cujete Linn., commonly known as Calabash Tree, had previously been reported to possess antimicrobial, anti-inflammatory, analgesic, antioxidant, and antidiabetic properties. It had been widely used as an herbal medicine in rural areas of the Davao region in the Philippines. The present study aims to investigate the in vitro antidiabetic potential of the different solvent extracts from the air-dried leaves of Calabash Tree. **Methods:** Secondary metabolites of the crude ethanol, partitioned ethanol/ hexane, and ethyl acetate extracts of the air-dried leaves of Crescentia cujete Linn. were assessed qualitatively. In vitro α-amylase and α-glucosidase inhibition assays were conducted. **Results:** Qualitative screening of the secondary metabolites showed the presence of flavonoids, saponins, alkaloids, saponins, reducing sugars, and condensed tannins in all the solvent extracts. Notably, the ethyl acetate solvent extract exhibited significant inhibition of 75.43% against α-amylase and ethanol solvent extract demonstrated significant inhibition of 57.12% against α-glucosidase. **Conclusion:** This study demonstrates that air-dried leaves of Crescentia cujete Linn. could serve as a valuable source of secondary metabolites capable of combating α-amylase and α-glucosidase activities.

Keywords: Phytochemical Screening, Calabash Tree, Diabetes Mellitus

PA9

Chlorophytum alismifolium Baker Ameliorates Hyperglycaemia: Correlation Between Blood Glucose Levels and Some Biomarkers

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ABSTRACT

Introduction: Medicinal plants are widely utilized in the management of many ailments. *Chlorophytum alismifolium* Baker ameliorates diabetes mellitus and its complications. This study focused on the antihyperglycaemic effect of *C. alismifolium* and the correlation of blood glucose level with some biomarkers using the Enzyme-linked Immunosorbent Assay (ELISA) technique. **Method:** Diabetes was induced in rats using streptozotocin. The rats were administered graded doses (150, 300 and 600 mg/kg) of the various extracts and fraction of *C. alismifolium* daily for 28 days. Blood glucose was measured weekly and serum dipeptidyl peptidase 4, (DPP-4) and peroxisome proliferator activated receptor gamma (PPAR-γ) levels were evaluated at the end of the study using ELISA technique. SPSS Version 20 was used for the analyses. Data were expressed as Mean ± Standard Error of the Mean (S.E.M.) and the differences between means were analyzed using One way and Repeated Measure ANOVA followed by Bonferoni's post hoc. Pearson's correlation analysis was performed to measure the association of blood glucose levels on day 28 with serum PPAR-γ, AR and DPP-4. Values of $P \leq 0.05$ were considered statistically significant. **Results:** Induction of diabetes significantly ($p<0.001$) raised the blood glucose level in hyperglycaemic rats compared to the normal control. Administration of the various extracts and fraction of *C. alismifolium* significantly ($p<0.05$) lowered the blood glucose levels compared to the hyperglycaemic control and over time. Ethyl acetate extract of *C. alismifolium* (EACA) produced the best glycaemic control and significantly ($p<0.05$) increased PPAR-γ expression and markedly ($p<0.05$) decreased serum levels of DPP-4. Pearson's correlation analysis revealed a significant negative correlation ($p<0.001$) between the blood glucose levels and PPAR-γ. However, a significant positive correlation was observed between the blood glucose levels and DPP-4 ($p<0.001$). **Conclusion:** EACA elicits antihyperglycaemic activity which is possibly mediated through increased PPAR-γ expression and decreased serum level of DPP-4.

Keywords: *Chlorophytum alismifolium*, Dipeptidyl peptidase 4, ELISA, Hyperglycaemia, Peroxisome proliferator activated receptor gamma

PA10

Assessment of a Self-Micro Emulsifying Drug Delivery System for Enhancing the Dissolution of Atorvastatin and Apigenin

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ABSTRACT

Introduction: Poorly water-soluble orally administered drugs often encounter dissolution challenges due to limited aqueous solubility. This study focuses on enhancing dissolution through self-micro emulsifying drug delivery systems (SMEDDS). It aims to improve the dissolution of atorvastatin and apigenin in fixed dose combination, both Biopharmaceutical Classification System Type 2 drugs, using well-optimized SMEDDS formulations in various vehicles. **Methods:** Lipid-based systems were meticulously developed for atorvastatin-apigenin, capable of forming Self-Micro Emulsifying Drug Delivery Systems (SMEDDS). These systems incorporated key components, including an oil phase (Capmul MCM and black seed oil) and a surfactant/co-surfactant or co-solvent phase (Cremophor RH40, Transcutol HP, PEG400). Evaluation of the SMEDDS encompassed critical quality attributes like size distribution and drug dissolution profiles, alongside assessments of dilution effects, transmittance, zeta potential, polydispersity index (PDI), and solubility profiles. **Results:** SMEDDS formulations yielded negatively charged dispersions, typically sized between 20-80 nanometers at varying dilutions. All SMEDDS showed rapid drug release, with about 90% released within 10-20 minutes, indicating improved release compared to unprocessed drug powder. Formulations remained stable upon dilution. Visual inspection, size distribution, and zeta potential of SMEDDS stored at 25°C and 40°C remained unchanged. Enhanced drug release in SMEDDS was attributed to stable nano-sized dispersions and high drug solubility within the formulation. **Conclusion:** The lipid-based systems incorporating Capmul MCM or black seed oil, along with Cremophor RH40, Transcutol HP, or PEG400, successfully generated Self-Micro Emulsifying Drug Delivery Systems (SMEDDS). These SMEDDS formulations resulted in a substantial improvement in the dissolution of both atorvastatin and apigenin, accompanied by optimized particle size, distribution, and long-term stability.

Keywords: Self-Micro Emulsifying Drug Delivery Systems (SMEDDS), Poor Water-Soluble Drug, Dissolution, Atorvastatin, Apigenin

PA11

In Vitro Antimicrobial and Anticancer Activities, and Identification of Rare Actinomycete Strains Isolated from Soil

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ABSTRACT

Introduction: Actinomycetes are among the most attractive microbial sources of new bioactive metabolites, and many genera within this group are being harnessed for their potential by the pharmaceutical industry. Unfortunately, the rate of discovering new compounds has decreased as ubiquitous species have already undergone extensive study. Currently, the discovery of new natural metabolites is focused on rare actinomycetes. **Methods:** Culture broth crude extracts of Strain TY052-011 and Strain TY028-004 were tested for antimicrobial activity using a preliminary method. Anticancer activity was tested using the Sulforhodamine B (SRB) assay. The strains were identified based on their 16S rRNA gene sequences. **Results:** Both strains exhibited antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*), but the extracts were inactive against Gram-negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*). Only Strain TY028-004 exhibited moderate anticancer activity against breast (*MCF-7*), ovarian (*SKOV-3*), and colorectal (*HT-29*) cancer cell lines, with IC_{50} values of 54.81, 66.03, and 74.36 ug/ml, respectively. Phylogenetic analysis indicated that Strain TY052-011 was most closely related to *Nonomuraea cypriaca* (98.37% homology), and Strain TY028-004 is closely related to *Microbispora bryophytorum* (98.56% homology). **Conclusion:** Nonomuraea sp. TY052-011 and Microbispora sp. TY028-004 are rare actinomycetes belonging to the family Streptosporangiaceae in the class Actinomycetia. Recent reports have indicated that new species of rare actinomycetes hold promise as sources of bioactive natural products with potential applications. Further investigation into the bioactive compounds produced by *Microbispora* sp. TY028-004 is promising, as it exhibits antimicrobial and anticancer activities.

Keywords: Rare Actinomycetes, *Nonomuraea, Microbispora*, Antimicrobial, Anticancer

PA12

Intracellular Trafficking of *Phyllanthus niruri* Extract-Loaded Chitosan Nanoparticles in Sertoli Cells

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ABSTRACT

Introduction: Ethanol extract of *Phyllanthus niruri* (*P. niruri*) leaves was previously loaded into chitosan nanoparticles, serving as a co-adjuvant therapy of hepatitis. However, these nanoparticles system showed reproductive toxicity towards Sertoli cells and spermatogenesis in mice. Understanding the mechanisms of cellular entry and the intracellular localization of nanoparticles is important for assessing their toxicity profiles. The present study aims to provide a comprehensive understanding of the reproductive toxicity associated with these nanoparticles. **Methods:** Chitosan nanoparticles encapsulating *P. niruri* extract were prepared by ionic gelation method. The cellular uptake mechanism and endosomal escape of cargo-loaded chitosan nanoparticles were examined through Confocal Laser Scanning Microscopy analysis. To investigate particle, rhodamine-labeled chitosan nanoparticles were employed. Sertoli cells were subjected to treatments involving sucrose, amiloride, or filipin III, while nucleus labeling was performed using Hoechst 33343. To assess the nanoparticles' ability to escape from endosomes, rhodamine-labeled chitosan nanoparticles were utilized, and endosomes were visualized by staining with Lysosensor. **Results:** The *P. niruri*-loaded chitosan nanoparticles exhibited a size of 185.70 ± 4.10 nm and a positive charge of +34.49 ± 2.10 mV. Interestingly, when *P. niruri* extract was replaced with rhodamine as the cargo, the size and charge of the particles remained consistent. The present results showed that these chitosan nanoparticles gain entry into the mouse Sertoli cells through mechanisms involving macropinocytosis and clathrin-dependent endocytosis. A significant increase in lysosomal colocalization of chitosan nanoparticles was observed after three hours, while significant cytosolic release from endosomes was noticed after five hours of incubation. **Conclusion:** The observed toxicity of *P. niruri* extract-loaded chitosan nanoparticles appear to be closely linked to the cellular uptake mechanism involving endocytosis, followed by subsequently release of their cargo within Sertoli cells.

Keywords: *Phyllanthus niruri*, Chitosan Nanoparticles, Uptake Mechanism, Endosomal Escape, Sertoli Cells

PA13

Fragment-Based *in Silico* Design of SARS CoV-2 Main Protease Inhibitors

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ABSTRACT

Introduction: 3CLpro is essential for SARS-CoV-2 replication and infection. Inhibition of 3CLpro using small molecules is, hence, a potential therapeutic strategy. In this study, a comprehensive crystallography-guided fragment-based drug discovery approach was employed to design new inhibitors for SARS-CoV-2 3CLpro. **Methods:** All small molecules co-crystallised with SARS-CoV-2 3CLpro and structures deposited in the Protein Data Bank were used as inputs. Fragments located within the binding pocket (87) were grouped into eight geographical types. These fragments were then interactively coupled using various synthetically reasonable linkers to generate larger molecules with divalent binding modes, taking advantage on interactions between two different fragments. **Results:** In total, 1,251 compounds were proposed, and 7,158 stereoisomers were screened using Glide (standard precision and extra precision), AutoDock Vina, and Prime MMGBSA. The top 22 hits having conformations approaching the linear combination of their constituent fragments were selected for MD simulation on Desmond. MD simulation suggested 15 of these compounds indeed adopted conformations very close to their constituent pieces, with far higher binding affinity than either constituent domain in isolation. **Conclusion:** These structures could provide a starting point for the subsequent development of SARS-CoV-2 3CLpro inhibitors with the potential of improved binding. Detailed structural information is provided for reference and further exploration.

Keywords: Coronavirus COVID-19, Fragment-Based Drug Discovery, Main Protease Mpro 3CLpro, Multivalency, SARS-CoV-2

PA14

Exploring Immunomodulation Effects of *Moringa oleifera* (Lam.) Leaves Extract on Normal and Immunocompromised Animal Model

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ABSTRACT

Introduction: Insufficient immunity can lead to poor immune system performance against antigens leading to chronic diseases via down-regulation in lymphocytes or stem cell proliferation. There is a surge in illnesses, particularly infectious diseases, that need effective body defence systems to regulate through the process of immunomodulation. As a result, the development of natural immunomodulators with desirable safety and efficacy profiles is crucial. This study aimed to investigate the immunoregulatory and regenerative capabilities of a standardized 70% ethanol extract of Moringa oleifera leaves (MoETE) on animal models. **Methods:** 5 groups (n=6) of SD of immuno-competent and induced immuno-suppressed rats were administrated with 150, 300 and 600 mg/kg of MoETE through oral gavage for 28 days whereas administration of water and levamisole served as negative and positive controls. Tail blood was withdrawn periodically and organs were analysed at the end of the study. **Results:** MoETE did not significantly affect the level of red and white blood cells in the healthy rats, immunosuppression group showed the supplementation of MoETE normalized the red and immune cell levels. Statistical analysis revealed a significant difference between the groups and days on the dependent variance WBC in both normal and immunosuppressed groups ($p < 0.05$ for α level). It was evident that the number of CD8+ T cells and regulatory T cells increased by MoETE. **Conclusion:** Supplementation of *Moringa Oleifera* (Lam.) improves the immune system function in immunocompromised animal models.

Keywords: Moringa oleifera, Immunomodulatory, Immune Cells

PA15

Development of Nanostructured Lipid Carrier as a Nanocarrier for Clove (*Syzygium aromaticum*) Essential Oil

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ABSTRACT

Introduction: The essential oil derived from clove (*Syzygium aromaticum*) has been reported to possess diverse pharmacological activities, including antioxidant, anticancer, and antimicrobial activities. However, the specific physicochemical properties of clove essential oil (CO), including high volatility, oxidation susceptibility, and potential irritation effects, limit the practical application of CO as a therapeutic agent. To overcome these challenges, the present research aimed to develop a nanostructured lipid carrier (NLC) to optimize the delivery of CO for enhanced health benefits. **Methods:** CO was incorporated into the NLC system using the emulsification-sonication method. Several formulation factors, including the selection of liquid lipid component, determination of surfactant concentration, and the selection of co-surfactant, were optimized. The optimized CO-NLC formulation underwent comprehensive characterization, including assessment of particle size and distribution, measurement of zeta potential, visualization of particle morphology, and computation of encapsulation efficiency. The stability of CO-NLC was rigorously assessed during the storage at 4 °C to ensure its reliability over time. **Results:** The optimized CO-NLC formulation displayed a spherical shape, homogenous particle distribution, and a hydrodynamic diameter of approximately 125 nm. These nanoparticles exhibited a negatively charged properties with an encapsulation efficiency of 97%. During the 14-day storage period, no significant change in the particle size of CO-NLC was observed. **Conclusion:** We succeeded in developing CO-NLC with both high encapsulation efficiency and stability, warranting further exploration and development in the pharmaceutical and cosmetic industries.

Keywords: Clove Essential Oil, Nanostructured Lipid Carrier, Antioxidant

PB1

In Vitro Anticancer Activities of *Derris microphylla* Extracts on Selected Cancer Cell Lines

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ABSTRACT

Introduction: Natural products, particularly those derived from plants, have been the mainstay of cancer chemotherapy for the past 40 years. Despite the advancement of current chemotherapy drugs, cancer cases continue to rise and the search for new chemotherapy drugs is still ongoing to combat drug resistance and minimise adverse effects in cancer patients. One such potential candidate is *Derris microphylla*, a plant species from the Fabaceae family, traditionally used by the Semai sub-ethnic for treating scabies. However, its potential in treating cancer has not been extensively studied or documented in scientific literature. The aim of this study is to evaluate the potential anticancer activity *in vitro* in *D. microphylla* aged 48 and 54 months. **Methods:** Plantlets were gathered from Ulu Geroh, Gopeng, Perak and cultivated at FRIM Research Station (SPF) in Selandar, Malacca. The leaves, stems and bark from 48- and 54-month-old trees were collected and subjected for ethanol extraction for 72 hours at room temperature. The resulting ethanolic extracts were then filtered, concentrated using a rotary evaporator and stored in 4°C. These ethanolic extracts were screened for in vitro anticancer activity against a panel of six human cancer cell lines (A2780, SKOV-3 – ovarian cancers, A375 – melanoma, HeLa – cervical cancer, HT-29 – colorectal cancer, MCF-7 – breast cancer) using Sulforhodamine B (SRB) assay. **Results:** The ethanolic extracts obtained from the stems of 48- and 54-month-old *D. microphylla* trees exhibited remarkably potent anticancer activity with IC_{50} values less than 10 µg/mL in all cancer cells tested. **Conclusion:** These findings offer valuable insights into the anticancer potential of *D. microphylla* extracts, thereby broadening treatment possibilities for numerous cancer cell lines that warrant further investigation.

Keywords: Derris microphylla, Traditional Medicine, In Vitro Anticancer, Cultivated, Semai

PB2

Design, Synthesis and Evaluation of Novel Enzalutamide Analogues as Potential Anticancer Agents

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ABSTRACT

Introduction: The androgen receptor inhibitor, Enzalutamide, has demonstrated effectiveness against castration-resistant prostate cancer, leading to clinical benefits and increased survival rates in men. However, the emergence of AR mutation (F876L) converts Enzalutamide from an antagonist to an agonist, indicating the rapid evolution of resistance. To overcome this resistance mechanism, our goal is to design and develop novel Enzalutamide analogues. **Methods:** We designed a dataset of Enzalutamide derivatives using the shape and electrostatic features of Enzalutamide to match the pharmacophoric features necessary for tight binding with the androgen receptor. Based on this design strategy, ten novel derivatives, including 5,5-dimethyl-3-(6-substituted benzo[d]thia/oxazol-2-yl)-2-thioxo-1-(4-(trifluoromethyl) pyridin-2-yl) imidazolidin-4-one (6a-j), were selected for synthesis. In-vitro evaluations of all compounds were performed on prostate cancer cell lines DU-145, LNCaP, and PC3. **Results:** Two compounds, 3-(6-hydroxybenzo[d]thiazol-2-yl)-5,5-dimethyl-2-thioxo-1-(4-(trifluoromethyl)pyridin-2-yl)imidazolidin-4-one (6c) and 3-(6-hydroxybenzo[d]oxazol-2-yl)-5,5-dimethyl-2-thioxo-1-(4-(trifluoromethyl)pyridin-2-yl)imidazolidin-4-one (6h), showed promising in-vitro antiproliferative activity against prostate cancer cell lines, with IC50 values ranging from 18.26 to 20.31µM. The binding mechanism of these potential androgen receptor inhibitors was further studied through molecular docking, molecular dynamics simulations, and MM-GBSA binding free energy calculations. The results of these analyses were found to be in agreement with the in-vitro studies, providing strong theoretical support for our hypothesis. **Conclusion:** Our study aimed to overcome resistance caused by the AR mutation in Enzalutamide treatment by designing novel analogues. Two compounds (6c and 6h) showed promising in-vitro antiproliferative activity against prostate cancer. Molecular docking and simulations supported our hypothesis, providing insights into the binding mechanism. Further research is needed to explore the therapeutic potential of these analogues in overcoming castration resistance.

Keywords: Hybrid Molecules, Imidazolidinone Derivatives, Molecular Docking

PB3

Protein Profiling of AIC250 Treated on Endothelial Cell Line EA.hy926 for Anti-angiogenesis Assessment

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ABSTRACT

Introduction: Early intervention of angiogenesis is crucial in halting tumour progression. Although VEGF-targeted anti-angiogenesis drugs exist, effectiveness and specificity challenges persist. Amid the quest for enhanced therapies, innovative strategies seek novel agents disrupting cancer angiogenesis via diverse mechanisms. Current inhibitors' limited efficacy and target reach underscore the need for new drug candidates affecting cancer angiogenesis proteins. Our previous finding discovered that active ingredient AIC250, a plant alkaloid from Simaroubaceae exhibited potent anti-angiogenic effects. Therefore, this study was undertaken to assess protein expression in non-treated and AIC250-treated endothelial cell line EA.hy926 to determine the compound's mechanisms of action in exhibiting anti-angiogenesis effect. **Methods:** Both non-treated and AIC250-treated-EA.hy926 cells were harvested, followed by protein extraction and trypsin digestion to produce peptides. The resultant peptide masses were subsequently acquired through Orbitrap-Easy nLCMS/MS analysis (Thermo Scientific, USA). The peptide masses were uploaded into PEAKS 7.5 software (Bioinformatics Solution Inc, Canada) for de novo sequencing. The results were subsequently compared against human protein database (SwissProt) for protein identification. **Results:** A total of 1057 proteins were identified. Among these, 66 proteins were differentially expressed with 0.5-fold changes where 48 proteins upregulated and 18 proteins downregulated. Preliminary protein analysis identified a diverse array of 8 distinct protein functional groups critical to cellular processes. These encompassed enzymes catalyzing diverse metabolic reactions, G-protein coupled receptors that influence signaling pathways, kinases central to cell communication, peptidases vital for protein degradation, transcription regulators shaping gene expression, translation regulators modulating protein synthesis, transporters managing molecule movement and a group of other proteins with unique functionalities. **Conclusion:** The protein analysis of non-treated and treated-AIC250 highlighted the potential mechanisms underlying AIC250's inhibition of angiogenesis. Further research is necessary to comprehensively elucidate AIC250's role as an anti-angiogenic agent and its specific impact on relevant protein networks.

Keywords: Anti-angiogenesis, Endothelial Cell Line EA.hy926, Proteome Profiling, Protein Family, Alkaloid

PB4

FDFT1 Mediates Cisplatin Resistance of Bladder Cancer and Is Targeted by miR-146b-5p

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ABSTRACT

Introduction: Chemotherapy against muscle-invasive bladder cancer is challenged by the increasing prevalence of chemoresistance. Farnesyl-diphosphate farnesyltransferase 1 (FDFT1), the first specific gene in the cholesterol biosynthesis pathway, has been identified as a tumour suppressor and chemoresistance modulator through a multiparametric screening on bladder cancer metastasis. In parallel to that, FDFT1 expression was reduced in our cisplatin-resistant bladder cancer cell line (T24R) compared to the parental, cisplatin-sensitive bladder cancer cell line (T24). Thus, this study aims to explore the role of FDFT1 in mediating the cisplatin resistance of bladder cancer cell lines. **Method:** Using both functional knockdown and ectopic overexpression, FDFT1 gene modifications were carried out in T24 and T24R cell lines and its regulation upon the cisplatin-induced cell apoptosis was assessed. **Results:** The siRNA knockdown of FDFT1 suppresses cisplatin-induced apoptosis in T24 cells and conversely, the overexpression of FDFT1 increases the cisplatin-induced apoptosis in T24R cells. Through bioinformatic analysis, an inverse correlation was found between miR-146b-5p and FDFT1 expression. This study has demonstrated for the first time that miR-146b-5p directly targets and downregulates the expression of FDFT1 along with decreasing the cisplatin sensitivity of T24 cells, of which could be restored by the forced expression of FDFT1. **Conclusion:** Taken together, these results indicate that FDFT1 is required at least in part in the regulation of cisplatin sensitivity of bladder cancer cells and might be modulated via miR-146b-5p activity, suggesting the FDFT1/miR-146b-5p axis as a promising potential target in tackling chemoresistance of bladder cancer.

Keywords: Bladder Cancer, Cisplatin Resistance, Cholesterol Biosynthesis Pathway, FDFT1, Mir-146b-5p

PB5

Anti-Angiogenic Properties of Postbiotics Derived from Lactic Acid Bacteria Against Colorectal Cancer *In Vitro*

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ABSTRACT

Introduction: The emergence of side effects and cancer resistance, which compromises the efficacy of current chemotherapy and targeted therapy against colorectal cancer (CRC), raises the need for alternative approaches to prevent and/ or manage CRC. Given that CRC is angiogenesis-dependent, the present study examined the anti-angiogenic potential of postbiotics derived from four unique strains of lactic acid bacteria (LAB; i.e., *Lactiplantibacillus plantarum* LAB1, *Pediococcus pentosaceus* LAB3, *P. acidilactici* LAB4 and *L. plantarum* LAB12) isolated from fermented food *in vitro*. **Methods:** The postbiotics derived from LAB were assessed for their anti-angiogenicity against human umbilical vein endothelial cells (HUVEC) using both scratch and tube formation assays. The effects of LAB-derived postbiotics against regulation of the expressions of RhoA, vascular endothelial growth factor (VEGF) and thrombospondin (TSP-1) in the HCT116 colorectal cancer cell line were then examined using immunocytochemistry. **Results:** Out of the four LAB postbiotics, LAB12-derived postbiotics possessed the best anti-angiogenic properties against migration and differentiation of HUVEC. Immunocytostaining of HCT116 cells treated with LAB12-derived postbiotics showed reduced formation of stress fibers, indicating inactivation of active RhoA. The immunocytochemistry findings also showed downregulation of VEGF but upregulation of TSP-1, indicating inhibition of the angiogenic switch in HCT116 cells. **Conclusion:** The present findings strongly implied the anti-angiogenic potential of LAB12-derived postbiotics and warrants further validation using *in vivo* models.

Keywords: Tumour Angiogenesis, Postbiotics, RhoA, Vascular Endothelial Growth Factor, Thrombospondin

PB6

Anti-Diabetic and Nephroprotective Activities of *Colocasia esculenta* (L.) Schott Leaf Decoction in Alloxan-Induced Albino Mice

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ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by hyperglycemia which may be aggravated by poor lifestyle. Variations in lifestyle with the application of therapeutic interventions may delay diabetes and its complications. For decades, plant-derived products have been used as an alternative source of medicine. This study was conducted to assess the anti-diabetic and nephroprotective activities of *Colocasia esculenta* (L.) Schott leaf decoction in alloxan-induced albino mice. **Methods:** The experimental subjects were made diabetic by injecting a single dose of 190 mg/kg alloxan monohydrate intraperitoneally in PBS (pH 7.4). Varying concentrations (400, 200, 100 mg/kg) of *C. esculenta* leaf decoction were prepared and administered to diabetic albino mice for 14 days. The nephroprotective activities were assessed by kidney coefficient (Kc), blood urea nitrogen (BUN), serum creatinine (SCr) levels, and histopathological analysis. **Results:** The anti-diabetic evaluations revealed that the interventions were correlated to the observation period ($p < 0.001$). The fasting blood glucose levels decrease with time. The reduced body weights were countered by the continuous uptake of *C. esculenta,* although no significant difference among the doses. This suggests that *C. esculenta* leaf improves blood glucose levels at all concentrations. The Kc values revealed insignificant differences among the test subjects with kidney enlargement in diabetic mice. Moreover, the BUN and SCr levels showed lower values compared to metformin-treated mice. This is accompanied by improved renal morphology and restoration of the glomerulus structure. **Conclusion:** The results of the study validate the effectiveness of the plant as a remedy for diabetes, however, further evaluation must still be carried out.

Keywords: Colocasia esculenta (L.) Schott, alloxan-induced albino mice, anti-diabetic, nephroprotective

PB7

7-Benzoylnimbocinol Isolated from *Azadirachta indica* Ameliorates Endoplasmic Reticulum (ER) Stress through the PERK Pathway

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ABSTRACT

Introduction: The endoplasmic reticulum (ER) is a large dynamic organelle present in eukaryotic cells, playing a vital role in processes such as protein synthesis, lipid metabolism and calcium storage. When the accumulation of misfolded proteins saturates the ER's natural capacity, it triggers ER stress, subsequently activating the unfolded protein response (UPR). This UPR mechanism is mediated through transmembrane signalling protein kinase R-like ER kinase (PERK). When the cell's coping mechanisms fall short, it can lead to apoptosis. *Azadirachta indica*, commonly known as Neem, is a fast-growing tree native to the Indian subcontinent and widely found throughout South Asia. A previous study from our research showed that Neem's crude extract could ameliorate ER stress by inhibiting the PERK pathway, with 7-benzoylnimbocinol identified as the major compound involved. The present study aims to evaluate the signalling pathway mediated by 7-benzoylnimbocinol in reducing ER stress in 3T3-L1 adipocyte. **Method:** ER stress in 3T3-L1 adipocytes was induced using 5 µg/mL of tunicamycin for 5 hours, prior to treating the cells with 10, 50 and 100 µM of 7-benzoylnimbocinol for 24 hours. Following treatment, protein extraction was performed using an appropriate lysis buffer. ELISA tests were conducted to determine the protein expression levels of PERK, ATF4, and GADD34. **Results:** The ELISA findings revealed that 7-benzoylnimbocinol effectively inhibited the expression of PERK and ATF4 proteins, while leading to an increase in GADD34 protein expression. **Conclusion:** The present results suggest that 7-benzoylnimbocinol has the potential to ameliorate ER stress by activating GADD34, while inhibiting PERK and ATF4, thus presenting a promising approach for further research in alleviating ER stress-related conditions.

Keywords: 7-benzoylnimbocinol, ER Stress, PERK Pathway, Neem, Diabetes

PB8

Anti-Inflammatory Potential of Rutin Isolated from *Physalis angulata*: A Promising Source for Therapeutic Intervention

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ABSTRACT

Introduction: *Physalis angulata*, an indigenous herb belonging to the Solanaceae family, has been recognised for its medicinal significance and is commonly found in tropical and subtropical regions. This study aimed to evaluate the anti-inflammatory activity of rutin, a flavonoid found in P. angulata. **Method:** A phytochemical analysis of the water fraction of the entire plant was conducted using chromatographic techniques, leading to the isolation and identification of rutin. The compound's structure was established through the interpretation of NMR spectroscopic data. The anti-inflammatory potential of the isolated rutin was assessed through various enzymatic inhibitory assays, including lipoxygenase, hyaluronidase, protein denaturation, xanthine oxidase and elastase. These enzymes are known to play essential roles in the inflammatory processes associated with various diseases. **Results:** The assayed compound exhibited promising anti-inflammatory properties with moderate inhibitory activity against elastase, lipoxygenase and xanthine oxidase but weakest in hyaluronidase inhibitory activity. **Conclusion:** We concluded that the tested flavonoid rutin has the potential to be a candidate for anti-inflammatory agents for the prevention and treatment of numerous diseases, that are caused by complex inflammatory processes.

Keywords: *Physalis angulata,* Rutin, Anti-inflammatory

PB9

Stability Study of A Nanoformulation of Standardised *Andrographis paniculata* (Burm.) Nees Aqueous Extract

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ABSTRACT

Introduction: Stability studies during product development are critical aspects that ensure the maintenance of product quality, safety, and efficacy throughout the shelf-life of a pharmaceutical product. Therefore, this study was designed to evaluate the impact of a lecithin phospholipid nanoformulation of standardised Andrographis paniculata aqueous extract (FAPAE) and *Andrographis paniculata* aqueous extract (APAE) during six months of accelerated stability testing. **Methods:** Lyophilized APAE was standardised for three main phytocompounds: andrographolide (AGP), neoandrographolide (NAG), and 14-deoxy-11,12-didehydroandrographolide (DDAG). Accelerated stability testing was conducted in three batches of the formulation and APAE. Samples were stored in a screw-capped transparent glass bottle. The study was conducted at 4°C, 30°C/75%RH, and 40°C/75%RH. Samples were randomly collected and analysed using FTIR and high-performance liquid chromatography (HPLC). **Results:** Quantification using HPLC revealed a significantly ($P < 0.05$) lower degradation of AGP and NAG in the formulation compared to APAE. FTIR spectra show decreased peak intensity with increasing time but no demonstrable additional peak was observed in both APAE and FAPAE. The formulation improved the shelf-life from 6.7 to 13.49 months for AGP and 7.72 to 9.74 months for NAG at 40°C/75%RH. Meanwhile, at 30°C/75%RH, the shelf-life was increased from 13.436 to 20.749 months for AGP and from 13.052 to 21.736 months for NAG. The shelf-life of DDAG was not determined since the compound increased with time. However, at 4°C, there was no significant change in the shelf-life of both preparations. **Conclusion:** FAPAE may be a useful nanoformulation for improving the shelf-life of the active compounds present in APAE.

Keywords: *Andrographis paniculata*, Andrographolide, Nanoformulation, Stability, Shelf-lifegeting

PB10

A Study on the Effect of Administration of 500mg/kg Tocotrienol-Rich Fraction (TRF) on Liver Membrane Protein Expression in Mice Using Label-Free Quantitative Proteomics

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ABSTRACT

Introduction: Tocotrienol-rich fraction (TRF) derived from palm oil is the most common preparation of tocotrienols that has superior antioxidant and biological effects. The objective of this study is to determine the effect of administration of 500mg/kg TRF on the liver membrane protein expression in mice. **Methods:** Adult male ICR white mice were divided into two groups: vehicle control (n = 6) and 500 mg/kg TRF-treated (n = 6). Mice in the vehicle control group were only administered the vehicle (corn oil) by oral gavage. The TRF-treated mice were administered 500 mg/kg of TRF dissolved in corn oil by oral gavage. The mice were treated once a day in the morning for 14 days. At day 15, the mice were sacrificed and their livers isolated. The livers were then homogenised, and the membrane fractions were analysed using the label-free quantitative proteomics method. **Results:** TRF significantly increased the expression of proteins involved in the upregulation of the fatty acid metabolism, amino acids metabolism, drug metabolism, metabolism of xenobiotics by cytochrome P450, glutathione metabolism, glycolysis/gluconeogenesis and biological oxidations pathways. TRF significantly decreased the expression of proteins involved in the downregulation of the steroid hormone biosynthesis, chemical carcinogenesis, retinol metabolism, starch and sucrose metabolism and cholesterol metabolism pathways. **Conclusion:** Treatment of mice with 500 mg/kg TRF for 14 days could improve the liver health, the liver antioxidant potential and the liver cytoprotective status compared to mice in the control group.

Keywords: Label-Free Proteomics, Vitamin E, Tocotrienol-Rich Fraction, Mice, Liver

PB11

Computational Platform for Natural Product Screening and Dereplication

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ABSTRACT

Introduction: The conventional process of natural drug development involves the purification and isolation of bioactive compounds from natural extracts, followed by manual structure elucidation using spectral data (e.g., NMR, MS). Two major challenges in this process are: (1) isolating and identifying new bioactive compounds in a reasonable time and cost, and (2) producing compounds with desired activity and toxicity profiles. Dereplication, which utilizes existing data on previously isolated bioactive compounds to identify the presence/absence of new compounds with minimal human intervention, offers a solution. With advancements in computer technology and machine learning, there is an opportunity to develop a computational platform integrating spectral data and experimental data of natural substances. **Methods:** A new database was curated consisting of various existing databases with the option of integrating future in-house isolated compound data, specifically for dereplication. Chemical compounds in the database were represented as tokens/vectors generated by calculating various chemical descriptors. Machine learning models, including naive Bayes classifier, support vector machines, random forest, and convolutional neural networks, were developed for structure elucidation in the dereplication process. The performance of these models was evaluated using precision, recall, F1-score, and AUC of the ROC curve. **Results:** The developed machine learning models were able to predict compound structures from preliminary spectral measurements with a precision of 78%, recall of 67%, an F1-score of 0.72, and an AUC of 0.79. These results are promising and the machine learning models can still be optimized further with additional data. **Conclusion:** The integration of machine learning algorithms with a curated database offers a promising approach for automating the dereplication process and discovery of active compounds from natural materials, potentially accelerating natural drug development. Future potential directions include external validation of the developed machine learning models using manual structure elucidation of natural product isolates.

Keywords: Machine Learning, Dereplication, Natural Products

PB12

Trans-Resveratrol Attenuates Fibronectin Deposition via Downregulation of TGFβ1-SMAD Pathway in Dexamethasone-treated Human Trabecular Meshwork Cells

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ABSTRACT

Introduction: TGF-β-SMAD pathway has been associated with increased extracellular matrix (ECM) in the trabecular meshwork (TM) leading to aqueous humour outflow resistance and elevation of intraocular pressure (IOP) in primary open-angle glaucoma (POAG). Elevated IOP remains the only modifiable risk factor and treatment target for POAG. Trans-resveratrol (TR), a polyphenolic compound has been shown to counteract steroid-induced increase in IOP. Its effect on ECM deposition mediated by TGF-β-SMAD pathway however, is unknown. This study explored the involvement of TGF-β-SMAD pathway in the reduction of fibronectin (FN) by TR in dexamethasone-treated human TM cells (HTMCs). **Methods:** Primary HTMCs were incubated with 12.5 μM TR, with or without 100 nM dexamethasone. Analysis of FN, TGF-β1, SMAD4 and SMAD7 were determined using cell lysate and culture media, collected after 3 and 7 days of incubation for gene and protein expressions using real-time polymerase chain reaction (RT-qPCR) and ELISA, respectively. **Results:** TR treatment downregulated both gene and protein expressions of FN by 1.3- and 76.72-fold, respectively; TGF-β1 by 0.89- and 75.61-fold, respectively and SMAD4 by 1.37- and 67.5-fold, respectively and upregulated the SMAD7 by 0.28- and 1.32-fold, respectively in comparison with dexamethasone-only treated group ($p<0.05$). **Conclusion:** Reduction of fibronectin by TR induced by dexamethasone in HTMCs involved the repression of TGF-β1 and SMAD4 and augmentation of the inhibitory SMAD7 signalling. These effects maybe the key to TR's ability in lowering IOP leading to ECM reduction and enhancing aqueous humour outflow in the TM. TR therefore has a significant potential as a future antiglaucoma agent. This study is supported by grant no. 600-RMC/GIP 5/3 (068/2022).

Keywords: Dexamethasone, Extracellular Matrix, Glaucoma, TGF-β, Trans-Resveratrol

PB13

Liposomes and Extracellular Vesicles: A Synergy for Wound Healing

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ABSTRACT

Introduction: Designing biomacromolecules and their assemblies is essential for advancing regenerative medicine, shedding light on molecular healing mechanisms, and facilitating innovative therapies for tissue regeneration and repair. Extracellular vesicles, nanoscale vesicles secreted by cells, are key players in cell-to-cell communication, tissue repair, and regeneration. Yet, their natural secretion is limited, impeding their therapeutic potential. **Methods:** Liposomal stimulation is explored to improve extracellular vesicles production. Dynamic light scattering and transmission electron microscopy were conducted to monitor the successful preparation of the different types of liposomes and collection of extracellular vesicles. Extracellular vesicles uptake and application of extracellular vesicles on scratch assay was performed. **Result:** Cholesterol-linoleic acid-liposomes significantly improved the secretion of extracellular vesicles from immortalized adipose-derived mesenchymal stem cells. Extracellular vesicle uptake was observed, and the cholesterol-linoleic acid-induced extracellular vesicles significantly enhanced the migration of human keratinocytes. **Conclusion:** Developing liposomes to enhance extracellular vesicle secretion could lead to new therapeutic approaches for wound healing, and understanding their secretion mechanisms could facilitate gene editing targeting their biogenesis pathway.

Keywords: Extracellular Vesicles, Immortalized AD-MSCs, Liposomes, Regenerative Medicine

PB14

Antioxidant Potency of *Moringa oleifera* Lam. Extract- and Fraction-Loaded Nanostructured Lipid Carriers

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ABSTRACT

Introduction: The generation of reactive oxygen species (ROS) on the skin can lead to oxidative stress and is believed to contribute to skin aging due to UV radiation. *Moringa oleifera* Lam. is one of the plants that contains various types of antioxidant compounds. This research aims to determine the antioxidant activity of ethanol extract and fractions from moringa leaves, as well as to formulate and characterize nanostructured lipid carriers (NLC) from the selected part of moringa leaves with the best antioxidant activity. **Methods:** Extraction was carried out using the maceration method with 96% ethanol as the solvent. Fractionation was done using liquid-liquid extraction with n-hexane, ethyl acetate, and water as solvents. Antioxidant activity tests on the extract and fractions were conducted using TLC-Bioautography and determination of IC_{50} values using the DPPH method. **Results:** The IC_{50} values for the extract, n-hexane fraction, ethyl acetate fraction, and aqueous-ethanol fraction were 199.79 ± 1.89, 194.96 ± 0.52, 85.77 ± 1.04, and 155.93 ± 2.51 µg/mL, respectively. The ethyl acetate fraction was chosen as the active ingredient for further preparation of NLC due to its best antioxidant activity. The obtained optimal formula consisted of 0.5% ethyl acetate fraction of moringa leaves, 1.8% glyceryl monostearate, 4.2% oleic acid, 4% Tween 80, and 1% TEGO® Care 165. The resulting NLC had a particle size of 253.63 ± 4.97 nm, polydispersity index of 0.34 ± 0.06, zeta potential of -35.59 mV, and encapsulation efficiency of 38.08 ± 15.94%. The antioxidant activity of NLC-Ethyl acetate fraction of moringa leaves increased compared to the IC_{50} of the ethyl acetate fraction, becoming 71.76 ± 0.81 µg/mL. The NLC-Ethyl acetate fraction formulation showed stability at temperatures of 2-8°C for 14 days. **Conclusion:** Ethyl acetate fraction of *Moringa oleifera* Lam. loaded NLC formula can be improved as a potential antioxidant.

Keywords: *Moringa oleifera* Lam., Antioxidant, DPPH, Nanostructured Lipid Carrier

PB15

Formulation of Biocompatible and Biodegradable Polymers as Andrographolide Carrier for Antidiabetic Therapy

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ABSTRACT

Introduction: The most convenient way to administer andrographolide for type II diabetes is through oral consumption. However, andrographolide has limited oral bioavailability due to its poor water solubility. Some strategies have been proposed to improve the solubility of andrographolide, one of which is by utilizing the polymeric nanocarrier. However, the use of non-biocompatible and non-biodegradable polymers has raised safety concerns. Therefore, we aim to develop a novel nanocarrier for andrographolide by utilizing biocompatible and biodegradable polymers, including polylactic-co-glycolic acid (PLGA), chitosan, and polyethylene glycol (PEG). **Methods:** The andrographolide-loaded polymeric nanocarrier was constructed using the solvent evaporation method. Several formulation factors were optimized. The resulting particles were characterized by measuring their size and distribution, zeta potential, morphology, and encapsulation efficiency. **Result:** The spherical and homogenous nanoparticles were obtained with a hydrodynamic diameter ranging from 200-300 nm. The encapsulation efficiency of andrographolide varied between 80% and 94%, depending on the initial concentration of andrographolide. Furthermore, it was found that the addition of PEG influences zeta potential and particle size. **Conclusion:** We successfully developed andrographolide-loaded polymeric nanocapsules using a combination of biocompatible and biodegradable polymers - PLGA, chitosan, and PEG. The nanoparticles represent a promising characteristic for improving the oral delivery of andrographolide.

Keywords: Andrographolide, Polymeric Nanoparticle, Poly Lactic-co-Glycolic Acid (PLGA), Polyethylene Glycol (PEG), Chitosan

PC1

Emergence of Bone Metastasis in Triple Negative Breast Cancers: Exploring the Role of MicroRNAs

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ABSTRACT

Introduction: Triple-negative Breast Cancer (TNBC) is highly combative among subtypes of breast cancer. Metastasis is the adverse complication of TNBC as it can be observed in ~45% of the TNBC cases, ultimately reducing the patient's survival. TNBC cells primarily target the bone for colonization and cause bone metastasis (BM). However, the underlying molecular mechanism remains underexplored to date. MicroRNAs and their roles are actively being studied in cancer progression and metastasis. In this study, we are aiming to study the MicroRNAs responsible for bone metastasis that may serve as potential biomarkers for TNBCs. **Methods:** The exploration of unique microRNAs was done using bioinformatic platforms in which both Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases were accessed. Selection of the unique microRNAs was done by comparing the read counts (RCs) and significance. Target prediction was also accomplished for selected microRNAs using the TargetScan. The validation of microRNAs was done using cell line studies. **Results:** Three unique miRNAs (miR-214, miR-149, and miR-363) were identified based on fold change from the TCGA database. Literature searches also suggested the role of these miRNAs, in bone-associated diseases, cancer, and metastasis. Hence, we further decided to determine their role in breast cancer cell lines. In MDA-MB-231, miR-214 was 3-fold downregulated and miR-363 was 2-fold upregulated as compared to the MCF-7 cell line ($p < 0.05$). No change in miR-149 was observed. **Conclusion:** These novel miRNAs (miR-214, miR-149, and miR-363) can be further explored in the bone metastasis- and bone cancer-specific cell line. Based on the bioinformatics and in vitro studies, miR-214 is found to be a promising candidate for further exploration. Subsequently, target prediction can be further explored to confirm the miRNA/mRNA axis's role in the development of bone metastasis in TNBCs.

Keywords: Triple-Negative Breast Cancer, Bone Metastasis, MicroRNAs

PC2

Evaluation of the Anti-Prostate Cancer Activity of Brazilin in C57BL/6 Mice

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ABSTRACT

Introduction: Prostate cancer, primarily originating from the prostate gland, manifests as prostate adenocarcinoma. Characterized by symptoms related to urination difficulties, this form of cancer is commonly diagnosed in individuals aged 65 and above. Prostate cancer also tends to progress slowly. Current research highlights the anticancer potential of natural compounds and their secondary metabolites in inhibiting the proliferation of various cancer cell types. One such compound that merits further exploration for its anticancer potential is brazilin. Brazilin is an isoflavonoid obtained from the Caesalpinia sappan heartwood. **Methods:** Acute toxicity of brazilin was assessed using the fix dose procedure method. In vitro cytotoxicity was performed on prostate cancer cell line using the WST-8 method. Syngeneic cancer animal model was used to study in vivo anticancer activity. Histopathological analyses of liver and kidneys were performed through the hematoxylin-eosin staining method. **Results:** The results confirmed the absence of heavy metal contamination (Cd, Cu, Pb, As, and Hg) in brazilin. The total plate count and mold/yeast count on brazilin adhered to established requirements, with values ≤ 10 and ≤ 10^3, respectively. Brazilin exhibited a favourable lethal dose 50 (LD_{50}) above 5,000 mg/kg bodyweight, categorizing it as practically non-toxic. The IC_{50} value of brazilin against DU prostate cancer cell line was determined to be 145 ± 17.25 mg/L. Histopathological examinations revealed no observable changes in the kidneys and liver. No metastatic developments were observed. In vivo studies demonstrated brazilin's prostate tumour inhibitory activity in C57BL/6 mice. **Conclusion:** Based on present data, brazilin demonstrated potential as a promising candidate for an anticancer drug. However, further research is required to unveil a comprehensive profile of its anticancer activity and underlying mechanisms.

Keywords: Brazilin, Prostate Cancer, Activity

PC3

Could Multiple Gene Mutations Among Asian Lung Cancer Patients Affect Response to EGFR-TKIs?

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ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) is a complex disease characterized by genetic alterations. EGFR mutations are the most common gene mutation in Asian population which ranged from 32.3% to 50.2% and this was followed by KRAS (8%), ALK (7.8%), RET (<5%), PIK3CA (2.9%), HER2 (2.1%), BRAF (1.6%), MET (1.3%), ROS1 (0.6%) and RET (0.6%). It has been reported EGFR mutations can co-occur with other gene mutations. The treatment landscape for EGFR mutation-positive NSCLC has evolved significantly with the introduction of tyrosine kinase inhibitors (TKIs) targeting the epidermal growth factor receptor (EGFR) pathway. These TKIs, spanning three generations, have become crucial advancements in managing the condition. However, it is important to note that not all EGFR mutation-positive patients exhibit a therapeutic response to these drugs. **Method:** This review is based on literature search performed using Google Scholar and PubMed between 2020 up to 2023. **Results:** There have been relevant reports on multiple-gene mutations affecting the response to EGFR-TKI drugs: EGFR with KRAS co-alteration showed no significant differences in the PFS; EGFR with ALK co-alterations showed poor responses to EGFR-TKIs; EGFR with PIK3CA co-alterations had significantly shorter progression-free survival (PFS); EGFR with HER2 co-alterations may contribute to resistance mechanisms against EGFR-TKIs. The above situations have been sporadically reported among the Asian population. **Conclusion:** It is believed that the presence of other gene mutations could have contributed to this clinical observation. Therefore, different subtypes of EGFR and co-gene mutations could impact the EGFR-TKI efficacy. One of the hypotheses being multiple gene mutations among Asian lung cancer patients affecting response to EGFR-TKIs. To date, a limited number of reports have provided indications supporting this perspective. Yet, a definitive conclusion has not been reached.

Keywords: NSCLC, Gene Mutation, Co-Gene Mutation, EGFR-TKI, Asian People

PC4

Design, Synthesis and Biological Evaluation of PROTAC for Its Anticancer Activity

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ABSTRACT

Introduction: Prostate cancer is a prevalent disease, affecting one in six men. The androgen receptor (AR) plays a vital role in prostate cancer progression. However, currently available AR inhibitors, such as Enzalutamide and Bicalutamide, face limitations in targeting undruggable proteins and developing resistance. To address these challenges, PROTACs (PROteolysis TArgeting Chimera) have emerged as a promising approach. In this study, we designed a novel PROTAC using Enzalutamide and Thalidomide as ligands, along with N-Boc-1,6-hexandiamine as the linker. Our innovative PROTAC has the potential to enhance anticancer activity by degrading the target protein and overcoming resistance caused by mutations. **Methods:** To design the PROTAC molecule, molecular docking (AutoDock 12.3) and molecular dynamics simulations (DESMOND module, Schrodinger) were performed using PDB IDs 7KHL and 7KHH. The synthesis involved four steps, including amidation reaction followed by acidification, which was monitored using techniques such as melting point determination, thin-layer chromatography (TLC), and infrared (IR) interpretation. The structure was confirmed using nuclear magnetic resonance (NMR) and mass spectrometry. **Results:** Molecular docking studies revealed promising dock scores of -10.65 kcal/mol (7KHL) and -12.511 kcal/mol (7KHH) for the designed PROTAC molecule. Characterization data confirmed the structure, with IR spectra displaying N-H stretching bands indicating the amide linkage. NMR spectra showed specific signals validating the presence of 2,6-dioxopiperidine and dimethyl-4-oxo-2-thioxoimidazolidin (Enzalutamide), and mass spectrometry confirmed the molecular weight. *In vitro* studies demonstrated significant anticancer activity of the PROTAC against MCF-7 cell lines, with a GI50 value below 10. **Conclusion:** The synthesis of enzalutamide-based PROTACs represents a novel and promising approach for targeted protein degradation. Enzalutamide, a well-known androgen receptor inhibitor primarily used in the treatment of prostate cancer, can be effectively utilized as a component in PROTACs to overcome drug resistance and enhance therapeutic efficacy. This study provides valuable insights into the potential of PROTACs in advancing the field of cancer treatment, specifically in targeting undruggable proteins and combating drug resistance in prostate cancer.

Keywords: PROTAC, Prostate cancer, Enzalutamide

PC5

Computer-Aided Discovery of Potential EGFR Inhibitors by Virtual Screening of Drug Bank, ADMET, Docking, DFT And MD Simulation Studies

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ABSTRACT

Introduction: Numerous malignancies, ranging from breast cancer, non-small cell lung cancer and chronic myeloid leukemia, are driven by aberrant tyrosine kinase signaling. Given the well-documented toxicity of current chemotherapeutic medicines, there is a great need and demand for the discovery of novel drugs that are either toxic-free or having low toxicity, while effectively targeting and inhibiting the growth of tumor cells. This work describes the in-silico examination of substances from the drug bank as potential EGFR inhibitors. **Methods:** Firstly, the drug bank was screened using the pharmacophore technique to select ligands. Erlotinib (DB00530) was used as a reference compound. The selected ligands were screened using ADMET analysis and the hit compounds were subsequently subjected to docking studies. The lead compound from docking studies was subjected to Density Functional Theory (DFT) and Molecular Dynamics (MD) simulation studies. **Results:** Using the pharmacophore technique, 23 compounds were identified through virtual drug bank screening. One hit molecule from ADMET prediction was the subject for docking study. According to the findings, DB03365 molecule fits to the EGFR active site by forming several hydrogen bond interactions with amino acids. DFT analysis revealed high reactivity of DB03365 compound within the binding pocket of the target protein, as indicated by ELUMO, EHOMO and band energy gap values. Furthermore, MD simulations for 100 ns revealed that the ligand interactions with the residues of EGFR protein were part of the essential residues for structural stability and functionality. **Conclusion:** DB03365 was found to be highly selective for EGFR, suggesting its potential as a therapeutic agent. However, further studies are warranted to thoroughly explore the therapeutic potentials of DB03365.

Keywords: EGFR inhibitor, Virtual Screening, Docking, Molecular Dynamic Simulation

PC6

In Silico Investigation of Quinozoline Derivatives as Potential EGFR L858R/T790M/C797S Mutant Inhibitor

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ABSTRACT

Introduction: EGFR is a transmembrane protein tyrosine kinase that is crucial for cellular signalling for cell growth, invasion, metastasis, apoptosis, and angiogenesis. Increased EGFR activity, such as EGFR overexpression and mutations, results in EGFR deregulation. Numerous malignant tumours, notably non-small cell lung cancer (NSCLC), are linked to elevated EGFR activity. A major obstacle in the development of EGFR tyrosine kinase inhibitors to combat treatment resistance in non-small cell lung cancer (NSCLC) is the targeting of L858R/T790M and L858R/T790M/C797S mutant EGFR. Therefore, it is essential to find new generation of EGFR tyrosine kinase inhibitors (TKIs). The objective of the study was to discover a novel quinazoline-3(4H)-one based potent EGFR-TK inhibitors to overcome the resistance of L858R/T790M/C797S mutant EGFR. **Methods:** Molecular docking of five quinazoline derivatives was performed followed by *in silico* ADMET and drug likeness study. **Results:** All the five derivatives showed higher interaction towards the wild type EGFR having CDOCKER interaction energy ranging from (-51.97 to -53.76 kcal/mol) than the L858R/T790M/C797S mutant type (-33.88 to -39.41 kcal/mol). The derivatives OD-5 and OD-4 showed the maximum interaction with the wild type and the mutant type respectively. *In silico* prediction depicted class 4 toxicity for all compounds (LD50 > 1500 mg/kg). Moreover, the physicochemical properties were suitable as drug candidate and passed the Lipinski's, Egan's, Veber's, Muegge's, and Ghose's rules for oral intake. **Conclusion:** The quinazoline derivatives demonstrated specific binding towards the wild type EGFR. Structural modification is required to increase selectivity towards the L858R/T790M/C797S mutant EGFR with further validation by *in vitro* experiments.

Keywords: Quinazoline derivatives, EGFR inhibitor, Docking, ADMET

PC7

Comparative Evaluation of the Antiglycation, Anti-diabetic, and Antioxidant Potential of Panyawan and Serpentina Capsules

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ABSTRACT

Introduction: The COVID-19 pandemic has prompted a surge in health-consciousness among the public, leading to increased consumption of dietary supplements. However, there is a common misconception that these supplements can cure diseases, often due to misleading labels like "No approved therapeutic claims" and "FDA-approved". Hence, there is a need to validate the efficacy of food supplements. In this study, we scrutinized two commercial food supplements for their potential antiglycation, anti-diabetic, and antioxidant properties. **Methods:** Various assays were conducted to assess the anti-glycation, anti-diabetic, and antioxidant effects of these food supplements. Additionally, treatments were performed on cultured and differentiated L6 skeletal muscle cells following standard protocols. Subsequently, these cells were lysed to extract proteins for the analysis of PI3K protein expression using ELISA. **Results:** Both capsules demonstrated the ability to preserve the secondary structure of bovine serum albumin (BSA). However, serpentina capsule may render better anti-diabetic activity as it exhibited moderately high anti-glycation activity compared to the positive control. Treatments using both dietary supplements also led to the downregulation of PI3K expression. Additionally, both supplements also exhibited moderate scavenging of DPPH radicals compared to the control. **Conclusion:** These results suggest that both panyawan and serpentina capsules possess promising anti-diabetic properties.

Keywords: Food Supplements, Anti-glycation, PI3K Protein Expression

PC8

Selected Mindanaoan Medicinal Plants as Potential Agents to Improve Insulin Resistance in Skeletal Muscle Cells Under Metabolic Stress

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ABSTRACT

Introduction:
Introduction: Type 2 diabetes is characterized by failure to control glucose homeostasis. Diabetes is predicted to more than double, affecting 7.8 million individuals in the Philippines by 2030. This represents a significant major threat to the nation's health and economy. This study offers strategies to help prevent the onset of the disease or to significantly slow its development and the onset of debilitating (and expensive to treat) complications. **Methods:** C2C12 mouse myotubes were incubated in standard tissue culture media, or media supplemented with 28 mM glucose, 200 µM palmitic acid, and 200 µM oleic acid as a glucolipotoxic cellular model of insulin resistance. Intracellular reactive species content was assayed using 2',7'-dichlorofluorescein diacetate dye, and glucose uptake was determined through 2-deoxy glucose-6-phosphate luminescence. Based on previous data (total phenolics and flavonoid content), ethanolic or decoction extracts of 6 out of 20 local tribe plants were further evaluated for antidiabetic screening. **Results:** Our data indicated that ethanol or decoction extracts of these six (6) plants, coded as (SMYLD, SKLD, MHLE, SELD, SGLD, and SMLE), significantly protect the cells from these deleterious reactive species. Importantly, two of these plants (SGLD and SMLE) were further evaluated and observed to elicit insulin-sensitizing effects or enhance insulin-dependent glucose uptake. **Conclusion:** These Mindanaoan plants could potentially be utilized as food supplements to deliver improved health to low-income families across the country with immediate effect. The use of locally available natural sources as potential anti-diabetic agents will make them more accessible and affordable to the communities and thus could offer a low-cost solution to a major healthcare problem.

Keywords: Glucolipotixicity, Oxidative Stress, Mindanao Medicinal Plants, Natural Products, Carnosine

PC9

Exploring the Potential Anti-Psoriatic Properties of A Semi-Synthetic 14-Deoxy-11,12-didehydroandrographolide Derivative

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ABSTRACT

Introduction: Psoriasis is a chronic skin disease characterized by inflammation and hyperproliferation that affects around 2% to 3% of the global population. Currently, no control is available for psoriasis and existing treatments have limitations due to side effects, necessitating the development of safer and more effective anti-psoriatic agents. This study was carried out to determine the anti-psoriatic activity of the 14-deoxy-11,12-didehydroandrographolide (DDAG) derivative via the inhibition of inflammatory pathways, such as nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK). **Methods:** Spontaneously immortalized human keratinocyte (HaCaT) and immortalized mouse macrophage (RAW264.7) cells, respectively were treated with andrographolide (AGP), DDAG, SRS49 (semi-synthesized DDAG), and gemcitabine (positive control). The cytotoxicity was evaluated via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. **Results:** AGP (IC_{50}: 3.03 μM) and gemcitabine (IC_{50}: 0.075 μM) exhibited high cytotoxicity against HaCaT cells, while DDAG did not exhibit any toxicity even at high concentration (100 μM). AGP and gemcitabine also displayed high cytotoxicity against RAW264.7 cells, with DDAG showing moderate cytotoxicity. SRS49 exhibit higher cytotoxicity against HaCaT cells (IC_{50}: 48.67 μM) compared to RAW264.7. SRS49 demonstrated anti-proliferative activity against HaCaT cells, indicating potential anti-psoriatic properties. Further studies will be conducted to investigate the effect of SRS49 against proteins involved in NF-κB and MAPK pathways through western blot analysis. **Conclusion:** SRS49 exhibited promising anti-psoriatic properties by selectively inhibiting HaCaT cell proliferation, making it a potential candidate for psoriasis treatment. However, additional studies are needed to determine whether SRS49 has anti-inflammatory activity in HaCaT cells induced with proinflammatory agents, such as tumour necrosis factor-alpha (TNF-α) and interleukin 17 (IL-17) to further support its efficacy against psoriasis.

Keywords: Psoriasis, 14-Deoxy-11,12-didehydroandrographolide, Anti-proliferative, Anti-inflammatory

PC10

In Vitro Neuroprotective Effects of *Centella asiatica*

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ABSTRACT

Introduction: The generation of free radicals and oxidative stress has been linked to several neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. The determination of free radical scavenging agents for the reduction of intracellular reactive oxygen species is one of the approaches used in the clinical management of neurodegeneration. *Centella asiatica* is an important medicinal plant with an extensive range of ethnomedicinal uses that have been reported to exhibit ranges of bioactivity. **Methods:** Bioassay-guided fractionation was performed in this study to determine the active fraction of *C. asiatica* which responds to neuroprotective effects on H_2O_2-induced neurotoxic neuroblastoma SH-SY5Y cell lines. The results were expressed as a percentage of cell viability which was determined using an MTT assay after each of the experiments. **Results:** In comparison to ethanolic extract, post-treatment with methanolic extracts at 0.49–125 g/ml demonstrated the best protection against H_2O_2-induced cells by enhancing cell viability. Six fractions were produced by further bioassay-guided separation of the methanolic extract. All fractions exhibit neuroprotective effects at concentrations ranging from 0.49 to 1 25 g/ml, which enhanced the vitality of H_2O_2-induced SH-SY5Y cells. **Conclusion:** The treatment of *C. asiatica* on H_2O_2-induced neurotoxicity showed promise, according to the findings. Taken together, this study may suggest *C. asiatica* as a potential therapeutic treatment for H_2O_2-induced neurotoxicity and further study should be done to investigate the active compound attributed to these neuroprotective effects.

Keywords: Medicinal plants, Centella asiatica, Neuroprotection, Hydrogen peroxide (H_2O_2)

PC11

Barriers To Psychiatric Illness Treatment in Saudi Arabia: A Population-Based Cross-Sectional Study

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ABSTRACT

Introduction: Mental illness is a disorder that can cause impairment and disability affecting mood, thinking, and behavior. Early intervention on mental illness could reduce morbidity. This study aims to evaluate the barriers associated with the family and society that prevent mental health patients from seeking consultation and treatment. **Methods:** A cross-sectional study was conducted upon 440 males and females between the ages of 26-40 years, between January and March 2020, in Saudi Arabia. Data were collected by an interview questionnaire which consisted of two parts. The first part included data about socio-demography, while the second part contains subsections of society/family, personal and medical barriers. **Results:** The results showed that 81.1% of the respondents indicated that society and family barriers impacted them. 70.3% of the respondents also believed that it was their own personal barriers that hindered them from seeking help. Medical barriers were opted by 63.5% of the respondents as a form of hindrance as well. Specifically, the respondents indicated that it is difficult to talk freely about the disease (39.5%) due to shame and stigma (25.9%), which is thus challenging for anyone to share about their feelings and emotions (34.3%). Our findings indicated a low level of trust in-hospital treatment, hence losing confidence in using medications. **Conclusion:** The findings of this study indicated that stigmatization from society and family could be the significant barriers that prevent most people from seeking mental health consultation.

Keywords: Mental Illness, Barriers, Stigma, Consultation

PC12

Association Between Frequency of Vegetable Intake and The Incidence of Depression in Indonesian General Population: Findings from The Indonesian Family Life Survey (IFLS-5)

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ABSTRACT

Introduction: A higher intake of fruits and vegetables has been linked to a reduced risk of certain chronic diseases, such as obesity, coronary heart disease, and several types of cancer. Consumption of fruits and vegetables has recently been connected to mental health, particularly depression. Depression is a mental disorder that involves a depressed mood or loss of pleasure or interest in activities for long periods. Depression prevalence in Indonesia has reached 3.7% of the population, or about 9 million people. Previous studies found that a higher frequency of vegetable intake could lower the risk of depression. This study investigates the association between the frequency of vegetable intake and the incidence of depression in the Indonesian general population. **Methods:** Retrospective data were obtained from the Indonesian Family Life Survey (IFLS-5) 2014, a national cross-sectional population-based survey in Indonesia. The respondents with CESD-10 depression scoring values and frequency of vegetable intake were included in this study. Age, gender, body mass index, marital status, employment status, type of employment, education level, location of residence, and province of the subject were considered sociodemographic factors. Binary logistic regression analysis was performed to find the association between the frequency of vegetable intake and the incidence of depression adjusted with sociodemographic factors. **Results:** A total of 18,412 respondent data were obtained. The findings showed that the frequency of vegetable intake once a week compared to seven times a week is associated with depression among respondents (aOR:1.658, 95%CI:1.023-2,687; p=0.040), adjusted by marital status, education level, and location of residence. **Conclusion:** Individuals who consume vegetables once a week compared to seven times a week have a higher risk of experiencing depression. Health promotion regarding increasing vegetable intake in the Indonesian general population should be performed to prevent depression.

Keywords: IFLS-5, Vegetable, Depression, CESD-10, Mental Health

PC13

Neuroprotective Potential of Astaxanthin Nanoemulsions in a Rat Model of Permanent Middle Cerebral Artery Occlusion (pMCAO): A Preliminary Study

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ABSTRACT

Introduction: Astaxanthin (ATX), a natural occurring carotenoid derived from the algae (*Haematococcus pluvialis*), exhibits abundant antioxidant and anti-inflammatory properties. Utilizing nanoemulsion technology potentially enhances its bioavailability by facilitating passage through the blood-brain barrier. This preliminary study investigated the impact of different concentration of ATX nanoemulsion in reducing the infarct volume and improving neurological function in a rat model of permanent middle cerebral artery occlusion (pMCAO). **Methods:** Twelve (12) Sprague Dawley rats were divided into four groups, each receiving different dosages of ATX nanoemulsion: Group A (160 mg/kg of body weight), Group B (320 mg/kg of BW), Group C (640 mg/kg of BW), and Group D (1280 mg/kg of BW). The administration of ATX nanoemulsion was carried out orally for 7 days before and 3 hours after pMCAO induction. Neurological function assessments and brain infarcted volume measurements were conducted 24 hours post-pMCAO. The rat was euthanized by cardiac puncture and the brain was collected for infarct volume analysis. The data were analysed by one-way ANOVA and post-hoc Tukey test, with a significance level set at $p < 0.05$. **Results:** The neurological scores and grid walking test showed significant differences ($p<0.05$) between group D with groups A and B. The rotarod test for group D was significantly higher ($p<0.001$) compared to groups A, B and C. Meanwhile, the infarct volume of group D was significantly lower ($p<0.001$) compared to groups A, B and C. **Conclusion:** This preliminary study showed that administration of ATX nanoemulsion at a concentration of 1280 mg/kg bodyweight to be optimal, as it significantly improved neurological function and reduced the infarct volume in the pMCAO rat model.

Keywords: Astaxanthin, Nanoemulsion, Stroke, Neuroprotection

PC14

The Effect of Temperature and Extraction Time on Antioxidant Activity in The Preparation of *Salvia officinalis* L. Extract

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ABSTRACT

Introduction: *Salvia officinalis* L., also known as sage. It belongs to the Lamiaceae family and is used for its pleasant fragrance and flavor. The aim of this study is to determine the optimum temperature and time for the preparation of sage water extract. **Methods:** Sage was mixed with water and exposed to different temperatures: 40°C, 60°C and 80°C. At each temperature, the extraction process took different lengths of time: 30 minutes, 1 hour, 2 hours, and 3 hours. After filtration, the antioxidant properties of the extract were evaluated using antioxidant assays. **Results:** Sage extract heated at a temperature of 40°C/ 3 hours can scavenge 66% of DPPH free radicals. In addition, heating at 40°C/ 30 minutes has a high FRAP value compared to the other parameters. In the TPC test, the highest TPC value was obtained at 40°C/ 1 hour. The MDA test showed that a temperature of 40°C/ 3 hours resulted in a lower MDA value. At 60°C, the percentage of DPPH inhibition is in the range of 15% - 63%. In addition, the 60°C/ 3 hours has a high FRAP value compared to the others. The highest TPC value was obtained at 60°C/ 2 hours. In addition, lower MDA values were obtained at 60°C/ 30 minutes. In contrast, the percentage of DPPH inhibition at a temperature of 80°C is in the range of 80% - 89%. The use of 80°C/ 2 hours parameter has a high FRAP value compared to others. The highest TPC value was measured at 80°C/ 30 minutes. However, a temperature of 80°C/ 1 hour resulted in lower MDA values than the other parameters. **Conclusion:** In conclusion, the optimal condition for the preparation of a sage extract with effective antioxidants is 60°C/ 30 minutes.

Keywords: Salvia officinalis, Antioxidant, DPPH, FRAP, MDA

PC15

Effects of Tocotrienol-doped Calcium Phosphate Cement on Bone Mineral Density, Bone Mineral Content and Biomechanical Strength in Tibia of Ovariectomised Rats with Bone Defect

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ABSTRACT

Introduction: Calcium phosphate cement (CPC) is used to fill in bone defects, but its practical application is restricted due to low mechanical properties and osteogenic potential. This study aimed to investigate the effects of CPC incorporated with palm tocotrienol (CPC/T3) on bone regeneration in ovariectomised rats with tibial bone defect. **Methods:** Female Sprague-Dawley rats were divided into four experimental arms: (a) sham-operated rats, (b) ovariectomised rats, (c) ovariectomised rats subjected to bone defect and implanted with CPC, as well as (d) ovariectomised rats subjected to bone defect and implanted with CPC/T3. The implantation was performed after 12 weeks of ovariectomy for a duration of 8 weeks. Rats were scanned with dual-energy X-ray absorptiometer for BMD and BMC at whole body and left tibia throughout the study. At the end of 20-week study, left tibias were harvested for biomechanical strength analysis. **Results:** Whole body BMD of the CPC/T3-filled group increased significantly after implantation (week 16 & 20) as compared to before implantation (week 0 & 12), which was not seen in other experimental groups. Higher left tibia BMC was also observed in the CPC- and CPC/T3-filled groups after implantation as compared to before implantation. The CPC/T3-filled group exhibited significantly higher bone stiffness as compared to the sham-operated and ovariectomised groups. **Conclusion:** The presence of tocotrienol in CPC potentially enhances BMD, BMC and bone stiffness suggesting that tocotrienol can be incorporated into CPC improve its properties.

Keywords: Calcium Phosphate Cement, Bone Defect, Ovariectomy, Tocotrienol, Vitamin E

PC16

Alternative To Animal Testing in Drug Regulatory Process: 3R As an Indispensable Approach

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ABSTRACT

Introduction: Regulatory studies have revolutionised over time. Today, the focus has shifted from animal to non-animal toxicity and efficacy testing. This move aligns with the international 3Rs (Replacement, Refinement and Reduction) principle and has also changed the regulator's perspective. The 3Rs principle has stimulated changes in policy, regulations, and new approaches to safety assessment in drug development in many countries. The 3Rs approach has resulted in the discovery and implementation of new technologies and human-relevant in vitro methods that reduce the reliance on animal testing. These include organoids, organ-on-a-chip and alternative assays such as the chick chorioallantoic membrane (CAM) assay. These advancements are not only valuable for research but also contribute to improved animal welfare by minimising the use of animals including non-human primates. Various international guidelines on the principles of regulatory acceptance of 3Rs testing approaches and regulatory testing approaches have been published to promote their application in pharmaceutical safety assessment. Additionally, in early 2023, US FDA passed a new legislation that does not require all new human drugs to be tested on animals, changing the current testing paradigm. The 3Rs alternative method is indispensable and has been widely adopted in various fields of biomedical research, applied in screening research for therapeutic targets, as well as preclinical toxicity testing. These 3Rs approaches are promising and might have drug development and discovery implication for future practice. **Method:** This presentation provides a current overview and future perspectives on 3Rs alternatives. **Results:** It was observed that there were no significant differences observed when comparing the legal framework, guidelines and standards across countries. **Conclusion:** Considering the persistent dedication to fostering global animal research and the international initiatives aimed at enhancing animal welfare, it is foreseeable that the landscape of laws, regulations and guidelines will continue to evolve.

Keywords: 3Rs Principles, Preclinical Studies, Organoids, Organ-On-A-Chip, Chick Chorioallantoic Membrane (CAM) Assay

PC17

In Vitro Cytotoxicity of Ruthenium (II) Polypyridyl Complex in Combination with PARP Inhibitor in A549 Lung Cancer Spheroids Model

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ABSTRACT

Introduction: The evaluation of drug effects in a 3D spheroids model can provide insight on the cytotoxicity and drug penetration. In addition, 3D spheroids often better recapitulate the tissue microenvironment in vivo as they mimic the complexity and heterogeneity of cellular organization in clinical tumors. Ruthenium (II) polypyridyl complexes (RPCs) have emerged as promising anticancer candidates due to their attractive DNA binding property. Inhibitors of poly(ADP-ribose) polymerase (PARP) are new small molecule drugs that show promising therapeutic effects. Previously, we have evaluated the rationale combination of the RPC $[Ru(dppz)2(PIP)]^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c] phenazine, PIP = 2-(phenyl)-imidazo[4,5-*f*][1,10]phenanthroline), "Ru-PIP" with PARP inhibitor Olaparib in 2D monolayer cell culture in which Ru-PIP/Olaparib synergy was shown. In the present study, we examine the identified synergistic Ru-PIP/Olaparib combination in 3D lung cancer spheroids to further elucidate synergy. **Methods:** A549 lung cancer spheroids were developed using hanging drop technique. Spheroids growth inhibition study and spheroids live/dead staining experiments were conducted to examine the cellular viability of the spheroids upon treated with Ru-PIP/Olaparib combination. **Results:** A549 cells formed spheroids that managed to grow in diameter in size and volume over 15 days, thereby qualifying them as a suitable 3D cell culture model. Our results show that the structural integrity of the A549 spheroids was lost after 12 days treatment with the combination, meanwhile single agents-treated spheroids remained structurally intact, although Ru-PIP single agent inhibited spheroids growth. Compared to single agents alone, the combination induced more cell death in A549 spheroids, as indicated by Calcein AM/PI staining. **Conclusion:** We demonstrate that the synergistic Ru-PIP/Olaparib combination is able to inhibit the growth of the more-resistant lung cancer spheroids model, showing promising therapeutic effects and merit further clinical assessment *in vivo*.

Keywords: Lung Cancer, Combination Therapy, Ruthenium, PARP inhibitor

PC18

In Vitro Cytotoxicity and Zebrafish Embryos Acute Toxicity Assessment of Ruthenium (II) Metal-based Complexes in Combination with PARP inhibitor

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ABSTRACT

Introduction: Poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi) target the key DNA repair enzymes of PARP and have emerged as small molecule drugs. Recently, ruthenium(II) polypyridyl complexes (RPCs) have shown promise as anticancer candidates due to their ability to form non-covalent (reversible) interactions with DNA. Previously, we have reported the RPC $[Ru(dppz)2(PIP)]^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine, PIP = 2-(phenyl)-imidazo[4,5-f][1,10]phenanthroline), "Ru-PIP" induce replication stress in cancer cells by stalling the DNA replication fork progression. Therefore, the rational combination of Ru-PIP with PARPi may show synergistic activities in cancer cells. In the present study, we examine the combination of Ru-PIP with the most successful PARPi to date, Olaparib for synergy in lung cancer cells. We additionally assess toxicity of the identified combination in a zebrafish embryo model. **Methods:** A549 cells were treated with Ru-PIP or Olaparib single agents alone or in combination and MTT assay was carried out. Synergy was determined using Chou and Talalay combination index (CI) method in which $C1 < 1$ represents synergy and synergy was further confirmed using clonogenic survival assay. Further acute toxicity test of the identified synergy combination on zebrafish embryos was then carried out. **Results:** Ru-PIP and Olaparib synergy was observed in A549 lung cancer cell line. Synergy was confirmed by loss in clonogenic potential. Moreover, acute zebrafish embryos toxicity studies revealed that this combination showed reduced toxicity compared to single-agent Ru-PIP. **Conclusion:** We demonstrate that the identified synergistic Ru-PIP/Olaparib combination may potentially reduce side effects observed in single-agent therapy and thus, demonstrate new promising therapeutic strategy to combat cancer.

Keywords: Lung Cancer, Combination Therapy, Ruthenium, PARP inhibitor