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CONFERENCE ABSTRACTS

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New medicines

A parallel duplex RNA hybrid platform for coordinated gene silencing and transcriptional activation: A next-generation multitarget nucleic acid therapeutic strategy

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Background: Multifactorial diseases arise from dysregulated gene networks rather than single gene abnormalities. Current nucleic acid therapeutics typically operate through either post-transcriptional gene silencing (e.g., RNA interference) or transcriptional activation (e.g., promoter-targeting small activating RNA), necessitating combination regimens to achieve bidirectional control. Such approaches increase formulation burden and frequently yield non-synergistic pharmacodynamic effects. We developed a Parallel Duplex RNA Hybrid (PDRH) platform that integrates a microRNA mimetic (miR-Mimic) strand and a promoter-targeting small activating RNA strand within a single duplex construct, enabling simultaneous cytoplasmic repression of a pathogenic gene and nuclear activation of a protective gene (Patent: 202410663042.7). The goal of this study is to evaluate whether PDRH achieves superior therapeutic efficacy compared with single-mechanism or combined nucleic acid approaches in models of acute myocardial infarction and solid tumours.

Method: Each PDRH construct comprises a 21-nucleotide microRNA mimetic targeting the 3' untranslated region of a disease-driving mRNA and a 21-nucleotide promoter-directed activating RNA designed against a transcription start site motif of a protective gene (Patent: 202410663042.7). Sequence architecture was optimised for thermodynamic asymmetry to favour selective strand loading, controlled GC content (40–65%), and promoter motif specificity verified by homology screening. Duplexes were lipid nanoparticle-formulated for in vivo delivery. Efficacy was evaluated in cardiomyocytes and cancer cell lines using quantitative reverse transcription polymerase chain reaction, Western blotting, calcium transient analysis, apoptosis and cell cycle assays. In vivo performance was assessed in murine myocardial infarction and xenograft tumour models, with functional, structural and molecular endpoints.

Results: The Meox1 × ATP2A2 PDRH reduced Meox1 protein by 78.4% within 12 hours while increasing ATP2A2 transcription 7.9-fold and SERCA2a protein 4.1-fold. Mechanistic analysis demonstrated compartment-specific action: Ago2-dependent cytoplasmic repression of Meox1 and promoter-directed nuclear activation of ATP2A2. In myocardial infarction models, PDRH reduced infarct size by 87% and improved ejection fraction by 88%, outperforming microRNA mimetic alone (18%), activating RNA alone (39%), and combined administration (65%). Fibrosis and apoptosis were markedly attenuated, calcium handling was normalised, and mitochondrial integrity preserved. In oncology models, the Kras × TP53 PDRH achieved 89% tumour growth inhibition, compared with 37% for Kras silencing alone, 29% for TP53 activation alone, and 71% for dual administration. PDRH induced p53-dependent cell cycle arrest, reduced invasion, and enhanced apoptosis. Across indications, therapeutic efficacy exceeded additive expectations, indicating true pharmacological synergy rather than parallel additive effects (Patent: 202410663042.7).

Conclusion: The Parallel Duplex RNA Hybrid platform represents a mechanistically integrated nucleic acid therapeutic strategy enabling bidirectional gene network reprogramming within a single molecular entity (Patent: 202410663042.7). By unifying RNA interference and transcriptional activation in one construct, PDRH simplifies combination therapy while delivering superior pharmacodynamic outcomes. This scalable platform provides

a framework for precision multitarget therapeutics across cardiovascular and oncological diseases and exemplifies next-generation RNA pharmacology.

The regulation of acupuncture in Brazil (LAW 15.345/2026) and the strategic role of the federal council of pharmacy's working group

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Background: The year 2026 marks a historic milestone for Brazilian public health with the enactment of Law No. 15,345/2026, which regulates the professional practice of acupuncture. For decades, the field faced intense legal disputes regarding professional boundaries. This summary analyzes the decisive role of the Federal Council of Pharmacy (CFF) and its Working Group (WG) on Traditional Chinese Medicine (TCM) and Acupuncture in securing the pharmacist's right to practice this specialty.

Method: Under the technical coordination of the WG, the CFF performed a vital role in the Brazilian Federal Deputy Chamber and the Federal Senate. Through active participation in several public hearings, the WG provided scientific and legal evidence to sustain acupuncture as a multiprofessional practice, successfully opposing attempts to grant exclusive rights to the medical category. The advocacy focused on the "Right to Health" and the "Freedom of Professional Exercise," ensuring that clinical pharmacists, equipped with specialized postgraduate training, could continue to offer high-quality TCM treatments.

The WG's contributions were twofold: Political and Technical. Politically, it articulated with lawmakers to ensure the text of Law 15,345/2026 recognized health specialists with proven certification. Technically, the WG established rigorous standards for pharmaceutical acupuncture, aligning national practice with World Health Organization (WHO) guidelines. This ensured that the transition to the new legal framework occurred without disrupting the services already integrated into the Unified Health System (SUS).

Results: The impact of this regulation is profound. It legitimizes the pharmacist as a primary healthcare provider in TCM, promoting the reduction of polypharmacy and offering non-pharmacological alternatives for chronic pain management and mental health. The Brazilian experience, spearheaded by the CFF's Working Group, serves as a global benchmark for the FIP, demonstrating how pharmaceutical institutions can lead the integration of traditional medicines into modern legal and clinical frameworks.

Conclusion: In conclusion, Law 15,345/2026 is a triumph for multidisciplinary health. The CFF's strategic intervention ensured that acupuncture remains a democratic and accessible tool, consolidating the pharmacist's clinical role in the global landscape of Integrative and Complementary Practices (PICS).

TJ3, a multi-target anti-ageing sesquiterpene lactone, reprogrammes ageing networks and preserves cognitive function

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Background: Ageing is driven by interconnected biological processes including oxidative stress, chronic inflammation, mitochondrial dysfunction and metabolic dysregulation, which collectively contribute to cognitive decline and neuroinflammation. Single-target approaches often fail to address this network complexity. We hypothesised that TJ3 (isoacoramone), a sesquiterpene lactone, acts as a multi-node network modulator capable of reprogramming ageing-associated redox, immune and metabolic pathways to preserve cognitive function. We therefore evaluated its effects using integrated omics and systems pharmacology approaches.

Method: Aged rodents received oral TJ3 (15 or 150 mg/kg) for 6–12 weeks. Cognitive performance was assessed using validated behavioural paradigms. Brain and serum samples underwent label-free proteomics and metabolomics. Differentially expressed proteins and metabolites were analysed by pathway enrichment and network modelling. Mechanistic validation was performed in lipopolysaccharide-activated microglia and Nrf2 reporter systems. Analyses focused on redox regulation, inflammatory signalling, mitochondrial function and metabolic remodelling.

Results: TJ3 significantly improved memory performance in aged animals and reduced microglial activation, indicating attenuation of neuroinflammation. Proteomic profiling demonstrated partial restoration of a youthful molecular signature. Age-associated upregulation of cytoskeletal remodelling and immune-recognition proteins, including actin-related protein 2 and lectin pathway components, was attenuated. Conversely, metabolic enzymes linked to glucose utilisation and mitochondrial function, including hexokinase domain-containing protein 1, were normalised.

Metabolomic analysis revealed reversal of key age-related shifts. TJ3 increased neuroprotective lipids, including docosahexaenoic acid and membrane phospholipids, enhanced amino acid balance, and reduced pro-inflammatory lipid mediators such as 12-hydroxyeicosatetraenoic acid. Notably, cystine and related glutathione precursors were

elevated, indicating strengthened antioxidant capacity. Mechanistically, TJ3 engaged multiple ageing-relevant pathways. It activated the Nrf2–Keap1 axis, inducing antioxidant enzymes such as haem oxygenase-1, NAD(P)H quinone dehydrogenase 1 and glutathione S-transferase, thereby restoring redox homeostasis. Concurrently, it suppressed IκB kinase–nuclear factor kappa B and mitogen-activated protein kinase signalling, reducing tumour necrosis factor alpha, interleukin 1 beta and interleukin 6 expression. Activation of AMP-activated protein kinase signalling improved mitochondrial efficiency and lipid oxidation. In vitro, TJ3 shifted microglia towards a less inflammatory phenotype and reduced markers associated with ferroptotic vulnerability.

Network modelling confirmed that TJ3 functions as a weak-affinity, multi-node regulator, modulating redox, inflammatory and metabolic hubs simultaneously rather than targeting a single pathway. Both tested doses were effective, with no observable toxicity.

Conclusion: TJ3 reprogrammes ageing-associated biological networks and preserves cognitive function through coordinated modulation of redox balance, immune activation and mitochondrial metabolism. By acting as a multi-target systems modulator rather than a single-pathway inhibitor, TJ3 exemplifies a network pharmacology strategy for age-related disorders. These findings support further translational development of TJ3 as a multi-mechanistic therapeutic candidate for cognitive ageing and systemic age-related decline.

A multitarget circular anti-microRNA oligonucleotide therapeutic platform for precision suppression of oncogenic microRNAs

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Background: Small RNA therapeutics such as microRNA mimics, small interfering RNA and antisense oligonucleotides are established gene-silencing strategies. However, most operate through a single-target mechanism. Given that complex diseases such as myocardial infarction and cancer are driven by multiple interacting pathogenic pathways, single-target interventions often produce limited therapeutic benefit due to compensatory signalling. A scalable multi-target nucleic acid strategy is therefore required to enhance pharmacological efficacy while maintaining molecular specificity. We developed a dual Mock-miR hybrid platform designed to simultaneously suppress two key pathogenic genes within a single small RNA duplex (Patent: 202411094331.6). We hypothesised that rational hybridisation of two disease-specific Mock-miR strands would produce synergistic gene suppression and superior therapeutic outcomes compared with individual strands or conventional approaches.

Method: For each disease model, two critical pathogenic genes were selected based on mechanistic relevance. Twenty-one nucleotide Mock-miR single strands were designed against target messenger RNA regions, optimised to achieve ≥65% complementary pairing potential. Two strands were synthesised and annealed at controlled temperature to form a double-stranded hybrid molecule (Patent: 202411094331.6). In a myocardial infarction model, Mock-miR strands targeting Carnitine Palmitoyltransferase 1B (Cpt1b) and Mitochondrial Ribosomal Protein S5 (Mrps5) were hybridised and delivered using lipid nanoparticle formulations. Gene expression, protein levels, cardiac function, infarct size, apoptosis and cardiomyocyte proliferation markers were assessed in vitro and in vivo. In oncology models, Mock-miR strands targeting Delta Like 1 Homolog (DLK1) and Neurotrophic Receptor Tyrosine Kinase (NTRK1) were hybridised and evaluated in hepatocellular carcinoma and pancreatic cancer cell lines and xenograft mouse models.

Results: In cardiomyocytes, the Cpt1b×Mrps5 hybrid reduced Cpt1b protein expression by 78.4% and Mrps5 protein by 64.1% compared with negative control sequences. In murine myocardial infarction, hybrid treatment significantly improved ejection fraction and fractional shortening, reduced infarct size, lowered serum lactate dehydrogenase levels and decreased apoptosis in the peri-infarct region from 7.6% to 1.4%. Cardiomyocyte proliferation markers (Ki67, phospho-histone H3 and 5-ethynyl-2'-deoxyuridine incorporation) were markedly increased, indicating reactivation of regenerative capacity. Mitochondrial structural integrity was preserved (Patent: 202411094331.6). In cancer models, the DLK1×NTRK1 hybrid reduced DLK1 and NTRK1 protein levels by 59.2% and 65.8%, respectively. Proliferation of hepatocellular and pancreatic cancer cells decreased by 72.1% and 66.6%, while apoptosis increased by over 50%. In xenograft models, tumour volume inhibition reached 72.7% and 65.3%, respectively, exceeding the effects observed with single-strand approaches (Patent: 202411094331.6).

Conclusion: The dual Mock-miR hybrid platform represents a multi-target small RNA therapeutic strategy that integrates specificity with coordinated pathway suppression (Patent: 202411094331.6). By combining two mechanistically linked targets within a single duplex molecule, this approach enhances therapeutic efficacy in both cardiovascular and oncological disease models. The platform provides a generalisable framework for the rational design of multi-target nucleic acid medicines suitable for complex diseases.

A six–IRES-like hexamer translation module enables chemically unmodified translatable circular mRNA for scalable protein replacement therapeutics

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Background: Messenger RNA therapeutics represent a major pharmaceutical innovation but require extensive chemical modification, capping, polyadenylation optimisation and purification to reduce instability and innate immune activation. These processes increase manufacturing complexity, cost and translational risk. Circular RNA offers enhanced nuclease resistance and reduced immune recognition; however, efficient cap-independent translation remains a limiting step. Although individual IRES-like hexamers have been identified in endogenous circular RNAs, rational engineering of a multimeric translation initiation module to systematically enable therapeutic protein expression has not been reported.

Purpose: We designed a six–IRES-like hexamer translation module to generate a translatable circular mRNA (tcmRNA) platform capable of chemically unmodified, high-efficiency cap-independent translation (Patent: 202410583877.1). We evaluated its pharmacological potential in two disease models: acute myocardial infarction (ATP2A2/SERCA2a replacement) and type 2 diabetes-associated impaired wound healing (GDF11 replacement).

Method: Six AU-rich IRES-like hexamers were rationally assembled and separated by 4-nucleotide linkers to form a combinatorial translation initiation module. Linear DNA templates encoding ATP2A2 or GDF11 were engineered with the hexamer module, transcribed in vitro, circularised, purified and treated with RNase R to remove residual linear RNA (Patent: 202410583877.1). No nucleotide chemical modifications were introduced. Circular constructs were delivered using lipid nanoparticles, including cardiomyocyte-targeted peptide-modified formulations for cardiac studies. In vitro protein expression was assessed in human AC16 cardiomyocytes and primary murine cardiomyocytes. In vivo efficacy was evaluated in a murine acute myocardial infarction model and a type 2 diabetic wound model. Cardiac function, infarct size, calcium handling, apoptosis and mitochondrial integrity were analysed. Wound closure and angiogenesis were quantified in diabetic mice.

Results: The six-hexamer configuration produced optimal translation efficiency; fewer hexamers significantly reduced protein output, whereas additional elements provided no further enhancement (Patent: 202410583877.1). In cardiomyocytes, ATP2A2-tcmRNA increased SERCA2a protein 4.3-fold at 12 hours and ATP2A2 mRNA 14.7-fold at 24 hours compared with circular constructs lacking the translation module ($p<0.001$). In vivo, myocardial ATP2A2 transcripts increased 7.4-fold and SERCA2a protein 9.8-fold within 24 hours. Infarct size was reduced by 85.4%, and ejection fraction improved by 92.2% compared with infarct controls ($p<0.001$). Calcium transient amplitude was restored, sarcoplasmic reticulum Ca^{2+} content increased, cytosolic Ca^{2+} overload decreased, and cardiomyocyte apoptosis was significantly reduced. Mitochondrial ultrastructure was preserved. In diabetic mice, GDF11-tcmRNA accelerated wound closure and enhanced angiogenesis. Compared with direct recombinant GDF11 protein administration, the tcmRNA approach produced a 2.7-fold greater therapeutic effect. Circular constructs lacking the IRES-like module showed no significant activity.

Conclusion: We establish a rationally engineered six–IRES-like hexamer translation module that enables chemically unmodified translatable circular mRNA for protein replacement therapy (Patent: 202410583877.1). This platform eliminates the need for nucleotide modification, reduces manufacturing complexity and enhances translational robustness. Demonstrated efficacy in cardiovascular and metabolic disease models highlights its scalability as a next-generation nucleic acid therapeutic strategy.

CCG4, a network-modulating anti-ageing compound, extends lifespan and reverses senescence signalling in C. elegans, HUVEC cells and aged mice

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Background: Ageing is a systems-level process driven by interconnected hallmarks including cellular senescence, chronic low-grade inflammation, mitochondrial dysfunction, and dysregulated nutrient sensing. Pharmacological strategies targeting single pathways have shown limited translational success, partly due to compensatory network adaptations. Increasing evidence supports the concept that weak-affinity, multi-node modulators capable of restoring network homeostasis may offer superior geroprotective efficacy. CCG4 is a newly characterised small molecule derived from natural product optimisation. Preliminary screening suggested lifespan extension activity. We hypothesised that CCG4 functions as a network-level regulator capable of attenuating senescence signalling and improving physiological resilience across biological scales.

Method: A translational multi-model framework was employed:
1.C. elegans lifespan assays were conducted across a broad concentration range (0.1–1000 μM) to evaluate longevity

effects and dose-response characteristics. 2.Endothelial senescence model: Human umbilical vein endothelial cells (HUVECs) were exposed to D-galactose to induce premature senescence. Protein levels of P16, P21 and P53 were assessed by Western blotting. Senescence-associated β-galactosidase staining quantified cellular ageing. AMPK and mTOR expression levels were examined to explore nutrient-sensing modulation. 3.Naturally aged C57BL/6 mice received oral CCG4 for up to three months. Functional outcomes included grip strength, balance beam performance, Barnes maze cognitive testing, fasting blood glucose, body weight monitoring, cardiac ultrasound parameters and survival trend analysis.

Results: Lifespan extension in invertebrates: CCG4 significantly increased mean lifespan in C. elegans, with maximal efficacy observed at low micromolar concentrations. Higher doses demonstrated attenuated or reversed benefit, indicating a hormetic dose-response pattern consistent with adaptive stress-modulating mechanisms. Suppression of endothelial senescence: In D-gal–induced HUVECs, CCG4 markedly reduced expression of P16 and P21, key cyclin-dependent kinase inhibitors that drive cell-cycle arrest. SA-β-gal positivity was significantly decreased, confirming functional reversal of the senescent phenotype. Although total AMPK and mTOR protein levels were not dramatically altered, an increased AMPK/mTOR ratio trend was observed, suggesting modulation of nutrient-sensing balance rather than direct pathway inhibition. Functional improvement in aged mice: In naturally aged mice, CCG4 improved muscle strength and enhanced motor coordination. Cognitive performance in the Barnes maze demonstrated improved spatial learning and memory retention compared with untreated aged controls. Importantly, these benefits occurred without adverse effects on fasting glucose, cardiac function, or body weight, supporting a favourable safety profile. Longevity trends indicated greater benefit at lower doses, consistent with the hormetic pattern observed in nematodes.

Conclusion: CCG4 extends lifespan in C. elegans, reverses endothelial senescence markers, and improves physical and cognitive function in aged mice. The consistent reverse dose-dependence across models suggests that CCG4 acts as a network-modulating geroprotective agent rather than a high-affinity pathway inhibitor. By attenuating senescence signalling and rebalancing nutrient-sensing pathways, CCG4 represents a promising multi-target candidate for age-related functional decline. Further mechanistic and pharmacodynamic studies are warranted to clarify its translational potential in human ageing interventions.

A multigene translatable circular RNA hybrid therapeutic platform enabling coordinated protein replacement and RNA modulation for advanced heart failure

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Background: Heart failure is characterised by defective sarcoplasmic reticulum calcium reuptake, mitochondrial bioenergetic insufficiency, maladaptive signaling activation and progressive cardiomyocyte loss. Reduced expression of ATP2A2 (SERCA2a), CAND1 and PGC-1α disrupts calcium cycling, proteostasis and mitochondrial biogenesis, forming a pathogenic network that cannot be adequately corrected by single-target therapy. While mRNA therapeutics enable protein replacement, linear constructs require nucleoside modification to improve stability and reduce innate immune activation, increasing manufacturing complexity and potential translational infidelity. Circular RNA offers intrinsic stability and reduced immunogenicity but lacks efficient translation initiation. We developed a multigene translatable circular RNA (MG-TcmRNA) hybrid platform designed to integrate multi-protein replacement, anti-microRNA de-repression and siRNA-mediated gene silencing within a single programmable therapeutic molecule (Patent: 202411042653.6).

Method: A synthetic AU-rich IRES-like pentamer translation module was engineered to enable cap-independent protein synthesis within circular RNA. Coding sequences for ATP2A2, CAND1 and PGC-1α were inserted sequentially into a circular backbone, each preceded by an independent translation module. Anti-miRNA sequences targeting miR-34a and miR-29 and siRNA targeting MTOR were incorporated. The construct was circularized without nucleoside modification, purified and encapsulated into cardiomyocyte-targeted lipid nanoparticles. Therapeutic efficacy was evaluated in human cardiomyocytes and in a transverse aortic constriction mouse model of heart failure.

Results: The MG-TcmRNA platform achieved robust, simultaneous multigene expression without chemical modification. In cardiomyocytes, the triple construct increased SERCA2a expression 13.1-fold, CAND1 16.8-fold and PGC-1α 10.5-fold within 24 hours, significantly exceeding single-gene constructs. Protein induction remained sustained and did not trigger detectable inflammatory activation. Functionally, enhanced SERCA2a expression normalized calcium handling, increasing calcium transient amplitude and accelerating relaxation kinetics while reducing cytosolic Ca^{2+} overload. PGC-1α overexpression restored mitochondrial respiratory capacity and preserved cristae integrity. CAND1 expression reduced apoptotic signaling and improved cellular viability. Anti-miRNA components suppressed miR-34a and miR-29 activity, further amplifying endogenous cardioprotective gene expression. Embedded siRNA

effectively downregulated MTOR signaling, reducing maladaptive hypertrophic pathways. In vivo, lipid nanoparticle–mediated delivery produced marked functional recovery. Ejection fraction improved to 91.2% in triple-gene therapy compared with 83.7% in dual-gene and 77.3% in single-gene constructs. Fractional shortening increased proportionally, while left ventricular dilation was significantly reduced. Cardiomyocyte apoptosis and interstitial fibrosis declined substantially. Circulating cardiac injury biomarkers decreased in parallel with structural and functional recovery. Across molecular, cellular and physiological endpoints, therapeutic efficacy followed a consistent hierarchy: triple > dual > single-gene constructs.

Conclusion: This multigene circular RNA hybrid platform enables coordinated restoration of calcium cycling, mitochondrial bioenergetics and survival signaling while simultaneously suppressing maladaptive pathways. By eliminating nucleoside modification and integrating multiple pharmacological mechanisms into a single construct, MG-TcmRNA represents a scalable next-generation RNA therapeutic strategy for complex cardiovascular diseases.

A dual mock-miR hybrid platform enables multi-target small RNA therapeutics for cardiovascular and oncological diseases

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Background: Small RNA therapeutics such as microRNA mimics, small interfering RNA and antisense oligonucleotides are established gene-silencing strategies. However, most operate through a single-target mechanism. Given that complex diseases such as myocardial infarction and cancer are driven by multiple interacting pathogenic pathways, single-target interventions often produce limited therapeutic benefit due to compensatory signalling. A scalable multi-target nucleic acid strategy is therefore required to enhance pharmacological efficacy while maintaining molecular specificity. We developed a dual Mock-miR hybrid platform designed to simultaneously suppress two key pathogenic genes within a single small RNA duplex (Patent: 202411094331.6). We hypothesised that rational hybridisation of two disease-specific Mock-miR strands would produce synergistic gene suppression and superior therapeutic outcomes compared with individual strands or conventional approaches.

Method: For each disease model, two critical pathogenic genes were selected based on mechanistic relevance. Twenty-one nucleotide Mock-miR single strands were designed against target messenger RNA regions, optimised to achieve ≥65% complementary pairing potential. Two strands were synthesised and annealed at controlled temperature to form a double-stranded hybrid molecule (Patent: 202411094331.6). In a myocardial infarction model, Mock-miR strands targeting Carnitine Palmitoyltransferase 1B (Cpt1b) and Mitochondrial Ribosomal Protein S5 (Mrps5) were hybridised and delivered using lipid nanoparticle formulations. Gene expression, protein levels, cardiac function, infarct size, apoptosis and cardiomyocyte proliferation markers were assessed in vitro and in vivo. In oncology models, Mock-miR strands targeting Delta Like 1 Homolog (DLK1) and Neurotrophic Receptor Tyrosine Kinase (NTRK1) were hybridised and evaluated in hepatocellular carcinoma and pancreatic cancer cell lines and xenograft mouse models.

Results: In cardiomyocytes, the Cpt1b×Mrps5 hybrid reduced Cpt1b protein expression by 78.4% and Mrps5 protein by 64.1% compared with negative control sequences. In murine myocardial infarction, hybrid treatment significantly improved ejection fraction and fractional shortening, reduced infarct size, lowered serum lactate dehydrogenase levels and decreased apoptosis in the peri-infarct region from 7.6% to 1.4%. Cardiomyocyte proliferation markers (Ki67, phospho-histone H3 and 5-ethynyl-2′-deoxyuridine incorporation) were markedly increased, indicating reactivation of regenerative capacity. Mitochondrial structural integrity was preserved (Patent: 202411094331.6). In cancer models, the DLK1×NTRK1 hybrid reduced DLK1 and NTRK1 protein levels by 59.2% and 65.8%, respectively. Proliferation of hepatocellular and pancreatic cancer cells decreased by 72.1% and 66.6%, while apoptosis increased by over 50%. In xenograft models, tumour volume inhibition reached 72.7% and 65.3%, respectively, exceeding the effects observed with single-strand approaches (Patent: 202411094331.6).

Conclusion: The dual Mock-miR hybrid platform represents a multi-target small RNA therapeutic strategy that integrates specificity with coordinated pathway suppression (Patent: 202411094331.6). By combining two mechanistically linked targets within a single duplex molecule, this approach enhances therapeutic efficacy in both cardiovascular and oncological disease models. The platform provides a generalisable framework for the rational design of multi-target nucleic acid medicines suitable for complex diseases.

Antioxidant properties and biological activity of natural compounds in fruit wines

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Background: Biologically active compounds from fruit and their derived products are essential for the human body. Berries are an interesting group of fruit, notable especially for their beneficial health effects. One representative of this group is the blueberry. Many products are derived from blueberries, and among them, wine stands out in particular.

Purpose: The aim of this study was to determine the phenolic profile, antioxidant activity, and in vitro activity of the analyzed fruit wine. In particular, it is important to emphasize the influence of blueberry wines on hyperglycemia prevention, as this problem is very common in the population today.

Method: Fruit wines were produced from blueberries, and different yeast strains were used to conduct controlled microvinification. Wines were produced with and without the addition of sugar and enzymatic preparation. Two different yeasts were used in separate fermentations. Identification and quantification of phenolic compounds were conducted by UPLC TQ-MS/MS, while antioxidant activity was measured by the FRAP method. Alpha-glucosidase inhibitory activity was measured in lyophilized blueberry wine dissolved in DMSO. In this method, alpha-glucosidase and the substrate solution, p-nitrophenyl alpha-D-glucopyranoside, were used.

Results: Blueberry wine samples were a source of phenolic acids and flavonoids. Among the phenolic acids, the most dominant were chlorogenic, protocatechuic, gallic, ellagic, and caffeic acids. Catechin and epicatechin were identified as the most dominant flavonoids. Antioxidant activity determined by the FRAP method showed that raspberry wine ranged from 72.47 to 90.21 mmol/L Fe2+. Blueberry wine demonstrated the ability to inhibit alpha-glucosidase activity, with results ranging from 25.51 to 45.28 µg/ml. Acarbose, with an inhibitory activity of 75.25 µg/ml, was used as a control in this method. Wines produced with the addition of sugar and enzymatic preparation showed higher phenolic compound content and better antioxidant activity. Higher inhibitory activity against alpha-glucosidase was also detected in these wine samples.

Conclusion: Blueberry wines proved to be good inhibitors of alpha-glucosidase compared to acarbose. The detected compounds in blueberry wines are responsible for this activity, along with many other biologically active principles. All these compounds, along with their synergistic and antagonistic effects, contribute to the antioxidant and inhibitory activity against alpha-glucosidase.

Phenolic extraction in Cabernet Sauvignon wines produced by thermovinification and carbonic maceration

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Background: Carbonic maceration is a winemaking technique where whole grape clusters ferment in a carbon dioxide–rich environment, resulting in wines that are fruity, soft, and low in tannins. Thermovinification is a technique used in red wine production where grapes are heated before fermentation to enhance the extraction of color, phenolic substances, and certain aromatic compounds.

Purpose: The aim of this study was to determine the influence of thermovinification and carbonic maceration on phenolic extraction.

Method: Grape variety Cabernet Sauvignon was harvested at optimal enological maturity from vineyards belonging to the experimental field “Radmilovac” of the Faculty of Agriculture in Zemun. For thermovinification, crushed grapes were heated at 60°C for one hour. After heating, the treated grapes were cooled to 27°C and inoculated with a pure yeast strain (BDX, Lallemand, Canada) to initiate alcoholic fermentation. Grapes intended for carbonic maceration were prepared by destemming without crushing. Dry ice was added and the vessel was covered with a lid. After four days, the must was inoculated with the same pure yeast strain (BDX, Lallemand, Canada). Total phenolic content in all wine samples was determined using the Folin–Ciocalteu method with gallic acid as a standard.

Results: The total polyphenol content in the carbonic maceration wine sample was 1220 ± 11.0 mg/L GAE, which was significantly lower than the total polyphenol content obtained by thermovinification (2800 ± 40.0 mg/L GAE).

Conclusion: The results indicate that the applied vinification technique significantly affects phenolic extraction. Thermovinification resulted in a considerably higher total polyphenol content compared to carbonic maceration, suggesting that heat treatment enhances the extraction of phenolic compounds during red wine production.

In silico and in vitro investigation of the molecular mechanisms of antiviral compounds for potentially inhibiting dengue virus NS2B-NS3 protease

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Background: The escalating global health threat posed by dengue virus (DENV), combined with the lack of effective antiviral therapies, and highlights the urgent need for novel therapeutic interventions. Dengue remains a major public health concern in tropical and subtropical regions, with increasing incidence and disease severity worldwide. Current management strategies are largely supportive, underscoring the importance of identifying targeted antiviral agents. The DENV NS2B-NS3 protease is a key enzyme involved in viral polyprotein processing and replication, making it an attractive and well-validated target for antiviral drug discovery. Targeting this protease offers a promising strategy for disrupting viral replication and disease progression.

Method: This study employed an integrated in silico and in vitro approach to identify potential synthetic inhibitors of the DENV NS2B-NS3 protease. A Pharmacophore model was first generated using six known inhibitors of the NS2B-NS3 protease, capturing essential molecular features required for effective inhibition. The validated Pharmacophore model was then used to virtually screen a library of 64,958 synthetic compounds obtained from ChemDiv Limited. Compounds exhibiting Pharmacophore fit scores greater than 55 were selected for further analysis. Twenty-five shortlisted compounds were subjected to molecular docking studies to evaluate their binding affinities and interaction profiles within the protease active site. The most promising candidates were further assessed using molecular dynamics simulations to examine complex stability and identify key amino acid residues involved in ligand binding. Finally, in vitro antiviral evaluation of selected lead compounds was performed using PCR-based analysis to assess their inhibitory effects on dengue viral replication.

Results: Results Pharmacophore-based virtual screening identified 25 compounds with high structural compatibility to known NS2B-NS3 protease inhibitors. Molecular docking analysis revealed three compounds—Y070-0848, Y070-0626, and G917-0162—as the most promising hits, exhibiting favourable binding energies and stable interactions within the protease active site. These compounds also demonstrated acceptable predicted pharmacological properties.
Molecular dynamics simulations further supported the stability of the ligand–protease complexes and highlighted the critical involvement of key residues, particularly Asp75, Ser135, and His51, in ligand binding and stabilization. These residues play essential roles in protease catalytic activity and inhibitor recognition.
In vitro evaluation revealed a significant reduction in viral RNA levels in treated samples compared to untreated controls, confirming antiviral activity. Among the three lead compounds, Y070-0848 exhibited the most potent inhibitory effect, followed by Y070-0626 and G917-0162, as evidenced by PCR analysis.

Conclusion: This study demonstrates the effectiveness of combining in silico and in vitro approaches for identifying novel antiviral compounds targeting the DENV NS2B-NS3 protease. The identified lead compounds, particularly Y070-0848, show strong potential as dengue antiviral agents. These findings provide a solid foundation for further optimization, toxicity assessment, and advanced biological evaluation, supporting their progression toward therapeutic development for dengue virus infection.

(+)-Borneol Potentiates Edaravone's Anti-neuroinflammatory Effect via P-gp-mediated Modulation of the CX3CL1/NF-κB Pathway in Cerebral Ischaemia/Reperfusion Injury

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Background: Cerebral ischaemia–reperfusion (CI/R) injury is a life-threatening neurological condition that can be more severe than cerebral ischaemic stroke. Edaravone-dexborneol, composed of edaravone and (+)-borneol, has emerged as a promising therapeutic option for CI/R injury. Several studies have shown that combining edaravone with (+)-borneol produces synergistic effects compared with edaravone alone. However, the specific mechanism underlying this synergy, particularly the role of (+)-borneol, remains unclear. Therefore, this study aimed to investigate whether (+)-borneol enhances brain delivery of edaravone to produce synergistic effects and to explore its potential binding targets.

Method: A rat middle cerebral artery occlusion/ischaemia–reperfusion (MCAO/IR) model and an oxygen–glucose deprivation/reoxygenation (OGD/R) cell model were established. Pharmacodynamic effects were evaluated by neurological scoring, infarct volume measurement, body weight change, triphenyltetrazolium chloride (TTC) staining and haematoxylin–eosin (HE) staining. Edaravone pharmacokinetics were studied using a rapid

high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method. Blood–brain barrier (BBB) integrity was assessed by Evans blue staining, while the permeability of fluorescein sodium salt (FLU) and horseradish peroxidase (HRP) was evaluated in vitro. Molecular docking and cellular thermal shift assay (CETSA) were performed to examine the binding interaction between (+)-borneol and transport proteins. Transcriptome sequencing combined with western blot and reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to investigate the anti-inflammatory regulatory mechanisms of P-glycoprotein (P-gp).

Results: Combined administration of edaravone and (+)-borneol significantly alleviated neuroinflammation both in vivo and in vitro. Co-administration of (+)-borneol increased edaravone exposure in the cerebral infarction area and enhanced its brain penetration. We further found that (+)-borneol helped to maintain blood–brain barrier (BBB) integrity. Elevated levels of tight-junction proteins indicated that the paracellular pathway played only a limited role in the increased cerebral edaravone concentration. (+)-Borneol up-regulated the expression of influx transporters (organic anion transporter 1 (OAT1) and OAT3) while down-regulating efflux transporters (P-gp and multidrug resistance-associated protein 1 (MRP1)) in both experimental systems. Moreover, (+)-borneol bound directly to P-gp and OAT3, thereby facilitating edaravone entry into brain tissue and reducing its efflux. Transcriptome analysis identified the C-X3-C motif chemokine ligand 1/nuclear factor kappa B (CX3CL1/NF-κB) signalling pathway as the key route through which (+)-borneol exerts its synergistic effect. The synergistic anti-neuroinflammatory activity of (+)-borneol and edaravone was almost completely abolished after P-gp silencing. Silencing of P-gp also down-regulated inflammatory cytokine expression and suppressed the CX3CL1/NF-κB signalling pathway.

Conclusion: (+)-Borneol enhances the protective effect of edaravone against cerebral ischaemia/reperfusion (CI/R) injury by promoting transcellular delivery of edaravone to the ischaemic region. The synergistic anti-neuroinflammatory effect of (+)-borneol and edaravone likely involves P-gp-mediated suppression of the CX3CL1/NF-κB signalling pathway.

Targeting inflammatory and fibrotic pathways with cannabidiol and its metabolites: An integrated cheminformatics and network pharmacology approach

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Background: Chronic inflammation and fibrosis are major contributors to morbidity across a range of non-communicable diseases, and current long-term therapies are often limited by adverse effects and insufficient efficacy. Although Cannabidiol (CBD) has shown therapeutic promise in inflammatory and fibrotic conditions, the contributions of its metabolites and their molecular mechanisms remain poorly characterised.

Purpose: This study aimed to elucidate the molecular basis of the anti-inflammatory and anti-fibrotic potential of CBD and its major metabolites by identifying key molecular targets and signalling pathways using integrated in silico drug discovery approaches, including network pharmacology techniques.

Method: CBD and a panel of its major metabolites were characterised using cheminformatics tools to predict physicochemical properties, pharmacokinetics, and toxicity. Potential protein targets were identified using SwissTargetPrediction and annotated against inflammation- and fibrosis-associated genes from GeneCards. Protein-protein interaction networks were constructed using STRING and analysed in Cytoscape to identify hub targets. Gene Ontology and KEGG pathway enrichment analyses were performed to assess biological relevance. Molecular docking was conducted to validate ligand-target interactions and estimate binding affinities.

Results: All compounds demonstrated favourable drug-like properties, high predicted gastrointestinal absorption, and acceptable toxicity profiles. Network pharmacology analysis identified overlapping targets associated with inflammation and fibrosis, revealing 10 shared hub proteins: TNF, IL6, STAT3, BCL2, EGFR, CASP3, MAPK3, PPARG, PTGS2, and MMP9. Functional enrichment analyses highlighted significant involvement in key signalling pathways, including MAPK, PI3K-Akt, EGFR, apoptosis, calcium signalling, and AGE–RAGE pathways. Molecular docking revealed strong binding affinities of CBD and its metabolites for these hub proteins, with one metabolite consistently demonstrating a more favourable interaction profile across multiple targets than the parent compound.

Conclusion: This integrated in silico analysis provides mechanistic insight into the multi-target therapeutic potential of CBD and its metabolites in modulating inflammatory and fibrotic pathways. The findings support

prioritising a CBD-derived metabolite as a promising lead candidate for further investigation. Ongoing computational validation, including molecular dynamics simulations, is being undertaken to further assess protein-ligand stability and interaction dynamics and to inform subsequent experimental studies.

The effect of boric acid sugar bait on Anopheles gambiae sensu stricto

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Background: Malaria is a life threatening disease that poses a major health concern globally. The disease burden has been attributed to several challenges encountered in the carrying out of vector control to decimate mosquito populations including insecticide resistance and change of anthropophagic mosquito feeding behaviour from endophagic to exophagic. This laboratory based study was done to determine the potential of boric acid incorporated in sugar bait to decimate adult mosquito populations by exploiting their sugar feeding behaviour where mortality, LD50, LD95 were determined and survivorship was analysed.

Method: This experimental study was done at the Tropical Pesticides Research Institute (TPRI) in Arusha, Tanzania from May to June 2018. Fresh mango juice was prepared, diluted with distilled water into 10% mango juice and then mixed with boric acid to obtain 2%, 1%, 0.5%, 0.25% and 0.175% concentrations which were each put into separate cages containing 45 (20 males and 25 females) laboratory reared adult Anopheles gambiae sensu stricto. Mortality was recorded after 24 hours intervals from 2 replicates per each boric acid concentration.

(i) Mortality was calculated using Abbot's Formula and found to be concentration dependent. After 24 hours mortalities of 48%, 55%, 60%, 80% and 96% were recorded for 0.175%, 0.25%, 0.5%, 1.0% and 2% boric acid respectively. After 48 hours the mortalities of 89%, 89%, 95%, 98% and 100% were recorded for 0.175%, 0.25%, 0.5%, 1.0% and 2% boric acid respectively. After 72 hours mortalities of 100% were recorded for all concentrations ranging from 0.175%, 0.25%, 0.5% and 1.0% boric acid.

(ii) Probit analysis at 95% Confidence Interval (CI) to obtain Lethal Dose 50% (LD50) and LD95 of boric acid for 24 hours and 48 hours intervals from SPSS software version 20 resulted into the following; LD50 for 24 hours and 48 hours intervals were 0.683% and 0.196% respectively, LD95 for 24 hours and 48 hours intervals were 2.733% and 0.490% respectively.

(iii) Kaplan-Meir survival analysis from Excel software resulted into the pattern which was interpreted as follows; The lowest survival probability was observed at boric acid concentration of 2% with the maximum of two days while the highest survival probability was observed at 0% which served the control with the maximum of five days.

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Conclusion: The findings of this study have shown that the boric acid sugar baiting method was effective in mosquito control even when used at low concentrations. The exploitation of this intervention which uses attractive toxic sugar bait is important in malaria vector control as it may circumvent the insecticide resistance problem and it is cheap to prepare. It should thus be incorporated in malaria vector control by utilizing boric acid soaked fruit peels and other plant parts which attract mosquitoes like blossoms of Acacia saligna, Tamarix jordanis and Polygonum equisetiforme. Further studies should be done on more vegetational mosquito attractants and the range of safety of boric acid to the environment. Boric acid sugar bait applicability to control other disease vectors such as house flies and tsetse flies should also be evaluated.

Selective suppression of the IRF7–type I interferon amplification axis by plant microRNAs miR-162 and miR-166 in LPS-stimulated microglial cells

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Background: Exosome-like nanoparticles (ELNs) derived from Atractylodes lancea rhizome exert anti-inflammatory effects in microglial cells and contain plant-derived microRNAs (miRNAs), including miR-162 and miR-166. Microglia play a pivotal role in neuroinflammation through the production of pro-inflammatory mediators and type I interferons. Dysregulated activation of interferon regulatory factor 7 (IRF7) and its downstream type I interferon amplification loop has been implicated in chronic neuroinflammatory and neurodegenerative disorders. Increasing evidence suggests that plant-derived miRNAs encapsulated within ELNs may regulate mammalian gene expression and influence innate immune signalling pathways, thereby contributing to cross-species molecular interactions; however, the molecular mechanisms mediated by individual miRNAs remain insufficiently characterised.

Method: BV-2 murine microglial cells were transfected with synthetic miR-162 or miR-166 mimics prior to stimulation with lipopolysaccharide (LPS). Transcriptome profiling was performed using next-generation sequencing to obtain comprehensive gene expression profiles. Differentially expressed genes (fold change ≥ 2) were analysed using Ingenuity Pathway Analysis (IPA) to predict upstream regulators and canonical pathways. Expression levels of IRF7 and inflammatory mediators, including inducible nitric oxide synthase (iNOS), C-X-C motif chemokine ligand 10 (CXCL10), interleukin (IL)-1β, C-C motif chemokine ligand 2 (CCL2), tumour necrosis factor alpha (TNFα) and IL-6, together with selected immune-regulatory genes (CCL12, haem oxygenase-1 [HO-1] and immune-responsive gene 1 [IRG1]), were quantified by real-time polymerase chain reaction. Statistical significance was defined as $p < 0.05$.

Results: Global transcriptomic analysis revealed coordinated suppression of interferon-responsive gene networks in both miR-162- and miR-166-treated cells, indicating broad modulation of innate immune activation. Upstream regulator analysis identified IRF7 as a markedly inhibited regulator in miR-162-treated cells compared with LPS alone (activation z-score −6.02), with a similar inhibitory pattern observed in miR-166-treated cells (z-score −4.75). Type I interferon-associated regulators, including interferon beta 1 (IFNB1), interferon alpha and beta receptor subunit 1 (IFNAR1), signal transducer and activator of transcription 1 (STAT1), stimulator of interferon genes (STING1), tyrosine kinase 2 (TYK2) and mitochondrial antiviral-signalling protein (MAVS), were coordinately suppressed. Canonical pathway analysis demonstrated significant inhibition of interferon-related signalling pathways, including ISGylation signalling (z-score −2.33, $p = 6.08 \times 10^{-8}$) and interferon alpha/beta signalling (z-score −2.83, $p = 5.81 \times 10^{-10}$). Real-time PCR confirmed significant reductions in IRF7 mRNA expression following miR-162 and miR-166 treatment ($p < 0.001$). LPS-induced expression of iNOS, CXCL10, IL-1β, CCL2, TNFα and IL-6 was significantly attenuated (all $p < 0.05$). HO-1 expression was significantly modulated, whereas IRG1 expression showed no significant change.

Conclusion: miR-162 and miR-166 attenuate LPS-induced inflammatory gene expression in microglial cells and selectively suppress the IRF7–type I interferon amplification axis. These findings provide mechanistic evidence that plant-derived miRNAs can regulate innate immune signalling pathways in mammalian cells. Targeted modulation of the interferon amplification cascade may represent a promising and biologically plausible strategy for controlling neuroinflammatory responses characterised by excessive type I interferon activation.

VDAC1 oligomerization-mediated mtDNA release under sublethal oxidative stress: A novel inflammatory mechanism in vitiligo

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Background: Oxidative stress is a critical initiating factor in vitiligo; however, the early molecular events linking redox imbalance to melanocyte immune activation remain unclear. Although high hydrogen peroxide (H2O2) concentrations induce melanocyte apoptosis or pyroptosis, the inflammatory consequences of sublethal oxidative stress—which more accurately reflect early disease stages—are poorly understood. This study investigates whether subtoxic H2O2

triggers a pro-inflammatory response in viable melanocytes via mitochondrial DNA (mtDNA) release and examines the underlying mechanisms involving voltage-dependent anion channel 1 (VDAC1) and the cGAS-STING signalling pathway.

Method: Human epidermal melanocytes (HEMCs) and keratinocytes (HEKCs) were exposed to subtoxic H2O2 (0.1 mM). Cell viability, mitochondrial integrity, and inflammatory profiles were assessed using MTT assays, LDH release, transmission electron microscopy, and western blotting. Cytosolic mtDNA release was quantified via qPCR and visualised using PicoGreen/MitoTracker staining. The roles of the mitochondrial permeability transition pore (mPTP) and VDAC1 oligomerisation were evaluated using cyclosporin A (CsA), the VDAC1 inhibitor VBIT-4, and siRNA-mediated gene silencing. The mtDNA-cGAS-STING axis was confirmed through mtDNA depletion (ethidium bromide), exogenous mtDNA transfection, and cGAS inhibition (RU.521). In vivo efficacy was evaluated in an H2O2-induced vitiligo mouse model using intradermal microneedle delivery of VBIT-4.

Results: Subtoxic H2O2 did not induce melanocyte death or mitochondrial damage but significantly upregulated pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-α/β) and chemokines (CXCL9/10), a response absent in keratinocytes. This inflammation was driven by selective cytosolic mtDNA release, activating the cGAS-STING pathway independently of TLR9 or pyroptosis. Mechanistically, mtDNA release required coordinated inner membrane permeabilisation via mPTP opening and outer membrane disruption through VDAC1 oligomerisation. Genetic VDAC1 knockdown, but not BAX/BAK knockdown, abolished mtDNA leakage. Pharmacological VDAC1 inhibition with VBIT-4 blocked mtDNA release, suppressed cGAS-STING activation, and attenuated downstream inflammatory cascades in vitro. In the H2O2-induced vitiligo mouse model, intradermal VBIT-4 dose-dependently restored melanin pigmentation, reduced cutaneous $CD8^+$ T cell infiltration, and decreased inflammatory mediator expression.

Conclusion: This study identifies a novel mechanism whereby sublethal oxidative stress drives non-apoptotic mtDNA release in melanocytes through coordinated mPTP opening and VDAC1 oligomerisation. The released mtDNA activates the cGAS-STING pathway, establishing a pro-inflammatory microenvironment that facilitates adaptive immune responses in vitiligo. Pharmacological targeting of VDAC1 oligomerisation with VBIT-4 disrupts this cascade, presenting a promising therapeutic strategy for early intervention in oxidative stress-driven pigmentary disorders.

RanolazIne for heart failure with preServed Ejection fraction: RISE-HFpEF trial protocol

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Background: Heart failure with preserved ejection fraction (HFpEF) is defined as a clinical syndrome characterized by signs and symptoms of heart failure, along with a left ventricular ejection fraction (LVEF) greater than 50%, as assessed by echocardiography. The key distinction between HFpEF and heart failure with reduced ejection fraction (HFrEF) is that left ventricular volume is typically normal in the former and increased in the latter. Although numerous therapies have been investigated for HFpEF, an optimal treatment demonstrating a clear mortality benefit has yet to be established with current medications. Ranolazine, an antianginal agent, has shown potential in improving diastolic dysfunction and myocardial relaxation in preclinical studies. This study aims to evaluate the efficacy and safety of ranolazine in patients with HFpEF.

Method: We planned to conduct a two-arm, parallel-group, randomized, double-blind, placebo-controlled, multicenter (8

centres) trial with a sample size of 545. The study protocol was approved by IEC, PGIMER, Chandigarh, registered with CTRI, India (Ref: CTRI/2022/02/040351) and financially supported by the ICMR, India (Ref: 65/01/2021/PD/BMS/PGIMER). The participants incudes adults between 18-75 years, both genders with symptomatic heart failure (NYHA class ≥ II), left ventricular ejection fraction ≤45%, elevated NT-proBNP (>125 pg/mL) are eligible. The participants are centrally randomized (1:1) using computer-generated block-stratified sequences by center and prior heart-failure hospitalization. Baseline assessments include 6-minute walk test, Kansas City Cardiomyopathy Questionnaire, echocardiography, NT-proBNP, and NYHA class, with follow-up visits at 15, 45, 120, and 150 days for adherence and safety, and efficacy reassessed at 3 and 6 months. This investigation builds upon our previous proof-of-concept randomized study (unpublished) involving 30 HFpEF patients, which demonstrated a statistically significant improvement in functional parameters with ranolazine compared with placebo. Expanding on these preliminary findings, the RISE-HFpEF trial is enrolling a larger cohort from a similar patient population to more robustly assess the efficacy and safety of ranolazine in HFpEF.

Results: The trial is currently in its active phase, with patient recruitment and scheduled investigations underway. Its primary objective is to evaluate the efficacy of ranolazine (500 mg twice daily for 24 weeks) compared with placebo in patients with HFpEF. Secondary outcomes include a composite of cardiovascular death, total heart-failure hospitalizations, non-fatal stroke, and non-fatal myocardial infarction over the same 24-week period. The additional secondary outcomes are: (i) percentage of Patients With 5-points or more improvement in KCCQ Clinical Summary Score (CSS) at Week 24; (ii) percentage of Patients With 10-points or more improvement in KCCQ CSS at Week 24; (iii) Change in echocardiographic parameters at baseline,12 weeks and 24 weeks.

Conclusion: The RISE-HFpEF trial is designed to address a critical unmet need in the management of HFpEF by evaluating a new pharmacological approach. The trial will provide clear evidence on the effectiveness of ranolazine in HFpEF.

Gut microbiota enrichment improves glucose and lipid regulation and attenuates blood pressure progression in an oestrogen-deficient rat model

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Background: Menopause-associated oestrogen depletion is strongly linked to increased cardiometabolic risk, characterised by impaired glucose regulation, dyslipidaemia, vascular dysfunction and low-grade systemic inflammation. These alterations contribute to the development of hypertension and cardiovascular disease in postmenopausal women. Although hormone replacement therapy can improve certain cardiovascular parameters, it does not fully restore metabolic homeostasis and its long-term use remains limited. Increasing evidence suggests that gut microbiota plays an important role in the regulation of metabolic pathways, lipid metabolism and vascular homeostasis through the production of bioactive metabolites that influence systemic inflammation and endothelial function. However, experimental evidence directly evaluating whether enrichment of gut microbiota can modulate cardiometabolic alterations associated with oestrogen deficiency remains limited. Understanding this interaction may contribute to the identification of new strategies to reduce cardiovascular risk during menopause.

Method: Female Wistar rats aged 12 months (n = 28) were randomly assigned to four experimental groups (n = 7 per group): sham-operated controls, ovariectomised rats (OVX), ovariectomised rats treated with oestradiol (5 micrograms per kilogram per day), and ovariectomised rats receiving probiotic-based microbiota enrichment (1×10^9 colony-forming units per kilogram per day). Ovariectomy was performed to induce experimental oestrogen depletion. Treatments were administered orally for 20 weeks. Metabolic and physiological assessments were conducted at weeks 5, 10 and 20. Biochemical analyses included blood glucose, total cholesterol, renal biomarkers (urea, creatinine and urea-to-creatinine ratio), and hepatic markers (alanine aminotransferase, alkaline phosphatase, bilirubin, albumin and globulins). Haematological parameters associated with inflammatory status and erythropoiesis were also evaluated. Cardiovascular assessment included systolic arterial pressure and diastolic arterial pressure as well as echocardiographic evaluation of left ventricular structure and systolic function.

Results: Ovariectomy induced progressive metabolic alterations characterised by impaired glucose regulation,

increased total cholesterol levels, changes in hepatic enzyme activity and shifts in haematological parameters associated with inflammatory status compared with sham controls. Ovariectomised animals also developed higher systolic and diastolic arterial pressure during the experimental period. Microbiota enrichment was associated with improved glucose levels and reduced total cholesterol compared with untreated ovariectomised animals. The intervention also modulated renal biomarkers, attenuated elevations in selected hepatic enzymes and improved inflammatory haematological indices. At week 20, systolic arterial pressure was lower in the microbiota-enriched group compared with untreated ovariectomised rats. Echocardiography demonstrated preserved systolic cardiac function across all experimental groups; however, animals receiving microbiota enrichment exhibited lower left ventricular weight, suggesting attenuation of early structural cardiac remodelling.

Conclusion: Enrichment of gut microbiota improved metabolic regulation and attenuated cardiovascular alterations induced by oestrogen depletion. Specifically, improved glucose and cholesterol levels were associated with reduced progression of systolic arterial pressure and lower left ventricular weight, suggesting early cardiovascular protection. Considering the central role of hyperglycaemia and dyslipidaemia in endothelial dysfunction and cardiac remodelling, these findings highlight the potential contribution of microbiota-mediated metabolic regulation to cardiovascular health during menopause. These results support the relevance of the gut–heart axis in cardiometabolic disease and provide experimental evidence that microbiota enrichment may represent a complementary strategy for reducing menopause-associated cardiovascular risk.

Evaluation of the antidiarrheal activity of dichloromethane-methanol crude extract of the aerial parts of Croton kinondoensis (Euphorbiaceae) in mice

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Background: Diarrhea remains a major global health concern, particularly in developing countries. Increasing resistance to conventional therapies highlights the need to investigate plant-based antidiarrheal agents. Croton kinondoensis, a traditional Kenyan remedy for gastrointestinal ailments, has yet to be scientifically validated. This study assessed its antidiarrheal and antimotility effects in vivo mouse models.

Method: Leaf extracts were prepared using dichloromethane/methanol (1:1). Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids and phenolics. Acute toxicity was assessed via OECD guideline 425. Antidiarrheal activity was tested using a castor oil-induced diarrhea model, and gastrointestinal motility was evaluated using the charcoal meal test. Mice were divided into five groups (n = 6): negative control (distilled water), positive control (loperamide 3 mg/kg), and three test groups receiving C. kinondoensis extract at doses of 100, 200, and 400 mg/kg). Statistical significance was determined using ANOVA and Tukey's post hoc test to determine significance ($p < 0.05$).

Results: The extract showed no toxicity at 2000 mg/kg. In the castor oil-induced diarrhea model, the C. kinondoensis extract at 400 mg/kg inhibited diarrhea by 25.57% ($p < 0.05$), compared to 71.43% inhibition by loperamide ($p < 0.001$). The charcoal meal transit test, 400 mg/kg of the extract reduced intestinal transit by 27.98% ($p < 0.01$), compared to 33.43% by loperamide ($p < 0.001$.

Conclusion: C. kinondoensis exhibits dose-dependent antidiarrheal and antimotility effects, supporting its traditional use. Although its efficacy was lower than loperamide, the extract demonstrated significant potential as a natural remedy for diarrhea likely attributable to the presence of flavonoids and tannins known to influence intestinal motility and secretion.

Diosgenin directly engages PIK3CA to rewire renal tubular epithelial cell death in acute kidney injury

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Background: Acute kidney injury (AKI) is a severe clinical condition associated with high mortality. Current therapeutic options are limited due to incomplete understanding of its pathogenesis and a shortage of specific druggable targets. This study aimed to investigate whether diosgenin, an active component derived from Polygonati Rhizoma, confers renal protection by directly targeting PIK3CA to regulate the fate of renal tubular epithelial cells. The primary objective was to identify and validate the direct interaction between diosgenin and the potential AKI target PIK3CA.

Method: An integrated research programme was employed. Network pharmacology, molecular docking, and molecular dynamics (MD) simulations were initially used to screen active compounds from Polygonati Rhizoma and predict their interaction with PIK3CA. The renoprotective effects of diosgenin were subsequently validated in vivo using cisplatin- and ischaemia-reperfusion injury (IRI)-induced murine AKI models, and in vitro using hypoxia/reoxygenation-exposed HK-2 cells. The specific binding between diosgenin and PIK3CA was confirmed through MD simulations, cellular

thermal shift assays (CETSA), and surface plasmon resonance (SPR). The underlying mechanism was investigated by assessing the PIK3CA/AKT signalling pathway and associated cell death markers using western blotting, immunofluorescence, and quantitative real-time PCR (qRT-PCR).

Results: Network pharmacology and molecular docking identified diosgenin as a compound that stably binds to PIK3CA. MD simulations, CETSA, and SPR analyses confirmed that diosgenin specifically targets the ILE459 residue of PIK3CA, stabilising its active conformation. In both AKI mouse models, diosgenin treatment significantly improved renal function, reduced histological damage, and suppressed inflammation, showing superior efficacy compared to curcumin. Mechanistically, the diosgenin-PIK3CA interaction promoted PIK3CA phosphorylation and activated the downstream AKT signalling pathway, which subsequently inhibited both apoptosis and pyroptosis in stressed renal tubular epithelial cells in vitro and in vivo.

Conclusion: This study identifies PIK3CA as a novel therapeutic target in AKI. By directly binding to PIK3CA, diosgenin effectively suppresses multiple programmed cell death pathways, presenting a promising new treatment strategy. These findings establish diosgenin as a potential lead compound and highlight the therapeutic potential of targeting PIK3CA in AKI. Further studies are warranted to evaluate the clinical translatability of this approach.

Increased uremic toxins due to CKD cause mesangial expansion, thereby accelerating CKD progression

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Background: The number of patients with chronic kidney disease (CKD) is increasing. Therefore, establishing reliable treatments for CKD is an urgent priority. This study examines the effects of the prebiotic lactulose on the gut microbiota and kidney function in an in vivo CKD model, and investigates the signals causing mesangial damage by uremic toxins in vitro.

Method and Results: CKD model rats were generated by performing 5/6 nephrectomy. They were compared to a treatment group administered lactulose for 8 weeks to regulate the intestinal flora. Compared to control rats, CKD rats significantly increased albuminuria and decreased creatinine clearance. In contrast, these renal dysfunctions were improved in the lactulose-treated group. Mesangial expansion in CKD rats was measured using Image-J (NIH). The results showed that mesangial expansion was improved in the lactulose-treated group. Analysis of fecal bacterial DNA using next-generation sequencing revealed an increase in the Proteobacteria and Lentispharae in the gut microbiota of CKD rats. Conversely, the lactulose treatment group exhibited a significant increase in the proportion of Bifidobacteria. These findings suggest that the increase in Bifidobacteria and improvement in gut flora induced by lactulose treatment may be involved in protective mechanisms for renal function. Adding indoxyl sulfate to the culture medium of cultured mesangial cells increased protein levels of type 4 collagen. Furthermore, indoxyl sulfate increased mRNA levels of TGF-β and protein levels of phosphorylation of Smad2/3.

Conclusion: Our findings show that in CKD, disruption of the gut microbiota leads to the activation of the TGF-β-Smad2/3-type 4 collagen pathway by urinary toxins, such as indoxyl sulfate. This activation induces mesangial expansion and drives the progression of CKD.

Curcumin- and naringin-derived carbon dots for targeting microglial neuroinflammation and blood-brain barrier penetration

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Background: Neurodegenerative diseases are closely associated with neuroinflammation driven by overactive microglia and excessive oxidative stress; therefore, ameliorating neuroinflammation is considered as an effective strategy for attenuating neurodegenerative disease progression. Carbon dots (CDs) have emerged as promising nanocarriers for neurodegenerative diseases because of their small size, favorable biocompatibility, and inherent ability to penetrate the blood-brain barrier (BBB), making them attractive for brain-targeted delivery. Curcumin and naringin are natural compounds renowned for their anti-neuroinflammatory and neuroprotective effects. However, their poor aqueous solubility, rapid metabolism, limited bioavailability, and inefficient BBB penetration restricted clinical translation. Natural product-derived carbon dots (NP-CDs), synthesized from bioactive compounds, are expected to enhance biocompatibility, BBB penetration, and bioactivity relative to their precursors. In the present study, curcumin- and naringin-derived carbon dots were synthesized, and their effects on neuroinflammation and BBB penetration were evaluated to assess their potential as brain-targeted therapeutics for neurodegenerative diseases and to elucidate underlying mechanisms of action.

Method: Curcumin- and naringin-derived carbon dots are synthesized via a thermal pyrolysis method and have been

physicochemically characterized in our previous studies. Briefly, the precursor compounds are heated to induce carbonization, followed by dissolution in alkaline buffer, ultrasonication, centrifugation, and dialysis purification. Network pharmacology analysis is conducted to predict potential multi-target interaction with neuroinflammatory hub genes. SIM-A9 microglial cells are pretreated with curcumin- and naringin-derived carbon dots at defined concentrations and incubation times prior to LPS stimulation. The secretion of nitric oxide (NO) and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, PGE2) is analyzed using Griess reagent and ELISA kits, respectively. Expression levels of iNOS, COX-2, arginase-1, CD206, signal transducer and activator of transcription 3 (STAT3), MAP kinases (MAPKs; ERK, JNK, and p38), nuclear factor-kappa B (NF-κB), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1) are analyzed by Western blotting. Intracellular and mitochondrial reactive oxygen species (ROS) production is assessed using ROS assay kits. All experiments are performed in triplicate to ensure reproducibility.

Results: Our experimental results showed that curcumin- and naringin-derived carbon dots attenuated the secretion of pro-inflammatory mediators and cytokines, including NO, IL-1β, IL-6, and TNF-α, and decreased the expression of iNOS and COX-2 in a dose-dependent manner. These carbon dots significantly inhibited the phosphorylation of MAPK pathways (p38, JNK, ERK1/2), STAT3, and NF-κB-p65 nuclear translocation. Network pharmacology analysis predicted that curcumin and naringin target hub genes (TNF, IL6, NFKB1, MAPK1/3, and PTGS2) enriched in neuroinflammation-related KEGG pathways, including NF-κB signaling, Toll-like receptor signaling, and neurodegeneration pathways. Protein-protein interaction (PPI) network analysis further identified these genes as central nodes associated with microglia-mediated neuroinflammation, supporting the experimental observations. The effects on PGE2, M2 markers (arginase-1, CD206), antioxidant enzymes (HO-1, NQO1), and intracellular and mitochondrial ROS production are currently being investigated. Potentially superior BBB permeability compared with their precursor compounds (curcumin and naringin) is also under evaluation.

Conclusion: In summary, curcumin- and naringin-derived carbon dots represent promising brain-targeted nanotherapeutics with potential anti-inflammatory activity. They may modulate multiple inflammatory signaling pathways, attenuate ROS, and affect BBB penetration, thereby highlighting their therapeutic potential for neurodegenerative diseases.

Foslinanib subverts multidrug resistance via HMMR-mediated cell cycle orchestration and programmed cell death

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Background: Multidrug resistance (MDR) accounts for over 90% of cancer-related mortalities. To date, no effective targeted therapies have been successfully developed to overcome this challenge because conventional strategies remain stymied by systemic toxicity or compensatory mutations. To address this therapeutic void, the novel 2-phenyl-4-quinolone derivative foslinanib has emerged as a promising candidate currently in Phase II clinical trials. Whilst recent studies have established that foslinanib promotes apoptosis, inhibits vasculogenic mimicry (VM), and modulates cell cycle inhibitory factors, its effectiveness in overcoming multidrug resistance remains unknown. Therefore, this study aims to investigate whether foslinanib subverts multidrug resistance in human cancer cells.

Method: Drug-sensitive (KB) and resistant (KB/Vin) cancer cells, along with normal Flp-In™-293 cells, served as experimental models to evaluate foslinanib efficacy through SRB cell viability and CFSE proliferation assays. Apoptosis induction and cell cycle distribution were analysed via flow cytometry. Central molecular targets were identified using RNAseq followed by weighted gene co-expression network analysis (WGCNA). All quantitative data were analysed using sigmaplot and expressed as mean ± standard error (SE). Comparisons between two experimental groups were performed using the unpaired Student's t-test. For analyses involving more than two groups, one-way analysis of variance (ANOVA) followed by Bonferroni post hoc correction was applied. Statistical significance is defined as a p value less than 0.05.

Results: Foslinanib exhibited potent cytotoxicity across cancer models, with IC50 values determined by SRB assays reaching 0.36 ± 0.02 μM in KB and 0.32 ± 0.01 μM in KB/vin cells. In normal Flp-In™-293 cells, the IC50 value was markedly higher (5.5 ± 0.04 μM), yielding selectivity indices of 15.3 for parental KB cells and 17.2 for resistant KB/vin cells. CFSE proliferation assays further confirmed that foslinanib inhibited cell growth in a time- and dose-dependent manner. Flow cytometric analysis revealed that foslinanib significantly modulated programmed cell death and cell cycle progression. Specifically, 48-hour treatment in KB cells increased the apoptotic population (early and late) from 1.78% to 10.73% at 0.1 μM and 23.83% at 0.2 μM. Notably, KB/vin cells exhibited a substantially more robust response, with

apoptotic rates rising from 2.42% to 18.28% and 43.6% under identical concentrations. Foslinanib also induced significant sub-G1 accumulation—peaking at 50% in KB and 20% in KB/vin cells—accompanied by prominent G2/M phase arrest in both cell lines. To elucidate the underlying molecular mechanisms, transcriptomic profiling via RNA-sequencing and WGCNA was performed on treated KB/vin cells, identifying HMMR as the unique hub gene significantly associated with both poor clinical prognosis and cell cycle orchestration.

Conclusion: Taken together, foslinanib subverts multidrug resistance by inducing robust programmed cell death and G2/M phase arrest. The identification of HMMR as a key hub gene highlights its central role in mitotic orchestration, establishing foslinanib as a promising candidate for MDR-targeted therapeutic interventions.

Development of a novel 2C-aminophenyl chromone derivative as a multi-target agent for triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) remains a clinically challenging breast cancer subtype because of the lack of effective targeted therapies. Despite current treatment with chemotherapy, radiotherapy, and immunotherapy, therapeutic outcomes remain limited. Therefore, the development of novel multi-target small molecules for TNBC treatment is warranted. Chromone is a promising scaffold in medicinal chemistry, and its derivatives have demonstrated anticancer potential. This study aimed to develop a novel 2C-aminophenyl chromone derivative, 6-((2-chlorobenzyl)oxy)-2-((3,4,5-trimethoxyphenyl)amino)-4H-chromen-4-one (TMAC), and to investigate its anti-tumour activity and underlying molecular mechanisms in TNBC.

Method: The anti-tumour effects of TMAC were evaluated in multiple TNBC cell lines using cell viability assays. Its combination effects with chemotherapeutic agents were further assessed by synergy analysis. To investigate the underlying mechanisms, cell-cycle distribution and apoptosis were analysed following TMAC treatment. In addition, a calcein-AM uptake assay was performed to determine whether TMAC inhibited P-glycoprotein (P-gp)-mediated drug efflux. RNA sequencing was subsequently conducted to further explore the molecular mechanisms associated with TMAC treatment. In vivo acute toxicity was assessed using zebrafish, and therapeutic efficacy was further validated in a zebrafish xenograft model.

Results: TMAC exhibited potent cytotoxic activity against multiple TNBC cell lines, including drug-resistant and BRCA-mutant models, with IC_{50} values ranging from 0.5 to 4 μM. It also showed significant synergistic effects with chemotherapeutic agents and olaparib in relevant TNBC models. Mechanistic studies demonstrated concentration-dependent induction of G2/M arrest and apoptosis, together with inhibition of P-gp-mediated efflux. Transcriptomic analysis further supported the involvement of pathways related to apoptosis, immune response, extracellular matrix organisation and adhesion, and MAPK/PI3K signalling. Notably, TMAC showed no significant acute toxicity in zebrafish and effectively suppressed tumour growth in the xenograft model.

Conclusion: Taken together, these findings suggest that TMAC is a promising multi-target agent for TNBC treatment, with potent anti-tumour activity, synergistic potential in combination therapy, and favourable in vivo safety and efficacy.

13-Week repeated dose toxicity study of Longkui Yinxiao soup in sprague dawley rats via oral gavage

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Background: Longkui Yinxiao Soup (LKYXS) is a traditional Chinese prescription commonly used for the treatment of psoriasis. However, limited toxicological information is available for LKYXS in preclinical animal model studies. The purpose of this study was to observe the toxic reactions of Sprague Dawley (SD) rats following 13 weeks of intragastric administration of LKYXS, evaluate the severity of these toxic reactions and identify the target organs of toxicity, thereby providing a basis for predicting potential clinical adverse reactions induced by repeated administration of the test substance.

Method: A total of 160 rats were randomly divided into 4 groups according to body weight: a control group and low-, medium-, and high-dose groups. There were 40 rats per group, with an equal number of males and females. Based on raw materials, the doses for the low-, medium-, and high-dose groups were 14.9 g/kg, 29.8 g/kg, and 59.6 g/kg, respectively, which were approximately equivalent to 19, 38,

and 76 times the human clinical dose. The rats were administered LKYXS by intragastric gavage at a volume of 10 mL/kg per dose, once daily, 7 days per week, for 13 consecutive weeks. The control group was administered the same volume of purified water, followed by a 28-day recovery period. During the administration period, general clinical signs, appearance, and behavioural activity were observed twice daily (before and after administration); during the recovery period, observations were conducted once daily. Body weight and food consumption were recorded once weekly. Necropsies were performed at the mid-administration point (10 rats per group), at the end of the administration period (20 rats per group), and at the end of the recovery period (10 rats per group). Blood samples were collected for the determination of haematology, serum biochemistry, and coagulation parameters. Histopathological examinations were carried out on all collected organs from the control and high-dose groups at each time point. Organ coefficients of the major organs were calculated for all animals.

Results: During the test period and subsequent recovery period, rats in all groups were in good general condition, with normal behavioural activity and respiration. No abnormal secretions were observed from glands such as the salivary glands and lacrimal glands. The fur was smooth, and no obvious abnormalities were noted in faeces or urine. There were no significant abnormalities in weight gain, food intake, haematological indices, serum biochemical indices, or urinary parameters. No obvious drug-related pathological changes were observed in the examined organs of the test animals.

Conclusion: No toxic target organs or toxic reactions related to LKYXS were observed in this study. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was determined to be 59.6 g/kg/day (calculated as raw medicine), which is approximately 76 times the human clinical dose.

Bioinformatic study reveals modulation of NRF2 pathway molecules by aristolactam AIIIA in oncogenic multidrug resistance

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Background: Activation of the NRF2 signaling pathway plays a critical role in reducing oxidative stress and contributes to the development of cancer multidrug resistance (MDR) during chemotherapy. However, NRF2 is the pivotal transcription factor in redox homeostasis for human. Direct NRF2 inhibition has shown to develop toxicities in vivo. Therefore, targeting downstream effectors of the NRF2 pathway may represent an alternative strategy. Aristolactam AIIIa (AAIIIa), an aporphine-type alkaloid isolated from the Fissistigma genus, has been reported to induce oxidative stress, yet its potential role in modulating the NRF2 pathway remains unclear.

Purpose: This study aims to investigate the mechanistic role of AAIIIa in reversing MDR in cancer.

Method: The parental KB cell line and its multidrug-resistant derivative KB/VIN were used as experimental models. RNA sequencing was performed to identify transcriptional changes associated with MDR and AAIIIa treatment. Functional enrichment and KEGG pathway analyses were conducted using Enrichr and Pathview. Gene expression and clinical relevance were further evaluated using public datasets from UALCAN and cBioPortal.

Results: Enrichment analysis demonstrated significant activation of NRF2 signaling and oxidative stress–related pathways in KB/VIN cells compared with KB cells, indicating that redox reprogramming contributes to MDR development. KEGG pathway mapping identified increased expression of NRF2 downstream effectors, including HO-1, NQO1, and thioredoxin reductase 1 (TXNRD1, or TrxR1), in KB/VIN cells. Notably, AAIIIa treatment reduced the elevated expression of these NRF2 target genes, suggesting that AAIIIa may attenuate NRF2 pathway hyperactivation without directly inhibiting NRF2. Analysis of TCGA datasets revealed that NQO1 and TXNRD1 expression increased with tumor stage in patients with head and neck squamous cell carcinoma. Higher expression levels of these genes were also associated with shorter disease-free survival, suggesting potential clinical relevance.

Conclusion: These findings suggest that AAIIIa may modulate NRF2 downstream effectors, including NQO1 and TXNRD1, and may counteract oncogenic multidrug resistance.

Phytochemical composition and antioxidant activity of bay laurel (laurus nobilis l.) leaf extracts and hydrolates from two Croatian geographical regions

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Background: Bay laurel (Laurus nobilis L.) is a Mediterranean plant widely used in traditional medicine and the cosmetic industry owing to its rich content of bioactive secondary metabolites. Polyphenolic compounds, including flavonoids and phenolic acids, are considered the primary contributors to its antioxidant, antimicrobial, and anti-inflammatory properties. Geographical origin is known to influence the biosynthesis of secondary metabolites through differences in climate, soil composition, and solar radiation. This study aimed to determine the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities, and quantitative levels of selected phenolic compounds in bay laurel ethanolic extracts and hydrolates from two geographically distinct Croatian localities and to assess whether geographical origin influences phytochemical composition.

Method: Leaf material was collected from northern (N) and southern (S) Croatian localities. Ethanolic extracts and hydrolates were analysed for total TPC using the Folin-Ciocalteu method (mgGAE/g for ethanolic extracts; mg/L for hydrolates) and TFC using the aluminium chloride colorimetric assay (mg/L). Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (mM TE/mL). Selected phenolic compounds were identified and quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis (mg/L).

Results: TPC was higher in the northern ethanolic extract (78.66 mgGAE/g) than in the southern (74.33 mgGAE/g). TFC was slightly higher in the southern ethanolic extract (3.87 mg/L vs. 3.15 mg/L). DPPH antioxidant activity was markedly higher in the northern ethanolic extract (14.49 ± 4.26 mM TE/mL) compared to the southern (7.65 ± 0.45 mM TE/mL). Hydrolates showed lower TPC (N: 257 mg/L; S: 234 mg/L), lower TFC (N: 0.61 mg/L; S: 0.46 mg/L), and weaker DPPH radical scavenging activity (N: 5.26 ± 0.21; S: 4.06 ± 0.04 mM TE/mL) compared with ethanolic extracts. None of the targeted phenolic compounds were detected in hydrolate samples by LC–MS/MS (below the limit of detection), consistent with the low volatility of most phenolic compounds, which limits their transfer into the aqueous phase during hydrodistillation. In ethanolic extracts, LC–MS/MS identified epicatechin as the dominant phenolic compound, with approximately 2.4-fold higher concentration in the northern extract (0.773 ± 0.048 mg/L) compared to the southern (0.323 ± 0.009 mg/L). Catechin was also higher in the northern sample (0.026 ± 0.003 mg/L vs. 0.018 ± 0.004 mg/L). Myricetin was exclusively detected in the northern extract (0.046 ± 0.0003 mg/L), while quercetin predominated in the southern extract (0.014 ± 0.002 mg/L vs. 0.006 ± 0.001 mg/L).

Conclusion: Bay laurel leaves from different geographical origins exhibited significant quantitative differences in phenolic composition and antioxidant activity. Northern samples showed higher antioxidant capacity, greater TPC, and elevated concentrations of epicatechin and catechin, while myricetin was detected exclusively in the northern extract, and quercetin predominated in the southern extract. Hydrolates also reflected geographical variability, with samples from the northern locality generally exhibiting higher TPC, TFC, and antioxidant activity than those from the southern locality. Overall, these findings suggest that geographical origin influences the accumulation of phenolic compounds in Laurus nobilis leaves, which may be relevant for the selection and standardisation of plant material for pharmaceutical applications.

Can chronic psychosocial stress increase dopaminergic vulnerability? Evidence from human dopaminergic neurons and a PINK1B9 Drosophila model of Parkinson's disease

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Background: Parkinson's disease is a common, age associated neurodegenerative disorder for which current therapies remain largely symptomatic, reinforcing the need to identify modifiable upstream risk factors that could inform prevention or disease modifying strategies. Stress is widely reported to worsen symptoms in people with Parkinson's disease, but it remains unclear whether chronic psychosocial stress can contribute to dopaminergic pathology in otherwise healthy individuals. To address this gap in our knowledge, we used complementary human cell culture and Drosophila models to test whether sustained stress signalling promotes dopaminergic vulnerability and Parkinsonian like outcomes, and whether effects depend on the specific stress mediator.

Method: For in vitro experiments, Lund human mesencephalic cells were differentiated into post mitotic dopaminergic neurons, with maturation confirmed by dopaminergic marker expression. Cultures were exposed for 7 days to either cortisol or corticotropin releasing factor (two

principal mediators of the stress response) in separate arms. Dopaminergic neuron viability, mitochondrial markers, and Parkinson's disease relevant protein expression profiles were assessed using immunohistochemistry and confocal microscopy. For in vivo experiments, wild type Drosophila melanogaster and a Parkinson's disease model (PINK1B9 mutant) underwent a 7 day unpredictable stress schedule: cold shock (day 1), heat shock (day 2), starvation (day 3), vibration (day 4), followed by continuous light cycle disruption/sleep deprivation (days 5–7). PD-relevant phenotypes such as survival rate, negative geotaxis motor performance, and immunohistochemical dopaminergic neuron counts in adult brains were quantified.

Results: In vitro, chronic cortisol exposure significantly reduced dopaminergic neuron viability and cell count, accompanied by changes in neuronal fibre density consistent with dopaminergic dysfunction. In contrast, chronic corticotropin releasing factor exposure produced an opposing profile, suggesting mediator specific effects on dopaminergic resilience. In vivo, the unpredictable stress schedule reduced survival rate and significantly altered motor performance in both wild type and PINK1B9 mutant flies. Stress exposure was also associated with reduced dopaminergic neuron counts in adult brains across genotypes. Overall, the data indicate that chronic stress can increase dopaminergic vulnerability, with divergent effects depending on the specific stress mediator.

Conclusion: Across human dopaminergic neurons and Drosophila models, sustained stress signalling was associated with dopaminergic toxicity and Parkinsonian like outcomes, and effects differed by mediator. These findings support stress linked pathways as plausible targets for mechanistic follow up and for prevention focused or disease modifying strategies in Parkinson's disease.

Synergistic enhancement of nitric oxide-mediated inhibition of platelet activation by luteolin

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Background: Excessive platelet activation contributes to both arterial and venous thrombosis, thereby increasing the risk of cardiovascular disease (CVD). Endogenous nitric oxide (NO) plays a critical role in inhibiting platelet aggregation; however, reduced NO bioavailability in CVD enhances platelet reactivity. Therefore, strategies aimed at restoring NO-mediated platelet inhibition are of significant clinical importance. Suppression of reactive oxygen species (ROS), phosphoinositide-3-kinase (PI3K), or cyclin-dependent kinase 2 (CDK2) signalling has been shown to potentiate NO-mediated platelet inhibition. Notably, luteolin, a naturally occurring antioxidant flavonoid widely present in fruits and vegetables, has been reported to inhibit both PI3K and CDK2 signalling pathways, suggesting its potential to enhance NO-mediated platelet inhibition. However, the precise mechanisms by which luteolin modulates NO-mediated platelet responses remain incompletely understood. This study aimed to investigate how luteolin modulates NO-mediated platelet inhibition through key signalling pathways.

Method: The blood samples were collected from healthy human donors and washed platelets were prepared. The platelets were divided into four groups: (1) vehicle group, (2) DEA/NONOate group, (3) luteolin group, and (4) DEA/NONOate plus luteolin group. Platelet functions were evaluated using 96-well plate aggregometry, light transmission aggregometry, ATP release assays, flow cytometry, and confocal microscopy. ROS generation and vasodilator-stimulated phosphoprotein (VASP) phosphorylation, as well as PI3K and CDK2 signalling were also analysed. In addition, rats were administered luteolin, and ex vivo platelet aggregation was evaluated using DEA/NONOate.

Results: Luteolin significantly enhanced DEA/NONOate-induced inhibition of platelet aggregation. The inhibitory effects of DEA/NONOate on ATP release, P-selectin expression, and activated GPIIb/IIIa were additionally augmented in the presence of luteolin. DEA/NONOate-mediated activation of VASP and suppression of ROS generation and AKT phosphorylation were further enhanced by luteolin. In addition, activation of CDK2 signaling with calyculin A reversed the synergistic inhibitory effect of luteolin and DEA/NONOate on platelet aggregation. Moreover, platelets from luteolin-treated rats showed enhanced inhibitory responses to DEA/NONOate.

Conclusion: Luteolin potentiates NO-mediated inhibition of platelet activation via ROS, PI3K, and CDK2 signalling pathways. These findings provide mechanistic insight into the regulatory role of luteolin in platelet function and thrombotic processes. Luteolin may therefore represent a promising candidate for the prevention of thrombotic events and could offer a potential therapeutic strategy for reducing thrombotic risk associated with cardiovascular diseases.

Evaluation of the anti-proliferative and immunomodulatory activities of pterostilbene in human melanoma and keratinocyte cell lines: In vitro and in silico studies

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Background: Malignant melanoma is one of the most aggressive and treatment-resistant forms of cancer, necessitating the development of novel pharmacological strategies. Pterostilbene, a natural dimethyl ether analogue of resveratrol found in berries and grapes, has emerged as a promising nutraceutical due to its high bioavailability and multifaceted biological activities, including antioxidant, anti-inflammatory, and chemopreventive effects. While its potential in various malignancies is documented, its specific impact on transformed skin cell proliferation and the cutaneous immune response requires comprehensive in vitro evaluation.

Purpose: The objective of this study was to investigate the anticancer activity of pterostilbene (2.5 – 60 µM) against human malignant melanoma cells (amelanotic C32 and melanotic A2058 cell lines) and transformed HaCaT keratinocytes. Specifically, the research assessed the ability of pterostilbene to modulate the secretion of pro-inflammatory cytokines, interleukin-6 (IL-6) and interleukin-8 (IL-8), under both basal and interleukin-1 (IL-) stimulated conditions. Furthermore, this study aimed to predict in silico the possible interactions of pterostilbene with related pathways, diseases, and genes.

Method: Cell proliferation was assessed using the sulforhodamine B assay after a 72-hour incubation period. For analysis of immunomodulatory activity of pterostilbene, the secretion of IL-8 and IL-6 was measured using a sandwich-type ELISA. To simulate inflammatory stress, cells were stimulated with IL-1. The in silico predictive study was performed using the ChemDIS-Mixture system.

Results: The findings revealed that pterostilbene significantly inhibited the proliferation of both melanoma cells and keratinocytes in a dose-dependent manner; however, the inhibitory effect was more pronounced in melanoma cell cultures. The results also demonstrated that pterostilbene modulated the secretion of the investigated cytokines. The analysis of the results regarding the effect of pterostilbene on the release of proinflammatory cytokines showed that this compound can inhibit or increase the secretion of IL-8 and IL-6, and the observed effect depends on the concentration and stimulation with proinflammatory cytokine.

Conclusion: Pterostilbene may have significant potential in the prevention and treatment of malignant melanoma and may become important in the future. It can also modulate immune functions of melanoma cells and keratinocytes through regulation of proinflammatory cytokines secretion. However, to ensure its effectiveness and safety, further research is necessary to understand the precise mechanisms of its action.

Peptidomimetic inhibitors of phosphatidylinositol-3 kinase as drug for trypanosomes

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Background: African trypanosomiasis is a neglected tropical disease, also known as sleeping sickness, that imposes significant health and economic burdens in sub-Saharan Africa, with limited therapeutic options and emerging drug resistance. The causative single-celled protozoan parasite, Trypanosoma brucei, is transmitted by the tsetse fly and can infect humans and mammals through a blood meal. T. brucei evades host antibodies through antigenic variation, which results from periodic switching of its variant surface glycoprotein (VSG) coat. The genome contains over 2,500 VSG genes, but only one VSG gene is transcribed at a time from a telomeric expression site (ES). Antigenic variation occurs by transcriptional switching between ESs or by recombination of VSG genes within the transcribed ES. Our lab's previous studies identified that some enzymes in the phosphatidylinositol (PI) signalling pathway are implicated in the regulation of VSG expression and switching. Phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P3] regulates VSG gene silencing and VSG switching, and disruption of this process is lethal to the parasite. This study aimed to determine whether PI(3,4,5)P3 synthesis mediated by phosphatidylinositol 3-kinase (PI3K) is essential for T. brucei survival and represents a potential drug target.

Method: Metabolomic analyses were performed to characterize phosphoinositide species produced by T. brucei. Recombinant T. brucei PI3K was expressed and purified from Escherichia coli, and enzymatic activity was assessed using PI and phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) as substrates. Peptidomimetic compounds were screened for inhibitory activity against bloodstream-form parasite growth. Target engagement was evaluated by PI3K overexpression in parasites and in vitro inhibition assays to determine the mechanisms of inhibition.

Results: T. brucei produced multiple phosphoinositides, including PI, PI3P and other phosphoinositides, with a low abundance of PI(4,5)P2 and PI(3,4,5)P3. Recombinant PI3K phosphorylated PI and PI(4,5)P2, generating PI3P and PI(3,4,5)P3, respectively. PI3K enzyme kinetics revealed a KM of 96.6 µM and a Vmax of 0.78 µmol·min^{-1} for PI, whereas PI(4,5)P2 exhibited a KM of 216 µM and a Vmax of 0.44 µmol·min^{-1}. Two peptidomimetics designed against PI3K, IPP-102 and IPP-103, inhibited bloodstream-form T. brucei growth with IC50 values of 5 µM and 10 µM, respectively. Both compounds also demonstrated antiparasitic activity against the related parasite Trypanosoma cruzi, with no significant cytotoxicity towards Vero cells, indicating selective toxicity towards parasites. Overexpression of PI3K increased resistance to both compounds, supporting on-target activity. In vitro, IPP-102 and IPP-103 inhibited recombinant PI3K activity as competitive (Ki = 4.4 µM) and uncompetitive (Ki = 3.2 µM) inhibitors, respectively. In vivo efficacy in murine infection models and antiparasitic activity against Leishmania spp. are currently being evaluated to further assess the translational potential of these compounds.

Conclusion: These findings demonstrate that PI3K-dependent PI(3,4,5)P3 synthesis is essential for T. brucei viability and identify PI3K as a promising therapeutic target. Peptidomimetic inhibitors represent a potential new class of antiparasitic agents and support further development of PI3K-targeting strategies for the treatment of African trypanosomiasis.

Neuroprotective effects of goji berry (Lycium spp.)

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Background: Neurodegenerative disorders are driven by oxidative stress, neuroinflammation, mitochondrial dysfunction, and neuronal apoptosis. Goji berries (Lycium barbarum, Lycium chinense) are rich in polyphenols and polysaccharides, which have demonstrated significant neuroprotective potential through multiple biological pathways.

Aim: To integrate current evidence on the neuroprotective effects of goji berry bioactive compounds and elucidate their underlying molecular and functional mechanisms.

Method: A structured analysis of four key studies (two experimental animal studies and two reviews) was performed, focusing on biochemical, molecular, and behavioral markers of neuroprotection, including oxidative stress, neurotrophic signaling, apoptosis, and neuroplasticity.

Results: Polyphenols (e.g., chlorogenic acid, quercetin, rutin) exhibit strong antioxidant and anti- inflammatory activity, reducing reactive oxygen species and protecting neurons from
oxidative damage. Polysaccharides (LBP) showed pronounced neuroprotective and psychotropic effects in ovariectomized rat models. They increased antioxidant enzyme activity (SOD, CAT, GPx), reduced lipid peroxidation (MDA), and enhanced expression of brain-derived neurotrophic factor (BDNF) and serotonergic receptors (5-HT2A), while decreasing neuronal apoptosis.
Behaviorally, LBP reduced anxiety and depression like symptoms, confirming functional neuroprotection. Additionally, goji berry supplementation improved neuroplasticity by enhancing dendritic morphology and synaptic protein expression in the hippocampus and prefrontal cortex, alongside reduced neuroinflammation and apoptotic markers.

Conclusion: Goji berry bioactive compounds exert neuroprotective effects through multiple complementary mechanisms, including antioxidant defense, modulation of neurotrophic factors, anti-inflammatory action, inhibition of apoptosis, and enhancement of neuroplasticity. These findings support their potential application in the prevention and management of neurodegenerative and mood disorders. However, further clinical studies are necessary to confirm their efficacy in humans.

Evaluation of the anti-oxidant properties and phytochemisrty of the methanol extract of the dried leaves of Rhizophora Racemosa (Rhizophoraceae)

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Background: Reactive oxygen species (ROS) generated during normal cellular metabolism can induce oxidative stress when produced in excess, leading to cellular damage and contributing to the development of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. The increasing limitations associated with

synthetic antioxidants, including safety concerns and reduced efficacy, have intensified the search for bioactive compounds from natural sources. Medicinal plants remain a rich reservoir of antioxidant agents; however, many species with potential therapeutic value are yet to be fully investigated. Rhizophora racemosa, a mangrove plant widely distributed in tropical regions, has been utilised in traditional medicine, but its antioxidant properties and chemical composition require further scientific validation.

Purpose: This study was designed to evaluate the antioxidant and free radical scavenging activity of the crude methanolic extract of Rhizophora racemosa leaves, characterise its phytochemical constituents, and determine its acute toxicity profile in an experimental animal model.

Method: Fresh leaves of Rhizophora racemosa were collected, air-dried, and pulverised before extraction using methanol through maceration. Preliminary phytochemical screening was conducted using standard qualitative methods to identify the presence of secondary metabolites. The antioxidant activity of the extract was assessed using the DPPH radical scavenging assay, and the inhibitory concentration (IC_{50}) was calculated to determine potency. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA). Acute toxicity evaluation was carried out in Wistar albino rats using Lorke's method to establish the safety profile of the extract. Additionally, gas chromatography–mass spectrometry (GC–MS) analysis was employed to identify and characterise the chemical constituents present in the extract.

Results: Phytochemical screening revealed the presence of flavonoids, terpenoids, saponins, phenolic compounds, and carbohydrates. The extract demonstrated significant antioxidant activity, with an IC_{50} value of 5 µg/mL, indicating strong free radical scavenging capacity when compared with ascorbic acid used as a reference standard. No mortality or observable signs of toxicity were recorded during the acute toxicity study, suggesting that the extract is relatively safe at the tested doses. GC–MS analysis identified several bioactive compounds, with 3,4-octadiene,7-methyl- identified as the predominant constituent, alongside other minor components.

Conclusion: The findings indicate that the methanolic leaf extract of Rhizophora racemosa possesses potent antioxidant activity and a favourable safety profile, supporting its potential as a natural source of therapeutic antioxidants. Further studies focusing on isolation, structural elucidation, and in vivo pharmacological evaluation of the active compounds are recommended to advance its development for clinical applications.

Probiotics and synbiotics affect oxidative stress and inflammation in HT-29 cells and APP/PS1 mouse model

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The human gut microbiome plays a crucial role in maintaining health by influencing various physiological processes through complex interactions with the host. Among the strategies to modulate this microbiome, probiotics and synbiotics have garnered significant attention for their potential health benefits. The current study designs optimize and test a novel probiotic and synbiotic formulation consisting of 3x10^9 CFU/ml three metabolically active probiotics, Lactobacillus plantarum, Lactobacillus fermentum, and Bifidobacteria infantis, together with 0.5% novel polyphenol-rich prebiotic, Triphala, for the synbiotic formulation. Additionally, the effects of such probiotics and synbiotics on HT-29 cells on oxidative stress and inflammation were studied. Moreover, the formulations were administered for 4 weeks in the 3-month-old female APP/PS1 mouse model to assess both oxidative stress and inflammation markers. The body weight was assessed daily. The results showed that after 24 hours, the synbiotic treatment increased HT-29 cell proliferation by 2-fold. Further, the reactive oxygen species (ROS) and IL-8 levels significantly decreased in HT-29 cells treated with synbiotics compared to the control. This result is in line with the findings in the APP/PS1 models. Altogether, the present study suggested that probiotic and synbiotic treatments were effective, as shown in the proliferation, oxidative stress, and inflammation levels in both human colon cancer cells and the APP/PS1 mouse model.

Selective Modulation of Fibrin Architecture Using AI-Designed Cyclic Peptides to Promote Endothelialization of Blood-Contacting Biomaterials

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Background: Failure of long-term endothelialization remains a central limitation preventing the clinical success of laboratory-manufactured small-diameter vascular grafts. Persistent fibrin deposition at the blood–material interface forms a dense barrier that impairs endothelial migration and promotes inflammatory responses despite systemic anticoagulation. Emerging evidence indicates that fibrin architecture, rather than fibrin presence alone, governs immune modulation and tissue regeneration. We hypothesized that selective modulation of fibrin polymerization could transform fibrin from a pathological

barrier into an in situ regenerative scaffold supporting endothelialization.

Method: An artificial intelligence–driven peptide discovery pipeline integrating RFDiffusion, ProteinMPNN, AlphaFold2, and Rosetta was implemented to design cyclic peptides targeting the fibrinogen hole B interface during clot assembly. Over 120,000 peptide candidates were computationally generated and sequentially filtered using energy scoring and structural validation. The top 100 peptides were chemically synthesized and evaluated in vitro using human fibrinogen and thrombin clot polymerization assays. Fibrin architecture was assessed through turbidity kinetics as a surrogate marker of clot porosity and network organization.

Results: Three cyclic peptides (CyFi25, CyFi49, and CyFi61) significantly increased fibrin turbidity in a dose-dependent manner, consistent with the formation of more porous fibrin networks. Half-maximal turbidity enhancement occurred at 5.7, 8.1, and 7.2 µM respectively, representing greater than ten-fold potency improvement compared with a reference PF4-derived hole B peptide binder (111 µM). Computational design enabled enhanced structural stability and target specificity while minimizing undesired interference with fibrin hole A interactions associated with clot destabilization.

Conclusion: Peptide-mediated modulation of fibrin assembly represents a novel therapeutic paradigm that preserves physiological coagulation while promoting regenerative immune responses at biomaterial interfaces. AI-designed cyclic peptides provide a scalable strategy to enhance endothelialization of vascular grafts and other blood-contacting medical devices, potentially addressing a longstanding barrier to translational cardiovascular biomaterials.

Investigating P. aeruginosa transcriptomes of hospital sink drain isolates and the influences of acid stress on copper's antimicrobial actions

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Background: Pseudomonas aeruginosa is a Gram-negative, opportunistic, multidrug-resistant pathogen frequently detected in healthcare sink drains and water systems, where it can act as a persistent reservoir for hospital-associated infection. In water systems, copper sink drain pipe materials are gaining renewed interest in healthcare facilities due to their antimicrobial properties. However, copper's mechanisms of action are challenging to resolve since this metal also acidifies its environment as copper concentrations increase (>0.5 mg/ml Cu^{2+}). This work compares transcriptomes of P. aeruginosa strains PAO1 to a French pathogen sink drain isolate, PA-8, which possesses a genomic island (GI-7) enriched with copper tolerance genes. Gene expression profile exposed to either 2 mg/ml $CuSO_4$ or to its equivalent acidified medium pH 5.5 is explored. A PA-8 ΔGI-7 knockout mutant is also included. We hypothesize that the GI-7 island confers significant copper tolerance as compared to PAO1, which lacks this element, and that acid stress genes overlap and contribute to copper's mechanism of action and gene expression profile at high (2mg/ml) copper concentrations.

Method: Copper sulfate ($CuSO_4$) and acid tolerance (pH 5.5) transcriptomes of three strains (PAO1, PA-8, and PA-8 ΔGI-7) were determined in Luria–Bertani bio-triplicate cultures grown to mid-log phase (OD_{600} = 0.5). Cultures were washed and then exposed to either 2 mg/mL $CuSO_4$ or pH 5.5 media for 3 hours. Cells were subsequently harvested, and mRNA was extracted for RNA-Seq transcriptomic analysis. Survival was assessed following 72-hour incubations of all three strains in sterile water with and without 600 µg/L $CuSO_4$.

Results: After 72 hours, PAO1 exhibited early copper susceptibility at 18 hrs, with no viable cells recovered following plating, whereas both PA-8 and PA-8 ΔGI-7 survived copper exposure up to 15 days. Transcriptomic analyses of Cu^{2+} and acid-exposed P. aeruginosa revealed that PAO1 showed Cu^{2+} exposure significantly increased differentially expressed genes (DEGs) as compared to pH 5.5 stress (up-regulated Cu^{2+}; 731 vs. pH 5.5; 348, and down-regulated Cu^{2+}; 376 vs pH 5.5; 44). In PA-8, 1461 and 251 DEGs were up- and down-regulated, respectively, after Cu^{2+} exposure as compared to pH 5.5 (356 up, 39 down). PA-8 ΔGI-7 exhibited fewer Cu^{2+} induced changes to DEGs (1,023 up and 178 down) but more acid-induced DEGs (450 up, 19 down). These findings suggest that Cu^{2+} exposure and stress trigger greater DEG responses than acid exposure. It also suggests the GI-7 region plays a notable role in Cu^{2+} tolerance, as its deletion reduced Cu^{2+} DEG responses. Analysis of gene functions reveals notable overlaps in acid- and Cu^{2+}-stress/tolerance genes under both acid and Cu^{2+}-treatments, suggesting that both gene-expression pathways contribute to copper resistance mechanisms.

Conclusion: The findings from our work indicate that copper induces broader transcriptional stress responses than acid alone and identify key genes involved in copper and acid stress, providing a foundation for elucidating mechanisms underlying copper tolerance and susceptibility in P. aeruginosa.

Natural anticoagulant potential of brassica oleracea: Bridging phytochemistry and pharmacy for cardiovascular health

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Background: Cardiovascular diseases remain one of the leading causes of morbidity and mortality worldwide. Thromboembolic disorders, resulting from abnormal activation of the coagulation cascade and platelet aggregation, significantly contribute to cardiovascular complications. Conventional anticoagulant drugs such as Heparin and Warfarin are widely used in clinical practice for the prevention and treatment of thrombosis. However, their use is often associated with several limitations including bleeding risk, narrow therapeutic index, and significant drug–food interactions. These challenges highlight the need for safer and more accessible therapeutic alternatives. Plant-derived bioactive compounds have attracted considerable attention due to their diverse pharmacological properties and relatively favourable safety profiles. Brassica oleracea (cabbage) is a commonly consumed cruciferous vegetable that contains a variety of phytochemicals such as flavonoids, phenolic compounds, glucosinolates, and anthocyanins. Bioactive flavonoids including Quercetin and Kaempferol have been reported to possess antioxidant, anti-inflammatory, and antiplatelet activities. Despite these promising biological properties, the anticoagulant potential of cabbage has not been extensively investigated. Therefore, the present study aims to evaluate the phytochemical composition and anticoagulant activity of Brassica oleracea extracts using established in vitro assays.

Method: Fresh green and red cabbage samples were collected from local markets in Hyderabad and authenticated prior to analysis. The plant material was washed, shade-dried, and powdered before extraction. Sequential extraction was carried out using solvents of increasing polarity including hexane, ethyl acetate, ethanol, and water. The resulting extracts were concentrated and preserved under suitable laboratory conditions for further evaluation. Preliminary phytochemical screening was performed to identify major classes of secondary metabolites including phenolics, flavonoids, tannins, coumarins, and alkaloids. Quantitative determination of total phenolic content and total flavonoid content was conducted using standard spectrophotometric techniques and HPLC analysis.

The anticoagulant activity of the extracts was evaluated using pooled citrated human plasma through standard coagulation assays. These assays included Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and Thrombin Time (TT), which assess the extrinsic, intrinsic, and common coagulation pathways respectively. Platelet aggregation studies were also performed using platelet-rich plasma stimulated with adenosine diphosphate, collagen, and arachidonic acid to evaluate antiplatelet activity.

Results: Phytochemical analysis confirmed the presence of flavonoids, phenolic compounds, and other secondary metabolites in the cabbage extracts. Quantitative estimation indicated appreciable levels of total phenolic and flavonoid content, suggesting the presence of bioactive constituents with potential pharmacological activity.
In vitro coagulation assays demonstrated that selected extracts of Brassica oleracea produced a concentration-dependent prolongation of PT, aPTT, and TT values compared with control samples. These findings suggest that the extracts may influence multiple pathways of the coagulation cascade. Platelet aggregation studies further indicated moderate inhibition of platelet aggregation induced by standard agonists, supporting potential antiplatelet activity.

Conclusion: The study demonstrates that Brassica oleracea contains bioactive phytochemicals with promising anticoagulant and antiplatelet properties. The observed prolongation of clotting parameters and inhibition of platelet aggregation suggest potential therapeutic relevance in the prevention of thromboembolic disorders. These findings highlight the importance of exploring plant-derived compounds for cardiovascular health applications and support further investigation through in vivo studies and clinical evaluation.

Polypharmacological approaches to pain management: Mapping evidence, clinical translation and research gaps through a scoping review

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Background: Pain management, particularly in chronic and neuropathic conditions, involves complex and overlapping biological pathways that are often inadequately addressed by single-target therapies. Polypharmacological drugs, which act on multiple targets within a single molecule, represent a promising strategy to improve analgesic efficacy and potentially reduce treatment burden. However, uncertainty remains regarding which polypharmacological analgesics have been systematically studied, how their mechanisms contribute to pain modulation, and the extent to which preclinical findings are supported by clinical evidence. A consolidated overview is therefore needed to inform future research and clinical decision-making.

The objective of this scoping review was to identify and characterise polypharmacological analgesics investigated in

pain management, assess the balance between preclinical and clinical evidence, and identify key gaps limiting clinical translation.

Method: A scoping review was conducted following the Arksey and O'Malley methodological framework and reported in accordance with PRISMA-ScR guidelines. Primary preclinical and clinical studies published in English between 2000 and 2025 were identified through systematic searches of PubMed, Embase, Web of Science, and Oria. After duplicate removal, 3,768 records were screened. Eligible studies were charted and analysed using inductive thematic analysis to synthesise mechanisms of action, pain models, and reported outcomes.

Results: Ten studies met the inclusion criteria: nine were preclinical, and one was clinical. Identified polypharmacological strategies included dual inhibition of fatty acid amide hydrolase and monoacylglycerol lipase, as well as modulation of transient receptor potential vanilloid 1 channels. The single clinical study evaluated tapentadol in patients with diabetic polyneuropathy. While all studies reported improved pain modulation, clinical evidence was scarce, and reporting of adverse effects, dose-response relationships, and interaction risks was limited.

Conclusion: Research on polypharmacological analgesics is currently dominated by preclinical investigations, with limited translation into robust clinical evidence. Methodological heterogeneity, non-standardised pain models, and insufficient safety data restrict clinical applicability. This work contributes to defining a future research agenda in pain pharmacotherapy by clarifying key evidence gaps and translational barriers, and by emphasising the need for coordinated mechanistic, translational, and clinical studies that bridge preclinical findings with real-world pain conditions and support the rational development and use of polypharmacological strategies.

Localised m1Ψ-engineered GDF11 messenger RNA lipid nanoparticle therapy reprogrammes the diabetic wound microenvironment through coordinated metabolic and immune restoration

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Background: Chronic diabetic wounds and foot ulcers remain refractory to currently available biologic therapies and account for a substantial proportion of preventable lower-limb amputations worldwide. The diabetic wound microenvironment is characterised by persistent inflammation, impaired angiogenesis, defective re-epithelialisation, excessive fibrosis, and profound metabolic dysfunction. Recombinant growth factor therapies have shown limited and inconsistent clinical benefit due to short biological half-life, rapid local degradation, and failure to correct underlying metabolic abnormalities that sustain chronic inflammation. Messenger RNA (mRNA) therapeutics provide transient, controllable in situ protein expression without genomic integration and may overcome these limitations. While mRNA therapeutics have achieved clinical success in vaccines and are advancing in oncology and metabolic disease, their application in chronic regenerative wound disorders remains unexplored. We therefore evaluated whether chemically stabilised GDF11 mRNA delivered via lipid nanoparticles could reprogramme the diabetic wound microenvironment and accelerate tissue repair.

Method: GDF11 mRNA was synthesised using N1-methyl-pseudouridine substitution with optimised 5′ cap and 3′ poly(A) structures to enhance translational efficiency and minimise innate immune activation. The transcript was encapsulated in lipid nanoparticles engineered to facilitate cellular uptake and endosomal escape following local administration. Transfection efficiency and protein expression were assessed in CFS and human umbilical vein endothelial cells and compared with liposomal delivery systems. Therapeutic efficacy was evaluated in murine dorsal wound models, streptozotocin-induced diabetic wound models, and diabetic rat foot ulcer models. Dose-ranging studies (0.0005–0.5 μg/mL) were conducted to determine optimal concentration and administration route. Single-cell RNA sequencing was performed to characterise cellular composition, differential gene expression, and pathway-level remodelling within treated wounds.

Results: m1Ψ-engineered GDF11 mRNA delivered via lipid nanoparticles achieved robust local protein expression with significantly greater efficiency than liposomal delivery and recombinant GDF11 protein. A single subcutaneous administration at 0.05 μg/mL resulted in sustained protein expression and significantly accelerated wound closure in diabetic models compared with phosphate-buffered saline and recombinant controls. Subcutaneous delivery demonstrated superior efficacy relative to topical administration, supporting the importance of local tissue bioavailability. Single-cell transcriptomic analysis identified 29 clusters re-annotated into 11 functional cell populations. Treatment increased keratinocyte, endothelial cell, and smooth muscle cell proportions, consistent with enhanced re-epithelialisation and vascular remodelling, while reducing excessive myeloid and plasma cell infiltration. Differential gene enrichment revealed 345 shared regulators associated with cytokine-mediated signalling, interferon-gamma response, antigen processing and presentation, leukocyte chemotaxis, and adaptive immune activation. Mechanistically, GDF11 mRNA therapy activated AMP-activated protein kinase, phosphatidylinositol-3-kinase/Akt, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha pathways. This enhanced insulin receptor substrate-1 phosphorylation, increased glucose transporter 4 expression, promoted glucose uptake and oxidative

phosphorylation, reduced hyperglycaemia-associated metabolic stress, improved mitochondrial activity, and attenuated collagen over-deposition and fibrotic remodelling.

Conclusion: Localised delivery of m1Ψ-engineered GDF11 mRNA via lipid nanoparticles induces coordinated metabolic and immunological reprogramming of the diabetic wound microenvironment and significantly accelerates tissue repair. By demonstrating dose optimisation, route-dependent efficacy, superiority over recombinant protein, sustained in situ expression, and systems-level cellular remodelling after a single administration, this work establishes localised messenger RNA-based protein replacement as a scalable and mechanistically grounded therapeutic platform for chronic regenerative disorders.