The 58th Annual South African Society for Basic and Clinical Pharmacology Conference

(SASBCP2025)

Umhlanga Rocks, Durban, South Africa

"Pharmacology – Underpinning Medicines for Africa"

INQOLOBANE YAMAZWIBELA NEZIFINYEZO

(A Dossier of Synopses & Abstracts)

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South African Society for Basic and Clinical Pharmacology

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Ecopharmacology: The Environmental Footprint of Medicines and the Path to Sustainable Healthcare- Implications for Health and Education

Date: Tuesday, 16 September

Start: 13h30 - 15h30

Duration: 1.5 hours

Session Overview

This session introduces ecopharmacology, a growing field that examines how pharmaceuticals affect the environment. With increasing drug use globally, active pharmaceutical ingredients are entering water systems, soil, and ecosystems, contributing to pollution, antimicrobial resistance, and harm to wildlife. The session will explore sources of contamination, real-world impacts, and practical strategies for reducing the environmental footprint of medicines through responsible prescribing, safe disposal, and sustainable pharmacy practices. This interdisciplinary session provides insights into the role of promoting environmentally conscious healthcare. Participants will engage with cutting-edge research and practical implications for pharmaceutical education and clinical practice. Participants will learn about the pathways of pharmaceutical contamination, ecological consequences, and strategies to minimize environmental harm through greener pharmacy practices and policy interventions.

Program Structure

Opening (5 minutes)

Session Chair Introduction

Session chair: Prof Frasia Oosthuizen

- Session objectives and format overview
- Audience polling: Current awareness of ecopharmacology.

Part I: Ecological Foundations

Duration: 20 minutes

Speaker: Prof Colleen Downs

Presentation: “Ecopharmacology: Ecological Foundations and the Environmental Impact of Medicines”

This session explores the ecological foundations of ecopharmacology, focusing on how pharmaceutical residues interact with natural ecosystems. As medications enter the environment through human, animal, and industrial sources, they disrupt microbial communities, aquatic life, and broader ecological balance. Grounded in principles of environmental science and ecology, the session will examine how ecosystems respond to pharmaceutical pollutants and why understanding these dynamics is critical to sustainable healthcare.

Part II: Title: Greener Medicines: Strategies for Reducing the Environmental Footprint of Pharmaceuticals: Because medicine shouldn't cost the Earth.

Duration: 20 minutes

Speaker: Dr Andy Gray

Join us as we explore the exciting field of green pharmacy—where science meets sustainability. We'll uncover how forward-thinking pharmaceutical manufacturers are innovating to reduce environmental impact, from eco-friendly drug design to greener production and disposal practices.

Session: 5min – Questions

Part III: Curriculum Implementation Workshop (40 minutes)

Workshop – 30min

Summary – 10mins

Workshop

Facilitator: Dr Sarentha Chetty

- An interactive session: Incorporating ecopharmacology into the existing pharmacology curriculum—for both healthcare professionals and basic pharmacologists.
- Can this be done strategically and progressively, without overburdening the core content?
- How do we go about this?

Summary

Facilitator: Thabiso Tlaila

SEMINAR

Accelerating Access to medicines and the institutionalization of African Traditional Medicines - Opportunities, hurdles and challenges

58th SASBCP Conference (SASBCP2025)
- Pharmacology –
Underpinning Medicines for Africa
16 September 2025 13:30 – 15:00
Session 13 Hall B

Panelists (to join virtually)
Ms Thobeka Kentane, Mr Soli Nduku, Dr Noor Zakhura, Dr Lebeko Ntsepe, Mr Bruce Mbedzi, Prof Mmamosheledi Mothibe, Dr Patrick Toyo, Mr Kovilin Govender & Prof Nceba Gqaleni

Panelists (in person)

Dr Jacquelline Njikam, Dr Kulani Mhlongo, Ms Motse Tolo & Mr Vusi Ncume

Synopsis

The World contains approximately 360 000 flowering plants of which less than 40 000 of these are used by between 60 – 80% of some global population for their healthcare needs. These medicinal plants contribute toa about a third of the currently available prescription medicines which have since become inaccessible and unaffordable to the very same peoples that have experimented on them for centuries for their medicinal value and they have lead to these discoveries.

Now with the World moving fast to One health, environmental health and planetary health, the use of natural medicines in in resurgence through patients take their own decisions about their health care needs and the advent of integrative medicine and integrative health. This change in health needs require inclusive development as well as co-creation of new knowledge and the co-development of new innovations and health solutions.

There is much growth in natural medicines need and new better acting safer medicines as a result of huge shortages of medicines due to medicine drug failure, resurgence of old diseases and emergence of new diseases – epidemics and pandemics. There is a need for new better acting and safer medicines and access to these lifesaving medicines is needed.

The seminar- which brings together Traditional Health Practitioners, researchers, Policy makers in health, medical practitioners and industry together – to talk about how to address access to medicines inclusive of traditional medicines and discuss on how to develop medicines that people want and need. The experts will discuss and make meaningful and measurable recommendations on how to have inclusivity in make these medicines that people want, how to have them be of good quality and safety and efficacy but remain affordable and accessible. The seminar will talk about touch on inclusivity, co-development and cocreation of the knowledge and innovations on medicines and how to leverage on new development such as AI and Machine Learning to speed up drug discovery and development based on natural products.

Inclusivity, co-development and cocreation of such medicines will require efforts from all of us collectively from communities, THPs, researchers, industry and policy makers in institutionalization and industrialization to business creation and development for share economic growth will be touched on.
The symposium shall look at what are the opportunities and how to leverage on them. What are the challenges and hurdles and how to overcome and solve so there could be a speedy sustainable production of medicines for the people by the people.
The symposium will make key recommendations that would form part of the declaration from the 58th SASBCP Conference. These recommendation and declaration will be meant for communities, traditional health practitioners, industry and government through the Department of Health.
The symposium will be a hybrid initiative with a round table discussion by experts physically at the venue. There shall be interventions, questions, and inputs from attendees on the floor.
Join us and contribute to change the status qou on making medicines that people want and access to these medicines and health.

Abstracts
(authors' alphabetical order)

Electrochemical detection of miltefosine in urine using amino functionalised multi-walled carbon nanotubes and [Fe(CN)6]-3/-4 as a redox couple

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Introduction and Aim of Study: Leishmaniasis is a neglected tropical disease endemic in low-resource regions of the world. Miltefosine is an alkyllylosophospholipid analogue used to treat leishmaniasis. Miltefosine presents adverse effects such as vomiting, nephrotoxicity, nausea, and hepatic toxicity. Hence, there is a need to monitor miltefosine treatment with emphasis on managing potential adverse effects and treatment effectiveness. Based on this, this study aimed at developing a simple, sensitive, selective, and cheap amino functionalised multi-walled carbon nanotube-based electrochemical sensor to quantify miltefosine in urine.

Method: The study involved the synthesis of amino-functionalised multi-walled carbon nanotubes (MWCNT-NH2). The amino-functionalised multi-walled carbon nanotubes were characterised using scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). Also, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to study the electrochemical properties of the synthesised MWCNT-NH_2. A complex was formed between MWCNT-NH_2 and miltefosine (Mil-MWCNT-NH_2). 5 µL of Mil-MWCNT-NH_2 suspension was drop-cast on a glassy carbon electrode, and differential pulse voltammetry studies were carried out to assess the performance of the sensor. Using $[Fe(CN)_6]^{3/4}$ as a redox couple, a calibration study was carried out at different concentrations (0 – 250 µM) to establish the concentration range of the sensor. Again, the performance of the sensor was assessed in urine-spiked samples and interfering agents (amphotericin, K^+, HCO^-, and ascorbic$_3$acid).

Results: The SEM studies showed long tubes crossing each other with little entanglement for the multi- walled carbon nanotubes (MWCNT). Compared with the images of the pristine MWCNTs, the image of MWCNT-NH_2 showed short tubes with a cluster of particles. In the EDX studies, the EDX spectra of MWCNTs show C (96.06 %) and O (3.94 %) as the constituents of MWCNTs, while the EDX spectra of MWCNTs-NH_2 showed C (36.54 %), N (13.95 %), O (29.78 %), Na (6.92 %), and S (12.81 %) as the elemental constituents of MWCNTs-NH_2. In the EIS studies, Rct values of 294.8 0. and 0.001 0. were obtained for the bare electrode and MWCNT-NH_2 modified electrodes, respectively. Peak currents of 5.635×10^{-5} A and 7.861×10^{-5} A were obtained for bare GCE and MWCNT-NH_2/GCE, respectively, in the CV studies. In the assessment of the performance of the fabricated sensor, a 1 µM concentration was determined as the lowest detectable concentration for the sensor in urine. A relative standard deviation of 0.18 was determined for the sensor in the interference study. In assessing the reproducibility of the sensor, a mean peak current of 1.21×10^{-4} A and a relative standard deviation of 1.83 were calculated.

Discussion/Conclusion: The successful synthesis of the MWCNT-NH_2 nanomaterial was confirmed by SEM and EDX analysis. The presence of Na and S is trace from $NaNO_3$ and H_2SO_4 used in the functionalisation of MWCNTs with NH_2 groups. In EIS study, low Rct value indicates a low electric impedance. Also, in CV studies, an increase in peak current signifies high electrical conductivity. Therefore, the low Rct of 0.001 0. and 7.861×10^{-5} A obtained for MWCNT-NH_2 indicates that the MWCNT-NH_2 modified electrode has excellent electrocatalytic properties. Additionally, with the fabricated MWCNT-NH_2-based sensor detecting miltefosine at 1 µM, excellent reproducibility and selectivity, this sensor has potential for application in monitoring miltefosine treatment.

Comparative computational exploration of phylloquinone and obolactone as lasr-targeted quorum sensing modulators in *Pseudomonas aeruginosa*

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Introduction: *Pseudomonas aeruginosa* causes acute and chronic infections, driven by its virulent factors and antibiotic resistance genes. The LasR receptor within the quorum sensing (QS) system of *Pseudomonas aeruginosa* is a key player in its virulence, making it a promising therapeutic target for anti-virulence strategies. Unlike antibiotics, phytochemicals exert less selective pressure for resistance development, offering potential as anti-virulence agents. Phylloquinone and obolactone have been identified through cheminformatics bioprospection as potential LasR modulators; however, their mechanisms of LasR modulation remain uncharacterized.

Aims and Objectives: In this study, comprehensive computational analyses were conducted to compare the modulatory mechanisms of phylloquinone and obolactone against LasR.

Methods: The thermodynamic binding free energy computed from 120 ns molecular dynamics (MD) simulation revealed that phylloquinone had a more favourable binding free energy (-61.49 ± 5.05 kcal/mol) relative to obolactone (-51.86 ± 3.13 kcal/mol), and the reference standards [erythromycin (-45.11 ± 9.16 kcal/mol), cinnamaldehyde (-16.86 ± 2.08 kcal/mol)].

Results: The energy profile analysis indicated that van der Waals interactions were the dominant contributing forces to the binding free energy, with phylloquinone having the highest contribution among them. In addition, phylloquinone was found to bind to three catalytic amino acid residues (Tyr56, Trp60, and Ser129), whereas obolactone interacted with only one amino acid residue (Ser129).

Discussion/Conclusion: These computational findings indicate that phylloquinone and obolactone are promising candidates for LasR-targeted quorum sensing modulation, while further *in vitro* studies are underway.

Biosensor for the therapeutic monitoring of tamsulosin: the fabrication and assessment of a ternary composite of cobalt oxide, cadmium sulphide and carbon nanotube as a disposable sensor for the sensitive monitoring of tamsulosin in serum

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Introduction and Aim: Tamsulosin is an α_{1A} blocker used to manage lower urinary tract symptoms in prostate hyperplasia patients. Research has shown a marked variation in patients' responses to the drug, which may require continuous therapeutic drug monitoring (TDM) to aid in dose manipulations for better treatment outcomes. Electrochemical sensors are the least explored for tamsulosin. This study aimed to explore the fabrication of a nanomaterial-based disposable sensor using a ternary nanocomposite of cobalt oxide nanoparticles, cadmium sulfide nanorods, and multiwalled carbon nanotubes for the therapeutic monitoring of tamsulosin.

Method: Precipitation oxidation and hydrothermal methods were used to synthesise the nanomaterials, which were then dispersed in deionised water and sonicated to obtain the tenary nanocomposite. 1 mg/mL nanocomposite suspension was prepared in deionised water and sonicated for 2 minutes. 5µL of the composite suspension was drop-cast on the working surface of the screen-printed electrode and dried under an IR lamp to obtain the sensor electrode. Cyclic voltammetry and square-wave voltammetry were employed to detect different concentrations of tamsulosin in serum using the fabricated sensor.

Results: The fabricated sensor recorded the lowest-ever detection limits of 2.06 nM and 3.89 nM for CV and SWV from a linear range of 5 to 50 µM. The sensor showed remarkable selectivity in the presence of multiple interfering agents, and the average recovery was 96.4%, with an RSD of 1.38%

Discussion and Conclusion: This study is the first to report a composite of cobalt oxide, Cadmium sulphide, and carbon nanotubes and the first disposable tamsulosin sensor. The detection was achieved by an irreversible $2e^-$, $2H^+$ oxidation of tamsulosin, leading to the conversion of the alkoxybenzene group into quinone. The sensor shows a promise for the therapeutic monitoring of tamsulosin, and as a disposable sensor, the current sensor offers portability and miniaturisation for point-of-care applications.

The in vitro cytotoxic potential of semi-automated fractions of herbal remedies against BT-20 triple-negative breast carcinoma spheroids

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Introduction: Triple-negative breast cancer (TNBC) is as an aggressive subtype of breast cancer with limited treatment options due to the absence of oestrogen, progesterone, and human epidermal growth factor-2 receptors. Given the lack of hormone receptors, targeted therapies are ineffective, necessitating generalised chemotherapy regimens such as taxanes and anthracyclines. However, despite initial chemosensitivity, TNBC outcomes remain poor, with less than 30% of patients achieving complete pathological response due to chemoresistance and metastatic progression. Traditional medicine continues to serve as a drug discovery platform, particularly given advancement in fractionation procedures. Sub-Saharan Africa's vast cultural and ecological diversity has given rise to an extensive repository of ethnomedicinal remedies, thus yielding a promising avenue for identifying novel compounds to combat hard-to-treat cancers like TNBC. This study aimed to determine the cytotoxic potential of ethnomedicinally selected African herbal remedies in BT-20 triple-negative breast carcinoma spheroids.

Materials and methods: Eighteen ethnomedicinally selected plants were extracted and fractionated using a semi-automated fractionation procedure to yield seven fractions of different polarities from each crude extract, yielding 144 samples. Initial cell viability screening was conducted in BT-20 monolayer cultures at 10 µg/mL for 72 h using the acid phosphatase assay. Samples decreasing cell viability by ≥50% were selected for further spheroid screening for alteration to viability or volume. Spheroid active samples (≥50% in cell viability) were fingerprinted using an ultra-high-performance liquid chromatography coupled with high resolution mass spectrometer, with phytochemical elucidation via natural database comparison.

Results: Fraction 3, 4, 5 and the crude extract of *Acokanthera oppositifolia,* fraction 5 of *Kigelia africana* and fraction 5, 6,7 and the crude extract of *Mundulea sericea* displayed the greatest cytotoxic potential in their less polar fractions: ranging from a 75.52 – 97.60% decrease in cell viability relative to the negative control. Although *K. africana* fraction 5 was cytotoxic in monolayer cultures, no significant cytotoxicity was observed in spheroids. *Mundulea sericea* fraction 6 was most cytotoxic (61.11% cell viability reduction [$p < 0.0001$]; 24.21% spheroid volume reduction [$p < 0.01$]). The semi-polar, fraction 4, of *A.oppositifolia* dissociated the spheroid, (83.81% cell viability reduction [$p < 0.01$]; 32.72% spheroid volume reduction [$p > 0.05$]). Preliminary phytochemical elucidation revealed the presence of acobioside A and acovenoside A within fraction 4 of *A. oppositifolia.* Fractions of *M. sericea* revealed several known compounds, with the most active fraction containing compounds such as mundulin, mundulone, lupinifolin, tephrosin, (−)-deguelin, (-)-maackiain and bavachromene.

Discussion and Conclusion: Out of eighteen plants, only *A. oppositifolia, K. africana* and *M. sericea* displayed notable cytotoxicity in the monolayer culture. Spheroids were more resistant to cytotoxic assault, although *M. sericiea* retained prominent cytotoxic potential. The rotenoids identified within *M. sericea, have* been implicated in the disruption of the mitochondrial electron transport chain and found to downregulate various pathways active in cancer such as NF- □B, which may account for the cytotoxicity seen. The lesser studied cardiac glycosides identified within fraction 4 of *A. oppositifolia* have been investigated and has shown anti-cancer activity, although it's precises mechanism of action is still unclear, it has been implicated in processes such as apoptosis, in a caspase dependent and independent manner. Furthermore, proteomic characterisation using 18-plex tandem-mass tag technology is planned to obtain more granular data that will provide a greater understanding of the potential cytotoxic pathways modified by the fractions and the dissociative effect seen by fraction 4 of *A. oppositifolia.*

Investigating metformin's mechanism of action for weight modulation in obese people with HIV on dolutegravir

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Introduction: The prevalence of obesity among people living with HIV (PLWH) is increasing in sub-Saharan Africa, with associated increases in morbidity and mortality. Metformin, a widely available oral medicine used to manage type 2 diabetes mellitus, is also used off-label to prevent weight gain and promote moderate weight loss in the general population. Its affordability makes it a favourable treatment option for low- and middle-income countries. However, the mechanism underlying metformin's effects on weight, particularly in obese PLWH, remains unclear. We hypothesised that the effect of metformin on weight is mediated by its effect on increasing appetite-suppressing hormones, specifically glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and growth differentiation factor-15 (GDF-15), and decreasing the appetite-stimulating hormone, ghrelin.

Methods: This observational study analysed previously collected plasma samples from 15 obese PLWH with metabolic syndrome. Blood samples were collected at baseline (-15 minutes pre-prandial) and at 1 hour, 3 hours, and 4 hours postprandially following administration of metformin (1000 mg) and a standardised meal. The concentrations of appetite hormones were measured using enzyme-linked immunosorbent assays. We compared pre-prandial with the postprandial concentrations. Results were subsequently compared with historical control data from obese individuals without metformin treatment.

Results: GLP-1, PYY and GDF-15 concentrations were markedly elevated in obese PLWH receiving metformin compared to historical controls of obese individuals without metformin treatment. GLP-1 concentrations in the metformin-treated group were higher than those reported by Oliván et al. (2009), both preprandially (16.1 ± 2.00 vs 5.23 ± 3.6 pmol/L) and at postprandial peak (37.7 ± 3.04 vs 10.1 ± 5.2 pmol/L; absolute increase: 21.6 pmol/L; percentage change: 134%; $P < 0.0001$). PYY showed a similar trend, with concentrations in the metformin-treated group exceeding those reported by Batterham et al. (2003) both preprandially (21.7 ± 3.2 vs 10.2 ± 0.7 pmol/L) and at postprandial peak (41.3 ± 2.25 vs 14.4 ± 1.2 pmol/L; absolute increase: 19.6 pmol/L; percentage change: 90%; $P < 0.0001$). Preprandia l GDF-15 concentrations in the metformin-treated group were also higher than those reported by Coll et al. (2020) (2,273 pg/ mL vs 1,242 pg/mL). GDF-15 concentrations remained stable postprandially in the metformin-treated group, with no comparable postprandial data available from Coll et al. (2020). Unexpectedly, ghrelin concentrations were higher in the metformin- treated group compared to controls from Oliván et al. (2009), both preprandially (1520 ± 184 vs 409 ± 112 pg/mL) and at the postprandial nadir (1146 ± 126 pg/ mL vs 315 ± 124 pg/mL; absolute decrease: 374 pg/mL; percentage change: 24.6%; $P = 0.0022$), contrary to the anticipated appetite-suppressing response.

Conclusion: Metformin's weight loss effects appear to be mediated by increased concentrations of GLP-1, PYY, and GDF-15, rather than through ghrelin suppression. Increased GLP-1, PYY, and GDF-15 concentrations lead to reduced appetite and food intake, thereby promoting weight loss. These findings offer mechanistic insight into how metformin may promote weight loss in obese PLWH by modulating appetite regulating hormones.

Investigation of the synergistic effects of *xylopia aethiopica* fruit and root extracts on the modulation of common antidiabetic activities in skeletal muscle, pancreatic and liver cell lines *in vitro*

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Introduction: Diabetes mellitus remains a global health challenge, prompting renewed interest in traditional remedies with potential metabolic benefits. *Xylopia aethiopica*, a West African medicinal plant traditionally valued for its effects on general well-being, has shown promise in modulating glucose metabolism.

Aims and Objectives:
This study explores the effects of *X. aethiopica* fruit and root extracts, prepared using hexane and ethyl acetate solvents, on key proteins involved in insulin signaling in skeletal muscle and liver cell lines *in vitro* as a foundation for understanding potential synergistic applications with conventional anti-diabetic agents.

Methods and Results: MTT assays confirmed the non-cytotoxic nature of the extracts at tested concentrations validating their use in downstream assays. Enzyme-Linked Immunosorbent Assays (ELISA) were conducted to quantify the expression of AKT and GLUT4 proteins. Preliminary results revealed upregulation in AKT expression and an increase in GLUT4 expression in treated cells compared to untreated controls. These findings indicate that *X. aethiopica* may modulate components of the insulin signaling cascade and enhance glucose uptake pathways.

Conclusion: While this study does not yet present data on combination treatments with insulin or metformin, it provides foundational insights into the plant's standalone effects, warranting further investigation into its potential as an adjunct therapy in diabetes management.

Synthesis, antischistosomal structure-activity relationship and physicochemical/DMPK profiling of *n*-pyridazin-3-ylbenzamides by *in silico* and *in vitro* approaches

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Introduction and Aims: Schistosomiasis is a neglected tropical disease and the second most fatal tropical disease. The 2024 World Health Organization (WHO) report indicates global mortality rates of 130,000 and 150,000 schistosomiasis deaths attributed to the causative agents *Schistosoma mansoni* and *S. haematobium,* respectively, both of which species are endemic to Zambia. Although praziquantel (PZQ) remains the almost exclusive standard of care drug, it has some shortcomings including ineffectiveness against immature parasites, challenges in paediatric dosing, a high adult dose and has shown drug resistance. Therefore, there remains an unmet medical need to develop other treatment options with different modes of action to circumvent the now well-known mechanism of resistance. This study was based on Medicines for Malaria Venture's compound **MMV687807**, which was preliminarily shown to possess promising *in vitro* antischistosomal activity but whose extensive structure-activity exploration was not undertaken yet. The study was also inspired by the desire to design analogues with better physicochemical properties such as logarithms of partition coefficient or distribution (Log*P* or Log*D*) and solubility. The aim of this study was to synthesize *N*-pyridazin-3-ylbenzamide (*N*-PdzBA) analogues and profile their hydrophobicity, solubility, cytotoxicity and antischistosomal activity.

Methods: This study was based on the structure-activity relationship (SAR) exploration results on **MMV687807**. The study introduced an *N*-pyridazin-3- yl heterocyclic ring in lieu of the *N*-phenyl carbocyclic ring thereby editing the *N*-phenylbenzamide (*N*-PhBA) scaffold of **MMV687807**, **MK1-11**, etc to the *N*-pyridazin-3-ylbenzamides (*N*-PdzBAs) seeing that *N*-PhBA hits were experimentally found poorly soluble. Six target compounds were successfully synthesized by carbodiimide-mediated amide coupling to the required purity (95%). LC-MS was used as the ultimate criterion of purity and to profile retention time (t_R) while UV-VIS, IR, ^{1}H and ^{13}C-NMR were used for characterization.

Results: Compared to the *N*-PhBA hits, *N*-PdzBAs showed slightly lower potency on *S. mansoni* adult worms and NTS but favourably lower cytotoxicity, better solubility and hydrophilicity. One candidate at 100 µg/mL even at highest dosage only showed 48.33% dead in 72 hrs activity on NTS which was still below the ≥ 50% activity threshold. In addition, other pharmacokinetic properties were found to be better (calculated Log*P* < 4.1 favourably way below 5 the Lipinski Ro5 cut-off) compared to the *N*-PhBAs (cLog*P* > 4.1) and calculated solubility (*S*) usually favourably way above 100 µM.

Discussion and Conclusion: PdzBA project compounds with favourably less cytotoxicity and less hydrophobicity even though the potency was unfavourably less could be rescued to anti-COVID19 and anti-Parkinson's agents as shown elsewhere.

Antibacterial potential of endophytic *Talaromyces spp.* isolated from ugandan medicinal plants against MRSA and ESBL producing *escherichia coli*

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Introduction: Endophytic fungi are ubiquitous microorganisms that reside within plant tissues without causing harm. When associated with medicinal plants, they exhibit significant pharmaceutical potential. Given the global challenge of antimicrobial resistance, which causes approximately 700,000 deaths annually, these fungi present a promising source for novel antimicrobial compounds.

Aim: To isolate and characterize endophytic fungi from selected Ugandan medicinal plants and assess their antibacterial activity against methicillin- resistant *Staphylococcus aureus* (MRSA) and extended-spectrum □-lactamase (ESBL)-producing *Escherichia coli.*

Methods: Leaves and stem barks were collected from healthy plants and surface sterilized fragments were inoculated on Potato dextrose agar (PDA). The fungi were identified using morphological traits and confirmed using ITS molecular barcode. Fermentation was conducted for up to 25 days using potato dextrose broth and PDA. Antibacterial screening was done using agar plug and cell free fermentation broth diffusion assays. Ethyl acetate extracts of bioactive *Talaromyces* isolates were tested using agar well diffusion and broth microdilution to determine MIC values.

Results: Four *Talaromyces spp*. isolates showed strong antibacterial activity. Ethyl acetate extracts exhibited MICs as low as 4.88 µg/ml against *S. aureus* and 9.76 µg/ml against *E. coli*. Specifically, isolates ALA and HSL2 were active against MRSA and ESBL-producing *E. coli*, with MICs of 156.25 µg/ml and 19.53 µg/ml, respectively.

Conclusion: Endophytic *Talaromyces spp*. from Ugandan medicinal plants produce metabolites with potent antibacterial activity, highlighting their potential as leads in the development of novel agents against drug-resistant pathogens. Further work is ongoing to isolate and characterize the bioactive metabolites responsible for the observed activity.

Targeted delivery of azacitidine using pegylated hyaluronic acid-functionalised liposomes for enhanced cancer therapy

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Introduction: Azacitidine (AZA), a DNA methyltransferase inhibitor, has shown promise as a treatment for myelodysplastic syndrome and breast cancer therapy. The high aqueous solubility and low lipophilicity (indicated by a negative log P) of AZA result in limited cancer cell permeation and hinder controlled release and clinical efficacy.

Aims and Objectives: To address these challenges, a pegylated hyaluronic acid-functionalised liposome (HA-PEG-LP) was designed, developed and optimised for the targeted delivery of azacitidine to cancer cell therapy and in vitro evaluation using HeLa, MDA-MB-231 and HEK cell lines.

Methods: The HA-PEG-LPs were prepared using a thin film hydration technique, and a characterisation study was performed using *FTIR*, TEM, and *DLS*. The optimised formulation was further subjected to particle size, surface charges, polydispersity index (PDI), drug loading, entrapment efficiency, in vitro drug release kinetics and haemolytic toxicity. The mean particle size of optimised AZA-HA-PEG-LP was 132 ± 1.305 nm, with a polydispersity index of 0.249 ± 0.015, and a favourable zeta potential of –26.1 ± 0.907 mV.

Results: An entrapment efficiency of 85.2% was achieved with the AZA-HA-PEG-LP formulation. The release rate of the bare azacitidine suggested a lack of pH controlled release due to the rapid burst at physiological pH 7.4, indicating a high potential for systemic side effects. Compared to the encapsulated drug, there was a significantly slower release. The AZA-HA-PEG-LP showed a biphasic release profile with an initial burst release then a gradual sustained release over time at pH 7.4 and pH 4.5. While the formulation showed a comparatively slower and more sustained release at pH 4.5, this not only proves the pH responsiveness of the formulation but also suggests the ability of the liposome to retain the AZA during systemic circulation comparatively improving drug delivery. With the aid of HA mediated targeting in the acidic environment, there is a potential for the formulation to be utilised for early systemic exposure followed by a sustained release at the tumour site.

Conclusion: Cell viability assays showed that AZA-HA-PEG-LP significantly enhanced internalisation and reduced viability in HeLa and MDA-MB_231 cells, particularly in CD44-overexpressing cancer cells, by inducing pro-apoptotic activity. Notably, AZA-HA-PEG-LP outperformed free AZA in targeting cancer cells with the highest concentrations of bare drug eliminating less cancerous cells compared to the formulation at the same concentration, suggesting an improved targeting and delivery.

Glycaemic control mechanisms of *Litchi chinensis* sonn. (litchi) peel ethyl acetate extract in a fructose/streptozotocin diabetic model of rats

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Introduction:
The glycaemic control potential and flavonoid profile of litchi have been documented for its hydroalcoholic extracts, while there is scarce information regarding its ethyl acetate extract.

Aims and Objectives: This study investigated the flavonoid profile, as well as the ameliorative potential and possible underlying mechanisms of litchi peel ethyl acetate extract on type 2 diabetes-related pathologies in a fructose/streptozotocin (STZ) model of diabetic rats.

Methods: Sprague-Dawley rats were induced with diabetes by administering 10% fructose for 2 weeks and a single i.p. injection of low-dose (40 mg/kg bw) STZ. Thereafter, the animals were orally treated with a low-dose (150 mg/kg bw) and high-dose (300 mg/ kg bw) of the extract (LDPE and HDPE, respectively) and metformin (200 mg/kg bw). Various diabetes related indices were assessed in the animals and their biological samples. The notable flavonoids in the extract determined using LC-MS.

Results: Compared to untreated diabetic rats (AUC = 1004 mg.h/dL), the HDPE significantly ($p < 0.05$) improved glucose tolerance (AUC = 847 mg.h/dL), which was statistically comparable ($p \square 0.05$) to the effect of metformin (AUC = 903 mg.h/dL). Serum insulin and pancreatic histology data showed that the STZ-induced pancreatic damage and insulin depletion was improved by the HDPE, which could be linked to the observed ameliorative effect of the extract on pancreatic lipid peroxidation and SOD and catalase activity. The extract further improved liver and muscle glycogen storage, as well as muscle hexokinase activity and Akt phosphorylation, suggesting that the extract exerts glycaemic control by enhancing glycogen storage and modulating insulin-mediated signalling of glucose uptake and utilization. LC-MS data and documented reports suggest that flavonoids, such as epicatechin, cinnamtannin B2, procyanidin B5, and proanthocyanidin A2, are the possible influencing compounds.

Discussion and Conclusion: The ethyl acetate extract of litchi peel could be a source of bioactive flavonoids that can potentiate glycaemic control in diabetes and mitigate oxidative stress-related pathologies.

Factors influencing the uptake of doxorubicin into BT-20 triple-negative breast carcinoma spheroids

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Introduction: Triple-negative breast cancer is one of the most aggressive forms of cancer, and is characterised by a lack of oestrogen, progesterone, and human epidermal growth factor receptors. Doxorubicin is often used chemotherapeutically, however, its uptake is influenced by cellular transport, the drug's ionisation state and membrane permeation. Understanding the contribution of these factors may help elucidate doxorubicin's permeability within spheroid models. The study aimed to determine the cytotoxicity of doxorubicin in combination with compounds that alter membrane integrity (egtazic acid [EGTA]), P-glycoprotein transporter functions (verapamil) and environmental pH (□-cyano-4-hydroxycinnamate [CHC]) in BT-20 triple-negative breast carcinoma spheroids.

Materials and methods: The 72-hour cytotoxic effects of three concentrations of doxorubicin, EGTA, verapamil, and CHC were evaluated using morphological analysis, planimetric measurements, and acid phosphatase activity. The combinational effects of 10 µM doxorubicin with 1.5 mM EGTA, 50 µM verapamil, or 0.25 mM CHC were assessed based on morphological changes. Fluorescence microscopy was performed on spheroids treated with these combinations to visualise uptake into the spheroid. Western blot analysis was conducted to assess the expression of E-cadherin and to generate a general cadherin expression profile, including N-, R-, K-, P-, E-, and VE-cadherin.

Results: Doxorubicin significantly reduced spheroid volume by 27.5% and viability by 39.9% at 10 µM, and volume by 50.6% and viability by 69.9% at 25 µM. EGTA (2 mM) disrupted spheroid integrity, while verapamil and CHC showed minimal effects. Fluorescence microscopy revealed a slight increase in doxorubicin accumulation in spheroid cores after pre-treatment with 1.5 mM EGTA, 50 µM verapamil, or CHC (0.4, 0.5, 1 mM), compared to doxorubicin alone. Western blotting showed differential cadherin profiles between 2D and 3D cultures, and a slight, non-significant decrease in E-cadherin expression in 3D as compared to 2D cultures.

Discussion and Conclusion: These findings suggest that altering membrane integrity, P-glycoprotein function and environmental pH may alter the response towards doxorubicin, however, further investigation is needed to ascertain to what degree this can be modulated.

Secretomic profiling of triple-negative breast cancer media using mag-net™ HP

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Introduction: Secretomics provides a real-time view of tumor biology by profiling proteins that cancer cells release into their microenvironment and circulation. In breast and other cancers, secreted factors can serve as predictive and prognostic markers of disease state and treatment response. However, conventional mass-spectrometry workflows often miss low-abundance cytokines, growth factors, and signalling mediators masked by highly abundant plasma proteins. Directly standardizing markers from patient samples is further complicated by inter- and intra-patient variability. Ex vivo secretome analyses in controlled culture conditions allow reproducible characterization of secreted proteomic signatures, which can then guide targeted validation in larger clinical cohorts. Mag-Net™ enrichment, as demonstrated by Wu et al., offers cost-effective capture of extracellular vesicle–linked, low-abundance proteins from plasma, enhancing downstream MS sensitivity.

Methods: BT-20 cells (1×10^5 cells/well) were cultured in 24-well plates and treated in triplicate with doxorubicin (0.3, 0.7 or 1.5 µM), or DMSO. After 72 h, conditioned media were collected and incubated with Mag-Net™ HP beads for secretome enrichment. Vesicle capture, clean up and digestion was performed in a semi-automated manner on a KingFisher™ Flex using the Mag-Net™ HP kit. Peptides were loaded onto Evotips and analysed using an Evosep One coupled to a Bruker timsTOF HT system.

Results: Mag-Net™ enrichment yielded identification of over 4,800 proteins per condition. Secretome profiling revealed more than 100 proteins with dose-dependent upregulation ($r > 0.8$, $p < 0.05$). Principal component analysis segregated doxorubicin-treated samples from controls and further resolved them by dose. Significantly up-regulated proteins (302–366 per contrast) included cell-cycle regulators, extracellular matrix modulators, and stress-associated secreted factors.

Conclusions: Mag-Net enrichment markedly improves detection of low-abundance secretome proteins in doxorubicin-treated TNBC cells. This streamlined approach enhances MS-based secretome workflows and supports discovery of clinically relevant biomarkers. Ongoing studies extend this pipeline to additional cell lines and patient-derived organoids.

The in *vitro* anti- proliferative effects of *cannabis sativa l.* On MCF-7 breast cancer cell lines

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Introduction: Breast cancer remains a leading cause of cancer-related mortality among women worldwide, underscoring the need for alternative therapeutic strategies.

Aims and Objectives: This study investigated the antiproliferative effects of *Cannabis sativa* L. methanol, ethanol, and hexane extracts on MCF-7 breast cancer cells and elucidated the underlying mechanisms.

Methods: Cytotoxicity was assessed using the MTT assay against MCF-7 and normal Hs27 fibroblasts, with doxorubicin as a positive control. Apoptosis was evaluated through morphological examination and Annexin V-FITC/propidium iodide (PI) flow cytometry, while cell cycle effects were analysed via PI staining. Protein expression of p53, caspase-3, and caspase-8 was determined by Western blotting, and caspase-9 activity was measured using a colorimetric assay.

Results: The methanol and ethanol extracts demonstrated potent, dose-dependent inhibition of MCF-7 proliferation with higher selectivity indices compared to hexane extract. Both extracts significantly induced early and late apoptosis, triggered Go/G1 and S-phase arrest, upregulated pro-apoptotic proteins, and enhanced caspase-9 activity.

Discussion/Conclusion: These findings suggest that *C. sativa* exerts its anticancer effects primarily through activation of intrinsic apoptotic pathways and cell cycle modulation. In conclusion, *C. sativa* contains bioactive compounds with promising potential as plant-derived agents for breast cancer therapy, warranting further in vivo validation and compound isolation studies

The development and validation of an LC-MS/MS method for the quantification of anidulafungin in plasma

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Background: Candidemia is a severe fungal infection primarily caused by *Candida* species and remains a major concern in hospitalized patients owing to its high morbidity and mortality. The global rise in antifungal resistance has increased the need for effective therapeutic strategies and robust drug monitoring methods. Anidulafungin (ANF), an echinocandin antifungal, is frequently used to treat invasive candidiasis. To optimize dosing and limit resistance development, this study aimed to develop a Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method for the quantification of anidulafungin in plasma to support a pharmacokinetic study.

Methods: A Shimadzu 8040 triple quadrupole LC-MS/MS in positive ion mode was used to monitor the transitions *m/z* 1140.5000→1122.5000 for ANF and 1146.4000→1128.4500 for Anidulafungin-d6 as an internal standard. Sample preparation involved protein precipitation with acetonitrile, followed by chromatographic separation using a C18 (3.00 × 100 mm, 3µm) column with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as the mobile phase. The calibration curve (0.0625–16 µg/mL) fitted a quadratic regression of 1/c (c = concentration) for ANF. The method was validated for sensitivity, specificity, stability, recovery, matrix effects, and process efficiency.

Results: The method demonstrated excellent intra- and inter-day precision and accuracy for calibration standards, with values ranging from 99.2–103.2% (CV% between 4.5–14.6%). The quality controls had an average accuracy ranging from 95.7 to 102.6% (CV% between 5.1% and 9.7%) for ANF. The analyte was stable in the matrix on bench for 4 hours and had 24-hour autosampler stability at 8 °C. The average % recovery was 88.7%, with no matrix effects observed in six different sources of plasma. The validation met the FDA (2018) and EMA (2011) guidelines and was applied to samples in a clinical trial.

Conclusion: This validated LC-MS/MS method provides a sensitive and robust approach for quantifying ANF in plasma, supporting therapeutic drug monitoring and pharmacokinetic studies in hospitalized patients with candidemia.

Evaluating the effect of stage 1 and 6 load-shedding on the stability and function of insulin analogues

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Purpose: The recurring power cuts occurring nationwide in South Africa introduce an unprecedented challenge in many diabetic patients, hindering insulin stability and jeopardising glycaemic control. Optimal storage conditions should be maintained to preserve its biological function, stability and pharmaceutical quality. These properties are essential for the effective therapy of the patient and therefore adequate control of their glycaemia. Temperatures outside of the recommended temperature ranges negatively affect insulin efficacy and stability which subsequently affect the management of blood glucose levels in diabetic patients. This research adopts the South African load-shedding model to imitate controlled temperature fluctuations to assess the effects of that environmental change on the stability and efficacy of four insulin analogues (aspart, detemir, glargine and glulisine).

Method: Each insulin analogue (aspart, detemir, glargine and glulisine) was stored in three different conditions: refrigeration (2-8°C), which serves as a control and a simulated stage 1 and 6 load-shedding schedule as the investigatory condition. The third condition, room temperature (25°C), served as a possible alternative storage condition. The conditions mentioned above were conducted over six weeks for each stage, and pharmacological assays were performed in two-week intervals. Insulin concentration recovered was measured each week using ELISA. The refrigerator temperature was monitored before and after power interruptions Insulin-stimulated glucose utilization and GLUT 4 translocation of skeletal muscle cells over the six weeks were monitored using In-cell ELISA.

Results: Over the six weeks, the visual appearance of all insulin analogue samples remained constant and exhibited no evidence of turbidity. Insulin concentration, for all four analogues stored at room temperatureandstoredinstage 1 and 6 load-shedding conditions, decreased progressively compared to week 0 baseline results from refrigerated control samples. Results for insulin-stimulated glucose utilization and GLUT 4 translocation remained relatively the same over the six weeks. For the control samples insulin concentration decreased slightly over time while insulin-stimulated glucose utilization and GLUT 4 translocation remained relatively the same during storage.

Conclusion: The decrease in concentration signals a discrepancy in the physical stability of the insulin analogues exposed to deviating storage techniques and in response to load-shedding. In contrast, the visual appearance shows no evidence of physical instability. The results of glucose utilization and GLUT 4 translocation suggest maintenance of the function of the insulin analogues. Further investigation of the efficacy of insulin should be carried out by assessing AKT phosphorylation.

The ability of UKZN pharmacy and medical students to apply drug and dosage adjustments in the case of renal dysfunction

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Background/Introduction: Renal dysfunction is experienced by many patients and encountered by many health professions. Adjustments are made to the type or dose of the medicines prescribed to patients with renal impairment using estimated creatinine clearance (eCL_{cr}), estimated glomerular filtration rate (eGFR) and/or serum creatinine (S_{cr}) levels. The two groups being studied undergo different tuition types with medical students receiving a structured guided tutorial with examples to practice, and with pharmacy students receiving lecture-based tuition with notes and formula for estimating creatinine clearance (CL_{cr}). Both groups will evaluate and/or make prescribing decisions and so must be competent at calculating and evaluating CL_{cr} levels, choosing appropriate drugs and dosages and making dosage adjustments or switching to a safer drug when necessary.

Aim: To determine the level of competence of medical and pharmacy students of UKZN to deliver quality care relating to making prescribing decisions in renal impairment, and to determine whether different educational experiences and types of tuition, or different home languages (English versus non-English) predict student success.

Method: This study assessed UKZN medical and pharmacy students after receiving tuition involving calculations and/or prescribing in renal impairment. Each group received the same set of scenarios comprising three fictitious patients, relevant pathology laboratory reports and/or excerpts of treatment guideline dosage information. They calculated eCL_{cr} (for two patients) and/or chose from a range of drug/dose adjustments (four prescribing decisions). The medical students' results were analysed retrospectively as they had received the questions in a formal assessment, while the pharmacy students were invited, after a routine lecture, to participate in the study and complete the assessment (which they were told would not form part of their formal assessment). Data regarding home language was also collected. Data was then analysed to attempt to determine whether students could conduct calculations and dosage and drug adjustments successfully, which group had more success and whether language had an impact on success. First, this study calculated the percentage of students deemed fully competent (answering all questions correctly), those calculating eCL_{cr} competently (both calculations correct) and those making competent prescribing decisions (all four drug/dose adjustments correct), amongst other factors. Then binary logistic regression was used to determine whether medical or pharmacy school tuition or home language predicted success.

Results: Of classes of 247 medical and 103 pharmacy students, 123 and 74 agreed to participate (49.8% and 71.8%) respectively. If the three fictitious cases each student had managed had been real, 369 and 222 patients would have been treated, respectively. Treatment would have been appropriate in 50.7 % of cases treated by medical students and 60.8% of cases treated by pharmacy students. For medical students, 13.8% were deemed fully competent (answering all questions correctly), 65.9% calculated eCL_{cr} competently (both calculations correct) and 16.3% made competent prescribing decisions (all four drug/dose adjustments correct). These measures of success for pharmacy students were 13.5%, 85.1% and 17.6% respectively. Pharmacy students were statistically significantly more likely to get at least half of the six questions correct (50% competence), all the calculations questions correct, and at least half of the prescribing questions correct (50% prescribing competence), p-value < 0.05. For both student groups, students having English as a home language demonstrated significantly more full competence and full calculation competence than students whose home language was a language other than English. For medical students, English mother tongue speakers were also more likely to get at least three of the six questions correct.

Conclusion: In conclusion, though there were some benefits to having the language of instruction and of reference materials as a home language, and some tuition differences that favoured pharmacy students, most students in both groups were underprepared for providing pharmacological care to patients with renal impairment. Remedical action is needed in order to improve their abilities.

Polymeric micelles for the delivery of novel synthesized Isatin-Linezolid hybrid compound against cervical cancer

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Introduction: Despite the advances made in cervical cancer treatment, limitations persist. Consequently, the development of novel therapeutics with enhanced properties (target-specificity and improved efficacy) is a crucial step in the pursuit of overcoming cervical cancer.

Aims and Objectives: Herein, we report the synthesis and encapsulation of a novel Isatin-Linezolid hybrid compound in PLGA polymeric micelles to target cervical cancer.

Methods: Computational studies of binding dynamics and thermodynamics were determined, and quercetin was used as a control against Bc12. The Isatin-Linezolid hybrid compound (C8) was synthesized in a four-step reaction and its formation was confirmed by 1H NMR, 13C NMR, and FTIR. C8 was further incorporated into PLGA polymeric micelles via the nanoprecipitate method, and physiochemically characterized. TEM was used to confirm its formation, morphology, and size. The stability of PLGA-C8 was tested, after freeze-drying and storing the nanosystem for 30 days. The in vitro cytotoxicity of PLGA-C8 was evaluated against HEK293 and HeLa.

Results: The computational studies results showed C8 had a binding affinity of -9.2 kcal/mol and quercetin had -7.0 kcal/mol. Free energy calculations further confirmed the superior binding affinity of Isatin-Linezolid (-40.64 kcal/mol) over quercetin (-39.55 kcal/mol). PLGA-C8 had a particle size, polydispersity index, zeta potential, and entrapment efficiency of 157.0 ± 0.36 nm, 0.216 ± 0.001, -16.9 ± 1.00 mV and 98%, respectively. In vitro drug release at pH 7.4 demonstrated that the encapsulation of C8 in PLGA improved the release with 83% after 48 hours while the bare had released 52%. Stability studies results showed the prepared PLGA-C8 to be stable. The in vitro hemolysis test identified PLGA-C8 as a non-hemolytic formulation.

Discussion/Conclusion: MTT assay results of novel Isatin-Linezolid hybrid compound and PLGA-C8 revealed less toxicity to HEK293 cells, as opposed to HeLa cells, which showed cell viability of less than 10%, emphasizing the efficacy of both C8 and PLGA-C8 against cervical cells. Therefore, based on the obtained results, the synthesized novel Isatin-Linezolid hybrid compound and the prepared PLGA-C8 polymeric micelles could serve as potential source of newly discovered chemotherapeutic agent for the treatment of cervical cancer.

Underpinning the melamine-complex conundrum - the potential for nephrotoxicity

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Background: Nutritional supplements are widely consumed by competitive athletes, recreational users, and the general public that spans all ages, from infants to the elderly. The global industry's estimated market value as at 2018 being US$115 billion. However, concerns have emerged over the presence of undeclared adulterants, such as melamine—a compound implicated in kidney failure and deaths in animals and infants, particularly during the 2008 Chinese milk scandal. The extent of melamine contamination and/or adulteration in supplements is not well understood. Additionally, melamine and its analogues, including cyanuric acid, uric acid, and melamine cyanurate, may pose nephrotoxic risks through interactions with the calcium-sensing receptor (CaSR), potentially causing kidney cell damage.

Methodology: A total of 138 nutritional supplement products were collected via retail, consumer contributions, and direct from suppliers in South Africa. These were analyzed for melamine content using Tandem Liquid Chromatography Mass Spectrometry. In a separate in silico study, molecular docking was conducted using Schrödinger's Maestro software to evaluate the CaSR binding profiles of melamine and its analogues, employing advanced docking processes such as molecular mechanics and molecular dynamics, for accurate binding affinity and stability prediction.

Results: Of the 138 products tested, 47% were positive for melamine. Among South African products, 82% contained melamine, compared to 58% of imported products. The median concentration across all products was 6.0 µg/g—below the WHO Tolerable Daily Intake of 200 µg/g. Molecular modelling revealed that cyanuric acid, uric acid, and melamine cyanurate showed stronger CaSR binding than melamine, with melamine cyanurate having the highest binding affinity and stability, supporting its role in nephrotoxicity.

Conclusion: While detected melamine levels in supplements are within safe limits as currently defined, potential health risks arise from chemical interactions with analogues forming harmful complexes. Ongoing monitoring and investigation into adulteration and chemical complex formation in supplements are recommended.

Ethanolic extracts of *carpobrotus edulis* leaf juice, and leaves and stems blend: Strong inhibitors of carbohydrates digestion enzymes

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Introduction and Aim: *Carpobrotus edulis* is used in traditional medicine to combat diabetes mellitus. However, there are limited scientific studies explaining the possible biomolecular mechanisms for its therapeutic claims. One approach to managing type 2 diabetes mellitus is to reduce postprandial hyperglycaemia by inhibiting carbohydrate digestion enzymes. This study investigated the effects of *Carpobrotus edulis* ethanolic extracts on digestive enzymes inhibition.

Methods: *C. edulis* leaf juice, leaves and stems, and roots extracts were macerated in 70% ethanol. The obtained extracts were analysed qualitatively for their polyphenols content using high-performance liquid chromatography (HPLC). Cytotoxicity of the extracts was tested on human embryonic kidney (HEK-293) cells. The ability of the extracts to inhibit the enzymes alpha-amylase, alpha-glucosidase and sucrase was assessed, using acarbose as the positive control.

Results: Quercetin and vanillic acid were identified from the *C. edulis* leaf juice, and leaves and stems extracts. Only quercetin was identified from the *C. edulis* roots extract. The extracts exhibited low cytotoxicity to HEK-293 cells at concentrations up to 100 µg/ml. The *C. edulis* leaf juice, and leaves and stems extracts exhibited strong □-amylase inhibition, with IC_{50} values (3,61 ± 0.21 µg/ml and 13.17 ± 0.02 µg/ ml respectively) statistically ($p \leq 0.05$) comparable to acarbose (8.25 ± 1.63 µg/ml). The *C. edulis* roots extract yielded a significantly higher IC_{50} value than acarbose. All extracts demonstrated stronger □-glucosidase inhibition (IC_{50} values = 2.50 ± 0.36, 2.47 ± 0.06 and 15.68 ± 0.76 µg/ml for *C. edulis* leaf juice, leaves and stems, and roots extracts, respectively) than acarbose (IC_{50} = 173.69 ± 14.92 µg/ml). The extracts demonstrated significantly less sucrase inhibition than acarbose.

Conclusion: The results indicate that *C. edulis* extracts could combat diabetes by inhibiting enzymes involved in carbohydrates digestion. With further research, this could lead to developing more effective digestive enzyme inhibitors for treatment of diabetes by preventing postprandial hyperglycaemia.

Treatment of dyslipidaemia and type 2 diabetes mellitus in diabetic dyslipidaemia in the Ilembe district, KwaZulu-Natal: A retrospective study

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Background: Type 2 diabetes mellitus (T2DM) is a growing epidemic in South Africa. It is associated with dyslipidaemia, and dyslipidaemia treatment is also given to diabetic patients at risk of heart disease to lower their risk. Treatment guidelines guide caregivers in monitoring target glucose and lipid levels and recommending drug treatment regimens. This study focused on patients with T2DM in rural KwaZulu-Natal. It aimed first to determine whether their blood level monitoring and drug treatment aligned with glucose and lipid control treatment guidelines. Second, it aimed to determine whether adherence to glucose and lipid control treatment guidelines improved glucose and lipid control, respectively. Thirdly, it aimed to explore whether glucose control predicted lipid control.

Aims:

- To assess what proportion of patients with Type 2 diabetes mellitus and dyslipidaemia are treated in alignment with recommended national treatment guidelines for glucose and lipid control in a South African public sector rural setting hospital
- To assess whether the alignment of management of these patients with recommended national treatment guidelines is associated with improved glucose and lipid control treatment targets.
- To determine if there is a relationship between glucose control and lipid control in these patients.

Method: This retrospective descriptive cross-sectional chart review was conducted among T2DM patients attending Montebello District (M) and General Justice Gizenga Mpanza Regional Hospital (G) in the Ilembe District, KwaZulu-Natal. Patient demographics, diabetes and diabetic dyslipidaemia medicines, blood glucose, HbA1c and lipid levels were collected over five weeks, and assessed against treatment guidelines. The percentage of patients treated in adherence with lipid and glucose control treatment guidelines, and of those achieving glucose and lipid control, was calculated. Using chi-squared testing, associations between adherence to treatment guidelines and glucose and lipid control, and between glucose and lipid control were sought.

Results: There were 65 participants at M and 300 at G. Over 50% (n=36 and 198 in the M and G studies, respectively) of participants were treated according to diabetes mellitus treatment guidelines. There were fewer than 17% % (n=34 and 47 in the M and G studies, respectively) of patients who had controlled HbA1c levels. Compliance with DM STGs predicted glucose control at the M and G studies; p = 0.004618 and p = 0.01623, respectively.
Over 40% (n=26 and 191 in the M and G studies, respectively) of participants were treated according to dyslipidaemia treatment guidelines. The most prevalent lipid abnormality was LDL-C (n=14 and 79 in the M and G studies, respectively). Of the 20 patients with LDL-C levels, dyslipidaemia guideline compliance predicted LDL-C control at M. The M and G studies found that complying with dyslipidaemia STGs predicted improved TC (n=34 and 249) and TG levels (n=28 and 179), respectively.
At M and G, 84.6% and 87.7% of patients received dyslipidaemia treatment, respectively. No association was found between glucose and lipid control.

Conclusion: Adherence to glucose and lipid control treatment guidelines, although suboptimum, predicts glucose control and some measures of improved lipid control. Treatment guideline adherence should be prioritised, and access to all lipid control medicines improved.

Evaluating the claimed antidiabetic effects of the commercial "Sela blood sugar" tea select *in vitro* confirmatory mechanistic studies

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Background: Type 2 diabetes (T2DM) is a major health issue due to limited safe and effective treatments and cure. "Sela blood sugar" (SBS) tea, an herbal medicinal blend, is marketed for lowering blood sugar level only based on traditional use backing its therapeutic claims.

Aim: This study evaluated *in vitro* cytotoxicity, antidiabetic potential of SBS tea extracts and its combined effect with acarbose to provide some scientific evidence supporting its therapeutic claims.

Methodology: SBS tea was extracted using water and a mixture of ethanol-water (60:40, v/v). Chang liver cells were used to assess the cytotoxicity of the obtained extracts. The antidiabetic activity of SBS tea extracts alone or in combination with standard drug (acarbose) was investigated by evaluating the inhibition of carbohydrates digestive enzymes (□-amylase and □-glucosidase).

Results: SBS tea extracts showed no cytotoxicity on Chang liver cells by maintaining a cell viability greater than 80% at the highest tested concentration (480 µg/mL). The aqueous and hydroethanolic extracts inhibited □-amylase activity with IC_{50} values of 1784.07 ± 383.12 and 1620.67 ± 254.51 µg/mL, and inhibited □-glucosidase activity with IC_{50} values of 4.88 ± 0.60 and 5.89 ± 0.36 µg/mL respectively. When combined with acarbose, led to a significant greater □-amylase (443.30 ± 24.64 and 463.70 ± 56.64 µg/mL) and □-glucosidase (1.34 ± 0.03 and 0.71 ± 0.11 µg/mL) inhibition activity.

Conclusion: These findings could in part support the traditional claims of SBS tea as adjuvant therapy for T2DM management. However, additional studies are required to provide a more comprehensive understanding of its antidiabetic mechanisms.

Rifampicin therapeutic drug monitoring and patient outcomes: a single center experience

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Background: Rifampicin forms the cornerstone of both the intensive and continuation phase of drug-sensitive tuberculosis (TB) treatment. However, the recommended weight-based or weight-banded dosing of rifampicin may not lead to therapeutic concentrations needed for TB cure as reported in previous literature. Additionally, the area under the concentration-time curve over 24 hours (AUC_{0-24}) of >41.1 mg.h/L has been consistently reported as a more robust target than peak concentration > 8 mg/L as defined in literature. The objectives of our study were to describe serum rifampicin concentrations and AUC_{0-24} in both adult and paediatric patients at a single South African centre, and to correlate the concentrations with disease outcome.

Methods: We conducted a retrospective review of all rifampicin therapeutic drug monitoring (TDM) data between 2017 and 2023 at our centre. TDM concentrations were used to calculate AUC using a limited regression formula.

Results: We included 15 adults and 7 paediatric participants. Prevalent co-morbid conditions amongst adults were HIV (42.9%) and chronic gastrointestinal disease (42.9%), with malnutrition (50%) predominating in the paediatric group. Most adult (10/15, 66.67%) and paediatric (5/6, 83.33%) participants did not reach adequate peak concentration. Where calculable, the AUC_{0-24} in adults (n=8) was reached in 4 participants (4/8, 50%) while AUC_{0-24} in paediatrics (n=5) was reached in 1 participant (1/5, 20%). Of the 6 adult participants dosed above the recommended dose of 12 mg/kg, 5 improved clinically (83.33%). All four paediatric participants who were dosed at or above the recommended dosage of 20 mg/kg improved.

Conclusion: We found suboptimal rifampicin serum concentrations in both adult and paediatric participants at our centre. However, the majority of paediatric participants did not achieve the preferred target of rifampicin AUC_{0-24}, likely related to chronic gastrointestinal disease and malnourishment. Higher rifampicin dosing and consideration for earlier TDM should be considered in “at-risk” participants to ensure adequate rifampicin exposure and improved clinical outcomes.

Release monitoring and detection of formulated solid nanoparticle–conjugated nicotine in blood and urine using electrochemical technique

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Introduction: Tobacco use is a global health pandemic, causing approximately 8 million deaths annually, with 7 million attributed to direct nicotine use and 1.3 million to secondhand exposure. Smokeless tobacco products contribute to over 300 million morbidities, including chronic kidney illnesses, with predicted mortality rate increases of over 100% by 2050.

Aims and Objectives: To address this challenge, we developed a cost-effective, carbon-based silver sensor for rapid nicotine analysis. Characterized using EDX, FTIR, SEM, DLS, and XRD, the sensor demonstrated significant sensitivity, specificity, and discriminating power, with detection and quantification limits of 2.283 × 10−9 M and 0.761 × 10−8 M, respectively.

Results and Conclusion: The sensor achieved an average recovery rate of 96.26% and was successfully applied in human urine and serum samples. This novel electrochemical approach holds potential for monitoring doping, nicotine release, diagnostics, and quality control, offering a timely solution for public health concerns.

Non-adherence to treatment among patients attending a public primary healthcare setting in South Africa: Prevalence and associated factors

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Abstract: In underdeveloped nations, treatment non-adherence continues to be a significant barrier to effective disease management. It has a major impact on patients and healthcare systems in public primary healthcare settings. Patients who do not take their medications as prescribed may be at higher risk for negative health consequences. Polypharmacy, side-effects, and drug-related problems are factors contributing to non-adherence. Additional patient-related issues include multimorbidity, lack of support, chronic-drugs, and health-literacy.

Aim: To ascertain the prevalence and contributing factors of treatment non-adherence among patients presenting to a public primary healthcare setting in South Africa.

Methodology: Cross-sectional quantitative research using structured questionnaires was carried out with one hundred patients who were chosen using random sampling. Self-reports from patients were used to assess non-adherence to therapy. A standardized questionnaire administered by the interviewer was used to gather data, and IBM SPSS version 29 was used for analysis. Patients aged 18 years and older who were using prescribed medications were included. The characteristics of the participants were obtained using descriptive statistics, and 95% confidence intervals (CIs) are reported for Odds ratios (ORs). Associations between related factors and treatment non-adherence were obtained using the Pearson Chi-square test; a P-value of less than 0.05 was deemed statistically significant.

Results: Of the 100 patients interviewed, 35% were males and 65% females., with the majority in the age-range of 60- 80 years and the majority having a high school level of education. Demographic characteristics associated with non-adherence to treatment were gender and age with P = 0.03. Chronic conditions, alcohol consumption, recreational drug use, use of medication reminders, waiting time to get treatment and support from healthcare providers all were statistically significant with P-values < .001, time to get to the clinic (P = 0.02), mode of transport (P = 0.01), alcohol consumption (OR 22.25 [95% CI: 8.539–57.977], P < .001) and recreational drugs use (OR 8.73 [95% CI: 5.01–15.98], P <.001) were also examined.

Conclusion: Patient medication non-adherence is a major medical problem globally. Though patient education is the key to improving compliance, use of compliance aids, proper motivation and support is also shown to increase medication adherence.

Effectiveness of *Jena DM*® herbal formulation as complementary therapy to conventional oral hypoglycemic agents in type-2 diabetes mellitus: a quasi-experimental study

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Introduction: Most Artemisia *spp* have clinically established anti-diabetic effects (Sessani et. al, 2022). Sesquiterpene Lactones (SLns) – a major class of terpenoid in Artemisia plants, may improve insulin sensitivity (Wang et. al, 2011) however clinical evidence for *A. annua* extracts is limited.

Aim: To evaluate the clinical efficacy of a de-artemisinized *Artemisia annua*-based poly-herbal formulation (*Jena DM*®) in type-2 diabetes mellitus (T2DM).

Methods: The quasi-experimental study enrolled 118 participants diagnosed with T2DM. Following ethical approval, participants were randomly assigned to two non-equal groups, *i.e.*, Oral hypoglycemics - OHA (55) Vs. oral OHA+ *Jena DM*® (63) group, based on whether they used *Jena DM*® or not at the point of enrolment. Follow-up was done for 12 weeks with periodic glycemic (Hb A1C), anthropometric, adverse event, insulin metabolism (HOMA2-IR, HOMA2-Beta), and blood pressure measurements. We used Independent samples t-test and Pearson chi-square tests for comparisons with *P* value threshold < 0.05.

Results: There was neither significant glycemic control benefits (0.1 [95% CI: -0.56, 0.80] %; p=0.798) nor improved insulin metabolism between groups. However, reduction in total body weight (2.0 [95% CI: 0.73, 3.28] kg; p=0.002) and the overall adverse event frequency were higher in the OHA+ *Jena DM*® group (p=0.001).

Discussion: Similar findings on glycemic control have been reported (Sesani et. al). Compared to *Artemisia drancunculus* and *Artemisia princeps*, *A. annua* extracts have comparable limited amounts of potent anti-hyperglycemic phytochemicals *e.g.*, flavonoids and coumarins (Balza and Towers 1984; Ryu et. al, 2013). SLns effect on body weight through energy intake (Fu et. al, 2020), adipocyte free fatty acid metabolism, hepatic PPAR gene expression (Goto et. al, 2010), and immunomodulatory effects on chronic inflammation (Cai et. al, 2016) has been reported. *Jena DM*® has a role in the clinical management of T2DM. However, there is need for more robust studies with longer participant follow-up.

Development of a platform for rational diagnostic design using a venom-antibody model

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Background: Snakebite envenoming is a significant global health threat, disproportionately affecting rural populations in sub-Saharan Africa. The administration of antivenom is crucial for neutralizing the effects of venom, especially in cases of potentially fatal neurotoxic venom. However, this treatment can also have severe side effects such as anaphylaxis, inflammatory responses, and serum sickness. Therefore, it is essential to quickly identify the source of envenoming to make informed decisions about antivenom administration. Currently, there is no reliable diagnostic test available for African snake species to help identify the source of envenomation in this region.

Aim: The aim of this project was to identify *Naja nivea* (cobra) toxins and antibody-binding sites by mapping epitopes and paratopes, and to use this information to create a pipeline for the development of aptamers that can differentially detect cobra venom toxins and/or toxin antibodies from a biological matrix.

Methods: A combination of analytical laboratory techniques and computational informatics was used to achieve this aim. The antibody-toxin binding epitopes within the complexes were determined by limited proteolysis, chemical crosslinking, and analysis usinghigh-resolutionliquidchromatography tandem mass spectrometry (HR-LC-MS/MS). Based on analytical results, computational informatics was used to identify peptide aptamers with optimal binding affinity to the toxins.

Results: Crucial epitopes have been identified, and computational peptide aptamers have been constructed. The binding affinity and specificity were evaluated by conducting computational molecular dynamic simulations. Two lead aptamers bound snake venom toxins with high affinity.

Conclusion: Crosslinking mass spectrometry and computational informatics offer a cost-effective and robust approach to rapidly identify recognition molecules for *N. nivea* venom toxins. Lead aptamers can be synthesized for further analysis and used in the development of point-of-care diagnostic tests for snake envenoming.

Experimental and computational mechanistic insights on citrus essential oils' metabolites for diabetes retinopathy intervention

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Introduction: Despite the increase in exploration of natural products as alternative medications to mitigate diabetes prevalence, citrus, a common fruit crop in South Africa remains underexplored. Although citrus essential oils' (CEOs) antidiabetic potential has been documented, their modulatory mechanism on aldose reductase (ADR), an enzyme implicated in diabetes retinopathy (DR) is limited.

Aims and Objectives: This study evaluated the modulatory effect of six CEOs (*C. aurantifolia, C. bergamia, C. limon, C. paradisi, C. reticulata,* and *C. sinensis*) on ADRusing *invitro* and *insilico* approaches. The half-maximal inhibitory concentration (IC_{50}) of ADR reference standard (ranirestat) (0.1327 mg/mL) was higher than the CEOs investigated with *C. limon* (0.0129 mg/mL) presenting the least IC_{50} among the oils.

Results: Chemical profiling of CEOs revealed the presence of varying amounts and types of metabolites, mainly terpenes. Structural-activity relationship showed that while benzyl-benzoate (-23.26 kcal/mol) and alpha-bergamotene (-22.99 kcal/mol) had the most significant binding free energy ($.6G_{bind}$) among CEOs metabolites, ranirestat had -44.97 kcal/mol. Similar to ranirestat, benzyl benzoate presented and sustained interactions with the catalytic residues (TYR48, HIS110, and CYS 298) of ADR which are critical for its inhibition; thus, corroborating its lower $.6G_{bind}$ relative to other top metabolites. The lower $.6G_{bind}$ of ranirestat relative to the metabolites might be due to the presence of more hydrogen bonds which enhanced its binding affinity and stability with ADR.

Discussion/Conclusion: Insights from the experimental and computational study of the putative leads are indicative of significant inhibitory effects of CEOs on ADR, which may however, require further modification to enhance their therapeutic relevance in the management of DR.

In-vitro investigation of drug-induced mitochondrial dysfunction and its effects on insulin sensitivity

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Background: Mitochondrial dysfunction has been implicated in the development and progression of insulin resistance - the hallmark of Type II Diabetes Mellitus (T2DM). Insulin resistance impairs the glucose uptake in the skeletal muscles and adipocytes, induces gluconeogenesis in the liver and promotes lipolysis in adipose tissue. Recent evidence has shown an increase in drug-induced mitochondrial dysfunction, characterized by elevated production of reactive oxygen species (ROS), impaired mitochondrial respiration, altered mitochondrial permeability transition, mitochondrial DNA damage or inhibition of beta-oxidation of fatty acids.

Study aims and objectives: This study aimed to evaluate the effect of 4 commonly used drugs: tenofovir disoproxil fumarate (TDF), simvastatin, doxorubicin and amitriptyline, in C2C12 cells on selected insulin sensitivity, oxidative stress, mitophagy and mitochondrial dynamics markers

Methods: All C2C12 preparations were completed in 96 well plates for this study. Following cytotoxicity evaluation using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, working concentrations of 12.5, 25, 50 and 100µM were selected for all the drugs. To ascertain mitochondrial damage, unconventional biochemical markers for mitochondrial functioning and mitophagy were assessed by in-cell ELISA. The markers used included microtubule-associated protein 1 light chain 3 (LC3), mitofusin 1 (MFN1), matrix Metalloproteinase (MMP1), p62 and Tafazzin. Luminometry was used to investigate oxidative stress. Glucose utilisation was assessed by media glucose uptake, and ELISA was used to examine GLUT 4 translocation and phosphor-AKT expression.

Results: The exposure to TDF and doxorubicin resulted in modest increases in protein expression of mitochondrial proteins LC3, MFN1, MMP1, p62, and Tafazzin. In contrast, simvastatin and amitriptyline slightly decreased the expression of these mitochondrial proteins, with statistically significant decreases in p62 expression after exposure to amitriptyline. These observations may indicate that the selected drugs are unlikely to be involved in the mitochondrial pathways associated with the proteins examined. Therefore, traditional biochemical markers for mitochondrial functioning such as ATP, mitochondrial membrane potential, PGC-1, citrate synthase leakage and mitochondrial complex activity will be further assessed. This will validate whether the mitochondria is implicated or not in response to the drugs. Intracellular ROS levels were slightly increased by simvastatin, TDF and amitriptyline, with significant decreases by doxorubicin. Furthermore, the effect of these potential mitotoxic agents was examined on insulin sensitivity markers. Non-significant increases in Glut 4 translocation and phospho-AKT were however observed. While simvastatin moderately decreased glucose uptake, amitriptyline, doxorubicin and TDF exposure resulted statistically significant decreases in glucose uptake.

Conclusions: Although majority of these results were not statistically significant, this study contributes to the scarce literature on the selected mitochondrial markers in response to the chosen mitotoxins. The trends identified may be biologically relevant and warrant further investigations. They may provide context for understanding drug-induced mitochondrial toxicity and inform future research directions.

Hyperglycaemia-induced oxidative stress: Investigating the antioxidant potential of *Artemisia Afra* and *Monsonia burkeana* plant extracts in Jurkat T-cells.

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Introduction: Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder characterised by chronic hyperglycaemia, oxidative stress, mitochondrial dysfunction and impaired cellular repair. These pathological features contribute to tissue damage and long-term complications. The escalating prevalence of T2DM imposes a substantial economic burden worldwide. While conventional therapies like metformin are effective, interest has grown in exploring more cost-effective, accessible therapies with minimal long-term side effects. Consequently, the exploration of plant-based interventions has gained attention due to their rich phytochemical content and potential antioxidant activity. This study aimed to investigate the effects of aqueous extracts of *Artemisia afra* (AA) and *Monsonia burkeana* (MB) compared to metformin, in modulating mitochondrial function, oxidative stress, antioxidant responses, and DNA repair mechanisms in hyperglycaemic Jurkat T-cells.

Methods: Jurkat T cells were cultured under normoglycaemic (NG) and hyperglycaemic (HG) conditions and treated with aqueous extracts of AA (25, 50 µg/mL of *Artemisia afra* extract), MB (25, 50 µg/mL of *Monsonia burkeana* extract), or metformin (1 mM). Cytotoxicity was assessed using MTT and LDH assays. Mitochondrial function (.6□m, ATP) was measured using luminometry. Oxidative stress (MDA, NO) and antioxidant markers (GSH, Catalase, SOD2, GPx1) were quantified via spectrophotometry, western blotting and luminometry. DNA repair activity was assessed by qPCR quantification of OGG1 expression. CYP450 3A4 activity was measured to evaluate metabolic processing.

Results: AA and MB treatments were not cytotoxic to hyperglycaemic Jurkat cells. Both extracts significantly lowered ATP production while increasing mitochondrial membrane potential (.6□m). AA slightly elevated MDA levels, suggesting a mild lipid peroxidation, whereas M. burkeana reduced MDA. Both plant extracts, like metformin, reduced NO concentrations, with AA50 producing a significant decrease. Treatment of hyperglycaemic cells significantly suppressed antioxidant enzymes GSH, catalase and SOD2, while GPx1 levels were modestly increased across all treatments. Notably, MB, like metformin, downregulated OGG1 expression, a marker of DNA repair, while AA significantly upregulated it, suggesting activation of a genomic protective response.

Conclusion: The results suggest that AA and MB extracts modulate mitochondrial function and redox balance differently in hyperglycaemic Jurkat cells. Treatments with AA increased mitochondrial polarisation and activated DNA repair pathways, suggesting pro-oxidant signalling. The MB extract demonstrated antioxidant potential by reducing NO and stabilising .6□m. These extracts exhibit distinct mechanisms of action that may inform future phytopharmacological strategies for managing diabetic oxidative stress.

Effect of geographical location on the phytochemical composition and biological activities of monsonia angustifolia collected from two provinces in South Africa

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Introduction and aim(s) of the study: The use of plants for human and animal healthcare dates to ancient times, with their medicinal value linked to secondary metabolites or phytochemicals. In South Africa, up to 60% of the population, especially in rural areas, relies on traditional healers for herbal remedies. Plant collection practices expose species to varying environmental conditions, potentially influencing their phytochemical content. *Monsonia angustifolia*, known for treating erectile dysfunction and enhancing libido, grows in different geographical regions, yet the effect of location on its phytochemical composition remains unclear.

Methods: This study aimed to determine the impact of geographical location on the phytochemical profile of *M. angustifolia* collected from Gauteng and Limpopo provinces. Whole plants were dried, powdered, and sequentially extracted with hexane, dichloromethane, acetone, and methanol. Phytochemical composition was analyzed using TLC, UV-Vis spectrophotometry, chemical screening tests, and GC-MS. Quantitative assays measured total phenolic, flavonoid, tannin, saponin, and alkaloid contents. Antioxidant activity was evaluated through DPPH, hydrogen peroxide reduction, and ferric chloride reduction assays, while antibacterial properties were assessed via micro-titer plate MIC tests.

Results: Methanol produced the highest extraction yield, while acetone yielded the least. TLC revealed more compound bands in the Gauteng sample. Both locations showed similar phytochemicals, but quantities differed: Limpopo samples had higher phenolic, tannin, and saponin levels, whereas Gauteng samples had higher flavonoid and alkaloid contents. GC-MS showed location-specific compounds, with Limpopo having more amines and alcohols, and Gauteng more hydrocarbons, fatty acids, and carbonyl compounds. Limpopo samples generally had stronger antioxidant activity (lower ECso values) except in FeCl3 reducing power. Antibacterial activity was similar between sites, with a slight advantage for Gauteng against *Streptococcus pyogenes*.

Discussion and Conclusion: The findings suggest that abiotic stress from differing geographical conditions influences the quantity, but not the type, of phytochemicals in *M. angustifolia*, affecting antioxidant but not antibacterial properties.

Anti proliferative effects of *Cannabis sativa* dichloromethane extract through oxidative stress-related pathways and the potential inhibition of the migration and invasiveness of human breast cancer cells (MDA-MB 231 and MCF-7)

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Introduction: Breast cancer is a leading cause of cancer-related morbidity and mortality worldwide, underscoring the need for urgent exploration of novel therapeutic strategies.

Aims and Objectives: This study investigated the effect of *Cannabis sativa* extract on oxidative stress, apoptosis and the inhibition of invasion in human breast cancer cells. The researched focused on key biomarkers that included antioxidant enzymes (Superoxide Dismutase (SOD), Glutathione (GSH)), the antioxidant protein transcription factor Nuclear factor erythroid 2related factor 2 (Nrf2), apoptotic protein (p53, caspases 8 and 9), and metalloproteinase (MMP-1 and MMP-9).

Methods: Cytotoxicity of Dichloromethane extract of *Cannabis sativa* was assayed using the MTT on MDA-MB 231 and MCF-7 breast cancer cell lines as well as on normal skin fibroblast cell (HS27) to evaluate the selectivity. Enzymatic assays and ELISA kit were used to quantify oxidative stress markers. The apoptotic pathway was determined using the Western blotting as well as the anti-metastasis activity.

Results: The results showed that the Dichloromethane extract of *Cannabis sativa* (DCME) strongly induced MDA-MB 231 and MCF-7 cell death in a concentration-dependent manner, with IC_{50} values of 75.46 ± 0.132 µg/mL and 78.68 ± 0.50 µg/mL at 24 h, respectively. The extract notably decreased SOD and GSH levels while increasing the expression and activity of caspase 8, 9 and p53 proteins in MDA-MB231, leading to cancer cell apoptosis and cell death. Furthermore, DCME suppressed the Nrf2 protein level and inhibited cellular migration, motility and invasion in a concentration-dependent manner by downregulating MMP-1, MMP-2 and Transforming Growth Factor Beta (TGF-□).

Discussion/Conclusion: *Cannabis sativa* extract inhibits cell proliferation, induce cell apoptosis, induce oxidative stress and inhibit metastatic in MDA-MB 231 and MCF-7 cell lines *in vitro*. This study underscores the potential of *Cannabis sativa* as a therapeutic agent in breast cancer treatment, warranting further investigation into its mechanisms and efficacy in clinical settings.

Ellagitannins from pomegranate peel ameliorates insulin resistance, pro-inflammatory signaling and oxidative stress linked to fructose-induced metabolic disorders in rats

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Introduction and Aim of the Study: Chronic fructose consumption has been implicated in metabolic and cardiovascular impairments. Pomegranate fruit is known to benefit oxidative and vascular health, which has been linked to some constituent ellagitannins. Increasing evidence has shown the peel is rich in ellagitannins. This study investigated the ameliorative potential of pomegranate peel ellagitannins on fructose-induced metabolic, oxidative and inflammatory alterations in rats.

Methods: Ellagitannins-rich fraction was recovered from the peel of pomegranate (Wonderful var.) using an Amberlite® XAD16N resin. The fraction was subjected to LC-MS analysis. The fraction (200 mg/kg bw) was administered to Sprague-Dawley rats receiving 20% fructose solution in place of drinking water for 6 weeks. Food intake, body weight, blood glucose and blood lipids were measured. Thereafter, insulin level, glycogen content, oxidative stress and inflammatory markers, hepatic histology and HOMA-IR were determined. Hepatic Akt phosphorylation was also determined.

Results: Fructose consumption suppressed food intake and increased weight gain. It further increased insulin levels, impaired glucose tolerance (AUC = 246 vs 208 mg.h/dL) and insulin action (HOMA-IR = 5.8 vs 2.0), altered blood lipids (triglycerides = 4.2 vs 1.7 mmol/L; LDL-cholesterol = 3.0 vs 1.4 mmol/L; HDL-cholesterol 0.4 vs 0.8 mmol/L), caused hepatic histological abnormalities and inflammation (IL- 1□ elevation) and increased hepatic and systemic oxidative stress. Ellagitannins treatment improved glucose tolerance (AUC = 230 mg.h/dL), insulin resistance (HOMA-IR = 4.0; improved hepatic Akt phosphorylation), blood lipids (triglycerides, LDL-cholesterol and HDL-cholesterol = 3.7, 1.9 and 0.6 mmol/L, respectively), hepatic histology and inflammation antioxidant status (reduced lipid peroxidation and increased SOD and catalase enzyme activity). LC-MS showed the presence of bioactive ellagitannins (punicalagin, corilagin and pendunculagin) and catechins.

Discussion and Conclusion: Ellagitannins from pomegranate peels may be useful dietary polyphenols to prevent or manage sugar-induced metabolic, cardiovascular and oxidative disorders.

Drug-likeness and anticancer potential of hexane volatile compounds from *viscum continuum*: an integrated *in vitro* and *in silico* study

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Background: *Viscum continuum*, a South African mistletoe species, has limited pharmacological data despite its ethnomedicinal use. This study evaluated the cytotoxic potential and drug-likeness of volatile compounds from its *n*-hexane extract using integrated *in vitro* and *in silico* approaches.

Methods: Gas chromatography–mass spectrometry (GC-MS) identified volatile constituents. Cytotoxicity was assessed against MCF7 (breast) and A549 (lung) cancer cell lines. SwissADME predicted pharmacokinetic profiles. SwissTargetPrediction identified potential protein targets, followed by molecular docking. QSAR modelling of PPARG inhibitors (AID 743199 dataset) employed a random forest algorithm.

Results: Eucalyptol and conjugated linoleic acid (CLA) ester were identified as major constituents. The *n*-hexane extract displayed selective cytotoxicity toward MCF7 and A549 cells. SwissADME predicted favourable drug-likeness for both compounds. Target prediction indicated CYP19A1, an oestrogen biosynthesis enzyme, as eucalyptol's top target (docking score: –2.7 kcal/mol), suggesting relevance in hormone-dependent breast cancer. CLA ester showed highest affinity for PPARG (–4.9 kcal/mol), a nuclear receptor implicated in lung cancer, with additional predicted targets (ALOX5, SCD, PPARA). QSAR modelling achieved high accuracy (AUC = 0.99), with ExtFP663 and ExtFP956 as key predictive features.

Conclusion: Eucalyptol and CLA ester are promising anticancer candidates with selective cytotoxicity and favourable pharmacokinetics. Their predicted targeting of CYP19A1 and PPARG supports potential therapeutic applications in hormone-dependent breast and lung cancers, meriting further preclinical investigation.

Cannabinoid-Cisplatin combinations induce apoptosis and mitotic arrest in cervical cancer cells

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Background: Cervical cancer remains a leading cause of cancer mortality among women, particularly in low-resource settings. This study evaluated cytotoxic and synergistic effects of cannabinoids (THC and CBD) with cisplatin in cervical cancer cells.

Methods: HeLa and SiHa cervical cancer cells and non-tumorigenic MCF-12A mammary epithelial cells were treated with varying concentrations of THC, CBD, cisplatin, and their combinations. Cytotoxicity was assessed using sulforhodamine B assay. Drug interactions were evaluated via checkerboard assays and Bliss Independence modeling. Cell morphology was assessed using light microscopy. Cell cycle distribution and apoptosis were assessed by flow cytometry. Autophagic activity was assessed using immunofluorescence and confocal microscopy

Results: THC, CBD, cisplatin combinations decreased viability in HeLa and SiHa cells by 48% and 46%, respectively. Morphological analysis showed increased apoptotic bodies (6.3%), cell shrinkage (6.5%), and rounded cells (79.7%) in HeLa cells exposed to THC-CBD-cisplatin combination. HeLa and SiHa cells exhibited metaphase arrest with reduced interphase populations however, MCF-12A cells maintained normal morphology. Flow cytometry confirmed increased sub-G_1 populations and cell cycle disruptions in cancer cells after THC-CBD-cisplatin exposure. Annexin V analysis revealed shifts toward early (31.33% and 37%) and late apoptosis (21.67% and 21.33%) in THC-CBD-cisplatin combination, with reduced viable cell fractions of 94.7% vs 40.3% and 94.3% vs 32% in HeLa and SiHa cells. The combination of THC and CBD induces significant puncta formation in cancerous HeLa and SiHa cells, indicating selective cytotoxic or autophagic activity, while sparing non-cancerous MCF-12A cells. In contrast, cisplatin alone exhibits minimal effect but demonstrates moderate enhancement when used in combination.

Conclusion: THC and CBD combined with cisplatin enhances cytotoxicity and promotes mitotic arrest and apoptosis in cervical cancer cells, while minimally affecting non-cancerous cells, suggesting potential for cannabinoid-based adjunct therapies.

Screening of pharmacological activities of *Amarathus hybridus linn* leaf extracts *in vitro*

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Introduction: Functional food play an important role in maintaining good health through disease prevention and improvement of physical wellbeing. *Amaranthus hybridus (A. hybridus)* is one of the leafy vegetables, that is rich in bioactive compounds and can be a potential agent for nutraceutical development.

Aims and Objectives: This study aimed to screen *A. hybridus L.* for antidiabetic and antibacterial activities *in vitro*.

Methods: Aqueous and methanol extracts of *A. hybridus* were screened for their potential to inhibit of carbohydrate metabolising enzymes (□-amylase and □-glucosidase) *in vitro*. The extracts were also screened for antibacterial activity using disc diffusion assay and the minimum inhibitory concentration (MIC) was determined using broth microdilution method. The extracts were subjected to LC-MS for Phytochemical profiling.

Results*: A. hybridus* aqueous and methanol extracts showed potential to inhibit carbohydrate metabolising enzymes with higher inhibition towards □-glucosidase activity than □-amylase activity. Inhibition of □-glucosidase by methanol extract ranged from 52% to 65% while water extract inhibition for □-glucosidase was 46% to 57% at the concentration range of 0.03125 mg/ml to 0.5 mg/ ml. The extracts demonstrated varied antimicrobial activities against *E. coli* and *S. aureus*, with methanol extract showing the MIC of 0.3125 mg/ml for *E. coli* and 0.625mg/ml for *S. aureus*. The MIC for water extracts was 1.25 mg/ml for both *E. coli* and *S. aureus*. LC-MS revealed the presence flavonoids such as rutin and quercetin 3-galactoside, phenolic acids (caftaric acid, protocatechuic acid, phaseolic acid) and coumarin derivatives such as coumarin sulfate.

Discussion/Conclusion: The findings of this study demonstrated the potential of *A. hybridus* to inhibit carbohydrates metabolising enzymes which may lead to a reduction in postprandial hyperglycaemia, hence it may be a potential agent for a nutraceutical to be used in diabetes management. In addition, the extracts showed potential antibacterial activities against *E. coli* and *S. aureus* and may be beneficial in combating infectious diseases associated with these species.

Design, synthesis, and evaluation of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine derivatives as *E. coli* DNA gyrase inhibitors

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The global pipeline for novel antibiotics has stalled, coinciding with an estimated 700,000 annual deaths from antimicrobial resistance (AMR) [1]. Innovative drug scaffolds are urgently needed to outpace emerging resistant pathogens, as they may offer favourable options to overcome antibiotic resistance due to their unique properties and pathways [2] . The aim of this study was to design, synthesise, and evaluate a novel series of 1,2,4-triazolo[1,5-a][1,3,5] triazine analogues (**7a–7t**) for *in vitro* antimicrobial activity, and to validate their mechanism via *E. coli* DNA gyrase enzyme inhibition. Compounds **7a–7t** were characterised by Fourier-Transformed Infrared (FTIR), High Resolution Mass Spectroscopy (HRMS) and Nuclear Magnetic Resonance (NMR). Antitubercular efficacy was assessed against Mycobacterium tuberculosis H*37*Rv, multidrug-resistant tuberculosis (MDR-TB), and extensively drug-resistant tuberculosis (XDR-TB) strains, alongside a panel of 9 bacterial pathogens, using a 96-well broth microdilution assay to determine Minimum Inhibitory Concentration (MIC) values. Most active derivatives underwent *Escherichia coli* (*E. coli*) DNA gyrase enzyme inhibition assays to determine their IC_{50} values. Complementary *in-silico* molecular docking to the *E. coli* Gyrase B active site was performed with AutoDock Vina [3]. Several analogues demonstrated dual antitubercular and antibacterial potency. Compounds **7e**, **7g**, **7h**, **7k**, **7l**, and **7m** inhibited H*37*Rv and MDR-TB at MIC 31.25 µg/mL. Notably, **7h** was highly active against *Enterobacter hormaechei* (MIC 0.98 µg/mL); **7l** against *E. coli* (MIC 3.9 µg/mL); and **7j**, **7r**, **7s** against *E. coli* were in the 7.8–15.6 µg/mL range. Derivatives **7j** and **7l** inhibited clinical Methicillin-resistant Staphylococcus *aureus* (MRSA) isolates (MIC 7.82 and 31.25 µg/mL). *E. coli* DNA Gyrase assays yielded IC_{50} values for **7j** (0.093 µM), **7l** (0.091 µM) and **7s** (0.049 µM), exhibiting significant enzyme inhibition of 97.6 % and 96.2 %, respectively. The *in silico* confirmed strong binding affinities to *E. coli* DNA gyrase B, supporting the experimental results for **7j** (–9.3 kcal/mol), **7l** (–9.1 kcal/mol), **7s** (–8.7 kcal/mol), and **7r** (–8.5 kcal/mol) comparable to ciprofloxacin (–8.2 kcal/ mol). Triazolotriazine derivatives exhibit potent *in vitro* antimicrobial activity, supported by *E. coli* DNA gyrase enzyme inhibition and strong computational binding. These triazolotriazine scaffolds warrant further development as leads against multidrug- resistant pathogens.

In silico molecular modelling of paclitaxel and its derivatives targeting microtubules in breast cancer

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Background: Most breast cancer mortalities are due to metastasis, where microtubules are a key regulator of migration and invasion. Paclitaxel, a tubulin-targeting chemotherapeutic, remains effective but is limited by drug resistance and significant toxicity. This study aimed to determine the binding affinity, binding free energy and molecular dynamics (MD) simulations of paclitaxel, and its *in silico* derivatives in breast cancer cells.

Methods: The ChEMBL database was screened for small molecules inhibitors with ≥85% structural similarity to paclitaxel. Four tubulin isoforms (5LXT, 4I4T, 6SES and 1TUB) were selected for molecular docking using Glide, Maestro. Thereafter, molecular mechanics with generalised born surface area (MM/GBSA) binding free energy calculations were conducted. The best performing isoform was taken further for docking followed by MM/GBSA. A 100-nanosecond MD simulation was performed using Desmond.

Results and Discussion: A total of 323 compounds were obtained from ChEMBL. The target, PDB: 6SES, was the best performing isoform based on MM/GBSA calculations and was used for subsequent docking. Among the screened compounds, 7-epi-paclitaxel and 2'-Acetyltaxol demonstrated intermediate and weak binding, with docking scores of –0.014 kcal/ mol and –5.220 kcal/mol, and MM/GBSA binding free energies of –64.53 kcal/mol and –60.70 kcal/mol, respectively. Root mean square deviation (RMSD) analysis of 7-epi paclitaxel showed values of 2.8Å for protein and ligand, indicating a stable protein-ligand complex.

Conclusion: The findings showed moderate and weak stability of 7-epi paclitaxel and 2'-Acetyltaxol in the active site, respectively. Additionally, these findings support the use of 7-epi paclitaxel as an improved therapeutic candidate. Findings prompt evaluation of these compounds as breast cancer therapies.

Ethnopharmacology and ethnomedicinal information on commonly used indigenous medicine in the Eastern Cape- A case for integrative medicine

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Introduction: Integrative medicine is described as healing oriented medicine that takes account of the whole person and makes use of all appropriate therapies. The goal of integrative medicine is to facilitate health within complex systems, from the individual to the communities and the environment. The WHO has provided guidance over the years for incorporation of indigenous medicines (IM) into the public health services, and encouraged member states to implement strategies that may lead to inclusion of indigenous medicines into their EML. The increased consumption of medicinal plants and herbal products globally (including IM) has been shown to correlate with prevalence of non-communicable diseases. In SA, one of the cited drawbacks for formal recognition and incorporation of IM in public health care is that there is no evidence-based and/or science-based information on the medicines, that would inform decision-making for successful health care delivery. This still prevails in the midst of widespread research being conducted on indigenous medicines in various institutions, therefore indicating a dearth in the translation of the research into beneficial outcomes for health care.

Aim: The research aimed to identify commonly used plant-based indigenous medicines in the Eastern Cape, and provide evidence-based ethnopharmacology information on the IM that would demonstrate their potential role in integrative health.

Method: The data was collected through structured interview-based questionnaires from 2 different communities, and patients attending primary health clinics in Makhanda, Eastern Cape. Search engines including Google scholar, Scopus, Web of Science, and PubMed were used to source ethnopharmacological, ethnomedical and other relevant information on the commonly used IM.

Results: A list of more than 10 plant-based traditional medicines was generated from the survey, that are commonly used for a range of conditions including gastrointestinal disorders, infections, pain relief, skin and respiratory system disorders. The systematic review revealed various pharmacological activities of some of the medicines, including anti-inflammatory, antioxidant, antibacterial, antifungal, anti-diarrhoeal and antidiabetic activities. Contrasting the IM with current conventional medicines revealed potential for herb-drug interactions (synergy, antagonism).

Conclusion: The findings will serve as a resource towards contextual strategic approaches that would fastrack integration of IM within primary health care, the development of relevant policies and protocols, as well as identification of relevant research gaps for follow-up studies.

The effect of *Musa SPP* (banana) isolated tyrosinase by kojic acid and extracts from *Artemisia spp*

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Background and Objectives: Melasma is a hyperpigmentation condition whereby grey-brown patches appear on the face or body, usually where the skin is exposed the most to the sun. This skin condition is much more common in women, thought to be related to hormones and pregnancy, and is made worse by UV exposure. The formation of melanin is dependant on the bio-catalytic action of tyrosinase which converts L-DOPA into dopachrome as a precursor for the melanogenesis. *Artemisia afra* has been reported to possess antityrosinase properties and with this, melasma could be managed by a tyrosinase inhibitor such as kojic acid and *A.afra*. The aim of this study was to investigate the effect of kojic acid and *A.afra* on tyrosinase extracted from *Musa spp* (modelling agent) in order to manage melasma.

Methodology: L-DOPA was prepared at a concentration of 0.2 $mg.ml^{-1}$, 0.4 $mg.ml^{-1}$, 0.6 $mg.ml^{-1}$, 0.8 $mg.ml^{-1}$ and 1.0 $mg.ml^{-1}$ in 0,50 $mol.dm^{-3}$ potassium phosphate buffer at pH 4,5. The tyrosinase enzyme was extracted from banana peel as well as the pulp and, prepared at a concentration of 1.0 $mg.ml^{-1}$ in the phosphate buffer with a 1% and 2% kojic acid prepared separately with the same potassium phosphate buffer. *A.afra* extracts were prepared through maceration and filtered with the filtrate being used in incubations.

Results: Various concentrations from 1% and 2% kojic acid solution were mixed with L-DOPA and tyrosinase extracts. The percentage inhibition for kojic acid on the peel and pulp averaged to 48% and 52% respectively. From this it was confirmed that kojic acid has an inhibitory effect on the modelling agent and would act as a positive control in determining the effect of *A.afra* on tyrosinase activity. From the data collected on *A.afra* extracts, it was noted that the stem showed a constant inhibitory effect of 50% on the peel while data collected on the pulp showed that the stem had an IC50 of 50% while the leaf showed a 12% inhibition.

Conclusion: From the study, it can be concluded that *A.afra* extracts possess antityrosinase properties, which are comparable to kojic acid, a recognised skin lightening agent and treatment for melasma. However, further investigations need to be conducted especially for its clinical application

The development and validation of an LC-MS/MS method for the quantification of ertapenem in human plasma: application to XDR-TB paediatric case series

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Background: Recently, carbapenems (ertapenem) have been repurposed to treat extensively drug-resistant TB, which requires prolonged therapy. However, clinical pharmacokinetic data are lacking for optimal dosing, especially in children, where doses depend on age and weight. Since the efficacy of carbapenems depends on time above the minimum inhibitory concentration, concentration-time profiles must be monitored. This study developed a therapeutic drug monitoring assay for ertapenem to measure plasma concentrations. The data obtained will be applied to the development of population pharmacokinetic model for model-informed dosing.

Methods: A Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed in the positive ion mode with transition ions 476.0500→114.0000 for ertapenem. A gradient of 10 to 70% B, using mobile phases A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid), was used for the retention and elution. A solid-phase extraction was used to extract ertapenem and [$^2H_4$]-ertapenem from 25 µL of plasma. Before storage, 2-(N-Morpholino)ethanesulfonic acid was added in a 1:1 ratio to ensure storage stability. Intra- and inter-day validations, including stability assessments under various storage conditions, were performed.

Results: The developed LC-MS/MS method had a retention time of 2.8 minutes for ertapenem and [$^2H_4$]-ertapenem. The stock solutions of ertapenem remained stable without MES, under ambient light and temperature for 24 hours, at 4 °C and - 20 °C for three days, and at - 80 °C for up to 7 months. A full validation was completed wherein the accuracy of the calibrator standards of ertapenem ranged between 98.2% and 101.6%, with % CVs between 1.9% to 8.6%. The quality controls had accuracies ranging between 96.2% and 101.0%, with % CVs between 5.6% and 13.3%. Matrix effects did not affect the quantification of ertapenem. The average recovery and process efficiency of the methods were 97.1% and 96.6%, respectively. Autosampler stability was shown for 29 hours at 4 °C. Frozen ertapenem plasma samples remained stable with and without MES for up to 2 months at - 80 °C and through three freeze- thaw cycles. Ertapenem was stable in whole blood for up to two hours on-bench, and the presence of 2% haemolysis did not affect the quantification. This method was applied to a pharmacokinetic evaluation of ertapenem in three case series of XDR- TB paediatrics.

Conclusion: An LC-MS/MS method was developed and validated for the quantification of ertapenem from human plasma, over a calibration range of 0.391 to 300 µg/mL. The developed method for ertapenem was used to determine the concentration-time profiles of three paediatric patients with extensively drug-resistant TB, which showed that target concentrations were achieved after weight-adjusted IV doses.

Investigating the *in-vitro* effects of *Withania somnifera* extracts on the progression of Glioblastoma multiforme

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Introduction: Glioblastoma multiforme (GBM) is a highly aggressive brain tumor or cancer. It is the most prevalent adult primary malignant and is extremely combative in men. Several factors are involved in development of cancer such as genetic alteration through mechanism of loss of cell cycle regulation and inhibition of apoptosis, while its inactivation is associated with invasion of cancer. Tumor also spreads through the angiogenesis process. Despite advance surgical techniques and chemotherapy, the prognosis of GBM remains poor with survival rate of approximately 15 months. Limitations of current treatment have sparked interest in exploring alternative and complimentary approaches, including the use of medicinal plants such *Withania somnifera* (WS) which has demonstrated a strong inhibitory effect on angiogenesis in both in vitro and in vivo studies.

Aim: To investigate the anti-angiogenic effects of WS extracts and their potential to induce apoptosis on U-87MG cell line.

Methods: The cytotoxicity effects of WS extracts (Methanol crude extract, Aqueous, Dichloromethane and hexane fractions), and temozolomide were evaluated on normal brain cell, Human Astrocyte (HA) cell line using MTT, while the antiproliferative activity was assessed on U-87MG cell line and colony formation was assesed. The mechanism of cell death was determined by annexin V-FITC using flow cytometry the anti-angiogenic effects of the plant extracts will also be assessed using Chick chorioallantonic membrane (CAM) model and Isocitrate dehydrogenase activity.

Results: WS methanol crude extract, and hexane and aqueous fractions did not show toxicity on normal cell (HA) with an exception of DCM fraction which was moderate non-toxicity along with temozolomide with recorded IC_{50}'s of 140.6µg/ml and 195.8µg/ml respectively. Aqueous and hexane fractions showed more anti-proliferative activity on U-87MG cell line compared to control drug with IC_{50} values of 178.2µg/ml,189.6µg/ml and 210µg/ml respectively. Exposure of the cells to methanol crude extract induced early apoptosis in 15.6% and late apoptosis in 18.4%, whereas fractions were Aqueous (10.6%, 14.6%), DCM (17.2%, 12.8%), and hexane (14.7%, 15.4%), and the standard drug Temozolomide were 12.4% and 16.6% of U-87MG cells as determined by annexin V-FITC and PI staining. DCM and Hexane fractions showed a significant inhibitory effect of colony formation on U-87MG cell line

Conclusion: These preliminary results showed that WS extract have anti-proliferative activity and apoptosis effect on U-87MG cell line. These findings suggests that the Aqueous and hexane fractions might contain bioactive compounds with anticancer characteristics that require additional characterisation and isolation

Exploring the role of mitophagy in drug-induced insulin resistance in muscle cells, *in vitro*

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Introduction: Some drugs, due to their toxicity such as mitochondrial dysfunction, cause drug-induced insulin resistance. If drug-induced insulin resistance is left untreated for an extended period of time, it may progress to Diabetes mellitus Type 2. A balance must always be maintained between mitochondrial dynamics and mitophagy, as any alterations may contribute to the pathogenesis of metabolic diseases such as diabetes mellitus. However, if damaged mitochondria are not removed, their accumulation leads to increased production of reactive oxygen species and causes cell apoptosis. Exploring the implications of mitophagy pathways and mitochondrial dynamics on drug-induced insulin resistance could lead to new approaches that can be used to mitigate insulin resistance associated with different classes of medication.

Aims and Objectives: The study aimed to investigate the role of mitophagy in drug-induced insulin resistance in skeletal muscle (C2C12) exposed to rifampicin, clarithromycin, tenofovir, and simvastatin. The cytotoxic profile of these drugs was determined. Additionally, to determine the development of an insulin-resistant state, activated AKT and GLUT 4 translocation are assessed. Inflammatory cytokine, TNF-□, was also quantified. Lastly, the study also assessed the effect of different drug concentrations on mitochondrial dynamics and mitophagy markers p62, LC3, and MFN1 expression.

Methodology: In this study, skeletal muscle cells were used and seeded in a 96 well plate. A cell viability study was conducted using the MTT assay by exposing cells to different drug concentrations (25,50,100,200, and 400 µM) for 24 hours. An Incell Elisa was then performed to determine the effect of drugs on the AKT phosphorylation, GLUT4 translocation, MFN1, LC3, TNF-□, and p62 expression after exposing the cells to drug concentrations 12.5,25,50 and 100µM for 24 hours.

Results: As the concentration of the drugs increased, the cell viability decreased. Cells treated with 25,50, and 100µM concentrations remained viable after 24 hours, hence used for further assays. By comparison to the insulin-treated control, the pre-treatment of cells with selected drugs decreased insulin sensitivity, as evidenced by a decrease in AKT phosphorylation and GLUT 4 translocation. A dose-dependent increase in the expression of LC3 and TNF-□ was observed in the C2C12-treated cells. All the drugs increased the expression of MFN1 and p62, with the exception of clarithromycin, which decreased the expression.

Conclusion: All 4 drugs studies demonstrate non cytotoxicity, up to a concentration of 100µM in C2C12 cells. Mito toxicant drugs potentially induce insulin resistance arising from mitochondrial dysfunction, inflammation, and impaired mitochondrial dynamics and mitophagy. The observations taken together warrant further investigation of the relationship between drug-induced insulin resistance and mitochondrial dynamics and mitophagy.

In vitro evaluation of anticholinesterase activity of cold ethanolic extract and fractions from *Cannabis sativa* L.: Therapeutic implications for Alzheimer's disease

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Background: Neurodegenerative diseases such as Alzheimer's disease are linked to impaired cholinergic transmission, primarily caused by the breakdown of acetylcholine. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are key enzymes involved in this process. Inhibiting their activity is a well-established therapeutic strategy. *Cannabis sativa*, known for its rich phytochemical content, has shown potential in neurological applications. This study investigates the phytochemical composition and cholinesterase inhibitory activity of *C. sativa* extracts.

Aim: This study investigates the phytocannabinoid composition and the *in vitro* cholinesterase inhibitory effect of *C. sativa* L. cold ethanolic extract and its derived fractions.

Methodology: Cold ethanolic extract of *C. sativa* L. flowers (CE) was fractioned by solid phase extraction using different solvent systems: acetonitrile (F1), acetonitrile: acetone (75:25 v/v) (F2), acetone (F3), acetonitrile:acetone (25:75 v/v) (F5) and water (F5). The phytocannabinoid composition of the cold ethanolic extract and its five fractions were analysed by thin layer chromatography (TLC) using cannabigerol (CBG), tetrahydrocannabivarin (THCVA), cannabinol (CBN) and Cannabidiol (CBD) as standards. Thereafter, the plant extract and fractions were tested for their potential to inhibit AChE and BChE enzymes using the Ellman method.

Results: TLC analysis revealed the presence of CBN and CBD in CE and fraction F1. Although chromatographic spots were also observed in fractions F2 through F5, these did not correspond to any of the reference standards, suggesting the presence of unidentified or less characterized phytoconstituents. The extract and fractions demonstrated a dose-dependent inhibition of AChE and BuChE at concentrations ranging from 10 to 100 µg/mL. Fraction F5 exhibited the highest AChE inhibition with inhibition rates increasing from 45% at 10 µg/mL to 93% at 100 µg/mL. Fraction F4 demonstrated the most potent BChE inhibition, exceeding 70% inhibition even at the lowest tested concentration (10 µg/mL). However, all fractions displayed efficacy compared to physostigmine, the standard drug, which inhibited AChE by 93% and BChE by 96 % at 10 µg/mL.

Conclusion: The findings suggest that *Cannabis sativa* contains bioactive compounds capable of inhibiting both AChE and BChE, supporting its neuropharmacological potential. These results provide scientific validation for its traditional use and warrant further investigation into its isolated constituents for possible application in the management of neurodegenerative diseases.

Resurrection tea may have cardiometabolic beneficial effect through a potential inhibition of renin-angiotensin system, cholesterol synthesis, and regulation of sugar transport

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Introduction: Cardiovascular diseases (CVDs) are the leading cause of death globally. They are caused by multiple cardiometabolic risk factors including dysglycemia, dyslipidemia and hypertension. There are some sound claims of cardiometabolic benefits in some of herbal teas made from indigenous South African plants. In collaboration with indigenous communities, the African Medicines, Innovations and Technologies Development (AMITD) platform has engaged in a series of scientific research to develop commercial herbal teas with beneficial effects on the incidence of CVDs.

Aim: This study evaluated *in vitro* the inhibitory effects of Resurrection tea extract on renin-angiotensin system (RAS) enzymes, on hydroxymethylglutaryl- coenzyme A reductase (HMG-CoA), a blood cholesterol level regulator and on sugar transport.

Methods: Resurrection teabag was steeped in pre-boiled distilled water. The resulting filtrate was freeze-dried. The inhibitory action of the freeze-dried tea extract on angiotensin converting enzyme (ACE), renin enzyme and HMG-CoA reductase was assessed *in vitro*. Further, the effect of the tea extract on glucose and fructose transport regulation was assessed through a yeast cell sugar transport model at various concentrations of sugar.

Results: Resurrection tea showed a dose-dependent inhibition of ACE, renin and HMG-CoA reductase enzymes with IC_{50} values of 172.53± 20.43, 58.49 ± 1.36 and 58.22 ±6.18 µg/mL respectively. Yeast cells treated with tea extract showed significant reduction of glucose and fructose transport compared to the control cells. However, this effect of tea extract on sugar transport across yeast cells tended to be reduced when the sugar concentration was higher (20 mM).

Conclusion: This study provides some insights in the potential benefit of Resurrection tea on cardiometabolic risk factors prevention. However further pre-clinical and clinical studies are needed to validate this hypothesis.

Novel folic acid-functionalized smart liposomes for the targeted delivery of quercetin against cancer

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Aim: This study aimed to design, formulate, and evaluate the physicochemical properties of folic acid-functionalized eicosapentaenoic acid liposomes for the delivery of quercetin (FA-EPA-Lip-QUE) against cervical and liver cancerous cells.

Methodology: The liposomes were prepared using the thin-film hydration method and characterized for particle size, polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency, FTIR, and cryo-TEM. Quercetin release profiles *in vitro* was performed using simple dialysis bag method, and monitored over 96 hours using RT-HPLC. *In vitro* hemolysis assays for evaluation of the biocompatibility of the formulation was performed. Cytotoxicity was assessed using the MTT assay on healthy (HEK293) and cancerous cell lines (HeLa and HepG2), and *in vitro* apoptotic activity was also evaluated using the real time annexin V kit. Storage stability was examined at 4°C and 25°C over a period of 60 days.

Results: FA-EPA-Lip-QUE liposomes demonstrated a uniform spherical morphology with a particle size of 106.4 ± 0.458 nm, a PDI of 0.198 ± 0.006, and a zeta potential of -16.30 ± 0.600 mV. The encapsulation efficiency of quercetin was observed to be 92.69 ± 0.25%. The prepared liposomes exhibited a significantly higher quercetin release at pH 5.0, compared to pH 7.4, indicative of sustained and selective drug release in acidic tumour microenvironments. Hemolysis assay results showed FA-EPA-Lip-QUE to be non-hemolytic across all tested concentrations, supporting its safety for intravenous administration. Cytotoxicity studies showed that FA-EPA-Lip-QUE had significantly lower toxicity toward healthy (normal) HEK293 cells, while demonstrating potent cytotoxicity against HeLa and HepG2 cancer cells, with IC_{50} values of 3.76 µg/mL and 6.42 µg/mL, respectively. This cytotoxic effect was significantly higher than that of free QUE. Apoptosis was assessed for free QUE and FA- EPA-Lip-QUE using the RealTime-Glo Apoptosis and Necrosis Assay, revealing that FA-EPA-Lip- QUE induced significant apoptotic cell death while exhibiting minimal to no necrotic effects.

Conclusion: The prepared FA-EPA-Lip-QUE formulation showed enhanced efficacy, reduced toxicity, and achieved greater cancer cell death compared to free QUE. These findings underscore the potential of FA-EPA-Lip-QUE as an effective and safe targeted drug delivery system for QUE in cancer therapy.

Rituximab in Refractory Dermatomyositis: Clinical Insights from South Africa – A Case Series Study

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Introduction: Inflammatory myopathies such as dermatomyositis (DM) and juvenile dermatomyositis (JDM) are rare autoimmune conditions characterized by skin manifestations, proximal muscle weakness, and systemic involvement. These disorders are often challenging to manage, with variable responses to corticosteroids and immunosuppressants. Rituximab, a B-cell-depleting monoclonal antibody, has emerged as a promising therapeutic option in refractory cases, though evidence in low-resource settings remains limited.

Aims and Objectives: To evaluate the clinical effectiveness of rituximab in patients with DM unresponsive to conventional therapy.

Methodology: A retrospective case review was conducted at Inkosi Albert Luthuli Central Hospital (IALCH). Records of patients who received rituximab-based therapy on a named-patient basis were analyzed. Clinical and laboratory data were extracted from the MediTech™ system and reviewed in relation to patient admissions and disease course. Ethical approval was obtained from the University of KwaZulu-Natal and relevant authorities.

Results:

Case A: Juvenile dermatomyositis

A 16-year-old Grade 10 scholar, previously healthy, presented in 2021 with a hyperpigmented rash, quadriparesis, dysphagia, and systemic features consistent with juvenile dermatomyositis. She showed poor response to initial therapy, which included azathioprine 100 mg once daily, methotrexate 7.5 mg weekly, and prednisone 25 mg twice daily with adjunctive medications. Due to persistent symptoms, rituximab was initiated, and after three weekly doses of 500 mg, the patient demonstrated marked functional recovery. She regained the ability to walk, perform daily activities, and swallow safely. Laboratory markers also improved significantly, although anaemia and proximal muscle weakness persisted. She remains under ongoing management, with consideration of tacrolimus as an additional therapeutic option for sustained disease control.

Case B: Adult-onset dermatomyositis

A 43-year-old female with a history of systemic lupus erythematosus (SLE) and type 2 diabetes mellitus (T2DM) was diagnosed with adult-onset dermatomyositis. She was initially treated with prednisone 12.5 mg daily, later reduced to 5 mg daily following the initiation of rituximab, in combination with methotrexate 20 mg weekly and azathioprine 150 mg daily. Despite being refractory to standard immunosuppressive therapy, the introduction of rituximab resulted in marked improvement. She received four weekly doses of 500 mg in February 2019, followed by two additional cycles of 1 g each in August and September 2019. This regimen led to a significant reduction in creatine kinase (CK) levels, from 10,326 U/L at baseline to 238 U/L by 2021, accompanied by notable improvement in muscle strength. The patient remains clinically stable with reduced disease activity, although residual mild muscle weakness persists.

Discussion/Conclusion: Both cases highlight the challenge of managing corticosteroid- and immunosuppressant-refractory dermatomyositis, where rituximab was associated with meaningful biochemical and clinical improvement. The adolescent case demonstrated a rapid functional recovery following rituximab therapy, although some morbidity, including persistent weakness and anaemia, remained. In contrast, the adult case showed gradual stabilization, with a marked reduction in creatine kinase levels and sustained improvement in muscle strength over time. Despite these encouraging outcomes, shared challenges included delayed diagnosis, fluctuating disease activity, and the need for long-term management of residual weakness and disability. These findings support rituximab as a viable therapeutic option in refractory inflammatory myopathies, particularly in resource-limited settings. However, personalized dosing strategies and further studies are necessary to optimize long-term outcomes and to evaluate cost-effectiveness within African healthcare systems.

The *in vitro* investigation of SARS-CoV-2 main protease (M^{PRO}) exposure on glucose handling in skeletal muscle and liver cells

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Introduction: Although there is growing evidence suggesting a link between SARS-CoV-2 infection and the onset of diabetes mellitus, current scientific data remain insufficient to clearly define this relationship. The occurrence of multi-organ failure in COVID-19 patients emphasizes the need to better understand the cellular and molecular pathways through which SARS-CoV-2 maycontributetodiabetesdevelopment. Existing research points to a connection between obesity, inflammation, and physiological stress—all of which are known to contribute to insulin resistance and the eventual development of type 2 diabetes. Given that COVID-19 can trigger these factors, it is important to further investigate its long-term health consequences. Studies have also shown that SARS- CoV-2 can cause lasting damage to organs due to widespread inflammation throughout the body. As such, the pandemic's influence should be taken into account when assessing future trends in diabetes prevalence, especially considering that diabetes rates were already climbing before COVID-19 emerged. More research is needed to understand how SARS-CoV-2 impacts glucose regulation and insulin responsiveness. In light of this, the current in vitro study aims to investigate the possible link between SARS-CoV-2 and diabetes by examining how the virus's main protease (M^{pro}) affects insulin- responsive cells such as C2C12 and HepG2.

Methods: This investigation was a laboratory-based in vitro experimental study using C2C12 (skeletal muscle) and HepG2 (liver) cell lines. The study was structured into seven key parts. The first section assessed the impact of SARS-CoV-2 Main protease (M^{pro}) on the viability of both skeletal muscle and liver cells. The second part examined how Mpro influenced both basal and insulin-stimulated glucose uptake. In the third section, the study evaluated the expression and movement of Glucose Transporter Type 4 (GLUT4) in C2C12 cells to determine potential signs of insulin resistance. The fourth segment focused on analyzing the expression of Protein Kinase B (AKT) in both C2C12 and HepG2 cells. Following this, the fifth section explored the effects of Mpro on the expression of DPP4, MMP1, and IL-6 in both cell types, including measuring their levels in the surrounding cell culture medium. The sixth part investigated oxidative stress by measuring intracellular reactive oxygen species (ROS) and quantifying malondialdehyde (MDA) levels in the medium.

Results: The baseline and insulin-stimulated glucose uptake was impaired in both HepG2 and C2C12 cell lines. M^{pro} also compromised GLUT4 translocation and expression in the C2C12 cell line. Insulin-stimulated AKT was also significantly altered in the presence of M^{pro} for the HepG2 cell line. Cellular DPP4 and IL-6 expression was also affected by M^{pro} in both cell lines. An increase in ROS was observed in C2C12 cells, while MDA levels were elevated in both C2C12 and HepG2 cells, indicating increased oxidative stress.

Discussion and conclusion: These findings indicate that SARS-CoV-2 M^{pro} may be contributing to the development of insulin resistance, leading to impaired glucose regulation and potentially playing a role in the emergence of new-onset diabetes mellitus observed in individuals after COVID-19 infection. The disruptions noted in glucose uptake, GLUT4 movement, and inflammatory marker levels suggest that M^{pro} likely exerts its effects indirectly by interfering with key intracellular signaling pathways involved in insulin action rather than through direct interaction with insulin. This idea is reinforced by the weak binding observed in the solid-phase assay and the high binding energy seen in molecular docking simulations. However, more research is needed to fully understand the mechanisms linking SARS-CoV-2 infection to diabetes onset, as current evidence is not sufficient to confirm M^{pro} as the sole causative factor.

SARS-CoV-2 main protease induced glucose handling impairments in skeletal muscle and hepatic cells *in vitro*

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Introduction: Since COVID-19 is known to induce these conditions, further investigation is necessary to fully understand its long-term effects on human health. Moreover, research indicates that SARS- CoV-2 infection is associated with persistent damage to organ systems due to the systemic inflammatory response. Consequently, it is essential to consider the effect of the COVID-19 pandemic when predicting the prevalence of diabetes mellitus in the future, especially since the incidence of diabetes mellitus was already on the rise before the pandemic. Additional research is required to fully comprehend the impact of SARS-CoV-2 infection on glucose tolerance and insulin sensitivity. The study aimed to explore the perceived relationship between SARS- CoV-2 and diabetes by utilizing the SARS-CoV-2 Main protease (M^{pro}) to investigate its effects of key insulin- sensitive cells, including C2C12 and HepG2. The aim is to uncover specific molecular mechanisms that may contribute to the development of the new- onset diabetes mellitus observed in patients post- COVID-19.

Methodology: This study was an *in vitro* laboratory-based experimental investigation that utilized C2C12 (skeletal muscles) and HepG2 (liver) cell lines. This study was divided into seven distinct sections. The first part focused on examining how the viability of skeletal muscle and liver cell lines will be impacted by the exposure of SARS-CoV-2 M^{pro}. In the second section, the study investigated how M^{pro} affected the baseline glucose uptake and insulin-stimulated glucose uptake. The study further explored the expression and translocation of Glucose transporter type 4 (GLUT4) in the C2C12 cell line therefore assessing any potential insulin resistance. Next, the study focused on the expression of Protein Kinase-B (AKT) in HepG2 and C2C12 cell lines. Thereafter, the study investigated the effect of M^{pro} on DPP4, MMP1 and IL-6 in C2C12 and HepG2 cells, including an assessment of the level of these proteins in cell culture medium. The sixth part of this study examined the reactive oxygen species (ROS) through the quantification of medium malonaldehyde (MDA) and an assessment of intracellular ROS. A solid-phase assay was conducted to assess any potential binding between M^{pro} and insulin.

Results: The baseline and insulin-stimulated glucose uptake was impaired in both HepG2 and C2C12 cell lines. M^{pro} also compromised GLUT4 translocation and expression in the C2C12 cell line. Insulin-stimulated AKT was also significantly altered in the presence of M^{pro} for the HepG2 cell line. Cellular DPP4 and IL-6 expression was also affected by M^{pro} in both cell lines. An increase in ROS was observed in C2C12 cells, while MDA levels were elevated in both C2C12 and HepG2 cells, indicating increased oxidative stress.

Conclusion: These observations suggest that the SARS-CoV-2 M^{pro} may be inducing an insulin-resistant state, contributing to the dysregulation of glucose metabolism and therefore potentially being one of the contributors of new-onset diabetes mellitus seen in patients post-COVID-19.

Mitochondria-mediated apoptotic effects of *aloe vera* methanol leaf extract and its fractions in colorectal cancer *in vitro*

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Introduction: Colorectal cancer (CRC) remains a major global health concern, with rising incidence and limited effectiveness of conventional treatments due to adverse side effects. This has led to growing interest in medicinal plants like *Aloe vera*, which is rich in bioactive compounds and shows promise as a safer, more targeted anticancer agent. Its traditional use in treating gastrointestinal symptoms commonly linked to CRC further highlights its potential role in colorectal health and therapy.

Aim: The study aims to elucidate the potential mechanisms by which *A. vera* methanol leaf extract and its fractions could induce apoptosis in HT29 and HCT15 in CRC cell lines.

Methods: The cytotoxic effects of *A. vera* methanol leaf extract and its fractions, namely the hexane fraction (AHL), butanol fraction (ABL), and aqueous fraction (AAL), 5-fluorouracil 5FU (control), were evaluated on normal human skin fibroblasts (HS27). Antiproliferative activity was assessed on CRC cell lines HT29 and HCT15 using the MTT assay. To further investigate cell death mechanisms, HT29 and HCT15 cells treated with active fractions were analysed for cell cycle arrest, mitochondrial membrane potential loss, and reactive oxygen species (ROS) generation.

Results: *A. vera* extract, along with the ABL and AAL fractions, exhibited minimal cytotoxicity (IC_{50} >200 µg/mL) towards normal HS27 cells. The AHL fraction was more selective (SI: 0.9) towards the normal cells compared to cancer cells. Both the crude extract & AHL fraction displayed moderate antiproliferative activity against the HT29 CRC cell line, with IC_{50} values of 54.6 µg/mL and 55.5 µg/mL, respectively. In the more resistant HCT15 cell line, only the AHL fraction showed notable inhibitory effects, achieving an IC_{50} of 72.6 µg/mL. Mechanistic studies indicated that both the crude extract and the AHL fraction induced apoptosis primarily through the mitochondria-mediated pathway, evidenced by increased ROS generation, disruption of mitochondrial membrane potential, and elevated apoptotic cell populations.

Conclusion: The *A. vera* crude extract and AHL fraction demonstrate promising anticancer activity against CRC cell lines while exhibiting selectivity toward non-cancerous cells. The AHL fraction, in particular, shows potential as a candidate for further development in colorectal cancer therapy, warranting careful dose optimization to minimize potential toxicity.

Nanohydrogel of curcumin/berberine co-crystals induces apoptosis via dual covalent/noncovalent inhibition of caspases in endometrial cancer cell lines: The synergy between pharmacokinetics and pharmacodynamics

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Background: Endometrial cancer (EC) poses a significant therapeutic challenge owing to tumor heterogeneity and resistance to standard treatments. Caspase-3, a central mediator of apoptosis, is a promising target for therapeutic intervention. However, the clinical translation of natural compounds, such as curcumin (CUR) and berberine (BBR), is limited by their poor solubility and bioavailability.

Objective: This study aimed to develop a curcumin/berberine (CUR-BBR) co-crystal-loaded chitosan nanohydrogel (CUR-BBR/CSNH) that enhances drug solubility and delivery and facilitates dual-site inhibition of caspase-3 to induce apoptosis in EC cells.

Methods: CUR-BBR co-crystals were synthesized by liquid-assisted grinding and characterized by PXRD, NMR, FTIR, SEM, TEM, DSC, and TGA. They were then encapsulated in a chitosan nanohydrogel. The physicochemical properties of the nanoparticles were evaluated using DLS and drug release assays at pH 7.4 and 4.5. Cytotoxicity and caspase-3/7 activation were assessed in HEC-59 endometrial cancer cells. Covalent and noncovalent docking, molecular dynamics simulations, and MM/GBSA binding energy analyses were performed to validate the dual-targeting mechanisms.

Results: CUR-BBR co-crystals exhibited >170-fold improved CUR solubility. The CUR-BBR/CSNH system demonstrated high encapsulation efficiency (99.68%), pH-responsive sustained release, and potent cytotoxicity (ICso = 12.36 µg/mL), outperforming free drugs, co-crystals, and camptothecin. Computational results confirmed CUR's covalent binding to caspase-3's active site and BBR's noncovalent allosteric inhibition, with synergistic binding energies and protein conformational stability.

Conclusion: This study introduces a novel nanohydrogel-based delivery system that leverages dual-site caspase-3 inhibition. The CUR-BBR/CSNH formulation offers a promising platform for overcoming therapeutic resistance in EC via the synergistic enhancement of both pharmacodynamics and pharmacokinetics.

Evaluation of anti-cancer properties of the *Schinus molle* fruit isolates

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Introduction and aim: Cervical cancer remains a major cause of morbidity and mortality, particularly in South Africa. There is an urgent need for novel, plant-based therapeutics with minimal side effects. *Schinus molle* L. (peppertree) is a medicinal plant traditionally used across Africa and South America to treat a range of ailments. Its reported content of flavonoids, phenolic acids, and essential oils suggests potential anti-cancer activity, though scientific evaluation remains limited. This study aimed to evaluate the cytotoxic properties of *Schinus molle* fruit extracts, with particular focus on their effects against ME-180 cervical cancer cells.

Methods: The fruits of *Schinus molle* were dried, powdered, and subjected to serial exhaustive extraction using hot water and four organic solvents of varying polarity in a shaker incubator. Phytochemical screening was performed using thin layer chromatography. Cytotoxicity was determined using the MTT assay on ME-180 cervical cancer cells and MRC-5 normal human fibroblasts.

Results: Cytotoxicity studies using the MTT assay revealed dose-dependent inhibition of ME-180 cervical cancer cell proliferation, with IC_{50} values ranging above 70 ug/ml. These values are consistent with preliminary analysis. Importantly, the extracts were also tested on MRC-5 normal human fibroblast cells and exhibited no significant toxicity, demonstrating their safety for normal cells.

Discussion and Conclusion: Although the crude extracts of *Schinus molle* demonstrated mild cytotoxicity against cervical cancer cells, they still support the plant's traditional medicinal use. These findings contribute to the early-stage exploration of *S. molle* as a potential source of bioactive compounds for cervical cancer research, warranting further studies involving compound isolation and mechanism-specific assays.

Deciphering the cytotoxicity, antidiabetic and antihypertensive bioactivities of *Tephrosia capensis* root extract through *in vitro* and in silico investigative models

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Introduction & Aim(s) of the Study: Tephrosia capensis, one species of the Tephrosia genus native to Southern Africa, has been reportedly employed in traditional medicine to manage certain ailing conditions in the African region. This study aimed to determine antidiabetic and antihypertensive potentials of the plant's root via in vitro investigations while also using computer-aided simulation studies of its phytoconstituents with relevant proteins.

Methods: Dried root extract of the plant from 70% ethanol extraction was subjected to LC-MS and GC-MS chemical analysis. The effect of the root extract on □-amylase, □-glucosidase, ACE, renin, DPP-4, and HMG-CoA reductase activities was determined using established assay protocols. The cytotoxic and protein glycation inhibitory capacities of the extract were also investigated. The pharmacokinetic properties of compounds in the extract were predicted using the Swiss model online tools, while their molecular affinities with the assayed proteins were computed via molecular docking analysis.

Results: The root extract competes favourably with acarbose and aliskiren in inhibiting □-glucosidase and renin enzymes, respectively. Though it has lower activities than the tested standard drugs, it displayed concentration-dependent inhibition of DPP-4, ACE and HMG-CoA reductase enzymes. The root sample significantly reduced protein glycation and lowered 3T3 adipocyte cell viability at the 5-160 µg/mL test concentrations. Compounds including semiglabrin, tephrorianin, and lupeol detected in the extract showed strong affinities with the various protein targets. Though the pharmacokinetic data revealed that these compounds have high gastrointestinal absorption, some were predicted to inhibit CYP3A4.

Discussion and Conclusion: This study presents, for the first time, pharmacological evidence to support the traditional use of Tephrosia capensis as a medicinal plant. There is a need to investigate further the plant's toxicity based on its observed effect on the normal adipocytes.

Comparative efficacy of fractionated Cape Cobra (*Naja nivea*) venom on breast and cervical cancer cell lines

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Introduction: Cancer remains a major global cause of mortality, with chemotherapy being the most widely used treatment. However, its lack of specificity and severe side effects highlight the need for more targeted therapies. Snake venom contains bioactive components, such as proteins, enzymes and peptides, that have shown selective cytotoxicity toward cancer cells. Some venom-derived compounds have already been FDA approved for treating conditions, like hypertension.

Aims and Objectives: This study investigates the therapeutic potential of fractionated Cape Cobra (*Naja nivea*) venom against breast and cervical cancer cell lines.

Methods: High resolution-liquid chromatography-mass spectrometry (HR-LC-MS/MS) was used to identify protein composition of the venom fractions. Six fractions were initially screened for cytotoxic effects, thereafter the two most potent fractions were selected for further evaluation. These were tested on estrogen receptor positive breast (MCF- 7 and T47D) and HPV-16 cervical (CaSki and SiHa) cancer cell lines and non-malignant epithelial cells (FG0 and ARPE-19) using the *in vitro* cell viability MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. The study calculated IC_{50} values and Selectivity Indices (SI) to evaluate potency and selectivity. The long-term effects were evaluated on cell lines that were successfully established in the short-term assays, using clonogenic and migration assays. Changes in EMT markers (□-catenin, □-actin, Vimentin, E-cadherin) were analysed through western blotting. High-performance liquid chromatography (HPLC) was used to assess the long-term stability of the venom fractions.

Results: Venom fractions 5 and 6 demonstrated the highest efficacy and moderate selectivity, significantly reducing short-term viability in breast (MCF7 and T47D) and cervical cancer (CaSki and SiHa) cell lines with favourable selectivity indices. MCF7 and SiHa cells treated with increasing concentrations of the fractions (1/2 IC_{50}, IC_{50} and 2X IC_{50}), demonstrated a dose-dependent reduction in colony formation compared to vehicle control. In contrast, the effect of snake venom fractions 5 and 6 effects on non-malignant cell lines (FG0 and ARPE-19) were minimal, with cell survival rates remaining above 70%. HR-LC-MS/MS identified that fraction 5 contained Cobra venom factor and Cytotoxin 5 while fraction 6 contained Cytotoxin 1a.

Discussion/Conclusion: These findings suggest that Cape cobra venom fractions exhibit promising anti-cancer activity, with selective effects on breast and cervical cancer cell lines

Proteomic characterisation of processed extracellular matrix scaffolds for wound healing

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Background: Secondary intent wounds, such as burns or ulcers, are challenging to treat due to significant tissue loss and prolonged healing times.[1,2] The standard treatments, autografts or allografts, are often limited by patient conditions, skin availability, and immune rejection.[2,3] Acellular dermal scaffolds (ADS) offer an alternative by using donor or animal skin cells to reduce rejection risks.[4] This study investigated the effects of an ADS combined with autologous platelet-rich plasma (PRP) on wound healing in a porcine model.

Methods: An optimized ADS was developed using detergent and supercritical carbon dioxide extraction and implanted into full-thickness porcine wounds. Three treatments were tested: ADS only, ADS with PRP, and a standard treatment control. Biopsies were taken on days 3, 5, 8, 10, 12, and 16 post-wounding for histological and protein activity analysis using immunohistochemistry (IHC) and mass spectrometry-based proteomics.

Results: Histological analysis revealed that wounds treated with ADS showed faster healing than the negative control, with quicker cellular infiltration and granulation tissue formation. By day 16, wounds treated with ADS and the ADS-PRP combination were mostly healed, resembling healthy tissue. Mass spectrometry-based proteomic analysis revealed that both the ADS-only and the ADS-PRP treatment groups upregulated biological pathways linked to ECM remodelling and inflammatory regulation compared to the control. The ADS-only and ADS-PRP treatments progressed at an accelerated rate compared to the standard treatment control.

Discussion and Conclusion: The advancement of healing with the combination treatment aligns with studies showing PRP promotes wound healing by enhancing re-epithelialisation, angiogenesis, wound contraction and collagen arrangement.[5] ADS treatment alone improved healing faster than control but slower than combination treatment. This supports the practice of “filling the gap” in secondary intent wounds.[6,7] This study confirmed an optimised ADS production method and showed that ADS with PRP enhanced wound healing. The combination of ADS and PRP treatment offers promising regenerative therapy for secondary intent wounds.

Geographical influence on the antioxidant and antibacterial activity of *Peltophorum africanum*

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Background: *Peltophorum africanum* is widely used in African traditional medicine. Its therapeutic potential may be affected by geographical factors influencing phytochemical composition.

Methods: Leaf extracts from Limpopo (LP) and Mpumalanga (MP) were screened for antioxidant activity (dot plot, TLC bioautography, DPPH, hydrogen peroxide scavenging, reducing power) and antibacterial activity against *Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Enterococcus faecalis* (disc diffusion, TLC bioautography, MIC).

Results: Antioxidant profiling revealed MP samples with more distinct active bands and generally lower IC_{50} values. LP crude extracts showed stronger overall antibacterial inhibition, while MP contained more unique antibacterial-active compounds. MIC analysis indicated LP extracts were more effective against *A. baumannii* and *S. aureus* in crude form, whereas MP had unique inhibition patterns against *E. coli* and *S. aureus*.

Conclusion: Geographical origin markedly influences *P. africanum*'s bioactivity. LP plants appear better suited for direct use as crude antibacterial agents, while MP plants show greater potential for isolating novel bioactive compounds for pharmaceutical development.

Breastmilk tenofovir levels and breastmilk macronutrient–growth relationships in HIV-exposed uninfected infants: Insights from the UmbiBaby cohort

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Introduction & Aim(s) of the Study: In South Africa, where ~25% of women of reproductive age are living with HIV, HIV-exposed-uninfected (HEU) infants remain at risk for growth faltering despite maternal antiretroviral therapy (ART). To better understand the pharmacological underpinnings of maternal– infant drug exposure in African contexts, this study assessed the detectability of tenofovir disoproxil fumarate (TDF) in breastmilk. It also compared breastmilk macronutrient composition between women living with HIV (WLHIV) on TDF-based ART and HIV-uninfected mothers, and examined the growth in HEU infants compared to HIV-unexposed-uninfected counterparts.

Methods: Data and breastmilk samples from the published UmbiBaby prospective cohort (Tshwane District) were analysed, including 90 mother–infant pairs, of whom 25 were WLHIV on TDF-based ART and 65 were HIV-uninfected controls. Breastmilk collected at multiple lactation stages was tested for TDF, tenofovir (TFV), and tenofovir-diphosphate (TFV-DP) via LC–MS/MS. Breastmilk macronutrient content (protein, fat, carbohydrate, energy) was measured using the Miris Human Milk Analyzer. Infant growth was analysed from 6 weeks to 24 months using WHO weight-for-age (WAZ) and length-for-age (LAZ), weight-for-length and body mass index-for-age z-scores.

Results: The prodrug TDF and parent drug TFV were detectable in several breastmilk samples; the intracellular active metabolite TFV-DP was not detected. Compared to breastmilk from HIV-uninfected mothers, milk from WLHIV had significantly higher protein levels at 6 weeks and 18 months, and higher fat, energy, and carbohydrate levels at 12 and 18 months (all $p<0.05$). Despite this, HEU infants, exposed to both HIV and ART, had significantly lower WAZ at 6 and 14 weeks and lower LAZ at 6 and 14 weeks, and 9 and 24 months (all $p<0.05$).

Conclusion: Tenofovir was detectable in breastmilk of women on TDF-based ART. Elevated breastmilk macronutrient levels did not prevent early and sustained growth deficits in HEU infants, highlighting the need for further pharmacokinetic–nutritional research in this population.

Antibiotic Prescribing Patterns in Patients with Respiratory Tract Infections at Berea and Motebang Hospitals, the Kingdom of Lesotho

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Background: The emergence of resistant pathogens is a global threat to the healthcare system. Availability and access to scientific evidence regarding utilisation patterns of antimicrobials is essential for the implementation of robust antimicrobial stewardship programmes and to effectively combat antimicrobial resistance. However, there is insufficient scientific knowledge regarding prescribing trends of systemic antibiotics for the treatment of respiratory tract infections in the Kingdom of Lesotho.

Aims and Objectives: The study investigated the prescribing trends of systemic antibiotics used to treat respiratory tract infections at Berea and Motebang hospitals, the Kingdom of Lesotho.

Methods: This was a cross-sectional and retrospective audit of medical records of patients diagnosed with and treated for respiratory infections at Berea and Motebang Hospitals between January 2015 and December 2024.
The data extracted included demographics, diagnosis, antibiotic prescriptions, comorbidity, and guidelines. Descriptive statistical analysis was performed with SPSS (IBM) version 20. The study protocol received full ethics approval from the UKZN ethics committee (Ref. No.: BREC6827/2024) and the Lesotho Ministry of Health Research and Ethics Committee (Ref. No.: ID 195-2024).

Results: At Berea Hospital, there were 53% (n = 783) males and 47% (n = 681) females % while in the Motebang government hospital, there were 55% (n = 775) males and 45% (n = 640) females, who met the inclusion criteria of this study. A total of 2 458 systemic antibiotics were prescribed for respiratory infections at Berea Hospital medical wards during the period of this study. The mostly prescribed (33.2%, n = 816) antibiotics were the third-generation cephalosporins; followed by the penicillins (26.6%, n = 654), macrolides (17.5%, n = 430), sulphonamide and trimethoprim (11.3%, n = 278), and aminoglycoside (6.3%, n = 154). Fluoroquinolones were prescribed less frequently (0.1%, n = 2). At Motebang Hospital, a total of 2 383 antibiotics and mostly prescribed (44.2%, n = 1 054) antibiotics were penicillins, followed by the third-generation cephalosporins (19.1%, n = 455), aminoglycosides, (13.2 %, n = 314), sulphonamides (12.2 %, n = 291) and macrolides (7.8%, n = 185). Imidazole derivatives (2.8%, n = 67) and fluoroquinolones (0.6%, n = 15) were prescribed less frequently.

Conclusion: The findings of this study suggest over-utilisation of at Berea and Motebang public hospitals in the Kingdom of Lesotho. Therefore, there is a need for a well-coordinated antimicrobial stewardship programme in the Kingdom of Lesotho to combat the excessive utilisation of antibiotics and to prevent the emergence of resistant pathogens.

From molecule to medicine use: Older people and pharmacists' perspectives on the barriers and facilitators to pharmacological effectiveness through medicine adherence in Makhanda, Eastern Cape, South Africa

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Introduction and Aim of the Study: Pharmacological efficacy depends not only on the molecular properties of medicines but also on how they are accessed, understood, and used in daily life. In the South African health system, chronic disease management increasingly relies on long-term pharmacotherapy. However, social, economic, and systemic factors can limit medicine adherence and reduce the real- world impact of pharmacological interventions. This study explored the perspectives of older people and pharmacists in Makhanda, Eastern Cape, focusing on the barriers and facilitators that affect medicine adherence in the management of chronic conditions.

Methods: This qualitative study formed part of a broader investigation into community-based approaches to medicine access and use among older populations. Four focus group discussions were conducted with older people aged 60 and above from both public and private healthcare sectors, recruited from health facilities in Makhanda. Participants were purposively selected based on their use of chronic medication. Following this Pharmacists across the Eastern Cape were interviewed telephonically to offer an overview of the challenges older people face and the strategies employed to improve adherence. Data were audio-recorded, transcribed verbatim, and analysed thematically using the World Health Organization's five-dimensional framework of medicine adherence: health system-related, therapy-related, patient-related, social/economic-related, and condition-related factors.

Results: Participants across both public and private sectors were managing multiple chronic conditions such as hypertension, diabetes, asthma, and HIV, often requiring complex daily regimens. While many expressed trust in their healthcare providers specifically (pharmacists and doctors), several barriers to adherence were reported. These included long clinic waiting times, communication gaps, adverse side effects, low health literacy, polypharmacy, and forgetfulness, particularly among those living alone. A lack of food was frequently cited as a reason for missed or delayed doses, affecting the effectiveness of treatment.

Facilitators of adherence included pharmacist support through clear instructions, counselling, and use of adherence aids such as pill boxes and blister packaging. Adherence tools such as pillboxes and alarms were helpful where available, although not universally accessible. Family caregivers were reported essential in reminding patients to take medication, helping in preparing meals, and offering emotional support. Community support and routine-based strategies, for example, placing pills near water or using alarms, also helped. However, access to these tools was not universal. Pharmacists reported that non-adherence was often hidden, with patients stockpiling unused medication or taking doses inconsistently. They emphasised the value of proactive counselling, medication reviews, and collaboration with prescribers to address financial and regimen-related challenges. Medicine adherence was shaped by a complex interplay of individual, social, and systemic factors, with pharmacist and caregiver involvement emerging as key enablers.

Discussion: In both public and private sectors, adherence was undermined by many factors; however, public sector patients were disproportionately affected by structural and socioeconomic barriers such as long clinic queues, inconsistent health care provider communication, and food insecurity directly compromising drug absorption and therapeutic outcomes. In contrast, private sector patients experienced fewer access barriers but were more likely to report intentional non-adherence, driven by side effects, scepticism, or preference for alternative therapies. This highlights that resource availability alone does not guarantee adherence, especially when beliefs and understanding of treatment are lacking. Pharmacists emerged as pivotal actors in promoting pharmacological effectiveness. Their role extended beyond dispensing to include patient education, regimen simplification, identification of non-adherence, and collaboration with prescribers. Importantly, pharmacists in both sectors reported that non-adherence often remains hidden, with patients continuing to collect but not consume medication. This reflects a critical gap between prescribing and use, where clinical intent fails to translate into therapeutic effect. From a systems perspective, the pharmacist's accessibility and continuity with patients position them as key enablers of adherence. Yet, their potential is constrained by inadequate resources, lack of integration into multidisciplinary teams, and limited service coverage, particularly in the public sector. The transition "from molecule to medicine use" is shaped not only by drug properties but also by human behaviour, health system functionality, social conditions, and therapy-related factors. Addressing medicine adherence among older people thus requires a holistic, patient-centred approach, one in which pharmacists play a central, yet currently underutilized, role in closing the gap between treatment and health outcome.

Conclusion: These findings highlight the need to contextualize pharmacology within the lived experiences of patients. Medicines may be pharmacologically sound, but their real-world effectiveness depends on a web of social, economic, and relational factors. Strengthening pharmacy services, supporting caregiver involvement, and addressing structural barriers such as food insecurity are vital to underpinning pharmacological success in South Africa.

Effect of oral hypoglycemic drugs on arterial elasticity and growth factors in black type 2 diabetes mellitus patients

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Background: In type 2 diabetes mellitus (T2DM) and atherosclerosis, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-□1) are reported as the chief positive regulators of angiogenesis. VEGF and TGF-□1 are also the key proangiogenic factor activating phossphati- dylinositol-3-kinase/Akt and thus, responsible for cell migration, survival and proliferation. Akt has been shown to affect the long-term regulation of vessel growth through phosphorylation of endothelial nitric oxide synthase, resulting in activation of persistent calcium-independent enzyme and nitric oxide synthesis. In T2DM patients angiogenic impulse is ischaemia leading to narrowed vessels because of atherosclerotic plaque.

Aim: To investigate the effects of an eight-month treatment with metformin alone and in combination with glimepiride on arterial elasticity, VEGF and TGF-□1 in black type 2 diabetes mellitus patients.

Methods: The AtCor SphygmoCor® system (AtCor Medical, Inc., Sydney, Australia) was used to measure PWV and Aix. VEGF and TGF-□1 levels were measured using Multiplexing with Bio-Plex Pro™ human inflammation panel I assay. Type 2 diabetic treatment naïve participants were divided into two groups: metformin (M) (*n* = 10) and metformin glimepiride (MS) (*n* = 14). After treatment initiation study participants were followed up at 4 and 8 months.

Results: The results of the study show no significant changes in plasma levels of VEGF and TGF-□1 after 8-month treatment period. The results also show no significant changes in PWV and Aix. PWV was directly correlated to VEGF (r^2=0.15558; P=0.0525). There was no correlation between Aix and VEGF (r^2=0.05326; P=0.5090 and TGF-1□ (r^2=0.01872; P=0.8166).

Conclusion: Metformin alone and in combination with glimepiride showed no significant changes in growth factors, PWV and Aix over the period of eight months treatment. There was positive correlation between PWV and VEGF levels indicating possible improvement of arterial elasticity with longer-term T2DM treatment in black participants.

The impact of knowledge and attitudes of healthcare providers on antibiotic stewardship practices in selected public hospitals in Lesotho.

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Introduction: Antibiotic resistance is a global health crisis mainly associated with the misuse and overuse of antibiotics. The highest burden of antibiotic resistance is observed in low- and middle-income countries where antibiotic stewardship programmes are still in the early phases of implementation.

Aims: This study aimed to assess the impact of knowledge and attitudes of healthcare providers on antibiotic stewardship practices in selected public hospitals in Lesotho.

Methods: A descriptive cross-sectional study was conducted among 59 healthcare providers in four purposively selected hospitals in Lesotho. Data was collected using a questionnaire between November 2023 and February 2024. Data was analysed using Statistical Package for Social Sciences Version 29. Categorical variables were summarised using proportions. The chi-square ($\square^2$) test with a significance level of $p < 0.05$ was used to explore antibiotic stewardship attitudes across the four hospitals. Spearman's rho was used to assess the correlation between knowledge, attitudes, and practice scores.

Results: The majority of participants believed that Antibiotic Resistance was important to influence antibiotic choice. Although the participants agreed that antibiotic resistance was a problem in Lesotho (n=36; 61.0%), in their hospitals (n=37; 62.7%) and their ward/unit (n=33; 5%), almost half reported not knowing the level of resistance to commonly used first line antibiotics (n=26; 44.1%) The overall attitude and knowledge scores were high (n=50; 84.7) and (n=41; 69.5%) while practice scores were generally fair (n=29;49.2%). Participants with higher attitudes and knowledge scores were more likely to have high practice scores.

Discussions: Healthcare providers in the study sites had good knowledge, attitudes and perceptions regarding antibiotic stewardship and resistance, although they had inadequate practice scores. The gaps in their practices may be addressed through the provision of additional education, as expressed by participants. Strict implementation of antibiotic stewardship guidelines to improve rational antibiotic use is recommended.

Potential antihypertensive effect of *Asystasia gangentica* (L.) leaves extracts targeting the renin–angiotensin system

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Background: Hypertension is a fast-growing health concern, especially in low- and middle-income countries like South Africa. The inhibition of renin-angiotensin-aldosterone system is an important therapeutic target for a new drug development. *A. gangetica*, a traditional medicinal plant shows promise for reducing blood pressure, but further research is needed to support this health claim.

Aim: This study aimed to investigate *in vitro* the angiotensin converting enzyme (ACE) and renin enzyme inhibitory effects of *A. gangentica* (L.) leaf extracts.

Methodology: *A. gagentica* (L) leaf extracts were prepared by sequential extraction using hexane, DCM, DCM/methanol, methanol and water. Cytotoxicity of the extracts was tested on *Vero* cells using the MTT assay. The anti-hypertensive potential of the plant extracts were investigated *in vitro* through angiotensin-converting enzyme (ACE) and renin enzyme inhibition assays.

Results: The *A. gangetica* leaf extracts exhibited no cytotoxicity to *Vero* cells by maintaining a cell viability above 50% at 200 µg/mL of extracts. The extracts exhibited concentration-dependent increase in the inhibition of ACE and renin enzyme activity. DCM and DCM/MeOH extracts demonstrated the strongest ACE inhibitory activity with IC_{50} values of 53.12 ± 4.74 µg/mL and 58.00 ± 2.70 µg/mL respectively. MeOH, DCM/MeOH extracts showed the strongest renin inhibitory activity with IC_{50} values of 24.31 ± 2.80 µg/mL and 29.91 ± 2.45 µg/mL respectively. Microsoft Excel was used to determine the IC_{50}.

Conclusion: This study provides some scientific evidence of antihypertensive potential of *A. gangentica* (L.) leaf extracts by inhibiting ACE and renin enzymes. However, further investigations are needed in different cell models to strengthen these findings.

The effect of mitotoxicants exposure on cellular and media dipeptidyl peptidase 4 concentrations in hepatic (HEPG-2) cell line

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Introduction: Dipeptidyl peptidase (DPP4) is a multifunction protein discovered in 1966. The pleiotropic roles can be attributed but not limited to the following: interaction with the extracellular matrix, association with adenosine deaminase, modulation of intracellular signalling pathways, acting as a coreceptor for viral entry and the protease activity. Literature evidence has suggested that DPP4 expression and shedding is increased by several stimuli including inflammation, hypoxic conditions, and hyperglycaemia. In this study, we sought to examine the effect of drug-induced mitochondrial dysfunction on the the expression and shedding of DPP4.

Aims and objectives: The aim of the study was to investigate the effect of mitotoxicants exposure on cellular DPP4 expression and media concentration in hepatic (HEPG-2) cell lines. The objectives of the study were to assess the effect of mitotoxicants (rotenone, antimycin, simvastatin, tenofovir) on cell viability and cellular expression and media concentrations of DPP4, interleukin-1, metalloproteinase-1 and tafazzin.

Methods: Hepatic (HEPG-2) were seeded in 96 wells plates until ready for experimentation. The cells were exposed to selected mitotoxicants (rotenone, antimycin, simvastatin and tenofovir) at 25, 50 and 100µg/ml for 24 hours. Therefter, the cytotoxicity studies were performed. In addition, the cellular expression, and media concentration DPP4, metalloproteinase 1 (MMP-1), interleukin (IL-6) and taffazin (TAZ) were assessed using ELISA technique.

Results: The concentrations of agents used in this study were not cytotoxic as confirmed by cell viability percentage similar to the untreated cells. The mitochondrial disturbance was confirmed by a reduction in TAZ cellular concentrations. In HEG-2 cells in particular, simvastatin induced an increase in the expression of DPP4, whilst all other agents demonstrated a decrease in DPP4 expression. Interestingly, for media analysis, only rotenone demonstrated a significant increase in media DPP4 concentration, suggesting an increase in DPP4 shedding. We further examined the association of this observations with inflammation through looking at IL-6 and MMP-1 concentrations. No effect was demonstrated on cellular MMP-1 whilst a slight increase was observed in media MMP-1 concentrations. Furthermore, the exposure to mitotoxicants demonstrated a reduction in cellular IL-6 concentrations, however an increase in media IL-6 concentrations was observed.

Conclusion: In conclusion, the study highlights the possibility of an increase expression and shedding of DPP4 through the exposure to mitotoxicants agents in particular rotenone and simvastatin. The elevated media MMP-1 and IL-6 could further support the role of the inflammation on DPP4 shedding. Considering the involvement of DPP4 shedding in several pathologies, further studies which explore the effect of various stimuli on DPP4 expression and shedding should be encouraged.

Computational evidence of melamine and its analogues binding to angiotensin receptors: Insights from molecular docking and dynamics simulations

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Background and Aim: Melamine and its analogues are documented adulterants in commercial protein supplements and are linked to nephrotoxicity due to renal accumulation. Angiotensin receptors, abundant in the kidneys, regulate blood pressure and fluid balance via the renin–angiotensin system. However, their potential interaction with melamine and related compounds has not been investigated. This study evaluated the binding interactions between melamine, its analogues, and angiotensin receptors using in silico molecular docking and molecular dynamics (MD) simulations.

Methods: The Schrödinger Release 2023-2 docking suite (Maestro version 13.6.122; Schrödinger, LLC, New York, USA) was used for all analyses. Crystal structures of angiotensin II type 1 receptor (AT1R) and type 2 receptor (AT2R) were retrieved from the Protein Data Bank and prepared for docking studies. Test ligands included melamine, cyanuric acid, and melamine-cyanurate, ~~while~~ angiotensin II, losartan, and PD123319 served as controls.

Results: Docking studies revealed that melamine and its analogues had docking scores comparable to the control compounds, which are established modulators of AT1R and AT2R. Melamine cyanurate demonstrated the highest predicted binding affinity for both receptors, with a docking score of approximately –37.3 kcal/mol. MD simulations confirmed the stability of these ligand–receptor complexes, showing consistent hydrogen bonding, ionic interactions, and hydrophobic contacts with key catalytic residues.

Conclusion: These findings suggest that melamine and its analogues may modulate angiotensin receptor function, potentially contributing to their nephrotoxic effects. Further in vitro studies are required to determine the biological implications of these interactions.

In vitro evaluation of anti-diabetic potential of *Helianthus tuberosus* L. (*Jerusalem artichoke*) plant extracts

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Introduction and Aim of the study: Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia due to insulin resistance or impaired insulin secretion. Existing antidiabetic medications often have gastrointestinal side effects and limited accessibility, highlighting the need for safer, plant-based alternatives (Zhao et., 2023). *Helianthus tuberosus* L. is a tuber rich in inulin, phenolic acids, and flavonoids, which have demonstrated promising antidiabetic effects in preliminary studies (Mariadoss et al., 2021). This study aimed to evaluate the *in vitro* antidiabetic activity of *H. tuberosus* extracts.

Methods: Extracts of *H. tuberosus* were sequentially prepared using solvents of varying polarity. The inhibitory effects of the extracts were evaluated using *in vitro* □-amylase and □-glucosidase assays (Mokhele et al., 2020) and the activity was quantified spectrophotometrically, and percentage inhibition was calculated relative to controls.

Results: The dichloromethane (DCM) extract showed the most potent □-amylase inhibitory activity closely matching the standard drug acarbose. Ethyl acetate and hexane extracts exhibited moderate to low activity. Acarbose demonstrated the highest potency against □-glucosidase activity, followed by the DCM and ethyl acetate extracts, while the hexane extract remained the least effective.

Conclusion: These findings suggest that medium polarity solvents such as DCM and ethyl acetate effectively extract bioactive compounds from *H. tuberosus* with significant enzyme inhibitory potential. Ongoing HR-LC-MS/MS phytochemical profiling will identify the specific secondary metabolites that may be responsible for the observed activity, highlighting *H. tuberosus* as a promising source of natural antidiabetic agents.

Flavonoids allosteric modulation of ACE2 and its implications for COVID-19: Computational insights and experimental perspectives on the membrane and mitochondrial effects of rutin

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Introduction and Aim: There has been a compelling need to identify new therapeutic agents and targets due to the increased complexity of disease mechanisms in recent times. The angiotensin-converting enzyme 2 (ACE2) plays a crucial role in the pathophysiology of the highly morbid COVID-19 that threatens the efficacy of numerous therapeutic agents, making ACE2 a promising target for therapeutic intervention. Flavonoids, known for their diverse therapeutic activities, have the potential to modulate ACE2.

Methods: This study used molecular docking, molecular dynamics (MD) simulation, and MM/GBSA using Amber tools to investigate the interactions between ACE2 and flavonoids. The most promising flavonoid was evaluated for cytotoxicity through MTT assay, CYP3A4 activity to assess metabolic effects and ATP, MMP, and LDH activities for cellular stress, metabolic function, and membrane integrity.

Results: The MD simulations showed the key allosteric binding residues including ARG255, PRO328, GLU357, GLU384, GLU388, and ARG500, involved in conformational modulation of ACE2. Rutin exhibited the strongest binding affinity to ACE2, followed by isoquercetin. Both flavonoids formed strong interactions and stable ACE2-flavonoid complexes. In vitro testing of rutin on A549 cells showed no significant cytotoxicity at concentrations up to 500 µM. Rutin reduced LDH release, enhanced ATP production and mitochondrial function without affecting membrane integrity, indicating its potential to modulate cellular energy metabolism and mitigate oxidative stress in the milieu of COVID-19 and cardiometabolic conditions linked to mitochondrial dysfunction and metabolic stress.

Discussion/Conclusion: These findings provide a mechanistic basis for allosteric modulation of ACE2 and position rutin as a promising candidate for further development, given its strong binding affinity, favourable cellular safety and metabolic effects.

Development and validation of LC-MS/MS analysis method for biomonitoring of glyphosate and its metabolites in human serum

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Background: Glyphosate is approved in >100 countries for its broad efficacy and low cost. Its widespread use raises environmental and health concerns. Inadvertent exposure can be monitored by quantifying glyphosate and its metabolites, aminomethylphosphonic acid (AMPA) and methylphosphonic acid (MPA). Due to its small size and highly polar nature, glyphosate typically requires derivatization, complicating sample preparation and method development. This study presents the first validated LC-MS/MS method in Southern Africa for simultaneous quantification, without derivatization, of glyphosate, AMPA, and MPA in human serum and its application to clinical samples from farming-intensive areas of the Western Cape for exposure monitoring.

Methods: Calibration standards and quality controls were prepared by spiking working solutions into pesticide-free human serum to final concentrations of 15.0–760 ng/mL for glyphosate, 25.00–1600 ng/ mL for AMPA, and 0.75–48 ng/mL for MPA. Sample preparation used Oasis MAX cartridges. Quantification was performed on a Sciex QTRAP 6500+ LC-MS/MS system with a Phenomex Luna C8 column and gradient elution. Validation followed SANTE/2021 guidelines, evaluating linearity, LOQ, selectivity, matrix effects, recovery, reproducibility, and autosampler stability. Deidentified serum samples (n = 261) from Western Cape communities with high pesticide usage were analysed for glyphosate and metabolite detection.

Results: The method showed a quadratic regression with 1/x2 weighting. Precision across all STDs and QCs remained within the acceptable threshold of ≤20%. It demonstrated high selectivity, with no interference from endogenous compounds at analyte retention times. Matrix effects and recovery, assessed using six independent serum sources, were within acceptable limits. A signal-to-noise threshold of ≥25 indicated detectable levels in 31/261 samples, with 3 showing quantifiable concentrations.

Conclusion: A robust LC–MS/MS method was developed and validated for simultaneous quantification of glyphosate, AMPA, and MPA in human serum. The method detected glyphosate and metabolites in 11.9% of patient samples from farming-intensive regions of the Western Cape

Molecular and Binding Analysis of Naringenin as a Potential Inhibitor of Bromodomain Containing Protein 4 (BRD4)

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Introduction: Bromodomain-containing protein 4 (BRD4) is an epigenetic regulator implicated in several pathological conditions, making it an attractive therapeutic target. BRD4 is crucial in gene transcription and is implicated in diseases like inflammation and cancer. It is essential for controlling gene transcription specific to keratinocytes, affecting cellular differentiation and proliferation, and preserving skin homeostasis. A possible therapeutic approach is to target BRD4 with small molecule, especially to overcome resistance in diseases linked to BRD4.

Aims and Objectives: In this study, we performed molecular docking calculation, molecular dynamics (MD) simulations and comprehensive binding energy analysis to compare the binding affinity and stability of BRD4 complexes with naringenin and the reference compound JQ1.

Methods: The MD simulations were conducted over 400 ns, and post-simulation analyses, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (RoG), and solvent-accessible surface area (SASA), were carried out to assess the structural dynamics and stability of the complexes.

Results: The BRD4-naringenin complex exhibited a lower average RMSD (1.22 Å) compared to the BRD4-JQ1 complex (1.40 Å), indicating greater structural stability. Similarly, lower RMSF values suggested reduced flexibility of the naringenin complex. The radius of gyration and SASA analyses further supported the compactness and solvent exposure consistency of the naringenin complex. Binding energy analysis revealed a significantly lower binding free energy (.6G_{bind}) for naringenin (-46.2 kcal/mol) compared to JQ1 (-28.7 kcal/mol), driven primarily by more favorable van der Waals interactions and enhanced gas-phase binding energy. Residue- wise energy decomposition analysis identified key interacting residues, highlighting stronger interactions with residues such as ILE105, MET91, and ASN99 in the naringenin complex.

Discussion/Conclusion: Collectively, these findings demonstrate that naringenin exhibits a strong binding affinity and stability compared to JQ1, making it a promising candidate for BRD4 inhibition. This study provides valuable insights into the molecular interactions and stability profiles of BRD4-ligand complexes, potentially guiding the development of novel therapeutics targeting BRD4.

Distinct toxicological profiles of pesticide metabolites compared to parent compounds: Evidence from a zebrafish model

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Introduction: The imperative to ensure food security for a rapidly growing global population has driven the intensification of agricultural practices [1], which in turn has led to a marked increase in the use of pesticides [2]. Among countries in Sub-Saharan Africa, South Africa reports the highest application rates of pesticide active ingredients [3]. Given that pesticides are inherently designed to disrupt biological systems,often through mechanisms that result in morbidity or mortality in target organisms,their widespread use raises significant concerns regarding human and ecological health.

Agricultural workers and farmers, who experience the mostdirectandfrequentcontactwiththese chemicals, face particularly elevated health risks. Furthermore, indirect exposure pathways pose additional hazards to the general population, wildlife, and ecosystems [4].To mitigate occupational health risks, regulatory frameworks have established Acceptable Operator Exposure Levels (AOELs), which are primarily derived from animal toxicology data [5]. However, a critical gap remains in our understanding of pesticide metabolite toxcicity, and the environmental persistence of pesticide metabolites

Aims and Objectives: This study addresses this knowledge gap by evaluating the toxicity profiles of four widely used pesticides, glyphosate, imidacloprid, chlorpyrifos, and mancozeb,alongside their principal metabolites. Zebrafish (*Danio rerio*), a well-established vertebrate model for toxicological research, were employed to assess and compare the acute and longterm toxicity of these compounds..

Materials and Methods: To determine acute toxicity healthy zebrafish larvae (94 hpf) were exposed to five concentrations of each pesticide and metabolite in 6-well plates (20 larvae/well, 5 mL E3 medium per well). A negative control (E3 only) and a positive control (3,4-dichloroaniline) were included. After 24 hours of exposure (118 hpf), survival was assessed via heartbeat observation under a light microscope as shown in Figure 1.

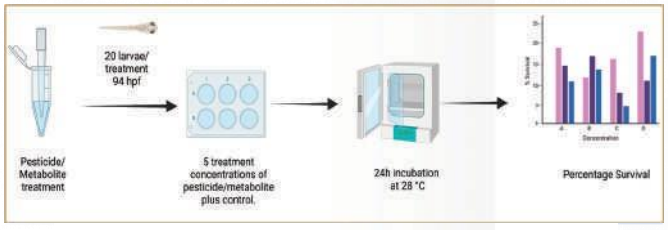
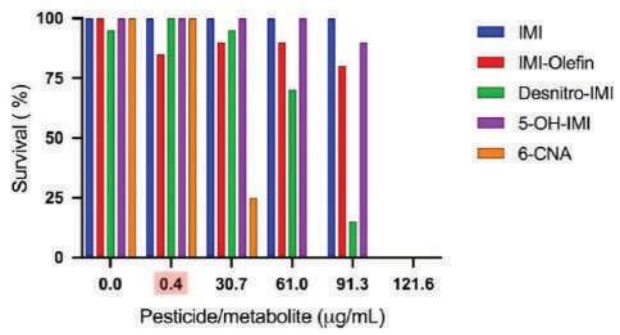


Figure 1: Workflow for the acute toxicity experiment

To assess teratogenesis, zebrafish embryos (≤3 hpf) were exposed to pesticides in 30 mL E3 medium (50 embryos/dish), with solutions refreshed once after 24 hours and maintained until 118 hpf. Teratogenicity was assessed through daily visual inspection and quantified at 118 hpf via locomotor activity using the DanioVision system and EthoVision XT 15 (Noldus, Netherlands). Larvae (up to n = 24 per group) were placed individually in 96-well plates and subjected to a light–dark transition test (LDTT). Imidacloprid, chlorpyrifos, and mancozeb followed a 40-minute LDTT (20-min dark acclimation, 10-min light, 10- min dark), while glyphosate used a 24-minute version (20-min dark, 2-min light, 2-min dark).

Results and Discussion: Given the broad scope of endpoints and the extensive panel of pesticides and metabolites analysed, this report presents only a subset of the results, focusing on imidacloprid and its metabolites.

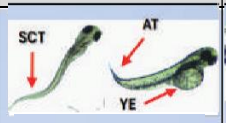
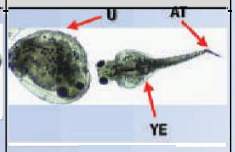
Acute toxic effects of imidacloprid and metabolites: Desnitro-IMI and 6-CNA demonstrated significant toxicity at concentrations only marginally exceeding the AOEL adjusted for zebrafish, indicating that these metabolites may present a higher toxicological risk than their parent compound, seen if figure 2.



Teratogenic effects of imidacloprid and metabolites: Teratogenicity assessments showed that imidacloprid had no effect on larval locomotion under either basal or stimulated conditions. In contrast, all tested concentrations of IMI metabolites significantly reduced locomotor activity. Morphological analysis revealed distinct developmental defects following exposure to 6-CNA and IMI olefin, with the most severe effects observed at 40% of the AOEL. Specifically, 6-CNA induced pronounced S-shaped tail deformities, while IMI olefin caused developmental delays, yolk sac edema, and abnormal tail morphology (Table 1).

These findings indicate that IMI metabolites may be more toxic than the parent compound.

Table 1: *Developmental deformities of larvae exposed to 6-CAN and imidacloprid olefin at 40% AOEL was depicted. The AOEL for IMI was 0.4 µg/mL. Observations of abnormal tails (AT), yolk sac edema (YE), S-shaped tails (SCT) and/or underdevelopment (U), Abbreviations: 6-CNA, 6-chloronicotinic acid; AOEL, acceptable operator exposure level.*

Control	6-CNA	Imida-cloprid Olefin
		

Conclusions: The findings indicate that pesticide metabolites often exhibit greater toxicity than their parent compounds, potentially causing significant developmental effects even at low exposure levels. This highlights the need for risk assessments to consider both parent chemicals and their metabolites to better protect human health and environmental integrity.

Availability of quality, safety and efficacy data on *Tribulus terrestris* for completion of a common technical document

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Background: *Tribulus terrestris Linn*. is widely used as an herbal testosterone supplement, particularly by athletes and individuals with androgen deficiencies. However, in South Africa, the lack of regulation for herbal medicines poses risks such as contamination, adulteration, and unknown adverse effects. Recent legislative changes mandate the registration of herbal medicines with the South African Health Products Authority (SAHPRA), requiring submission in the Common Technical Document (CTD) format, which includes quality, safety, and efficacy data. This study aimed to conduct a review of the available data on the testosterone-boosting effects of *Tribulus terrestris* to inform the preparation of Module 2 of a CTD.

Methods: A narrative review was performed using PubMed, Scopus, Web of Science, EBSCOhost, and the Cochrane Library. English-language, full-text articles evaluating the androgenic effects of *Tribulus terrestris* extracts and supplements were included. Data were extracted and organised according to the quality, non-clinical, and clinical sections required for a CTD summary.

Results: The review revealed significant variability in the chemical composition of *Tribulus terrestris*, influenced by factors such as growth conditions, plant part selection, and extraction methods. Differences in study designs, phytochemical standardisations, and the use of polyherbal formulations further complicated comparisons across studies. While some studies demonstrated potential androgenic effects, others showed limited or no efficacy. Safety data were sparse, with few studies systematically evaluating adverse effects.

Conclusion: The findings highlight the need for standardisation in the preparation and evaluation of *Tribulus terrestris* products. Variability in study outcomes reflects inconsistencies in methodologies and preparation techniques. Further research, particularly in African populations, is crucial to address these gaps. In the interim, regulatory measures by SAHPRA should focus on ensuring the quality control and safe manufacturing practices of herbal products to safeguard consumer health.

The investigation of the effects of *Trifolium burchellianum Ser*. and Wild fern- The development towards cardiovascular disease mitigation particularly in heart failure.

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Introduction: Cardiovascular diseases are a leading cause of morbidity and mortality worldwide, with heart failure being a major contributor. Conventional drugs used to treat cardiovascular diseases like heart failure, hypertension and stroke are very expensive, in accessible and have many intolerable side effects, with very small therapeutic windows, mostly are prescription only drugs and thus rarely available to the people living in the developing countries. Therefore, people have shifted to the practice of medicinal plants as an alternative strategy to manage cardiovascular diseases and other related diseases.

Aim: This study is aimed at investigating the possible safety and efficacy activities of *T. burchellianum* and *Wild fern* extracts on cardiovascular diseases as well as its possible mechanism of action on heart failure.

Methods: To evaluate the cytotoxic effects of the plant extracts, MTT assay was used. The Presence of antioxidant constituents was determined using DPPH scavenging. Angiotensin-converting enzyme inhibitory assay was used to measure the plant extracts 'ability to convert angiotensin I to angiotensin II. Antithrombin screening kit was used to determine the ability of the plant extracts to inhibit thrombin. CAM assay was used to evaluate the ability of the plant extracts on inducing vascularisation.

Results: The cytotoxicity assay results revealed that *T. burchellianum* and Wild fern exhibited CC_{50} values of 25 µg/ml and 50 µg/ml respectively, indicating relatively safe concentration ranges for the plant extracts. The antioxidant activity as measured by the DPPH assay, yielded an EC_{50} value of 14.08µg/ml for *T. burchellianum* and 23 µg/ml for Wild fern indicating strong antioxidant properties. Additionally, the plant extract demonstrated ACE inhibitory activity with an IC_{50} of 25 ± 2.08 µg/ml *T. burchellianum* and 13,65 ±1,98 for Wild fern, which may contribute to blood pressure regulation. The antithrombin activity assay of *T. Burchellianum* showed an IC_{50} value of 6.85 ± 1.192ug/ml suggesting potential benefits in preventing blood clot formation. The wild fern extract induced vascularisation at a concentration of 500 µg/ml while *T. Burchellianum* induced vascularisation at 1 µg/ml. These findings suggest that *T. burchellianum* and Wild fern may provide a promising therapeutic approach for the treatment of cardiovascular related diseases such as heart failure. However, *T. burchellianum* and *Wild fern* still need further investigated to fully understand the mechanisms of action of the plants in exerting their cardioprotective effects.

Antibacterial effect of herbal extract and homeopathic preparation of *Calendula officinalis* on South African ESKAPE pathogens in vitro

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Aim: Medicinal plants possess considerable potential for discovering new phytochemicals that can be a solution in fighting multi-resistant pathogens. *Calendula officinalis* (C. officinalis) is used worldwide due to its antimicrobial properties. This pilot study aimed to assess the antibacterial activity of herbal extract and homeopathic preparation of *C. officinalis* flowers against South African ESKAPE pathogens.

Methods: Different ethanol concentrations of herbal extract (50%, 60%, and 90%) and homeopathic preparation (62%) were tested against South African ESKAPE pathogens using the Kirby-Bauer disc diffusion method (6.0mm disk diameter).

Findings: The different concentrations tested showed minimal inhibitory effects. he ethanol herbal extract, *C. officinalis* (20µl), showed some antibacterial activity against ESKAPE pathogens compared to homeopathic-prepared *Calendula officinalis*. However, the 50% ethanol herbal extract *C. officinalis* (20µl) showed significant antibacterial activity against Staphylococcus species as compared to Homeopathic prepared *Calendula officinalis*.

Conclusion: This study serves as a baseline for further studies on medicinal plants as alternative antibiotics. Medicinal plants, in different preparations, possess phytochemicals that can penetrate the cell membranes of resistant pathogens.

Evaluating the Anticancer Potential of *Camellia sinensis* Teas

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Background: *Camellia sinensis*, the botanical source of green, white, oolong, and black teas, is widely consumed globally and recognized for its rich polyphenolic content. These polyphenols exhibit antioxidant properties and have been implicated in cancer chemoprevention. While preclinical and observational studies suggest potential anticancer benefits, the clinical efficacy of tea consumption in oncology remains inconclusive.

Aim and Objectives: This study aims to systematically evaluate the clinical impact of *Camellia sinensis* tea consumption in cancer patients receiving standard anticancer therapy. It investigates the effects on disease progression, remission rates, quality of life, and safety outcomes through a comprehensive review of the literature. The objective is to synthesize current evidence and assess the therapeutic potential of tea as an adjunctive intervention in oncology.

Methods: A systematic review and meta-analysis were conducted to assess the therapeutic impact of *Camellia sinensis* tea consumption among patients diagnosed with various malignancies. Comprehensive searches were performed across CENTRAL, EMBASE, MEDLINE, PubMed Central, Scopus, and reference lists of relevant reviews, covering literature from January 1, 1980, to July 1, 2024. Study selection adhered to PICOS criteria and PRISMA guidelines. Risk of bias was evaluated using the JBI SUMARI tool, and data synthesis was performed using a random-effects model. Results were visualized via forest plots and summarized in Excel.

Results: A total of 100 studies met inclusion criteria. Green tea demonstrated superior outcomes compared to other *Camellia sinensis* variants, with consumption exceeding two cups per day associated with a reduced overall cancer incidence (summary RR = 0.83; 95% CI: 0.65–1.07). Fourteen meta-analyses were conducted comparing green tea plus standard anticancer therapy versus standard therapy alone, encompassing endpoints such as cancer prevention, risk reduction, and adverse events. None of the pooled effect sizes reached statistical significance ($p > 0.001$), and heterogeneity was low ($I2 < 50\%$), indicating methodological consistency across studies.

Conclusion: Green tea appears to be a safe and well-tolerated adjunct with potential benefits in reducing cancer risk. However, current evidence does not support definitive clinical efficacy. Further large-scale, long-duration prospective studies are warranted to elucidate underlying mechanisms and to evaluate the effects of other tea types, including oolong, white, and black teas.

Improved longitudinal adherence to antiretroviral therapy in virally suppressed people with HIV reduces development of serious non-AIDS events

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Background: Non-communicable diseases, especially serious non-AIDS events (SNAEs) like cardiovascular disease (CVD) and death, are increasing in people with HIV (PWH). SNAEs are linked to persistent inflammation, and recent studies suggest that imperfect adherence to ART, even in the context of viral suppression, is associated with higher risks of SNAEs, highlighting the need for targeted interventions beyond viral suppression.

Objective: To determine the time-varying effect of self-reported antiretroviral therapy (ART) adherence on the incidence of the SNAEs of death and CVD in PWH who are virally suppressed at baseline.

Methods: We performed a secondary analysis of anonymized data of participants that were enrolled in the prospective observational Advancing Clinical Therapeutics Globally for HIV/AIDS and Other Infections (ACTG) A5001 and A5322 studies. Eligibility criteria for this analysis included PWH without study-defined SNAEs at baseline. The baseline was defined as the time at the first viral suppression to <200 copies/mL after treatment initiation. Adherence to ART was assessed using the periodically administered ACTG self-report questionnaire, with percentage adherence calculated as the proportion of prescribed doses taken over the preceding four days. Data were analyzed using inverse probability of censoring weights (IPCW), and sensitivity analyses were conducted using generalized estimating equations (GEE) models.

Results: Among 2,932 participants with viral suppression at baseline followed for a median of 4.3 years, 260 SNAEs occurred, comprising 125 deaths and 135 cardiovascular events. Nearly all participants (95.8%) remained virally suppressed to <200 copies/mL over the full duration of follow-up. Higher ART adherence was significantly associated with a reduced risk of first SNAE using IPCW: each 10% increase in adherence decreased risk by 9% (adjusted hazard ratio 0.991, 95% confidence interval 0.982–0.999). Associations remained significant in the sensitivity analysis when first and subsequent SNAEs were considered. Increased age and higher viral load were significantly associated with greater SNAE risk, while former smokers or those who never smoked had significantly lower risk compared to current smokers.

Conclusions: Higher ART adherence reduces SNAE risk even with viral suppression, underscoring the need for continued adherence support to improve long-term health outcomes in HIV care.

Valorization of Biomass Waste for Medicinal Purposes

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Introduction: Fossil fuels, whose negative effects on the environment are well-recognized, are a major feedstock for producing pharmaceutical agents. In line with this observation is the accumulation of huge volumes of solid waste, of which organic waste, with cellulose as a structural component, is contributing largely.
An alternative and "green" approach is producing pharmaceuticals from waste cellulose, a process that aligns with circular economy principles. Cellulose, the most abundant organic polymer on Earth, which is found in agricultural waste, paper, wood, and textiles, can be converted into valuable drugs or active pharmaceutical intermediates, replacing fossil fuels as feedstock. The key processes to convert cellulose into pharmaceuticals include enzymatic (cellulase) hydrolysis of cellulose into glucose, which could be fermented by engineered microbes to produce bio-based pharmaceutical ingredients such as penicillin precursors and steroids. Cellulose could also be developed as a platform chemical, such as succinic acid for antimalarials or lactic acid for the formation of a biodegradable drug carrier.
Researchers at the Department of Pharmacology and Therapeutics at the Sefako Makgatho Health Sciences University (SMU) are investigating the use of cellulose from wastepaper, sawdust, peanut shells, and banana peels as a renewable feedstock for bio-product development and the formation of a nano-cellulose drug carrier.

Aim: To bioconvert the cellulose section of organic waste materials with cellulase enzymes into glucose, a renewable feedstock for bio-pharmaceutical synthesis.

Methods: Standard waste-cellulose to sugar conversion procedure: Incubation mixture
Waste cellulose substrates: Wastepaper, sawdust, peanut shells (50 mg).
Enzymes: Cellulase from *Aspergillus niger* and garden snails (200 ul ; 2.0 $mg.ml^{-1}$).
Buffer solution: Tris (800 ul ; 0,5 M, pH 5,0).
Incubation temperature: 50 °C.
Incubation time: 2 h.
Sugar determination: Spectrophotometrically (DNS-method).

Results: The cellulose content of wastepaper, sawdust, and peanut shells showed different susceptibilities for saccharification by cellulase from *Aspergillus niger* and garden snails. The percentage saccharification obtained during a standard conversion procedure varied between 8 – 10 % depending on the type of organic waste and nature of the cellulase enzyme. More sugar was produced by changing the incubation conditions, with the glucose used to produce bio-acetic acid and lactic acid. The non-degraded cellulose section is used in the preparation of a nanocellulose drug carrier.

Conclusions: Solid organic waste such as sawdust, wastepaper, and peanut shells has the potential to be developed as a renewable feedstock for the biosynthesis of pharmaceuticals. Chemical substances derived from waste cellulose can serve as platform chemicals to produce renewable pharmaceuticals. The process of developing waste cellulose is environmentally benign, limits the use of fossil fuels, decreases the high volumes of organic waste produced annually, and can be applied in the process of evolving the concept of Green Pharmacology.

Surveillance, frequency and nature of anti-doping rule violations in South Africa: an annual review

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Introduction: As an extension of the World Doping Anti-doping Agency, the South African Institute for Drug-Free Sport aims to reduce the prevalence of non-therapeutic drug use (doping) through a combination of detection, deterrence and education.

Aims and Objectives: This paper describes an annual frequency profile and nature of prohibited substance use and anti-doping rule violations (ADRVs) among South African sports persons who qualify to be tested.

Methods: An anonymized dataset of testing records as captured and released in the 2023/2024 Annual Report of the South African Institute for Drug-Free Sport (SAIDS) for the period 1 April 2023 - 31 March 2024, was extracted from the SAIDS Anti-Doping Administration and Management System. The dataset was examined to identify doping transgressions and adverse analytical findings (AAFs) by sport, gender, doping class and sanction applied. Data was subsequently analysed categorically, with descriptive nominal frequency counts and inferentially, using non-parametric chi-square and odds ratios with $p \leq 0.05$.

Results: A total of 2335 tests were conducted, comprising urine (n=1594; 68.27%), blood (n=322; 13.79%), EPO-receptor agonists (n=297; 12.72%); carbon isotope ratio mass spectrometry (n=43; 1.84%) and growth hormone releasing factor (n=79; 3.38%). A total of 47 individuals were found to be in violation of anti-doping roles, with 4.26% being guilty of test evasion and 45 persons (95.74%) testing positive for a total of 54 AAFs. Results from intelligence-based testing (tip-off information), rendered a 53.13% yield of ADRVs. There were significantly ($p \leq 0.0001$) more males (n=29; 72.5%) than females (n=11; 27.5%) among the ADRVs (positive tests) - while 6.38% of positive tests were in minors (<18 yrs. old). The class of banned substances making up the significant ($p \leq 0.0001$) majority AAFs were anabolic agents (55.56%), diuretics and masking agents (12.96%) and hormone modulators (9.26%) – with cannabinoids and stimulants making up 7.41% each. Peptide hormones (3.7%), glucocorticoids and narcotics (1.85% each), made up the remaining minority of banned substances. In the cases of ADRVs among minors, 66.66% were for anabolic agents and 33.33% for a stimulant. The recorded frequency of anabolic agent use was significantly ($p \leq 0.0001$) more for males (n=21; 75.0%) than females (n=7; 25.0%). However, there was no significant difference in the odds between males and females for anabolic use vs. other substances (OR 3.00; 95 % CI: 0.73 to 12.39; $p = 0.13$). Overall, the significant ($p \leq 0.0001$) greatest frequency of AAFs were found in athletics (n=16; 29.63%) and bodybuilding (n= 13; 24.07%) – followed by mountain-bike cycling (n=5; 9.26%), powerlifting and rugby with 7.41% each, while mixed martial arts made up 5.56% of the 54 AAFs. The highest diagnostic yield for AAFs (positives vs. tests done) was for bodybuilding with 130%, followed by mixed martial arts (12.12%), powerlifting (10.81%) and weightlifting (8.70%). In contrast, despite relatively high frequencies of AAFs – relatively low diagnostic yields found for cycling (2.41%), athletics (1.96%) and rugby (1.01%). The typical sanction applied was a 3-to-4-year ban (n=26; 59.09%), while 22.73% (n=10) were sanctioned for ≥5 years. likely reflecting repeat offences.

Conclusion: The use of androgenic anabolic agents and attempts to mask their presence - is the most common form of ADRVs in South Africa, among both males and females, adults and minors, particularly in body-building and strength-based sports. A good yield in detecting ADRVs through intelligence-based testing, prompted by anonymous public-surveillance “tip-offs”, indicates a desire to keep sport clean and free of doping. Besides the typical four-year mandated ban from competition imposed by SAIDS as a deterrent for ADRVs, pharmacists also need to play a key role through vigilance and education, to change athlete-behaviour in the use of non-therapeutic sports performance enhancing drugs.

Primum non nocere et utilitarius: an ethical elucidation of anti-doping

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Introduction, Aims and Objectives: The use of banned substances in sport is commonplace and problematic. Accordingly, the World Anti-Doping Agency (WADA) was established. In conjunction with other national anti-doping agencies throughout the world, such as the South African Institute for Drug-Free Sport (SAIDS), WADA aims to achieve international harmonisation and improvement of standards and practices in anti-doping. While *primum non nocere* is an ethical cornerstone taught to health scientists for over 2000 years, this principle is not universally adopted. Sport is sometimes used as a drug-discovery laboratory with anti-doping agencies being one step behind manufacturers of new undetectable substances with pharmacological properties similar to those already available on the market. Similarly, while self-harm is typically considered a form of cognitive dissonance, some philosophers believe an individuals' autonomy of choice is paramount, suggesting that doping should not be banned. This leads to contemplating potential coercive agents for the development and use of performance enhancing drugs, whether to test or not to test, as well as its ethical justifications.

Methods: There are two methodological frameworks developed and applied in this paper. First, the operational definition of doping refers to the non-therapeutic use of specific listed substances and methods which are banned because they meet at least two of the three following criteria: i) they enhance performance; ii) pose a threat to athlete health; or iii) create an unfair advantage – thus violating the spirit of sport. Secondly, in addition to the fundamental ethical principle of non-maleficence, this paper uses a utilitarian framework, within consequentialist philosophy, as a means for arguing the ultimate moral justification for elucidating and supporting the anti-doping lobby.

Thesis: Although the use of banned performance enhancing substances (doping) is indeed documented not to be good for one's health, some Kantian philosophers focussing on individual human rights, find no morally compelling arguments to restrict drug use by athletes - as long as such a choice is informed (of risk) and fully voluntary - the athlete chooses, for himself or herself (values which are permissible in a free society). They further claim that restriction of choice attempts to impose alternative values on the drug-choosing athlete and if choice is restricted, we deny the athlete the values of self-reliance, personal achievement, and autonomy. Thus, it is argued that testing for drugs in sport comprises a form of moral accounting depriving someone of a freedom and a potential violation of their autonomy. By extension, pharmacologists using the sporting landscape as a backdrop in conjunction with the vulnerabilities or gullibility of individuals to push the boundaries and develop new undetectable substances – will argue that they are merely expanding the horizons of drug discovery but the choice to dope, invariably lies with the individual. However, most logical individuals are unlikely to use a banned substance if they have the cognition to discern the risks to their health. Thus, what are the coercive agents at play for them to override their logic and better knowledge? Through the metamorphosis of contemporary sport and its media entertainment appeal, professional sport has become a viable means of earning an income. Accordingly, their choice to dope is more than an egotistical wish to be victorious, albeit by an unfair and reprehensible advantage at the cost of fair-play in competition. The better the performance the greater the potential earnings for the individual through prize-money, bonusses, sponsorship endorsements and appearance fees as well as for colluding pharmacologists active in the field of doping. By deduction - individuals are prepared to risk their health via doping as the monetary rewards are a strong coercive agent to do so! By extension, in the South African context, this coercive impulse is even stronger among both mature and young historically and racially marginalized individuals from resource-constrained environments - for whom sport and its potential financial rewards, has become a means of social and economic mobility to emancipate themselves from poverty and oppression.

In reviewing doping violations world-wide and locally, it is evident that doping transgressions are not limited to adults. Along these lines, even Kantian philosophers honour the moral principle of paternalism in a soft paternalistic approach, conceding that there is a moral obligation to ban the use of performance enhancing drugs among minors, and if there is no action then that is avoiding moral duty. This is motivated on the basis that interference is necessary because the youth lack the rational skills to act in their own best interests. Accordingly, soft paternalists dictate that minors should be prevented from doping and that adults should be allowed to practice doping. One could agree with this dictum - that because the use of performance-enhancing substances is potentially harmful to the user, does not necessarily mean they must be banned for adults as they may feel "it's my life, my body, and I should be at liberty to do with it whatever I want to". However, hard paternalists take issue with the belief of soft paternalists who argue that testing for and sanctions against doping in sport, should be limited to youths or those not fully voluntary because the person is not fully informed or competent. As such,

hard paternalism suggests that interference is sometimes justified even if the person is rationally competent, as assumed in the case of adulthood – especially if their behaviour and choices are potentially harmful. This point of view is expounded by the utilitarian philosophy of ethical decision making espoused by Bentham and Mills, which support the interference in another's personal liberty in order to act in what you believe is their best (individual) interest and leads to the best outcome for all (community). As such the utilitarian approach of paternalism on the grounds of harm to others - implies that it is wrong to harm others or to coerce them into potentially harmful situations. In the context of doping, this approach challenges sports libertarians who claim that banning performance-enhancing substances is an unjustified paternalistic action that violates the principle of autonomy. In truth the subtle coercion or influence of sporting role models can be a powerful persuasive influence to act. If an impressionable young athlete perceives that success is only attainable through a particular practice of a sports-hero, such as the use of banned substances, then the practice, which may be harmful to the role-model, becomes potentially harmful to others (youths).

Conclusion: Health-care practitioners, pharmacists and pharmacologists are expected to exercise public safety through pharmacovigilance, Thus, while respecting autonomy, the principles of non-maleficence, beneficence and justice deserve emphasis in the fight against doping. This is justified by the utilitarian ethical imperative of paternalism (behaviour modification) on the grounds of harm to others (youths). Accordingly, there is a need for more cognisance, education, and support in this sphere, against the use of banned substances in sport.

Safety and efficacy of psilocybin in the management of treatment-resistant depression

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Background: Major depressive disorder (MDD), is recognised globally as one of the most widespread debilitating diseases, affecting approximately 264 million people world-wide. Only 60-70% of patients who suffer from depression reach remission, unfortunately, the rest are left to suffer from treatment-resistant depression (TRD). As a result, there is a need to develop novel treatments that provide rapid relief of depressive symptoms with a minimum number of side effects, particularly in TRD patients. The phenomenon of psychedelics as therapeutic agents has entered a new era, characterised by a sudden surge of interest in their therapeutic benefits. This has brought about a positive change to not only laws governing access to psychedelics, but also the public's perceptions and uses. With depression having had tremendous health and economic burden to society globally due to multiple factors including its undertreatment, there is a need for fast-acting medications such as Psilocybin. Psilocybin provides a glimmer of hope for patients who may have grown weary and have given up on successful alleviation of depressive symptoms

Aims: This study was aimed to assess the safety and efficacy of Psilocybin in the management of TRD. The findings of this study will contribute knowledge on these aspects, as well as identifying effective interventions that have been successful in the management of the condition. Furthermore, it will provide guidance to policy-makers in the development and implementation of evidence-based practices in the management of TRD.

Methods: A systematic review and meta-analysis of clinical trials were conducted to compare the safety and efficacy of psilocybin with other antidepressant treatments for the management of TRD. The Preferred Reporting Items for Systematic Reviews (PRISMA) and JBI Manual for Systematic Reviews of Effectiveness were followed.
Databases including PubMed, MEDLINE, Cochrane Collaboration's CENTRAL trials registry, PsycINFO and EMBASE were searched between January 2014 and January 2025 for clinical trials on psilocybin in comparison to other antidepressants in the management of treatment-resistant depression; both published and unpublished records were included. A significant number of publications were identified after removing duplicates. Subsequent to the title and abstract screening, full text screening was conducted, and selected studies were included in the review.

Results: Preliminary results have shown that psilocybin is more effective in the management of TRD compared to some of the conventional antidepressants. On completion of the study, we also expect it to have a better safety profile compared to various classes of the aforementioned drugs.

Discussion/Conclusion: The final interpretation of study findings is currently pending, as data analysis is still ongoing. Once complete, this section will examine the effects of psilocybin at varying doses (25 mg, 10 mg, and 1 mg (control)) on treatment resistant depression severity, safety profiles, and participant response patterns. The discussion will contextualize findings within existing literature and address clinical implications, limitations, and directions for future research.

Monitoring antimicrobial use through wastewater-based epidemiology: An Alternative Antimicrobial Monitoring System

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Abstract: Background: Wastewater-based epidemiology (WBE) has become a useful tool for monitoring population health, including antimicrobial resistance (AMR). This research investigates how WBE can monitor antimicrobial use (AMU) to support traditional surveillance systems that often have data gaps, lack standardization, and underreport cases.

Objective: This study aims to examine the current state of WBE for monitoring AMU through a scoping review. It also evaluates the relationship between antibiotic residues in wastewater and antimicrobial use data from healthcare and community sources.

Methods: A scoping review in line with PRISMA-ScR guidelines to identify studies using WBE for antimicrobial monitoring. Concurrently, an observational study to measure antimicrobial residues in hospital and municipal wastewater using gas chromatography–mass spectrometry (GC-MS) was conducted. These concentrations will be compared to antimicrobial consumption data gathered through the WHO Antimicrobial Consumption (AMC) tool.

Preliminary Findings: The scoping review shows a growing interest worldwide in using WBE for AMU monitoring, but important methodological and ethical issues remain unresolved. Initial experimental data from local hospital waste and wastewater treatment plant inflow indicate possible links between residue levels and reported antibiotic use patterns.

Conclusion: WBE is a promising method for AMU monitoring, particularly in places with limited resources. While the scoping review lays the groundwork for understanding current practices, the experimental part provides practical insights into feasibility and implementation. Future efforts will aim to improve analytical methods, standardize interpretation, and explore how to incorporate WBE into antimicrobial stewardship programs.

Investigating the effects of 4OH-estrone on glucose metabolism in skeletal muscle and liver cells.

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Introduction: Estrogen hormones are important in controlling glucose homeostasis and pancreatic cell function (1). However, the relationship between estrogen metabolites and glucose homeostasis is not fully elucidated. Just recently, scientists have identified that a metabolite of estrogen, 4-hydroxyestradiol can form adducts with insulin, which can trigger the production of antibodies against these adducts (2,3). Although the effects that 4OH-estradiol has on diabetes mellitus have been highlighted, there remains a notable lack of comprehensive data the other estrogen metabolites, especially 4OH-estrone, have on glycemic control.

Aims and Objectives: The study aimed to assess the cytotoxic effects of 4OH-estrone and to elucidate any resulting changes in glucose uptake, the expression of GLUT 4 and Akt, autophagy and mitochondrial function in hepatic (HepG2) and skeletal muscle (C2C12) cell lines. Additionally, the effects of co-incubating 4OH-estrone with different insulin analogues (aspart, detemir, glargine and glulisine) by assessing changes in GLUT4 and Akt expression as well as glucose uptake was examined.

Methodology: In this study, skeletal muscle (C2C12) and liver (HepG2) cell line were used. A cell viability study was conducted after 24hour exposure of C2C12 and HepG2 to 4OH-estrone at (0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 µM) using MTT assay. Thereafter, differentiated C2C12 and HepG2 cell preparations were exposed to 4OH-estrone at different concentrations (0.2, 0.4, 0.8, and 1.6µM) for 24 hours to evaluate glucose uptake. Furthermore, the effects of 4Oh-estrone were assessed on AKT, GLUT-4, MFN1, Rab7, LC3 and DPP4 expressions as well as GLUT 4 translocation. Thereafter, 4OH-estrone was co-incubated with the insulin analogs (aspart, detemir, glargine and glulisine) and the change in insulin activity was assessed by evaluating GLUT 4 in C2C12 cells and AKT expression and glucose uptake in C2C12 and HepG2 cells.

Results: C2C12 and HepG2 cells treated with 4OH-estrone at concentrations (0.2, 0.4, 0.8, and 1.6µM) were viable after 24 hours. It was observed that GLUT4 and AKT expression and AKT phosphorylation in C2C12 cells decreased in the presence of 4OH-estrone, comparedto the control. GLUT4 translocation increased at lower 4OH-estrone concentrations but decreased at higher concentrations. A dose-dependent increase in DPP4, LC3, MFN1 and Rab7 expression was observed in C2C12 cells treated with 4OH-estrone. Co-incubation of insulin analogues with estrone revealed an enhanced GLUT 4 expression with detemir, a significant impairment with glulisine and a concentration-dependent reduction in GLUT 4 expression with aspart and glargine.

Conclusion: 4OH-estrone is non-cytotoxic up to 1.6 µM in both C2C12 and HepG2 cells. 4OH-estrone impairs mitochondrial integrity, through suppression of MFN1 and tafazzin. 4OH-estrone induces autophagy in both cell lines, with evidence of dose-dependent LC3 activation. These findings suggest that elevated levels of 4OH-estrone, which can occur due to altered estrogen metabolism, may impair glucose uptake and insulin signaling in muscle and liver cells, potentially worsening insulin resistance and glycemic control. This highlights the need to consider estrogen metabolite profiles when selecting insulin analogues for personalized diabetes management.