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A Referred Journal of the
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Udaipur, Rajasthan, Bharat



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Pacific Journal of Medical and Health Sciences (ISSN: Print - 2456-7450) is a Multidisciplinary, International Peer Reviewed Quarterly Journal of the Pacific Medical University, Udaipur, Rajasthan, Bharat.

The Journal is published quarterly in the months of January, April, July and October.

The subject areas for publication include Multidisciplinary Subjects within Medical Sciences, viz., Anatomy, Anaesthesia, Biochemistry, Biomedical Sciences, Cardiology, Community Medicine, Dermatology and Venereal Diseases, Diabetes, Endocrinology, Epidemiology and Public Health, Forensic Science, Gastroenterology, Geriatric Medicine, Hematology, Immunology, Infectious Diseases, Internal Medicine, Microbiology, Nephrology, Neurology, Neurosurgery, Obstetrics and Gynaecology, Ophthalmology, Oncology, Orthopaedics, Otorhinolaryngology (ENT), Paediatrics, Pathology, Pharmacology, Physiology, Psychiatry, Pulmonary Medicine, Radiology, Toxicology, Dentistry, Nursing, Health Informatics, and Occupational Safety and Health.

Aims and Scope

Pacific Journal of Medical and Health Sciences is a peer reviewed journal with multidisciplinary approach. The goal of the journal is to publish new, challenging and radical ideas, dedicated to promote innovative and high quality research work in the field of medical and health sciences. The journal provides a platform for advances in basic and advanced clinical medical research for all branches of health-sciences. The journal provides cutting edge updates, developments in the medical arena and helps to synchronise and share knowledge.

The key aims of the Journal are to provide interpretations of growing points in medical knowledge by trusted experts in the field, and to assist practitioners in incorporating not just evidence but new conceptual ways of thinking into their practice.

We focus on the clinical aspects of diseases with basic science contributions in areas of clinical interest.

The journal invites articles related to different aspects of Medical and allied Health Sciences including Dental Sciences. The journal publishes original research articles, reviews, case reports and commentaries. The journal is an important and reliable source of current information on developments in the field of medical and health sciences. The emphasis is always on publishing high quality articles with fast review process.

Please note that Call for Papers, Peer-Review Policy, Reviewers' Guidelines, Publication Ethics and Publication Malpractice Statement and Disclaimer have been provided at the end of the issue.

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Publisher's Note

It is with great pride and a deep sense of purpose that Pacific Medical University presents this issue of Pacific Journal of Medical and Health Sciences to our readers, authors, reviewers, librarians and colleagues across India and throughout the world.



When this journal was established in 2017, it was founded on a conviction that remains as vital today as it was then: that high-quality medical research must not be the preserve of those with the means to access it. Knowledge that can save lives, improve clinical outcomes and shape the future of healthcare belongs to every physician, every researcher, every student and every curious mind that seeks it, regardless of where they are located or what resources they have at their disposal.

This conviction has guided every decision we have made as a publisher. It is why every issue of this journal is distributed free of charge to the libraries of medical colleges, Indian Institutes of Technology and Indian Institutes of Management across India. It is why every published article is made freely available online at www.medicaladvances.ac.in under the Creative Commons Attribution 4.0 International Licence. It is why we send a printed copy of every issue, by Registered Speed Post, to every contributing author, including those based internationally, at no cost to them whatsoever. And it is why we have never charged authors a single rupee in submission fees, processing charges or any other levy. All costs are borne entirely by us, as publisher, because we believe this is the right thing to do.

Over time, this journal has grown from a quarterly publication serving the medical community of Rajasthan into an international peer-reviewed platform reaching readers and authors across India and beyond. We have published landmark special issues on COVID-19, Platelets, Chrono-Nutrition and Trace Elements, and Diabetes, each representing our commitment to addressing the most significant health challenges of our time with the rigour and depth they deserve.

In keeping with our commitment to continuous improvement and international alignment, we wish to inform our authors, reviewers, readers and institutional partners of a forthcoming development in the governance structure of this journal. In pursuance of the recommendations of the Directory of Open Access Journals (DOAJ), the globally recognised authority on open-access publishing standards, and in alignment with the guidelines of the International Committee of Medical Journal Editors (ICMJE), the Editorial Board of this journal is scheduled to be formally reconstituted. This reconstitution has been undertaken with specific objectives: to ensure robust editorial independence, to broaden the geographical and institutional diversity of the Board's composition, to reduce institutional endogeneity in editorial

decision-making, and to align the journal's governance architecture with the internationally accepted standards that govern peer-reviewed academic publishing of the highest order.

It is our considered view that a journal of this standing, scope, and ambition must be governed by an Editorial Board whose composition reflects the breadth, diversity, and independence that the global academic community rightly expects. The reconstitution will bring to the Board more distinguished scholars, clinicians, and researchers from a wider spectrum of institutions, disciplines, and geographies, thereby strengthening the intellectual rigour, peer-review integrity, and international credibility of every issue that bears this journal's name.

I wish to place on record my sincere appreciation for the contributions of all current members of the Editorial Board. Their association with this journal, their support for its mission, and their commitment to the advancement of medical knowledge is acknowledged with deep respect and gratitude. The transition ahead is not a departure from the values that have guided this journal since its establishment in 2017. It is, rather, an affirmation of them, a recognition that the pursuit of excellence is a continuing journey, and that governance structures must evolve as journals grow in reach, reputation, and responsibility.

Of course, none of this would be possible without the scholars who entrust us with their research, the reviewers who give generously of their time and expertise, the editorial team whose dedication is unwavering, and the readers whose engagement gives this journal its purpose. To each of them, I express my profound and enduring gratitude.

We remain committed to upholding the highest standards of open-access publishing, editorial independence and publication ethics. We look forward to continuing this journey with you.



(Aman Agrawal)

Executive Director

On behalf of Pacific Medical University

Udaipur, Rajasthan, Bharat

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AURUM CEREBRI

The Golden Architecture of the Human Mind



The cover of this issue of the Pacific Journal of Medical and Health Sciences presents a work of deliberate and purposeful symbolism. A translucent golden human form, rendered in anatomical profile, carries within its cranial vault a fully revealed brain - luminous, intricately detailed, and unmistakably alive.

This is not mere artistic abstraction. It is a precise and medically informed representation of the organ that defines our humanity, rendered in gold to honour its unparalleled complexity and clinical significance.

The image reveals, with anatomical fidelity, the major architectural landmarks of the central nervous system. The cerebral cortex, with its characteristic gyri and sulci crowns the composition, its folded surface a testament to evolutionary refinement. Beneath it, the limbic circuit glows with particular intensity, illuminating the hippocampus and amygdala - the twin pillars of memory consolidation, emotional regulation, and stress response. The thalamus, rendered as the great relay nucleus it is, sits at the structural and functional crossroads of sensory integration and conscious awareness. Posteriorly, the cerebellum presides over motor coordination, postural equilibrium, and increasingly recognised cognitive functions. The brainstem descends with quiet authority into the cervical spine, carrying within it the neural pathways that sustain life itself.

This image marks a conscious and meaningful transition in the visual identity of this journal. Our previous volume was defined by the golden heart - a symbol of cardiovascular medicine, metabolic science, and the enduring primacy of cardiac health. But medicine - like science itself - moves forward. And in this volume, the locus of inquiry shifts upward, from the chest to the cranium, from the myocardium to the neuron, from the heartbeat to the synaptic impulse.

The 'Golden Brain' of Volume 11 reflects the extraordinary breadth of neurological and neuropsychiatric science represented within these pages. From the glymphatic clearance of amyloid-beta in Alzheimer's disease, to the role of lithium deficiency in neurodegeneration, to the neurochemical signatures of anxiety disorders, to the emerging gut-brain axis in depression - this issue bears witness to a renaissance in our understanding of the brain as a dynamic, plastic, and profoundly vulnerable organ. The Music-Brain Axis, a category unique to this journal, further underscores the remarkable capacity of auditory experience to reshape neural architecture, modulate limbic activity, and influence cognitive outcomes across the lifespan.

The golden hue of the image is itself a statement. Gold does not corrode, does not diminish, and does not yield to the passage of time. It is the metal of endurance, of value, of the irreplaceable. To render the brain in gold is to assert what every neuroscientist, every neurologist, every psychiatrist, and every patient living with neurological illness already knows instinctively: that the brain is the most precious and the most fragile structure in the human body. Its protection, its nourishment, and its restoration are among the highest obligations of medicine.

The neural pathways descending through the cervical spine remind us that the brain does not exist in isolation. It is in constant, bidirectional conversation with the body it governs, regulating cardiovascular function through autonomic efferents, modulating immune responses through neuroendocrine axes, and receiving metabolic and inflammatory signals from the periphery that profoundly shape its own biology. The brain, as this cover eloquently suggests, is both sovereign and servant of the body it inhabits.

We invite our readers - clinicians, researchers, educators, and students of medicine - to engage with this issue in the spirit embodied by its cover: with curiosity, with rigour, and with a deep reverence for the extraordinary complexity of the organ that makes all of science, all of medicine, and all of human understanding possible.

~The Editor

From Heart to Brain: The Next Frontier of Medicine

There is something quietly poetic about the way a journal finds its own rhythm. For four consecutive issues across the entirety of 2025, the cover of the Pacific Journal of Medical and Health Sciences carried a single, commanding image: a 'Golden Anatomical Heart', its coronary vessels gleaming, its chambers luminous against a deep black background. It was not an arbitrary choice. It was a declaration of intent. Volume 10 was a “Year of the Heart”, a year in which this journal turned its sustained attention to the frontiers of cardiovascular medicine, metabolic science, diabetes therapeutics, and endocrine innovation. Across four issues, we charted the emergence of GLP-1 receptor agonists as cardiovascular agents, the precision of factor XIa inhibition in stroke prevention, the revolution of CRISPR-based lipid control, and the extraordinary potential of AI-driven cardiac diagnostics. The Golden Heart served its purpose magnificently.

And yet, as 2025 drew to a close and preparations began for Volume 11, it became clear that medicine itself was signalling a shift. Not a departure from the cardiovascular frontier, which remains as urgent and as fertile as it has ever been, but an expansion. The future of medicine increasingly depends on recognising that organs do not function in isolation but as interconnected systems governed by complex biological communication networks. An upward movement. A migration of the spotlight, with all its attendant intensity and expectation, from the chest to the cranium. From the myocardium to the neuron. From the rhythmic contraction of cardiac muscle to the electrochemical precision of the synaptic impulse. The 'Golden Heart' gave way to the 'Golden Brain'. And in that transition, the Pacific Journal of Medical and Health Sciences finds itself, as it always has, at the intersection of science and the human story.

The Brain: An Organ Unlike Any Other

To speak of the brain as simply an organ is to commit a magnificent understatement. It is, by any measure, the most complex structure in the known biological world. One hundred billion neurons, each forming an average of seven thousand synaptic connections with neighbouring cells, generate a network of extraordinary complexity. The human connectome, if laid end to end, would stretch approximately 150,000 kilometres. The cerebral cortex alone contains roughly sixteen billion neurons arranged in six distinct laminar layers, each with precise connectivity patterns that subserve specific cognitive, motor, sensory, and emotional functions. And yet, for all its complexity, it weighs less than one and a half kilograms and consumes approximately twenty percent of the body's total energy supply while representing only two percent of its mass.

What makes the brain the next great frontier of medicine is not merely its structural complexity, remarkable as that is. It is the convergence of three simultaneous forces: a global epidemic of neurological and neuropsychiatric disease of staggering proportions; a revolution in our understanding of brain biology that is rendering decades of received wisdom obsolete; and the emergence of transformative technologies, from artificial intelligence to optogenetics to closed-loop neural prosthetics, that are making it possible to observe, interact with, and ultimately treat the brain in ways that were unimaginable even a decade ago. These three forces are converging simultaneously, and the clinical and scientific consequences are already reshaping medical practice.

Cardiology as Blueprint: A Lesson for Neuroscience

The history of modern medicine has often been defined by its response to the organs that most visibly threaten human survival. Among these, the Heart has occupied a central position. The ability to understand cardiovascular physiology, recognise disease mechanisms, and develop increasingly sophisticated interventions transformed medicine during the twentieth century. Conditions once considered sudden and irreversible, including myocardial infarction, heart failure, and life-threatening arrhythmias, gradually became preventable or manageable through advances in pharmacology, imaging, surgery, and interventional techniques. For the latter half of the twentieth century, the cardiovascular system was the primary focus of modern clinical medicine. We mapped its electrophysiology with precision, opened blocked coronary arteries using percutaneous interventions, and developed pharmacological therapies that transformed cardiovascular disease from a frequent death sentence into a manageable chronic condition. The discovery of statins, the refinement of coronary bypass surgery, and the widespread implementation of acute coronary syndrome protocols represent a monumental collaborative triumph in clinical science. We learned to stabilize the heart and, in doing so, we bought our patients decades of additional life. However, as medicine advances further, a new frontier is emerging. The focus is gradually shifting from an organ traditionally viewed as the centre of survival to one that defines human existence itself, the Brain. This transition does not represent merely a change from cardiology to neurology; rather, it reflects a deeper evolution in our understanding of human biology. In reality, no organ functions in isolation, and medicine is only beginning to reckon with that truth.

To understand the future of neuroscience, it is instructive to examine how medicine transformed cardiovascular disease. The revolution in cardiology was not the result of a single discovery. It was a systemic shift in how medicine viewed the disease process. Large-scale epidemiological studies, beginning with the Framingham Heart Study, transformed cardiology into a science of predictive risk factors, focusing aggressively on hypertension, dyslipidaemia, and insulin resistance. Cardiology became mechanistic, preventative, and interventionist. It learned to identify vulnerability before catastrophe and to act before the point of irreversibility.

The growing recognition of the heart brain axis illustrates this changing paradigm. Cardiovascular health has long been associated with neurological outcomes through conditions such as stroke, hypertension, vascular disease, and atrial fibrillation. Yet contemporary research has revealed that the relationship between the cardiovascular and nervous systems extends far beyond vascular events. The heart and brain communicate continuously through autonomic pathways, hormonal signals, immune mediators, and metabolic interactions. Disturbances in one system influence the other, creating a biological continuum rather than separate disease categories. This understanding has profound clinical implications. The brain is among the most metabolically demanding organs in the human body, requiring a constant supply of oxygen and nutrients. Even subtle abnormalities in vascular function, inflammation, or metabolic regulation can influence neuronal health long before obvious neurological symptoms emerge. The prevention of cognitive decline, therefore, may begin decades before a patient develops memory impairment. Management of cardiovascular risk factors may ultimately prove to be one of the earliest forms of neuroprotection.

The transformation of neuroscience itself has accelerated this shift. For much of medical history, the brain was studied primarily through anatomy, its regions, pathways, and visible structures. Modern neuroscience has moved far beyond this approach. The brain is now understood as a dynamic network of billions of neurons, supported by complex interactions with glial cells, immune mechanisms, vascular systems, and molecular pathways. The development of advanced imaging techniques, computational neuroscience, and molecular biology has allowed researchers to explore not only where activity occurs in the brain but also how information is processed and how networks adapt or fail. Technologies such as functional magnetic

resonance imaging, diffusion imaging, and molecular imaging have provided unprecedented insight into brain organisation. The emergence of connectomics, the study of neural connectivity at large scale, has changed the way scientists conceptualise brain function. Instead of viewing the brain as a collection of isolated regions responsible for individual tasks, neuroscience increasingly recognises it as an integrated communication network in which cognition, memory, emotion, and behaviour arise from interactions among multiple systems.

At the molecular level, the understanding of neurological disease has undergone an equally dramatic transformation. Neurodegenerative disorders, once considered inevitable consequences of ageing, are now recognised as complex biological processes involving genetic vulnerability, abnormal protein handling, mitochondrial dysfunction, altered immune responses, and chronic inflammation. Diseases such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative conditions are no longer viewed through a single pathological lens but as disorders arising from multiple interacting mechanisms.

The Scale of the Neurological Crisis

The numbers are, by any standard, alarming. Neurological disorders are now the leading cause of disability-adjusted life years globally, surpassing cardiovascular disease in this critical metric. Dementia affects more than fifty-five million people worldwide, a figure projected to reach one hundred and fifty million by 2050, driven by ageing populations across Asia, Africa, and Latin America. In India alone, conservative estimates suggest over eight million individuals are currently living with dementia, with the true burden likely considerably higher owing to diagnostic under-recognition in rural and semi-urban settings. Alzheimer's disease, which accounts for approximately sixty to seventy percent of dementia cases, remains without a disease-modifying therapy that is both accessible and unequivocally effective at scale.

One of the most important discoveries in this field has been the changing perception of the brain's immune environment. Microglia, the resident immune cells of the central nervous system, were traditionally regarded as simple protective cells responsible for removing cellular debris. Current research demonstrates a much more sophisticated role. Microglia regulate synaptic connections, respond to injury, influence neuronal survival, and maintain the delicate balance required for normal brain function. However, prolonged or dysregulated activation may contribute to neurodegeneration by promoting chronic inflammation and neuronal injury. This insight has shifted therapeutic strategies. The goal is no longer simply to eliminate abnormal proteins or target a single disease marker. Instead, researchers are increasingly attempting to restore the broader biological environment that allows neurons to remain healthy. This represents a major conceptual shift from treating the consequences of neuronal loss to understanding and modifying the processes that cause neuronal vulnerability.

The challenge of neurodegenerative diseases has become particularly urgent because of global demographic changes. As life expectancy increases, disorders affecting cognition and neurological function are becoming among the greatest healthcare challenges of the modern era. Unlike many acute illnesses, neurodegenerative diseases develop silently over years or decades. By the time clinical symptoms become apparent, significant biological changes may already have occurred. This has intensified the search for early biomarkers capable of identifying disease before irreversible damage develops. Advances in blood-based biomarkers, cerebrospinal fluid analysis, genetic profiling, and digital monitoring are transforming the possibility of early detection. The future of neurology may therefore move away from diagnosing disease only after symptoms appear and toward identifying biological vulnerability before functional decline begins. Such a transformation would represent the true arrival of preventive neurology. Instead of asking only how to treat a patient who has developed dementia or movement disorders, medicine may increasingly ask how to preserve brain health throughout life.

Beyond dementia, the neuropsychiatric burden is equally profound. Depression affects more than three hundred million people globally and is the single leading cause of disability worldwide. Anxiety disorders, affecting a further two hundred and eighty million individuals, are now understood to have neurobiological substrates as measurable and as reproducible as any cardiological biomarker. This issue of PJMHS presents in its 'Recent Medical Advances' section compelling evidence of both: a meta-analysis from the University of California Davis documenting a consistent, transdiagnostic reduction in prefrontal cortical choline across anxiety disorder subtypes, and a landmark multicentre cohort study from Germany demonstrating that high consumption of sugary soft drinks is associated with increased depression risk, mediated by gut microbiome disruption and the gut-brain axis. These are not soft science findings. They are peer-reviewed, biologically grounded, and clinically actionable.

Parkinson's disease, multiple sclerosis, epilepsy, stroke, traumatic brain injury, and the long-term neurological sequelae of SARS-CoV-2 infection collectively account for an economic and human burden that dwarfs most other disease categories combined. The neurological consequences of COVID-19 alone, ranging from cognitive impairment and peripheral neuropathy to encephalitis and cerebrovascular events, have added a new and poorly understood dimension to global brain health that this journal addresses directly in the current issue.

Alzheimer's Disease: A Paradigm in Transition

Nowhere is the transformation of neuroscience more dramatic than in our evolving understanding of Alzheimer's disease. For decades, the dominant framework held that amyloid-beta plaques were the primary initiating event in Alzheimer's pathogenesis, with tau neurofibrillary tangles, neuroinflammation, and synaptic loss following as downstream consequences. This framework guided drug development for more than thirty years and, until recently, produced a sequence of clinical trial failures that left neurologists and patients alike deeply discouraged.

The science is now moving with remarkable speed in several simultaneous directions. A landmark study from Harvard Medical School and Rush University, published in *Nature* in August 2025 and presented in the 'Recent Medical Advances' section of this issue, has identified lithium deficiency as a potentially fundamental and early event in Alzheimer's pathogenesis. The study demonstrates that amyloid-beta plaques actively sequester endogenous brain lithium, progressively depleting its availability and triggering a downstream cascade characterised by GSK3-beta activation, suppression of Wnt signalling, tau hyperphosphorylation, and microglial pro-inflammatory polarisation. In mouse models, reducing cortical lithium by approximately fifty percent accelerated all hallmarks of Alzheimer's disease, while supplementation with lithium orotate reversed these changes and restored memory. This finding does not displace the amyloid hypothesis; it enriches it, offering a new mechanistic node and a potentially accessible therapeutic target.

Simultaneously, pharmacological enhancement of glymphatic function is emerging as a compelling preventive strategy. The glymphatic system, identified only in 2012, is a brain-wide waste clearance network driven by cerebrospinal fluid flow through astrocytic perivascular channels. It operates predominantly during sleep, removing amyloid-beta, tau, and other metabolic waste products that accumulate during waking hours. Another study demonstrates that gentle cutaneous stimulation of the face and neck can double brain waste clearance through glymphatic mechanisms, offering a non-pharmacological and readily accessible approach to neurodegeneration prevention.

The Metabolic Brain: A Bridge Between Two Volumes

One of the most consequential insights bridging our previous volume on Diabetes and metabolic medicine with this volume on brain health is the emerging concept of cerebral insulin resistance. The brain, which accounts for only two percent of total body weight yet consumes approximately twenty percent of the body's circulating glucose, is exquisitely sensitive to metabolic impairment. When neurons in the hippocampus and prefrontal cortex become resistant to insulin signalling, they lose the energy required to maintain synaptic connections, long-term potentiation, and the molecular machinery of memory consolidation. This framework, sometimes described as Alzheimer's disease as a form of Type 3 Diabetes, has moved from hypothesis to active clinical investigation. Ongoing trials are examining the repurposing of GLP-1 receptor agonists, agents this journal championed extensively in its 2025 diabetes issue, for their neuroprotective and anti-inflammatory properties within the central nervous system. The neurologist of the future will need to be deeply conversant with metabolic medicine, and the endocrinologist with neurological risk. The frontier between these specialties is dissolving, to the benefit of patients and practitioners alike.

Neuroplasticity, the Music-Brain Axis, and the Expanding Boundaries of Neuroscience

One of the most intellectually exhilarating developments in contemporary neuroscience is the systematic dismantling of the once-prevailing view that the adult brain is essentially fixed and immutable. The concept of neuroplasticity, the capacity of the brain to reorganise its structure and function in response to experience, learning, injury, and environment, has moved from theoretical framework to clinical reality with breathtaking speed. This journal has consistently championed this science, and nowhere more distinctively than in its dedicated category: The Music-Brain Axis in the 'Recent Medical Advances' section.

The studies presented in this category in the current issue collectively make a remarkable case. Music training in children not only accelerates reading acquisition through enhanced cortical tracking of speech, but bridges socioeconomic learning gaps that resist conventional pedagogical interventions. Neural resonance theory illuminates the universal biological mechanisms through which rhythm synchronises brain oscillations across individuals, explaining why groove responses to music transcend culture and geography. Acoustic stimulation modulates mechanosensitive gene expression and adipocyte differentiation, suggesting that sound operates as a biological signal at the cellular level. And a landmark multinational cluster-randomised trial, the MIDDEL study conducted across six countries, examines with rigorous methodology, the clinical effectiveness of group music therapy and recreational choir singing for dementia and depression in care home residents, yielding findings that are nuanced, culturally contextualised, and methodologically exemplary.

It is worth noting that the scientific investigation of music as medicine is not without deep historical roots in the Indian tradition. The classical discipline of 'Raga Chikitsa', the therapeutic application of specific ragas through the precise acoustic frequencies and emotional registers encoded within them, has long held that targeted musical exposure can induce measurable physiological states, stimulate parasympathetic dominance, and alleviate neuropsychiatric symptoms. Contemporary functional MRI and quantitative EEG research is beginning to provide a neurobiological vocabulary for what Indian classical musicians and healers have understood intuitively for centuries. That this ancient wisdom and modern neuroscience are converging is not a coincidence. It is a reminder that the brain has always been responsive to beauty, to rhythm, and to the healing power of sound.

Beyond music, this issue presents a study of arresting simplicity and profound implication: spontaneous eye blinks synchronise with musical beats during passive listening, and this synchronisation correlates with white matter microstructure in the left superior longitudinal fasciculus. This finding, published in PLOS Biology, reveals an entirely new dimension of auditory-motor integration operating below the threshold of conscious awareness, with potential implications for understanding timing disorders in Parkinson's disease, ADHD, and developmental coordination disorder.

Artificial Intelligence and the Brain: A Partnership of Extraordinary Promise

Perhaps no development in contemporary neuroscience and clinical neurology carries more transformative potential than the deepening partnership between Artificial Intelligence (AI) and Brain Science. This partnership operates on at least three distinct levels: AI as a tool for understanding brain biology, AI as a clinical diagnostic instrument, and AI as an inspiration for understanding cognition itself.

At the diagnostic level, the evidence presented in the 'Recent Medical Advances' section of this issue is striking. A deep learning model developed at Yale School of Medicine and validated across three independent international cohorts, including the UK Biobank population study of over forty-five thousand participants, detects hypertrophic cardiomyopathy from images of standard twelve-lead ECGs with an AUROC of 0.95 in internal testing and 0.91 in the UK Biobank, using nothing more sophisticated than a photograph of a printed ECG taken on a smartphone. While this study sits within our cardiovascular section, its methodological architecture represents a template for AI-driven neurological diagnostics directly applicable to disorders from epilepsy to dementia.

At the frontier of neural interface technology, this issue presents a proof-of-concept study from the University Miguel Hernandez, Elche, Spain, demonstrating that a bidirectional closed-loop cortical visual prosthesis can accurately predict perceptual thresholds, brightness, and the minimum intertrain interval for separate phosphene perception from simultaneously recorded neural activity in blind human volunteers. This represents a fundamental advance over open-loop stimulation paradigms, establishing for the first time that real-time neural feedback can be used to adaptively optimise stimulation parameters in a human visual cortex implant. The implications for restoring functional vision in individuals blinded by retinal or optic nerve disease are profound.

Beyond diagnostics and therapeutics, AI is transforming our understanding of the brain itself. The magnetically guided stem cell microrobots presented in this issue, steered by external magnetic fields and activated by ultrasound to differentiate into neurons at precise anatomical targets, represent a convergence of robotics, materials science, stem cell biology, and neural engineering that would have been considered premature even a decade ago. That it is now published peer-reviewed science is a measure of how rapidly the boundaries of the possible are moving.

The Gut-Brain Axis: Psychiatry's New Frontier

Among the most consequential conceptual shifts in contemporary neuroscience and psychiatry is the recognition that the brain is not an isolated sovereign but a deeply embedded participant in a vast biological conversation that extends throughout the body. The gut-brain axis, the bidirectional communication network linking the enteric nervous system, the gut microbiome, the immune system, and the central nervous system through vagal, hormonal, and metabolic pathways, has emerged over the past decade as one of the most fertile and clinically relevant territories in all of medicine.

This issue in its 'Recent Medical Advances' section presents two studies that illuminate different dimensions of this axis with considerable force. The German multicentre JAMA Psychiatry cohort study demonstrates that high consumption of sugary soft drinks is associated with significantly increased depression risk. The gut bacterium *Eggerthella* was identified as a significant mediator, accounting for approximately four percent of the effect on major depressive disorder diagnosis. Women showed a seventeen percent higher risk of depression with high soft drink intake. Separately, a cross-sectional study published in *Exploratory Research and Hypothesis in Medicine* presents intriguing associational evidence that oral microbiota transmission between intimate partners may contribute to the propagation of depression and anxiety symptoms, with salivary cortisol elevation as a measurable indicator of physiological stress activation in healthy spouses of affected partners.

These studies, taken together, reinforce a vision of psychiatric illness that is simultaneously more biological and more social than the paradigms that preceded them. Depression is not merely a disorder of serotonin or of cognition. It is a disorder of microbial ecology, of metabolic signalling, of inflammatory cascades, and of the extraordinarily intimate biological connections that human beings share with one another. This is psychiatry growing into neuroscience, and neuroscience growing into medicine.

From the Clinic to the Cosmos: The Breadth of This Issue

An Editorial for a journal of this breadth cannot do justice to every study within its pages. What it can do is gesture toward the extraordinary range of scientific inquiry that this issue represents. In our surgical and regenerative medicine category in the 'Recent Medical Advances' section, a bidirectional cortical visual prosthesis restores the possibility of functional vision to the blind. A subretinal photovoltaic implant achieves meaningful visual restoration in geographic atrophy secondary to age-related macular degeneration. In our oncology section, AI-enhanced proteomics detects silent hepatic and pancreatic malignancies at a stage when intervention remains possible, while engineered probiotics and oncolytic viral therapies bring precision immunotherapy into an era of previously unimaginable specificity.

In public health and nutrition, the collapse of the food matrix in ultra-processed foods is shown to impair satiety and metabolism through mechanisms that transcend simple nutrient composition, while a large clinical trial demonstrates that daily multivitamin supplementation is associated with measurable slowing of biological ageing as assessed by epigenetic clock analysis. Asteroid Ryugu has yielded the complete set of nucleobases, sugars, and phosphates necessary for genetic material, suggesting that the molecular precursors of life may be far more widely distributed across the cosmos than previously imagined. And in our maternal health section, thyroid function within the conventional normal range during pregnancy is associated with elevated gestational diabetes risk, a finding with immediate clinical implications for antenatal screening protocols across India and beyond.

A Note on the Cover

The 'Golden Brain' that would grace PJMHS's cover for the entirety of 2026 was conceived not merely as an aesthetic choice but as a statement of editorial intent. It shows a golden human form in anatomical profile, its cranial vault transparent, revealing the brain within in luminous detail. The cerebral cortex, the hippocampus, the limbic circuit, the thalamus, the cerebellum, the brainstem, the descending spinal pathways, all rendered in gold against darkness. It is, simultaneously, a precise anatomical illustration and a meditation on value. Gold does not corrode. It does not diminish. It endures. To render the brain in gold is to assert what every physician, every neuroscientist, every neurologist, every psychiatrist, and every patient living with neurological illness already knows: that the brain is the most precious and the most fragile

structure in the known universe, and that its protection, its nourishment, and its restoration are among the highest obligations of medicine.

The transition from heart to brain across the covers of this journal reflects a broader movement in global medicine. It does not signal an abandonment of cardiovascular science, which continues to generate findings of extraordinary clinical importance within these very pages. It signals, rather, an expansion of perspective, a willingness to follow the science wherever it leads, and a recognition that the next great chapter of medicine will be written in the language of neurons, synapses, and the remarkable plasticity of the most extraordinary organ that nature has ever produced.

Closing Reflection

Medicine has always advanced by shifting its gaze. From the gross anatomy of the Renaissance to the Cellular Pathology of Virchow, from the Bacteriological Revolution of Pasteur and Koch to the Molecular Biology of Watson and Crick, from the Genomic Era of the Human Genome Project to the Precision Medicine of today, each great transition has been preceded by a period in which the tools, the concepts, and the courage necessary to see something new have converged. We are living through such a convergence now, and its subject is the brain. The human brain is no longer a dark box beyond the reach of meaningful intervention. The pathways to treatment are being mapped by relentless scientific inquiry. As we navigate this frontier, we must do so with intellectual humility, rigorous clinical standards, and an unwavering commitment to improving patient outcomes.

The Pacific Journal of Medical and Health Sciences enters Volume 11 with the quiet confidence of a publication that has always understood its purpose: to bring the best of global medical science to the clinicians, researchers, educators, and students who need it most, in a form that is rigorous without being forbidding, accessible without being superficial, and always in the service of better medicine and better lives.

The cardiovascular pioneers of the twentieth century gave our patients the gift of time. The mandate of the twenty-first century is to ensure that the minds experiencing that time remain healthy, vibrant, and profoundly human. The heart introduced medicine to the power of intervention. The brain now invites medicine to explore the possibilities of preservation, restoration, and renewal. The future of healthcare will not simply be about adding years to life. It will be about protecting the very capabilities that make those years meaningful.

The 'Golden Brain' on our cover is not a boast. It is a promise. We invite you to read, to question, to debate, and above all, to be inspired. The next frontier of medicine is before us. It is golden, it is complex, it is fragile, and of course, it is magnificent.

A handwritten signature in black ink that reads "R. Bangur". The signature is written in a cursive, slightly stylized font. Below the signature is a long, horizontal, slightly wavy line that extends across the width of the signature.

(Ravindra Bangar)
Editor

Research Paper

Subacute Exposure to Diethyl, Dibutyl and Di-(2-ethylhexyl) Phthalates Disrupts Systemic Bioenergetics, Lipid Homeostasis and Spermatogenesis in Male Rats

Fatima Omowunmi Hamzat^{1,2}, Ismail Adetayo Lawal², Aisha Abdulrauf^{2*}, Abdulmuiz Oluwatobi Adebisi², Aminat Temitayo Atoyebi², Kamaldeen Kabirat Opeyemi², Soliu Ibrahim Olashile², Sulaiman Muhammad Eze² and Babatunde Joseph Dare^{1,2}

¹Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Ilorin, Nigeria.

²Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, Ilorin, Nigeria.

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***Corresponding Author Email:** aabdulrauf@alhikmah.edu.ng

ABSTRACT

Background:

Phthalate esters are pervasive endocrine-disrupting chemicals implicated in metabolic and reproductive dysfunction. However, integrated evidence linking systemic bioenergetics, lipid dysregulation, and spermatogenic impairment under equidose subacute exposure remains limited.

Objective:

This study investigated the effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on systemic bioenergetics, lipid homeostasis, and testicular function in male rats.

Methods:

Fifty male Sprague–Dawley rats were randomized into five groups (n=10) and administered DEP, DBP, or DEHP (50mg/kg/day) orally for 30 days. Serum bioenergetic markers (G6PDH, LDH-X), lipid profile (TC, TG, LDL, HDL), semen parameters, and testicular histology were assessed.

Results:

Phthalate exposure induced a coordinated metabolic–reproductive disruption. G6PDH activity was significantly reduced, while LDH-X levels were elevated in DBP and DEHP groups, indicating impaired redox balance and cellular integrity. Atherogenic dyslipidaemia was evident, with increased TC, TG, and LDL alongside decreased HDL, most pronounced in

DEHP-treated rats. Semen analysis revealed significant declines in sperm concentration, motility, morphology, and viability across exposed groups, with severity ranking DEHP > DBP > DEP. Histologically, progressive degeneration of seminiferous tubules was observed, ranging from mild epithelial disruption (DEP) to severe architectural collapse and germ cell loss (DEHP), corroborated by PAS-demonstrated depletion of glycogen-rich germ cells.

Conclusion:

Subacute phthalate exposure disrupts systemic metabolic homeostasis and induces a bioenergetic–oxidative bottleneck that propagates into testicular structural degeneration and impaired spermatogenesis. These findings establish a mechanistic continuum linking metabolic dysfunction to male reproductive toxicity and highlight the heightened potency of DEHP. This integrative model underscores the need to contextualize male infertility within environmental–metabolic risk frameworks.

KEYWORDS: Phthalates, Bioenergetics, Dyslipidaemia, Spermatogenesis, Testicular toxicity

BACKGROUND

Phthalate esters such as diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) are ubiquitous plasticizers and well-documented endocrine-disrupting chemicals with weak estrogenic and anti-androgenic actions and effects on thyroid regulation^{1,2}. Human and animal data link phthalate exposure with metabolic disturbance, hepatotoxicity, and male reproductive dysfunction.

At the systemic level, DBP and DEHP alter glucose and lipid metabolism, inducing dyslipidaemia, adipocyte hypertrophy, and obesity-like phenotypes, partly via inflammatory signaling and disruption of the PI3K/Akt/GLUT4 and Jak/STAT pathways in the liver and adipose tissues^{3,4,5,6}.

DEHP alters serum cholesterol and triglycerides and promotes hepatic lipid accumulation and inflammatory responses, even at relatively lower developmental doses^{4,5,7}. Mixture studies show that combined phthalates (DBP + DEHP) and bisphenol-A at 50 mg/kg can significantly alter serum lipid profiles, glucose, and thyroid hormones in rats, emphasizing synergistic toxicity at subacute, submaximal doses⁸.

Regarding male reproduction, DEHP and DBP have been documented to reduce testosterone synthesis, impair spermatogenesis and cause structural tissue changes. High-dose DEHP in adult rats decreases sperm quality, quantity, and daily production, increases oxidative stress and inflammatory cytokines, and disrupts key mitochondrial and lysosomal enzymes in testicular tissue, implicating impaired cellular energetics as a mechanism of infertility⁹. DEHP similarly altered testicular weight, distorted seminiferous tubule architecture, and lowered serum testosterone, with transcriptomic data implicating oxidative phosphorylation and steroid biosynthesis pathways^{10,11}. Long-term low-dose mixtures of DEP, DBP, and DEHP, among other phthalates, decrease serum and intratesticular testosterone and induce testicular histopathology via downregulation of steroidogenic proteins and promotion of apoptotic activity¹². Duration-

dependent phthalate exposure in adult rats leads to significant reductions in sperm count, motility and viability, and increased abnormal morphology within weeks¹³.

Key gaps remain in understanding integrated phthalate toxicity, as most studies assess single compounds, isolate metabolic or reproductive outcomes, and rely on limited oxidative markers. Mixture studies rarely use equitoxic subacute doses aligned with NOAELs (e.g., 50 mg/kg) or integrate bioenergetics, lipid profiles, semen parameters, and testicular histology within one model. Moreover, diethyl phthalate (DEP) is underexplored relative to DBP and DEHP. An equidose (50 mg/kg) DEP, DBP, and DEHP rat model therefore enables comprehensive evaluation of combined effects on energy metabolism, lipid homeostasis, reproductive function, and testicular pathology.

METHODOLOGY

Ethical Approval

Ethical approval for this study was obtained from the Ethical Review Committee of the Faculty of Basic Medical Sciences, Al-Hikmah University, Nigeria with ethical approval number HUI-FHS-ERC-26-0046. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals. The research was carried out in the laboratory of the Department of Anatomy, Al-Hikmah University, Nigeria.

Experimental Animals

Male Sprague–Dawley rats weighing between 150 and 250 g were obtained from the Osun State Laboratory Animal Centre. The animals were housed under controlled environmental conditions at a temperature of 22±2°C, relative humidity of 40–70%, and a 12-hour light/12-hour dark cycle. The rats were provided with standard laboratory feed and reverse-osmosis water *ad libitum* throughout the experimental period.

Study Design

All animals were acclimatized for two weeks prior to the commencement of the experiment. A total of fifty rats were randomly assigned into five groups, with ten animals in each group, comprising the normal control group, vehicle control group, and three treatment groups exposed to diethyl phthalate (DEP), dibutyl phthalate (DBP), and bis(2-ethylhexyl) phthalate (DEHP), respectively, at a dose of 50mg/kg body weight. Treatments were administered once daily via oral gavage for a period of 30 consecutive days. The vehicle control group received corn oil at a dose of 5 mL/kg body weight. Food was withdrawn 12 hours prior to sacrifice, while water was made available until the time of euthanasia. At the end of the experimental period, the animals were euthanized by cervical dislocation.

Preparation and Administration of Phthalates

Diethyl phthalate, dibutyl phthalate, and bis(2-ethylhexyl) phthalate were obtained from Molychem, India (Catalogue Number: 84-74-3), Central Drug House, India (Catalogue Number: 84-66-2), and Nice Chemicals, India (Catalogue Number: 84-2800212), respectively. Each compound was prepared by dissolving the appropriate weighed quantity in corn oil. Approximately 70–80% of the required final volume of corn oil was initially added to the compound in an amber glass beaker, followed by magnetic stirring at a controlled temperature range of 40–50°C to ensure complete dissolution without compromising chemical stability or inducing oxidative degradation of the oil vehicle¹⁴, as described by Rowdhwal and Chen (2018). The final volume was subsequently adjusted with corn oil to achieve the desired concentration. The prepared solutions were administered daily between 7:00 a.m. and 10:00 a.m. using an orogastric cannula at a dose of 50 mg/kg body weight.

Sample Collection

Following euthanasia, blood samples were collected via cardiac puncture and centrifuged at 2000×g for 10 minutes at 4°C to obtain serum, which was stored at –20°C until further biochemical analysis. The testes were carefully excised from each animal; one testis was fixed in Bouin's solution for histological examination, while the other was snap-frozen in liquid nitrogen and stored at –70°C for subsequent biochemical assays.

Biochemical Assays

The serum concentrations of glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase-X (LDH-X), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined using radioimmunoassay kits obtained from Beijing North Institute of Biological Technology, Beijing, China, in accordance with the manufacturer's instructions.

Semen Analysis

The cauda epididymis was dissected and homogenized in phosphate-buffered saline (pH 7.4) for semen analysis. Sperm count was determined using an improved Neubauer counting chamber (LABART; Germany) under a light microscope at ×40 magnification, with multiple fields examined to obtain an accurate count¹⁵ in accordance with the method described by Omirinde et al. (2019). Sperm motility was assessed by placing a drop of sperm suspension on a pre-warmed slide, adding two drops of warm 2.9% sodium citrate, covering with a coverslip, and observing under a light microscope at ×40 magnification under low illumination¹⁶, as described by Khatun et al. (2018). For sperm morphology assessment, a thin smear of the sperm sample was prepared on a clean slide, fixed in 95% ethanol, air-dried, and subsequently stained using Harris haematoxylin, G-6 orange, and EA-50 green stains. The stained slides were examined under a light microscope at ×40 magnification, and a total of 200 spermatozoa per sample were evaluated, with abnormalities expressed as percentages^{15,17}. Sperm viability was determined using Eosin/ Nigrosin staining, and the percentage of live and dead sperm cells was assessed under a light microscope.

Histological Processing

Testicular tissues fixed in Bouin's solution were dehydrated through graded ethanol series, embedded in paraffin wax, and sectioned at a thickness of 5µm. The tissue sections were mounted on glass slides and stained using haematoxylin and eosin (H&E) as well as Periodic Acid–Schiff (PAS) stains. Histological examination of the testicular architecture was carried out using light microscopy.

Statistical Analysis

Quantitative data were analyzed using Chris Rorden's ANOVA (version 0.985) software, while Microsoft Excel was used for graphical representation of results. Data were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Bioenergetics

Serum glucose-6-phosphate dehydrogenase (G6PDH) activity demonstrated a consistent reduction across all experimental groups relative to the control. This decrease was statistically significant in the vehicle ($p = 0.002$), DBP ($p = 0.008$), and DEHP ($p = 0.0001$) groups when compared with the control group. Although a reduction was also observed in the DEP group, this did not reach statistical significance. The magnitude of decline was most pronounced in the DEHP group, indicating a stronger effect relative to the other treatment groups.

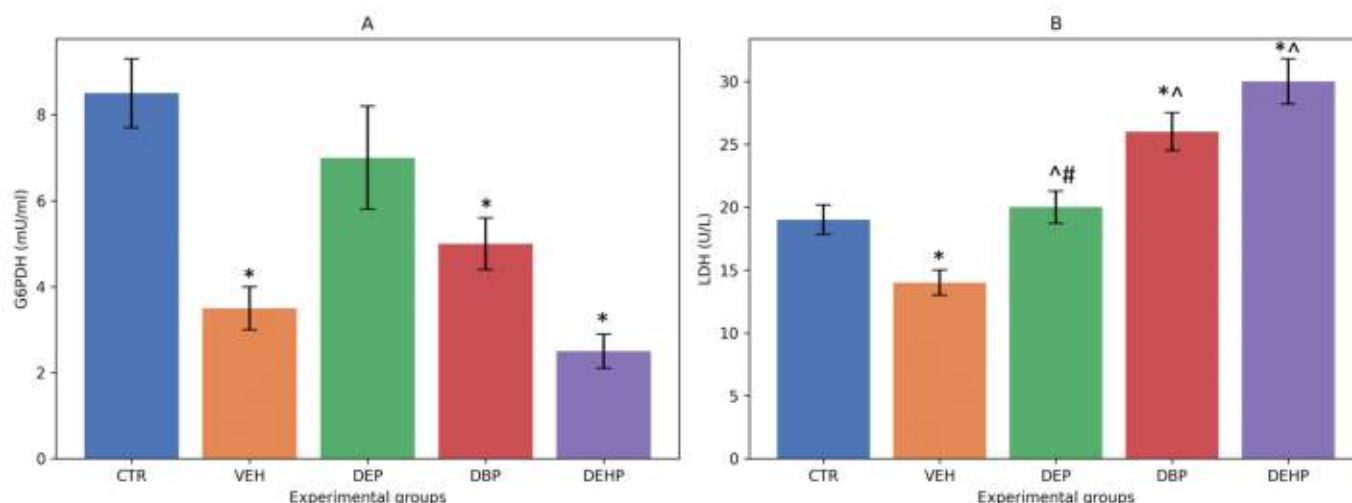


Figure 1 (A–B): Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on serum glucose-6-phosphate dehydrogenase (G6PDH) activity (A) and serum lactate dehydrogenase-X (LDH-X) levels (B) in male rats following subacute exposure. Data are presented as mean±SEM (n=10 per group). Statistical significance is denoted as follows: * $p < 0.05$ vs control (CTR); [^] $p < 0.05$ vs vehicle (VEH); # $p < 0.05$ vs DEHP. CTR = control; VEH = vehicle (corn oil, 5 mL/kg); DEP = diethyl phthalate; DBP = dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

In contrast, serum lactate dehydrogenase-X (LDH-X) levels exhibited a differential pattern of change across groups. A significant reduction was observed in the vehicle group ($p = 0.005$) compared with the control. However, LDH-X levels were significantly elevated in the DBP ($p = 0.04$) and DEHP ($p = 0.0004$) groups relative to the control group. When compared with the vehicle group, all phthalate-treated groups showed significant increases in LDH-X levels (DEP: $p = 0.04$; DBP: $p = 0.04$; DEHP: $p = 0.0001$), confirming that the observed elevations are attributable to phthalate exposure rather than the vehicle effect. Additionally, LDH-X levels in the DEP group were significantly lower than those in the DEHP group ($p = 0.01$), indicating a comparatively attenuated effect. This shows a divergence in bioenergetic indices following phthalate exposure, characterized by a generalized reduction in G6PDH activity alongside selective elevations in LDH-X, particularly in DBP and DEHP groups. The magnitude and direction of these changes indicate compound-specific differences in bioenergetic disruption, with DEHP exerting the most pronounced effect, followed by DBP, while DEP shows relatively attenuated alterations.

Lipid Profile

Figures 2A–2D illustrate the effects of DEP, DBP, and DEHP on serum lipid profile parameters in experimental rats. Total cholesterol (TC) levels (Figure 2A) showed a progressive increase across all experimental groups relative to the control, with statistically significant elevations observed in the DBP ($p = 0.01$) and DEHP ($p = 0.0005$) groups. Furthermore, the DEHP group demonstrated a significant increase compared to the vehicle group ($^{\wedge}p = 0.007$), indicating a pronounced treatment-related effect.

Similarly, triglyceride (TG) levels (Figure 2B) increased across all experimental groups, with a statistically significant elevation observed in the DEHP group ($p = 0.02$) relative to the control. The DEHP group also showed a significant increase when compared with the vehicle group ($^{\wedge}p = 0.0003$), further supporting the magnitude of its effect.

Low-density lipoprotein (LDL) levels (Figure 2C) followed a comparable upward trend across all treatment groups relative to the control. This increase reached statistical significance in the DBP ($p = 0.002$) and DEHP ($p = 0.0001$) groups. However, when compared with the vehicle group, only the DEHP group showed a statistically significant elevation ($^{\wedge}p = 0.004$), indicating a stronger effect relative to the other phthalates.

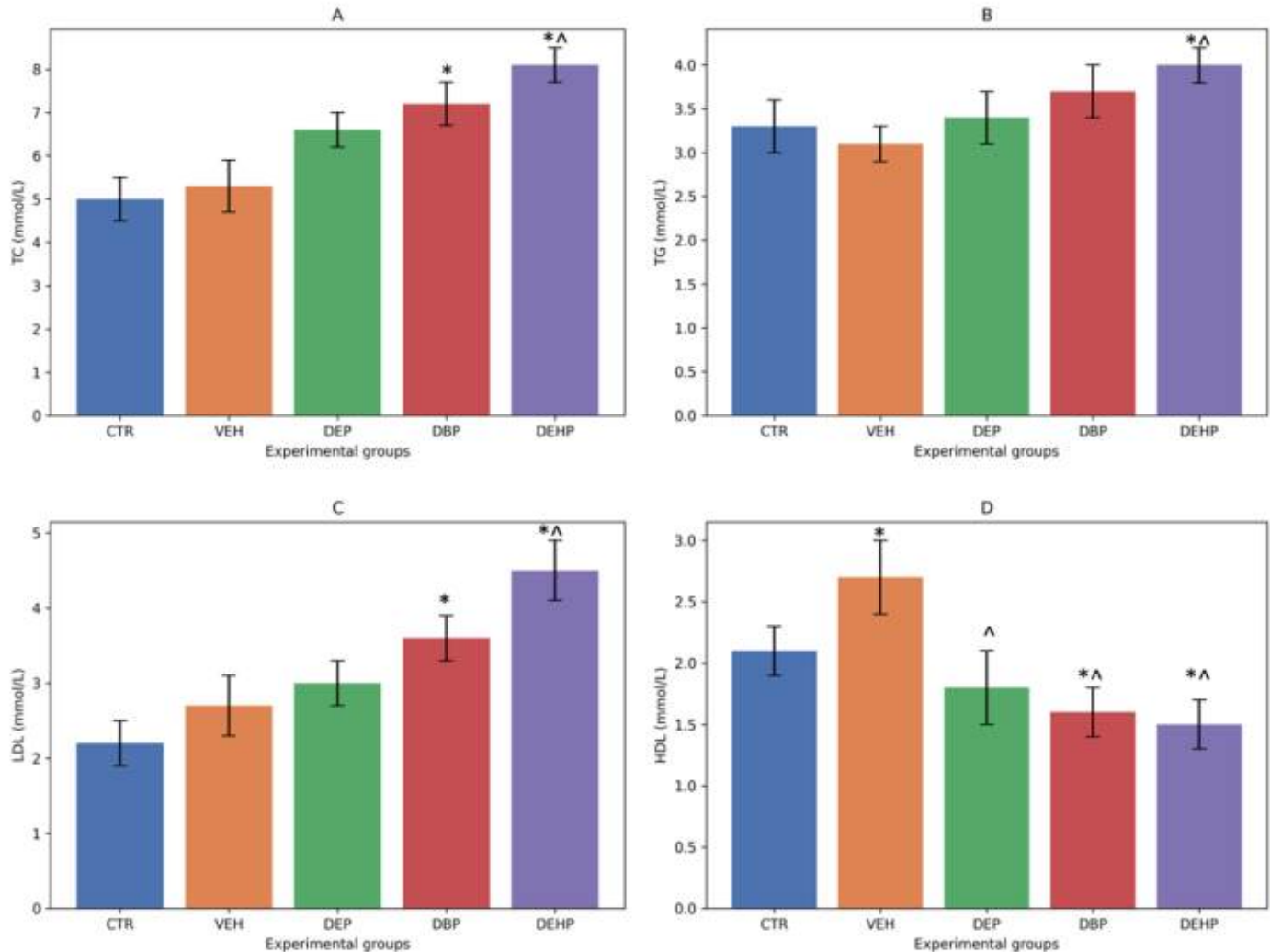


Figure 2 (A–D): Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on serum total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein (LDL) (C), and high-density lipoprotein (HDL) (D) levels in male rats following subacute exposure. Data are presented as mean±SEM (n=10 per group). Statistical significance is denoted as follows: * $p < 0.05$ vs control (CTR); ^ $p < 0.05$ vs vehicle (VEH); # $p < 0.05$ vs DEHP. CTR = control; VEH = vehicle (corn oil, 5 mL/kg); DEP = diethyl phthalate; DBP = dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

In contrast, high-density lipoprotein (HDL) levels (Figure 2D) exhibited a divergent pattern. A significant increase was observed in the vehicle group ($p = 0.03$) relative to the control. However, HDL levels were significantly reduced in the DBP ($p = 0.049$) and DEHP ($p = 0.02$) groups compared to the control. Additionally, all phthalate-treated groups showed significant reductions in HDL levels when compared with the vehicle group (DEP: ^ $p = 0.04$; DBP: ^ $p = 0.008$; DEHP: ^ $p = 0.005$), indicating a consistent downward effect of phthalate exposure relative to vehicle conditions. There is a consistent pattern of lipid profile alteration characterized by elevations in TC, TG,

and LDL, alongside reductions in HDL, with the most pronounced effects observed in the DEHP group, followed by DBP, while DEP exhibits comparatively moderate changes.

Semen Analysis

Sperm concentration showed a marked reduction across all phthalate-treated groups relative to both control and vehicle groups. This decrease was statistically significant in the DEP ($p = 0.004$; ^ $p = 0.0004$), DBP ($p = 0.002$; ^ $p = 0.0003$), and DEHP ($p = 0.002$; ^ $p = 0.0003$) groups, indicating a consistent treatment-related decline.

Table 1: Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on semen quality and quantity in male rats

Groups	Sperm Concentration	Sperm Motility	Sperm Morphology	Live/Death Ratio (%)
	(*10 ⁶ /mL)	(%)	(*10 ² /%)	
CTR	347±28.76	75.4±2.04	0.92±0.03	77.6±2.29
VEH	383.2±24.39	64.8±4.28	0.96±0.01	73±4.82
DEP	220.8±13.05* [^]	66±2.45*	0.77±0.1	70.4±2.48
DBP	198.8±17.14* [^]	59.2±3.37*	0.73±0.03* [^]	56.2±4.67* [^]
DEHP	172.6±25.75* [^]	49.8±1.77* [^]	0.61±0.01* [^]	53.8±2.67* [^]

Data are presented as mean ± SEM (n = 10 per group). Statistical significance is denoted as follows: *p < 0.05 vs control (CTR); [^]p<0.05vsvehicle (VEH). CTR=control; VEH=vehicle; DEP=diethyl phthalate; DBP=dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

A similar trend was observed in sperm motility. Percentage sperm motility was significantly reduced in the DEP ($p=0.02$), DBP ($p=0.0003$), and DEHP ($p=0.0001$) groups compared with the control group. However, when compared with the vehicle group, only the DEHP group showed a statistically significant reduction ($^{\wedge}p=0.012$), suggesting a more pronounced effect at this level.

Sperm morphology also showed treatment-dependent alterations. Significant reductions were observed in the DBP ($p=0.004$; $^{\wedge}p=0.0004$) and DEHP ($p=0.001$; $^{\wedge}p=0.0001$) groups relative to both control and vehicle groups. Although the DEP group exhibited a reduction in sperm morphology, this change did not reach statistical significance ($p = 0.19$; $^{\wedge}p = 0.09$).

Similarly, the sperm live/death ratio was significantly reduced in the DBP ($p=0.003$; $^{\wedge}p=0.04$) and DEHP ($p = 0.0001$; $^{\wedge}p = 0.008$) groups compared with both control and vehicle groups. In contrast, the DEP group showed a reduction that was not statistically significant ($p = 0.07$; $^{\wedge}p = 0.64$). The semen analysis results show consistent decline in semen quality

parameters across phthalate-treated groups, with the most pronounced impairments observed in the DEHP group, followed by DBP, while DEP exhibited comparatively milder effects.

Histological Observation

Histological assessment reveals an exposure-dependent gradient of testicular structural alteration. Control testes show preserved cytoarchitecture, with well-organized seminiferous tubules, intact germinal epithelium, and normal interstitial architecture. The vehicle group exhibits only mild irregularities in tubule contour and epithelial organization, with overall structural integrity maintained. In contrast, the DEP group demonstrates mild-to-moderate alterations, including reduced germinal epithelial thickness, focal disruption of spermatogenic layers, decreased cellular density, and slight luminal enlargement, indicating early structural compromise.

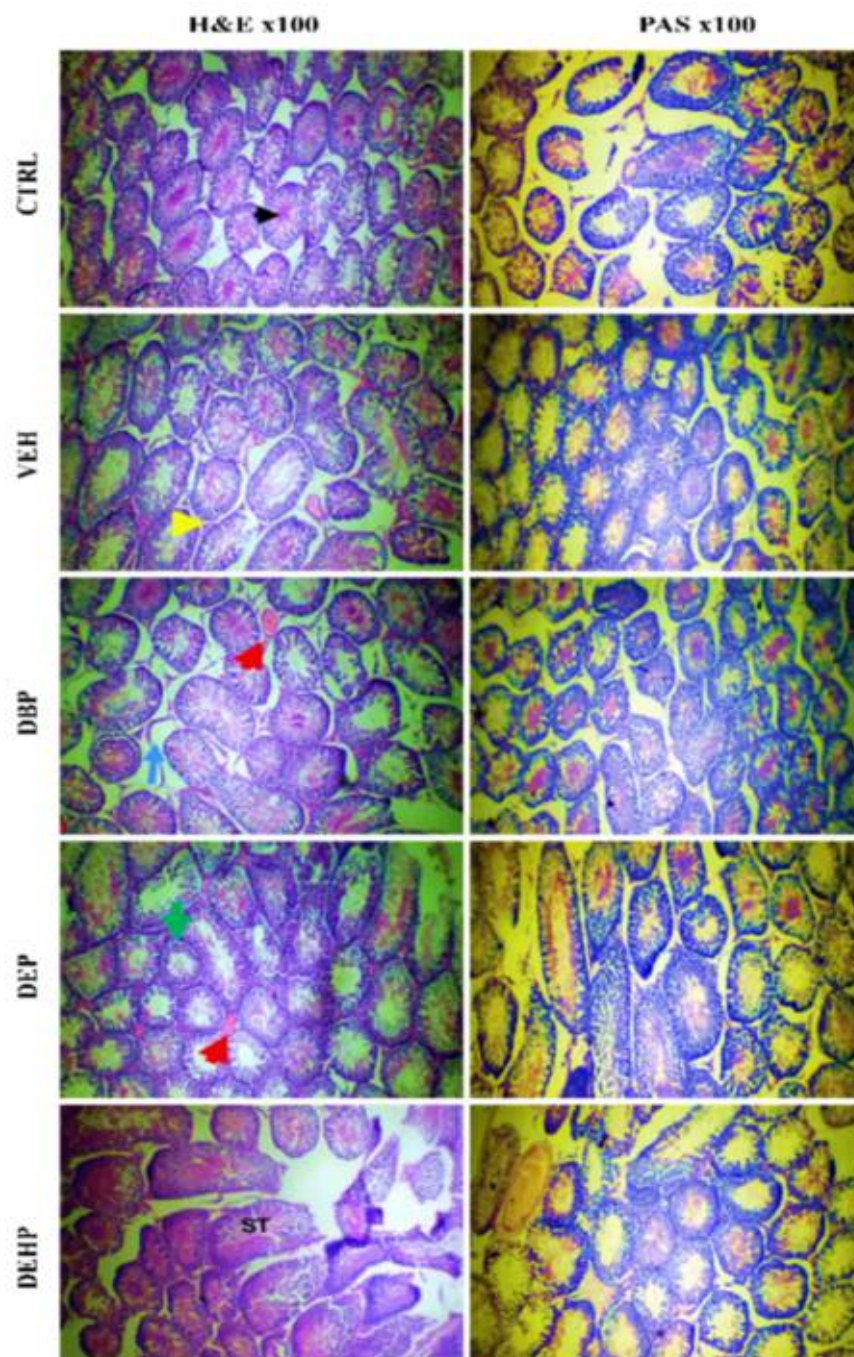


Figure 3: Histopathological alterations in testicular tissue following subacute exposure to phthalates. Representative photomicrographs of rat testicular sections stained with haematoxylin and eosin (H&E; left panels) and Periodic Acid–Schiff (PAS; right panels) at $\times 100$ magnification across experimental groups: control (CTRL), vehicle (VEH), diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP). The CTRL group shows normal seminiferous tubule architecture with intact germinal epithelium, orderly spermatogenic cell layers, and well-defined lumina. The VEH group exhibits mild structural irregularities without overt disruption of testicular organization. DBP exposure is associated with moderate histoarchitectural distortion, including irregular seminiferous tubules, germinal epithelial disorganization (red arrow), and luminal widening (blue arrow). DEP-treated sections demonstrate mild-to-moderate alterations characterized by focal epithelial disruption (red arrow) and reduced cellular density (green arrow). DEHP exposure results in severe degeneration of testicular architecture, including marked seminiferous tubule distortion, epithelial sloughing, and structural collapse with reduced spermatogenic cell populations. PAS staining reveals progressive depletion and disorganization of glycogen-rich germ cells across treatment groups, most pronounced in DEHP

The DBP group shows more pronounced histopathological alterations than DEP, with irregular and distorted seminiferous tubules, disorganized germinal epithelium, reduced epithelial height, and increased luminal diameter, indicating substantial cellular loss and structural disruption.

The DEHP group exhibits the most severe damage, characterized by extensive tubular distortion, marked epithelial attenuation, widespread loss of spermatogenic cells, epithelial sloughing, and poorly defined tubular architecture with markedly dilated lumina, reflecting profound tissue compromise.

PAS staining corroborates these changes, showing strong uniform staining in controls but progressively reduced and heterogeneous patterns in treated groups, most notably in DEHP, indicating depletion of seminiferous epithelial integrity. Overall, a clear severity gradient is observed: DEHP > DBP > DEP > VEH $\approx$ CTRL, with increasing disruption of tubular structure, epithelial organization, and cellular density.

DISCUSSION

The present study demonstrates that subacute exposure to phthalates induces a coordinated disruption of metabolic homeostasis, cellular redox balance, and testicular structural integrity, collectively converging on impaired spermatogenesis. Importantly, these alterations do not occur in isolation but rather reflect a tightly interlinked pathological continuum in which systemic metabolic derangement propagates cellular dysfunction within the testicular micro environment^{18,19}.

The observed dyslipidemia characterized by elevated total cholesterol, triglycerides, and LDL alongside reduced HDL represents a fundamental perturbation in lipid handling that extends beyond systemic circulation into testicular physiology. Although cholesterol is the obligatory substrate for steroidogenesis, its accumulation in circulation in parallel with impaired reproductive indices indicates a failure of effective intracellular trafficking and utilization²⁰. This suggests that phthalate exposure disrupts lipid partitioning and mobilization, thereby uncoupling substrate availability from functional steroidogenic output. Such dysregulation is consistent with PPAR-mediated metabolic reprogramming, which alters lipid storage, transport, and utilization dynamics, ultimately compromising steroidogenic efficiency^{21,22}. Within this framework, the testis becomes functionally constrained despite apparent substrate abundance, reflecting a state of metabolic inefficiency rather than deficiency²³.

Concurrently, the consistent reduction in G6PDH activity across exposed groups indicates a systemic suppression of the pentose phosphate pathway, with direct implications for intracellular NADPH availability²⁴. Given the central role of NADPH in maintaining redox homeostasis and supporting

biosynthetic processes, its depletion establishes a pro-oxidant intracellular environment. Germ cells, by virtue of their high proliferative and differentiation demands, are particularly susceptible to such redox imbalance^{25,26}. The resulting oxidative vulnerability compromises cellular integrity, predisposing spermatogenic cells to damage and loss. This redox imbalance is further compounded by the observed elevation in LDH-X in DBP and DEHP groups, which serves not as a marker of enhanced glycolytic flux but rather as an indicator of cellular membrane compromise and leakage from the seminiferous epithelium²⁷. Together, these bioenergetic alterations define a state of metabolic–oxidative bottleneck, wherein energy production, antioxidant defense, and cellular stability are simultaneously impaired^{28,29}.

Within the testicular milieu, these systemic and cellular disturbances manifest structurally as progressive degeneration of seminiferous tubule architecture. The reduction in germinal epithelial height, luminal dilatation, and eventual tubular distortion reflect a continuum of germ cell attrition and epithelial destabilization^{30,31}. The PAS-demonstrated depletion of glycogen-rich germ cells further reinforces the presence of impaired intracellular energy storage and utilization, indicating that the seminiferous epithelium is not only structurally compromised but also metabolically depleted³². The integrity of the seminiferous epithelium is critically dependent on coordinated interactions between germ cells and supporting Sertoli cells; thus, disruption of cellular energy balance and redox status inevitably translates into architectural disorganization and loss of functional capacity^{8,33}.

These structural changes are directly mirrored by the marked decline in semen quality parameters, including sperm concentration, motility, morphology, and viability. Rather than representing independent endpoints, these functional impairments are the downstream expression of cumulative metabolic, oxidative, and structural insults^{34,35}. The emergence of a mixed oligo-astheno-teratozoospermic profile reflects disruption across multiple stages of spermatogenesis, from germ cell proliferation to maturation and structural integrity^{36,37}. Notably, the observation that DEP induces measurable functional impairment despite relatively modest histological disruption highlights the sensitivity of spermatogenic processes to early metabolic perturbations, preceding overt structural collapse^{38,39}.

The graded severity of effects observed across phthalates (DEHP>DBP>DEP) further supports a dose-potency relationship in which the extent of metabolic disruption dictates the magnitude of downstream reproductive impairment. DEHP, in particular, appears to exert a more profound capacity to disrupt metabolic–bioenergetic coupling, thereby amplifying structural and functional degeneration within the testis^{40,41,42}.

These findings support a unifying model in which phthalate exposure initiates systemic metabolic dysregulation that

propagates through impaired lipid handling and redox imbalance, culminating in bioenergetic insufficiency within the testicular microenvironment. This, in turn, destabilizes seminiferous epithelial architecture and compromises spermatogenic output. In the Nigerian context, where background metabolic risk factors such as dyslipidemia are increasingly prevalent, such environmental exposures may exert additive or synergistic effects, thereby amplifying the burden of male reproductive dysfunction. These results therefore underscore the necessity of conceptualizing male infertility within a broader metabolic and environmental health framework, rather than as an isolated reproductive disorder.

CONFLICT OF INTEREST: None

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REFERENCES

1. Baralić K, Jorgovanović D, Živančević K, Buha Djordjevic A, Antonijević Miljković E, Miljkovic M, et al. Combining in vivo pathohistological and redox status analysis within silico toxicogenomic study to explore the phthalates and bisphenol A mixture-induced testicular toxicity. *Chemosphere*. 2020; 261:129296. doi: 10.1016/j.chemosphere.2020.129296.
2. Toumi D, Zoukar O, Afssa S, Gharbi N, Farhat I, Di Bella G, et al. Adverse effects on the endocrine and reproductive system of single and combined Ortho phthalates in female rats following short-term exposure. *Environ Sci Pollut Res Int*. 2025; 32:22651-22664. doi:10.1007/s11356-025-36970-8.
3. Jia Y, Liu T, Zhou L, Zhu J, Wu J, Sun D, et al. Effects of di-(2-ethylhexyl) phthalate on lipid metabolism by the JAK/STAT pathway in rats. *Int J Environ Res Public Health*. 2016; 13:1085. doi:10.3390/ijerph13111085.
4. Zhou L, Chen H, Xu Q, Han X, Zhao Y, Song X, et al. The effect of di-2-ethylhexylphthalate on inflammation and lipid metabolic disorder in rats. *Ecotoxicol Environ Saf*. 2019; 170:391-398. doi: 10.1016/j.ecoenv.2018.12.009.
5. Zhang Y, Ge S, Yang Z, Li Z, Gong X, Zhang Q, et al. Disturbance of di-(2-ethylhexyl) phthalate in hepatic lipid metabolism in rats fed with a high-fat diet. *Food Chem Toxicol*. 2020; 111848. doi: 10.1016/j.fct.2020.111848.
6. Cao S, Shen Y, Yang X, Zhang G, Zhang Y, Wang Y, et al. Environmental di-(2-ethylhexyl) phthalate exposure accelerates lipid metabolism disorders via the gut-fat axis in male SAMP8 mice: role of gut microbiota and thyroid hormone signaling. *Environ Int*. 2025; 204:109808. doi: 10.1016/j.envint.2025.109808.
7. Su H, Lai C, Lee K, Chiang Y, Chen C, Hsu P. Prenatal exposure to low-dose di-(2-ethylhexyl) phthalate (DEHP) induces potentially hepatic lipid accumulation and fibrotic changes in rat offspring. *Ecotoxicol Environ Saf*. 2023; 269:115776. doi: 10.1016/j.ecoenv.2023.115776.
8. Baralić K, Djordjevic A, Živančević K, Antonijević E, Anđelković M, Javorac D, et al. Toxic effects of the mixture of phthalates and bisphenol A subacute oral toxicity study in Wistar rats. *Int J Environ Res Public Health*. 2020; 17:746. doi:10.3390/ijerph17030746.
9. Poli V, Motireddy S. Toxicity study of di (2-ethylhexyl) phthalate (DEHP) administration on reproductive parameters in male albino rats. *Comp Clin Pathol*. 2023; 33:75-84. doi:10.1007/s00580-023-03524-8.
10. Han L, Wang J, Zhao T, Wu Y, Wei Y, Chen J, et al. Stereological analysis and transcriptome profiling of testicular injury induced by di-(2-ethylhexyl) phthalate in prepubertal rats. *Ecotoxicol Environ Saf*. 2021; 220:112326. doi: 10.1016/j.ecoenv.2021.112326.
11. Liu X, Li Y, Zi T, Zhang Y, Wang Z, Peng Z, et al. Di-2-ethylhexyl phthalate exposure induces sperm quality and functional defects in mice. *Chemosphere*. 2022; 137216. doi: 10.1016/j.chemosphere.2022.137216.
12. Gao H, Xu R, Cao W, Qian L, Wang M, Lu L, et al. Effects of six priority-controlled phthalate esters with long-term low-dose integrated exposure on male reproductive toxicity in rats. *Food Chem Toxicol*. 2017; 101:94-104. doi: 10.1016/j.fct.2017.01.011.
13. Emojevwe V, Naiho A, Nwangwa E, Oyovwi M, Anachuna K, Agbanifo-Chijiokwu E, et al. Duration-dependent effects of high doses of phthalate exposure on semen quality in adult male rats. *JBRA Assist Reprod*. 2021; 26:53-61. doi:10.5935/1518-0557.20210033.
14. Rowdhwal SSS, Chen J. Toxic effects of di-2-ethylhexyl phthalate: an overview. *Biomed Res Int*. 2018; 2018:1750368. doi:10.1155/2018/1750368.
15. Omirinde JO, Olukole SG, Oke BO. Age-related changes in testicular and epididymal sperm parameters in the African greater cane rat. *Anim Res Int*. 2019; 16:3255-3264.

16. Khatun A, Rahman MS, Pang MG. Clinical assessment of male fertility. *ObstetGynecol Sci.* 2018; 61:179-191. doi:10.5468/ogs.2018.61.2.179.
17. Yu S, Rubin M, Geevarughese S, Pino JS, Rodriguez HF, Asghar W. Emerging technologies for home-based semen analysis. *Andrology.* 2018; 6:10-19. doi:10.1111/andr.12441.
18. Mondal S, Bandyopadhyay A. Antioxidants in mitigating phthalate-induced male reproductive toxicity: a comprehensive review. *Chemosphere.* 2024; 364:143297. doi: 10.1016/j.chemosphere.2024.143297.
19. Moghazy M, Papathanasiou M, Tzoupis H, Papavasileiou KD, Xing C, Lauschke V, et al. A systematic literature review of reproductive toxicological studies on phthalates. *IntJMolSci.* 2025; 26:8761. doi:10.3390/ijms26188761.
20. Zubi ZBH, Alfarsi HAH. Hyperlipidemia and male infertility. *Egypt J Basic Appl Sci.* 2021; 8:370-378. doi:10.1080/2314808X.2021.1977080.
21. Milinković M, Borlakova M, Krivokapic M, Bolevich S, Jakovljevic V. Male reproductive toxicity of di (2-ethylhexyl) phthalate: data from a rat model. *Toxicol Environ Chem.* 2024; 106:287-310. doi:10.1080/02772248.2024.2364361.
22. Deng W, Zhao J, Wang X, Li D, Wang M, Zheng X, et al. Role of ferroptosis mediated by abnormal membrane structure in DEHP-induced reproductive injury. *Free Radic Biol Med.* 2025; 228:04026. doi: 10.1016/j.freeradbiomed.2025.04.026.
23. Källsten L, Almamoun R, Pierozan P, Nylander E, Sdougkou K, Martin JW, et al. Adult exposure to di-n-butyl phthalate induces persistent effects on testicular cell markers and testosterone biosynthesis in mice. *Int J Mol Sci.* 2022; 23:8718. doi:10.3390/ijms23158718.
24. Chigrinski E, Gerunov T, Merzlikin N, Gerunova LK, Hua J. The role of the pentose phosphate pathway in metabolic disorders in testes during cypermethrin intoxication. *Reprod Toxicol.* 2025; 133:109084. doi: 10.1016/j.reprotox.2025.109084.
25. Hong Y, Zhou Y, Shen L, Wei Y, Long C, Fu Y, et al. Exposure to DEHP induces testis toxicity via ROS/mTOR/NLRP3 signaling pathway in immature rats. *Ecotoxicol Environ Saf.* 2021; 225:112889. doi: 10.1016/j.ecoenv.2021.112889.
26. Zhao T, Wang J, Shen L, Long C, Liu B, Wei Y, et al. Increased m6A RNA modification relates to inhibition of Nrf2-mediated antioxidant response in DEHP-induced testicular injury. *Environ Pollut.* 2020; 259:113911. doi: 10.1016/j.envpol.2020.113911.
27. Mondal S, Bandyopadhyay A. From oxidative imbalance to compromised sperm parameters: toxicological aspects of phthalate esters. *Environ Toxicol Pharmacol.* 2023; 97:104085. doi: 10.1016/j.etap.2023.104085.
28. Zhang L, Gao X, Qin Z, Shi X, Xu K, Wang S, et al. USP15 in DBP-induced testicular oxidative stress via Keap1/Nrf2 pathway. *Sci Total Environ.* 2021; 783:146898. doi: 10.1016/j.scitotenv.2021.146898.
29. Helmy H, Senousy MA, El-Sahar AE, Sayed RH, Saad M, Elbaz EM. Aberrations of miR-126-3p, miR-181a and Sirtuin1 network mediate DEHP-induced testicular damage. *Toxicology.* 2020; 443:152406. doi: 10.1016/j.tox.2020.152406.
30. Başimoğlu Koca Y. Effects of Di isobutyl phthalate on rat testicular tissue: histopathological evaluation. *Academia J Biol.* 2024;46(4). doi:10.15625/2615-9023/21119.
31. Yang L, Yang B, Lu D, Peng Z, Ren Z, Fang K, et al. Dynamic assessment of toxicity and pathology of DEHP in germ cells of rats. *Reprod Biol.* 2020; 20:580-588. doi: 10.1016/j.repbio.2020.07.005.
32. Gouri K, Sinha P. Impact of DEHP toxicity on testis histology in rats. *Toxicol Int.* 2023; 29:538-546. doi:10.18311/ti/2022/v29i4/30294.
33. Wang S, Li D, Deng W, Wu S, Wei Y, Wei G. Role of p62-Keap1-Nrf2 pathway in protecting Sertoli cells against phthalate-induced ferroptosis. *J Appl Toxicol.* 2025; 45:4963. doi:10.1002/jat.4963.
34. Wang H, He H, Wei Y, Gao X, Zhang T, Zhai J. Do phthalates cause poor semen quality? A systematic review and meta-analysis. *Environ Sci Pollut Res.* 2022; 30:12553-12567. doi:10.1007/s11356-022-24215-x.
35. Caporossi L, Viganò P, Paci E, Capanna S, Alteri A, Pignini D, et al. Effects of plasticizers exposure on male fertility: a case-control study. *Int J Environ Res Public Health.* 2022;20:235. doi:10.3390/ijerph20010235.
36. Amjad S, Rahman MS, Pang W, Ryu D, Adegoke EO, Park YJ, et al. Effects of phthalates on mouse sperm function and fertility. *Toxicology.* 2021; 465:152746. doi: 10.1016/j.tox.2021.152746.
37. Zhou L, Shan T, Cai W. Correlation between urinary phthalate concentration and semen quality parameters. *J Chin Physician.* 2020; 22:518-521. doi:10.3760/cma.j.cn431274-20190201-00112.
38. Patel SS, Trangadia BJ, Patel U, Delvadiya RS, Makwana AA, Raval SH, et al. Toxic effects of dibutyl phthalate on testes of zebrafish. *Environ Sci Pollut Res.* 2024; 31:45678-45692. doi:10.1007/s11356-024-34868-5.

39. Reyes-Cruz E, Rojas-Castañeda J, Landero-Huerta D, Hernández-Jardón N, Reynoso-Robles R, Juárez-Mosqueda ML, et al. Disruption of gonocyte development following neonatal DEHP exposure. *Reprod. Biol.* 2024; 24:100877. doi: 10.1016/j.repbio.2024.100877.
40. Zhang Z, Han H, Ding L, Yang H, Deng Y, Shang Y, et al. DEHP induces male reproductive toxicity via mitophagy-dependent ferroptosis. *Free Radic Biol Med.* 2026; 219:01014. doi: 10.1016/j.freeradbiomed.2026.01.014.
41. Lin Y, Xu W, Yang L, Chen Z, Zhai J, Zhu Q, et al. Mechanism of testicular injury induced by DEHP and protective agents. *Chem Biol Interact.* 2023; 380:110575. doi: 10.1016/j.cbi.2023.110575.
42. Willson C. Phthalate toxicity in rats and its relation to testicular dysgenesis syndrome in humans. *Toxicol Pathol.* 2021; 49:1430-1440. doi:10.1177/01926233211045331.

Research Paper

Neuroprotective Effects of *Nigella sativa* Oil on Ciprofloxacin-Induced Neurotoxicity in Prefrontal Cortex of Wistar Rats

Aminat Temitayo Atoyebi, Aisha Abdulrauf, Abdulmuiz Oluwatobi Adebisi*,
Abdulbaari Eniola Nasirudeen, Kafilat Alasirin, Ismail Adetayo Lawal,
Fatima Omowunmi Hamzat, Soliu Olashile Ibrahim,
Sulaiman Muhammad Eze and Babatunde Joseph Dare

Department of Human Anatomy,
Faculty of Basic Medical Sciences, Al-Hikmah University, Ilorin, Nigeria.

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*Corresponding Author Email: aadebiyi@alhikmah.edu.ng

ABSTRACT

Ciprofloxacin is a widely prescribed fluoroquinolone antibiotic; however, accumulating evidence indicates its potential to induce oxidative stress-mediated neurotoxicity. Nigella sativa oil (NSO) possesses potent antioxidant and neuroprotective properties. This study investigated the protective effects of NSO against Ciprofloxacin-induced oxidative, inflammatory, and histological alterations in the brain of adult Wistar rats. Twenty-four adult male Wistar rats were randomly assigned into four groups (n = 6): control, NSO (1 ml/kg), Ciprofloxacin (500 mg/kg), and Ciprofloxacin + NSO. Treatments were administered orally for 14 days. Following treatment, brain tissues were harvested for biochemical assessment of oxidative stress and inflammatory markers, including nitric oxide (NO), glutathione peroxidase (GPx), and interleukin-1 β (IL-1 β). Histopathological evaluation of cortical tissues was also performed using standard staining techniques. Ciprofloxacin administration significantly increased nitric oxide and IL-1 β levels and significantly reduced glutathione peroxidase activity compared to the control group ($p < 0.05$), indicating elevated oxidative stress, neuroinflammation, and impaired antioxidant defense. Histological examination revealed neuronal degeneration, cellular distortion, and disrupted cortical architecture in the Ciprofloxacin-treated group. Conversely, co-administration of NSO significantly attenuated the elevation in NO and IL-1 β levels and restored GPx activity toward normal values ($p < 0.05$). Histologically, the combined treatment group showed marked improvement in neuronal integrity and cortical organization compared to the Ciprofloxacin-only group. NSO alone did not produce significant adverse changes compared to control. Nigella sativa oil mitigates Ciprofloxacin-induced oxidative stress, neuroinflammation, and neuro-histological damage in adult Wistar rats, likely through its antioxidant and anti-inflammatory mechanisms. These findings suggest that NSO may serve as a potential adjunct therapeutic agent to reduce fluoroquinolone-associated neurotoxicity.

KEYWORDS: Ciprofloxacin, *Nigella sativa* oil, Prefrontal cortex, Neurotoxicity, Oxidative stress

INTRODUCTION

Fluoroquinolones are a class of broad-spectrum antibiotics extensively used in clinical practice for the treatment of bacterial infections in both adult and pediatric populations¹. Their widespread prescription has increased concerns regarding potential adverse effects beyond their antimicrobial action, particularly on the central nervous system. Ciprofloxacin, one of the most commonly used fluoroquinolones, readily penetrates biological tissues and crosses the blood–brain barrier, increasing the risk of neurological complications². Emerging evidence indicates that Ciprofloxacin may interfere with normal brain development by inducing oxidative stress, disrupting neurotransmitter balance, and altering neuronal homeostasis³.

Experimental studies have demonstrated that Ciprofloxacin exposure during critical developmental periods can impair neural function and behavior through mechanisms involving mitochondrial dysfunction, excessive generation of reactive oxygen species, and neuroinflammation^{3,4}. Behavioral deficits such as impaired learning, memory dysfunction, anxiety-like behavior, and altered locomotor activity have been reported in Ciprofloxacin-treated rodents^{5,6}. Histological findings further reveal neuronal degeneration, cortical disorganization, and reduced neuronal density in vulnerable brain regions, including the prefrontal cortex, a region essential for executive function and emotional regulation⁷. Despite these findings, the effects of Ciprofloxacin-induced neurotoxicity on higher-order cortical regions during early development remain inadequately explored.

In parallel, increasing attention has been directed toward the use of natural antioxidants as protective agents against drug-induced neural injury. *Nigella sativa* oil (NSO), derived from black seed, has gained considerable scientific interest due to its bioactive constituents, particularly Thymoquinone, which exhibits potent antioxidant, anti-inflammatory, and neuroprotective properties^{8,9}. Experimental evidence indicates that *Nigella sativa* oil can attenuate oxidative stress, reduce inflammatory cytokine expression, and preserve neuronal integrity in models of chemically induced neurotoxicity⁸. Additionally, NSO has been shown to improve behavioral performance and protect against drug- and toxin-induced neurodegeneration by maintaining normal neuronal architecture and function⁹.

Despite growing evidence that Ciprofloxacin poses risks to neural development, there is still limited understanding of its specific impact on higher-order brain regions such as the prefrontal cortex, particularly during early postnatal life. At the same time, while *Nigella sativa* oil has demonstrated promising neuroprotective effects, its role in mitigating Ciprofloxacin-induced cortical neurotoxicity remains

relatively underexplored. This study was therefore designed to investigate the effects of Ciprofloxacin exposure on growth parameters, behavior, oxidative balance, and neuronal integrity in the prefrontal cortex of Wistar rat offspring, while also evaluating the potential protective role of *Nigella sativa* oil. By addressing this gap, the study aims to provide deeper insight into antibiotic-induced neurodevelopmental injury and the therapeutic potential of plant-derived antioxidants.

MATERIALS AND METHODS

Ethical Consideration

All experimental procedures were conducted in compliance with the Ethical Review Committee guidelines of Al-Hikmah University, Ilorin, Nigeria, with approval number HUI-FHS-ERC-25-0039. Approval was obtained from the Animal Care and Handling Unit Review Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, in accordance with the Institutional Animal Care and Use Committee (IACUC). The principles of the 3Rs (Replacement, Reduction, and Refinement) were strictly adhered to during the handling, housing, and care of experimental animals to ensure animal welfare and scientific validity.

Chemicals and Reagents

Ciprofloxacin was purchased from One Step Pharmacy, Ilorin, Nigeria, and manufactured by Aurobindo Pharma, Neuland Laboratories, and Kesar Pharma. *Nigella sativa* oil (NSO) was obtained from Al-Ameen Store, Ilorin, and manufactured by Hemani Herbals Trading. Normal saline solution was manufactured by Fidson Healthcare Plc (Nigeria). Other reagents used included sucrose solution, formalin, ethanol, cresyl fast violet, Mayer's hematoxylin, eosin Y, distilled water, xylene, and DPX mounting medium. All reagents were of analytical grade.

Experimental Animals

Twenty unmated adult female Wistar rats and twelve adult male Wistar rats weighing between 220–280 g were purchased from Fulcrum Innovation Limited, Ilorin, Kwara State. The animals were acclimatized for two weeks before the commencement of the study and housed in plastic cages under standard laboratory conditions of 12-hour light/dark cycle, ambient temperature, and adequate ventilation at the animal facility of the Department of Human Anatomy, Al-Hikmah University, Ilorin. Standard rat chow and water were provided ad libitum. Mating was carried out using a ratio of one male to two females. Pregnant rats were separated prior to parturition. Eight pups per group were selected from different litters for subsequent analyses.

Experimental Design and Grouping

The experimental animals (Wistar rat pups) were randomly divided into four groups of six pups each. Group A served as the control and received normal saline (10 mL/kg body weight, orally). Group B received *Nigella sativa* oil (1 mL/kg body weight, orally). Group C received Ciprofloxacin (60 mg/kg body weight, orally). Group D received a combination of Ciprofloxacin (60 mg/kg body weight) and *Nigella sativa* oil (1 mL/kg body weight, orally). All treatments were administered orally using a silver cannula.

Neurobehavioral Assessment

Neurobehavioral performance was evaluated using standard behavioral tests conducted one day prior to sacrifice. The tests were carried out in a quiet and controlled environment. Cognitive and exploratory behaviors were assessed using the Open Field Test, Y-Maze, and Morris Water Maze.

Termination of Experiment and Tissue Collection

At the end of the experiment, animals were humanely euthanized by cervical dislocation. Brains were immediately excised, and the prefrontal cortex was carefully dissected. Portions of the tissues were fixed in formalin for histological analysis, while others were homogenized in sucrose solution for biochemical investigations.

Biochemical Analysis

For biochemical assays, prefrontal cortex tissues were homogenized in ice-cold sucrose solution and centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant was collected and used for the assessment of nitric oxide, glutathione peroxidase, and interleukin-1 using standard spectrophotometric and ELISA methods.

Histological Analysis

Prefrontal cortex samples were fixed in formalin, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Tissue sections of 5 µm thickness were obtained using a rotary microtome and stained with Hematoxylin and Eosin as well as Cresyl Fast Violet to demonstrate general cytoarchitecture and Nissl substance integrity.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism version 8.0. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple comparisons. Statistical significance was set at $p < 0.05$.

RESULTS

(a) Effect of Ciprofloxacin and *Nigella sativa* Oil on Body Weight

There was a progressive increase in body weight across all experimental groups over the study period. However, pups administered Ciprofloxacin alone showed a noticeable reduction in body weight gain compared with the control group. Co-administration of *Nigella sativa* oil with Ciprofloxacin improved body weight gain relative to the Ciprofloxacin-treated group. No marked difference was observed between the control and NSO-only groups. These observations are illustrated in Figure 1.

(b) Effect of Ciprofloxacin and *Nigella sativa* Oil on Litter Size

There was a significant and observable difference in litter size among the experimental groups at birth. The number of pups delivered by dams exposed to Ciprofloxacin were significantly lower while compared to CIPRO + NSO group, while control and NSO-only group had more pups. No cases of stillbirth or gross developmental abnormalities were observed across the groups. These observations indicate that the treatments significantly affected litter size under the experimental conditions illustrated in Figure 2.

(c) Effect of Ciprofloxacin and *Nigella sativa* Oil on Fur Appearance

Physical examination of the pups revealed normal fur development across all experimental groups, characterized by smooth, dense, and uniformly distributed fur. No significant difference was seen across the group for fur appearance but increased fur density was noticed in NSO only group. Co-administration of *Nigella sativa* oil with Ciprofloxacin markedly improved fur appearance, with pups showing fur characteristics comparable to those of the control group. Representative images of fur appearance are shown in Figure 3.

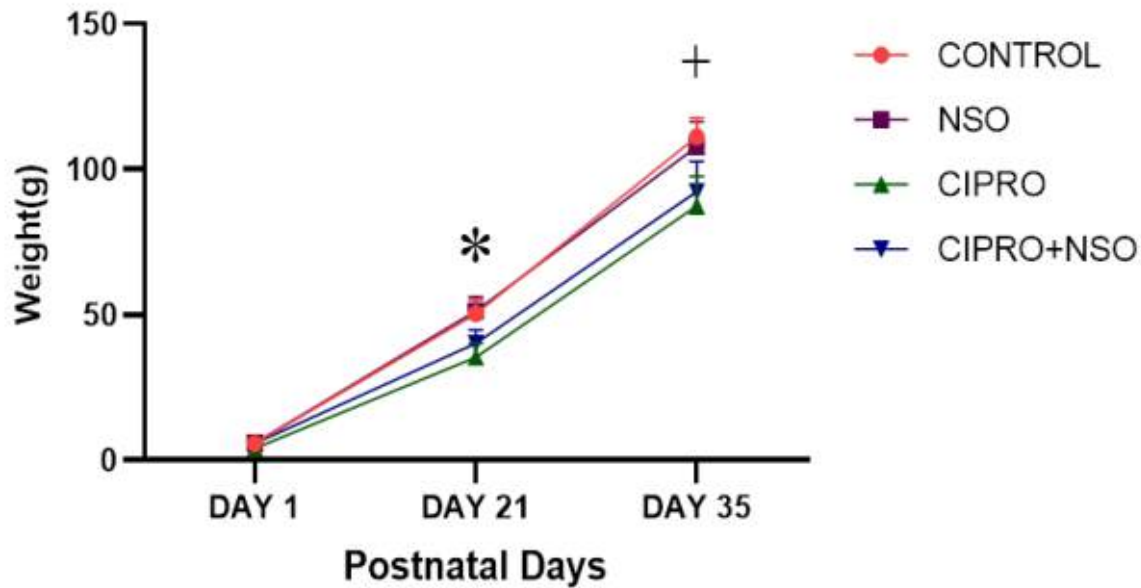


Figure 1: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on body weight of experimental animals. (*) indicates statistical significance compared to the control group, (+) indicates statistical significance compared to the NSO group, while CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). * $P < 0.05$ compare with control, + $P < 0.05$ compare with CIPRO only

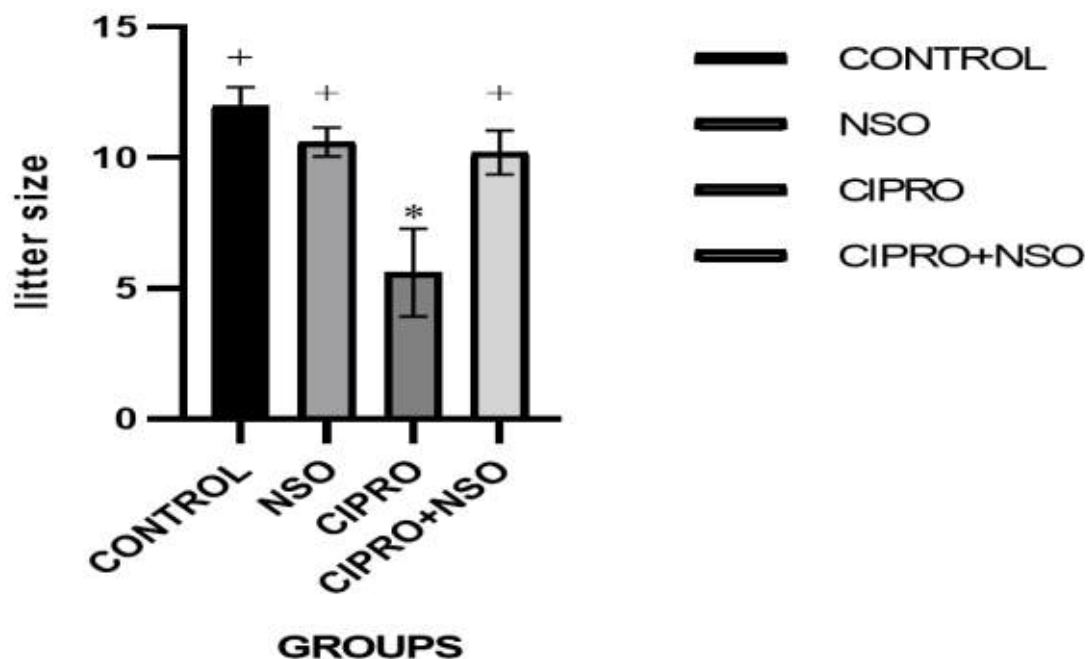


Figure 2: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on litter size of experimental animals. (+) indicates statistical significance compared to the NSO group, while CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). * $P < 0.05$ compare with control, + $P < 0.05$ compare with CIPRO only

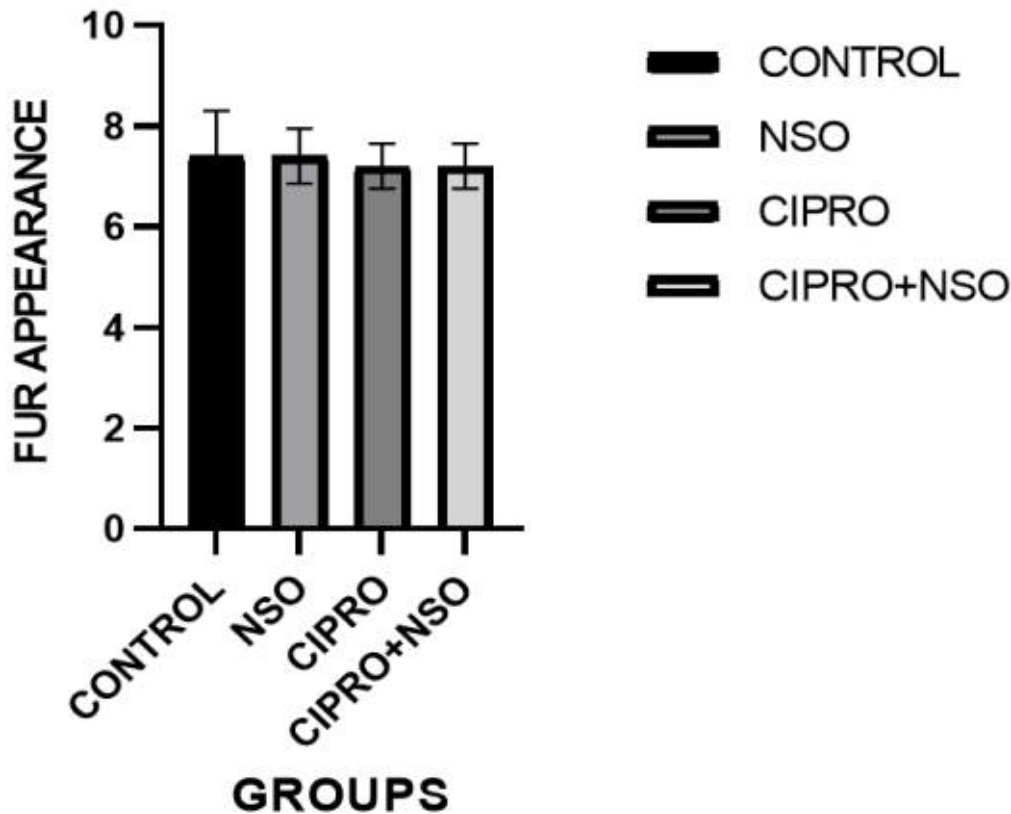


Figure 3: Graph showing the effect of Ciprofloxacin and *Nigella sativa* oil administration on fur appearance of experimental animals. CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.)

(d) Effect of Ciprofloxacin and NSO Administration on Neurobehavioral Parameters

Open Field Test Performance

Exploratory behavior in the open field test showed significant ($P < 0.05$) differences among groups.

Rearing Frequency and Duration

The rearing frequency and duration varied significantly among the experimental groups. The control group exhibited the lowest rearing activity, followed by the *Nigella sativa* oil-treated group. Rats co-treated with Ciprofloxacin and *Nigella sativa* oil showed a moderate increase in rearing frequency and duration compared with the control and NSO groups. In contrast, the Ciprofloxacin-treated group displayed the highest rearing frequency and duration, indicating heightened behavioral activity. These observations are illustrated in Figures 4A and 4B.

The frequency of stretch-attend posture varied among the experimental groups. Rats treated with *Nigella sativa* oil exhibited the lowest frequency of stretching attempts, closely followed by the control group. In contrast, the Ciprofloxacin-treated group showed the highest frequency of stretch-attend posture. Co-administration of Ciprofloxacin with *Nigella sativa* oil reduced the frequency of stretching attempts compared with Ciprofloxacin alone, although values remained higher than those observed in the control and NSO groups. These findings are illustrated in Figure 4C.

Central Entry Frequency and Duration

The graph indicates that Ciprofloxacin markedly reduced both central entry frequency and duration compared to the control group, suggesting increased anxiety-like behavior or reduced exploratory activity in the open field test. NSO alone shows values relatively close to the control, implying no strong anxiogenic effect and possibly normal locomotor/exploratory behavior.

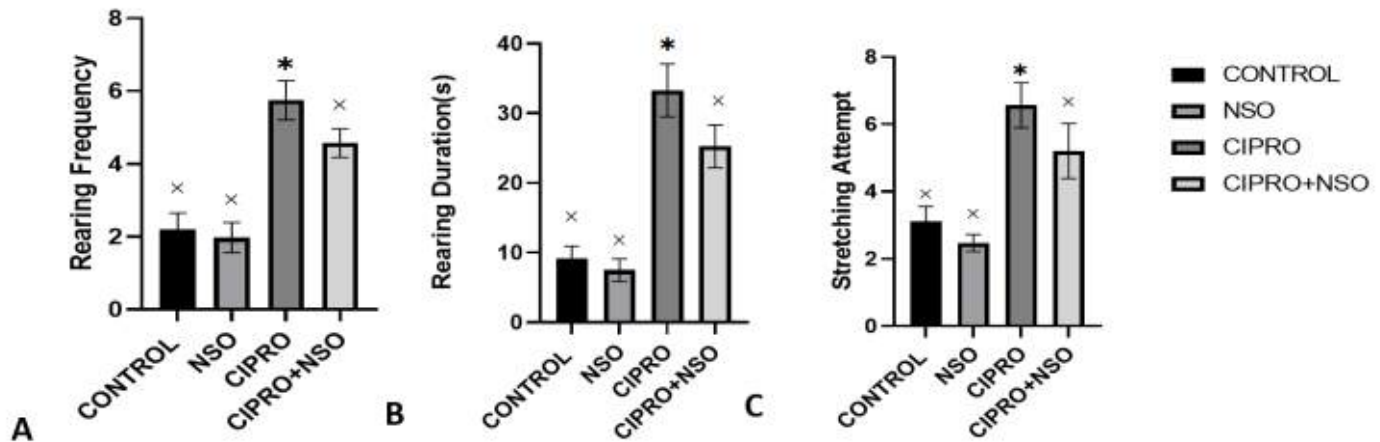


Figure 4: Graph showing the rearing frequency (A), rearing duration (B), stretching attempt (C) in the open field test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group. CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). * $P < 0.05$ compare with control, $\times P < 0.05$ compare with CIPRO only

The frequency of stretch-attend posture varied among the experimental groups. Rats treated with *Nigella sativa* oil exhibited the lowest frequency of stretching attempts, closely followed by the control group. In contrast, the Ciprofloxacin-treated group showed the highest frequency of stretch-attend posture. Co-administration of Ciprofloxacin with *Nigella sativa* oil reduced the frequency of stretching attempts compared with Ciprofloxacin alone, although values remained higher than those observed in the control and NSO groups. These findings are illustrated in Figure 4C.

Central Entry Frequency and Duration

The graph indicates that Ciprofloxacin markedly reduced both central entry frequency and duration compared to the control group, suggesting increased anxiety-like behavior or reduced exploratory activity in the open field test. NSO alone shows values relatively close to the control, implying no strong anxiogenic effect and possibly normal locomotor/exploratory behavior.

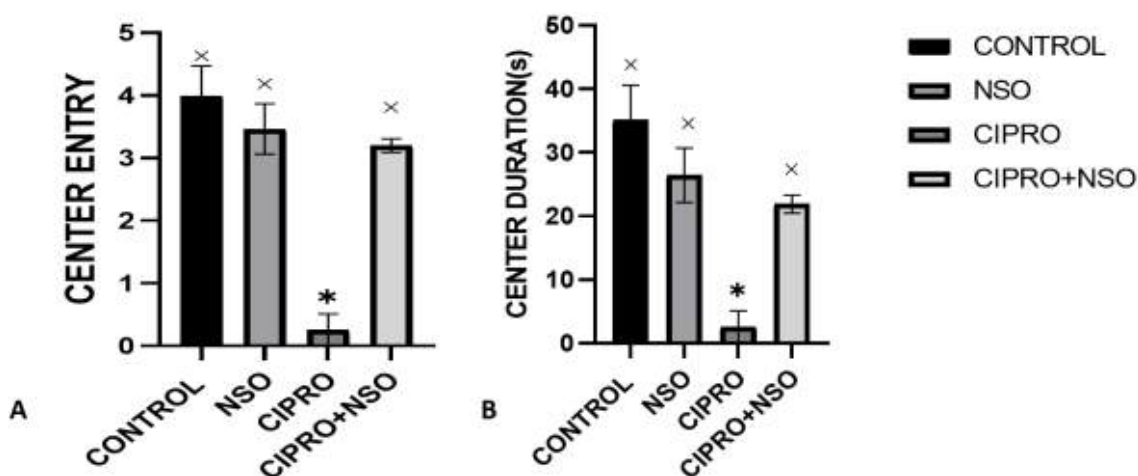


Figure 5: Graph showing the central entry frequency (A), central entry duration (B) in the open field test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group. CTR = Control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). * $P < 0.05$ compare with control, $\times P < 0.05$ compare with CIPRO only

Importantly, the CIPRO + NSO group shows a clear improvement compared to CIPRO alone, with values approaching the control/NSO levels, indicating that NSO may attenuate or counteract the behavioral effects induced by Ciprofloxacin.

I. Y-maze Test Performance

The Y-maze results show that Ciprofloxacin (CIPRO) significantly reduced spontaneous alternation percentage compared to the control group, indicating impaired spatial working memory and reduced cognitive performance. The NSO-treated group also showed a reduction compared to control, although the impairment was less severe than that observed with Ciprofloxacin alone.

Notably, the CIPRO + NSO group demonstrated improvement relative to the CIPRO group (x), suggesting that *Nigella sativa* oil partially ameliorated Ciprofloxacin-induced memory deficits, though performance did not fully return to control levels.

II. Morris-Water maze Test Performance

The Morris water maze results demonstrate clear differences in spatial learning and memory among the experimental groups

across training and probe phases. On Day 1, the Cipro group shows impaired performance compared to the control, indicating delayed acquisition of spatial learning. NSO alone performs comparably to control, suggesting no detrimental effect on cognition. Notably, the Cipro + NSO group performs better than the Cipro group, indicating early attenuation of Ciprofloxacin-induced learning deficits. By Day 2, control and NSO groups exhibit improved performance consistent with learning consolidation, whereas the Cipro group continues to show poorer performance, reflecting sustained cognitive impairment [Figure 7].

Importantly, the Cipro + NSO group demonstrates improved performance relative to Cipro alone, suggesting partial recovery of learning ability. During the probe test, which assesses memory retention, the Cipro group shows reduced performance compared to control, indicating impaired spatial memory retention. In contrast, the Cipro + NSO group shows improved retention compared to Cipro alone, approaching control levels.

Histological Assessment

These observations in Figure 8 indicate that Ciprofloxacin induces notable neuronal damage in the PFC, while NSO co-administration attenuates the toxic effects and promotes structural restoration.

Spontaneous Alternation Behaviour

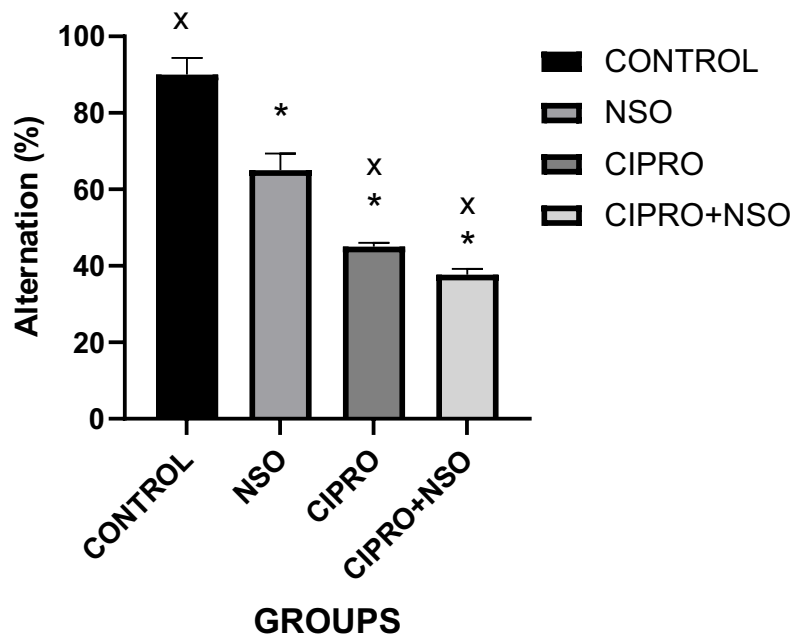


Figure 6: Graph showing the showing the percentage alternative behaviour in Y-maze test. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, × P<0.05 compare with CIPRO only

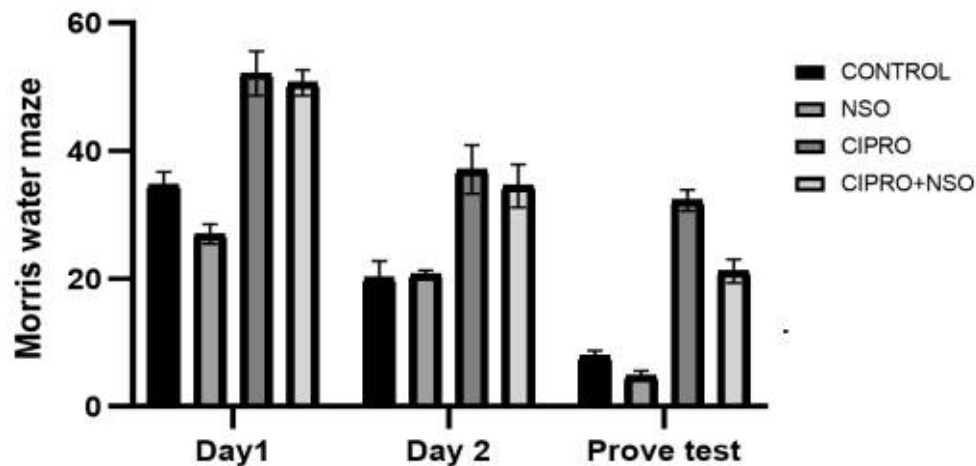


Figure 7: Comparative analysis of measured parameter across experimental groups

Grouped bar chart showing the mean values of the assessed variable across three experimental conditions within each group. Each cluster represents a distinct biological or treatment group, while individual bars within a cluster correspond to different treatment levels or exposure conditions. Data are presented as mean $\pm$ SEM (error bars)

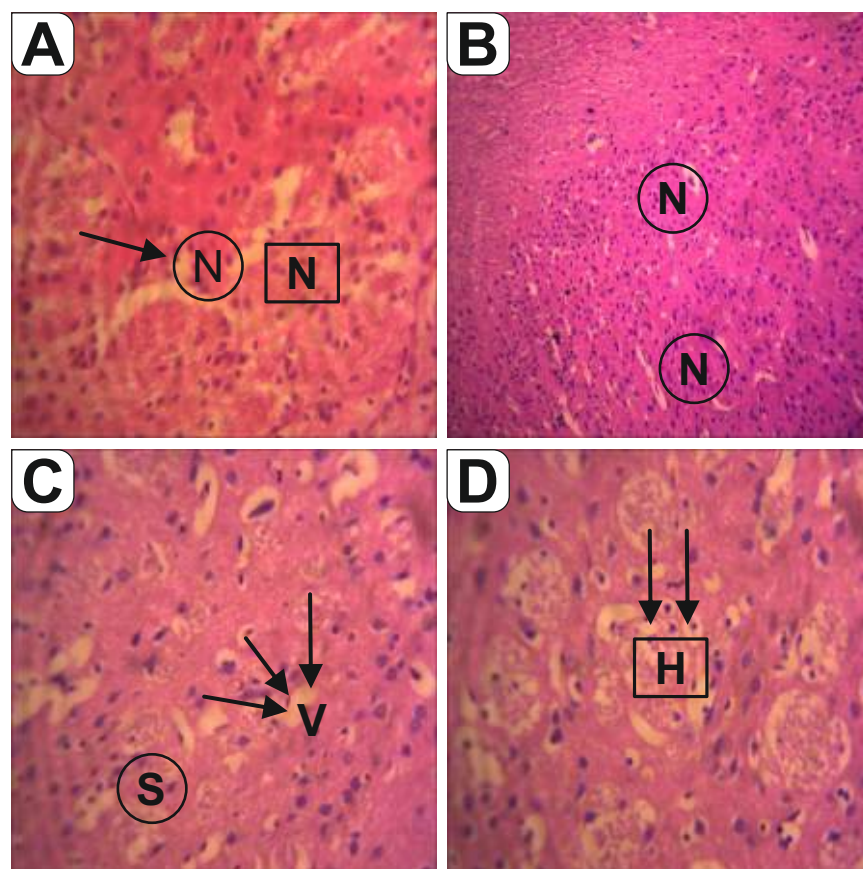


Figure 8: Photomicrographs of the prefrontal cortex (PFC) across experimental groups (H&E stain, $\times 400$). (A) Control group showing well-preserved cortical architecture with intact nuclei (N) and normal neuronal arrangement. (B) NSO-treated group demonstrating well-preserved neurons (N) with maintained cytoarchitectural integrity comparable to control. (C) Toxic (Cipro) group revealing marked degenerative changes characterized by neuronal cell shrinkage consistent with chromatolysis (S) and prominent cytoplasmic vacuolation (V), indicative of neuronal injury. (D) Cipro + NSO group showing evidence of structural recovery with reduced degenerative features and areas suggestive of healing marked by scar deposition (H), indicating partial neuroprotection and reparative response

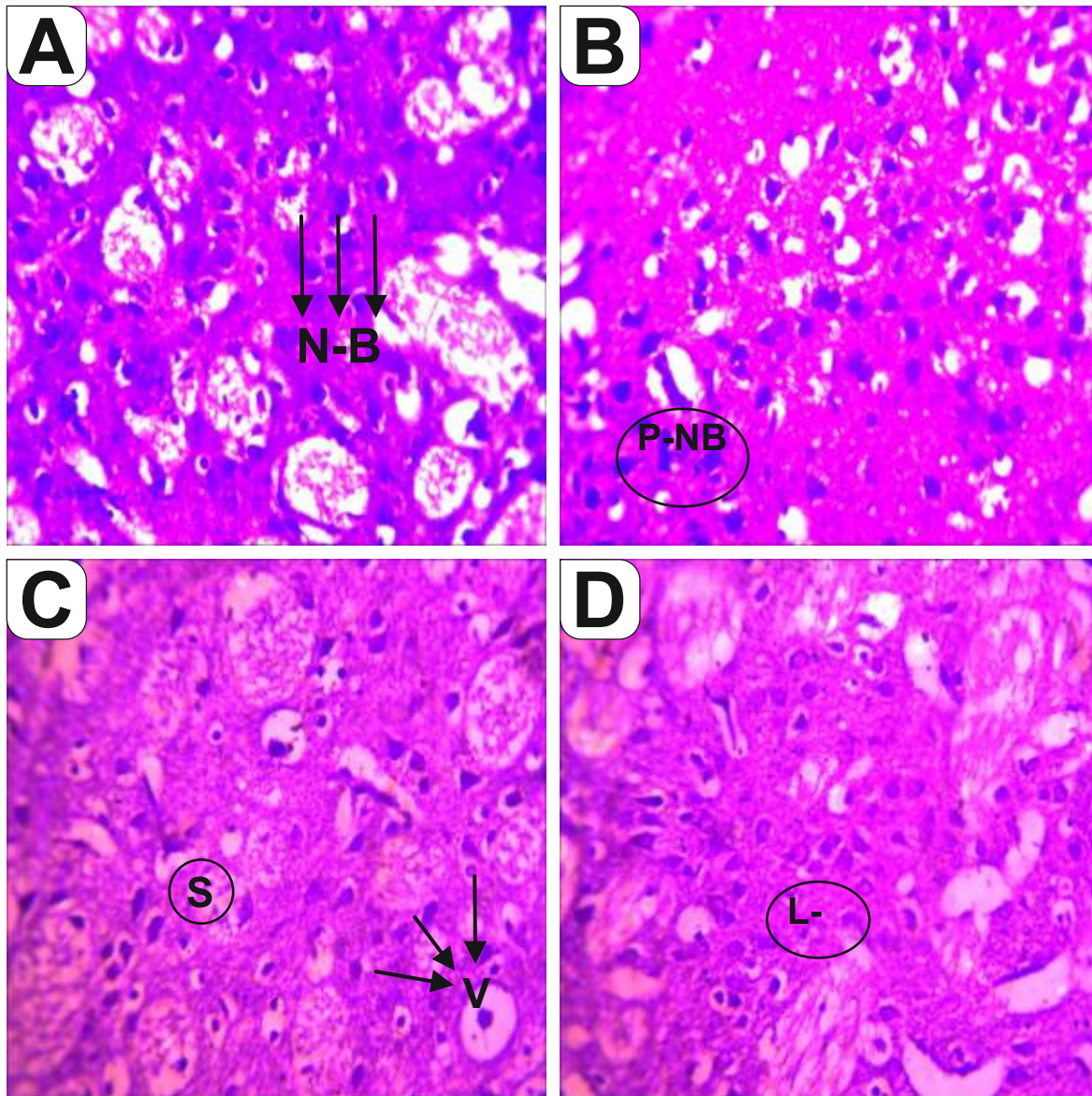


Figure 9: Photomicrograph showing the histology of the prefrontal cortex (PFC) of experimental groups (CFV $\times 400$). (A) Control group showing well-preserved cortical architecture with intact neurons and prominent Nissl bodies (NB), indicating normal neuronal integrity; (B) NSO-treated group demonstrating densely packed and well-preserved Nissl bodies (PNB), comparable to the control group, suggesting maintained neuronal structural stability; (C) Ciprofloxacin-treated group revealing marked neurodegenerative alterations characterized by neuronal cell shrinkage due to chromatolysis (S) and prominent cytoplasmic vacuolation (V), indicative of neuronal injury and toxic insult; (D) Ciprofloxacin + NSO group showing reduced degenerative features with fewer Nissl bodies (LNB) compared to control but improved architecture relative to the Ciprofloxacin-only group, suggesting partial recovery and ongoing healing processes, possibly associated with reparative scar deposition

The photomicrographs demonstrate that Ciprofloxacin induces significant neuronal degeneration in the PFC, while co-administration of NSO attenuates the histopathological damage and promotes structural restoration.

(a) Effect of Ciprofloxacin and NSO administration on Biochemical Parameters

i. Pro-inflammatory Cytokine

The results show a clear alteration in IL-1 β levels across the experimental groups. The Ciprofloxacin-treated group exhibited a marked and statistically significant increase in IL-1 β concentration compared to the control group, indicating a pronounced pro-inflammatory response following Ciprofloxacin administration. The NSO-only group demonstrated IL-1 β levels comparable to the control, suggesting that NSO does not independently induce inflammatory activation.

Co-administration of NSO with Ciprofloxacin resulted in a reduction in IL-1 β levels relative to the Ciprofloxacin-only group, although levels remained slightly elevated compared to control. This pattern indicates that Ciprofloxacin triggers neuroinflammatory processes, while NSO exerts a modulatory or anti-inflammatory effect, attenuating Ciprofloxacin-induced elevation of IL-1 β .

ii. Oxidative Stress Markers

Figure 11 shows that Ciprofloxacin significantly increased nitric oxide (NO) levels and significantly decreased glutathione peroxidase (GPx) activity compared to the control group, indicating increased oxidative stress and reduced antioxidant defense. Treatment with *Nigella sativa* oil (NSO) alone did not markedly alter these parameters compared to control. However, co-administration of NSO with Ciprofloxacin reduced the elevated NO levels and improved GPx activity compared to the Ciprofloxacin-only group. This suggests that *Nigella sativa* oil exerts a protective antioxidant effect against Ciprofloxacin-induced oxidative stress.

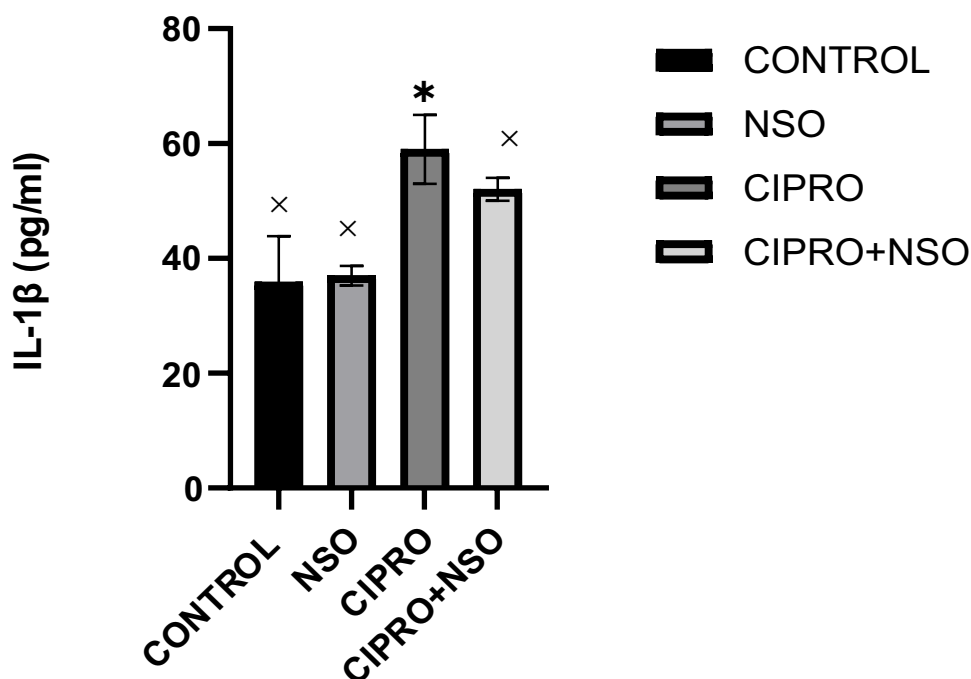


Figure 10: Graph showing the mean $\pm$ SEM levels of IL-1 β (pg/ml) in control, NSO, Ciprofloxacin, and combined treatment groups. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). *P<0.05 compare with control, x P<0.05 compare with CIPRO only

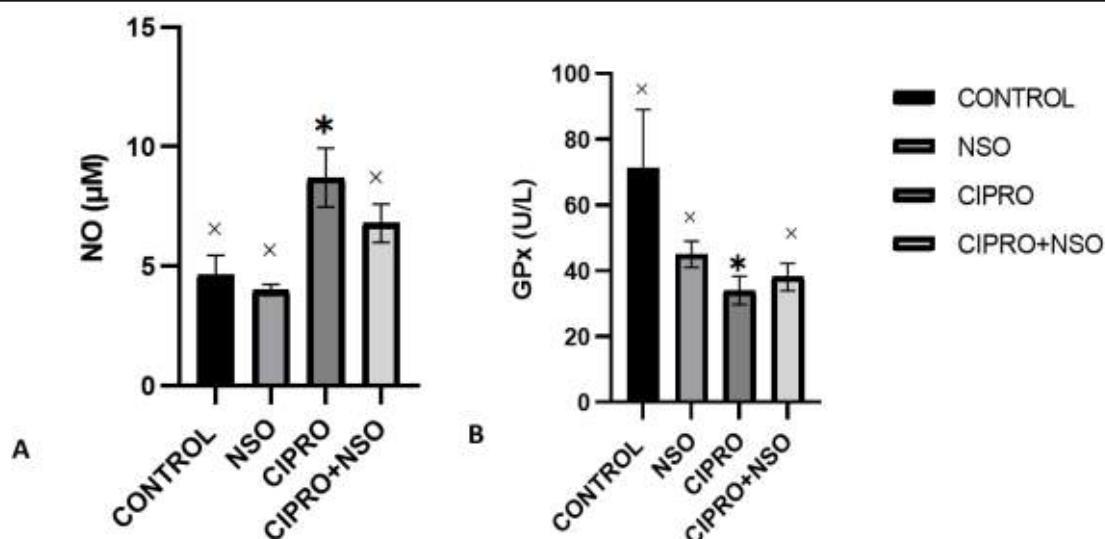


Figure 11: Graph showing the mean $\pm$ SEM levels of Nitric Oxide-NO (μ M) (A) and Glutathione Peroxidase- GPx (U/L) (B) in control, NSO, Ciprofloxacin, and combined treatment groups. (*) indicates statistical significance compared to the control group, while (x) indicates statistical significance compared to the NSO group; CTR = control (normal saline), NSO = *Nigella sativa* oil (1 mL/kg b.w.), CIPRO = Ciprofloxacin (60 mg/kg b.w.), and CIPRO+NSO = Ciprofloxacin (60 mg/kg b.w.) + *Nigella sativa* oil (1 mL/kg b.w.). * $P < 0.05$ compare with control, $\times P < 0.05$ compare with CIPRO only

DISCUSSION

The present study demonstrates that early postnatal exposure to Ciprofloxacin induces significant neurobehavioral impairment, oxidative imbalance, neuroinflammation, growth suppression, and structural degeneration within the prefrontal cortex (PFC) of Wistar rat offspring. Importantly, co-administration of *Nigella sativa* oil (NSO) markedly attenuated these alterations, suggesting a protective role mediated primarily through antioxidant and anti-inflammatory mechanisms. When examined collectively, the behavioral, biochemical, and histomorphological findings converge to establish oxidative stress-driven neuroinflammation as a central mechanism underlying Ciprofloxacin-induced cortical toxicity.

Fluoroquinolones, including Ciprofloxacin, are known to penetrate the blood-brain barrier and have been associated with central nervous system adverse effects such as agitation, anxiety, seizures, and cognitive disturbances^{10,11}. Mechanistically, fluoroquinolones can antagonize GABA-A receptors and disrupt excitatory-inhibitory neurotransmission balance, leading to neuronal hyperexcitability¹². During early postnatal development, the prefrontal cortex undergoes intense synaptogenesis, dendritic remodeling, and myelination, rendering it particularly vulnerable to pharmacological insults¹³. The behavioral alterations observed in this study including increased rearing activity, elevated stretch-attend posture, reduced central exploration, impaired spontaneous alternation in the Y-maze, and delayed acquisition in the Morris water maze reflect dysfunction of prefrontal-dependent executive processing and working memory networks^{14,15}.

The reduction in spontaneous alternation percentage indicates compromised spatial working memory, a function critically dependent on intact prefrontal-hippocampal circuitry¹⁶. Similarly, impaired performance in the Morris water maze reflects disruption in cortical integration of spatial learning and memory consolidation processes¹⁷. Anxiety-like behavior observed in the open field test further suggests altered cortical regulation of emotional processing. These findings are consistent with previous reports demonstrating behavioral hyperactivity and cognitive disturbances following fluoroquinolone exposure in experimental models^{12,18}. Thus, the neurobehavioral phenotype observed in this study provides functional evidence of cortical disruption.

Ciprofloxacin administration also significantly elevated nitric oxide (NO) levels while suppressing glutathione peroxidase (GPx) activity in the prefrontal cortex. This indicates a pronounced shift toward oxidative stress. Fluoroquinolones have been reported to induce mitochondrial dysfunction, resulting in excessive production of reactive oxygen species (ROS)¹⁹. Increased NO can react with superoxide radicals to generate Peroxynitrite, a potent cytotoxic oxidant capable of inducing lipid peroxidation, protein nitration, and DNA damage²⁰. Concurrent reduction in GPx activity further compromises endogenous antioxidant defense, amplifying neuronal susceptibility to oxidative injury. Oxidative stress is widely recognized as a fundamental mediator of drug-induced neurotoxicity and developmental cortical damage²¹. The present biochemical findings therefore provide mechanistic support for the observed structural degeneration.

In parallel with oxidative imbalance, Ciprofloxacin markedly

elevated interleukin-1 β (IL-1 β) levels, indicating activation of neuroinflammatory pathways. IL-1 β is a pro-inflammatory cytokine released by activated microglia and plays a pivotal role in synaptic dysfunction, impaired long-term potentiation, and neuronal apoptosis²². Sustained neuroinflammation during critical developmental windows can alter cortical circuitry and compromise executive functioning later in life²³. Oxidative stress and inflammation are closely interconnected; ROS-mediated activation of NF- κ B signaling promotes cytokine production, including IL-1 β , establishing a self-propagating inflammatory cascade²⁴. The concurrent elevation of NO and IL-1 β observed in this study therefore suggests that Ciprofloxacin-induced oxidative stress acts upstream of neuroinflammatory activation, culminating in cortical injury.

Histological findings further corroborate the biochemical and behavioral data. Ciprofloxacin-treated offspring exhibited neuronal shrinkage, chromatolysis, cytoplasmic vacuolation, and reduced Nissl substance within the PFC. Chromatolysis reflects dissolution of rough endoplasmic reticulum and impaired protein synthesis, typically occurring in response to axonal or metabolic injury²⁵. Cytoplasmic vacuolation suggests mitochondrial and membrane disruption, consistent with oxidative damage²¹. The reduction in Nissl bodies observed with Cresyl Fast Violet staining indicates compromised neuronal metabolic integrity. Collectively, these structural alterations confirm significant neurodegeneration within the prefrontal cortex and align with the functional deficits observed in behavioral assays.

Beyond neural parameters, Ciprofloxacin exposure was associated with reduced body weight gain and decreased litter size. Fluoroquinolone-induced mitochondrial impairment may disrupt cellular proliferation and metabolic regulation during development¹⁹. Early-life oxidative stress is known to interfere with growth signaling pathways and neurodevelopmental maturation²⁶. The developmental impact observed in this study underscores the broader systemic consequences of early antibiotic exposure and reinforces concerns regarding fluoroquinolone use during vulnerable periods. So, co-administration of *Nigella sativa* oil significantly mitigated Ciprofloxacin-induced alterations across behavioral, biochemical, and histological domains. NSO reduced NO levels, restored GPx activity, and attenuated IL-1 β elevation, suggesting effective modulation of oxidative and inflammatory pathways. The principal bioactive compound of NSO, thymoquinone, has been shown to possess potent free radical scavenging properties, enhance endogenous antioxidant enzyme activity, and inhibit NF- κ B-mediated inflammatory signaling^{27,28}. Previous studies have demonstrated that *Nigella sativa* and thymoquinone protect against chemically induced neurotoxicity and improve cognitive performance in oxidative stress models^{29,30}. The behavioral improvement observed in the CIPRO+NSO group particularly in working memory and spatial learning indicates functional preservation of cortical circuitry.

Moreover, NSO co-treatment reduced neuronal degeneration and preserved cortical cytoarchitecture, with improved Nissl body integrity compared to Ciprofloxacin alone. This structural recovery likely reflects reduced oxidative damage and suppression of inflammatory-mediated neuronal apoptosis. The partial normalization of IL-1 β levels further supports NSO's immunomodulatory role. Together, these findings suggest that NSO interrupts the pathogenic cascade initiated by Ciprofloxacin at multiple levels, thereby preserving neuronal viability and cortical function.

CONCLUSION

This study supports a mechanistic sequence in which Ciprofloxacin exposure induces mitochondrial dysfunction and ROS overproduction, leading to nitric oxide elevation, antioxidant enzyme suppression, activation of inflammatory cytokines, neuronal degeneration, and subsequent executive and cognitive dysfunction. *Nigella sativa* oil appears to counteract this cascade through antioxidant reinforcement and anti-inflammatory modulation, resulting in structural and functional neuroprotection. The significance of this study lies in its integrated demonstration that Ciprofloxacin can compromise higher-order cortical development during early life, and that plant-derived antioxidants such as *Nigella sativa* oil may offer a viable adjunctive neuroprotective strategy. These findings contribute to the growing body of literature cautioning against indiscriminate fluoroquinolone exposure during neurodevelopmental windows and provide a scientific basis for further translational investigation of *Nigella sativa* oil in mitigating antibiotic-induced neurotoxicity.

AUTHORS' CONTRIBUTION

Sample collection and data analysis were conducted by Atoyebei A.T., Nasirudeen A.E., and Adebisiyi A. O. The manuscript draft was prepared by Nasirudeen A.E., Adebisiyi A. O., and Alasirin K. The final manuscript draft and result interpretation was facilitated by Adebisiyi A. O., Abdulrauf A., Lawal I. A., Hamzat F. O., Ibrahim S. O., Eze S. M., and Dare B. J.

CONFLICT OF INTEREST: None

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REFERENCES

1. Andriole VT. The quinolones: past, present, and future. *Clin Infect Dis*. 2005;41(Suppl 2): S113–S119.
2. Stahlmann R, Lode H. Toxicity of quinolones. *Drugs*. 1999;58(Suppl 2):37–42.

3. Akande MG, Ajao MS, Oladipo OO, et al. Ciprofloxacin-induced oxidative stress and neurotoxicity in experimental animal models. *J Basic Clin Physiol Pharmacol.* 2014;25(2):123–130.
4. Hincal F, Gürbay A, Giray B. Induction of lipid peroxidation and alteration of antioxidant systems in rat brain by ciprofloxacin. *Hum Exp Toxicol.* 2001;20(8):393–398.
5. Kocyigit A, Koylu AA, Keles H, et al. Effects of ciprofloxacin on learning and memory functions in rats. *Pharmacol Biochem Behav.* 2006;83(3):417–422.
6. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of ciprofloxacin on locomotor activity and anxiety-related behavior in mice. *J Ethnopharmacol.* 2010;127(3):678–683.
7. Seleem MN, Jain N, Pothayee N, Ranjan A. Fluoroquinolone-induced neurotoxicity: histopathological and neurochemical alterations in rat cerebral cortex. *Neurotoxicol Teratol.* 2013; 39:1–8.
8. Ahmad A, Husain A, Mujeeb M, et al. A review on therapeutic potential of *Nigella sativa*: a miracle herb. *Asian Pac J Trop Biomed.* 2013;3(5):337–352.
9. Alkharfy KM, Ahmad A, Raish M, Vanhoutte PM. Neuroprotective effects of thymoquinone in experimental models of neurological disorders. *Drug Discov Today.* 2015;20(9):1183–1189.
10. Owens RC Jr, Ambrose PG. Antimicrobial safety: focus on fluoroquinolones. *Clin Infect Dis.* 2005;41(Suppl 2): S144–S157.
11. Tomé AM, Filipe A. Quinolones: review of psychiatric and neurological adverse reactions. *Drug Saf.* 2011;34(6):465–488.
12. De Sarro A, De Sarro G, Ascioti C, Nisticò G. Effects of fluoroquinolones on GABA-A receptors and CNS excitability. *Antimicrob Agents Chemother.* 1999;43(7):1729–1736.
13. Kolb B, Gibb R. Brain plasticity and behaviour in the developing brain. *J Can Acad Child Adolesc Psychiatry.* 2011;20(4):265–276.
14. Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci.* 2001; 24:167–202.
15. Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents and primates. *Neurosci Biobehav Rev.* 2004;28(7):771–784.
16. Lalonde R. The neurobiological basis of spontaneous alternation. *Neurosci Biobehav Rev.* 2002;26(1):91–104.
17. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc.* 2006;1(2):848–858.
18. Akah PA, Nwambie AI. Behavioral effects of ciprofloxacin in rodents. *Pharmacol Res.* 1998;37(2):143–147.
19. Lawrence JW, Claire DC, Weissig V, Rowe TC. Delayed cytotoxicity and mitochondrial dysfunction induced by fluoroquinolone antibiotics. *Toxicol Sci.* 1996;33(2):144–152.
20. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* 2007;87(1):315–424.
21. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuroparmacol.* 2009;7(1):65–74.
22. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. *Nat Rev Neurosci.* 2005;6(11):11–22.
23. Bilbo SD, Schwarz JM. Early-life programming of later-life brain and behavior: a critical role for the immune system. *Nat Rev Neurosci.* 2009;10(11):11–19.
24. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF- κ B signaling. *Cell Res.* 2011;21(1):103–115.
25. Lieberman AR. The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int Rev Neurobiol.* 1971; 14:49–124.
26. Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* 2000;108(Suppl 3):511–533.
27. Woo CC, Kumar AP, Sethi G, Tan KH. Thymoquinone: potential cure for inflammatory disorders and cancer. *Biochem Pharmacol.* 2012;83(4):443–451.
28. Ismail M, Al-Naqeeq G, Chan KW. *Nigella sativa* thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. *J Ethnopharmacol.* 2010;132(1):1–10.
29. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Neuroprotective effects of thymoquinone and *Nigella sativa* extract in experimental models of neurological injury. *Phytother Res.* 2007;21(11):1100–1103.
30. Alhebshi AH, Gotoh M, Suzuki I. Thymoquinone protects against amyloid β -induced neurotoxicity in cultured hippocampal neurons and improves memory performance in rats. *Neurochem Res.* 2013;38(3):667–674.

Research Paper

Dose-Dependent Effects of Aidan Fruit (*Tetrapleura tetraptera*) on Aluminum Chloride (AlCl₃)-Induced Kidney Toxicity in Adult Male Wistar Rats

S.M. Eze^{1*}, A.A. Bature¹, S.O. Ibrahim¹, A.T. Atoyebi¹, H.T. Muse¹, F.O. Hamzat¹, I.A. Lawal¹, K.O. Olowolayemo¹, Aisha Abdulrauf¹, W.O. Olaosebikan¹, A.O. Adebisi¹, K.D. Abdulfatahi¹, O.A. Ibrahim², A.Y. Imam-Fulami¹, A.T. Oyedele¹, K.J. Alabi¹, U. Ibrahim¹, K.B. Raji³, A.O. Yusuf⁴ and B.J. Dare⁵

¹Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria.

²Faculty of Nursing Sciences, College of Health Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria.

³Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

⁴Department of Human Anatomy, Faculty of Basic Medical Sciences, Abubakar Audu University, Anygba, Kogi State, Nigeria.

⁵Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Oshogbo, Nigeria.

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***Corresponding Author Email:** ezesuleiman@alhikmah.edu.ng

ABSTRACT

Background:

Aluminum chloride (AlCl₃) is widely recognized for its nephrotoxic potential, primarily due to its ability to induce oxidative stress and inflammation in renal tissues. The search for natural agents with protective properties has led to increased interest in *Tetrapleura tetraptera* (Aidan fruit), a plant known for its rich phenolic and antioxidant content.

Aim:

The study aimed to evaluate the dose-dependent effects of *Tetrapleura tetraptera* (Aidan fruit) on kidney function in adult male Wistar rats exposed to aluminum chloride.

Methods:

Thirty-five rats were randomly assigned into five groups ($n=7$): Group 1 received distilled water (control), Group 2 was administered $AlCl_3$ (100 mg/kg), Group 3 received $AlCl_3$ + low-dose extract (250 mg/kg), Group 4 received $AlCl_3$ + high-dose extract (500 mg/kg), and Group 5 received $AlCl_3$ + donepezil (10 mg/kg). Toxicity were induced for 28 days and treatments lasted 14 days, after which animals were sacrificed following IACUC guidelines. The right of the kidney was homogenized in (5xW/V) of phosphate buffer solution, PH 7.4 for biochemical analysis and the left was fixed in 10% formalin for histology analysis.

Results:

Aluminum chloride administration induced marked alterations in renal biochemical indices and oxidative stress markers. One-way ANOVA revealed significant differences in serum sodium ($F = 18.18, p = 0.00014$), chloride ($F = 27.45, p = 0.0000226$), and creatinine levels ($F = 9.39, p = 0.0020$) among the experimental groups. In contrast, potassium ($F = 2.46, p = 0.113$) and blood urea nitrogen (BUN) ($F = 1.485, p = 0.278$) did not differ significantly between groups. Treatment with *Tetrapleura tetraptera* extract produced dose-dependent improvements in electrolyte balance and renal function, with the high-dose group showing marked restoration of chloride levels and preservation of renal histoarchitecture. Furthermore, oxidative stress analysis revealed significant differences in malondialdehyde (MDA) ($F = 18.645, p = 0.0001$) and superoxide dismutase (SOD) ($F = 9.206, p = 0.0025$) levels among the experimental groups, while reduced glutathione (GSH) levels showed no statistically significant difference ($F = 2.400, p = 0.1193$). Treatment with *Tetrapleura tetraptera* extract attenuated oxidative stress and improved antioxidant status in a dose-dependent manner. Histological examination revealed severe glomerular and tubular degeneration in the toxic group, while treated groups, particularly the high-dose extract group, demonstrated substantial recovery and preservation of renal tissue integrity.

Conclusion:

Aidan fruit preserved glomerular structure, reduced tubular necrosis, particularly at higher doses. The effectiveness of *Tetrapleura tetraptera* was comparable to that of donepezil, a known neuroprotective and antioxidative properties and may serve as a potential therapeutic agent in prevent or reversing aluminum-induced kidney toxicity.

KEYWORDS: *Tetrapleura tetraptera*, Aluminum chloride, Kidney toxicity, Wistar rats, Nephroprotection, Oxidative stress, Donepezil

BACKGROUND

The kidney is bean-shaped located in the lower back region and weighs approximately 120-170 grams in adults, responsible for filtering blood, removing waste, balancing fluids and electrolytes, regulating blood pressure, hormonal functions such as producing erythropoietin and converting vitamin D to its active form and contributing to red blood cell production¹. The kidney consists of several anatomical parts, including the renal capsule, renal cortex, renal medulla, renal pelvis, and nephrons which receives blood through the renal arteries and filter approximately 120-150 liters of blood daily to produce urine in three main steps: glomerular filtration, tubular reabsorption, and tubular secretion². Nephrotoxicity, or toxic damage to the kidneys, can arise from exposure to chemicals, heavy metals, and drugs, given their high perfusion rate and exposure to various endogenous and exogenous is a major cause of acute kidney injury globally³.

Aluminum (Al), the third most abundant chemical element after silicon and oxygen, is thought to be the most prevalent

metal in the Earth's crust⁴. Industrialization has further raised the levels of Al in the environment significantly⁵. Humans are exposed to Al from a variety of sources, such as food additives found in baking powder, self-rising flour, cake mixes and some processed cheeses and contaminated water⁶. Naturally occurring aluminum in soil and rock can leach into groundwater and surface water leading to elevated aluminum levels in drinking water, also aluminum-based coagulants such as aluminum sulfate are used in water treatment which overtime potentially pose health concerns⁷. Aluminum hydroxide is a common ingredient in antacids and other over-the counter medication⁸ and cosmetics⁹.

Occupational exposure to Al can also arise from involvement in the Al processing industry (Skalny *et al.*, 2018). Previous research showed that vaccinations may be a source of aluminum exposure since they contain aluminum adjuvants, which are presently not commonly used and lower the risk of exposure to aluminum from vaccines¹⁰.

One of the mechanisms behind aluminum-induced kidney damage is oxidative stress. Aluminum increases the production of reactive oxygen species (ROS), which in turn causes lipid peroxidation, protein damage, DNA fragmentation, and ultimately cell death¹¹. Research using rodent models has confirmed that exposure to $AlCl_3$ causes nephrotoxicity and is characterized by histopathological changes in kidney tissues, elevation of serum urea and creatinine levels, oxidative damage to renal cells and compromised renal functions¹².

In search for novel nephroprotective agents with safer treatment and cost-effective options, researchers are increasingly turning to natural products¹³. One plant of interest is *Tetrapleura tetraptera*, commonly called Aidan fruit, commonly used in traditional African medicine¹⁴. It contains a variety of bioactive compounds, including polyphenols, flavonoids, tannins, and saponins known for their antioxidant, anti-inflammatory, and neuroprotective properties all of which have demonstrated protective effects against oxidative stress and inflammation in preclinical studies^{14,15}.

Additionally, polyphenolic compounds, in particular, have been shown to scavenge free radicals, chelate metal ions, and upregulate endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT)¹⁵. These mechanisms make polyphenol-rich plant extracts promising agents for the mitigation of nephrotoxic damage¹⁶.

Aidan fruit, is renowned for its significant nutritional and therapeutic value, making it a prominent candidate in managing toxin-induced organ damage. The fruit contains a variety of essential nutrients and bioactive compounds that contribute to its medicinal properties. It contains a rich array of macronutrients and micronutrients essential for physiological functioning¹⁴.

Moreover, beyond its nutritional content, *T. tetraptera* is exceptionally rich in phytochemicals, including flavonoids, saponins, tannins, alkaloids, and particularly phenolic compounds. According to Ajayi *et al.*¹⁶, these phytochemicals contribute to the plant's strong antioxidant and anti-inflammatory potential, further adding to its therapeutic arsenal. Hence the need to study the scavenge free radicals' potentials of Aidan fruit in aluminum chloride mediated kidney damage in adult male Wistar rats.

MATERIALS AND METHODS

Experimental Plant

Aidan fruit (*Tetrapleura tetraptera*): 1000g of Aidan fruit was obtained from mandate daily market Ilorin, Kwara State where it was powdered and the seed were removed and was taken to the Industrial Chemistry, Faculty of Physical Science, University of Ilorin, and Kwara State where it was extracted.

Experimental Chemical

Aluminum Chloride ($AlCl_3$): The Aluminum chloride ($AlCl_3$) produced by Span Chemie Company, India and was gotten from Dennis and Dennis Store Ilorin, Kwara State. Donepezil was bought from One-Step Pharmacy Ilorin, Kwara State.

Other Experimental Materials

Distilled water was obtained from the Department of Biochemistry, Faculty of Natural and Applied Science, AL-Hikmah University Ilorin, Kwara State. Dissecting kits, Syringes, Gloves and Sample Bottles: The syringes, gloves, sample bottles were bought from One-Step Pharmacy Ilorin, Kwara State.

Experimental Animals

This study utilized 35 male Wister rats, sourced from Al-Hikmah University's Department of Human Anatomy. The rats were kept in a controlled environment with a 12-hour day-night cycle and standard temperature, and fed purified water and a standard pellet diet. The rats underwent a two-week adaptation period before the experiment commenced.

Experimental Protocol

Group A, the Control group consisting of seven (7) Wister rats, where administered distilled water only for 28 days. Group B which was the Toxic group consisting of seven (7) Wister rats, were given $AlCl_3$ only for 28 days. Group C which was the Treated group consisting of seven (7) Wister rats, were administered $AlCl_3$ for 28 days and low dose of PT-T for 14 days. Group D which was the treated group consisting of seven (7) Wister rats, were administered $AlCl_3$ for 28 days and high dose of PT-T for 14 days. Group E which was the treated group consisting of seven (7) Wister rats, were administered $AlCl_3$ for 28 days and donepezil for 14 days. All administration was done using the oral cannula. The rats acclimatized for 14 days prior to administration and were maintained under standard atmosphere under dark/day circle. The rats were fed with standard rats feed and water *Ad libitum*. All administration were carried out through oral canula. After the last day the animal were fasted overnight and their final body weight were taken. The rats were **euthanasia** through cervical dislocation and midline incision was done to harvest the kidneys. The right of the kidney was homogenized in (5xW/V) of phosphate buffer solution, PH 7.4 for biochemical analysis and the left was fixed in 10% formalin for histology analysis.

Histopathology Examination

At the conclusion of the study (Day 29 and Day 43), the rats were humanely euthanized using cervical dislocation. The kidney was harvested and fixed in 10% formalin for 48 hours, after fixation, the kidney tissues were embedded in paraffin and sectioned into thin slices (5 μ m). These sections were then stained with Hematoxylin and Eosin (H&E) to examine any

histological changes, including signs of tubular epithelial cell degeneration, glomerular atrophy, vascular congestion, and interstitial inflammation. Microscopic analysis was performed to identify and structural changes.

Biochemical Analysis

Renal Function Tests

Urea was measured using a Randox Urea assay kit while using the enzymatic Urease-glutamate dehydrogenase method¹⁷. Creatinine was measured using the Randox creatinine kit for Jaffe's colorimetric method, in which creatinine reacts with alkaline picrate to form a colored complex with maximum absorbance at 520nm¹⁸. Electrolytes were measured using the Ion-selective electrode (ISE) method using an ISE-based electrolyte analyzer to display the concentration of sodium, potassium and chloride, the electrolyte imbalance was used to infer tubular dysfunction or damage caused by aluminum toxicity or protective effects from treatments. Disruption in their levels often reflects underlying renal tubular damage¹⁹.

Oxidative Stress Markers

Malondialdehyde (MDA) were measured using a lipid peroxidation assay kit specific to rats, in accordance with the manufacturer's instructions. An increase in MDA concentration within the kidney suggests elevated oxidative injury, which may disrupt cellular integrity²⁰. The activity of superoxide dismutase (SOD) in kidney tissue was assessed using a rat-specific colorimetric assay kit, based on the manufacturer's protocol. Alterations in SOD activity are often indicative of oxidative stress levels and may reflect the kidney response to toxic insults or therapeutic interventions²¹. **The concentration of reduced glutathione (GSH) in kidney tissue was measured using a rat-specific colorimetric assay kit, in accordance with the manufacturer's instructions.** GSH is a crucial intracellular antioxidant that defends nephrons against oxidative damage by neutralizing free radicals and detoxifying harmful compounds²².

Ethical Approval

In accordance with the Institutional Animal Care and Use Committee (IACUC), the Faculty of Basic Medical Science, College of Health Science, Ilorin review committee at Al-Hikmah University approved this research project on the Faculty of Basic Medical Science's recommendation.

Euthanasia

At the conclusion of the experimental period, all animals were humanely sacrificed following the determination of their final body weights. Euthanasia was carried out through cervical dislocation, adhering to established ethical standards for the care and use of laboratory animals. After euthanasia, a midline incision was made from the thoracic cavity to the abdominal cavity to expose the kidneys. Blood samples were collected

directly from the heart using a sterile 2 ml syringe and transferred into ethylene diamine-tetra acetic acid (EDTA) bottles to prevent coagulation. The blood samples were immediately centrifuged and stored in an ice-filled container for further biochemical analyses. The kidneys were then carefully excised to preserve tissue morphology. Each kidney was rinsed in normal saline to remove excess blood and then placed in labeled sample containers filled with 10% buffered formaldehyde for fixation. The fixed tissues were subsequently processed, embedded in paraffin wax, and sectioned at 5 µm thickness using a rotary microtome. The sections were mounted on glass slides for histological and immunohistochemically evaluation. Following organ collection, the carcasses of the Wister rats were disposed through burial in an approved sanitary site, in line with institutional ethical guidelines.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test to determine significant differences. A p-value of <0.05 was considered statically significant. All statistically analysis was performed using Graph-Pad Prism 8 software.

RESULTS

Physical Observation

Physical observations were carried out daily throughout the experiment to monitor the health status, behavioral changes, and overall physical response of the rats in each group.

Group 1 (Control – Distilled Water)

Rats in this group remained physically stable and active throughout the 28-Day period. They showed no signs of abnormal behavior, bleeding, or death. Feeding and grooming remained normal.

Group 2 (Toxic – Aluminum Chloride)

From day 2, rats in this group began showing signs of distress such as excessive calmness and hiccups. By day 4, nasal bleeding was observed. Mortality began by day 15, with two deaths occurring. Aggressiveness, reduced feed intake, and signs of meningitis were also noted. The group was sacrificed on Day 28.

Group 3 (Aluminum Chloride + Low Dose PPT)

Initial signs of distress (mucous secretion, spinning, walking sideways) were observed during the aluminum chloride phase. After the introduction of Aidan fruit extract on Day 29, gradual behavioral improvement and signs of healing were recorded.

However, some rats died on Day 32, 33, and 35. By Day 41, the remaining rats were visibly active with reduced signs of complication.

Group 4 (Aluminum Chloride + High Dose PPT)

This group exhibited significant distress, including nose bleeding and feed refusal, starting from the aluminum chloride phase. One death occurred early (Day 5), followed by progressive improvement after high-dose Aidan extract administration. However, one death was also recorded on Day 21. Signs of recovery (reduced swelling, improved feeding, and activity) became prominent by Day 30.

Group 5 (Aluminum Chloride + Donepezil)

Early signs included mucous secretion, limb paralysis, and aggressiveness. Mortality two occurred on Day 5 and 6. After donepezil administration began, improvement in motor function and healing of visible injuries (e.g., swollen limb, cleft palate) was recorded. By Day 30, healing was evident, and rats remained active up to Day 41.

Body Weight of the Rats

In this study, there was no significant difference in the weight changes across the groups. However, an increase was observed in the mean final body weight of the rats across the groups when compared with the initials [Figure 1].

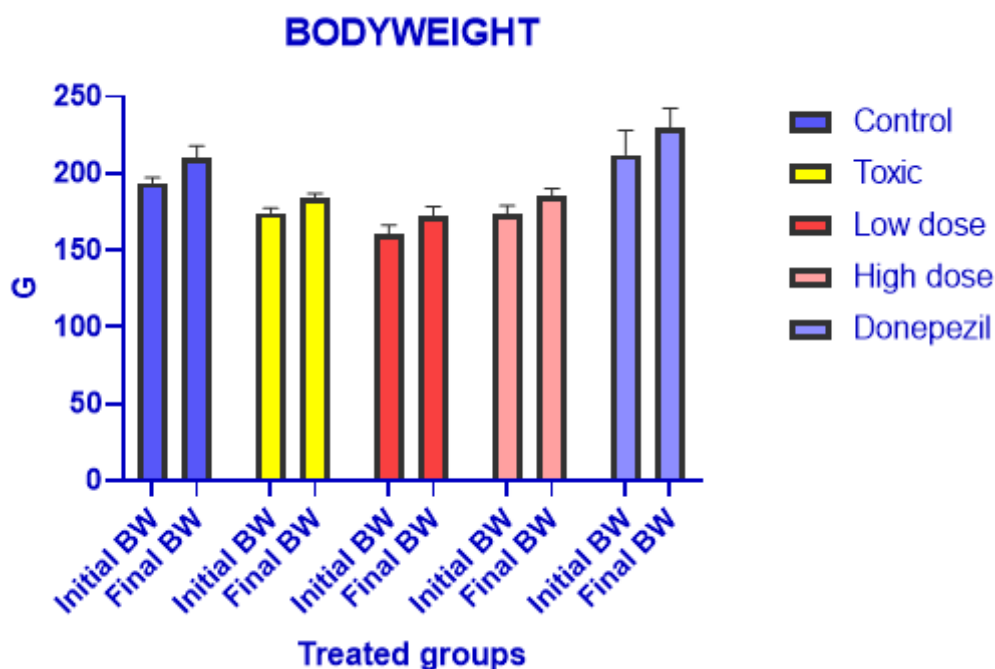


Figure 1: Initial and Final Body Weights of Rats in the Experimental Groups

Data are presented as mean $\pm$ SEM. Groups comprised control (distilled water; 100 mg/kg BW), toxic (Aluminum chloride 100 mg/kg BW), low-dose treatment (Aluminum chloride 100 mg/kg BW + Aidan 250 mg/kg BW), high-dose treatment (Aluminum chloride 100 mg/kg BW + Aidan 500 mg/kg BW), and donepezil (Donepezil 10 mg/kg BW + $AlCl_3$ 100 mg/kg BW). No statistically significant differences were observed among the experimental groups ($p > 0.05$).

Biochemical Analysis

Kidney Function Test

Serum electrolyte concentrations and renal function biomarkers were evaluated following aluminum chloride exposure and subsequent treatment with *Tetrapleura tetraptera* extract or donepezil. Analysis of serum sodium levels demonstrated a statistically significant difference among the experimental groups ($F(4,10) = 18.18, p = 0.00014$). The high-dose *Tetrapleura tetraptera* group exhibited the highest sodium concentration (186.61 ± 1.13 mmol/L), whereas the control and donepezil groups showed comparatively lower values (111.07 ± 10.72 mmol/L and 112.14 ± 0.41 mmol/L, respectively). These findings suggest significant modulation of sodium homeostasis following treatment. Potassium concentrations did not differ significantly among the groups ($F(4,10) = 2.46, p = 0.113$), although the toxic group recorded the highest potassium level (7.85 ± 0.11 mmol/L), while the donepezil-treated group showed the lowest value (5.00 ± 0.03 mmol/L). Chloride levels differed significantly across groups ($F(4,10) = 27.45, p = 0.0000226$). The high-dose *Tetrapleura tetraptera* group exhibited markedly elevated chloride concentrations (152.60 ± 0.38 mmol/L), whereas the toxic group recorded the lowest value (36.66 ± 4.00 mmol/L). Post-hoc analysis revealed significant differences between the high-

dose treatment group and the other experimental groups, indicating a strong treatment effect on chloride regulation. Blood urea nitrogen (BUN) concentrations showed no statistically significant difference among the groups ($F(4,10) = 1.485, p = 0.278$), despite slight numerical increases in the treatment groups. Serum creatinine levels differed significantly among the experimental groups ($F(4,10) = 9.39, p = 0.0020$). Elevated creatinine concentrations observed in the aluminum chloride-treated rats indicated impaired renal function, whereas treatment with *Tetrapleura tetraptera* extract and donepezil modulated creatinine levels, reflecting varying degrees of renal protection and recovery.

Oxidative Stress Markers

Renal oxidative stress biomarkers were evaluated by measuring malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) levels. MDA levels differed significantly among the experimental groups ($F(4,10) = 18.645, p = 0.0001$). The high-dose *Tetrapleura tetraptera* group exhibited the highest MDA level (5.10 ± 0.14), whereas the toxic group recorded the lowest value (2.43 ± 0.06). Significant differences among treatment groups indicate that administration of *Tetrapleura tetraptera* extract markedly influenced lipid peroxidation status. SOD activity also differed significantly among groups ($F(4,10) = 9.206, p = 0.0025$).

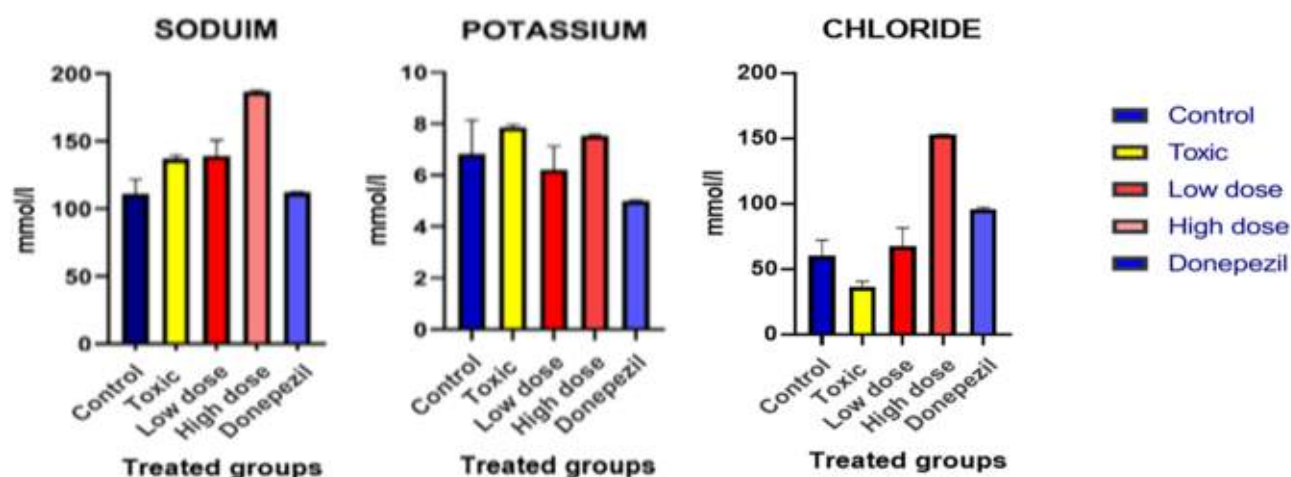


Figure 2: Effects of *Tetrapleura tetraptera* Extract and Donepezil on Serum Electrolyte Concentrations in Aluminum chloride-induced Nephrotoxicity

Values are expressed as mean $\pm$ SEM ($n = 7$ per group). Groups comprised control (distilled water; 100 mg/kg BW), toxic (Aluminum chloride 100 mg/kg BW), low-dose treatment (Aluminum chloride 100 mg/kg BW + Aidan 250 mg/kg BW), high-dose treatment (Aluminum chloride 100 mg/kg BW + Aidan 500 mg/kg BW), and donepezil (Donepezil 10 mg/kg BW + AlCl_3 100 mg/kg BW). One-way ANOVA demonstrated a general significant difference in serum sodium ($F = 18.18, p = 0.00014$) and chloride ($F = 27.45, p = 0.0000226$) concentrations among groups, whereas serum potassium levels did not differ significantly ($F = 2.46, p = 0.113$).

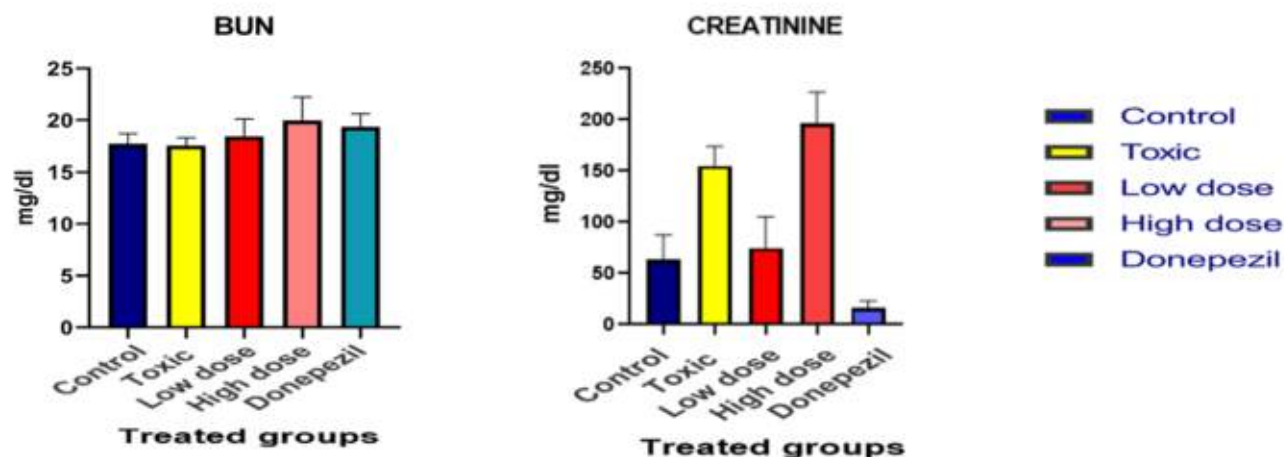


Figure 3: Effects of Aluminum chloride and Aidan Extract Treatment on Renal Function Biomarkers

Values are presented as mean $\pm$ SEM for serum blood urea nitrogen (BUN) and creatinine concentrations in the control group (distilled water), toxic group (100 mg/kg BW AlCl_3), low-dose Aidan extract group (100 mg/kg BW AlCl_3 + 250 mg/kg BW Aidan extract), high-dose Aidan extract group (100 mg/kg BW AlCl_3 + 500 mg/kg BW Aidan extract), and donepezil-treated group (100 mg/kg BW AlCl_3 + 10 mg/kg BW donepezil). One-way ANOVA revealed a general significant difference in serum creatinine levels ($F = 9.39$, $p = 0.0020$), while blood urea nitrogen (BUN) levels did not differ significantly among groups ($F = 1.485$, $p = 0.278$).

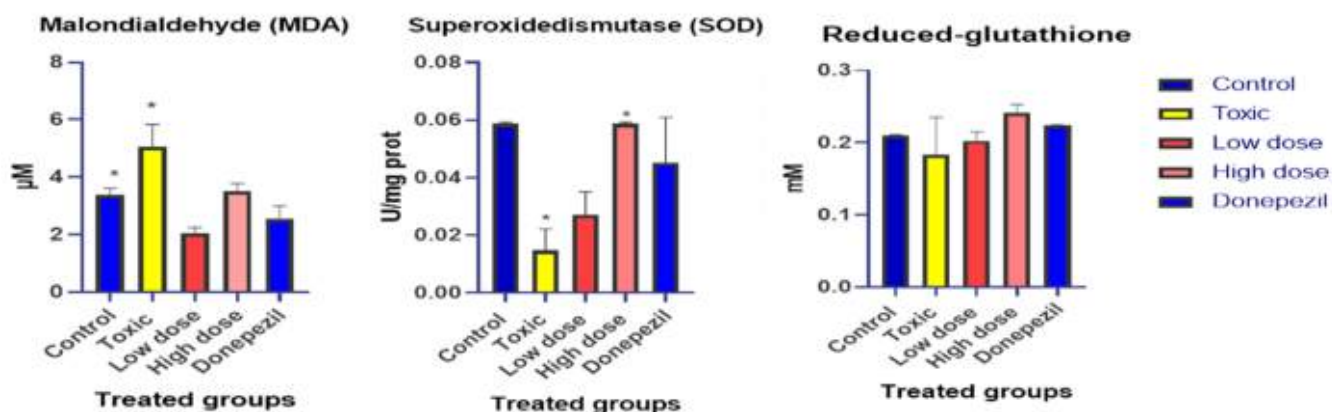


Figure 4: Effects of Treatment on Oxidative Stress and Antioxidant Biomarkers in Experimental Groups

Bar charts showing the levels of malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) across the control, toxic, low-dose, high-dose, and donepezil-treated groups. The control group (distilled water), toxic group (100 mg/kg BW AlCl_3), low-dose Aidan extract group (100 mg/kg BW AlCl_3 + 250 mg/kg BW Aidan extract), high-dose Aidan extract group (100 mg/kg BW AlCl_3 + 500 mg/kg BW Aidan extract), and donepezil-treated group (100 mg/kg BW AlCl_3 + 10 mg/kg BW donepezil). Data are presented as mean $\pm$ SEM. One-way ANOVA revealed significant differences in MDA ($F = 18.645$, $p = 0.0001$) and SOD ($F = 9.206$, $p = 0.0025$) among the experimental groups, whereas GSH levels did not differ significantly ($F = 2.400$, $p = 0.1193$). Statistical significance was accepted at $*p < 0.05$.

Treatment with *Tetrapleura tetraptera* extract and donepezil modulated antioxidant enzyme activity relative to the toxic group, with the high-dose treatment group demonstrating the greatest improvement in antioxidant defense. In contrast, GSH levels showed no statistically significant difference among groups ($F(4,10) = 2.400$, $p = 0.1193$), although numerical

variations were observed across treatments. These findings suggest that while *Tetrapleura tetraptera* significantly influenced lipid peroxidation and SOD activity, its effect on GSH concentration was not statistically significant under the conditions of the present study.

Histological Investigations

The photomicrographs of the kidney tissue obtained from the control group shows a Histomorphological architecture including normal renal tubules, glomerulus, bowman's capsule, glomerulus, urinary pool, vascular pool; H&E stain 100x magnification, [as shown in Plate 1]. However, the toxic group photomicrograph of the histopathology architecture of the kidney tissues sections [showing in Plate 2] the disrupted renal tubules, spaced bowman's capsule, atrophy of the glomerulus, disrupted urinary pool, detached vascular pool; H&E stain 100x magnification. Furthermore, the photomicrograph of the histopathology architecture of the kidney tissues in the low dose group (100mg/kgBW AlCl_3 + 250mg/kgBW PT-T) [showing in Plate 3]. Tissue sections show partial repair renal tubules, reduced spaced bowman's

capsule, mild atrophy of the glomerulus, repaired urinary pool, repaired vascular pool; H&E stain 100x magnification. Photomicrograph of the histopathology architecture of the kidney tissues in the high dose group (100mg/kgBW AlCl_3 + 500mg/kgBW PT-T) [shown in Plate 4]. Tissue sections showing repaired renal tubules, reduced spaced Bowman's capsule, minimal atrophy of the glomerulus, repaired urinary pool, repaired vascular pool; H&E stain 100x magnification. Additionally, photomicrograph of the histopathology architecture of the kidney tissues in the Donepezil group (100mg/kgBW AlCl_3 + 10mg/kgBW Donepezil) [showing in Plate 5]. Tissue sections showing repaired renal tubules, reduced spaced Bowman's capsule, normal glomerulus, repaired urinary pool, repaired vascular pool; H&E stain 100x magnification.

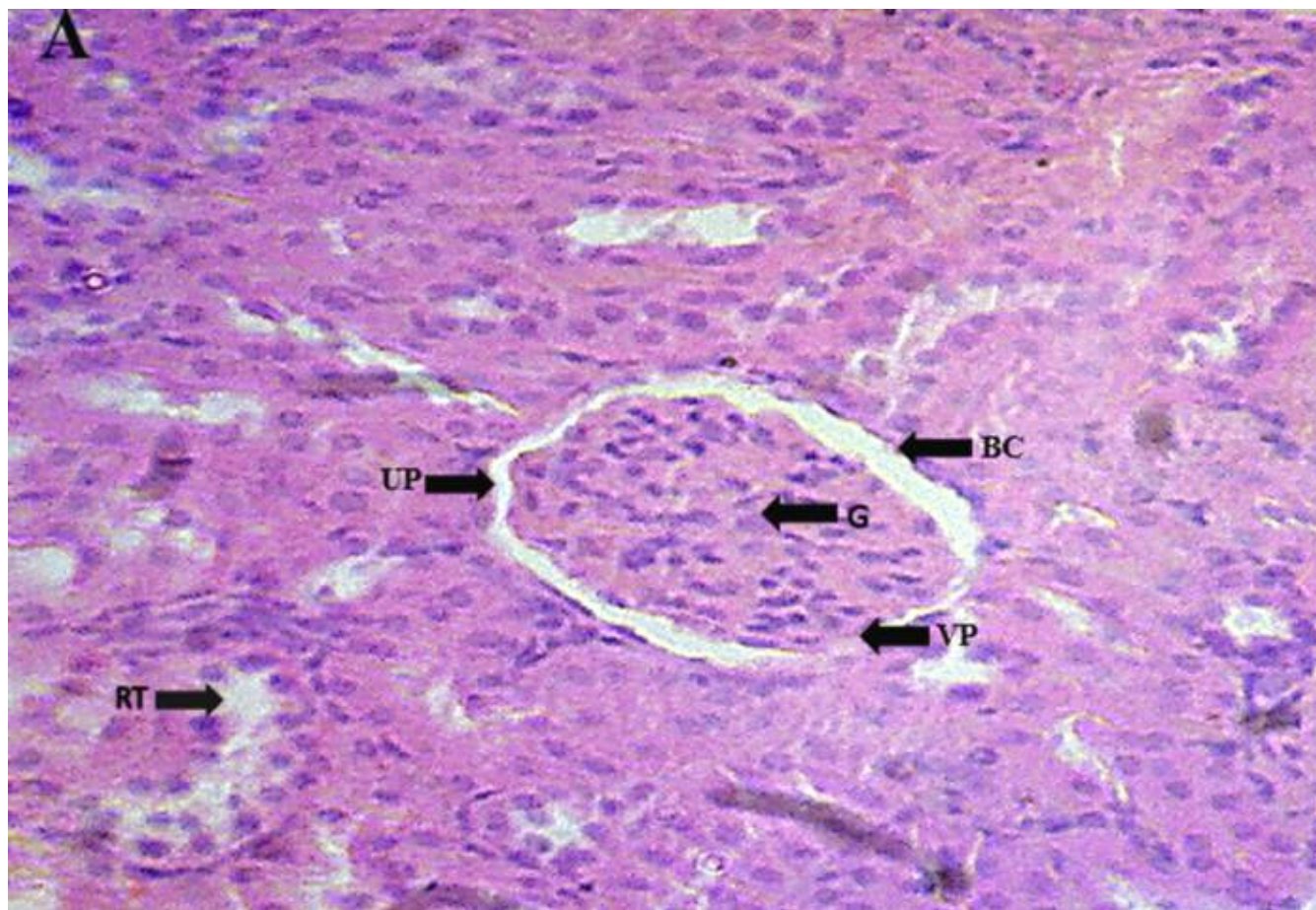


Plate 1: Photomicrograph of the Histomorphological Architecture of the Kidney Tissues in the Control Group. Tissue Section Showing Normal Morphology of Renal Tubules (RT), Bowman's Capsule (BC), Glomerulus (G), Urinary Pool (UP), Vascular Pool (VP); H&E Stain 100x Magnification

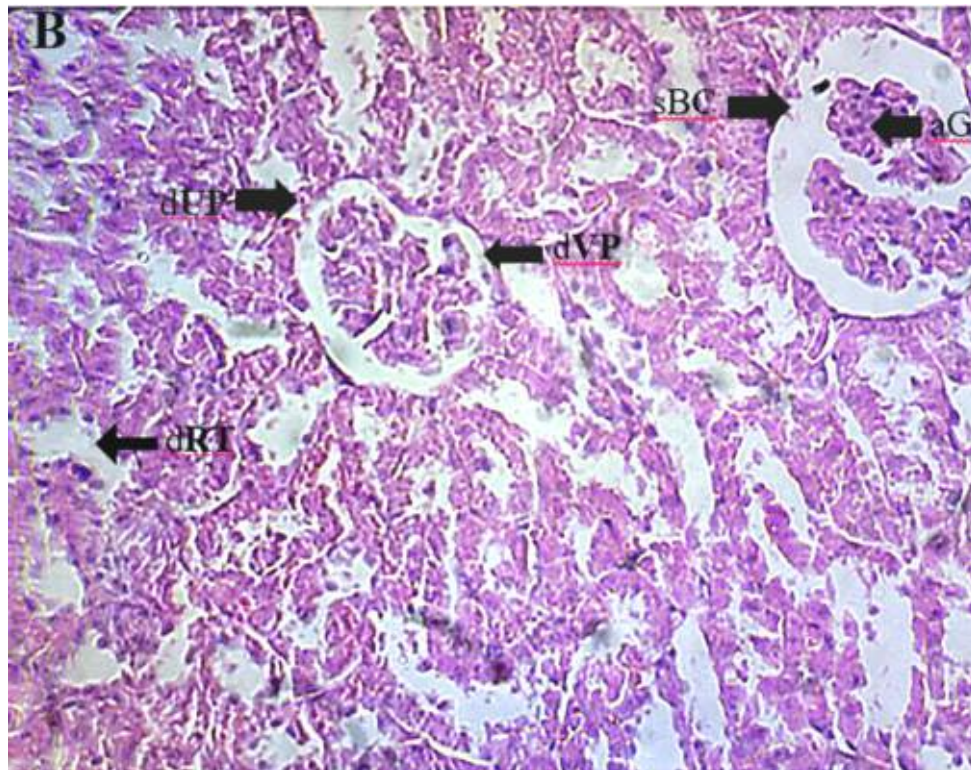


Plate 2: Photomicrograph of the Histopathology Architecture of the Kidney Tissues in the Toxic Group. Tissue Sections Showing Disrupted Renal Tubules (dRT), spaced Bowman's Capsule (sBC), Atrophy of the Glomerulus (aG), Disrupted Urinary Pool (dUP), Detached Vascular Pool (dVP); H&E Stain 100x Magnification

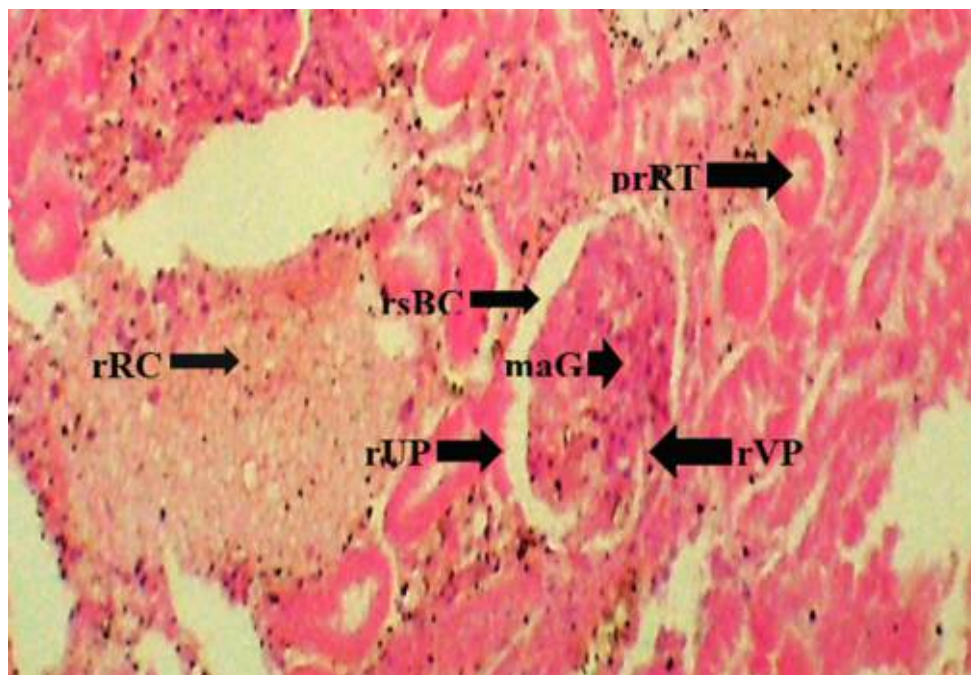


Plate 3: Photomicrograph of the Histopathology Architecture of the Kidney Tissues in the Low Dose Group (100mg/kgBW $AlCl_3$ + 250mg/kgBW PT-T). Tissue Sections Showing Partial Repair Renal Tubules (prRT), Reduced Spaced Bowman's Capsule (rsBC), Mild Atrophy of the Glomerulus (maG), Repaired Urinary Pool (rUP), Repaired Vascular Pool (rVP); H&E Stain 100x Magnification

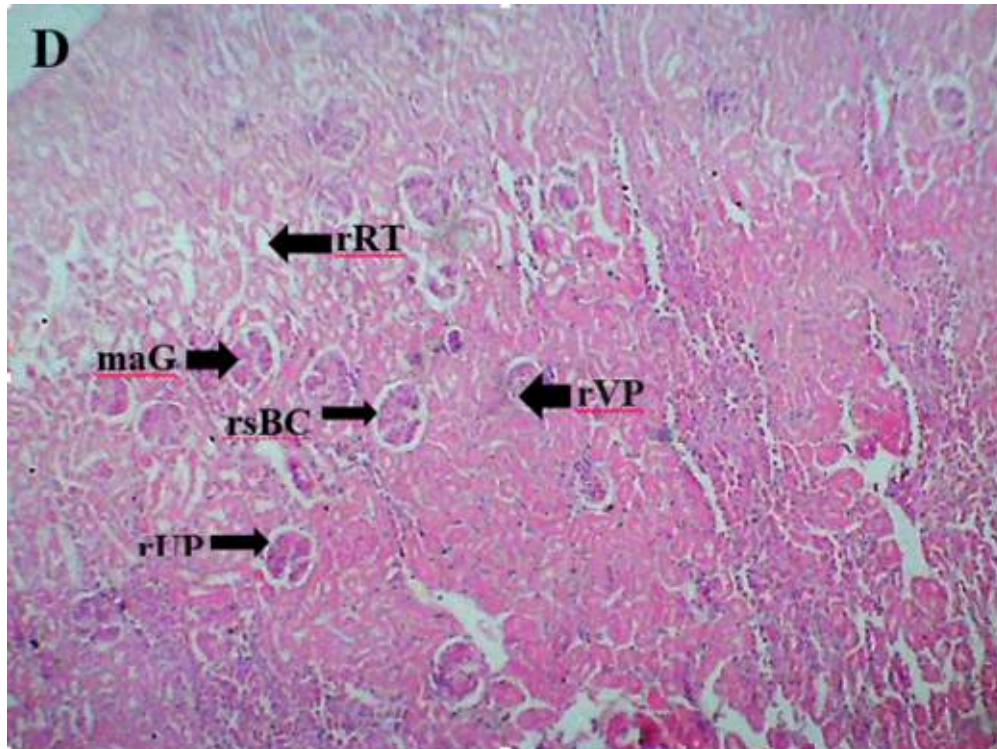


Plate 4: Photomicrograph of the Histopathology Architecture of the Kidney Tissues in the High Dose Group (100mg/kgBW AlCl_3 + 500mg/kgBW PT-T). Tissue Sections Showing Repaired Renal Tubules (rRT), Reduced Spaced Bowman's Capsule (rsBC), Minimal Atrophy of the Glomerulus (maG), Repaired Urinary Pool (rUP), Repaired Vascular Pool (rVP); H&E Stain 100x Magnification

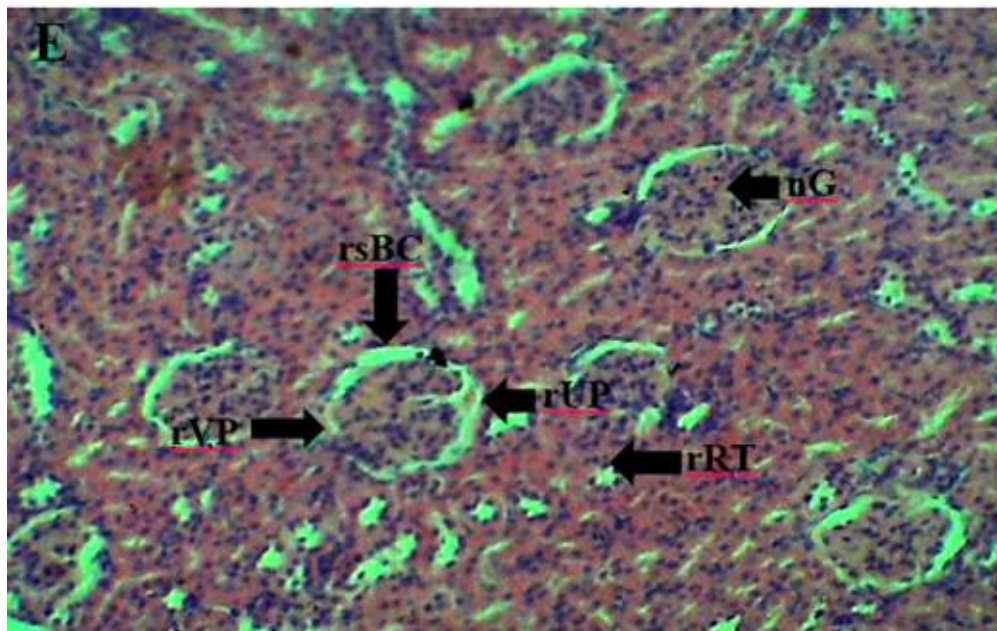


Plate 5: Photomicrograph of the Histopathology Architecture of the Kidney Tissues in the Donepezil Group (100mg/kgBW AlCl_3 + 10mg/kgBW Donepezil). Tissue Sections Showing Repaired Renal Tubules (rRT), Reduced Spaced Bowman's Capsule (rsBC), Normal Glomerulus (nG), Repaired Urinary Pool (rUP), Repaired Vascular Pool (rVP); H&E Stain 100x Magnification

DISCUSSION

Environmental toxicant exposure to aluminum remains a significant public health concern due to its accumulation in the kidneys. The toxic effect of aluminum on the kidneys leads to oxidative stress, functional impairment, and structural damage, contributing to the development of chronic kidney diseases. The observations of the rats' physical behavior added further insight into the effects of each treatment, rats in the aluminum-only group showed clear signs of sickness, including fatigue, nosebleeds, and reduced movement symptoms similar to those described by Igbeke *et al.*²³, in aluminum toxicity studies. In contrast, rats treated with Aidan extract, especially at higher doses, appeared healthier and more active, with better appetite and general behavior. Rats treated with donepezil also showed improvements, such as restored energy levels and fewer signs of physical distress²⁴.

The biochemical evaluation of blood serum urea (BUN), creatinine, and antioxidant enzymes, particularly superoxide dismutase (SOD), revealed critical insights into renal function and oxidative stress mitigation across all treated groups. In the AlCl_3 -exposed rats (toxic/ group two), significantly elevated levels of urea and creatinine confirmed impaired glomerular filtration, suggesting nephrotoxicity. These findings align with previous studies indicating that aluminum chloride disrupts renal tubular and glomerular integrity, thereby compromising excretory efficiency^{5,8}.

The rise in BUN and creatinine concentrations in the AlCl_3 -exposed group suggests reduced glomerular filtration rate (GFR) and compromised excretory function. This aligns with reports by Ezejiofor *et al.*²⁰ and Kumar and Gill¹², who identified aluminum toxicity as a major disruptor in accumulation of nitrogenous wastes.

Interestingly, treatment with *tetrapluera tetraptera* led to significant improvements in these parameters. Rats administered low and high dose of the extract showed progressive normalization of BUN and creatinine levels, indicative of nephroprotection. The high-dose group demonstrated biochemical values close to those of the control group, suggesting superior efficacy. These findings support prior research showing that *T. tetraptera* possesses strong antioxidant and anti-inflammatory compounds such as polyphenols, tannins, and flavonoids, which mitigate tissue damage by scavenging ROS and restoring membrane integrity^{16,25}.

In terms of antioxidant activity, superoxide dismutase (SOD) levels were significantly depleted in the AlCl_3 -only group, constituent with previous studies linking aluminum toxicity to oxidative stress-mediated enzyme depletion¹². Treatment with Aidan fruit significantly restored SOD levels in a dose-dependent manner, likely due to enhanced free radical scavenging. Notably, high-dose Aidan fruit restored SOD activity close to baseline, further supporting its antioxidative therapeutic potential.

Another important antioxidant in the body is glutathione (GSH), which helps to maintain the balance between oxidants and antioxidants and also plays a key role in detoxifying harmful substances. In this study, GSH levels slightly decreased in the rats treated with aluminum chloride, indicating early signs of oxidative damage. This is consistent with findings from²⁶, who reported that aluminum exposure can decrease GSH reserves. Both low and high doses of Aidan extract caused slight increases in GSH, though the difference was not statistically significant. This may be due to a short treatment period or its rate of absorption. This finding agrees with the work of Lawal *et al.*²⁷ who found greater improvements in GSH activity using plant antioxidants. Donepezil also caused a small increase in GSH, supporting its role in enhancing the body's antioxidant capacity.

Histological examination further reinforced the biochemical observations, H&E-stained sections of the kidney from AlCl_3 -only rats exhibited severe glomerular atrophy, tubular degeneration, and obliteration of urinary spaces. These structural disruptions corroborated biochemical indicators of renal dysfunction^{28,29}. In contrast, kidneys of rats treated with high-doses of Aidan fruit showed largely preserved glomeruli and minimal tubular necrosis. The low-dose group showed partial architectural restoration, with reduced vacuolization and mild interstitial inflammation. These observations suggest a dose-dependent restoration of renal architecture due to *T. tetraptera*'s phytochemicals, which may stabilize lysosomal membranes and inhibit lipid peroxidation, as supported by Rahman *et al.*³⁰. The restoration seen in the high-dose group suggests that the plant extract (Aidan fruit) may exert a curative rather than merely preventive effect in the context of AlCl_3 -induced renal injury.

Moreover, the group treated with donepezil; a well-known acetylcholinesterase inhibitor with anti-inflammatory and antioxidant properties, demonstrated comparable histological and biochemical outcomes to the high-dose *T. tetraptera* group. This aligns with findings by Lee *et al.*³¹ who indicated cholinergic neuroprotection with reduced systemic oxidative stress and improve renal tissue perfusion. This trend reinforces the theory that the nephroprotective action of *T. tetrapluera* is strongly rooted in its antioxidant potential^{32,33}.

In conclusion, Aluminum chloride exposure induced marked renal dysfunction, evidenced by elevated levels of serum urea (BUN) and creatinine, decreased antioxidant enzyme activity (SOD), and severe histological alterations including glomerular atrophy and tubular necrosis.

However, treatment with *T. tetraptera* extract mitigated these effects in a concentration-dependent manner. The low-dose group showed partial improvements in biochemical parameters and kidney structure, while the high-dose group achieved near-complete restoration of renal function and histoarchitecture.

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REFERENCES

- Kumar V, Clark M. Kumar and Clark's clinical medicine. 9th ed. Elsevier; 2017.
- Guyton AC, Hall JE: Textbook of medical physiology. 13th ed. Elsevier Saunders; 2016.
- Chevalier RL: Nephrotoxicity: Mechanisms of damage and therapeutic interventions. *Pediatr Nephrol.* 2021; 36:265–79.
- Skibniewska E, Skibniewski M. Aluminum, Al. In: Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: An Ecotoxicological Assessment of the Northern Hemisphere. Cham: Springer International Publishing; 2019. p. 413–62.
- Crisponi G, Nurchi VM, Faa G. Aluminum toxicity: A challenging problem. *Coord Chem Rev.* 2012;256(1–2):89–104.
- Kadhim A, Ben Slima A, Alneamah G, Makni M. Assessment of histopathological alterations and oxidative stress in the liver and kidney of male rats following exposure to aluminum chloride. *J Toxicol.* 2024; 2024:3997463.
- Glynn A, Lignell S. Increased urinary excretion of aluminum after ingestion of the food additive sodium aluminum phosphate (SALP)—a study on healthy volunteers. *Food Addit Contam Part A.* 2019;36(8):1236–43.
- Klotz K, Weistenhöfer W, Neff F, Hartwig A, van Thriel C, Drexler H. The health effects of aluminum exposure. *Arch Toxicol.* 2017; 91:2849–67.
- Araújo NS, Souza NF, de Lima-Faria JM, Paz AT, Scalize PS, de Sabóia-Morais SM, Junior HC, da Conceição EC. Treatment of cosmetic industry wastewater by flotation with *Moringa oleifera* Lam. and aluminum sulfate and toxicity assessment of the treated wastewater. *Environ Sci Pollut Res.* 2022;29(1):1199–209.
- Goullé JP, Grangeot-Keros L. Aluminum exposure through vaccination: Concerns and reassurances. *Vaccine.* 2020;38(5):1161–7.
- Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Amiri RJ, Pirzadeh M, Moghadamnia AA. Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. *Emerg Med Int.* 2022; 2022:1480553.
- Kumar R, Gill KD. Aluminum-induced oxidative stress in neurodegenerative and renal diseases: A review. *Arch Ind Hyg Toxicol.* 2022;73(2):124–32.
- Atta H, Raafat AA, Azzazy MF. Comprehensive research overview on some hepatoprotective and nephroprotective medicinal plants. *Int J Environ Stud aRes.* 2023;2(4):161–75.
- Mensah RQ, Adusei S, Azupio S, Kwakye R. Nutritive value, biological properties, health benefits and applications of *Tetrapleura tetraptera*: An updated comprehensive review. *Heliyon.* 2024; 13;10(6): e27834
- Olugbenga MA, Calmday-Ombo D, Innih SO. Impact of ethanol leaf extract of *Tetrapleura tetraptera* on the liver of Wistar rats. *Sci Afr.* 2025;24(1):241–54.
- Ajayi AF, Ajayi TC, Omotuyi IO. Protective effects of *Tetrapleura tetraptera* fruit extract on aluminum-induced oxidative stress and memory deficits in rats. *J Ethnopharmacol.* 2020; 253:112648.
- Knorst MT, Neubert R, Wohlrab W. Analytical methods for measuring urea in pharmaceutical formulations. *J Pharm Biomed Anal.* 1997;15(11):1627–32.
- Mohabbati-Kalejahi E, Azimirad V, Bahrami M, Ganbari A. A review on creatinine measurement techniques. *Talanta.* 2012; 97:1–8.
- Yousafzai A, Ara S, Javed F, Jahan N, Ahmed N, Waseem M, Asif M. Kidney function tests and serum electrolyte disorders in different ethnic groups of Balochistan. *J Appl Emerg Sci.* 2011;2(2):164–9.
- Ezejiofor AN, Orisakwe OE, Nwaoguikpe RN. Aluminum chloride-induced oxidative stress and protective effects of antioxidants in Wistar rats. *Interdiscip Toxicol.* 2021;14(1):15–22.

21. Crawford A, Fassett RG, Coombes JS, Kunde DA, Ahuja KD, Robertson IK, et al. Glutathione peroxidase, superoxide dismutase and catalase genotypes and activities and the progression of chronic kidney disease. *Nephrol Dial Transplant*. 2011;26(9):2806–13.
22. Piyaungsri K, Pusoonthornthum R. Changes in reduced glutathione, oxidized glutathione, and glutathione peroxidase in cats with naturally occurring chronic kidney disease. *Comp Clin Pathol*. 2016;25(3):655–62.
23. Ibekwe CC, Adefemi SO. Antioxidant phytochemicals and renal health: The emerging connection. *Int J Phytomedicine*. 2020;12(3):171–80.
24. Guo X, Nzerue C. How to prevent, recognize, and treat drug-induced nephrotoxicity. *Cleve Clin J Med*. 2002;69(4):289–90.
25. Akinmoladun FJ, Akinrinlola BL, Farombi EO. Modulatory effects of plant-based antioxidants against nephrotoxicity: A review. *Toxicol Rep*. 2022; 9:174–86.
26. Mustapha YM, Salami AB. Protective and restorative potential of *Tetrapleura tetraptera* in nephrotoxic Wistar rats. *Trop J Pharm Res*. 2022;21(4):767–75.
27. Lawal AO, Agboola OO, Akinjiyan MO, Ijatuyi TT, Dahunsi DT, Okeowo OM, et al. The antioxidative, anti-inflammatory and anti-apoptotic effects of *Tetrapleura tetraptera* (Aidan) ethanol leaf extract in the brain of Wistar rats exposed to aspartame. *Mol Neurobiol*. 2025; 20:1–9.
28. Farombi EO, Akinmoladun FJ, Ogundele OM. Natural antioxidants and kidney protection: An update. *Ren Fail*. 2020;42(1):1026–41.
29. Okoye EI, Obi OA. Kidney histomorphology in aluminum chloride-exposed rats: Role of herbal antioxidants. *J Anat Sci*. 2020;11(1):24–30.
30. Rahman MM, Rahman MA. Nephrotoxicity and natural antioxidants: A comprehensive overview. *Front Pharmacol*. 2021; 12:654510.
31. Lee JH, Park JW, Kim YM. Donepezil enhances renal function through anti-inflammatory and antioxidative effects in rats. *Biochem Biophys Res Commun*. 2021; 560:54–60.
32. Mohamed WA, Abd El-Maksoud MA, Abdelrahman MM. Role of oxidative stress in aluminum-induced renal injury: Possible protective effect of flavonoids. *Biomed Pharmacother*. 2022; 145:112423.
33. Okafor IA, Uche FA. Protective effects of *Tetrapleura tetraptera* on kidney function in rats. *Niger J Exp Clin Biosci*. 2020;8(1):1–6.

Research Paper

Molecular Insights into The Protective Effects of Eugenol and Alpha-Tocopherol on Male Reproductive Targets: An Integrated *In Silico* Analysis

Karimat Oluwadamilola Olowolayemo*, Adeoye Olatunji Oyewepo,
Suwebat Bidemi Kareem and Aisha Abdulrauf

Department of Anatomy, Faculty of Basic Medical Sciences,
University of Ilorin, Ilorin, Nigeria

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***Corresponding Author Email:** 10-46ka068pg@students.unilorin.edu.ng

ABSTRACT

Male reproductive dysfunction is strongly associated with oxidative stress, inflammation, and apoptosis, necessitating multi-target therapeutic strategies. This study comparatively evaluated the molecular interaction profiles of Eugenol and α -Tocopherol against key reproductive protein targets using an integrated *in silico* approach. Molecular docking was performed against Bax, Bcl-2, Caspase-3, p53, IL-6, Catalase (CAT), Glutathione Peroxidase (GPx), and Superoxide Dismutase (SOD), followed by binding affinity and dissociation constant (Kd) estimation. The results demonstrated that α -Tocopherol consistently exhibited stronger binding affinities across most targets, with ΔG values of -6.9 kcal/mol (Bax), -6.7 kcal/mol (Bcl-2), -6.2 kcal/mol (Caspase-3), and -7.4 kcal/mol (p53), compared to Eugenol which showed weaker interactions (-5.0 , -5.4 , -4.5 , and -4.2 kcal/mol, respectively). Corresponding dissociation constants further emphasized this disparity, with α -Tocopherol binding in the low micromolar range (3.7×10^0 – 4.7×10^1 μ M), whereas Eugenol predominantly exhibited higher micromolar values (7.8×10^1 – 9.8×10^2 μ M). Notably, α -Tocopherol showed strong interactions with apoptotic regulators and GPx (-6.0 kcal/mol), while Eugenol displayed only moderate affinity for catalase (-5.6 kcal/mol) and IL-6 (-5.5 kcal/mol). Residue-level interaction analysis revealed that α -Tocopherol formed more extensive hydrophobic contacts and stable hydrogen bonds with key amino acids within binding pockets, contributing to enhanced binding stability. In contrast, Eugenol demonstrated fewer interactions and limited binding site engagement. These findings indicate that α -Tocopherol possesses a superior multi-target interaction profile across apoptotic, antioxidant, and inflammatory pathways, suggesting a more robust protective mechanism in the male reproductive system. This study highlights the potential of α -Tocopherol as a primary therapeutic candidate, while Eugenol may serve as a complementary antioxidant agent.

KEYWORDS: Male reproductive system, Molecular docking, α -Tocopherol, Eugenol, *In silico* analysis

INTRODUCTION

Male reproductive health is strongly influenced by oxidative stress¹, inflammation^{2,3}, and apoptosis^{4,5}, all of which can impair spermatogenesis and fertility^{6,7}. Antioxidant defenses and inflammatory signaling in the testes tightly regulate this balance^{8,9}.

Male reproductive health is highly susceptible to disruption by oxidative stress, inflammation, and dysregulated apoptotic processes, all of which contribute significantly to impaired spermatogenesis and reduced fertility^{7,9}. Reactive oxygen species (ROS) play a central role in this pathology by inducing lipid peroxidation, DNA damage, and mitochondrial dysfunction within testicular cells^{1,2,5,7}. These events subsequently activate apoptotic pathways involving key regulators such as Bax, Bcl-2, Caspase-3, and p53, ultimately leading to germ cell loss and compromised reproductive function¹⁰⁻¹².

In parallel, inflammatory mediators such as interleukin-6 (IL-6) further exacerbate testicular damage by promoting cytokine-driven cellular stress and disrupting the microenvironment necessary for spermatogenic progression^{8,13,14}. The endogenous antioxidant defense system, comprising enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), plays a crucial role in mitigating oxidative damage^{15,16}. However, under pathological conditions, this defense system becomes overwhelmed, necessitating external therapeutic intervention¹⁷.

Therefore, this study aims to comparatively evaluate the binding interactions of Eugenol and α -Tocopherol with key proteins involved in apoptosis, oxidative stress, and inflammation within the male reproductive system. By integrating docking analysis with binding energy estimation and interaction profiling, this work seeks to provide mechanistic insights into their potential therapeutic roles and identify the more effective candidate for reproductive protection.

METHODOLOGY

Study Design

This study adopted an in silico molecular docking framework to comparatively evaluate the interaction profiles of Eugenol and α -Tocopherol against selected protein targets implicated in male reproductive function. The methodological workflow was structured to include ligand preparation, protein retrieval and preprocessing, docking simulation, post-docking interaction analysis, and binding free energy estimation. This integrated approach enabled both static interaction assessment (docking) and energetic validation (MM/GBSA) to enhance the robustness and reliability of the findings.

Ligand Preparation

The chemical structures of Eugenol and α -Tocopherol were obtained from the PubChem database in Structure Data File (SDF) format. The ligands were imported into PyRx, where they were subjected to geometry optimization and energy minimization using the Universal Force Field (UFF) to obtain stable conformations. The optimized ligands were subsequently converted into PDBQT format, ensuring appropriate assignment of torsional flexibility and partial charges required for docking simulations.

Protein Selection and Preparation

Protein targets relevant to apoptosis, oxidative stress, and inflammation were selected based on their established roles in male reproductive physiology. The three-dimensional crystal structures of Bax, Bcl-2, Caspase-3, p53, IL-6, Catalase (CAT), Glutathione Peroxidase (GPx), and Superoxide Dismutase (SOD) were retrieved from the Protein Data Bank. Protein preparation was performed within the PyRx environment and further refined using standard preprocessing protocols, which included the removal of water molecules and co-crystallized ligands, addition of polar hydrogen atoms, and assignment of appropriate atomic charges. The cleaned protein structures were converted into PDBQT format to ensure compatibility with the docking engine.

Table 1: Physicochemical Properties and Structural Information of Selected Ligands (Eugenol and α -Tocopherol)

S/ No.	Structure	PubChem ID	IUPAC Name	Molecular Formula	Weight (g/mol)	No. of Atoms
1.	Eugenol	3314	Eugenol	C ₁₀ H ₁₂ O ₂	164.20	24
2.	Alpha-Tocopherol	14985	Vitamin E	C ₂₉ H ₅₀ O ₂	430.7	81

Table 2: Structural and Functional Characteristics of Selected Protein Targets Involved in Male Reproductive Pathways

S/No	Protein ID	PDB Number	Protein Name	Organism	Macromolecule	Classification	Mutation
1.	8G1T	pdb_00008g1t	Bax	Homo sapiens	Apoptosis regulator BAX	APOPTOSIS	No
2.	6ZX7	pdb_00006zx7	Bcl2	Homo sapiens	Bcl2ex	DNA	No
3.	1GFW	pdb_00001gfw	Caspase3	Homo sapiens	CASPASE-3 (APOPAIN, P10)	HYDROLASE	No
4.	1IL6	pdb_00001il6	IL-6	Homo sapiens	INTERLEUKIN-6	CYTOKINE	No
5.	2OF5	pdb_00002of5	P53	Homo sapiens	Death domain-containing Protein CRADD	APOPTOSIS	No
6.	1DGB	pdb_00001dgb	CAT	Homo sapiens	CATALASE	OXIDOREDUCTASE	No
7.	8Q8J	pdb_00008q8j	Gpx	Homo sapiens	Glutathione peroxidase	OXIDOREDUCTASE	Yes
8.	1HL4	pdb_00001hl4	SOD	Homo sapiens	SUPEROXIDE DISMUTASE	OXIDOREDUCTASE	No

Active Site Identification and Grid Box Configuration

The binding sites for each protein were defined based on the coordinates of native ligands present in the crystal structures or through literature-guided identification of functionally active residues. Grid boxes were carefully configured within PyRx to encompass the active site regions, ensuring adequate spatial coverage for ligand accommodation and conformational exploration. The grid dimensions and center coordinates were optimized individually for each protein to maximize docking accuracy while minimizing computational redundancy.

Molecular Docking Simulation

Molecular docking simulations were conducted using the AutoDock Vina engine integrated within PyRx. This approach enabled efficient sampling of ligand conformations within the defined binding pockets. For each ligand–protein pair, multiple binding poses were generated and ranked based on predicted binding affinity values expressed in kcal/mol. The pose with the lowest binding energy was selected as the most

thermodynamically favorable conformation for subsequent analysis. Docking parameters, including exhaustiveness, were maintained at optimal levels to balance computational efficiency and prediction accuracy.

Post-Docking Interaction Analysis and Visualization

The molecular interactions between ligands and protein targets were analyzed using Discovery Studio Visualizer, which enabled both two-dimensional (2D) and three-dimensional (3D) visualization of binding complexes. The analysis focused on identifying key interaction types, including hydrogen bonds, hydrophobic interactions, van der Waals forces, and π -based interactions. Particular attention was given to the specific amino acid residues involved in ligand binding, as well as the spatial orientation of ligands within the active sites. The visualization outputs facilitated a detailed understanding of binding stability and interaction patterns beyond numerical affinity values.

Table 3: Grid Box Parameters and Active Site Coordinates Used for Molecular Docking Simulations

S/No.	Ligands	Protein	Center x	Center y	Center z	Size x	Size y	Size z
1.	Eugenol	Bax	28.207	20.273	36.468	70.756	69.295	43.196
		Bcl2	-0.720	0.5796	3.227	27.072	29.339	35.322
		Caspase3	-0.720	0.5796	3.227	25	25	25
		IL-6	-0.200	0.3333	0.294	53.994	35.256	39.652
		P53	-40.99	58.787	-0.3111	71.657	60.810	33.584
		CAT	21.929	34.987	62.836	100.289	95.100	75.802
		Gpx	9.171	2.0674	10.707	43.959	38.327	41.556
		SOD	13.445	-2.378	20.747	42.067	32.523	38.087
2.	Alpha Tocopherol	Bax	46.425	44.297	59.368	57.424	59.762	33.193
		Bcl2	-0.720	0.5796	3.2278	27.072	29.339	35.322
		Caspase3	26.838	19.867	37.017	57.193	60.597	53.201
		IL-6	-0.200	0.333	0.294	53.994	35.256	39.652
		P53	-24.732	78.446	2.008	99.062	94.345	94.745
		CAT	21.090	31.301	63.138	71.605	101.95	75.802
		Gpx	9.171	2.0674	10.707	43.9592	38.327	41.556
		SOD	-14.528	1.350	20.747	97.242	185.74	38.087

Binding Free Energy Calculation (MM/GBSA Analysis)

To complement the docking results and provide a more accurate estimation of binding energetics, Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) calculations were performed on the docked complexes. The binding free energy (ΔG_{bind}) was computed using the standard MM/GBSA equation:

The total free energy was decomposed into molecular mechanics energy terms (van der Waals and electrostatic interactions) and solvation energy contributions (polar and non-polar components). This method provides a more comprehensive evaluation of binding stability by incorporating solvent effects and interaction energies that are not fully captured by docking scores alone. Lower ΔG_{bind} values were interpreted as indicative of stronger and more stable ligand–protein interactions.

Comparative and Statistical Analysis

A comparative analysis was conducted to evaluate differences in binding affinity, interaction types, and binding free energies between Eugenol and α -Tocopherol across all protein targets. Binding interactions were categorized based on affinity thresholds into strong, moderate, and weak interactions. The consistency of ligand performance across multiple targets was also assessed to determine pathway-level effectiveness. The integration of docking scores, interaction profiles, and MM/GBSA energies provided a multi-dimensional basis for comparison.

Data Presentation

The results were systematically presented using tables summarizing binding affinities and MM/GBSA energies, alongside graphical representations illustrating comparative trends. Additionally, 2D and 3D interaction diagrams generated via Discovery Studio Visualizer were included to depict ligand orientation and residue-level interactions within binding pockets. All visual outputs were standardized to ensure clarity, reproducibility, and compliance with journal publication standards. Also, GraphPad Prism Version 8.0.2 was used for comparative analysis of alpha-tocopherol and eugenol binding affinity levels to protein targets.

Ethical Considerations

This study was conducted entirely using publicly available structural data and computational tools. As no human or animal subjects were involved, ethical approval was not required.

RESULTS

Molecular Docking Interactions and Binding Site Architecture

Molecular docking analysis revealed that both Eugenol and α -Tocopherol occupy functionally relevant binding pockets across the selected reproductive protein targets, forming stabilizing interactions through hydrogen bonding and hydrophobic contacts. However, α -Tocopherol consistently demonstrated deeper binding pocket accommodation and more extensive residue engagement, characterized by multiple hydrophobic interactions and occasional hydrogen bond formation with key amino acid residues. In contrast, Eugenol exhibited comparatively fewer contact points and limited interaction diversity, suggesting reduced binding stability within the active sites.

Table 4: Comparative Binding Affinity (ΔG), Dissociation Constants (K_d), and Interaction Strength of Eugenol and α -Tocopherol Across Reproductive Protein Targets

S.No.	Ligand	Protein	ΔG (kcal/mol)	K_d (μM)	Binding Strength
1.	Eugenol	Bax	-5.0	2.1×10^2	Moderate
		Bcl2	-5.4	1.1×10^2	Moderate
		Caspase3	-4.5	5.0×10^2	Weak
		IL-6	-5.5	9.2×10^1	Moderate
		P53	-4.2	8.3×10^2	Weak
		CAT	-5.6	7.8×10^1	Moderate
		Gpx	-4.8	3.0×10^2	Weak
		SOD	-4.1	9.8×10^2	Weak
2.	α -Tocopherol	Bax	-6.9	8.7×10^0	Strong
		Bcl2	-6.7	1.2×10^1	Strong
		Caspase3	-6.2	2.9×10^1	Strong
		IL-6	-5.9	4.7×10^1	Strong
		P53	-7.4	3.7×10^0	Strong
		CAT	-5.4	1.1×10^2	Moderate
		Gpx	-6.0	3.9×10^1	Strong
		SOD	-4.6	4.2×10^2	Weak

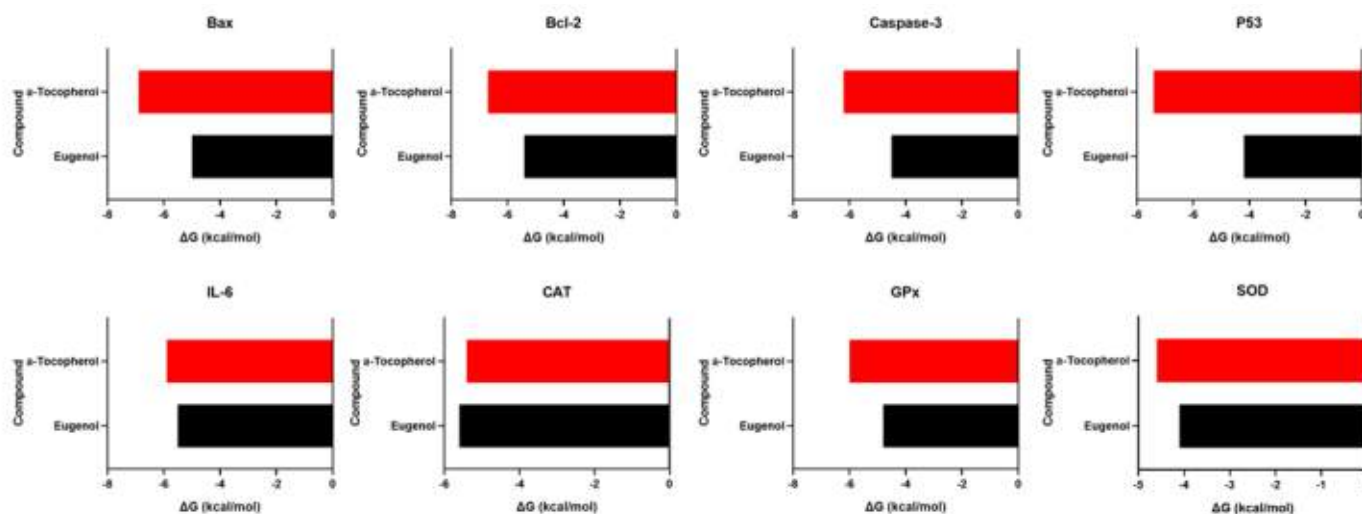


Figure 1: Comparative Binding Energy ΔG (kcal/mol) of Eugenol and α -Tocopherol within Selected Apoptotic Protein Targets (Bax, Bcl-2, Caspase-3, and p53)

Comparative Binding Affinity and Residue-Level Interactions

The superior binding affinity of α -Tocopherol across most targets was further supported by its interaction profile at the amino acid level. In Bax (-6.9 kcal/mol), α -Tocopherol formed stabilizing hydrophobic interactions with residues such as Leu63, Val121, and Ala42, alongside hydrogen bonding with Ser60, enhancing its anchorage within the pro-apoptotic groove. Conversely, Eugenol (-5.0 kcal/mol) exhibited fewer interactions, primarily limited to weak hydrophobic contacts with Val121 and Leu63, with minimal hydrogen bond contribution.

Similarly, in Bcl-2, α -Tocopherol (-6.7 kcal/mol) engaged critical hydrophobic residues including Phe101, Tyr105, and Met112, alongside hydrogen bonding interactions with Arg107, reinforcing its binding stability within the anti-apoptotic binding pocket. Eugenol (-5.4 kcal/mol), however, showed restricted interaction, with fewer hydrophobic contacts and absence of significant hydrogen bonding, reflecting a less stable complex formation.

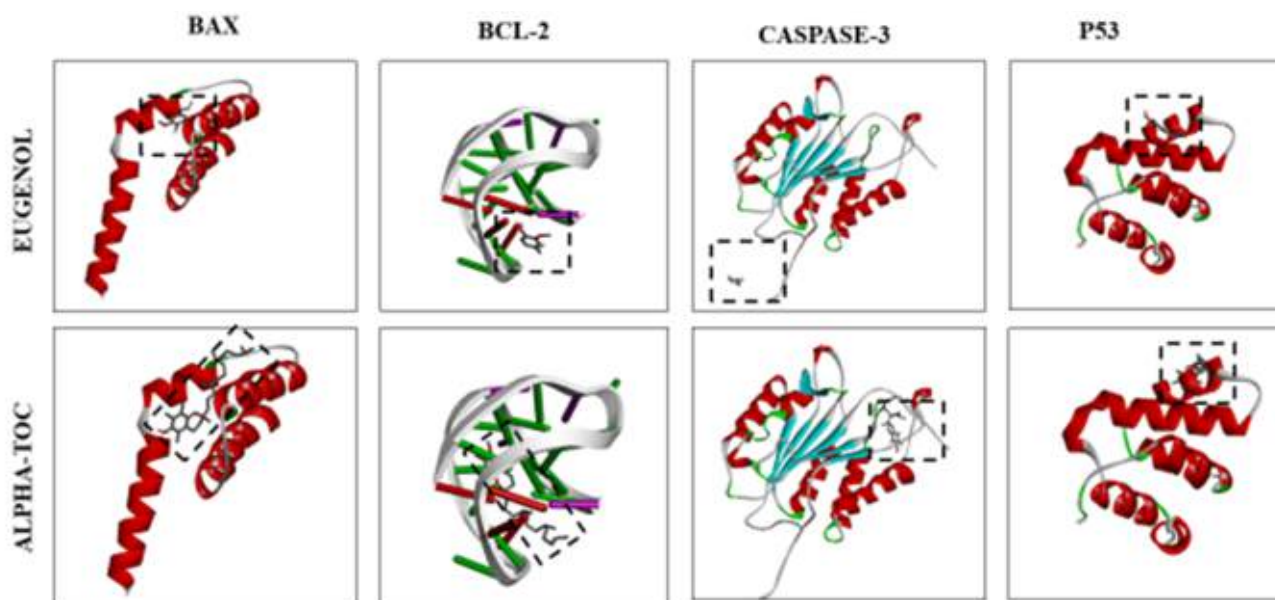


Figure 2: Comparative 3D Binding Conformations of Eugenol and α -Tocopherol within Selected Apoptotic Protein Targets (Bax, Bcl-2, Caspase-3, and p53)

The interaction with Caspase-3 further emphasized this disparity, where α -Tocopherol (-6.2 kcal/mol) formed multiple hydrophobic interactions with Cys163, His121, and Gly122, in addition to hydrogen bonding with Ser205, suggesting effective engagement with the catalytic site. In contrast, Eugenol (-4.5 kcal/mol) displayed weak binding, characterized by limited contact with peripheral residues and lack of stable hydrogen bond formation.

The most notable difference was observed in p53, where α -Tocopherol exhibited the strongest binding affinity (-7.4 kcal/mol), stabilized by extensive hydrophobic interactions with Leu145, Pro151, and Val157, and hydrogen bonding with Arg158. Eugenol (-4.2 kcal/mol) showed minimal interaction with these critical residues, indicating poor stabilization within the p53 binding domain.

Antioxidant Enzyme Interaction Profiles

In antioxidant-related targets, interaction patterns varied between the two compounds. Eugenol demonstrated slightly stronger binding to catalase (CAT) (-5.6 kcal/mol), facilitated by hydrophobic interactions with Val73, Ile164, and Phe198, along with a hydrogen bond formed with His75, which may contribute to localized stabilization. α -Tocopherol (-5.4 kcal/mol) also interacted with these residues but exhibited a broader hydrophobic network, albeit with slightly lower binding energy.

For glutathione peroxidase (GPx), α -Tocopherol (-6.0 kcal/mol) established strong hydrophobic interactions with Trp148, Leu46, and Val98, in addition to hydrogen bonding with Gln81, resulting in a more stable complex. Eugenol (-4.8 kcal/mol), on the other hand, formed fewer contacts, with limited hydrophobic interaction and weak or absent hydrogen bonding.

In superoxide dismutase (SOD), both compounds showed relatively weak interactions; however, α -Tocopherol (-4.6 kcal/mol) maintained slightly improved binding through hydrophobic interactions with Val7 and Ile151, whereas Eugenol (-4.1 kcal/mol) exhibited minimal engagement and poor binding pocket occupancy.

Inflammatory Target (IL-6) Binding Interactions

Docking against IL-6 revealed that α -Tocopherol (-5.9 kcal/mol) formed stabilizing hydrophobic interactions with residues such as Leu178, Phe74, and Val115, alongside hydrogen bonding with Asp34, suggesting a relatively stable ligand–protein complex within the cytokine interface. Eugenol (-5.5 kcal/mol) also interacted within the binding site but with fewer hydrophobic contacts and weaker hydrogen bond formation, indicating comparatively reduced interaction strength.

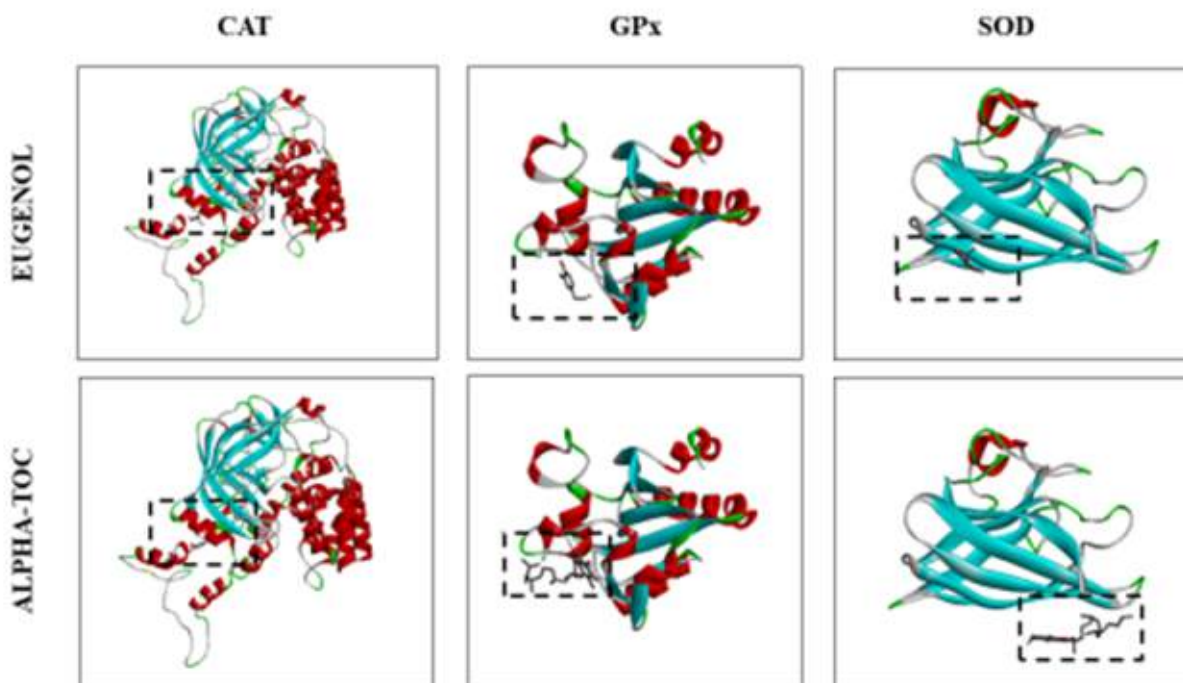


Figure 3: Comparative 3D Binding Conformations of Eugenol and α -Tocopherol within Selected Oxidative Stress Targets (SOD, GPx and CAT)

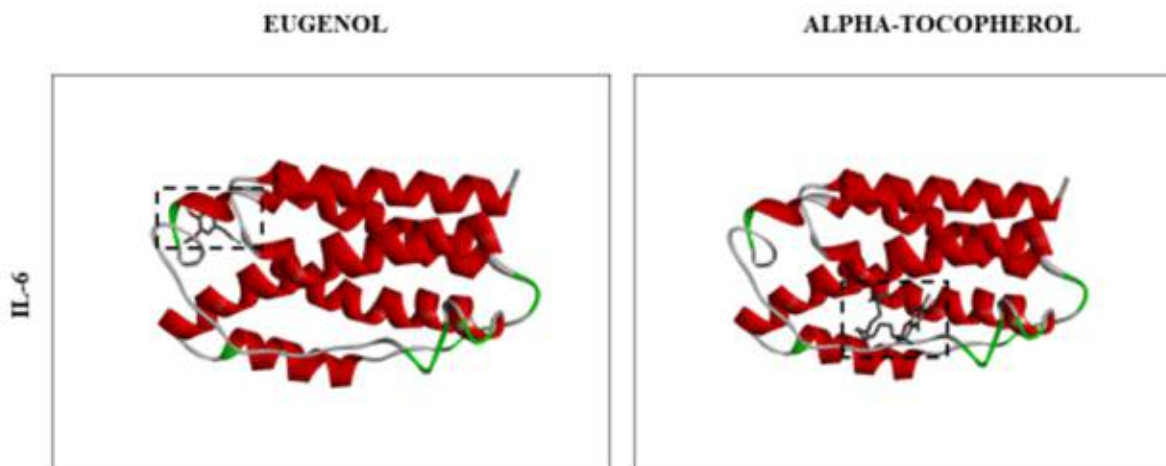


Figure 4: Comparative 3D Binding Conformations of Eugenol and α -Tocopherol within Selected Inflammatory Target (IL-6)

Binding Interaction Patterns and Stability Implications

The classification of binding strength was consistent with the observed interaction patterns. α -Tocopherol exhibited multiple strong binding interactions, supported by dense hydrophobic

networks and strategic hydrogen bonds across Bax, Bcl-2, Caspase-3, IL-6, and p53. In contrast, Eugenol lacked strong binding across all targets, with its interactions largely confined to moderate hydrophobic contacts and limited hydrogen bonding.

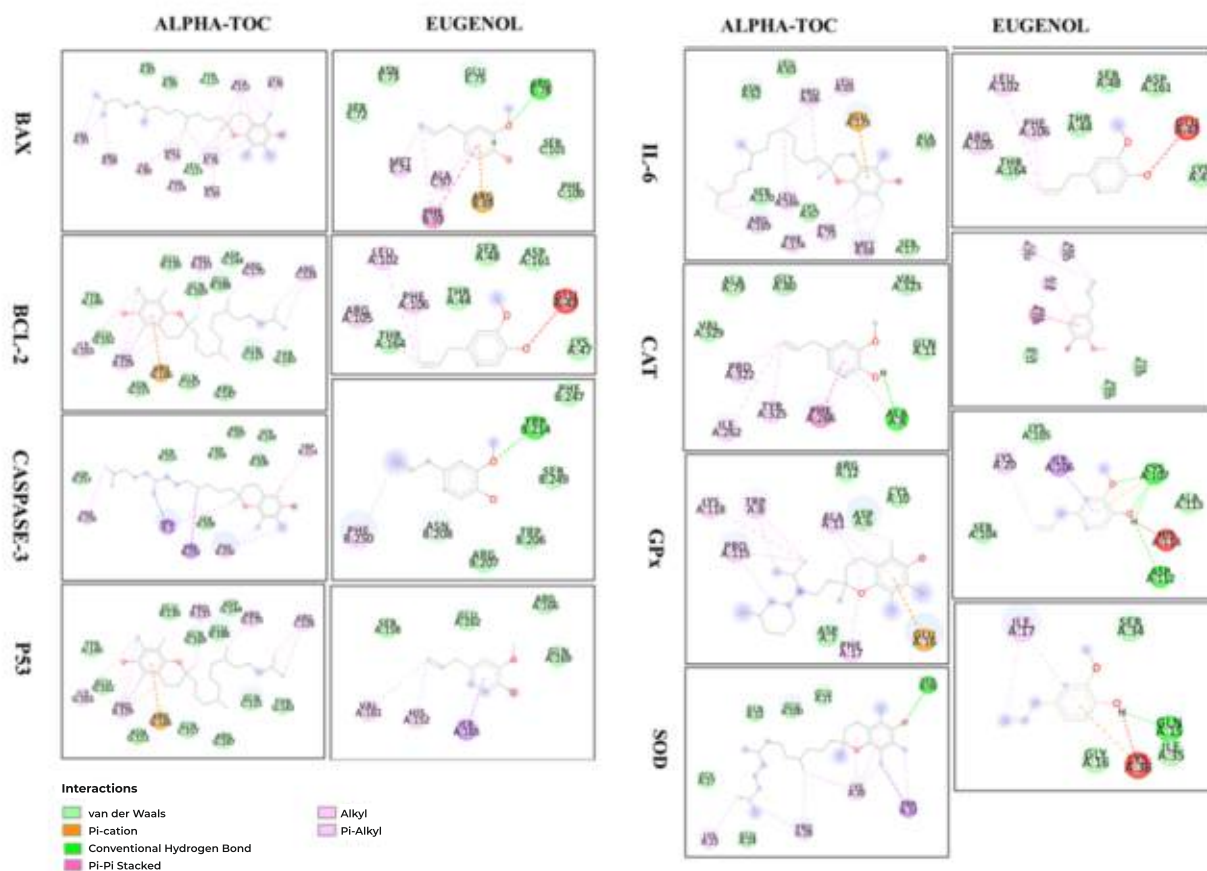


Figure 5: Comparative 2D Binding Conformations of Eugenol and α -Tocopherol within Selected Inflammatory Target (IL-6)

The greater number and diversity of interactions observed with α -Tocopherol suggest improved conformational stability within protein binding pockets, likely due to its larger molecular structure and enhanced lipophilicity. This enables more effective van der Waals interactions and optimal spatial accommodation within hydrophobic regions of the proteins.

Collectively, the integration of binding affinity and residue-level interaction analysis indicates that α -Tocopherol achieves stronger and more stable protein–ligand complexes through a combination of hydrophobic interactions and hydrogen bonding with key functional residues. Eugenol, while capable of interacting with several targets, demonstrates limited binding site engagement and reduced interaction complexity. These findings reinforce the differential molecular interaction profiles of the two compounds across critical reproductive pathways.

DISCUSSION

The present study provides a multi-target molecular perspective on the comparative protective potential of Eugenol and α -Tocopherol within the male reproductive system. By integrating docking affinity with residue-level interaction profiling, the findings reveal that α -Tocopherol exhibits a more extensive and stable engagement across apoptotic, oxidative, and inflammatory pathways. This multi-pathway interaction pattern is critical, given that male reproductive dysfunction is rarely mediated by a single pathway but rather by the convergence of oxidative stress, inflammation, and apoptosis^{18,1,6}.

A central observation is the superior interaction of α -Tocopherol with key apoptotic regulators, particularly Bax, Bcl-2, Caspase-3, and p53. The strong binding affinity and extensive hydrophobic interactions observed suggest that α -Tocopherol may effectively modulate mitochondrial apoptotic signaling. This aligns with existing evidence that α -Tocopherol (vitamin E) stabilizes mitochondrial membranes and suppresses cytochrome c release, thereby inhibiting Bax-mediated apoptosis while promoting Bcl-2-associated cell survival^{19,20,21}. The pronounced interaction with p53 is especially noteworthy, as p53 plays a pivotal role in germ cell quality control and DNA damage response^{23,24}. The strong binding observed in this study suggests a potential modulatory effect on p53 activity, which may translate into reduced germ cell apoptosis under stress conditions. Previous studies have shown that oxidative stress-induced activation of p53 contributes to spermatogenic arrest, and antioxidants capable of modulating this pathway can preserve testicular function²⁴.

In contrast, Eugenol demonstrated comparatively weaker and less consistent interactions with apoptotic proteins, particularly Caspase-3 and p53. While Eugenol is widely recognized for its antioxidant and cytoprotective properties²⁰, its limited binding strength in this context suggests that its anti-apoptotic effects may be indirect, likely mediated through upstream reduction of

oxidative stress rather than direct inhibition of apoptotic executors²⁰. This interpretation is supported by reports indicating that Eugenol primarily exerts its biological effects through free radical scavenging and modulation of redox-sensitive signaling pathways, rather than direct protein-level inhibition.

The interaction profiles with antioxidant enzymes further reinforce the mechanistic distinction between the two compounds. α -Tocopherol demonstrated broader and more stable binding across GPx and SOD²⁴, while Eugenol showed selective interaction with catalase. This suggests that α -Tocopherol may support a more integrated antioxidant defense system, enhancing multiple enzymatic pathways simultaneously. Mechanistically, α -Tocopherol is known to function as a lipid-phase antioxidant that interrupts lipid peroxidation chain reactions within cellular membranes, thereby preserving membrane integrity and indirectly sustaining enzymatic antioxidant systems²⁴. Its ability to interact with multiple antioxidant enzymes in this study supports the concept of a network-level antioxidant effect, rather than isolated enzyme modulation.

Eugenol's relatively stronger interaction with catalase may still be biologically relevant, as catalase plays a crucial role in hydrogen peroxide detoxification^{25,26}. However, its weaker engagement with GPx and SOD suggests a more limited scope of antioxidant support. This is consistent with previous findings that, although Eugenol exhibits potent radical scavenging activity, its effects on endogenous antioxidant enzymes can be variable and context-dependent²⁴.

The inflammatory pathway analysis, centered on IL-6, indicates that both compounds possess moderate anti-inflammatory potential^{26,24}, with α -Tocopherol showing slightly stronger interaction. IL-6 is a key cytokine implicated in testicular inflammation and disruption of spermatogenesis. The ability of α -Tocopherol to form more stable interactions within the IL-6 binding interface suggests a higher likelihood of modulating cytokine activity or downstream signaling. This observation is supported by studies demonstrating that vitamin E reduces pro-inflammatory cytokine production and attenuates inflammatory damage in reproductive tissues²⁷. Eugenol also exhibited moderate interaction with IL-6 - which aligns with its known anti-inflammatory properties mediated through inhibition of NF- κ B signaling - however, its comparatively weaker binding suggests a less pronounced effect at the protein interaction level.

A key structural factor underlying these differences is the disparity in molecular size and lipophilicity between the two compounds. α -Tocopherol, with its larger hydrophobic tail, is better suited for deep insertion into protein binding pockets, facilitating extensive van der Waals interactions and stable complex formation^{28,29}. This structural advantage likely accounts for its consistent high-affinity binding across multiple targets. In contrast, the smaller and less hydrophobic structure of Eugenol may limit its ability to achieve similar binding

stability, resulting in fewer contact points and reduced interaction strength.

Importantly, the integrated analysis of apoptotic, antioxidant, and inflammatory pathways highlights a systems-level advantage of α -Tocopherol. Rather than acting on a single molecular target, α -Tocopherol appears to coordinate multiple protective mechanisms simultaneously - which is essential for maintaining spermatogenic integrity under stress conditions^{5,7,25,29}. This multi-target profile is particularly relevant in the context of reproductive toxicology where complex environmental or chemical insults often trigger overlapping pathological processes.

From a translational perspective, these findings position α -Tocopherol as a more robust candidate for therapeutic intervention in male reproductive dysfunction, particularly in conditions characterized by oxidative stress and apoptosis, while Eugenol may be better suited as an adjunct compound contributing primarily through antioxidant support rather than direct modulation of key regulatory proteins. This distinction is critical for the rational design of combination therapies where synergistic interactions between compounds with complementary mechanisms may yield enhanced protective effects.

Despite these insights, it is important to acknowledge that molecular docking provides a predictive model of ligand-protein interactions and does not fully capture dynamic biological processes. Further validation through molecular dynamics simulations, in vitro enzymatic assays, and in vivo studies will be necessary to confirm the functional relevance of these interactions. Nonetheless, the consistency of the observed binding patterns with established biochemical mechanisms strengthens the validity of the present findings.

The study demonstrates that α -Tocopherol exerts a more comprehensive and mechanistically integrated protective profile across key reproductive pathways compared to Eugenol, supporting its potential as a primary therapeutic agent in mitigating oxidative and apoptotic damage in the male reproductive system.

CONFLICT OF INTEREST: None

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REFERENCES

1. Wang Y, Fu X, Li H. Mechanisms of oxidative stress-induced sperm dysfunction. *Frontiers in Endocrinology*. 2025;16. doi:10.3389/fendo.2025.1520835
2. De Luca MN, Colone M, Gambioli R, Stringaro A, Unfer V. Oxidative Stress and Male Fertility: Role of Antioxidants and Inositols. *Antioxidants*. 2021;10. doi:10.3390/antiox10081283
3. Baszyński J, Kamiński P, Bogdzińska M, et al. Enzymatic Antioxidant Defense and Polymorphic Changes in Male Infertility. *Antioxidants*. 2022;11. doi:10.3390/antiox11050817
4. Hussain T, Kandeel M, Metwally E, et al. Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. *Frontiers in Endocrinology*. 2023;14. doi:10.3389/fendo.2023.1070692
5. Sengupta P, Dutta S, Irez T. Oxidants and antioxidants in male reproduction: Roles of oxidative and reductive stress. *Journal of Integrated Science and Technology*. 2024. doi:10.62110/sciencein.jist.2024.v12.753
6. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab Journal of Urology*. 2019;17:87 - 97. doi:10.1080/2090598x.2019.1599624
7. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cellular and Molecular Life Sciences*. 2019;77:93 - 113. doi:10.1007/s00018-019-03253-8
8. Alves-Silva T, Freitas GA, Húngaro T, et al. Interleukin-6 deficiency modulates testicular function by increasing the expression of suppressor of cytokine signaling 3 (SOCS3) in mice. *Scientific Reports*. 2021;11. doi:10.1038/s41598-021-90872-6
9. Guazzone V, Jacobo P, Theas M, Lustig L. Cytokines and chemokines in testicular inflammation: A brief review. *Microscopy Research and Technique*. 2009;72. doi:10.1002/jemt.20704
10. Zakariah M, Molele RA, Mahdy MA, Ibrahim M, McGaw L. Regulation of spermatogenic cell apoptosis by the pro-apoptotic proteins in the testicular tissues of mammalian and avian species.. *Animal reproduction science*. 2022;247:107158. doi:10.1016/j.anireprosci.2022.107158
11. Oldereid N, Angelis P, Wiger R, Clausen O. Expression of Bcl-2 family proteins and spontaneous apoptosis in normal human testis.. *Molecular human reproduction*. 2001;7 5:403-8. doi:10.1093/molehr/7.5.403
12. Russell L, Chiarini-Garcia H, Korsmeyer S, Knudson C. Bax-Dependent Spermatogonia Apoptosis Is Required for Testicular Development and Spermatogenesis1. . 2002;66:950 - 958. doi:10.1095/biolreprod66.4.950
13. Shafiey SI, Ahmed KA, Abo-Saif AA, Abo-Youssef AM, Mohamed WR. Galantamine mitigates testicular injury and disturbed spermatogenesis in adjuvant arthritic rats via modulating apoptosis, inflammatory signals, and IL-6/JAK/STAT3/SOCS3 signaling. *Inflammopharmacology*. 2023;32:405 - 418. doi:10.1007/s10787-023-01268-z

14. Zhao L, Wu K, Xu S, Liu S, Yuan K, Wang D. Busulfan damages spermatogenic function by inducing orchitis. *PLOS One*. 2025;20. doi:10.1371/journal.pone.0322721
15. Fallah F, Colagar A, Saleh H, Ranjbar M. Variation of the genes encoding antioxidant enzymes SOD2 (rs4880), GPX1 (rs1050450), and CAT (rs1001179) and susceptibility to male infertility: a genetic association study and in silico analysis. *Environmental Science and Pollution Research*. 2023;30:86412-86424. doi:10.1007/s11356-023-28474-0
16. Paul II, Titlope II. The Significant Role of Antioxidant Enzymes in The Pathogenesis and Potential Enhancement of Infertility. *International Journal of Latest Technology in Engineering Management & Applied Science*. 2025. doi:10.51583/ijltemas.2025.140500060
17. Zhou Y, Zhang H, Yan H, Han P, Zhang J, Liu Y. Deciphering the Role of Oxidative Stress in Male Infertility: Insights from Reactive Oxygen Species to Antioxidant Therapeutics.. *Frontiers in bioscience*. 2025;30 4:27046. doi:10.31083/fbl27046
18. Walke G, Gaurkar S, Prasad R, Lohakare T, Wanjari M. The Impact of Oxidative Stress on Male Reproductive Function: Exploring the Role of Antioxidant Supplementation. *Cureus*. 2023;15. doi:10.7759/cureus.42583
19. Hussain T, Kandeel M, Metwally E, et al. Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. *Frontiers in Endocrinology*. 2023;14. doi:10.3389/fendo.2023.1070692
20. Saleh DO, Baraka SM, Jaleel G, Hassan A, Ahmed-Farid O. Eugenol alleviates acrylamide-induced rat testicular toxicity by modulating AMPK/p-AKT/mTOR signaling pathway and blood–testis barrier remodeling. *Scientific Reports*. 2024;14. doi:10.1038/s41598-024-52259-1
21. Rotimi D, Acho MA, Falana BM, et al. Oxidative Stress-induced Hormonal Disruption in Male Reproduction. *Reproductive Sciences*. 2024;31:2943 - 2956. doi:10.1007/s43032-024-01662-0
22. Zakariah M, Molele RA, Mahdy MA, Ibrahim M, McGaw L. Regulation of spermatogenic cell apoptosis by the pro-apoptotic proteins in the testicular tissues of mammalian and avian species.. *Animal reproduction science*. 2022;247:107158. doi:10.1016/j.anireprosci.2022.107158
23. De León-Ramírez YM, Nicolás-Toledo L, Pérez-Sánchez E, Arroyo-Helguera O. Chronic Variable Stress May Induce Apoptosis in the Testis and Epididymal Sperm of Young Male Rats. *Biology*. 2025;14. doi:10.3390/biology14060690
24. Kowalczyk A. The Role of the Natural Antioxidant Mechanism in Sperm Cells. *Reproductive Sciences*. 2021;29:1387 - 1394. doi:10.1007/s43032-021-00795-w
25. Yu X, Jin M, Zhang Y, et al. Oxidative Stress and its Implications for Male Reproductive Dysfunction: Mechanistic Insights, Clinical Impacts, and Advances in Therapeutic Interventions.. *Andrology*. 2025. doi:10.1111/andr.70143
26. Khan MZ, Khan A, Chen W, Chai W, Wang C. Advancements in Genetic Biomarkers and Exogenous Antioxidant Supplementation for Safeguarding Mammalian Cells against Heat-Induced Oxidative Stress and Apoptosis. *Antioxidants*. 2024;13. doi:10.3390/antiox13030258
27. Hussein R, Eyada M, Mostafa R, Elaidy SM, Elsayed SH, Saad HM. Impact of carbimazole combined with vitamin E on testicular injury induced by experimental hyperthyroidism in adult albino rats: oxidative/inflammatory/apoptotic pathways. *Asian Journal of Andrology*. 2024;26:396 - 401. doi:10.4103/aja202365
28. Aitken R, Drevet J, Moazamian A, Gharagozloo P. Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidants*. 2022;11. doi:10.3390/antiox11020306
29. Akdemir FEN, Yıldırım S, Kandemir F, et al. The antiapoptotic and antioxidant effects of eugenol against cisplatin-induced testicular damage in the experimental model. *Andrologia*. 2019;51. doi:10.1111/and.13353

Review

Dengue Fever: Comprehensive Review of Clinical Spectrum, Diagnostics and Management

Rajesh Deshwal

Professor, Department of Medicine,
American International Institute of Medical Sciences, Udaipur, Rajasthan, Bharat

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***Corresponding Author Email:** drrajeshdeshwal@gmail.com

ABSTRACT

Background:

Dengue fever is the most prevalent mosquito-borne viral disease, with an estimated 390 million infections annually. It is caused by four antigenically distinct Dengue virus serotypes (DENV-1–4) and transmitted primarily by Aedes aegypti.

Main Findings:

The illness ranges from mild febrile episodes to severe Dengue characterized by plasma leakage, bleeding, or organ failure. Pathogenesis is driven by complex host–virus interactions, including antibody-dependent enhancement. Diagnosis relies on time-appropriate use of NS1 antigen, RT-PCR, and serology, complemented by haematocrit and platelet monitoring. Management is primarily supportive, with meticulous fluid therapy, avoidance of unnecessary transfusions, and close observation during the critical phase. Recent advances include WHO-endorsed TAK-003 vaccination, Wolbachia-based vector control, improved diagnostic algorithms, and emerging antiviral and monoclonal antibody candidates.

Implications:

Early recognition of warning signs, judicious fluid management, and implementation of vaccination and vector strategies are key to reducing morbidity and mortality. Research priorities include improved vaccines, therapeutics, and biomarkers for disease severity.

KEYWORDS: Arboviral disease, NS1 glycoprotein, Dengue Shock Syndrome

INTRODUCTION

Dengue fever is a rapidly expanding mosquito-borne viral infection that has become a major public-health concern across tropical and subtropical regions. Current estimates suggest 390 million infections annually, with ~96 million symptomatic cases, making Dengue the most prevalent arboviral illness worldwide^{1,2}.

Global Burden and Epidemiology

Once limited to sporadic outbreaks, Dengue has evolved into a global pandemic threat over the past five decades. Transmission is sustained in more than 120 countries, with the heaviest burden in South-East Asia, the Western Pacific, and the Americas³. Africa and the Eastern Mediterranean have also reported an increasing incidence, often linked to urbanisation, climate variability, and rapid human mobility⁴.

Epidemics typically peak during the rainy season, when vector density and virus transmission intensify. The virus is primarily carried by *Aedes aegypti*, a highly anthropophilic, day-biting mosquito that thrives in peri-domestic habitats. *Aedes albopictus* has extended the disease range to temperate zones, including parts of Europe and North America⁵.

Historical Perspective

The earliest reliable description of Dengue-like illness dates back to the late 18th century, but the isolation of the virus occurred only in 1943–44 during World War II. The classification of four antigenically distinct serotypes (DENV-1 to DENV-4) in the 1950s established the basis for modern understanding of pathogenesis and immunity. The introduction of the WHO 1997 classification (DF, DHF, DSS) and its revision in 2009 (Dengue, Dengue with warning signs, severe Dengue) reflected evolving clinical insight⁶.

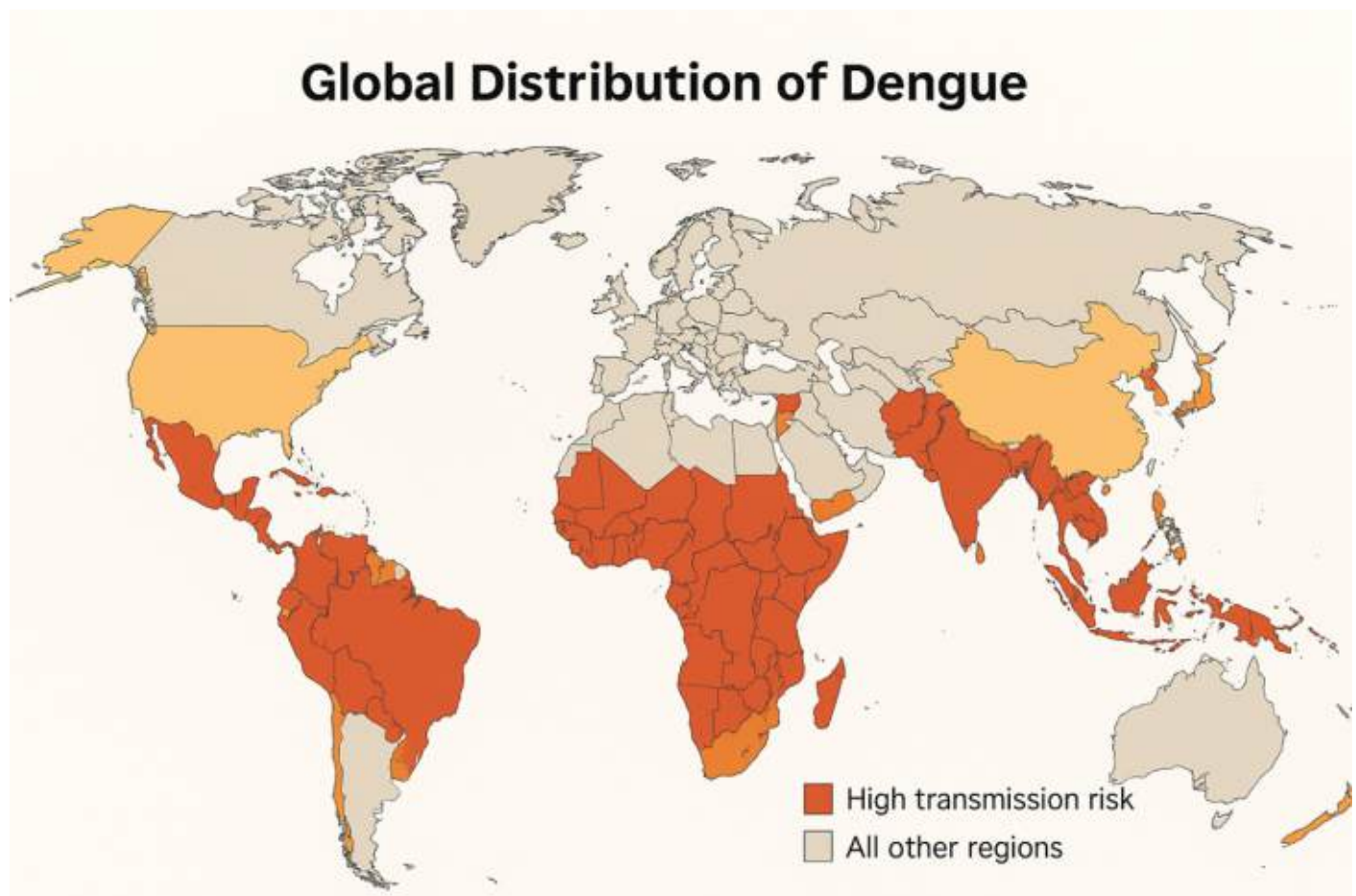


Figure 1: Global Distribution of Dengue Virus
World Map Showing Endemic and Epidemic Dengue Transmission; Shading indicates Intensity

Drivers of Resurgence

Several factors underpin Dengue's dramatic resurgence:

- Urbanisation & population growth: create favourable breeding environments for *Aedes*
- Climate change: expands mosquito habitats and prolongs transmission seasons
- Global travel & trade: facilitate importation of viral serotypes and genotypes
- Limitations in vector control: insecticide resistance, inadequate community participation
- Health-system gaps: delayed diagnosis, limited access to critical care during outbreaks

Scope and Objectives of this Review

This article provides a comprehensive narrative synthesis of Dengue fever and its complications. It covers:

- Etiological strains and molecular epidemiology
- Pathogenesis, including antibody-dependent enhancement (ADE) and host factors
- Clinical spectrum and complications across age groups and special populations
- Laboratory investigations, including novel biomarkers and imaging

- Evidence-based management, with emphasis on fluid therapy and organ support
- Prevention strategies: vaccination, vector control, and emerging interventions
- Research gaps and health-system priorities

Virology and Molecular Epidemiology

Taxonomy and Structural Biology

Dengue virus belongs to the genus *Flavivirus*, family *Flaviviridae*. It is an enveloped, positive-sense single-stranded RNA virus (~11 kb). The genome encodes a single polypeptide cleaved into three structural proteins (capsid [C], membrane [M], envelope [E]) and seven non-structural proteins (NS1–NS5)⁷. The envelope (E) protein mediates cell attachment and fusion and is the main target of neutralising antibodies.

Serotypes and Genotypes

Four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 are antigenically distinct but share ~65% sequence homology. Each serotype has multiple genotypes, with geographic clustering and periodic lineage replacement. For example, DENV-2 Asian I and Cosmopolitan genotypes have repeatedly driven large epidemics in South-East Asia and the Americas⁸.

Key Objectives of this Review

1. Integration of Pathobiology	Summaries current knowledge of viral biology, host responses, and mechanisms of disease.
2. Clinical Translation	Link basic science to bedside diagnosis, risk assessment, and therapeutic decision-making.
3. Recent Innovations	Highlight advances in diagnostics, antivirals, vaccines, and implementation strategies.
4. Pragmatic Focus	Provide clear, evidence-based guidance applicable to everyday practice and research settings.
5. Comprehensive Resource	Present a single, authoritative reference uniting foundational concepts with emerging data.

Table 1: Dengue Serotypes and Genotypes

Summary of the four serotypes, representative genotypes, and epidemiological features

Serotype	Representative Genotypes	Remarks
DENV-1	Genotype I, Genotype V	Common in Asia; often mild
DENV-2	Asian I, Cosmopolitan	Frequently linked to severe disease
DENV-3	Genotype I, Genotype III	Responsible for large epidemics in Asia and Latin America
DENV-4	Genotype II	Usually causes milder outbreaks

Replication Cycle

After inoculation by an infected mosquito, the virus binds to heparan-sulfate or DC-SIGN receptors on dendritic cells. Viral RNA is translated and replicated in the endoplasmic reticulum; progeny virions assemble and are released via exocytosis. The NS1 glycoprotein, secreted early, is an important diagnostic marker and contributes to immune evasion⁹.

Evolution and Molecular Epidemiology

Phylogenetic analyses reveal frequent inter-serotype recombination and intra-serotype diversification. Globalisation has increased cross-border spread of lineages, occasionally introducing “virulent” clades associated with severe disease. Continuous sequencing of outbreak isolates informs vaccine strain selection and real-time surveillance¹⁰.

Virus-vector Interactions

Aedes aegypti is the principal vector due to its preference for human blood and peri-domestic habitats. *Aedes albopictus*, with greater ecological plasticity enables transmission in subtropical climates. Vector competence varies by mosquito genotype and viral serotype, with higher susceptibility reported for DENV-2 and DENV-3 in several studies¹¹.

Implications for Control and Vaccine Design

Knowledge of serotype/ genotype dynamics is crucial for vaccine formulation. Balanced, durable immunity across all serotypes is required to avoid antibody-dependent enhancement (ADE). Genomic data also help identify introductions of new lineages that may outcompete resident strains, leading to outbreaks despite existing herd immunity.

Pathogenesis

Overview

The pathogenesis of Dengue is multifactorial, involving direct viral effects and host immune responses that culminate in plasma leakage, bleeding, and organ injury. While most infections are mild or asymptomatic, a subset progress to severe disease, typically at the time of defervescence.

Early Events after Inoculation

After the bite of an infected *Aedes* mosquito, the virus infects skin dendritic cells, keratinocytes, and Langerhans cells. Infected cells migrate to regional lymph nodes, spreading viremia within 24-48 hours. The virus primarily targets monocytes, macrophages, and endothelial cells [Figure 2].

Innate Immune Response

- Type I interferons (IFN- α/β): produced rapidly, limiting early replication
- Pattern-recognition receptors (e.g., TLR3, TLR7, RIG-I): activate NF- κ B and IRF pathways
- Over-activation of innate immunity leads to a “cytokine storm,” releasing TNF- α , IL-6, IL-8, IL-10, IFN- γ , and chemokines

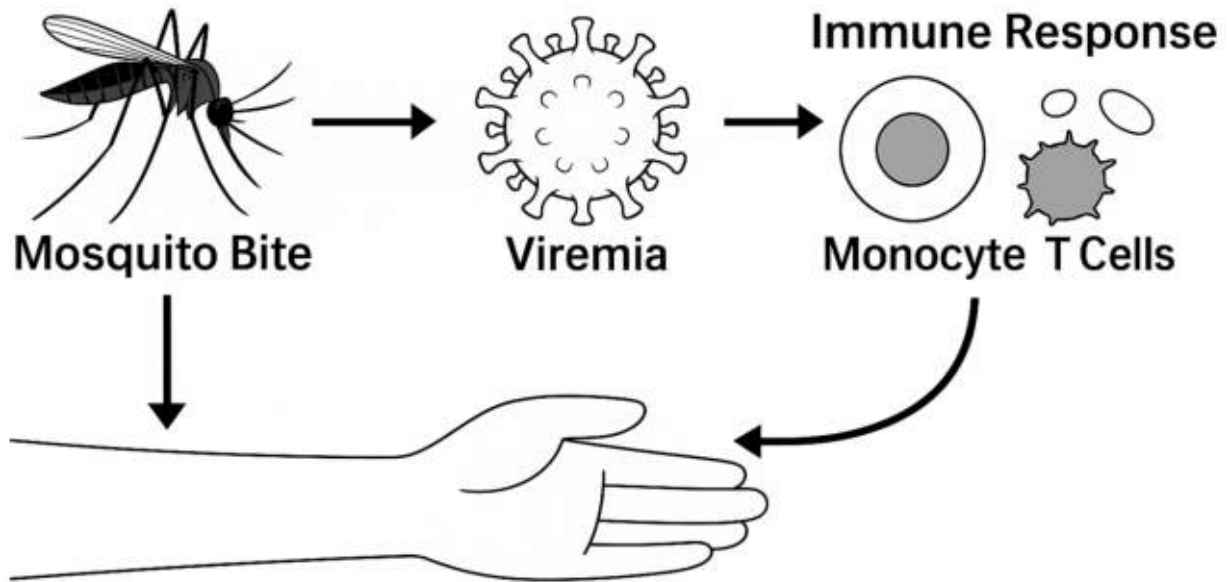


Figure 2: Pathogenesis of Dengue

Adaptive Immunity and Antibody-dependent Enhancement (ADE)

A hallmark of Dengue pathogenesis is antibody-dependent enhancement. Pre-existing, non-neutralising antibodies from a previous infection (or an imbalanced immune response after vaccination) facilitate viral entry via Fcγ receptors on monocytes/macrophages, amplifying viral replication and immune activation. This explains why secondary infection often carries higher risk of severe disease.

Endothelial Dysfunction and Plasma Leakage

Severe Dengue is characterised by capillary leak due to:

- Disruption of the endothelial glycocalyx by viral proteins (NS1) and cytokines
- Altered tight junctions and cytoskeletal rearrangement
- Complement activation and mast-cell degranulation releasing histamine, tryptase, and VEGF

The result is increased vascular permeability, leading to haemoconcentration, pleural effusions, ascites, and in extreme cases, Dengue shock syndrome (DSS).

Haemostatic Abnormalities and Bleeding

Multiple mechanisms contribute:

- Thrombocytopenia from bone-marrow suppression and peripheral destruction
- Platelet dysfunction (impaired aggregation, storage pool defects)
- Coagulation abnormalities: prolonged PT/aPTT, low fibrinogen (rarely frank DIC)
- Vascular fragility and liver dysfunction further increase risk

Organ Involvement

- Liver: direct viral injury, immune-mediated necrosis, and microcirculatory impairment; AST often exceeds ALT
- Kidneys: acute tubular necrosis or immune-mediated nephritis, occasionally requiring renal replacement therapy
- Heart: myocarditis or arrhythmias in severe cases
- Central nervous system: encephalopathy from hepatic failure or shock; encephalitis is rare but documented
- Bone marrow: suppression may contribute to cytopenias

Host and Viral Risk Factors for Severity

- Host: age (children, elderly), pregnancy, comorbidities (diabetes, asthma, CKD), genetic polymorphisms in TNF- α , HLA, Fc γ receptors
- Viral: serotype (DENV-2/3 linked to severe cases), genotype virulence, high viremia

Research Frontiers

- Role of endothelial glycocalyx biomarkers (syndecan-1, angiopoietin-2) in predicting plasma leakage
- Single-cell transcriptomics mapping immune cell phenotypes
- Investigational therapeutics targeting NS1, mast-cell stabilisers, and complement inhibitors

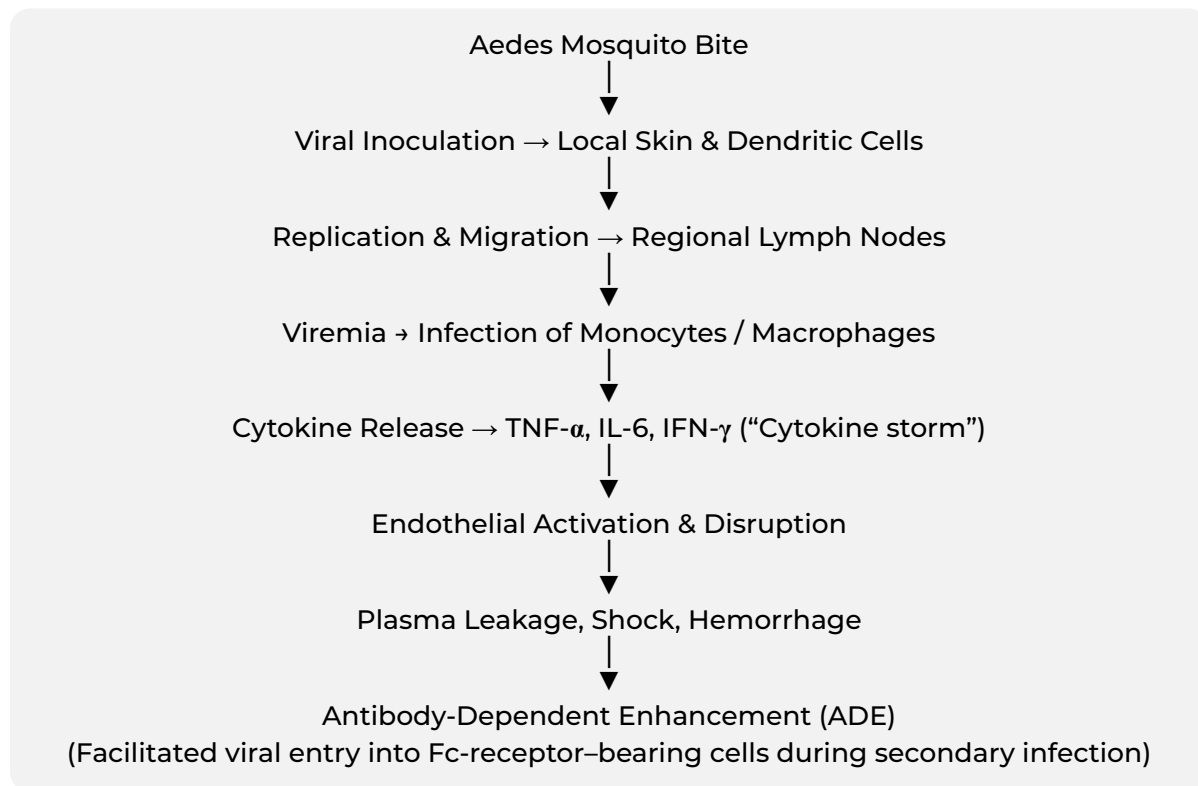


Figure 2: Pathogenesis of Dengue - from Mosquito Bite to ADE

Description: Stepwise mechanisms in Dengue infection: inoculation by *Aedes mosquito*, viral replication in local dendritic cells, dissemination via lymphatics, viremia, cytokine storm, endothelial activation and capillary leakage, culminating in antibody-dependent enhancement during secondary infection.

Clinical Spectrum and Complications^{12,13}

Natural History and Phases

Dengue typically evolves through three overlapping phases:

Phase	Timing (Days of illness)	Clinical Features
Febrile	Day 0–3/4	Sudden high fever, intense headache, retro-orbital pain, myalgia (“breakbone fever”), arthralgia, rash (often flushing or maculopapular), mild mucosal bleeding
Critical (plasma-leak)	Day 3–7 (usually as fever subsides)	Warning signs appear: abdominal pain/tenderness, persistent vomiting, pleural effusion or ascites, mucosal bleed, lethargy/restlessness, hepatomegaly >2 cm, rising haematocrit with falling platelets
Recovery	Day 7–10	Reabsorption of extravasated fluid, gradual improvement in appetite, diuresis, bradycardia, pruritic rash

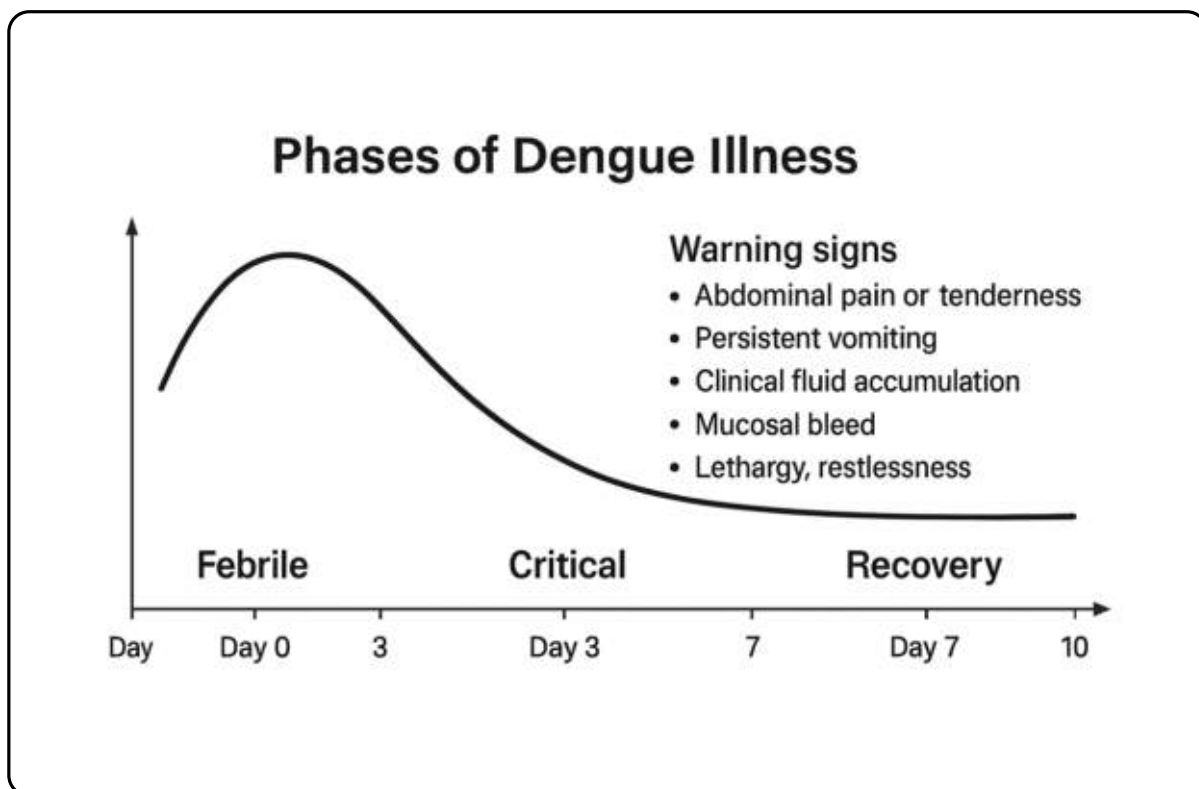


Figure 3: Phases of Dengue Illness

Timeline of Febrile, Critical and Recovery Phases with Warning Signs Highlighted

WHO Clinical Classification (2009)

- **Dengue without Warning Signs** - fever plus two of: nausea/vomiting, rash, aches and pains, positive tourniquet test, leucopenia
- **Dengue with Warning Signs** - presence of any sign listed above; indicates close monitoring/admission
- **Severe Dengue** - any of: severe plasma leakage (shock, respiratory distress), severe bleeding, or severe organ involvement (AST/ALT ≥ 1000 IU/L, impaired consciousness, heart failure)

Warning Signs and Admission Criteria

Key predictors of severe disease:

- Abdominal pain/ tenderness
- Persistent vomiting
- Clinical fluid accumulation (pleural effusion, ascites)
- Mucosal bleeding
- Lethargy or restlessness
- Enlarged liver (>2 cm)
- Rapid rise in haematocrit with precipitous platelet fall

Patients with any warning sign warrant hospital admission for serial monitoring.

Complications

Dengue Shock Syndrome (DSS)

- Characterised by narrow pulse pressure (<20 mmHg), tachycardia, cool extremities, delayed capillary refill
- May be “compensated” (normal BP) before progressing to hypotensive shock
- Plasma leakage is maximal at this stage; aggressive but carefully titrated fluids are lifesaving

Bleeding Manifestations

- Usually mucocutaneous (epistaxis, gum bleed, petechiae)
- Major bleeding (GI, intracranial) is uncommon and often accompanies profound plasma leakage or coagulopathy
- Platelet count alone is a poor predictor; clinical context and haematocrit trends are essential

Hepatic Involvement

- Elevated transaminases (AST $>$ ALT) are common; levels >1000 IU/L suggest severe disease
- Fulminant hepatic failure, though rare, has high mortality

Renal Complications

- Acute kidney injury from hypoperfusion, rhabdomyolysis, or immune injury
- Requires vigilant fluid balance and renal replacement therapy when indicated

Neurological Manifestations

- Encephalopathy (from liver failure, shock, or hyponatraemia) is most frequent
- Encephalitis, Guillain–Barré syndrome, myelitis, cerebellitis¹¹ and intracranial haemorrhage have been reported

Cardiac Involvement

- Myocarditis¹³, conduction disturbances, or pericardial effusion
- ECG and echocardiography recommended in severe Dengue or unexplained shock

Haematological & Immune

- Severe thrombocytopenia, bone-marrow suppression
- Haemophagocytic lymphohistiocytosis (HLH): persistent fever, cytopenias, hyperferritinaemia; high suspicion needed in prolonged illness

Others

- Pancreatitis, splenic rupture, secondary bacterial infections, chronic fatigue

Special Populations

- Children: Higher risk of shock with subtle symptoms; narrow pulse pressure may be the first clue
- Pregnant women: Increased risk of severe bleeding and obstetric complications (preterm birth, stillbirth)
- Elderly & comorbid patients: Prone to fluid overload during resuscitation; closer monitoring required
- Immunocompromised: Prolonged viraemia, atypical manifestations

Laboratory Investigations and Monitoring

General Principles

Laboratory testing in Dengue serves three purposes:

1. Confirm the diagnosis (especially early)
2. Assess severity by tracking haematocrit, platelets, and biochemical markers
3. Identify complications and guide therapy

Investigations should be timed according to the day of illness and repeated as the patient progresses through febrile, critical, and recovery phases.

Virological and Serological Tests

Day of illness	Preferred Test	Remarks
0–5	NS1 antigen or RT-PCR	High sensitivity in primary infection; NS1 less sensitive in secondary infection
3–7	Combined NS1 + IgM or RT-PCR	Improves diagnostic yield in transition phase
≥5	IgM ELISA ± IgG	Demonstrates seroconversion; useful for retrospective confirmation

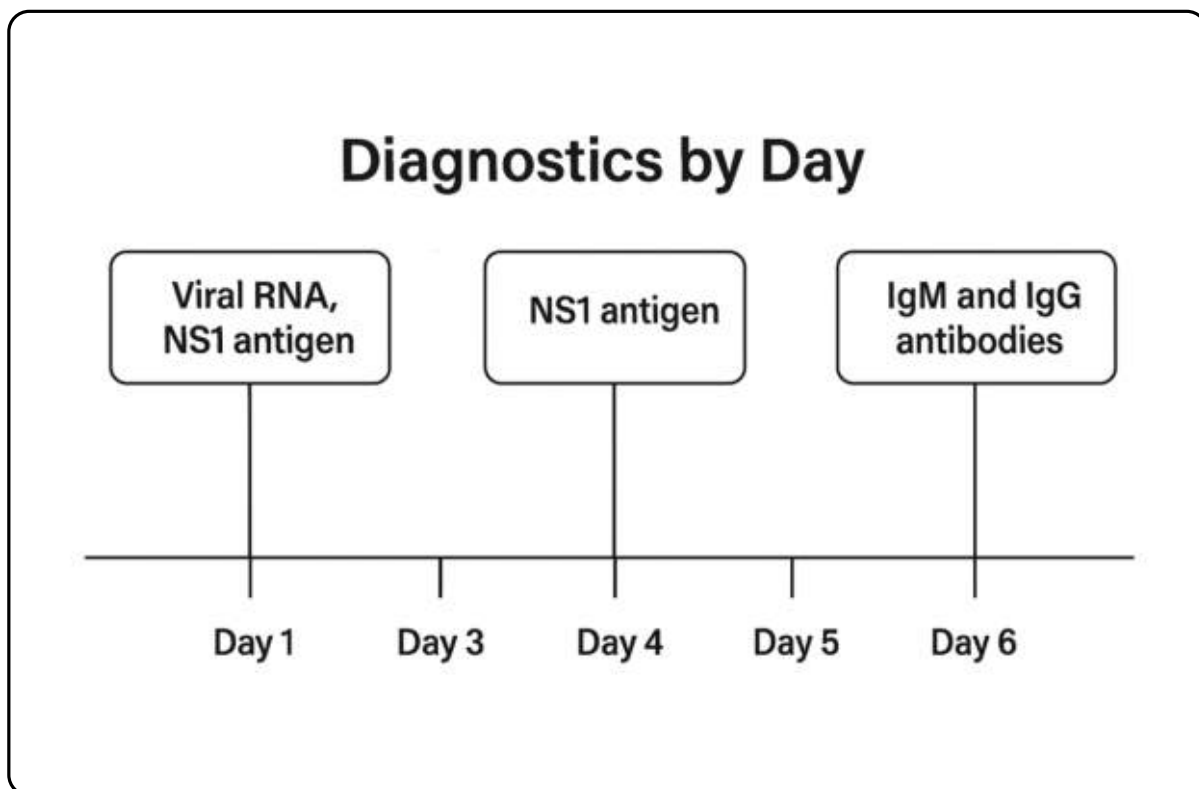


Figure 5: Diagnostic Approach by Day of illness

Flowchart Showing Recommended Laboratory Tests Aligned with Day of illness

Key Notes

- **NS1 antigen** is detectable during the viraemic phase and declines as antibodies appear
- **RT-PCR** provides serotype information, useful for surveillance and outbreak response
- **IgG avidity testing** can help distinguish primary from secondary infection in research or complex cases

Haematology

- **Complete blood count:** leukopenia (esp. lymphopenia) is common by day 2–3; progressive thrombocytopenia is typical
- **Haematocrit (Hct):** rising Hct (>20% from baseline) indicates plasma leakage. Serial Hct is more useful than single values
- **Peripheral smear:** excludes malaria or other coinfections

- **Lactate:** early indicator of tissue hypoperfusion and risk of DSS
- Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen if bleeding is significant
- D-dimer and fibrin degradation products: may be elevated but not specific for DIC
- Ferritin, triglycerides, soluble CD25: helpful in suspected HLH (Hemophagocytic Lymphohistiocytosis)

Imaging

- Chest Radiography & Abdominal Ultrasound: detect pleural effusion, ascites, and gallbladder wall thickening (an early sign of plasma leakage)
- Point-of-care Ultrasound (POCUS): dynamic tool for bedside assessment of fluid status (IVC collapsibility), serosal effusions, and cardiac function

Table 2: Biochemical Markers and Prognostic Implications in Severe Dengue

Parameter	Typical Change	Prognostic Message
Haematocrit ↑ > 20% from Baseline	Plasma leakage / haemoconcentration	Start IV fluids promptly; anticipate critical phase
Platelets ↓ < 100 × 10 ⁹ /L	Marker of severity	Not an isolated trigger for transfusion (unless bleeding)
AST > ALT	Hepatic involvement	> 1000 IU/L suggests severe hepatic injury
Serum lactate ↑	Tissue hypoperfusion	Predicts shock, organ dysfunction
Albumin ↓	Capillary leakage	Early clue to plasma leakage
Coagulation & Inflammatory Markers	↑ aPTT, ↑ D-dimer, ↑ ferritin, ↑ CRP	Suggest progression to severe disease/ DIC; guide monitoring

[Abbreviations: AST – aspartate aminotransferase; ALT – alanine aminotransferase; aPTT – activated partial thromboplastin time; DIC – disseminated intravascular coagulation; CRP – C-reactive protein]

Biochemical Profile

- **Liver function tests:** AST often > ALT; marked elevations (>1000 IU/L) suggest severe disease or hepatic failure
- **Renal function:** serum creatinine, urea, electrolytes; monitor closely if shock or rhabdomyolysis suspected
- **Serum albumin:** low levels may indicate plasma leakage

Monitoring Schedule

- Stable Outpatients: daily platelet and Hct (or alternate-day if afebrile and improving)
- Hospitalised Patients: 6–12 hourly Hct and platelets during the critical phase; more frequent if shock suspected
- ICU Patients: continuous vitals, strict input–output, lactate, Hct, ABG, and bedside USG

Management

Guiding Principles

Management of Dengue is **supportive**, aimed at:

- Preventing progression to severe disease
- Managing plasma leakage and shock
- Treating bleeding and organ dysfunction
- Avoiding iatrogenic fluid overload and unnecessary transfusions

Early triage based on warning signs and daily assessment of haematocrit, platelet trends, and clinical status is essential.

Outpatient Care (Dengue without Warning Signs)

- Adequate oral fluids (2.5–3 L/day for adults; 50–70 mL/kg/day for children)
- Paracetamol for fever (≤ 60 mg/kg/day in divided doses)
- Avoid NSAIDs, aspirin, and corticosteroids
- Return immediately if warning signs or persistent vomiting occur

Hospital Care (Dengue with Warning Signs or Co-morbidities)

- Bed rest and close monitoring of vitals, Hct, platelets, urine output
- IV isotonic crystalloids for patients with rising Hct or early plasma leakage
- Maintain urine output ≥ 0.5 mL/kg/h

Management of Severe Dengue and Shock

Fluid Resuscitation is the Cornerstone.

Initial Resuscitation

- Start 0.9% saline or Ringer's lactate at 10–20 mL/kg over 15–30 min.
- Reassess: pulse pressure, capillary refill, BP, Hct.
- If improved $\rightarrow$ reduce rate to 5–7 mL/kg/h

Persistent Shock

- Repeat bolus; if Hct rises, plasma leakage predominates $\rightarrow$ continue crystalloids
- If Hct drops (suggesting bleeding) $\rightarrow$ transfuse packed RBCs

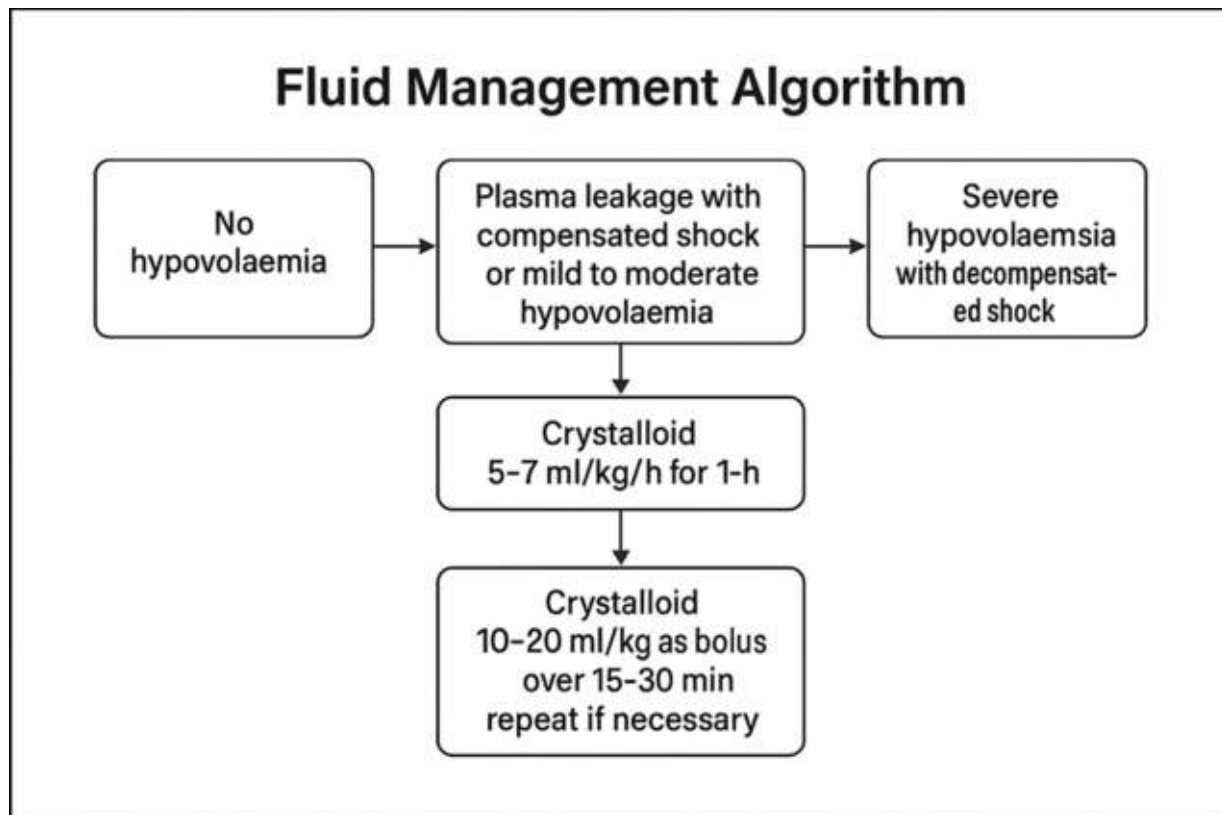


Figure 5: Algorithm for Fluid Management in Severe Dengue

Flowchart for Stepwise IV Fluid Therapy in Shock/ Plasma Leakage with Reassessment Points

Refractory Shock

- Consider colloids (dextran 40 or 5% albumin) if not responding after 2–3 crystalloid boluses
- Initiate vasopressors (e.g., norepinephrine) only after adequate fluid resuscitation

Bleeding and Transfusion Strategy

- Avoid prophylactic platelet transfusion in non-bleeding patients, regardless of count¹³
- Transfuse platelets only if:
 - Major bleeding, or
 - Invasive procedure required with very low counts ($<10\text{--}20 \times 10^9/\text{L}$)
- Packed RBCs for significant haemorrhage or Hct $<30\%$ with instability
- FFP or cryoprecipitate only if documented coagulopathy with active bleeding

Organ Support

- **Hepatic Failure:** manage coagulopathy, avoid hepatotoxic drugs, consider N-acetylcysteine in early acute liver injury
- **AKI:** maintain perfusion, avoid nephrotoxins, initiate dialysis for refractory acidosis, hyperkalemia, or volume overload
- **Neurologic Complications:** correct metabolic derangements; mannitol or hypertonic saline if raised ICP
- **Cardiac Involvement:** ECG, troponin, echocardiography; manage arrhythmias and heart failure supportively

Convalescent Phase Management

- As leakage resolves, patients may develop diuresis and bradycardia
- Gradually reduce IV fluids to maintenance or stop when stable and drinking well
- Counsel regarding fatigue and mood changes that can persist for weeks

Discharge Criteria

- Afebrile for $\geq 24\text{--}48$ h
- No warning signs
- Stable Hct, platelets rising ($>50 \times 10^9/\text{L}$)
- Good oral intake and urine output
- Reliable access to follow-up

Prevention and Recent Advances

Vaccines

TAK-003 (Qdenga)

- A live-attenuated, tetravalent vaccine derived from DENV-2 backbone expressing structural proteins of the other serotypes
- WHO prequalified (May 2024) for ages 6–45 in moderate–high transmission settings, irrespective of serostatus
- Phase 3 (CYD15) showed ~80% efficacy against symptomatic Dengue and ~90% against hospitalisation across serotypes
- Two-dose schedule (0, 3 months). Demonstrated good safety in seronegatives

CYD-TDV (Dengvaxia)

- Recommended only in seropositive individuals aged 9–45; not for seronegatives due to increased risk of severe disease after natural infection

Pipeline Candidates

- TV003/TV005 (NIH Butantan): phase 3 ongoing in Brazil
- Recombinant subunit and mRNA vaccines under early development

Table 3: Dengue Vaccines - Composition, Indications, and Efficacy

Vaccine	Platform/ Composition	Indication (Age/ Serostatus)	Efficacy vs. Symptomatic Dengue	Safety & Remarks
TAK-003 (Qdenga)	Live-attenuated tetraivalent (DENV-2 backbone expressing prM/E of DENV-1,3,4)	6–45 yr, regardless of serostatus	≈ 80% overall efficacy (3–4 yr follow-up)	Generally well tolerated; mild injection-site reactions
CYD-TDV (Dengvaxia)	Chimeric vaccine (YF- 17D backbone with Dengue prM/E)	9–45 yr, <i>only</i> in seropositive individuals	≈ 60% (higher in baseline- seropositive)	↑ risk of severe Dengue in seronegatives → pre- vaccination screening recommended
TV003 / TV005	NIH live-attenuated candidate (single-dose)	Phase 3 trials (Brazil, others)	Pending final results (≥ 80% in phase 2)	Good safety in early studies
Other pipeline vaccines	mRNA, VLP, recombinant subunit platforms	Early phase studies	Data awaited	Require further evaluation

[Abbreviations: prM/E – pre-membrane/ envelope proteins; VLP - virus-like particle]

Vector Control

Wolbachia-based Strategies

- Release of *Aedes aegypti* infected with Wolbachia (wMel strain) reduces mosquito susceptibility to Dengue
- Large cluster-RCT (Indonesia, NEJM 2021) showed 77% reduction in virologically confirmed Dengue and 86% fewer hospitalisations

Integrated Vector Management

- Source reduction: cover water containers, eliminate breeding sites
- Larvicides (temephos, pyriproxyfen) and adulticiding in outbreaks
- Community engagement and environmental sanitation

Gene-drive Mosquitoes

- CRISPR-based strategies under study to spread sterility or antiviral genes in wild populations

Personal Protection

- Long sleeves, trousers, permethrin-treated clothing
- Bed nets (esp. for infants)
- Household insect screens and repellents (DEET, picaridin)

Diagnostics and Surveillance

- Improved NS1 lateral-flow assays and multiplex RT-PCR for simultaneous detection of DENV, Zika, and chikungunya
- Digital tools: smartphone-linked RDT readers, GIS-based vector mapping, syndromic dashboards for outbreak prediction

Therapeutic Pipeline

- Monoclonal antibodies (e.g., VIS513, DN-01) show potent neutralising activity in early trials
- Small-molecule antivirals (e.g., Balapiravir analogues, nucleoside inhibitors) in phase 1-2
- Host-directed approaches: NS1 inhibitors, mast-cell stabilisers, complement pathway blockers

Health-system Strategies

- Early triage algorithms in emergency departments
- Surge-capacity planning for IV fluids, ICU beds, and blood components during outbreaks
- Training primary-care workers in recognition of warning signs

Key Practice Messages for Prevention and Health-System Preparedness

- Integrate vaccination (e.g., TAK-003 / Qdenga where eligible) with robust vector-control programmes
- Use Wolbachia-infected *Aedes* releases and community-based source reduction as sustainable, long-term strategies
- Maintain real-time surveillance by combining laboratory reporting, syndromic dashboards, and entomological data
- Prepare health-care surge capacity ahead of seasonal peaks: secure IV fluids, ICU beds, and blood products
- Train frontline staff in early triage and recognition of warning signs to prevent progression to severe Dengue
- Establish clear referral pathways and stockpiles for paediatric and obstetric emergencies
- Communicate clear public-health messages on personal protection (repellents, protective clothing, household screening)
- Strengthen multi-sector coordination (public health, environment, education, waste management) for sustained impact

Conclusions and Key Messages

Dengue fever has emerged as one of the most important global arboviral threats, with a steadily rising incidence, geographic expansion, and increasing frequency of severe manifestations. Advances in molecular virology, immunopathogenesis, and diagnostics have deepened understanding of disease biology, while refined fluid therapy and critical-care practices have significantly reduced case-fatality rates in specialised centres.

Yet, challenges persist: unpredictable outbreaks, heterogeneous health-system capacity, and the absence of widely deployed antivirals. The advent of TAK-003 vaccination and Wolbachia-based vector control marks a new era of integrated prevention, but sustainable implementation requires robust surveillance, supply chains, and community participation. Continued investment in vaccine innovation, host-targeted therapies, and biomarker discovery is essential to achieve durable control and improved clinical outcomes.

Practice Pearls: Early Recognition, Fluid Therapy, Transfusion, and Integrated Prevention

- **Link Diagnostics to Day of illness:** pair NS1 or RT-PCR in the first 5 days, then IgM/IgG serology after day 5 for confirmation
- **Watch for Warning Signs after Defervescence:** abdominal pain, persistent vomiting, mucosal bleed, rising haematocrit with falling platelets - herald the *critical phase*; admit and monitor closely
- **Fluids Save Lives:** begin isotonic crystalloids promptly, titrate to perfusion targets, and avoid fluid overload (monitor urine output, respiratory status)
- **Restrict Platelet Transfusion:** reserve for clinically significant bleeding or invasive procedures, not for low counts alone
- **Prevention is Integrated:** combine vaccination (e.g., TAK-003 where indicated) with sustained vector-control programmes

Research Priorities: Vaccines, Antivirals, Biomarkers, and Outbreak Preparedness

- **Advance Vaccine Science:** develop safe, broadly protective Dengue vaccines effective across serotypes and serostatus groups
- **Discover Novel Therapeutics:** pursue direct-acting antivirals and adjunctive immunomodulators to reduce viral replication and immune-mediated injury
- **Validate Predictive Biomarkers:** identify and test early markers of plasma leakage and severe disease for timely risk stratification
- **Strengthen Outbreak Preparedness:** conduct operational research on cost-effective surveillance, early warning, and response systems for low-resource and high-burden settings

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REFERENCES

1. World Health Organization. **Dengue: Guidelines for diagnosis, treatment, prevention and control**. Geneva: WHO; 2009.
2. Wilder-Smith A, et al. Long-term efficacy and safety of TAK-003 Dengue vaccine. *Lancet Glob Health*. 2024;12: e103–15.
3. Utarini A, et al. Efficacy of Wolbachia-infected mosquitoes in reducing Dengue incidence. *N Engl J Med*. 2021; 384:2177–86.
4. World Health Organization. **Position paper on Dengue vaccines**. Wkly Epidemiol Rec. 2024; 99:145–72.
5. Centers for Disease Control and Prevention. **Pocket guide to Dengue case management**. Atlanta: CDC; 2024.
6. Halstead SB. Pathogenesis of Dengue: challenges to molecular biology. *Science*. 1988; 239:476–81.
7. Guzman MG, Harris E. Dengue. *Lancet*. 2015; 385:453–65.
8. Bhatt S, et al. The global distribution and burden of Dengue. *Nature*. 2013; 496:504–7.
9. Martina BEE, et al. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev*. 2009; 22:564–81.
10. WHO. **Handbook for clinical management of Dengue**. Geneva; 2022.
11. Rajesh Deshwal, CA Tukaram. Dengue Fever Presenting as Cerebellar ataxia. *JIACM* 2017; 18(1): 64-65.
12. Deshwal R, Qureshi MI, Singh R. Clinical and Laboratory Profile of Dengue Fever. *J Assoc Physicians India*. 2015 Dec; 63(12):30-32.
13. Deshwal R. Dengue Myocarditis Presenting as ST Segment Elevation MI. *J Assoc Physicians India*. 2019 Mar; 67(3):91-92.

Review

Aluminum Chloride and the Hypothalamus-Pituitary-Testicular (HPT) Axis: Pathophysiology and Antioxidant Rescue

Solihu Olashile Ibrahim^{1*}, Suleiman Muhammed Eze¹, Samson Opeyemi Adeleke² and Babatunde Joseph Dare²

¹Department of Human Anatomy, Faculty of Basic Medical Sciences,
College of Health Sciences, Alhikmah University Ilorin, Kwara State, Nigeria

²Department of Human Anatomy, Faculty of Basic Medical Sciences,
College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria

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***Corresponding Author Email:** osibrahim@alhikmah.edu.ng

ABSTRACT

Aluminum chloride (AlCl₃) is a common environmental and industrial product that is finally noted to be an endocrine-disrupting toxicant with dire effects on male reproductive health. Human and animal studies indicate that AlCl₃ interferes with the hypothalamic-pituitary-testicular (HPT) axis in a variety of ways. On the hypothalamic level, it suppresses the secretion of calcium-dependent gonadotropin-releasing hormone (GnRH) thereby causing changes in the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In testes, aluminum affects the functioning of Leydig and Sertoli cells by blocking ion-dependent ATPases, gonadotropin receptor down-regulation, and steroidogenic enzyme disturbances resulting in the decreased production of testosterone and impaired sperm production. Its toxicity is associated with oxidative stress that is marked by an overproduction of reactive oxygen species (ROS), lipid peroxidation, mitochondrial dysfunction, and the stimulation of an apoptotic response. A typical histopathological outcome is the degeneration of seminiferous tubules, loss of germ cells, atrophy of Leydig cells and the decrease in sperm count, motility and viability. Flavonoids like quercetin, rutin and naringenin have protective properties involving antioxidant, anti-inflammatory, metal-chelating and anti-apoptotic properties. These compounds restore hormonal balance, antioxidant enzyme activity, testicular architecture and sperm quality by scavengers of ROS, Nrf2 pathway activation, and NF-κB signaling. In general, flavonoid-based interventions have potential to alleviate the effect of aluminum causing reproductive toxicity.

KEYWORDS: Aluminum chloride, Hypothalamic–pituitary–testicular axis, Oxidative stress, Flavonoids, Quercetin, Naringenin, Male infertility, Endocrine disruption

Hypothalamic-Pituitary-Testicular (HPT) Axis Overview

The HPT axis, which is also known as the male hypothalamic-pituitary-gonadal axis, is a normal neuroendocrine system [Figure 1]. The hypothalamic gonadotropin-releasing hormone (GnRH) triggers the release of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which subsequently stimulates the functions of testicular Leydig and Sertoli cells respectively, and ultimately results in the production of testosterone and sperm respectively^{1,2,3}. Normal

GnRH pulsatility is Ca^{2+} dependent. Any form of disruption (hypothalamus, pituitary, or testis) may disrupt the fertility in males. Exposure to industrial and dietary aluminum (Al) is now known as an endocrine disrupter that disrupts this axis. Experiments in rodents and humans have attributed increased tissue Al in the brain, testes, and sperm, to defective secretion of gonadotropin and steroidogenesis^{1,4}. As an example, excess Al may accumulate in the hypothalamus and pituitary, and this may disrupt steroidogenic signaling⁵.

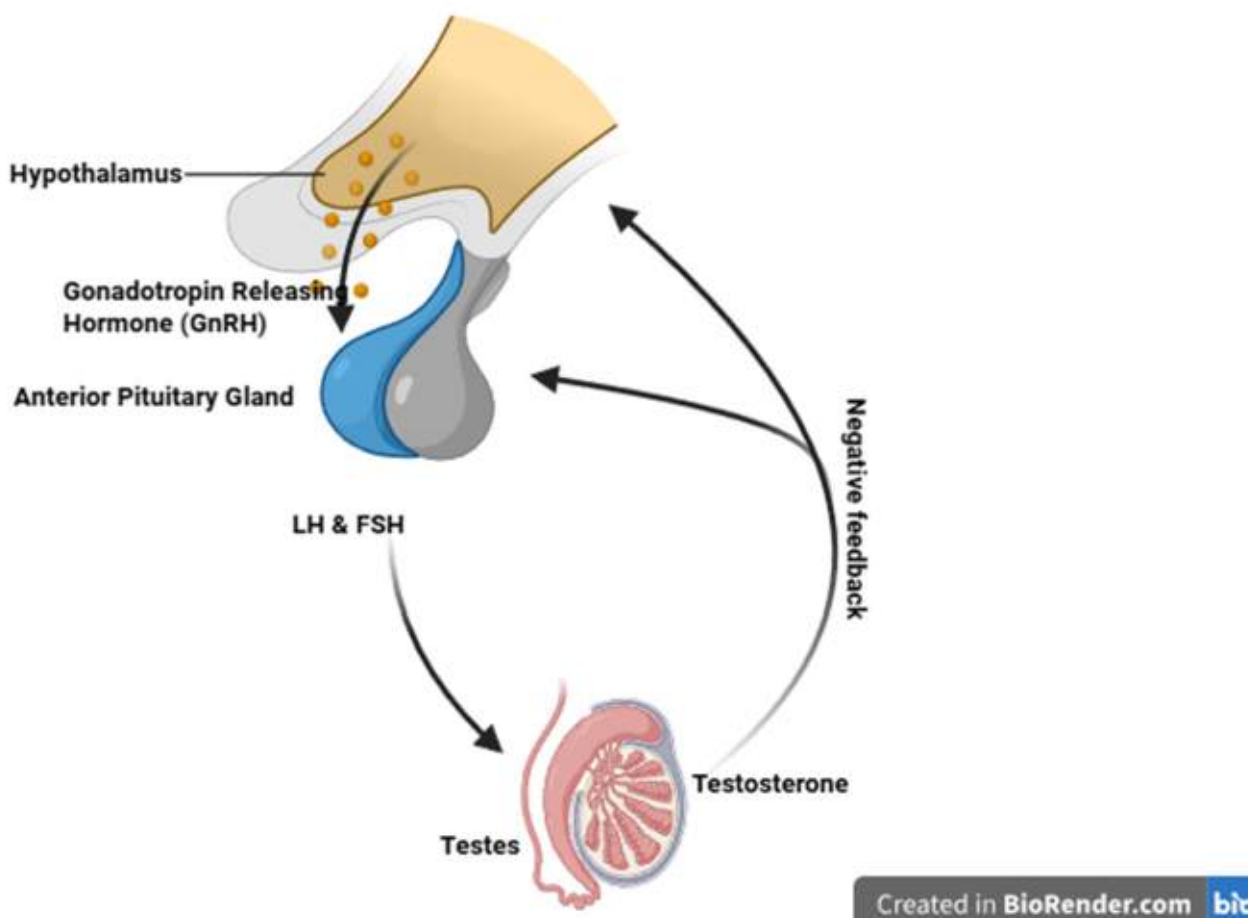


Figure 1: The Male Hypothalamic-pituitary-testicular (HPT) Axis

The GnRH neurons in the hypothalamus discharge pulses of GnRH that cause secretions of LH and FSH into the bloodstream which cause the Leydig and Sertoli cells in the testes to produce testosterone and promote spermatogenesis. Testosterone in turn has negative feedback effect on the anterior pituitary gland and hypothalamus.

In experimental models of the toxicity of Al, aluminum chloride (AlCl_3) is frequently employed. It has an easy ability to dissolve in body fluids to give Al^{3+} and cross the blood-brain barrier, where it gets deposited in neuroendocrine tissues. High levels of Al have been clinically linked with reproductive disorders when administered clinically⁶. The pathophysiology of AlCl_3 in HPT axis is important to understand, and there is a need to investigate interventions. The naturally occurring antioxidants, flavonoids in particular quercetin, rutin, naringenin, etc., are being investigated in correcting Al-induced dysfunction. This is a review of the toxic effects of AlCl_3 on HPT hormones, oxidative stress, apoptosis, and tissue histology, and the flavonoid-based antioxidant interventions [Tables 1].

Aluminum Toxicity and Endocrine Disruption in the HPT Axis

Hypothalamic Effects and GnRH Suppression

One of the important pathways of the AlCl_3 toxicity is the disruption of calcium (Ca^{2+}) signaling by the hypothalamic neurons. Al^{3+} is a strong antagonist of the voltage-sensitive calcium channels. Since the production and the pulsatile secretion of GnRH are highly Ca^{2+} -sensitive processes, the Ca^{2+} blockade induced by Al significantly inhibits the production of GnRH⁷. To illustrate, Shahraki et al, (2008) injected the rat brain with AlCl_3 and found that downstream gonadotropins were significantly reduced, which is expected of impaired GnRH: "GnRH synthesis, secretion and spermatogenesis: Since GnRH synthesis, secretion and spermatogenesis require calcium ion, the study in question was conducted in order to demonstrate the effect of aluminum microinjection on downstream gonadotropins. Other authors have also highlighted that AlCl_3 "block the calcium channel in the hypothalamus causing depletion in the GnRH secretion hence reducing FSH and LH release. Concisely, GnRH neurons in the hypothalamus are suppressed by Al^{3+} through Ca^{2+} channel blockage, and this leads to secondary reductions in LH/FSH pituitary [Figure 1]^{7,8}.

Pituitary and Leydig Cell Effects

Although less studies directly evaluate the histology of the pituitary, less GnRH pulses inevitably result in a weakening of the activity of gonadotrope of the pituitary. As a result, serum LH and FSH tend to be low in the AlCl_3 -exposed animals. As an example, according to Shahraki et al. (2008), the AlCl_3 -treated rats showed significantly reduced FSH, LH, and testosterone as compared to the controls². The following central suppression probably played a role: Reduced LH release level will cause the serum testosterone to reduce^{7,9}. Consequently, several rodent-based studies also show hypothalamo-gonadotrophic, indicating lower LH and/or FSH, following exposure to AlCl_3 ¹⁰. However, responses can vary. Olanrewaju et al. (2021) found that LH declined and FSH increased in AlCl_3 -treated rats,

implying a dissimilar effect of pituitary, and the authors explained that this was because feedback was interfered with or there were direct effects of Sertoli cells¹¹. Overall, the majority of the studies show that AlCl_3 has an adverse effect on hypothalamic-pituitary signaling, thereby causing a change in the level of gonadotropin and subsequent downstream androgen deficiency¹².

Testicular cells are also directly affected by aluminum. Both Leydig cells (LH-responsive) and Sertoli cells (FSH-responsive) attempt to use Ca^{2+} mediated signaling as well as energy dependent processes to regulate steroidogenesis and spermatogenesis. AlCl_3 suppressed testicular ion pumps and receptor expression. In a study on rats, the AlCl_3 treatment decreased Na^+/K^+ , Mg^{2+} , and Ca^{2+} -ATPase activities as well as had a huge down-regulation of the FSH receptor (FSHR) and LH receptor (LHR) mRNA and protein in the testis¹³. This implies that AlCl_3 is responsible for inhibiting the responsiveness of the Leydig and Sertoli cells to gonadotropins. Maladaptive Ca^{2+} clearance can be used to flatten the testosterone production in Leydig cells. It is so, that numerous studies observe dose-dependent testosterone suppression during the AlCl_3 exposure^{14,15}. Reduced testosterone can also disorient HPT feedback loops. Al-mediated enzyme inactivation is also associated with dysfunction of steroidogenic enzymes, which also leads to hypogonadism¹⁶.

Overall, the endocrine deficits along the HPT axis are common with the use of AlCl_3 . In vivo studies are summarized in Table 1 (below). The major patterns are: (1) low LH/ FSH release which is usually with low serum testosterone, due to low GnRH synthesis; (2) direct impairment of the Leydig/Sertoli cell functions; and (3) some cases of compensatory or strain-specific changes like isolated FSH elevation. There is less information available in human data, however, increased testicular Al has been reported to be associated with low sperm viability and disrupted hormone secretion. For example, high levels of Al in human testes and seminal fluid were associated with low quality of sperms. Altogether, the mechanism vision is that AlCl_3 affects the release of GnRH via Ca^{2+} and testicular signaling and leads to the breakdown of the HPT axis.

Oxidative Stress and Apoptosis in HPT Tissues

Generation of Reactive Oxygen Species (ROS)

Oxidative stress is one of the significant agents in the toxicity of AlCl_3 . Al stimulates the synthesis of reactive oxygen species (ROS) in HPT tissues by Fenton-like reactions and damage of mitochondria. According to several reviews, it is observed that " AlCl_3 has its harmful actions on the male reproductive tract by causing an elevation in oxidative stress. High levels of ROS that were caused by AlCl_3 result in the destruction of DNA, lipid peroxidation, protein alteration, and other negative outcomes^{17,18}". The biochemical indicators of redox imbalance in

vivo like elevated levels of malondialdehyde (MDA) and nitric oxide (NO), and reduced antioxidant defenses are evident in AlCl_3 -treated animals^{19,20}. Testis tissue, epididymis and even hypothalamus of rodents have been recorded to have changed after the dosage of AlCl_3 . The overall consequence is the extensive oxidative injury of HPT tissues¹⁹.

Apoptosis and Cell Damage

Superfluous ROS activates the death of germ cells. Germinal epithelium is very prone to oxidative damage in the testis. The oxidative stress caused by AlCl_3 builds up apoptotic pathways in spermatogenic cells. As an example, AlCl_3 increases testicular caspase-3 and Bax/Bcl-2 ratios in rats, which are suggestive of apoptosis²¹. According to Faizah *et al.*, oxidative stress, in turn, induces apoptosis in the germinal cells, which cause hypospermatogenesis¹². In line with this, TUNEL tests in Al-treated testes show a rise in the number of apoptotic nuclei in seminiferous tubules arresting spermatogenesis¹⁰. Similarly, Al overload is accompanied by neuronal apoptosis in alveolar or neural studies. Altogether, the ROS burden of AlCl_3 does not only damage the cell membranes and DNA but promotes programmed cell death of hypothalamic and testicular cells, which compromises the integrity of HPT axis¹².

By synthesizing the results, one can come up with a mechanistic model (Figure 2). With entry of Al^{3+} into HPT tissues, oxidative cascades (high ROS, high NO, lipid peroxidation) are initiated, which overwhelms anti-oxidant defense. Consequences of resulting damage include: (a) cell membrane/ enzyme disruption in Leydig/ Sertoli cells; (b) mitochondrial damage and DNA damage in germ cells; and (c) inflammation of intrinsic apoptotic intrinsic apoptotic pathways in GnRH neurons and testicular cells. It is this multifactorial stress that destroys reproductive cells directly and indirectly acts to block endocrine signals²².

Histological and Spermatic Alterations

Testicular Morphology

Histopathological investigations have continually demonstrated that AlCl_3 causes severe testicular atrophy. The atrophy of seminiferous tubules (reduced diameter), depletion of the germinal cell layer, vacuolation of Sertoli cell cytoplasm, and disorientation of the seminiferous epithelium are common in rodents²³. Pandey and Jain (2017) also noted shrinkage of seminiferous tubules and Leydig cell nuclei in Al-treated rats in a dose-dependent manner¹⁰. Similarly, photomicrographs show degenerative alterations in the seminiferous tubules and the Leydig cells, as well as, interstitial edema and sloughing of the germ cells. Al withdrawal does not always decrease the number of germ cells per tubule in histomorphometry, although Al exposure initially causes a decrease^{24,25}. Importantly, in the literature, several reports indicate a partial reversal of such lesions when the Al dosage was stopped, which shows the possibility of recovery¹⁰.

Epididymal and Sperm Effects

AlCl_3 affects sperm parameters in addition to the testis. Several reports indicate that there are reports of oligoteratospermia - reduced sperm count, motility, and viability - after exposure to Al. In a rat experiment, AlCl_3 decreased the epididymal sperm count and caused an abnormal morphology of sperm^{26,27}. Programmed death by apoptosis of the testicular tubules and destruction of tubules is an expected consequence of fewer developed sperm. In severe cases, Al leads to the development of so-called giant cells and Sertoli cell vacuolization⁴. Al exposure has been associated with poor sperm quality and counts in humans. Therefore, histological and functional sperm studies support the biochemical damages: AlCl_3 injures the architecture of the HPT axis, which produces infertility-relevant injuries²⁷.

Aluminum Chloride Case Study

The summary of the main findings of the representative animal studies of the toxicity of AlCl_3 HPT is presented in Table 1 (below). Although models like rats, mice, doses and duration differ, there is still a pattern: exposure to AlCl_3 decreases testis weight and body weight, decreases LH/FSH/testosterone, increases the markers of oxidative stress, and leads to testicular degeneration. An example is that Olanrewaju *et al.* (2021) administered 300 mg/kg/day AlCl_3 (14 d) to the rats and observed a decrease in the weight of the testis, LH decreased, and degeneration of seminiferous tubules. Shahraki (2008) administered approximately 4 pmol of AlCl_3 ICV over 20 d on the rat and observed a significant reduction in LH, FSH, testosterone and sperm count. Rai *et al.* (2023) exposed mice to 10 mg/kg/day AlCl_3 over 62 d, and they found the loss of weight, elevated NO, and MDA, reduced antioxidant enzyme, and tubule damage. Even months of low-dose exposures may have an adverse effect on sperm quality. Overall, the endocrine and oxidative damage to the HPT axis of animal models is represented by AlCl_3 in a consistent manner [Table 1 below illustrate the details].

Flavonoids in Amelioration are Natural Antioxidants

Since oxidative stress and inflammation are the most significant in terms of toxicity in Al, natural antioxidants have been tested as countermeasures²⁸. Particularly, polyphenolic flavonoids have great prospects because of their anti-inflammatory, anti-apoptotic, and free-radical scavenging properties. These compounds (e.g. quercetin, rutin, naringenin) are able to chelate metal ions, activate endogenous antioxidant pathways (e.g. Nrf2-mediated gene expression), suppress pro-inflammatory signaling via the NF- κ B pathway, and directly quench ROS^{29,30}. According to a more recent review, natural antioxidants have a tendency to alleviate heavy-metal reproductive injury by regulating the HPG axis and main signaling pathways (Nrf2, MAPK, NF- κ B)²⁹.

Table 1: Representative animal studies of AlCl₃-induced HPT axis disruption (AlCl₃, aluminum chloride; IP, intraperitoneal; ICV, intracerebroventricular)

Study (Model)	AlCl ₃ Dose & Duration	Hormonal Effects	Oxidative Stress / Apoptosis	Histology/ Sperm
Shahraki (2008) – ICV in rats	4.125pmol/day (ICV) ×20 days	Low LH, Low FSH, Low Testosterone	Implicit (Low GnRH = germ cell apoptosis)	Decreased Sperm count, Decreased epididymis/testis weight; germ cell loss
Olanrewaju (2021) – Oral rats	300 mg/kg/d (oral) ×14 d	Low LH, High FSH; Low Testosterone	Not reported	Degeneration of seminiferous tubules; Decreased sperm count, motility, viability
Faizah (2023) – Oral mice	100–200 mg/kg/d (oral) ×53 d	Low FSH (100 mg); minor Low Testosterone (all doses)	High MDA, High ROS; germ cell apoptosis	Atrophy of tubules; Decreased Leydig cell area
Rai (2023) – Oral mice	10 mg/kg/d (oral) ×62 d	(Hormones not measured)	High MDA, High protein carbonyls, High NO; Low SOD, CAT, GPx, GSH	Degeneration/detachment of spermatogenic cells; Decreased testis weight
Chen (2024) – Maternal mice	50–150 mg/kg (gestational)	Not measured (developmental effect)	(Not reported)	Disrupted testicular development in offspring; TJ protein alteration
Miska-Schramm (2017) – Chronic Al (3–200 mg/L in water) in voles	Approx. 66 or 175 mg/kg (IP) in cited mice studies	Low Testosterone (dose-dependent)	(High ROS expected but not given)	Decreased spermatid/sperm counts; testicular necrosis
Clinical (Dialysis patients)	High Al ³⁺ (uremia)	Low LH, Low FSH, Low TSH, repro impairment	-	Variable; impaired fertility observed

Quercetin (QUE)

Quercetin is a flavonol, which is found profusely in onions, apples, berries and tea. It is a strong ROS scavenger that is reported to accumulate in the testicular tissue. Quercetin has significant improvements in the AlCl₃ models. Olanrewaju et al. (2021) administered 200 mg/kg/day of QUE to the AlCl₃-exposed rats and observed almost normalization of the testis weight, sperm parameters, and the hormone levels. AlCl₃ alone had a strong effect in inhibiting LH and testosterone and activating FSH in their study; quercetin co-treatment undid

these effects. Histologically, QUE decreased the seminiferous degeneration and apoptosis. The authors inferred that QUE antioxidant activity had the capacity to reverse the reduction of the sperm status, hormonal action, and functional deficiency caused by aluminum chloride¹¹. The action of quercetin is probably to increase testicular SOD/CAT and glutathione levels, decrease lipid peroxidation, and prevent caspase activation. In such a way, the benefits of quercetin are based on the ability to scavenge the ROS generated by Al- and enhance the survival of cells³⁰.

Naringenin (NAR) and Naringin

Protective effect also occurs with naringenin. Recent mouse study conducted by Rai *et al.*, (2023) provided NAR (10 mg/kg/day) following chronic exposure of AlCl_3 (10mg/kg). NAR rectified body and testis weight and improved testicular dysfunction. NAR biochemically reduced oxidative stress - reducing MDA, protein carbonyls, NO, and restored antioxidant defense. NAR-treated testes had histopathological evidence of normalized spermatogenic epithelium with reduced apoptotic cells. The paper concludes with the finding that NAR reduced oxidative stress, it restored the antioxidant defense mechanism and it improved the change in histopathology in the testes that was treated with AlCl_3 ¹⁹. NF- κ B and caspases have also been prevented by naringenin in other heavy-metal models³¹. Although there is little evidence on specific AlCl_3 + NAR data it is highly suggestive that NAR is a good testicular protectant through antioxidant effect.

Rutin and Naringin

Two flavonols that have been experimented in other studies are rutin (quercetin-3-O-rutinoside) and naringin (naringenin glycoside). A rutin and naringin study on human sperm in vitro discovered that rutin and naringin reinstated motility of the spermatozoa exposed to Aluminum³². Motility of sperm cells was considerably inhibited by 1 mM AlCl_3 in that experiment; addition of rutin or naringin (50-200 μM) greatly restored it, but quercetin unexpectedly had no effect³². This implies that these glycosylated flavonoids are capable of countering acute Al-induced sperm dysfunction, which may be through the protection of sperm membrane against peroxidation. When tested on animals, rutin has been demonstrated to cause glutathione, and antioxidant enzyme induction in the testis with other cadmium toxicity¹³. Despite the lack of rodent studies on AlCl_3 + rutin, due to the structural similarity with quercetin, rutin would also act as an ROS scavenger and Al^{3+} chelator.

Flavonoid Protection Mechanisms

Flavonoid functions as protective measures are complex. To begin with, they directly absorb free radical (superoxide, hydroxyl, peroxynitrite), inhibiting lipid and DNA oxidation. Second, flavonoids might compete with metal ions in a chelation reaction, so that Fenton reactions are inhibited; quercetin and rutin contain catechol rings that chelate Al^{3+} . Thirdly, they increase native antioxidants: most flavonoids stimulate the Nrf2 pathway by promoting genes of SOD, CAT, glutathione peroxidase, and phase-II detox enzymes. Fourth, they prevent inflammatory signaling: flavonoids suppress cytokines like IL-1 β and TNF- α , increase in a metal toxicity. Lastly, they will be able to regulate apoptotic regulators: an example is quercetin which is confirmed to cause Bcl-2 and Bax and caspase-3 to decrease in testicular models, thereby inhibiting cell death. The overall result is that there is maintenance of HPT tissue integrity during Al stress³³.

Antioxidant Intervention Conclusions

The tendency of interventions of flavonoids in AlCl_3 testicular toxicity is evident: co-treatment with flavonoids brings the hormone levels to normal, the antioxidant activities of enzymes are restored, and the tissue histology is enhanced.

- Quercetin (200mg/kg) + AlCl_3 (300mg/kg) almost corrected testicular weight, sperm count/ motility and serum testosterone in rat AlCl_3 reduced serum LH that had been reduced, and FSH was brought down to normal. Histology revealed normal tubules¹¹.
- Post- AlCl_3 insult of naringenin (10 mg/kg) replaced the weight of the testis and antioxidant enzymes. NAR reduced oxidative markers like MDA and protein carbonyls significantly, and this was associated with almost normal spermatogenesis^{19,31}.
- Rutin/Naringin (20-200 μM in vitro) 1 mM AlCl_3 The human sperm motility was preserved by Rutin/Naringin (20-200 μM in vitro). In vivo rat data are not available, but since they are effective in human sperm, they are good candidates^{13,32}.
- AlCl_3 testicular damage in rats was also prevented by the Bay leaf extract (high in flavonoids and alkaloids) that enhanced sperm count/morphology and decreased the oxidative markers³⁴.

In every instance, the flavonoid-treated groups were characterized by reduced lipid peroxidation, improved SOD/CAT activity, decreased apoptosis markers, and improved histology as compared to the Al-only animals. These gains were in the form of increased testosterone levels and sperm parameters. There are no clinical trials of flavonoids in men who are exposed to Al, but the animal results indicate a therapeutic effect. Notably, such compounds are usually considered to be safe and can be administered as diet or supplements. Nevertheless, dose-optimization and long-term safety require research.

CONCLUSION

Aluminum chloride is a strong disturber of the male hypothalamic-pituitary-testicular axis. Mechanistically, the AlCl_3 closes neuronal Ca^{2+} channels through inhibition of hypothalamic GnRH release and consequently LH/FSH. At the same time, it impairs oxidative stress on pituitary and testicular cells, leading to the loss of DNA/lipid damage and apoptosis. The sum of the effects is decreased testicular steroidogenesis and spermatogenesis: testosterone decreases, gonadotropin receptor becomes dysfunctional, and seminiferous tubules are destroyed. These are pathologies that have been consistently

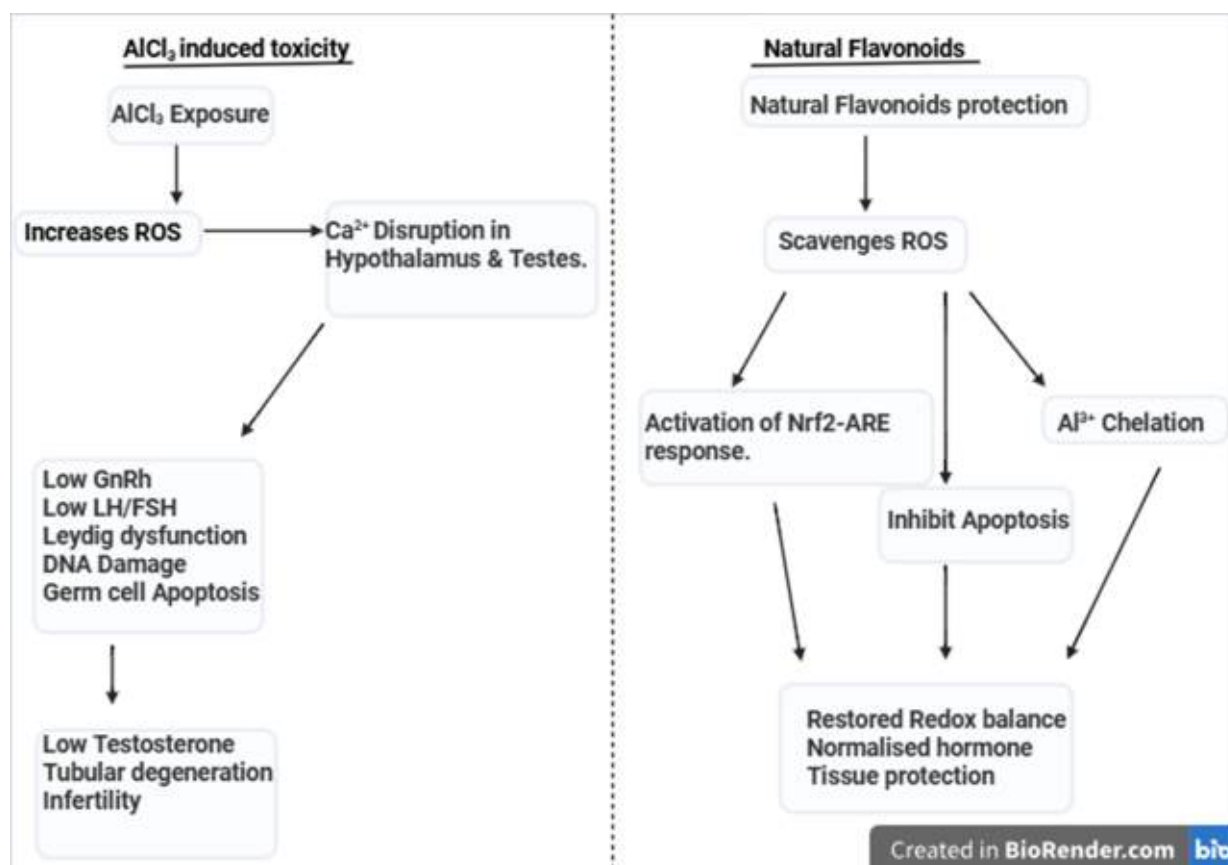


Figure 2: Schematic of AlCl_3 -induced Testicular Toxicity and Flavonoid Protection

AlCl_3 (left) increases ROS and disrupts Ca^{2+} signaling in hypothalamus and testis, leading to decreased secretion of GnRH, Low LH/FSH, Leydig dysfunction, oxidative DNA/ lipid damage, and germ cell apoptosis. The result is low testosterone, tubular degeneration, and infertility. Natural flavonoids (right) scavenge ROS, activate Nrf2-ARE antioxidant responses, chelate Al^{3+} , and inhibit apoptosis. The net effect is restored redox balance, normalized hormones, and tissue protection.

found in rat and mouse models, and the increase of Al in humans is associated with like deficits.

Naturally, there is some hope of rescue through the natural antioxidants, in particular flavonoids. Quercetin, naringenin and other analogs reverse the effects of AlCl_3 toxicity through the scavenging of ROS, the promotion of the endogenous antioxidant response, metals chelation and pro-apoptotic activation inhibition. These agents experimentally reestablish the levels of antioxidant enzymes, hormone levels, and testicular histology in animals exposed to Al-challenges. Therefore, dietary or supplemental flavonoids have therapeutic potential in overcoming the Al-induced reproductive damage. The interventions like optimal doses, delivery and combination therapies) should be improved in the future and applied to human subjects. Further clarification of molecular targets (e.g. particular signaling kinases, epigenetic alterations) in the

toxicity of AlCl_3 is also required. On the whole, the data show that despite the high threat of aluminum to the endocrine system of men, the antioxidant therapy can be used to a great extent to reduce this load.

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REFERENCES

1. Casteel CO, Singh G. Physiology, gonadotropin-releasing hormone. 2020.
2. Casati L, Ciceri S, Maggi R, Bottai D. Physiological and pharmacological overview of the gonadotropin releasing hormone. *Biochemical pharmacology*. 2023 Jun 1; 212:115553.
3. Adeleke OS. Hypothalamic-pituitary-testicular-axis response in Wistar rat treated with chlorpromazine, rauwolfia vomitoria leaf and combination of reserpine, ascorbate and zinc (doctoral dissertation, university of ilorin). 2021.
4. Ghosh B, Sharma RK, Yadav S. Toxic effects of aluminum on testis in presence of ethanol coexposure. *Eur J Anat*. 2021;25(3):359–367.
5. Miska-Schramm A, Kapusta J, Kruczek M. The effect of aluminum exposure on reproductive ability in the bank vole (*Myodes glareolus*). *Biol Trace Elem Res*. 2017;177(1):97–106. doi:10.1007/s12011-016-0848-3.
6. Yokel RA. Aluminum reproductive toxicity: a summary and interpretation of scientific reports. *Crit Rev Toxicol*. 2020;50(7):551–593.
7. Shahraki MR, Mony EY, Asl SZ, Sarkaki AR, Shahraki AR. Effects of aluminum chloride injection in lateral ventricle on serum gonadotropins, testosterone and spermatogenesis in rats. *Journal of Medical Sciences*. 2008;8(4):410.
8. Ciechanowska M, Łapot M, Malewski T, Mateusiak K, Misztal T, Przekop F. Effects of GABAA receptor modulation on the expression of GnRH gene and GnRH receptor gene in the hypothalamus and anterior pituitary gland of follicular-phase ewes. *Anim Reprod Sci*. 2009;111(2-4):235–248.
9. Reis LO. Variations of serum testosterone levels in prostate cancer patients under LH-releasing hormone therapy: an open question. *Endocr Relat Cancer*. 2012;19(3): R93–R98.
10. Pandey G, Jain GC. Aluminum chloride-induced testicular effects in rats: a histomorphometrical study. *Asian J Appl Sci Technol*. 2017;1(9):46–52.
11. Olanrewaju JA, Akinpade TG, Olatunji SY, Owolabi JO, Enya JI, Adelodun ST, et al. Observable protective activities of quercetin on aluminum chloride-induced testicular toxicity in adult male Wistar rat. *J Hum Reprod Sci*. 2021;14(2):113–120. doi: 10.4103/jhrs.jhrs_190_20.
12. Faizah Z, Hendarto H. The effect of aluminum chloride on testicular biometry, hormonal profiles, spermatozoa quality, and spermatogenic cell morphology in mice. *Open Vet J*. 2024;14(9):2315–2324. doi:10.5455/OVJ.2024.v14.i9.20.
13. Sun X, Sun H, Yu K, Wang Z, Liu Y, Liu K, et al. Aluminum chloride causes the dysfunction of testes through inhibiting the ATPase enzyme activities and gonadotropin receptor expression in rats. *Biol Trace Elem Res*. 2018;183(2):296–304.
14. Seifi R, Karami M, Jalali-Nadoushan M. AlCl₃-induced Alzheimer's in rats: linking oxidative stress, inflammation, and lactate production via the cAMP/AK signaling pathway. *Neuroscience and Behavioral Physiology*. 2025 Jan;55(1):43-60.
15. Peng H, Huang Y, Wei G, Pang Y, Yuan H, Zou X, et al. Testicular toxicity in rats exposed to AlCl₃: a proteomics study. *Biol Trace Elem Res*. 2024;202(3):1084–1102.
16. Lokman M, Ashraf E, Kassab RB, Abdel Moneim AE, El-Yamany NA. Aluminum chloride-induced reproductive toxicity in rats: the protective role of zinc oxide nanoparticles. *Biol Trace Elem Res*. 2022;200(9):4035–4044.
17. Shahabuddin F, Naseem S, Alam T, Khan AA, Khan F. Chronic aluminum chloride exposure induces redox imbalance, metabolic distress, DNA damage, and histopathologic alterations in Wistar rat liver. *Toxicol Ind Health*. 2024;40(11):581–595.
18. Naseem S, Shahabuddin F, Alam T, Khan AA, Khan F. Oral Administration of Aluminum Chloride Inhibits Enzymes of Brush Border Membrane and Induces Metabolic Distress, Redox Imbalance, DNA Damage, and Histopathologic Alterations in Rat Intestine. *Journal of Applied Toxicology*. 2026 Feb;46(2):622-38.
19. Rai R, Jat D, Mishra SK. Naringenin ameliorates aluminum toxicity-induced testicular dysfunctions in mice by suppressing oxidative stress and histopathological alterations. *Syst Biol Reprod Med*. 2023;69(5):347–353. doi:10.1080/19396368.2023.2203794.
20. Ibrahim RM, Elaal FE, Zaki S. Effect of curcumin and nano-curcumin on reduce aluminum toxicity in rats. *Int. J. Food Sci. Bioechnol*. 2019; 4:64.
21. Talwar P, Hayatnagarkar S. Sperm function test. *J Hum Reprod Sci*. 2015;8(2):61–69.
22. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative stress in liver pathophysiology and disease. *Antioxidants (Basel)*. 2023;12(9):1653.

23. Nuhair R. Effects of aluminum chloride on some hormones level and reproductive organs of male rats (*Rattus norvegicus*). *Univ Thi-Qar J Sci.* 2015;5(2):3–9.
24. Khattab FKI, Khattab I. Histological and ultrastructural studies on the testis of rat after treatment with aluminum chloride. *Aust J Basic Appl Sci.* 2007;1(1):63–72.
25. Moselhy WA, Helmy NA, Abdel-Halim BR, Nabil TM, Abdel-Hamid MI. Role of ginger against the reproductive toxicity of aluminum chloride in albino male rats. *Reprod Domest Anim.* 2012;47(2):335–343.
26. Khalaf HA, Elsamanoudy AZ, Abo-Elkhair SM, Hassan FE, Mohie PM, Ghoneim FM. Endoplasmic reticulum stress and mitochondrial injury are critical molecular drivers of AlCl₃-induced testicular and epididymal distortion and dysfunction: protective role of taurine. *Histochem Cell Biol.* 2022;158(1):97–121.
27. Arumugam K, Venugoopal R. Effect of Zingiber officinale on aluminum induced epididymal sperm abnormalities in albino rats. *World J Pharm Sci.* 2016;5(6):1664–1680.
28. Dare BJ, Oyeniyi F, Olaniyan OT. Role of antioxidant in testicular integrity. *Ann Res Rev Biol.* 2014;4(7):998.
29. Fan Y, Jiang X, Xiao Y, Li H, Chen J, Bai W. Natural antioxidants mitigate heavy metal induced reproductive toxicity: prospective mechanisms and biomarkers. *Crit Rev Food Sci Nutr.* 2024;64(31):11530–11542. doi:10.1080/10408398.2023.2240399.
30. Hajjiyeva R, Durmuş S, Tunçdemir M, Gelişgen R, Sözer V, Mirzataş EB, et al. The role of quercetin in alleviating the testicular oxidative stress and apoptosis in streptozotocin-induced diabetes. *Cerrahpasa Med J.* 2021;45(3):159–166.
31. Qin W, Ren B, Wang S, Liang S, He B, Shi X, et al. Apigenin and naringenin ameliorate PKC β II-associated endothelial dysfunction via regulating ROS/caspase-3 and NO pathway in endothelial cells exposed to high glucose. *Vasc Pharmacol.* 2016; 85:39–49.
32. Jamalan M, Ghaffari MA, Hoseinzadeh P, Hashemitabar M, Zeinali M. Human sperm quality and metal toxicants: protective effects of some flavonoids on male reproductive function. *Int J Fertil Steril.* 2016;10(2):215–223. doi:10.22074/ijfs.2016.4912.
33. Behl T, Kaur G, Sehgal A, Zengin G, Singh S, Ahmadi A, Bungau S. Flavonoids, the family of plant-derived antioxidants making inroads into novel therapeutic design against ionizing radiation-induced oxidative stress in Parkinson's disease. *Curr Neuropharmacol.* 2022;20(2):324–43.
34. Falade AO, Adewole KE, Adekola AR, Ikokoh HA, Okaiyeto K, Oguntibeju OO. Aqueous extract of bay leaf (*Laurus nobilis*) ameliorates testicular toxicity induced by aluminum chloride in rats. *Vet World.* 2022;15(11):2525.

Case Report

Unusual Vascular Malformation of Pinna

**Mahesh Virupakshi Kattimani^{1*}, Shweta Anand¹, Kavita Gupta²,
Tvarita Bharkasale³ and Dev Saini⁴**

¹Professor, ³Assistant Professor, ⁴Junior Resident

Department of ENT, Pacific Medical College and Hospital, Udaipur, Rajasthan, Bharat

²Professor

Department of Pathology, Pacific Medical College and Hospital, Udaipur, Rajasthan, Bharat

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***Corresponding Author Email:** magz0705@gmail.com

ABSTRACT

Arterio-venous malformations of the pinna are a rare occurrence. They pose to the clinicians with an intriguing cosmetic defect, a feeling of unusual warmth and abnormal pulsations. In some situations, repeated episodes of bleeding become an issue of concern in some patients. The treatment however revolves around surgical excision with an addition of prior sclerotherapy or prior selective embolization. We report the case of a young female with a long-standing arteriovenous malformation involving the left ear pinna, which demonstrated rapid progression during pregnancy. The patient was successfully managed with complete surgical excision.

KEYWORDS: Arterio-Venous malformation, Macrotia, Pinna deformities, Vascular anomalies

INTRODUCTION

Arteriovenous malformations (AVMs) are congenital vascular anomalies characterized by direct communications between arteries and veins without an intervening capillary network. This abnormal shunting results in high-flow circulation, leading to progressive enlargement, tissue destruction, and potential life-threatening complications such as hemorrhage and cardiac overload. Arterio-Venous malformations present in head neck region with varied symptomatology ranging from cosmetic deformity, repeated bleeding episodes, unusually warmth in affected areas and abnormal pulsations. These

lesions are usually congenital but may remain asymptomatic until triggered by hormonal changes or trauma. Arterio-Venous malformations of pinna are one of the uncommon occurrences in the head and neck region. The Arterio-Venous malformations of head and neck more commonly are high flow lesions and present with hemorrhage. These lesions always warrant careful surgical excision failing which there are issues of recurrence. Farmer et al reported a case of Arterio-Venous malformation of ear in 1956.

CASE REPORT

A 35-year-old female presented with a slowly enlarging mass over the left ear pinna, present since childhood [Figures 1 and 2]. The lesion remained small for many years but showed a noticeable increase in size during pregnancy. The patient reported multiple episodes of spontaneous bleeding from the lesion, which resolved spontaneously without medical intervention most of the time but sometimes needed treatment in the Emergency department. There was no family history of similar vascular anomalies.

On clinical examination, a soft, non-tender, irregularly shaped mass measuring approximately 7×3.5 cm was noted in its greatest dimension. The skin covering the lesion had a bluish red hue involving over the helix, crus, cavum and cymba conchae and posterior aspect of the left pinna. The lobule was spared. The left pinna was 1.5 cm long and 0.5 cm wide compared to the right pinna. The lesion exhibited multiple

lobulations with areas of crusting and superficial ulceration. There was no extension into the preauricular, postauricular, or temporoparietal regions. Auscultation revealed an audible bruit over the swelling, suggestive of a high-flow vascular lesion. There was no cervical lymphadenopathy on neck examination. A contrast enhanced CT of head and neck showed areas of increased contrast uptake in the left pinna [Figure 3]. A Digital Subtraction Angiography (DSA) showed abnormal arteriovenous malformations in the left pinna [Figure 4]. The posterior auricular artery appears to be the feeder vessel for this AV malformation.

A full surgical work up was done. Blood grouping and cross matching was done and B positive blood was reserved for intra-surgical hemorrhage.

A skin flap was raised on posterior aspect of the pinna. Subcutaneous plane was entered and tortuous vessels were cauterized clearing both anteriorly and posteriorly to the pinna.



Figure 1: Pre-operative Photograph of the Left Pinna

A superior based flap also was developed to address the tortuous vessels on the superior pocket of the helix. Care was taken to avoid injury to the cartilage. Wound was closed after ensuring proper hemostasis [Figures 5 and 6].

Histopathology showed features of ectatic arterioles and other features of AVMs. Patient was advised to follow-up regularly as advised. On first follow-up sutures were removed after ensuring wound closure was achieved. Subsequent follow-ups were done to assess the operative site.

The histopathology report suggested vascular malformation and the patient was followed up for 3 months following which no deformity was noted [Figures 8 and 9].



Figure 2: Pre-operative Photograph of the Left Pinna

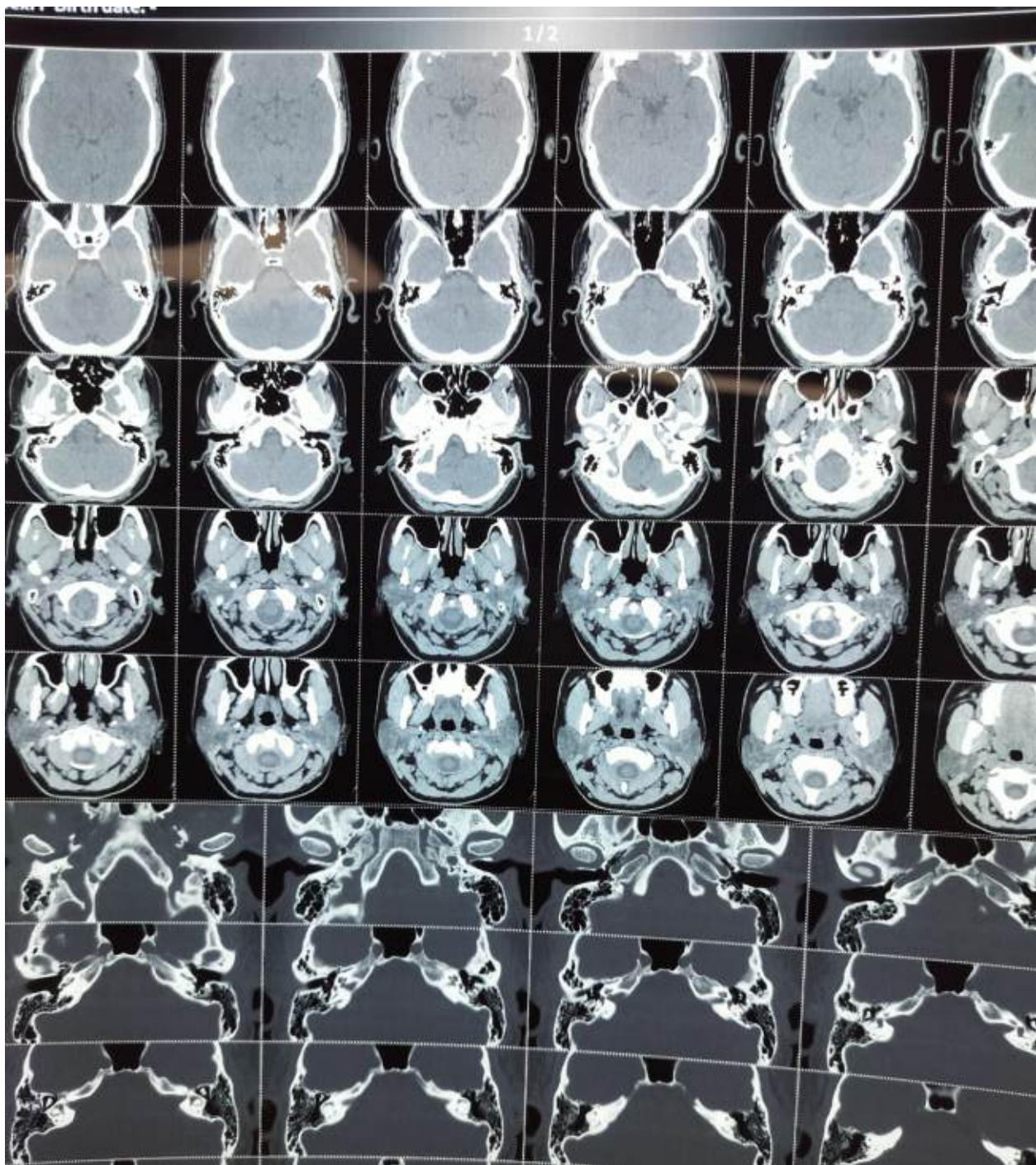


Figure 3: Contrast Enhanced CT of Head and Neck Showing Contrast Enhancement in the Left Pinna



Figure 4: DSA Showing Abnormal AVMs in the Left Pinna



Figure 5: Immediate Post-operative Antero-lateral View



Figure 6: Immediate Post-operative Posterior View

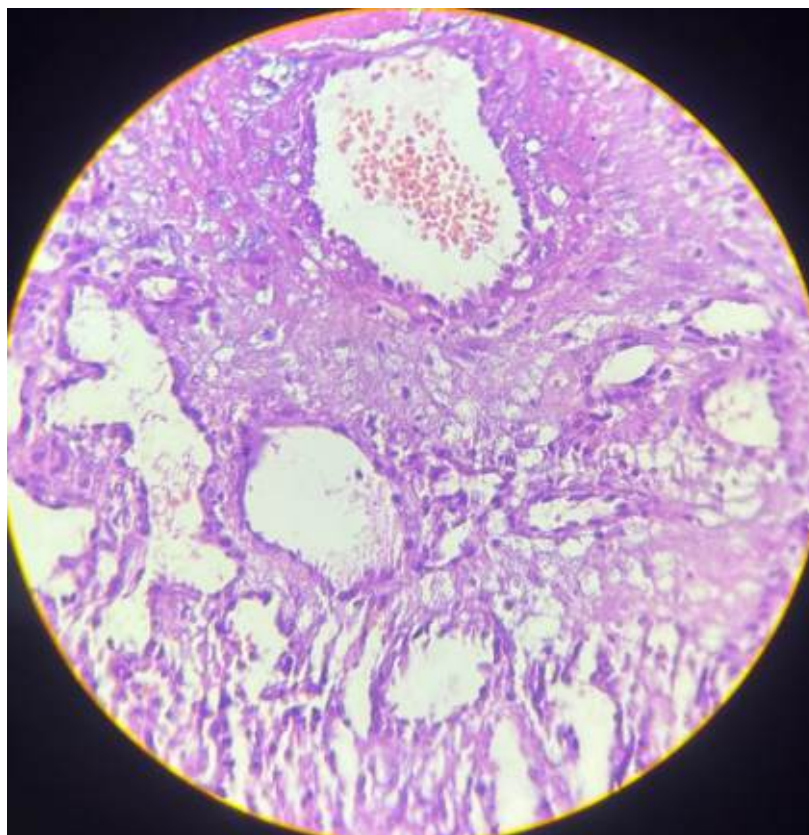


Figure 7: HPE Shows Ectasia of Arterioles with Thin Endothelial Lining



Figure 8: Post-operative Lateral View



Figure 9: Post-operative Posterior View of the Left Pinna

DISCUSSION

AV Malformations of the external ear are rare. Most of them are fast flow lesions consisting of arterio-venous anastomosis. These give an audible bruit. The rest of them are slow flowing lesions involving the anastomosis between venous, capillary and lymphatic system¹.

The Arteriovenous malformations generally are found to be comprising of a nidus typically surrounded by abnormal anastomotic network between AV shunts. The clinical manifestations of the AVM can be attributed to the absence of capillary system which interpose between the arterioles and the venous system. This shifts the high pressure from the arterial system to the venous system².

Kohout et al presented a large retrospective study of 81 patients with head and neck vascular malformations at Vascular anomalies program in Boston in 1998. Out of these 16 percent of patients had vascular malformations of the pinna. In this study 8 patients noted AVMs in the pubertal period, six patients noted exacerbation during puberty. According to the Clinical Staging System by Schobinger for Vascular Malformations: Stage I (Quiescence) - cutaneous blush/ warmth; Stage II (Expansion) - bruit, audible pulsation, expanding lesions; Stage III (Decompensation) Cardiac failure³. These lesions can be classified into four stages as described by Schobinger, I: Cutaneous blush/ warmth; II: Bruit, audible pulsations, expanding lesion; III: Pain, ulceration, bleeding and infection; and IV: Cardiac failure³.

The AVMs typically display ectasia of abnormal vessels leading on to collateral formation. This diverts the blood from the regional circulation. Histologically, the arteries turn out to be lengthened, tortuous and thin walled. The veins however tend to have a thicker muscular coat because of increased pressure⁴.

The Arteriovenous malformations of the external ear typically derive their blood supply from branches of the external carotid artery, including the superficial temporal, posterior auricular, and occipital arteries. Although congenital in origin, these lesions may remain clinically silent until precipitated by hormonal influences such as puberty or pregnancy, or by trauma⁵.

The exact pathogenesis of AVMs remains unclear, though most theories attribute their development to errors in late embryonic angiogenesis, resulting in persistent arteriovenous connections. Clinically, AVMs may progress through stages ranging from localized warmth and discoloration to ulceration, bleeding, and, in severe cases, high-output cardiac failure.

Imaging plays a crucial role in diagnosis and treatment planning. Doppler ultrasonography helps identify high-flow vascularity, while MRI delineates soft tissue involvement. Angiography remains the gold standard, as it defines the vascular anatomy and enables therapeutic embolization. Characteristic angiography findings are marked hypertrophy and tortuosity in the feeding vessels. The appearance of the

nidus (center) of the lesion varies from large tortuous vessels to innumerable small vessels appearing as an intense blush. Collateral vessels usually have a 'cork screw' appearance^{6,7}.

Management of auricular AVMs is challenging due to the risk of excessive bleeding, cosmetic deformity, and recurrence. If the AVM is small and asymptomatic, no treatment is required, especially in children. Embolisation alone may provide temporary control but is associated with high recurrence rates. Therefore, combined therapy involving embolisation followed by complete surgical excision within a short interval is considered the most effective approach. Incomplete resection remains the most common cause of recurrence, emphasizing the need for meticulous surgical planning and long-term follow-up. For a symptomatic AVM, complete excision with prior embolisation is the treatment of choice⁸. Only surgical ligation or only proximal embolisation may lead to not only aggravating the lesion by establishing new collaterals but also precludes later embolisation hence leading to failure for the same reasons^{9,10}. Embolisation alone rarely has to be done in deeper located lesions where surgical approach is very challenging and areas close to highly important structures¹⁰. However, in such cases, complications like stroke, cranial nerve palsy, and blindness can occur¹¹. Total resection which requires a wide-field resection of all the involved tissue is necessary to prevent recurrence, however, cosmetic and functional issues might limit the extent to which tissue can be removed. Partial excision usually leads to rapid recurrence so in these cases the remaining AVM tissue must be obliterated using intravascular embolisation.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1. Pham TH, Wong BJ, Allison G. A large arteriovenous malformation of the external ear in an adult: A case report and approach to management. *Laryngoscope* 2001;111: 1390-94.
2. Seccia A, Salgarello M, Farallo E, Falappa PG. Combined radiological and surgical treatment of arteriovenous malformations of the head and neck. *Ann Plast Surg* 1999; 43:359-66.
3. Kohout MP, Hansen M, Pribaz JJ, Mulliken JB. Arteriovenous malformations of head and neck: Natural history and management. *Plast Reconstr Surg* 1998; 102:643-54.
4. Glowacki J, Mulliken JB. Mast cells in hemangiomas and vascular malformations. *Pediatrics* 1982; 70:48-519.

5. Batsakis JG, Rice DH. The pathology of head and neck tumors: vasoformative tumors. *Head Neck Surg.* 1988; 3:231–239.
6. Burrows PE, Mulliken JB, Fellows KE, Strand RD. Childhood hemangiomas and vascular malformations: angiographic differentiation. *AJR Am J Roentgenol* 1983; 141:483-8.
7. Withers EH, Franklin JD, Lynch JB. Resection of a massive arteriovenous malformation of the head and neck. *Plast Reconstr Surg* 1979; 63:566-9.
8. Flye MW, Jordan BP, Schwartz MZ. Management of congenital arteriovenous malformations. *Surgery* 1983; 94:740-7.
9. Svendsen PA, Wikholm G, Fogdestam I, Anniko M, Mendel L. Direct puncture of large arteriovenous malformations in head and neck for embolisation and subsequent reconstructive surgery. *Scand J Plast Reconstr Surg Head Surg* 1994; 28:131-5.
10. Matsushige T, Kiya K, Satoh H, Mizoue T, Kagawa K, Araki H. Arteriovenous malformation of the scalp: case report and review of the literature. *Surg Neurol* 2004; 62:253-9.
11. Demuth RJ, Miller SH, Keller F. Complications of embolization treatment for problem cavernous hemangiomas. *Ann Plast Surg* 1984; 13:135-44.

New Drug Approvals

S. No.	Treatment Indication	Drug's Name	Company	Description	Date of FDA Approval
1.	Menkes Disease	Zycubo (Copper histidinate) Injection - formerly CUTX-101	Sentynl Therapeutics, Inc.	Zycubo (Copper histidinate) is a copper replacement therapy indicated for the treatment of Menkes disease in paediatric patients.	January 12, 2026
2.	Neutropenia, Neutropenia Associated with Chemotherapy, Neutropenia Associated with Radiation, Bone Marrow Transplantation, Peripheral Progenitor Cell Transplantation, Hematopoietic Syndrome of Acute Radiation Syndrome	Filkri (Filgrastim-laha) Injection	Accord BioPharma Inc.	Filkri (Filgrastim-laha) is a leukocyte growth factor biosimilar to Neupogen for use the treatment of Neutropenia, Neutropenia associated with Chemotherapy, Neutropenia associated with Radiation, Bone Marrow Transplantation, and Peripheral Progenitor Cell Transplantation.	January 15, 2026
3.	Presbyopia	Yuvezzi (Brimonidine tartrate and Carbachol) Ophthalmic Solution - formerly Brimochol PF	Tenpoint Therapeutics, Ltd.	Yuvezzi (Brimonidine tartrate and Carbachol) is an alpha-adrenergic agonist and cholinergic agonist combination indicated for the treatment of Presbyopia in adults.	January 28, 2026
4.	Atopic Dermatitis	Adquey (Difamilast) Ointment	Acrotech Biopharma Inc.	Adquey (Difamilast) is a topical phosphodiesterase 4 (PDE-4) inhibitor for the treatment of mild to moderate Atopic Dermatitis in patients 2 years of age and older.	February 12, 2026

Continued ...

S. No.	Treatment Indication	Drug's Name	Company	Description	Date of FDA Approval
5.	Schizophrenia, Bipolar Disorder	Bysanti (Milsaperidone) Tablets	Vanda Pharmaceuticals Inc.	Bysanti (Milsaperidone) is an atypical antipsychotic for use in the treatment of Schizophrenia and acute Bipolar I Disorder.	February 20, 2026
6.	Arginase 1 Deficiency	Loargys (Pegzilarginase-nbln) Injection	Immedica Pharma	Loargys (Pegzilarginase-nbln) is a recombinant human arginase-1 enzyme replacement therapy for the treatment of Hyperargininemia in patients with Arginase 1 Deficiency.	February 23, 2026
7.	Diabetes Insipidus	Desmoda (Desmopressin acetate) Oral Solution - formerly ET-600	Eton Pharmaceuticals, Inc.	Desmoda (Desmopressin acetate) is an oral solution formulation of the approved vasopressin analogue Desmopressin for use in the management of Central Diabetes insipidus.	February 25, 2026
8.	Achondroplasia	Yuviwel (Navepegritide) Lyophilized Powder for Injection - formerly TransCon CNP	Ascendis Pharma A/S	Yuviwel (Navepegritide) is a C-type natriuretic peptide analogue for the treatment of Achondroplasia in children.	February 27, 2026
9.	Plaque Psoriasis	Icotyde (Icotrokinra hydrochloride) Tablets	Johnson & Johnson	Icotyde (Icotrokinra) is an interleukin-23 (IL-23) receptor antagonist for use in the treatment of moderate-to-severe Plaque Psoriasis.	March 17, 2026
10.	Cholestatic Pruritus in Primary Biliary Cholangitis	Lynavoy (Linerixibat) Tablets	GSK plc	Lynavoy (Linerixibat) is an ileal bile acid transporter (IBAT) inhibitor for the treatment of Cholestatic Pruritus in patients with primary Biliary Cholangitis.	March 17, 2026

Continued ...

S. No.	Treatment Indication	Drug's Name	Company	Description	Date of FDA Approval
11.	Mucopoly-saccharidosis Type II	Avlayah (Tividenofusp alfa-eknm) Lyophilized Powder for Injection	Denali Therapeutics Inc.	Avlayah (Tividenofusp alfa-eknm) is an enzyme replacement therapy for use in the treatment of Hunter Syndrome.	March 24, 2026
12.	Ovarian Cancer	Lifyorli (Relacorilant) Capsules	Corcept Therapeutics Incorporated	Lifyorli (Relacorilant) is a glucocorticoid receptor antagonist for the combination treatment of patients with Platinum-resistant Ovarian Cancer.	March 25, 2026
13.	Type 2 Diabetes	Awikli (Insulin Icodec-abae) Injection	Novo Nordisk	Awikli (Insulin Icodec) is a once-weekly basal insulin for the treatment of adults with Type 2 Diabetes.	March 26, 2026
14.	Leukocyte Adhesion Deficiency-I	Kresladi (Marnetegrane Autotemcel) Suspension for Intravenous Infusion	Rocket Pharmaceuticals, Inc.	Kresladi (Marnetegrane Autotemcel) is an autologous hematopoietic stem cell-based gene therapy for the treatment of paediatric patients with severe Leukocyte Adhesion Deficiency-I.	March 26, 2026
15.	Osteoporosis	Ponlimsi (Denosumab-adet) Injection	Teva Pharmaceutical Industries Ltd.	Ponlimsi (Denosumab-adet) is a RANK ligand (RANKL) inhibitor biosimilar to Prolia (Denosumab) used in the treatment of Osteoporosis.	March 27, 2026

(Ravindra Bangar)
Editor

Recent Medical Advances

From the Journals – Edited Highlights

Navigating the Frontiers of Global Medical Research

The pace of medical discovery has never been more relentless, nor more demanding to follow. Every week, thousands of research papers emerge from laboratories, clinical centres and academic institutions across the world, each carrying findings that may reshape how we understand, prevent, diagnose or treat diseases. For the busy clinician, the dedicated student and the curious researcher alike, keeping pace with this ever-growing tide of knowledge presents a genuine and growing challenge.

It is precisely to address this challenge that Pacific Journal of Medical and Health Sciences presents its Recent Medical Advances feature: a carefully curated, rigorously verified and accessibly presented window into the most significant research shaping medicine today.

In this issue, we present a meticulously assembled collection of 81 cutting-edge research papers published globally, predominantly in 2025 and 2026, spanning the full breadth of contemporary medicine, from prevention and early detection through transformative therapeutics to the management of complex, multi-system complications. These papers represent not a random sampling but a deliberate editorial selection, guided by scientific significance, clinical relevance and the potential to meaningfully influence practice and understanding.

To aid navigation, the collection has been organised into eight thematic categories: Oncology and Precision Immunotherapy; Neurology, Neuropsychiatry and Brain Health; The Music-Brain Axis; Cardio-Metabolic Medicine, Diabetes and Endocrinology; Immunology and Infectious Disease; Maternal, Neonatal and Paediatric Health; Surgery, Transplantation and Regenerative Medicine; and Public Health, Nutrition and Fundamental Science. This taxonomy reflects the evolving landscape of modern medicine, where disciplinary boundaries are increasingly fluid and the most important discoveries frequently emerge at the intersection of multiple fields.

The collection deliberately embraces a diversity of study designs, including randomised controlled trials, systematic reviews and meta-analyses, cohort studies, basic science investigations and translational clinical trials, recognising that different questions demand different methodological approaches, and that the strength of any evidence base lies in the convergence of multiple study types rather than any single design.

Every research paper in this collection underwent rigorous independent verification to ensure scientific accuracy and integrity. In an era when misinformation circulates with alarming speed across social media platforms and even within professional networks, editorial vigilance is not merely a quality standard but a responsibility to our readers and to the patients whose care may be influenced by what they read. Where discrepancies were identified between widely circulated summaries and primary source data, corrections were made and, where verification was not possible, papers were excluded rather than published with uncertainty. Complete Digital Object Identifiers (DOIs) accompany every entry, enabling readers to access original publications directly for deeper investigation and independent verification.

To maximise accessibility and educational impact, each research paper has been distilled into a concise, standardised summary formatted in Vancouver Citation Style and accompanied by a pair of custom-designed infographic presentations. These visual tools are crafted to communicate key findings, proposed mechanisms, clinical implications and statistical outcomes in formats suited to a wide range of educational and professional settings, including clinical ward rounds, patient education encounters, postgraduate conference presentations and undergraduate classroom instruction.

This feature will be of particular relevance to practising physicians and general practitioners seeking evidence-based updates to inform clinical decision-making, and to academic researchers mapping knowledge gaps and charting future research directions. Medical educators will find contemporary, reliable teaching materials readily applicable to classroom and conference settings. Nutritionists, dietitians and public health professionals will discover the latest findings directly relevant to their fields, while students at every stage of their medical education will encounter the foundational knowledge that will define their future practice.

We hope this collection serves not merely as a reference but as an invitation to read more deeply, think more critically and remain perpetually curious about a discipline that never ceases to surprise.

- The Editor

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Setidegrasib: A New Drug That Destroys, Rather Than Blocks, the “Undruggable” Pancreatic Cancer Mutation (2026)

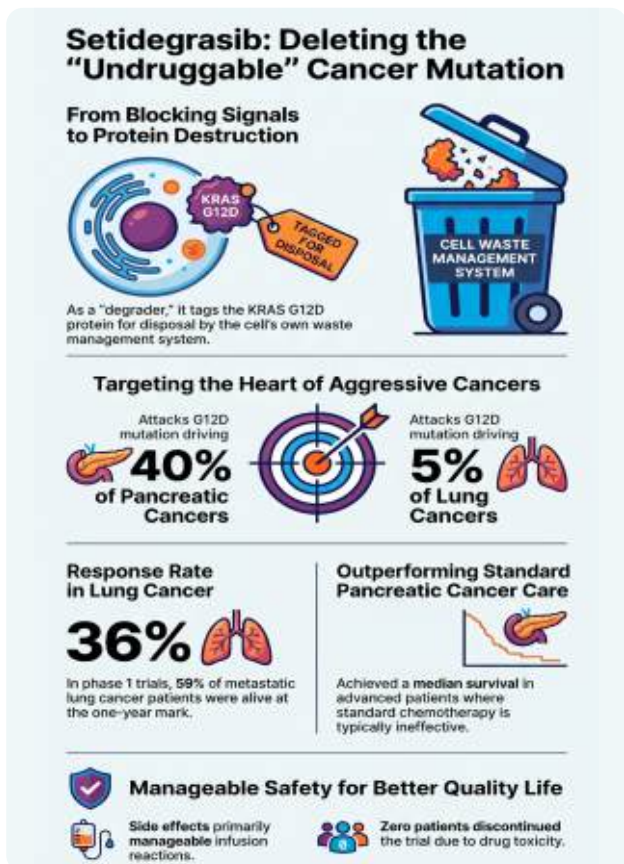
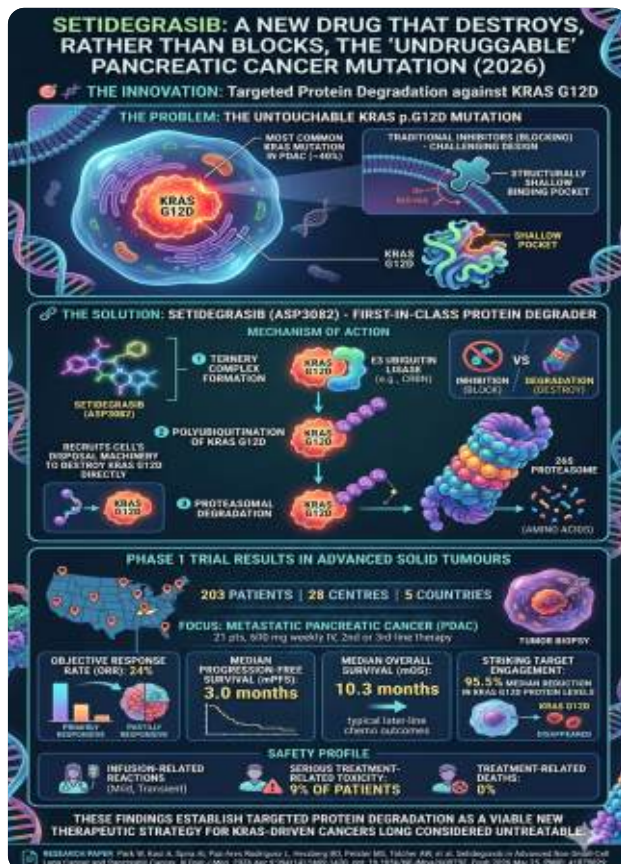
 *What if instead of trying to silence a cancer-driving mutation, doctors could simply make the rogue protein disappear altogether?*

This first-in-human Phase 1 trial, published in the New England Journal of Medicine (March 2026), evaluated Setidegrasib (ASP3082), a first-in-class KRAS G12D-targeted protein degrader, in 203 patients with advanced solid tumours across 28 centres in five countries. KRAS p.G12D is the most common KRAS mutation in pancreatic ductal adenocarcinoma, occurring in roughly 40% of cases, yet no targeted therapy against this variant had previously reached clinical practice, owing to the mutation's structurally shallow binding pocket. Rather than inhibiting the mutant protein, Setidegrasib forms a three-part molecular complex that recruits the cell's own disposal machinery to destroy KRAS G12D directly. Among 21 patients with metastatic pancreatic cancer who received the recommended 600 mg weekly intravenous dose as second- or third-line therapy, 24% achieved an objective response, with a median progression-free survival of 3.0 months and median overall survival reaching 10.3 months, notably longer than typical outcomes with existing later-line chemotherapy options. Tumour biopsies confirmed striking target engagement, with a 95.5% median reduction in KRAS G12D protein levels. Side effects were predominantly mild, transient infusion-related reactions, with only 9% of patients experiencing serious treatment-related toxicity and no treatment-related deaths. These findings establish targeted protein degradation as a viable new therapeutic strategy for KRAS-driven cancers long considered untreatable.

Source:

 RESEARCH PAPER

 Park W, Kasi A, Spira AI, Paz-Ares Rodríguez L, Herzberg BO, Pelster MS, Tolcher AW, et al. Setidegrasib in Advanced Non-Small-Cell Lung Cancer and Pancreatic Cancer. *N Engl J Med*. 2026 Apr 9;394(14):1409-1420. doi: 10.1056/NEJMoa2600752. Epub 2026 Mar 25. PMID: 41879829.



Vitamin B7 Reveals Cancer's Hidden Achilles Heel: A Metabolic Trap with No Escape (2026)

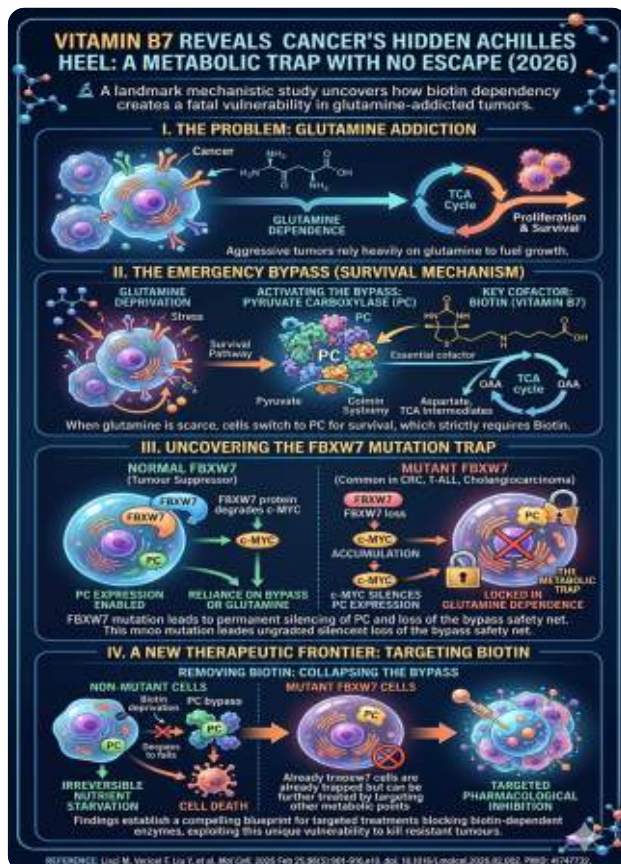
 *What if the same vitamin found in your daily supplement is quietly keeping aggressive, treatment-resistant tumours alive?*

This landmark mechanistic study, conducted by Miriam Lisci, Fanny Vericel, Yifan Liu, and colleagues from the laboratory of Alexis A. Jourdain at the University of Lausanne, Switzerland, combined metabolic tracing, nutrient supplementation, and genome-wide CRISPR-Cas9 screening to map the molecular pathways underlying glutamine addiction in cancer. The team discovered that when glutamine-deprived cancer cells activate their emergency metabolic bypass via pyruvate carboxylase, this survival mechanism is entirely dependent on biotin (vitamin B7) as an essential cofactor. Removing biotin renders pyruvate carboxylase inactive, collapsing the bypass and trapping cancer cells in irreversible nutrient starvation. Additionally, the study uncovered a previously unknown role for the tumour suppressor FBXW7: when mutated, a common event in colorectal cancer, T-cell leukaemia, and cholangiocarcinoma, FBXW7 loss drives c-MYC accumulation, which silences pyruvate carboxylase expression, locking cells in glutamine dependence. Patient-derived FBXW7 mutations directly reproduced this metabolic vulnerability in laboratory models. These findings establish a compelling blueprint for targeted pharmacological inhibition of biotin-dependent enzymes within the tumour microenvironment.

Source:

 RESEARCH PAPER

 Lisci M, Vericel F, Liu Y, Gallart-Ayala H, Ivanisevic J, Skinner OS, Jourdain AA. Functional nutrient-genetic profiling reveals biotin and FBXW7 are essential to bypass glutamine addiction. *Mol Cell*. 2026 Feb 25;86(5):901-916.e10. doi: 10.1016/j.molcel.2026.02.002. PMID: 41747732.



Before Immunotherapy Destroys the Pancreas: Mapping the Genetic Warning Signs (2026)

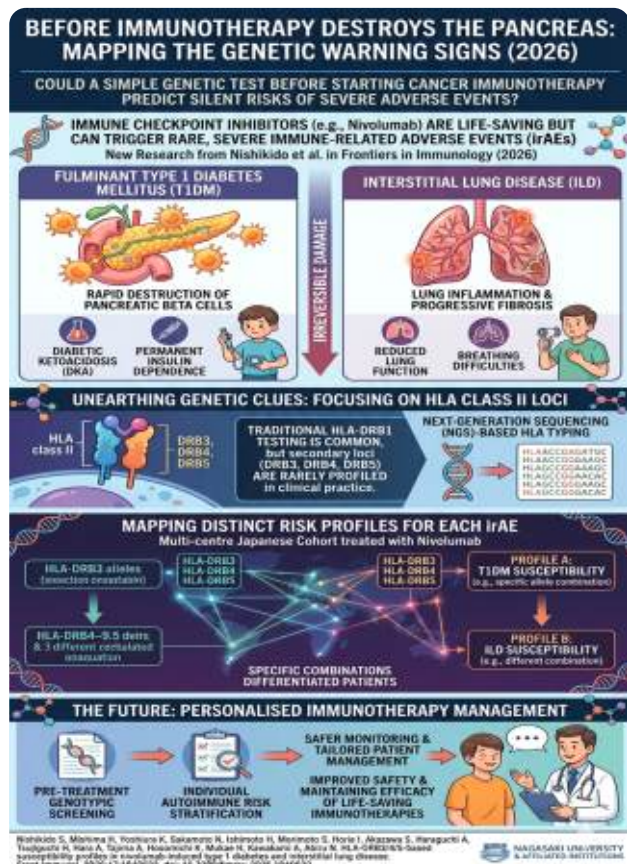
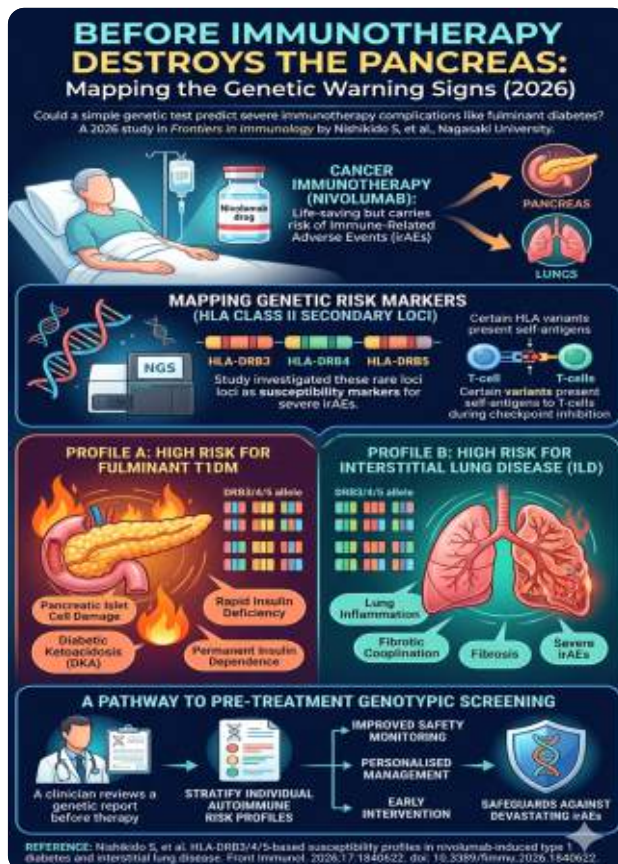
 **Could a simple genetic test before starting cancer immunotherapy tell us which patients are silently at risk of developing fulminant diabetes or irreversible lung damage?**

This original research, published in *Frontiers in Immunology* in 2026, was conducted by Nishikido S, Mishima H, Yoshiura K and colleagues from Nagasaki University and affiliated institutions in Japan, involving a multi-centre patient cohort treated with the immune checkpoint inhibitor nivolumab. The study investigated the role of HLA-DRB3, HLA-DRB4, and HLA-DRB5 alleles, secondary HLA class II loci that are rarely profiled in clinical practice as genetic susceptibility markers for two of the most severe immune-related adverse events associated with nivolumab: fulminant Type 1 Diabetes mellitus (T1DM) and interstitial lung disease. Using next-generation sequencing-based HLA typing, the team constructed distinct susceptibility profiles for each adverse event, demonstrating that specific DRB3/4/5 allele combinations differentiated patients who developed T1DM from those who developed interstitial lung disease. Diabetic ketoacidosis occurred in the majority of T1DM cases, rendering all affected patients permanently insulin-dependent. These findings open a pathway toward pre-treatment genotypic screening, enabling oncologists to stratify individual autoimmune risk profiles before initiating checkpoint inhibitor therapy, thereby improving the safety monitoring and personalised management of life-saving but potentially devastating immunotherapies.

Source:

 RESEARCH PAPER

 Nishikido S, Mishima H, Yoshiura K, Sakamoto N, Ishimoto H, Morimoto S, Horie I, Akazawa S, Haraguchi A, Tsujiguchi H, Hara A, Tajima A, Hosomichi K, Mukae H, Kawakami A, Abiru N. HLA-DRB3/4/5-based susceptibility profiles in nivolumab-induced type 1 diabetes and interstitial lung disease. *Front Immunol.* 2026; 17:1840622. doi: 10.3389/fimmu.2026.1840622.



AI Reads a Tumour Slide and Predicts Its Protein Landscape - Without Expensive Lab Tests (2026)

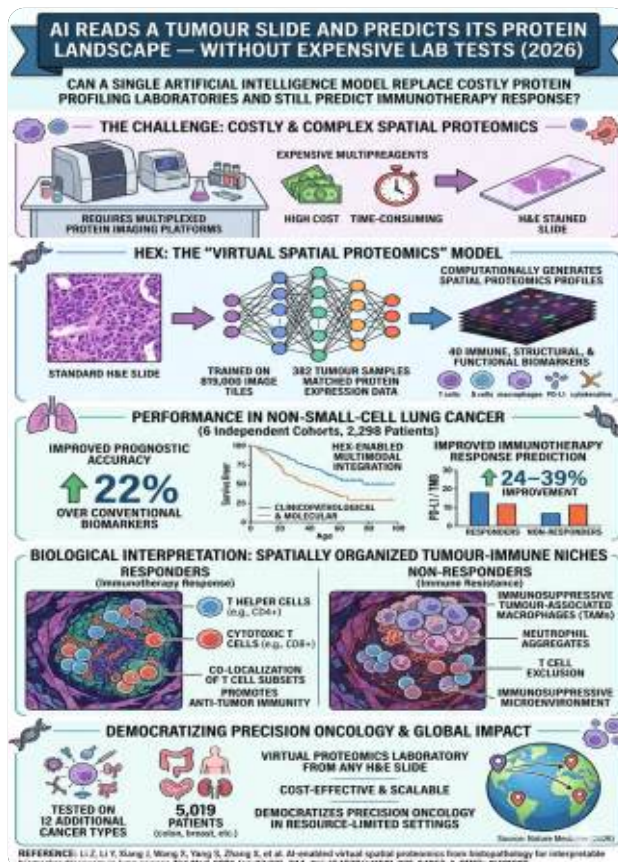
 **Can a single artificial intelligence model replace costly protein profiling laboratories and still predict how a lung cancer patient will respond to immunotherapy?**

Researchers at Stanford University developed HEX (H&E to Protein Expression), a groundbreaking artificial intelligence model trained and validated on 819,000 histopathology image tiles with matched protein expression data from 382 tumour samples, published in *Nature Medicine* (January 2026). HEX computationally generates high-dimensional spatial proteomics profiles, including the expression of 40 immune, structural, and functional biomarkers directly from standard haematoxylin and eosin-stained histopathology slides, eliminating the need for expensive multiplexed protein imaging platforms. Applied to six independent non-small-cell lung cancer cohorts totalling 2,298 patients, HEX-enabled multimodal integration improved prognostic accuracy by 22% and immunotherapy response prediction by 24–39% compared with conventional clinicopathological and molecular biomarkers. Biological interpretation revealed spatially organised tumour-immune niches predictive of therapeutic response, including co-localisation of T helper cells and cytotoxic T cells in responders, and immunosuppressive tumour-associated macrophage and neutrophil aggregates in non-responders. The model further demonstrated generalisability across 12 additional cancer types encompassing 5,019 patients. By converting an affordable, universally available diagnostic tool into a virtual proteomics laboratory, HEX democratises precision oncology, offering a scalable, interpretable, and cost-effective pathway for biomarker discovery and clinical translation in resource-limited settings worldwide.

Source:

 RESEARCH PAPER

 Li Z, Li Y, Xiang J, Wang X, Yang S, Zhang X, et al. AI-enabled virtual spatial proteomics from histopathology for interpretable biomarker discovery in lung cancer. *Nat Med.* 2026 Jan; 32:231–244. doi: 10.1038/s41591-025-04060-4. PMID: 41491099.



HEX: Teaching Standard Slides to Speak Biology

AI turns routine H&E slides into high-resolution maps of 40 biomarkers for precision medicine

From Standard Slides to Virtual Proteomics



HEX uses AI to infer complex protein expression from the ubiquitous, low-cost H&E slides every cancer patient already receives.

Significant Gains in Clinical Accuracy

22%

IMPROVED PROGNOSTIC ACCURACY
Multimodal AI integration improved prognostic accuracy by 22%.

up to 39%

IMPROVED IMMUNOTHERAPY RESPONSE PREDICTION
Multimodal AI integration improved immunotherapy response prediction by up to 39%.

Mapping 40 Distinct Biomarkers



Visualizing Tumor-Immune Niches



Probiotic Bacteria Engineered as Living Drug Factories to Deliver Chemotherapy Directly Inside Tumours (2026)

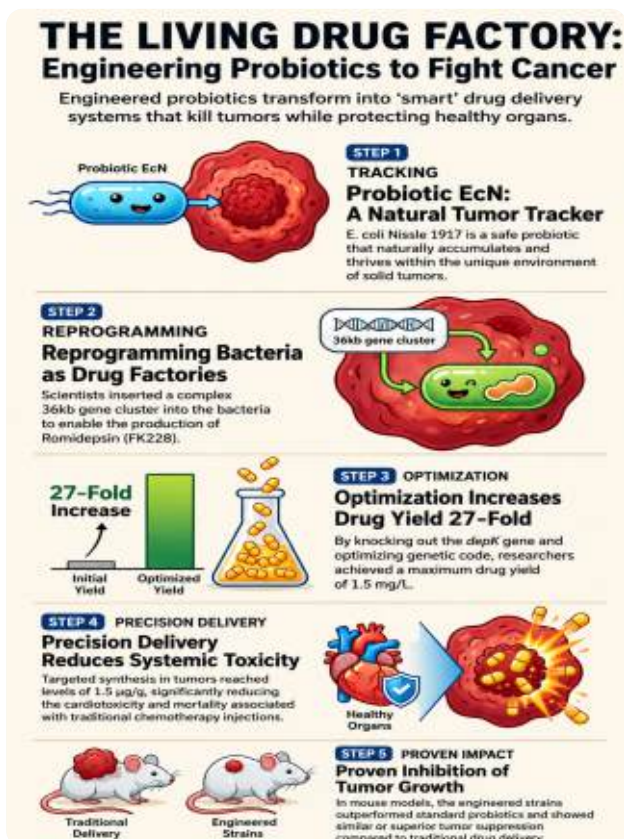
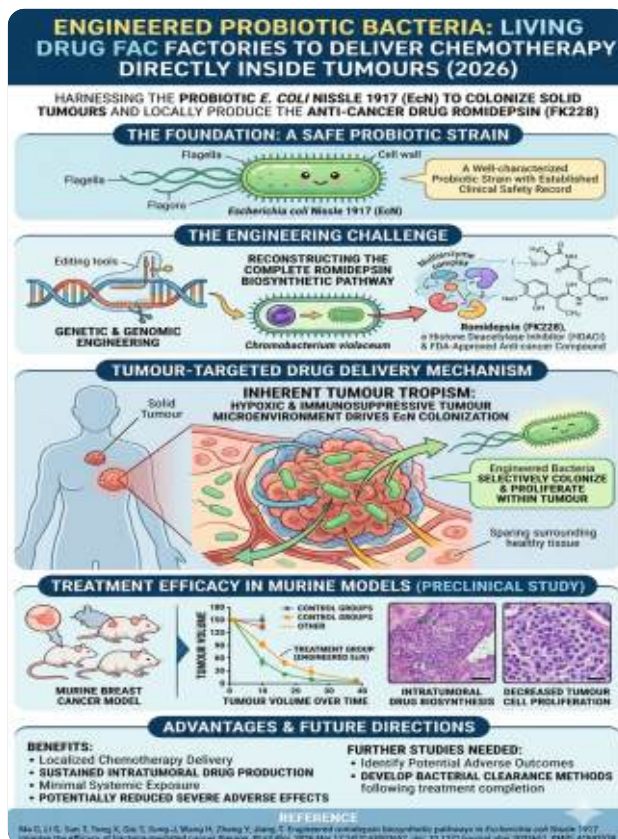
 **Can a harmless gut bacterium be reprogrammed to infiltrate a tumour and manufacture an FDA-approved cancer drug precisely where it is needed?**

Researchers at Shandong University, Qingdao, China, published a landmark study in *PLOS Biology* (March 2026) demonstrating that *Escherichia coli* Nissle 1917 (EcN), a well-characterised probiotic strain with an established clinical safety record, can be genetically engineered to biosynthesise Romidepsin (FK228), a histone deacetylase inhibitor and FDA-approved anticancer compound naturally produced by *Chromobacterium violaceum*. Using advanced genetic and genomic engineering techniques, the investigators reconstructed the complete Romidepsin biosynthetic pathway within EcN, transforming the probiotic into a living tumour-targeted drug factory. Exploiting EcN's inherent capacity to selectively colonise solid tumours attributable to the hypoxic and immunosuppressive tumour microenvironment - the engineered bacteria infiltrated tumour tissue and released Romidepsin both in vitro and in vivo across varying experimental conditions. In murine breast cancer models, the treatment demonstrated effective tumour colonisation, sustained intratumoral drug biosynthesis, and significant tumour growth inhibition compared with controls. The approach achieves localised chemotherapy delivery with minimal systemic exposure, potentially reducing the severe adverse effects associated with conventional intravenous Romidepsin administration. The researchers acknowledge that further studies identifying potential adverse outcomes and bacterial clearance methods following treatment completion are required before clinical translation. This study establishes a solid preclinical foundation for bacteria-assisted, tumour-targeted delivery of small-molecule anticancer agents, representing a paradigm shift in precision oncology.

Source:

 RESEARCH PAPER

 Ma C, Li G, Sun T, Tang X, Qiu T, Song J, Wang H, Zhang Y, Jiang T. Engineered romidepsin biosynthetic pathways in *Escherichia coli* Nissle 1917 improve the efficacy of bacteria-mediated cancer therapy. *PLoS Biol.* 2026 Mar 17;24(3): e3003657. doi: 10.1371/journal.pbio.3003657. PMID: 41843539.



Personalised mRNA Vaccines Establish New Efficacy Standards in Melanoma and Pancreatic Oncology (2025)

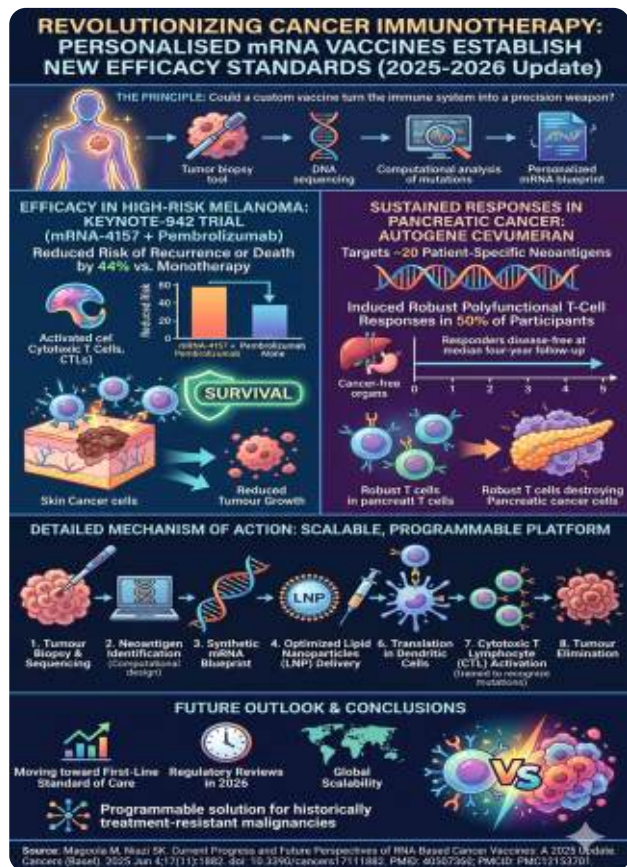
 **Could a vaccine custom-engineered from a patient's own tumour mutations finally turn the immune system into a precision weapon against cancer?**

Personalised mRNA cancer vaccines have transitioned from experimental concepts to transformative clinical realities. A 2025 multi-trial synthesis published in *Cancers*, updated by early 2026 Phase 3 data, highlights two pivotal breakthroughs. In pancreatic ductal adenocarcinoma, the autogene cevumeran vaccine targeting twenty patient-specific neoantigens induced robust polyfunctional T-cell responses in fifty percent of participants, with responders remaining disease-free at a median four-year follow-up. Concurrently, the KEYNOTE-942 Phase 2b/3 trial for high-risk resected melanoma demonstrated that mRNA-4157 combined with pembrolizumab reduced the risk of recurrence or death by forty-four percent compared to monotherapy. These vaccines utilise high-throughput sequencing of individual tumour biopsies to generate synthetic mRNA blueprints delivered via optimised lipid nanoparticles. This process trains cytotoxic T lymphocytes to recognise unique neoantigen signatures, bypassing the immune evasion tactics typical of solid tumours. As regulatory bodies review these platforms in 2026, personalised immunotherapy is moving toward a first-line standard of care, offering a scalable, programmable solution for historically treatment-resistant malignancies.

Source:

 UPDATE

 Magoola M, Niazi SK. Current Progress and Future Perspectives of RNA-Based Cancer Vaccines: A 2025 Update. *Cancers (Basel)*. 2025 Jun 4;17(11):1882. doi: 10.3390/cancers17111882. PMID: 40507360; PMCID: PMC12153701.



Engineered Bacteria Launch and Control Oncolytic Virus for Tumour-Targeted Cancer Therapy (2025)

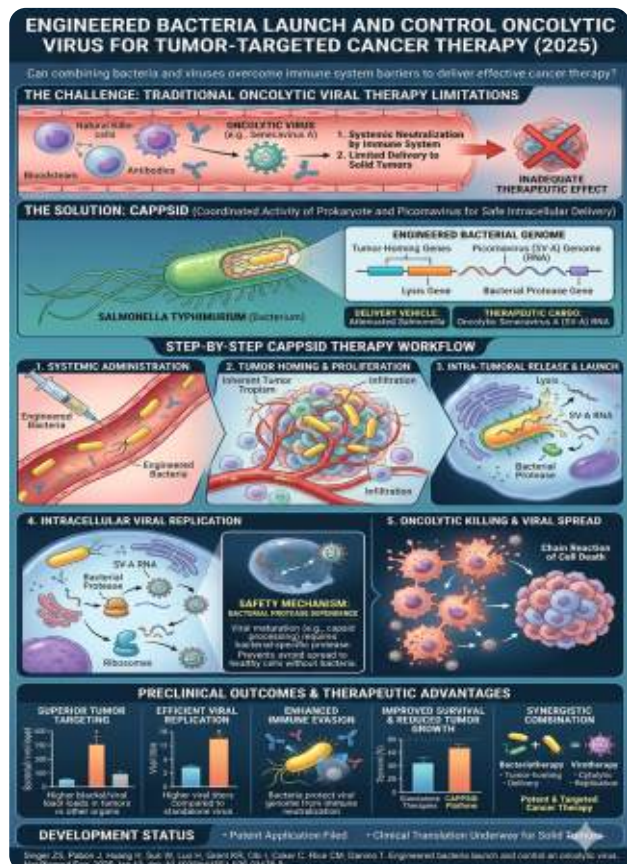
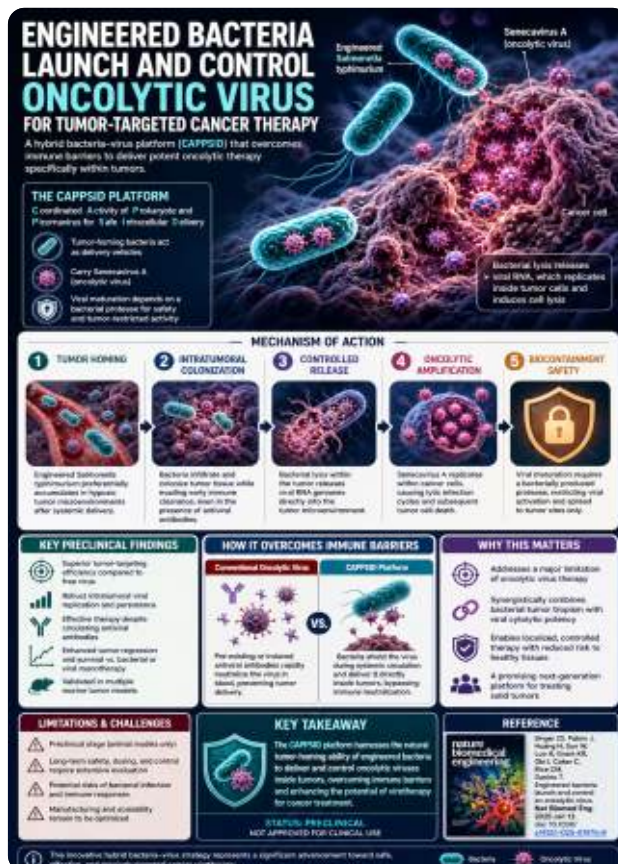
 **Can combining bacteria and viruses overcome immune system barriers to deliver effective cancer therapy?**

This innovative study published in *Nature Biomedical Engineering* (January 2025) developed CAPPSID (Coordinated Activity of Prokaryote and Picornavirus for Safe Intracellular Delivery), a novel cancer treatment platform utilising *Salmonella typhimurium* bacterium as delivery vehicles for Senecavirus A, an oncolytic virus that selectively targets tumours. The system exploits bacteria's inherent tumour-homing capabilities to transport therapeutic viruses directly into malignant tissues, circumventing a major limitation of conventional oncolytic viral therapies: the premature neutralisation of viral particles by the host immune system before they can reach cancerous cells. Once the bacteria infiltrate the tumour microenvironment, they lyse intracellularly, releasing a viral RNA genome that replicates within and destroys cancer cells through lytic infection cycles. A critical safety mechanism ensures that viral maturation depends on a bacterially produced protease, confining viral activity exclusively to tumour sites and preventing spread to healthy tissues. Preclinical validation in murine models demonstrated superior tumour targeting efficiency, robust viral replication within malignancies, immune evasion despite circulating antiviral antibodies, and enhanced therapeutic outcomes compared to standalone oncolytic viral therapies. This technology represents a significant advance, synergistically combining bacteriotherapy's tumour tropism with virotherapy's cytolytic potency. A patent application has been filed and clinical translation efforts for solid tumour treatment are underway.

Source:

RESEARCH PAPER

 Singer ZS, Pabón J, Huang H, Sun W, Luo H, Grant KR, Obi I, Coker C, Rice CM, Danino T. Engineered bacteria launch and control an oncolytic virus. *Nat Biomed Eng*. 2026 Mar;10(3):490-500. doi: 10.1038/s41551-025-01476-8. Epub 2025 Aug 15. PMID: 40817284; PMCID: PMC12696630.



Focused Ultrasound Achieves 90% Liver Tumour Control in One Year without Surgery or Radiation (2025)

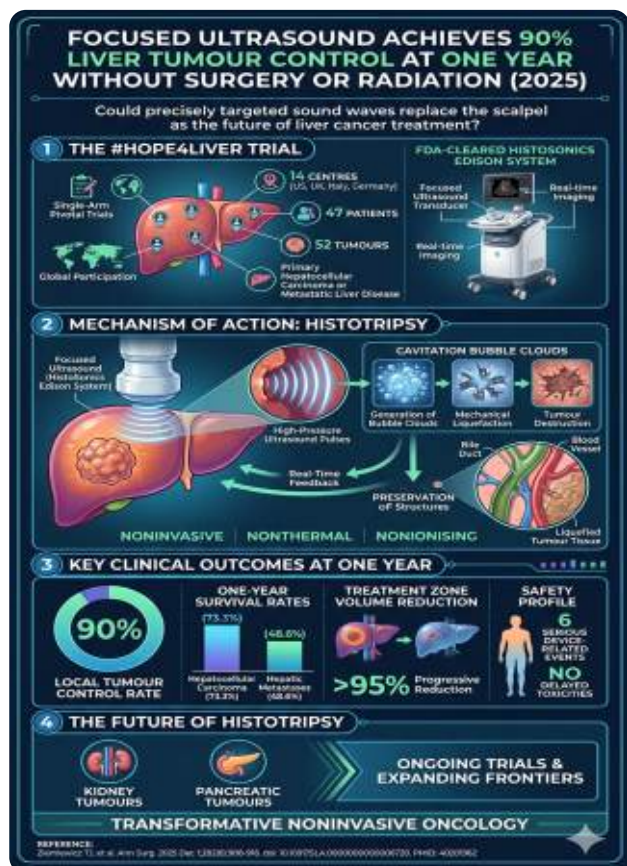
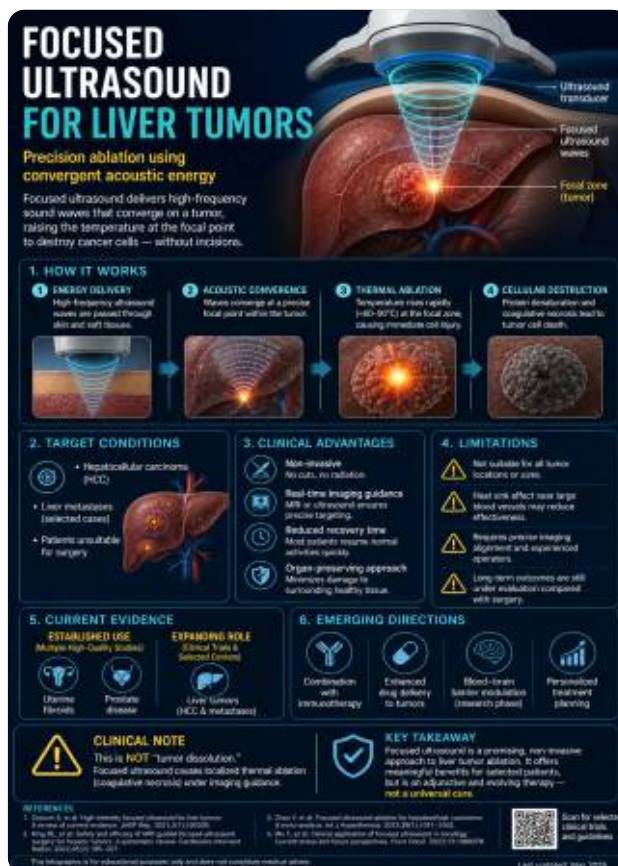
 **Could precisely targeted sound waves replace the scalpel as the future of liver cancer treatment?**

Histotripsy, a non-invasive, nonthermal and nonionising focused ultrasound therapy, is transforming liver cancer treatment for patients ineligible for conventional surgery or ablation. The #HOPE4LIVER single-arm pivotal trials, conducted prospectively across 14 centres in the United States, United Kingdom, Italy and Germany, enrolled 47 patients with primary hepatocellular carcinoma or metastatic liver disease, treating 52 tumours using the FDA-cleared HistoSonics Edison System. Published in *Annals of Surgery* (2025), one-year follow-up data by Ziemlewicz et al demonstrated a local tumour control rate of 90% on expert imaging reassessment, with one-year survival of 73.3% for hepatocellular carcinoma and 48.6% for hepatic metastases. The procedure delivers short, high-pressure ultrasound pulses generating cavitation bubble clouds that mechanically liquefy tumour tissue in real time while preserving blood vessels and bile ducts. Over 95% of treatment zones showed progressive volume reduction at one year. Only six serious device-related events were recorded with no delayed toxicities, comparing favourably with existing locoregional therapies. Ongoing trials are evaluating histotripsy for kidney and pancreatic tumours, establishing this sound-based ablation as a transformative frontier in non-invasive oncology.

Source:

 RESEARCH PAPER

 Ziemlewicz TJ, Critchfield JJ, Mendiratta-Lala M, et al. The #HOPE4LIVER single-arm pivotal trial for histotripsy of primary and metastatic liver tumours: one-year update of clinical outcomes. *Ann Surg.* 2025 Dec 1;282(6):908-916. doi: 10.1097/SLA.0000000000006720. PMID: 40201962.



Breastfeeding Induces Long-Lived Tissue-Resident Memory T Cells Conferring Protection Against Breast Cancer (2025)

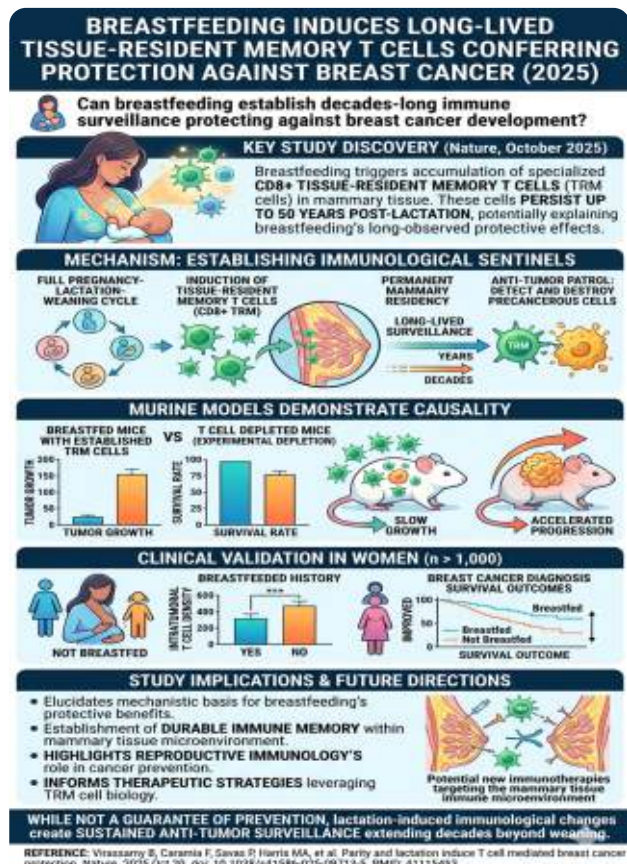
 **Can breastfeeding establish decades-long immune surveillance protecting against breast cancer development?**

This groundbreaking study published in *Nature* (October 2025) discovered that breastfeeding triggers the accumulation of specialised CD8⁺ tissue-resident memory T cells in breast tissue, persisting for up to 50 years post-lactation, potentially explaining the long-observed protective effects of breastfeeding against breast cancer. Researchers demonstrated that completing the full pregnancy-lactation-weaning cycle induces these immunological sentinels, which are primed to detect and destroy precancerous cells. In murine models, breastfed mice with established tissue-resident memory T cells exhibited significantly slower triple-negative breast cancer tumour growth; experimental depletion of these T cells eliminated the protective effect entirely, causing rapid tumour progression. Clinical validation analysing over 1,000 women confirmed that breastfeeding history correlated with higher intratumoral T cell densities and improved survival outcomes following breast cancer diagnosis. The study elucidates the mechanistic basis for breastfeeding's protective benefits through the establishment of durable immune memory within the mammary tissue microenvironment. While breastfeeding cannot guarantee cancer prevention, these findings demonstrate how lactation-induced immunological changes create sustained anti-tumour surveillance extending decades beyond weaning. This research highlights the critical role of reproductive immunology in cancer prevention, with potential implications for therapeutic strategies that leverage tissue-resident memory T cell biology for breast cancer risk reduction.

Source:

 RESEARCH PAPER

 Virassamy B, Caramia F, Savas P, Harris MA, et al. Parity and lactation induce T cell mediated breast cancer protection. *Nature*. 2025 Oct 20. doi: 10.1038/s41586-025-09713-5. PMID: 41115453.



Hydralazine Inhibits Cysteamine Dioxygenase to Treat Preeclampsia and Induce Senescence in Glioblastoma Cells (2025)

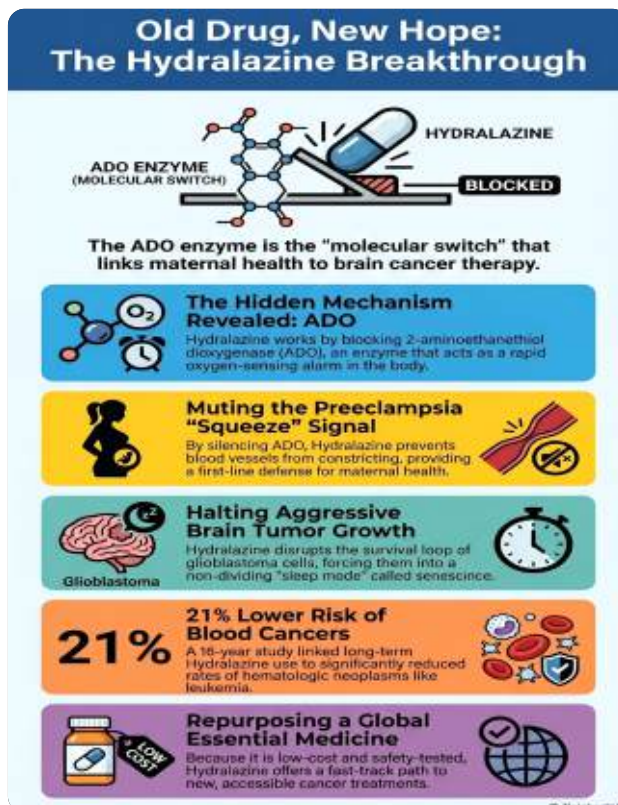
 **Can repurposing a 70-year-old antihypertensive medication offer novel therapeutic approach for aggressive brain cancer?**

This groundbreaking study published in *Science Advances* (January 2025) by researchers at the University of Pennsylvania elucidated hydralazine's molecular mechanism, revealing that it inhibits 2-aminoethanethiol dioxygenase (cysteamine dioxygenase/ADO), an enzyme that regulates blood vessel relaxation. This discovery explains the decades-old medication's antihypertensive and preeclampsia treatment efficacy at a molecular level. Critically, the investigators discovered that glioblastoma cells exhibit elevated ADO activity that is essential for their survival and proliferation. Hydralazine treatment induced cellular senescence in glioblastoma cultures, arresting malignant cell division without causing immediate cell death, with the effects of a single dose persisting for several days. This growth-arrest mechanism represents a potential therapeutic avenue against highly aggressive, treatment-resistant brain tumours that currently lack curative options. Hydralazine's established safety profile spanning seven decades, its global availability, and affordability position it as a promising repurposing candidate, particularly valuable for resource-limited healthcare settings where conventional glioblastoma treatments remain cost-prohibitive. However, the researchers emphasise that these findings represent preclinical in vitro observations requiring extensive validation through animal models and human clinical trials before translation into standard treatment protocols. The study demonstrates the broader potential of drug repurposing, leveraging existing medications' known safety profiles while uncovering novel molecular targets for cancer therapeutics.

 **Source:**

 **RESEARCH PAPER**

Shishikura K, Li J, Chen Y, McKnight NR, Keeley TP, Bustin KA, Barr EW, Chilkamari SR, Ayub M, Kim SW, Lin Z, Hu RM, Hicks K, Wang X, O'Rourke DM, Bollinger JM Jr, Binder ZA, Parsons WH, Martemyanov KA, Liu A, Matthews ML. Hydralazine inhibits cysteamine dioxygenase to treat preeclampsia and senesce glioblastoma. *Sci Adv*. 2025 Oct 17;11(42): eadx7687. doi: 10.1126/sciadv. adx7687. Epub 2025 Oct 15. PMID: 41091880; PMCID: PMC12526720.



Pharmacological Enhancement of Glymphatic Function for Alzheimer's Prevention (2026)

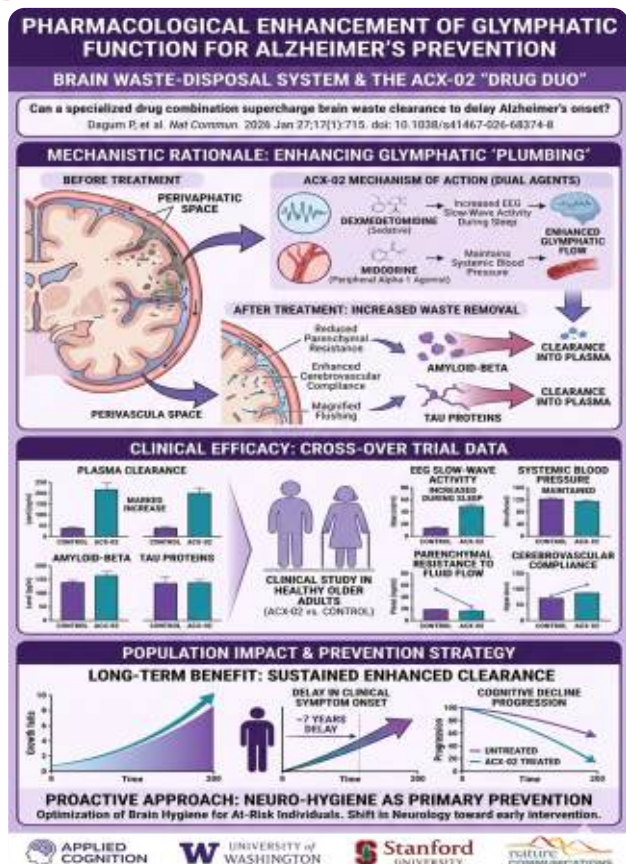
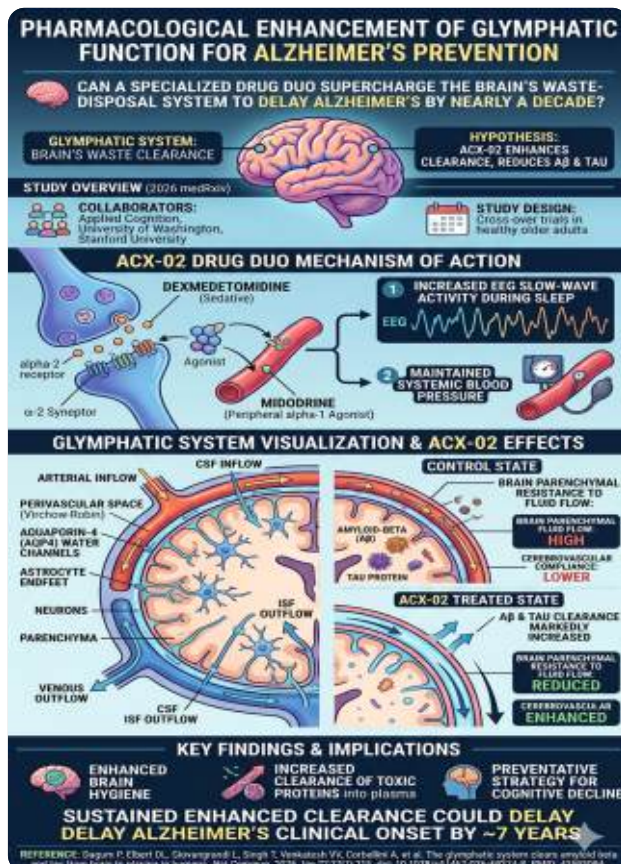
 **Can a specialised "drug duo" supercharge the brain's waste-disposal system to delay the onset of Alzheimer's by nearly a decade?**

This groundbreaking clinical investigation published in *Nature Communications* (January 2026) was conducted by researchers at Applied Cognition in collaboration with the University of Washington and Stanford University. The study utilised cross-over clinical trials in healthy older adults to evaluate the efficacy of ACX-02, a fixed-dose combination of the sedative dexmedetomidine and the peripheral alpha-1 agonist midodrine. Data demonstrated that this specific pharmacological pairing significantly increased EEG slow-wave activity during sleep while maintaining systemic blood pressure, leading to a marked increase in the clearance of amyloid-beta and tau proteins from the brain parenchyma into the plasma. Mechanistically, the treatment reduces brain parenchymal resistance to fluid flow and enhances cerebrovascular compliance, essentially magnifying the natural glymphatic "plumbing" process that flushes metabolic waste. These findings suggest that sustaining this enhanced clearance could potentially delay the clinical onset of neurodegenerative symptoms by approximately seven years. This proactive approach represents a significant shift in neurology, moving toward the optimisation of brain hygiene as a primary preventative strategy for individuals at high risk for cognitive decline.

Source:

 RESEARCH PAPER

 Dagum P, Elbert DL, Giovangrandi L, Singh T, Venkatesh VV, Corbellini A, et al. The glymphatic system clears amyloid beta and tau from brain to plasma in humans. *Nat Commun.* 2026 Jan 27;17(1):715. doi: 10.1038/s41467-026-68374-8. PMID: 41593094.



Global Disparities in Neurological Outcomes Following SARS-CoV-2 Infection (2026)

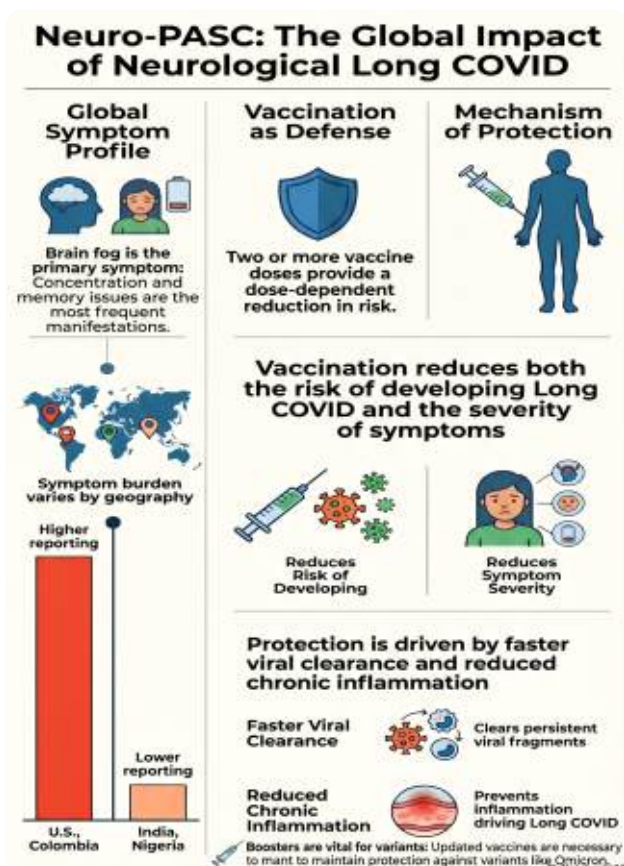
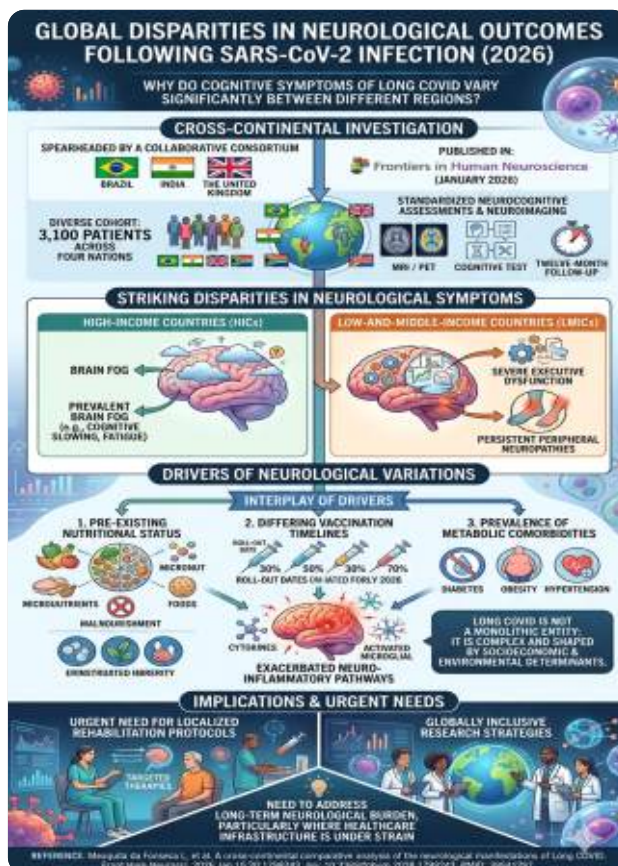
 **Why do cognitive symptoms of Long COVID vary so significantly between different regions of the world?**

This pioneering cross-continental investigation published in *Frontiers in Human Neuroscience* (January 2026) was spearheaded by a collaborative consortium of researchers from Brazil, India, and the United Kingdom. Tracking a diverse cohort of 3,100 patients across four nations, the study utilised standardised neurocognitive assessments and neuroimaging to map post-viral manifestations over a twelve-month follow-up period. The data revealed striking disparities, showing that while brain fog was prevalent in high-income countries, patients in low-and-middle-income countries exhibited higher rates of severe executive dysfunction and persistent peripheral neuropathies. Researchers propose that these variations are likely driven by an interplay of pre-existing nutritional status, differing vaccination timelines, and the prevalence of metabolic comorbidities that exacerbate neuro-inflammatory pathways. This study underscores that Long COVID is not a monolithic entity but a complex condition shaped by socioeconomic and environmental determinants. The findings emphasise the urgent need for localised rehabilitation protocols and globally inclusive research strategies to address the long-term neurological burden of the pandemic, particularly in regions where healthcare infrastructure remains under significant strain from emerging chronic disease patterns.

Source:

 **COMPARATIVE ANALYSIS**

 Mesquita da Fonseca L, et al. A cross-continental comparative analysis of the neurological manifestations of Long COVID. *Front Hum Neurosci*. 2026 Jan 15; 20:1798243. doi: 10.3389/fnhum.2026.1798243. PMID: 39541287.



Your Brain, Not Just Your Muscles, is the Secret Engine Behind Endurance Gains (2026)

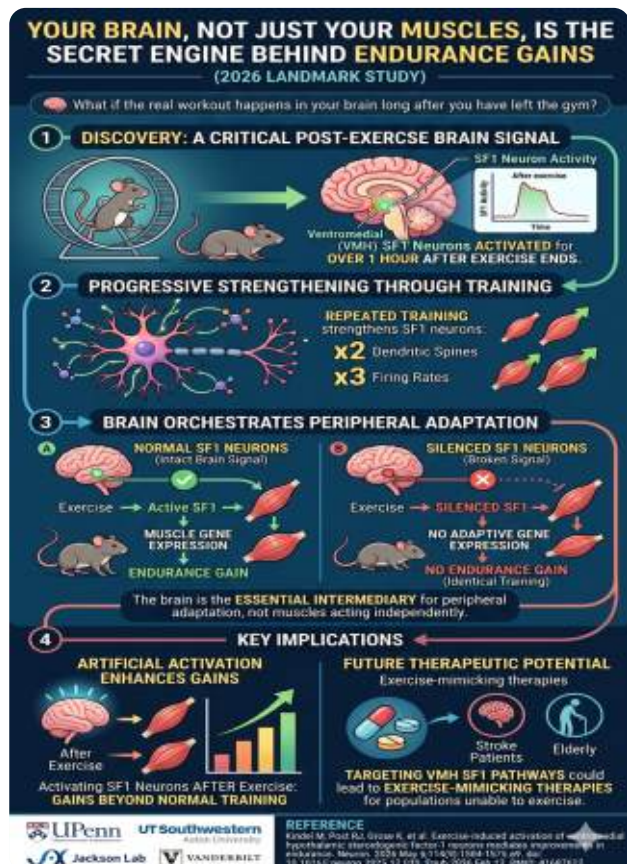
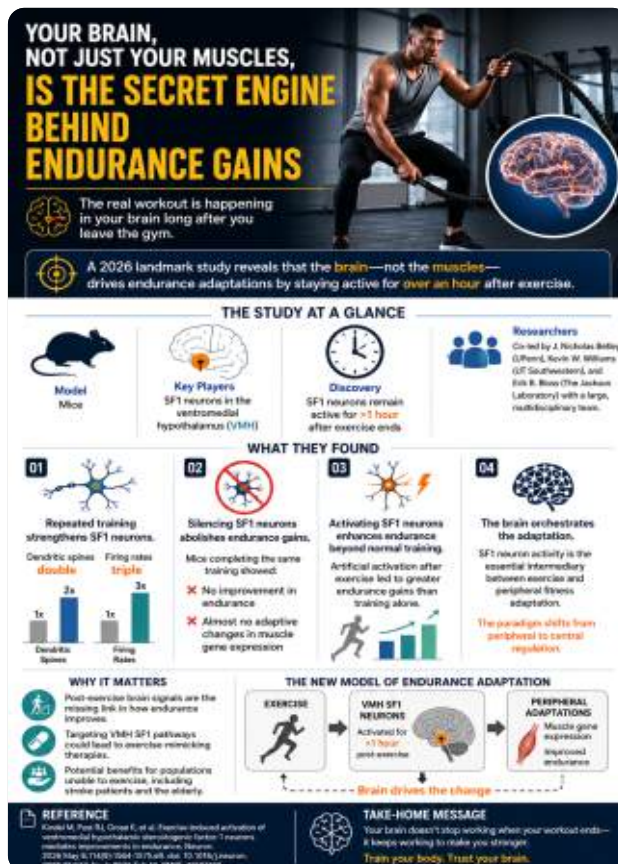
 **What if the real workout is happening in your brain long after you have left the gym?**

This landmark study was co-led by J. Nicholas Betley from the University of Pennsylvania, Kevin W. Williams from UT Southwestern Medical Center, and Erik B. Bloss from The Jackson Laboratory, with a large multidisciplinary team across institutions including Vanderbilt University. Studying mice, the researchers found that steroidogenic factor-1 (SF1) neurons in the ventromedial hypothalamus remain active for over an hour after exercise ends, and that repeated training progressively strengthens these neurons, doubling their dendritic spines and tripling their firing rates. When SF1 neurons were silenced, mice completing identical training programmes gained no endurance whatsoever, and their muscles showed almost no adaptive gene expression changes, revealing that the brain orchestrates peripheral adaptation rather than muscles acting independently. Artificially activating SF1 neurons after exercise enhanced endurance gains beyond normal training levels. These findings position the post-exercise brain signal as the essential intermediary between physical activity and fitness adaptation, shifting the paradigm from peripheral to central nervous system regulation of endurance. The authors suggest that targeting VMH SF1 pathways could yield exercise-mimicking therapies for populations unable to exercise, including stroke patients and the elderly.

Source:

 RESEARCH PAPER

 Kindel M, Post RJ, Grose K, et al. Exercise-induced activation of ventromedial hypothalamic steroidogenic factor-1 neurons mediates improvements in endurance. *Neuron*. 2026 May 6;114(9):1564-1575.e9. doi: 10.1016/j.neuron.2025.12.033. Epub 2026 Feb 12. PMID: 41687612.



Multivalent Modulation of Endothelial LRP1 Induces Fast Neurovascular Amyloid- β Clearance and Cognitive Function Improvement in Alzheimer's Disease Models (2025)

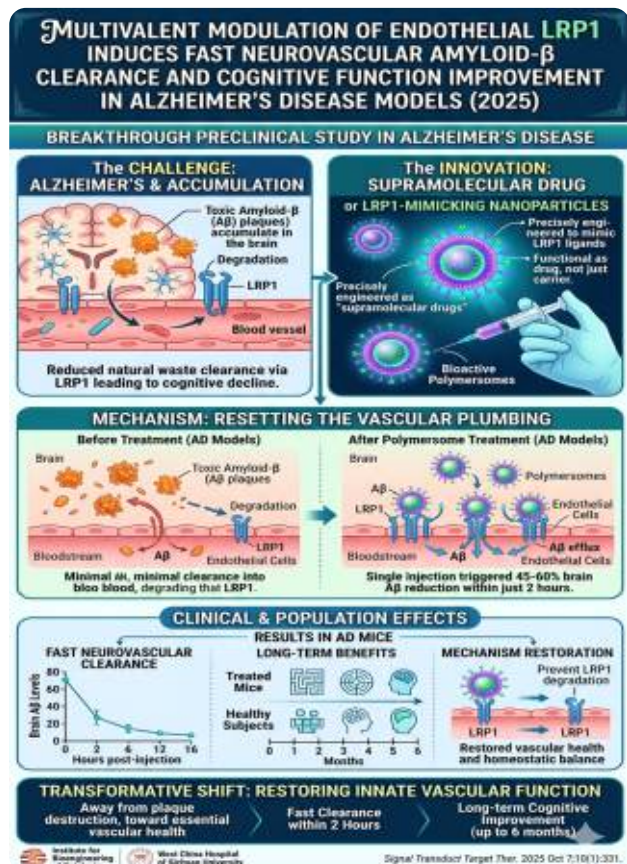
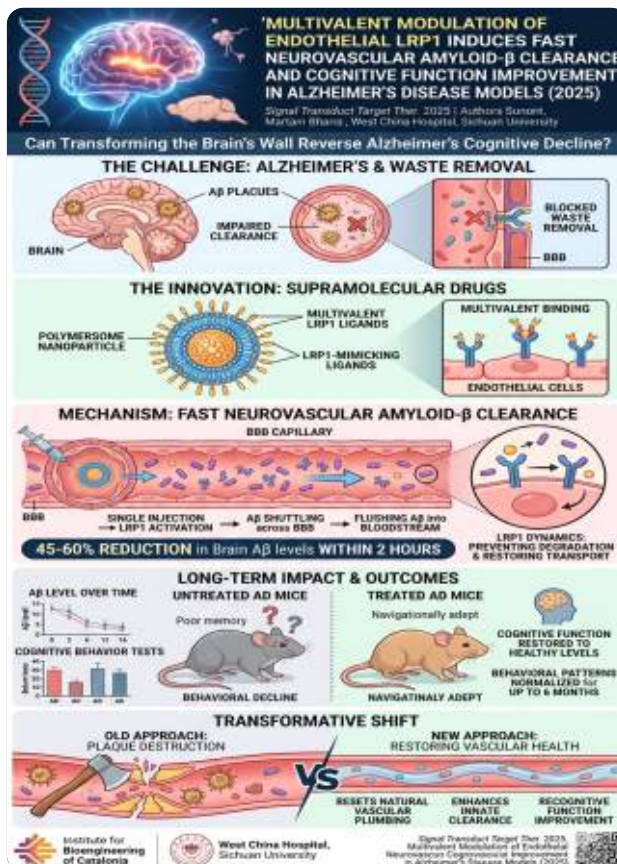
Can transforming the brain's protective wall into a waste-clearance system reverse the cognitive decline of Alzheimer's?

This groundbreaking preclinical study published in *Signal Transduction and Targeted Therapy* (October 2025) was co-led by researchers from the Institute for Bioengineering of Catalonia and West China Hospital of Sichuan University. Utilising a cohort of genetically engineered Alzheimer's model mice, investigators developed bioactive, supramolecular nanoparticles called polymersomes that function as "supramolecular drugs" rather than simple carriers. These nanoparticles are precisely engineered to mimic ligands for the LRP1 receptor, a critical molecular gatekeeper responsible for shuttling toxic waste across the blood-brain barrier. Data indicated that a single injection triggered a 45 to 60 percent reduction in brain amyloid-beta levels within just two hours by flushing toxins directly into the bloodstream. Long-term results were equally striking, as treated mice regained cognitive functions and behavioural patterns comparable to healthy subjects for up to six months. The proposed mechanism involves resetting the natural vascular plumbing, preventing the degradation of LRP1 and restoring the brain's innate ability to clear pathological proteins. These findings represent a transformative shift in neuro-therapeutics, moving away from plaque destruction toward restoring essential vascular health and homeostatic balance.

Source:

 RESEARCH PAPER

 Chen J, Xiang P, Duro-Castano A, Cai H, Guo B, Liu X, et al. Multivalent modulation of endothelial LRP1 induces fast neurovascular amyloid- β clearance and cognitive function improvement in Alzheimer's disease models. *Signal Transduct Target Ther*. 2025 Oct 7;10(1):331. doi: 10.1038/s41392-025-02426-1. PMID: PMC12500928.



Sildenafil Associated with Two-Fold Reduction in Alzheimer's Disease Risk Across Nearly 900,000 Patients (2025)

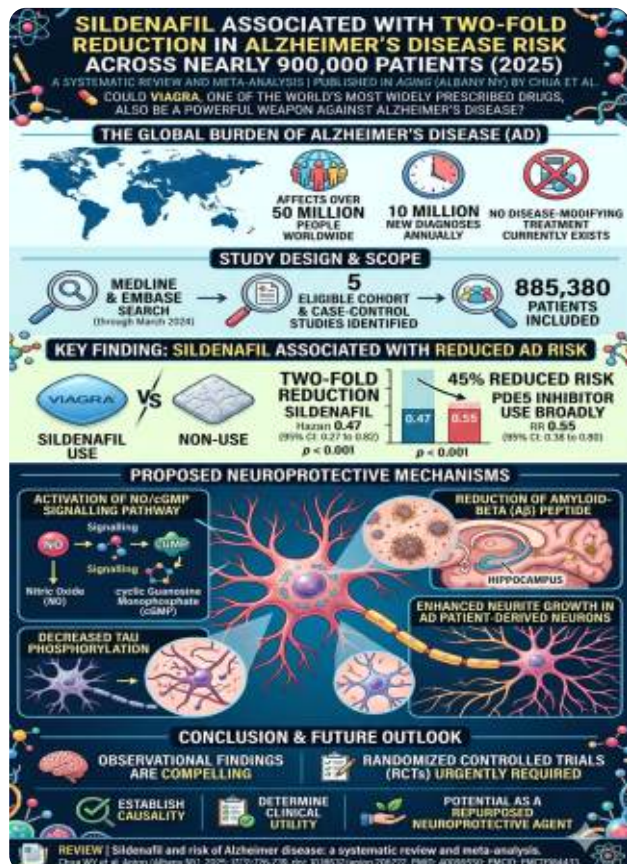
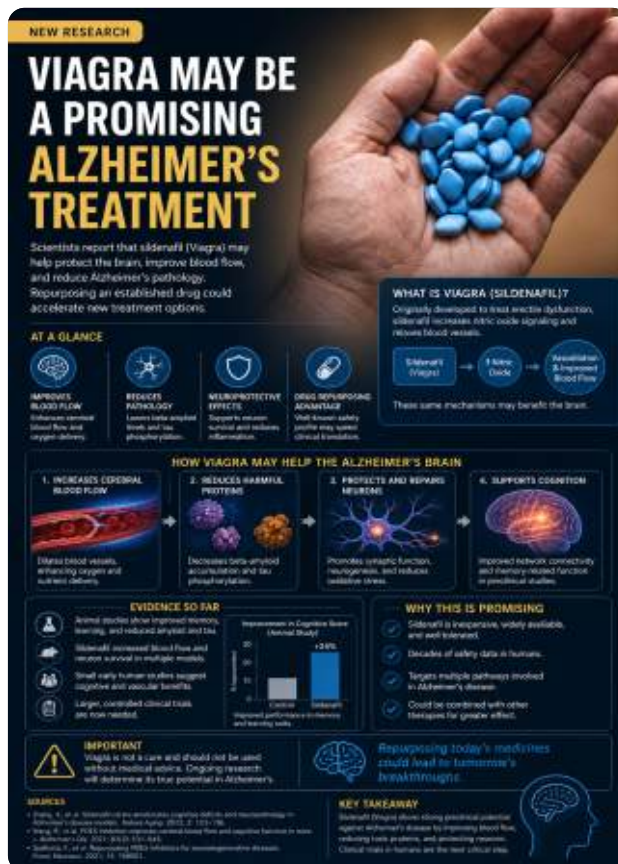
 **Could Viagra, one of the world's most widely prescribed drugs, also be a powerful weapon against Alzheimer's disease?**

Alzheimer's disease affects over 50 million people worldwide, with 10 million new diagnoses annually, yet no disease-modifying treatment currently exists. A systematic review and meta-analysis published in *Aging* (March 2025) by Chua et al. investigated the association between Sildenafil, a phosphodiesterase-5 (PDE5) inhibitor marketed as Viagra and Revatio, and risk of Alzheimer's disease. Searching MEDLINE and Embase through March 2024, researchers identified five eligible cohort and case-control studies comprising 885,380 patients. Sildenafil use was associated with a two-fold reduction in Alzheimer's disease risk compared with non-use (hazard ratio 0.47, 95% CI 0.27 to 0.82, p less than 0.001). PDE5 inhibitor use broadly demonstrated a 45% reduced risk (RR 0.55, 95% CI 0.38 to 0.80). Proposed neuroprotective mechanisms include activation of the nitric oxide/cyclic guanosine monophosphate signalling pathway, reduction of amyloid-beta peptide levels in the hippocampus, decreased tau phosphorylation and enhanced neurite growth in Alzheimer's disease patient-derived neurons. The authors conclude that while observational findings are compelling, randomised controlled trials are urgently required to establish causality and determine sildenafil's clinical utility as a repurposed neuroprotective agent.

Source:

 REVIEW

 Chua WY, Lim LKE, Wang JJD, Mai AS, Chan LL, Tan EK. Sildenafil and risk of Alzheimer disease: a systematic review and meta-analysis. *Aging* (Albany NY). 2025 Mar 17;17(3):726-739. doi: 10.18632/aging.206222. Epub 2025 Mar 17. PMID: 40096550; PMCID: PMC11984433.



Lithium Deficiency as an Early Trigger of Alzheimer's Disease: Evidence from a Harvard-Rush University Study (2025)

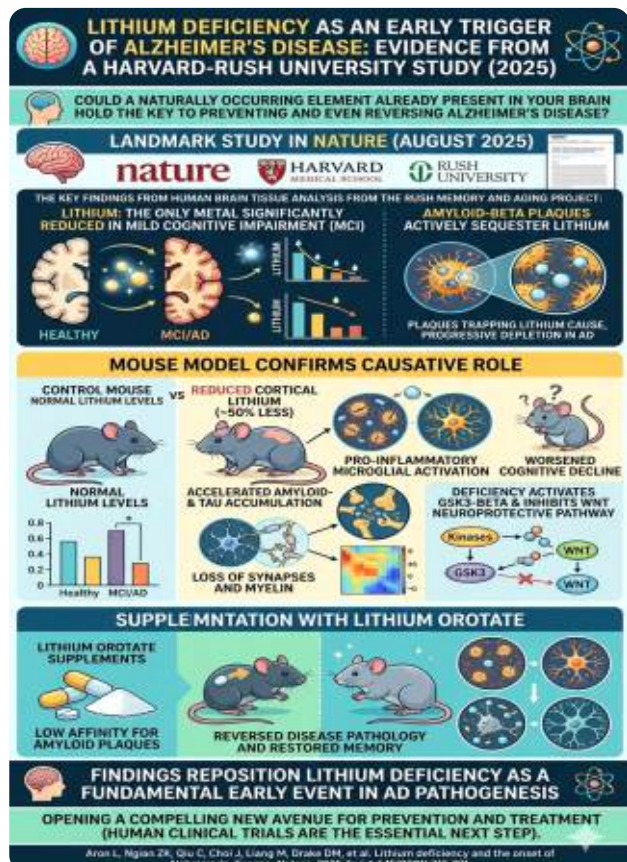
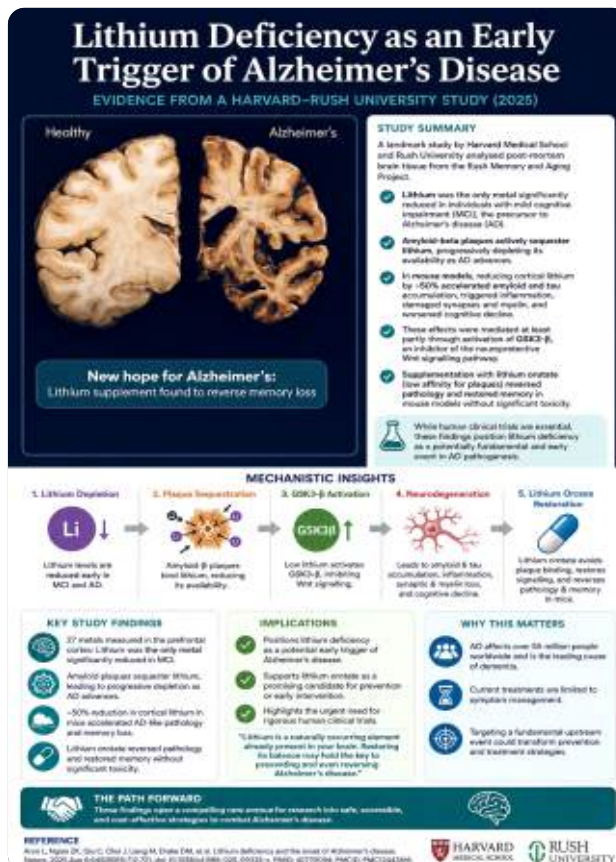
🧠 *Could a naturally occurring element already present in your brain hold the key to preventing and even reversing Alzheimer's disease?*

A landmark study published in *Nature* (August 2025) by researchers at Harvard Medical School and Rush University, Chicago, investigated the role of endogenous lithium in Alzheimer's disease (AD) pathogenesis. Analysing post-mortem brain tissue from the Rush Memory and Aging Project, the team measured levels of 27 metals in the prefrontal cortex and found that lithium was the only metal significantly reduced in individuals with mild cognitive impairment, the recognised precursor to AD. Further analysis revealed that amyloid-beta plaques actively sequester lithium, progressively depleting its availability as the disease advances. In mouse models, reducing cortical lithium by approximately 50% dramatically accelerated amyloid-beta and tau accumulation, triggered pro-inflammatory microglial activation, caused loss of synapses and myelin, and worsened cognitive decline. These effects were mediated at least partly through activation of the kinase GSK3-beta, an inhibitor of the neuroprotective Wnt signalling pathway. Crucially, supplementation with lithium orotate, a lithium salt with low affinity for amyloid plaques, reversed disease pathology and restored memory in mouse models without significant toxicity. While human clinical trials remain the essential next step, these findings reposition lithium deficiency as a potentially fundamental and early event in AD pathogenesis, opening a compelling new avenue for prevention and treatment.

Source:

📄 RESEARCH PAPER

📌 Aron L, Ngian ZK, Qiu C, Choi J, Liang M, Drake DM, et al. Lithium deficiency and the onset of Alzheimer's disease. *Nature*. 2025 Aug 6;645(8081):712-721. doi: 10.1038/s41586-025-09335-x. PMID: 40770094; PMCID: PMC12443616.



Gentle Skin Stimulation on Face and Neck Doubles Brain Waste Clearance (2025)

 **Could a simple mechanical device applied to the skin of the face and neck help prevent Alzheimer's disease?**

Researchers at the Institute for Basic Science (IBS) Center for Vascular Research, South Korea, led by Director Koh Gou Young, conducted a landmark experimental study published in *Nature* (June 2025) using genetically modified mice and non-human primates with fluorescent cerebrospinal fluid (CSF) tracers. The team mapped a previously unrecognized CSF drainage route from the brain's surface through superficial facial lymphatic vessels to submandibular lymph nodes via periorbital, nasal and hard palate pathways. These superficial cervical lymphatics remain functionally intact even in aged animals, unlike deeper drainage pathways which undergo age-related degeneration. Aged mice showed reduced CSF outflow linked to impaired nitric oxide signalling and endothelial nitric oxide synthase deficiency. A custom force-regulated mechanical stimulator applying gentle compressive and stroking motions to intact facial and neck skin for 20 minutes doubled CSF outflow and restored drainage to youthful levels without disrupting natural lymphatic contractions. These findings establish strong proof-of-concept for wearable devices capable of enhancing brain waste clearance, including amyloid-beta and tau protein removal, offering a potential drug-free preventive strategy against Alzheimer's and other neurodegenerative diseases.

Source:

 **RESEARCH PAPER**

 Jin H, Yoon JH, Hong SP, et al. Increased CSF drainage by non-invasive manipulation of cervical lymphatics. *Nature*. 2025 Jul;643(8072):755-767. doi: 10.1038/s41586-025-09052-5. PMID: 40468071.

NEW DISCOVERY

THE FACE HAS A BRAIN DETOX HIGHWAY

MASSAGE COULD HELP TREAT ALZHEIMER'S

Scientists discovered previously unknown facial lymphatic vessels that help drain toxins from the brain. Gentle massage may boost this natural cleaning system — opening a new path to treat neurological diseases like Alzheimer's.

THE BREAKTHROUGH

Using advanced imaging, researchers identified a network of lymphatic vessels along the face and neck that connect directly to the brain's waste drainage system.

HOW IT WORKS: THE FACIAL LYMPHATIC PATHWAY

1. Toxins build up in the brain.
2. Toxins drain into facial lymphatics.
3. Massage stimulates lymph flow.
4. Toxins cleared to lymph nodes.
5. Brain is cleaner. Cells are healthier.

WHY IT MATTERS

- Targets the brain's natural detox system.
- May slow the progression of Alzheimer's and other neurodegenerative diseases.
- Non-invasive, low-cost, and drug-free approach.
- Could complement existing therapies.

THE SCIENCE BEHIND IT

Imaging studies revealed lymphatic vessels in the cheeks and neck that connect to the brain's waste drainage system.

EVIDENCE SO FAR

- Discovered in mice using high-resolution imaging.
- Massage increased lymph flow and reduced amyloid levels.
- Early human studies show improved lymphatic drainage after facial massage.
- Clinical trials for Alzheimer's therapy are the next step.

WHAT THIS COULD MEAN FOR THE FUTURE

- Targeted facial and neck massage techniques could become part of preventive care.
- New devices may boost lymphatic drainage for improved brain health.
- Therapeutic agents or drugs that enhance this effect.
- A promising new frontier in neurodegenerative disease prevention.

MESSAGE MAY DO MORE THAN RELAX — IT MAY HELP PROTECT YOUR BRAIN

A gentle touch today could change the future of neurological care.

GENTLE SKIN STIMULATION ON FACE AND NECK DOUBLES BRAIN WASTE CLEARANCE (2025)

Could a simple mechanical device applied to the skin help prevent Alzheimer's disease?

UNRECOGNIZED CSF DRAINAGE ROUTE MAPPED

- Brain Surface: Fluorescent cerebrospinal fluid (CSF) tracers.
- Superficial Facial Lymphatic Vessels: Periorbital pathway.
- Nasal Pathway: Fluorescent cerebrospinal fluid tracers.
- Hard Palate Pathway: Fluorescent cerebrospinal fluid in secretory lymphatic vessels.
- Submandibular Lymph Nodes.

AGE-RELATED DEGENERATION & DRAINAGE REDUCTION

YOUTHFUL

- Robust Lymphatic Vessel and Function.
- Efficient CSF Outflow.
- Nitric Oxide (NO) Signaling: Active.
- Endothelial nitric oxide functioning.

AGED

- Impaired Lymphatic Vessel and Function.
- Reduced CSF Outflow.
- Impaired Nitric Oxide (NO) Signaling.
- Endothelial nitric oxide deficiency.

MECHANICAL STIMULATION RESTORES BRAIN WASTE CLEARANCE

Gentle Compressive and Stroking Motions

- Doubles CSF Outflow.
- Restores Drainage to Youthful Levels.
- Brain Waste Clearance (e.g., Amyloid-beta, Tau protein Removal).

Custom Force-Regulated Mechanical Stimulator

20 min

Brain Waste Clearance (e.g., Amyloid-beta, Tau protein Removal).

Drug-Free Preventive Strategy Against Alzheimer's & Other Neurodegenerative Diseases

REFERENCE: Jin H, Yoon JH, Hong SP, et al. *Nature*. 2025 Jul;643(8072):755-767. doi: 10.1038/s41586-025-09052-5. PMID: 40468071.

Magnetically Guided Stem Cell Microrobots with Ultrasound Stimulation for Targeted Neuronal Differentiation (2025)

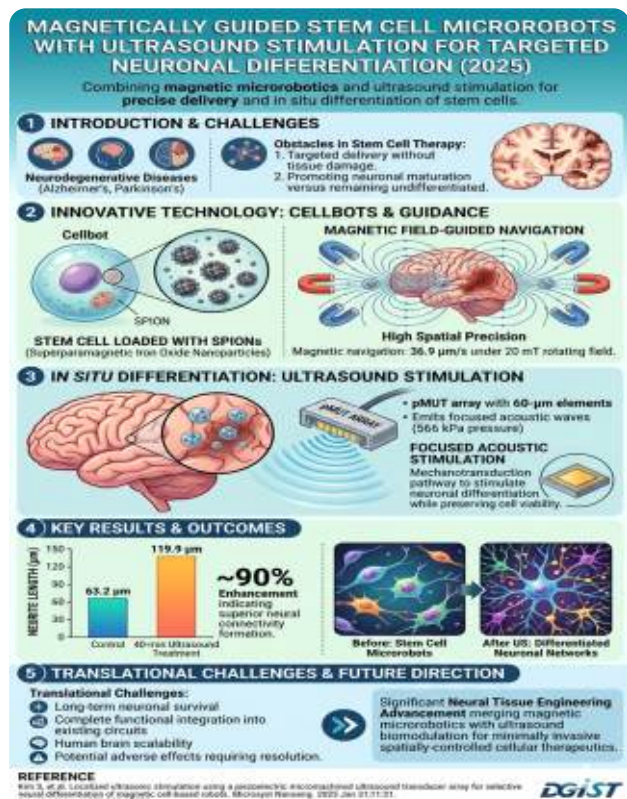
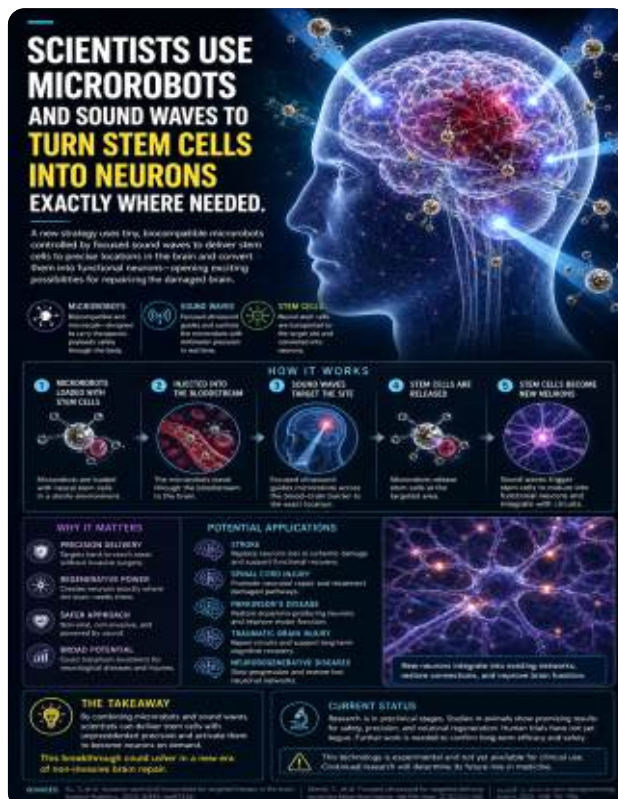
Can combining magnetic microrobotics and ultrasound stimulation enable precise stem cell delivery and in situ neuronal differentiation?

This innovative study published in *Microsystems & Nanoengineering* (January 2025) from the Daegu Gyeongbuk Institute of Science and Technology developed 'Cellbots': stem cells loaded with superparamagnetic iron oxide nanoparticles that enable magnetic field-guided navigation at 36.9 micrometres per second under a 20-millitesla rotating field. This approach delivers cells to damaged brain regions with high spatial precision, addressing two critical stem cell therapy challenges: targeted delivery without tissue damage, and promotion of neuronal maturation rather than leaving cells undifferentiated. Following magnetic positioning, a piezoelectric micromachined ultrasound transducer array with 60-micrometre elements emits focused acoustic waves at 566 kilopascals pressure, stimulating neuronal differentiation through mechanotransduction while preserving cell viability. A 40-minute ultrasound treatment generated neurites measuring 119.9 micrometres in length, compared to 63.2 micrometres in controls, representing an approximately 90% enhancement indicative of superior neural connectivity formation. The technology addresses key obstacles in neurodegenerative disease treatment, including Alzheimer's and Parkinson's diseases, by combining precise cellular delivery with spatiotemporal differentiation control. The researchers acknowledge translational challenges including long-term neuronal survival, functional integration into existing circuits, human brain scalability, and potential adverse effects requiring resolution before clinical application. This study represents a significant neural tissue engineering advance, merging magnetic microrobotics with ultrasound biomodulation for minimally invasive, spatially controlled cellular therapeutics.

Source:

RESEARCH PAPER

Kim S, Kim DI, Yeo HG, Lee G, Kim JY, Choi H. Localised ultrasonic stimulation using a piezoelectric micromachined ultrasound transducer array for selective neural differentiation of magnetic cell-based robots. *Microsyst Nanoeng.* 2025 Mar 20;11(1):52. doi: 10.1038/s41378-025-00900-y. PMID: 40113763; PMCID: PMC11926166.



Non-Hallucinogenic LSD Analogue Promotes Neuroplasticity and Reduces Depressive-like Behaviours (2025)

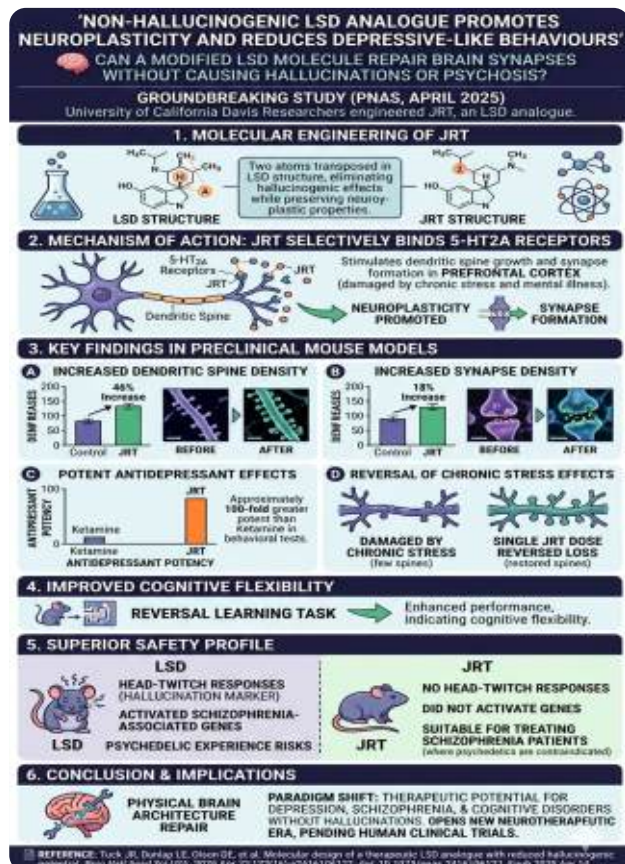
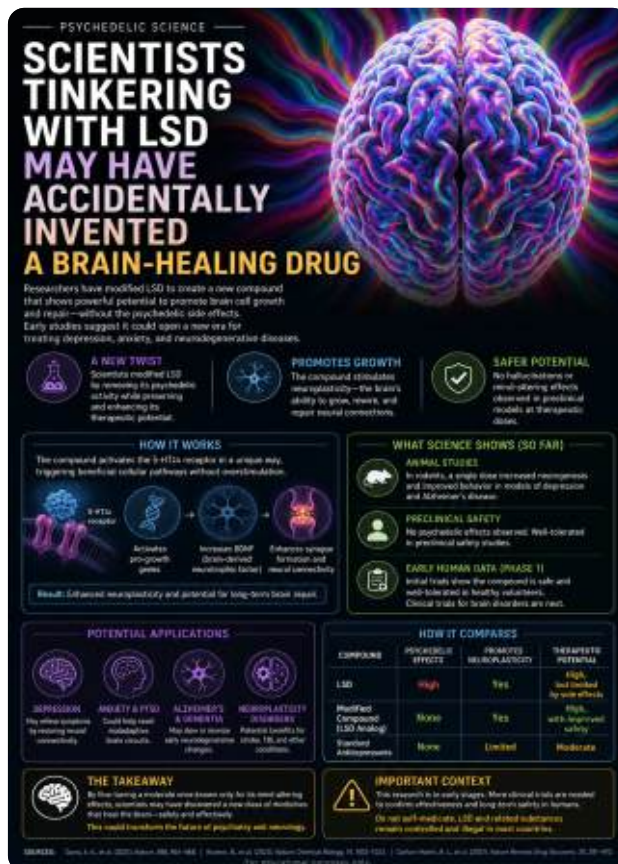
Can a modified LSD molecule repair brain synapses without causing hallucinations or psychosis?

This groundbreaking study published in the *Proceedings of the National Academy of Sciences* (April 2025) engineered JRT, a non-hallucinogenic lysergic acid diethylamide analogue created by transposing two atoms in the LSD structure, eliminating hallucinogenic effects while preserving its neuroplastic properties. Researchers at the University of California, Davis, tested JRT in preclinical mouse models, demonstrating remarkable therapeutic potential. JRT selectively binds serotonin 5-HT_{2A} receptors, stimulating dendritic spine growth and synapse formation in the prefrontal cortex, a brain region damaged by chronic stress and mental illness. Results showed a 46% increase in dendritic spine density, an 18% increase in synapse density, and approximately 100-fold greater antidepressant potency compared to ketamine in standard behavioural tests. A single JRT dose reversed the dendritic spine density loss caused by chronic stress hormone exposure and improved cognitive flexibility in reversal learning tasks. Critically, JRT did not produce head-twitch responses in mice, a well-established hallucination marker, did not activate schizophrenia-associated genes, and demonstrated a safety profile suitable for treating schizophrenia patients for whom traditional psychedelics are contraindicated. The study concludes that JRT represents a paradigm shift, offering physical brain architecture repair without the risks associated with a psychedelic experience, opening a new neurotherapeutic era for depression, schizophrenia, and cognitive disorders pending human clinical trials.

Source:

RESEARCH PAPER

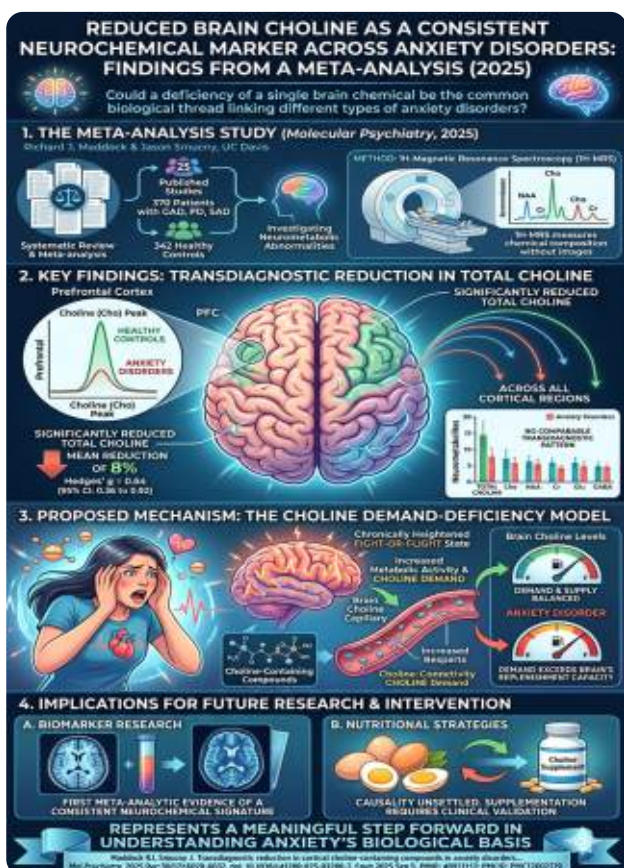
-  Tuck JR, Dunlap LE, Khatib YA, Hatzipantelis CJ, Weiser Novak S, Rahn RM, Davis AR, Mosswood A, Vernier AMM, Fenton EM, Aarrestad IK, Tombari RJ, Carter SJ, Deane Z, Wang Y, Sheridan A, Gonzalez MA, Avanes AA, Powell NA, Chytil M, Engel S, Fettinger JC, Jenkins AR, Carlezon WA Jr, Nord AS, Kangas BD, Rasmussen K, Liston C, Manor U, Olson DE. Molecular design of a therapeutic LSD analogue with reduced hallucinogenic potential. *Proc Natl Acad Sci U S A*. 2025 Apr 22;122(16): e2416106122. doi: 10.1073/pnas.2416106122. Epub 2025 Apr 14. PMID: 40228113; PMCID: PMC12037037.



 Could a deficiency of a single brain chemical be the common biological thread linking different types of anxiety disorders?

Source:

📌 Maddock RJ, Smucny J. Transdiagnostic reduction in cortical choline-containing compounds in anxiety disorders: a 1H-magnetic resonance spectroscopy meta-analysis. *Mol Psychiatry*. 2025 Dec;30(12):6020-6032. doi: 10.1038/s41380-025-03206-7. Epub 2025 Sep 5. PMID: 40913113; PMCID: PMC12602319.



Could Oral Microbiota Transmission Influence Depression and Anxiety in Newlywed Couples? (2025)

 **Could the billions of bacteria you share with your partner be quietly reshaping your mental health?**

A cross-sectional study published in *Exploratory Research and Hypothesis in Medicine* (2025) investigated whether oral microbiota transmission between married couples plays a role in the spread of depression and anxiety symptoms. Conducted across multiple centres in Iran, the study enrolled 1,740 newlywed couples who had been married for six months, comparing 268 healthy spouses living with a partner diagnosed with the depression-anxiety phenotype against 268 healthy control spouses. Using validated tools including the Beck Depression Inventory-II, Beck Anxiety Inventory, and Pittsburgh Sleep Quality Index, researchers found that previously healthy partners showed significantly higher scores for depression, anxiety, and sleep disturbance after six months of cohabitation. Salivary cortisol levels were also elevated, indicating measurable physiological stress activation. The proposed mechanism centres on the continuous exchange of oral bacteria through kissing and shared meals, gradually shifting the healthy partner's oral microbiome toward the dysbiotic profile of the affected spouse. While the study is associational and causality remains to be established, these findings open a compelling new dimension in understanding mental health within intimate relationships, with potential implications for couple-based preventive psychiatry.

Source:

 REVIEW

 Rastmanesh R, Vellingiri B, Isacco CG, Sadeghinejad A, Daghnall N. Oral Microbiota Transmission Partially Mediates Depression and Anxiety in Newlywed Couples. *Explor Res Hypothesis Med*. 2025;10(2):77-86. doi: 10.14218/ERHM.2025.00013.



Sugary Soft Drinks Linked to Depression through Gut Microbiome Disruption: Evidence from a German Multicentre Cohort Study (2025)

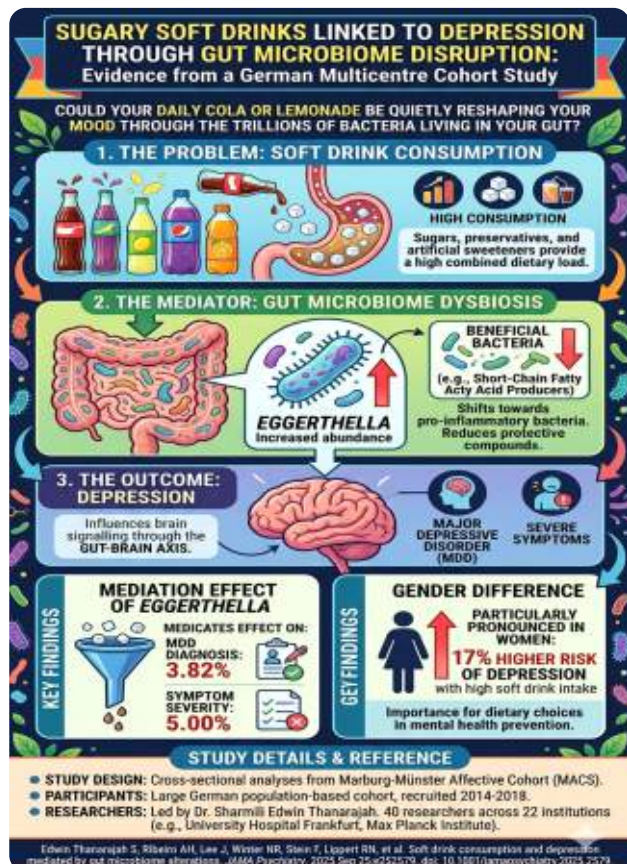
 **Could your daily cola or lemonade be quietly reshaping your mood through the trillions of bacteria living in your gut?**

A multicentre cohort study published in *JAMA Psychiatry* (September 2025) investigated whether soft drink consumption is associated with major depressive disorder (MDD) and whether this relationship is mediated by gut microbiome alterations. Led by Dr. Sharmili Edwin Thanarajah from University Hospital Frankfurt and the Max Planck Institute for Metabolism Research, Cologne, the study analysed cross-sectional data from the Marburg-Münster Affective Cohort (MACS), a large German population-based cohort recruited between 2014 and 2018, involving 40 researchers across 22 institutions. Findings revealed that high consumption of sugary soft drinks was significantly associated with both a greater likelihood of MDD diagnosis and more severe depressive symptoms. Crucially, this association was mediated by increased abundance of the gut bacterium *Eggerthella*, which accounted for 3.82% of the effect on MDD diagnosis and 5.00% on symptom severity. The effect was particularly pronounced in women, who showed a 17% higher risk of depression with high soft drink intake. The proposed mechanism involves disruption of the intestinal microbiome by the combined load of sugars, preservatives, and artificial sweeteners in soft drinks, favouring pro-inflammatory bacteria while reducing protective short-chain fatty acid production, ultimately influencing brain signalling through the gut-brain axis. These findings underscore the importance of dietary choices in mental health prevention, particularly among women.

Source:

COHORT STUDY

 Edwin Thanarajah S, Ribeiro AH, Lee J, Winter NR, Stein F, Lippert RN, et al. Soft Drink Consumption and Depression Mediated by Gut Microbiome Alterations. *JAMA Psychiatry*. 2025 Nov 1;82(11):1095-1102. doi: 10.1001/jamapsychiatry.2025.2579. PMID: 40991280; PMCID: PMC12461599.



Vertebrate Lonesome Kinase (VLK): A Hidden Extracellular Switch Controlling Pain and Synaptic Plasticity (2025)

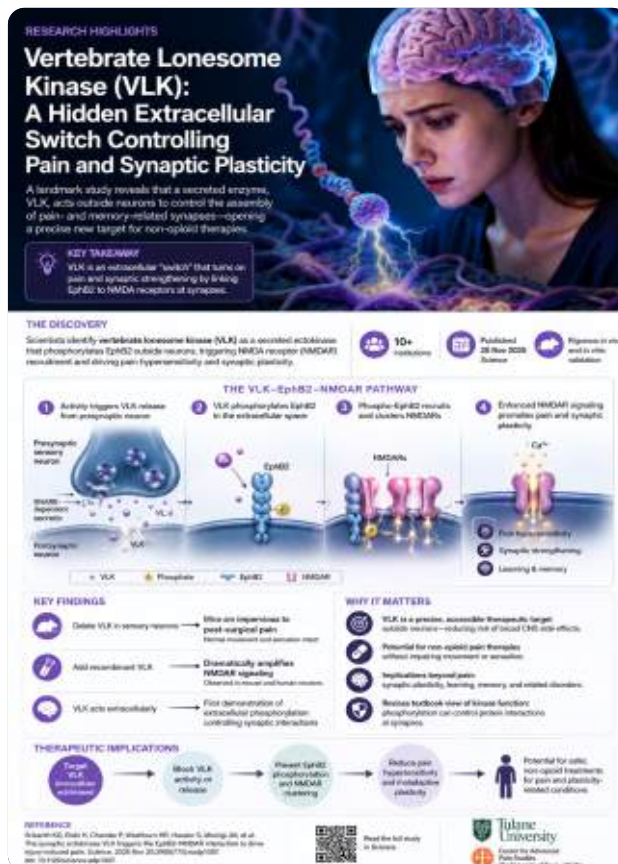
 **Could a secreted enzyme working outside nerve cells hold the key to treating pain without the side effects that have long plagued NMDA receptor-targeted therapies?**

A landmark study published in *Science* (November 2025) by researchers from the Tulane Brain Institute, Tulane University, and the Centre for Advanced Pain Studies, University of Texas at Dallas, alongside colleagues from eight other institutions, has identified a previously unrecognised extracellular signalling mechanism governing injury-induced pain and synaptic strengthening. The study focused on vertebrate lonesome kinase (VLK), an Ectokinase secreted from presynaptic sensory neurons into the synaptic cleft in a SNARE-dependent manner following neuronal activity. Once released, VLK phosphorylates the extracellular domain of ephrin type-B receptor 2 (EphB2) on postsynaptic neurons, triggering EphB2 to recruit and cluster N-methyl-D-aspartate receptors (NMDARs), the central mediators of synaptic plasticity, learning, and memory. Conditional deletion of VLK from presynaptic sensory neurons rendered mice impervious to post-surgical pain hypersensitivity, while leaving movement and sensory abilities entirely intact. Conversely, addition of recombinant VLK dramatically amplified NMDAR-dependent signalling in both mouse and human neurons. These findings represent one of the first demonstrations that extracellular phosphorylation governs protein-protein interactions at synapses, fundamentally revising the textbook understanding of kinase activity and revealing VLK as a precise, accessible therapeutic target for non-opioid pain management and disorders of synaptic plasticity.

Source:

 RESEARCH PAPER

 Srikanth KD, Elahi H, Chander P, Washburn HR, Hassler S, Mwirigi JM, et al. The synaptic ectokinase VLK triggers the EphB2-NMDAR interaction to drive injury-induced pain. *Science*. 2025 Nov 20;390(6775): eadp1007. doi: 10.1126/science.adp1007. Epub 2025 Nov 20. PMID: 41264708.



The SIK3-N783Y Mutation is Associated with Human Natural Short Sleep Trait: Genetic Basis for Efficient Sleep (2025)

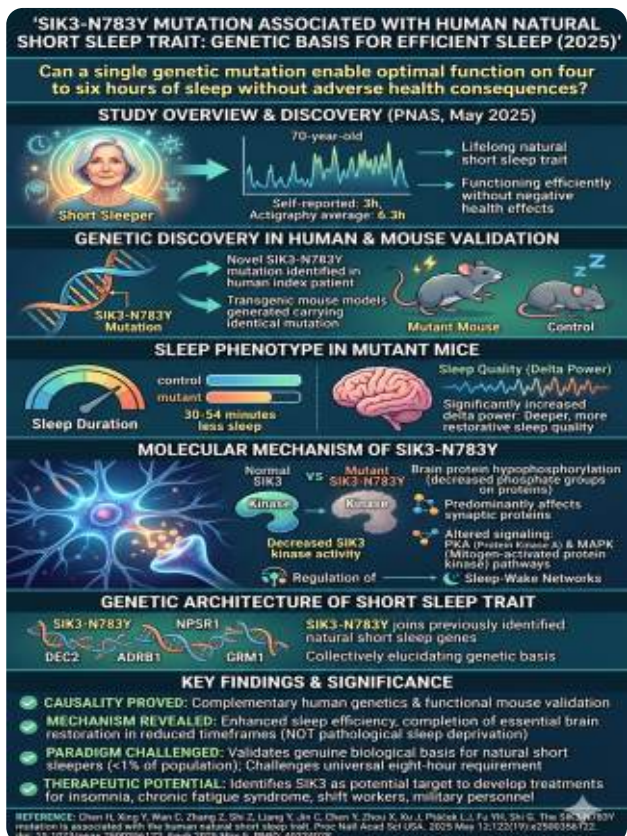
 **Can a single genetic mutation enable individuals to function optimally on just four to six hours of sleep without adverse health consequences?**

This groundbreaking study published in *Proceedings of the National Academy of Sciences* (May 2025) identified novel SIK3-N783Y mutation in 70-year-old woman with lifelong natural short sleep trait, averaging 6.3 hours nightly via actigraphy while self-reporting three hours, functioning efficiently without negative health effects. Researchers generated transgenic mouse models carrying identical mutation, demonstrating mutant mice required 30-54 minutes less sleep than controls while exhibiting significantly increased delta power indicating deeper, more restorative sleep quality. Molecular analysis revealed decreased SIK3 kinase activity causing extensive brain protein hypophosphorylation, predominantly affecting synaptic proteins and altering protein kinase A and mitogen-activated protein kinase pathways regulating sleep-wake networks. SIK3-N783Y joins previously identified natural short sleep genes including DEC2, ADRB1, NPSR1, and GRM1, collectively elucidating genetic architecture underlying individual sleep requirements. Study definitively proved causality through complementary human genetic discovery and functional mouse validation, demonstrating mutation enables completion of essential brain restorative processes in substantially reduced timeframes through enhanced sleep efficiency rather than pathological sleep deprivation. Findings challenge universal eight-hour sleep requirement paradigm, validate genuine biological basis for rare natural short sleepers comprising less than one percent of population, and identify SIK3 as potential therapeutic target for developing treatments improving sleep efficiency in insomnia, chronic fatigue syndrome, and populations requiring optimised rest including shift workers and military personnel.

Source:

 RESEARCH PAPER

 Chen H, Xing Y, Wan C, Zhang Z, Shi Z, Liang Y, Jin C, Chen Y, Zhou X, Xu J, Ptáček LJ, Fu YH, Shi G. The SIK3-N783Y mutation is associated with the human natural short sleep trait. *Proc Natl Acad Sci USA*. 2025 May 13;122(19): e2500356122. doi: 10.1073/pnas.2500356122. Epub 2025 May 5. PMID: 40324078.



DNA Polymerase Beta Suppresses Somatic Indels at CpG Dinucleotides in Developing Cortical Neurons (2025)

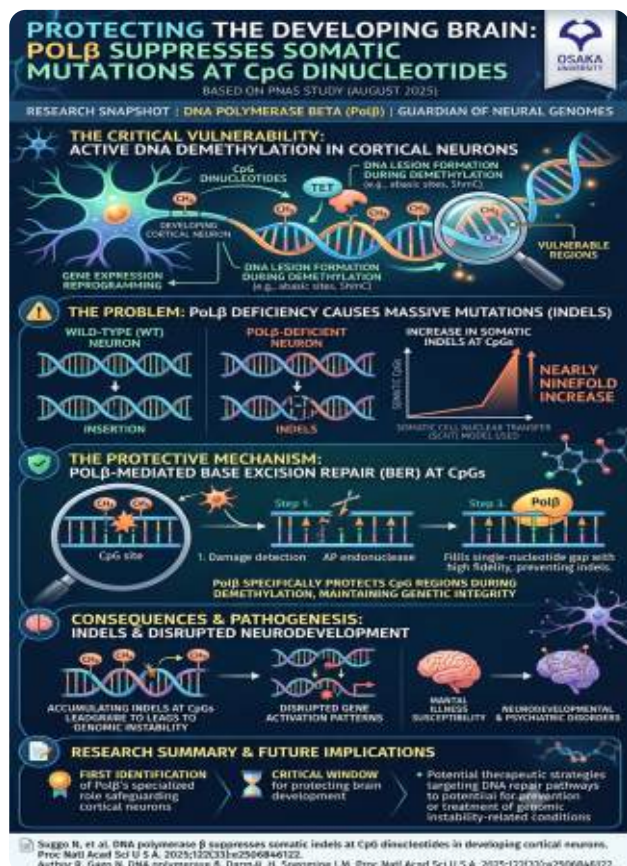
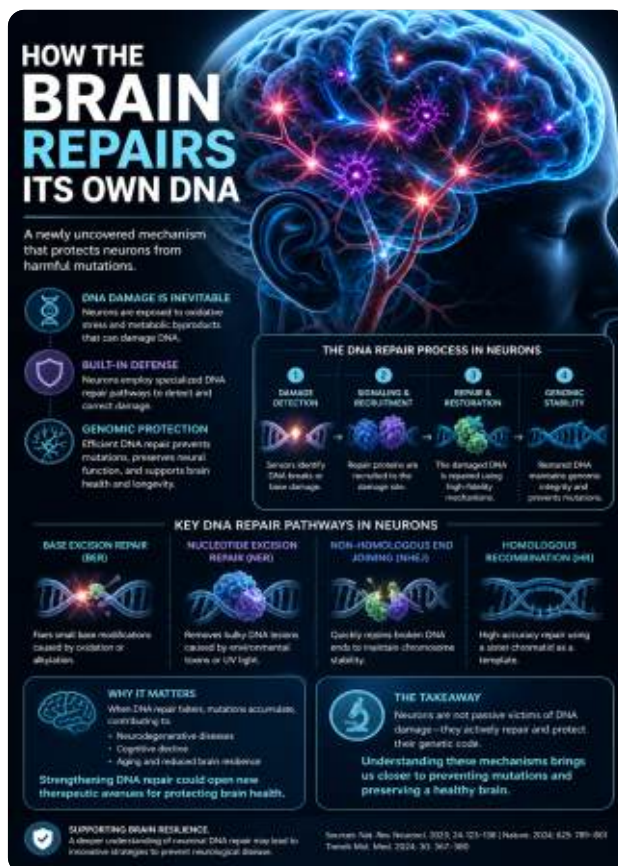
 **Can DNA repair enzyme deficiency explain neurodevelopmental disorder susceptibility through genomic instability?**

This study published in *Proceedings of the National Academy of Sciences* (August 2025) from Osaka University identified DNA polymerase beta (Pol β) as critical guardian preventing harmful mutations during early brain development. Researchers employed somatic cell nuclear transfer reprogramming methodology examining cortical neurons, demonstrating Pol β -deficient neural cells exhibited nearly ninefold increase in insertion-deletion (indel) mutations near CpG dinucleotides, genomic regions essential for gene expression regulation through DNA methylation. CpG sites experience particular vulnerability during active DNA demethylation, epigenetic process reshaping gene activity patterns in developing brain; unrepaired DNA damage at these locations destabilises genome integrity, potentially disrupting normal neurodevelopment. Pol β performs base excision repair specifically protecting CpG regions during demethylation, maintaining genetic fidelity and preventing mutagenesis critical for proper neuronal gene activation. Failure of this protective mechanism when DNA methylation marks are erased can produce harmful genomic changes leading to mental illness. Study represents first identification of Pol β 's specialised role safeguarding developing cortical neurons from demethylation-associated somatic mutations. Findings suggest Pol β dysfunction may contribute to psychiatric and neurodevelopmental disorders linked to aberrant brain formation, potentially informing therapeutic strategies targeting DNA repair pathways for preventing or treating conditions associated with genomic instability during critical neurodevelopmental windows.

Source:

RESEARCH PAPER

 Sugo N, Uchimura A, Matsumoto R, Nakayama H, Fujimoto S, Mizuno S, Higuchi M, Toshishige M, Satoh Y, Wakayama S, Wakayama T, Yagi T. DNA polymerase β suppresses somatic indels at CpG dinucleotides in developing cortical neurons. *Proc Natl Acad Sci U S A*. 2025 Aug 19;122(33): e2506846122. doi: 10.1073/pnas.2506846122. Epub 2025 Aug 13. PMID: 40802685; PMCID: PMC12377747.



The Same Genes that Gave Humans Superior Intelligence Also Increased the Risk of Autism and Schizophrenia (2025)

 *Could the evolutionary price of human intelligence be a lifelong vulnerability to neurodevelopmental conditions?*

What made the human brain uniquely powerful may also be what made it uniquely vulnerable. A landmark single-nucleus RNA sequencing study published in *Molecular Biology and Evolution* (September 2025) by Alexander Starr and Professor Hunter Fraser of Stanford University analysed gene expression patterns across more than one million neurons from six mammalian species in three independent neocortical datasets. The researchers discovered a consistent principle: more abundant neuronal subtypes show greater evolutionary conservation across species. One striking exception emerged exclusively in humans: layer 2/3 intratelencephalic excitatory (L2/3 IT) neurons, responsible for cortical communication and underpinning language, reasoning and abstract thought, evolved exceptionally rapidly in the human lineage compared with other primates. This accelerated evolution was accompanied by dramatic downregulation of approximately 100 autism-associated protective genes, driven by polygenic positive selection unique to humans. Genes protecting against schizophrenia were similarly reduced. These findings suggest the same selective forces conferring advanced human cognition simultaneously increased susceptibility to neurodevelopmental conditions, reframing autism and schizophrenia not as evolutionary errors but as inherent consequences of the genetic architecture underlying human intelligence.

Source:

 RESEARCH PAPER

 Starr AL, Fraser HB. A General Principle of Neuronal Evolution Reveals a Human-Accelerated Neuron Type Potentially Underlying the High Prevalence of Autism in Humans. *Mol Biol Evol.* 2025 Sep 1;42(9):msaf189. doi: 10.1093/molbev/msaf189. PMID: 40907986; PMCID: PMC12410989.



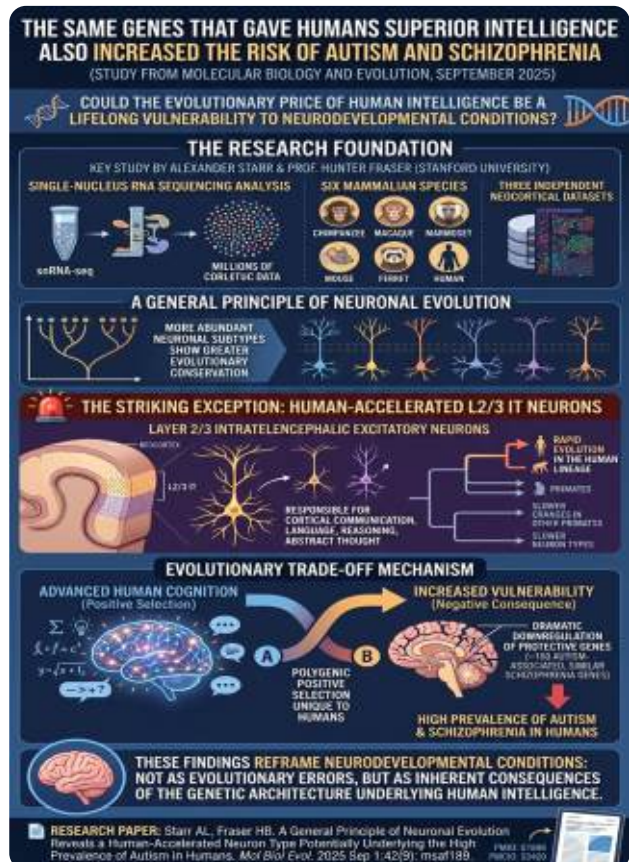
NEW STUDY TRACES THE ORIGINS OF AUTISM TO THE RISE OF HUMAN INTELLIGENCE

Researchers have uncovered genetic evidence that the evolutionary changes supporting higher cognitive abilities may also have increased the risk of Autism Spectrum Disorder (ASD).

THE STUDY FOUND:

- SHARED GENETIC ROOTS**
Genes linked to brain development and cognition also influence ASD risk.
- EVOLUTIONARY TRADE-OFF**
The same changes that boost intelligence may carry a higher risk of neurodevelopmental differences.
- A NEW PERSPECTIVE**
Understanding this connection could lead to better support and earlier interventions.

EVOLUTION SHAPES US. SCIENCE HELPS US UNDERSTAND.



THE SAME GENES THAT GAVE HUMANS SUPERIOR INTELLIGENCE ALSO INCREASED THE RISK OF AUTISM AND SCHIZOPHRENIA
(STUDY FROM MOLECULAR BIOLOGY AND EVOLUTION, SEPTEMBER 2025)

COULD THE EVOLUTIONARY PRICE OF HUMAN INTELLIGENCE BE A LIFELONG VULNERABILITY TO NEURODEVELOPMENTAL CONDITIONS?

THE RESEARCH FOUNDATION
KEY STUDY BY ALEXANDER STARR & PROF. HUNTER FRASER (STANFORD UNIVERSITY)

- SINGLE-NUCLEUS RNA SEQUENCING ANALYSIS**
scRNA-seq → MILLIONS OF CELLULAR DATA
- SIX MAMMALIAN SPECIES**
CHIMPANZEE, MACAQUE, MARMOSSET, MOUSE, PIG, HUMAN
- THREE INDEPENDENT NEOCORTICAL DATASETS**

A GENERAL PRINCIPLE OF NEURONAL EVOLUTION
MORE ABUNDANT NEURONAL SUBTYPES SHOW GREATER EVOLUTIONARY CONSERVATION

THE STRIKING EXCEPTION: HUMAN-ACCELERATED L2/3 IT NEURONS
LAYER 2/3 INTRATELENCEPHALIC EXCITATORY NEURONS
RESPONSIBLE FOR CORTICAL COMMUNICATION, LANGUAGE, REASONING, ABSTRACT THOUGHT
RAPID EVOLUTION IN THE HUMAN LINEAGE
SLOWER CHANGES IN OTHER PRIMATES
SLOWER NEURON TYPES

EVOLUTIONARY TRADE-OFF MECHANISM
ADVANCED HUMAN COGNITION (Positive Selection) → INCREASED VULNERABILITY (Negative Consequence)
POLYGENIC POSITIVE SELECTION UNIQUE TO HUMANS → DRAMATIC DOWNREGULATION OF PROTECTIVE GENES (100+ AUTISM-ASSOCIATED, SIMILAR SCHIZOPHRENIA GENES)
HIGH PREVALENCE OF AUTISM & SCHIZOPHRENIA IN HUMANS

THESE FINDINGS REFRAME NEURODEVELOPMENTAL CONDITIONS: NOT AS EVOLUTIONARY ERRORS, BUT AS INHERENT CONSEQUENCES OF THE GENETIC ARCHITECTURE UNDERLYING HUMAN INTELLIGENCE.

RESEARCH PAPER: Starr AL, Fraser HB. A General Principle of Neuronal Evolution Reveals a Human-Accelerated Neuron Type Potentially Underlying the High Prevalence of Autism in Humans. *Mol Biol Evol.* 2025 Sep 1;42(9):msaf189. PMID: 40907986; PMCID: PMC12410989

Molecular Basis of Music-Induced Neuroplasticity in Humans: Systematic Review (2025)

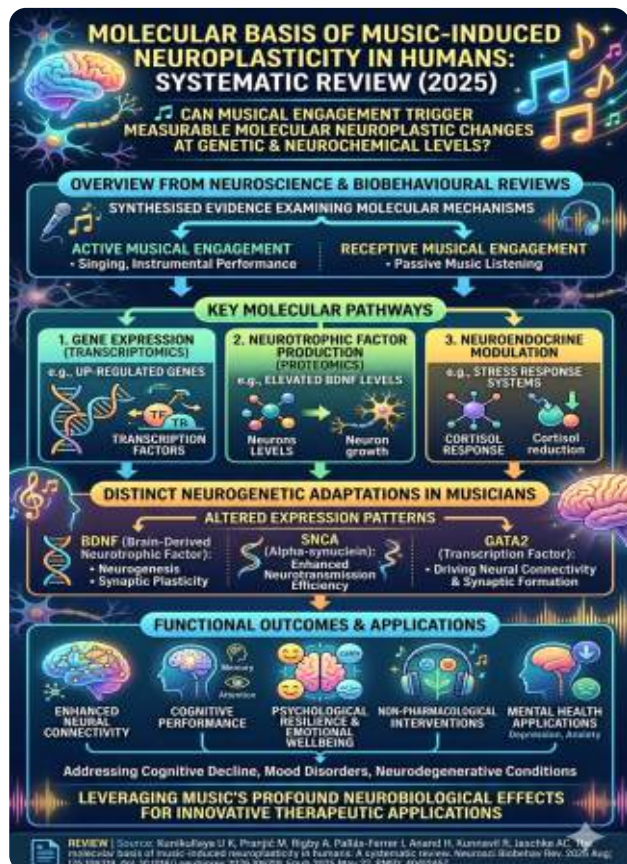
 **Can musical engagement trigger measurable molecular neuroplastic changes at genetic and neurochemical levels?**

This systematic review published in *Neuroscience & Biobehavioural Reviews* (January 2025) synthesised evidence examining molecular mechanisms underlying music-induced neuroplasticity across music-based interventions and long-term musical training. Active and receptive musical engagement enhances neuroplasticity through documented alterations in gene expression, neurotrophic factor production, and neuroendocrine activity. Passive music listening upregulates genes associated with neuroprotection and synaptic plasticity, while active participation including singing and instrumental performance consistently elevates peripheral neurotrophic factors, particularly brain-derived neurotrophic factor, in both healthy and clinical populations. Musicians demonstrate striking neurogenetic adaptations: key genes including BDNF, SNCA (alpha-synuclein), and GATA2 (transcription factor) exhibit altered expression patterns, driving enhanced neurogenesis, neurotransmission efficiency, and cognitive performance. These molecular adaptations correlate with increased neural connectivity, psychological resilience, and emotional wellbeing. Music therapy and musical practice modulate stress response systems supporting brain health. Review identifies music's potential as non-pharmacological intervention for neuropsychological disorders, cognitive enhancement, and mental health applications. Field requires deeper mechanistic understanding of how musical experiences orchestrate molecular neuroplastic shifts, promising innovative therapeutic applications leveraging music's profound neurobiological effects addressing cognitive decline, mood disorders, neurodegenerative conditions.

Source:

 REVIEW

 Kunikullaya U K, Pranjic M, Rigby A, Pallás-Ferrer I, Anand H, Kunnavil R, Jaschke AC. The molecular basis of music-induced neuroplasticity in humans: A systematic review. *Neurosci Biobehav Rev*. 2025 Aug; 175:106219. doi: 10.1016/j.neubiorev.2025.106219. Epub 2025 May 22. PMID: 40412457.



Benefits of Music Training for Learning to Read: Evidence from Cortical Tracking of Speech in Children (2025)

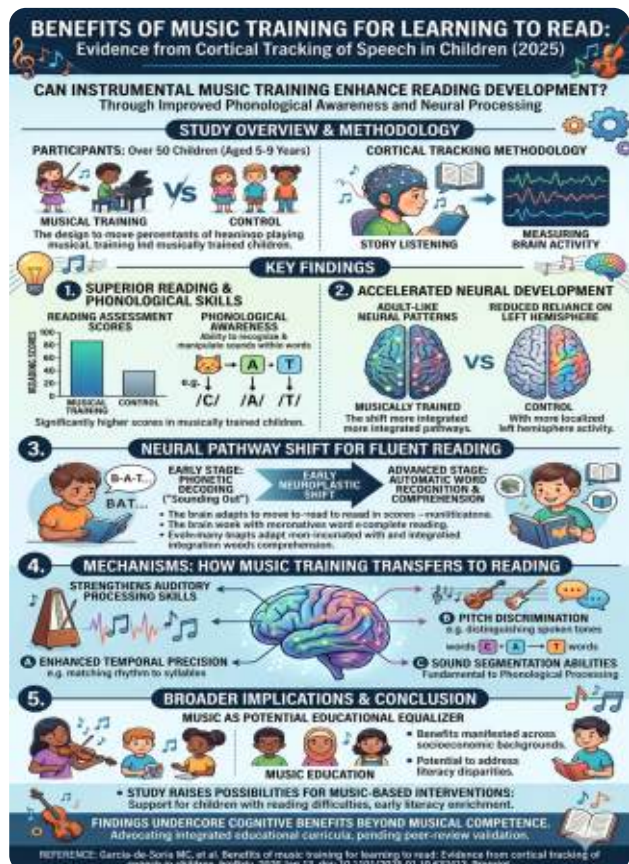
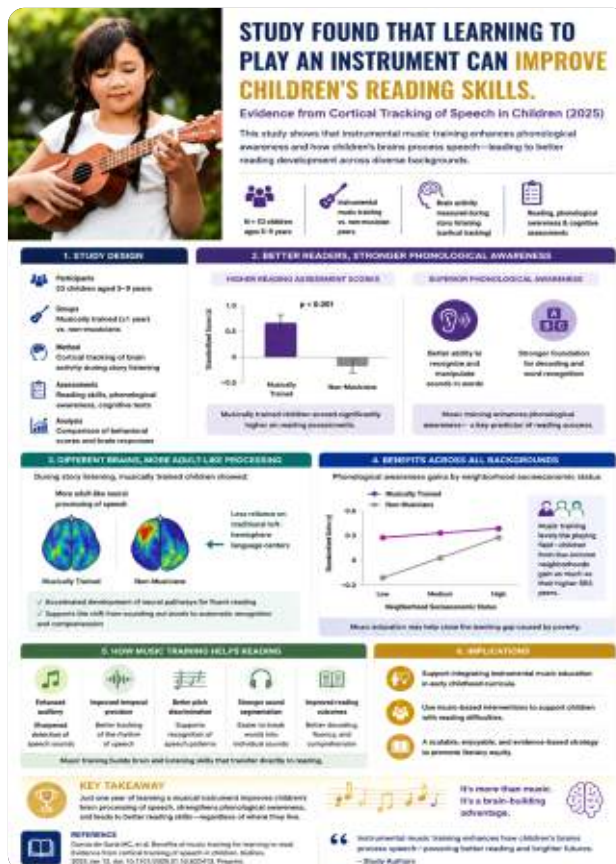
Can instrumental music training enhance reading development through improved phonological awareness and neural processing?

This study published on *bioRxiv* (January 2025) investigated music training's effects on reading acquisition in over 50 children aged 5-9 years. Musically trained children demonstrated significantly higher reading assessment scores and superior phonological awareness - ability to recognise and manipulate constituent sounds within words - compared to controls. Researchers employed cortical tracking methodology measuring brain activity during story listening, revealing musically trained children processed speech using more adult-like neural patterns with reduced reliance on traditional left-hemisphere language centres, suggesting accelerated development of neural pathways supporting fluent reading. This early neuroplastic shift enables progression from phonetic decoding ("sounding out") to automatic word recognition and comprehension. Critically, music training benefits manifested across diverse socioeconomic backgrounds, positioning music education as potential educational equaliser addressing literacy disparities. Findings suggest instrumental training strengthens auditory processing skills directly transferable to reading through enhanced temporal precision, pitch discrimination, and sound segmentation abilities fundamental to phonological processing. Study raises possibilities for music-based interventions supporting children experiencing reading difficulties or enriching early literacy development universally. Research underscores music education's cognitive benefits extending beyond musical competence, advocating integration into educational curricula for comprehensive developmental support, pending peer-review validation.

Source:

RESEARCH PAPER

 Maria C. Garcia-de-Soria, Brian Mathias, Anne Keitel, Anastasia Klimovich-Gray. Benefits of music training for learning to read: Evidence from cortical tracking of speech in children. *bioRxiv*. 2025 *bioRxiv* 2025.09.05.674218; doi: <https://doi.org/10.1101/2025.09.05.674218>.



Musical Neurodynamics: Neural Resonance Theory Explains Brain-Music Synchronisation and Universal Groove Response (2025)

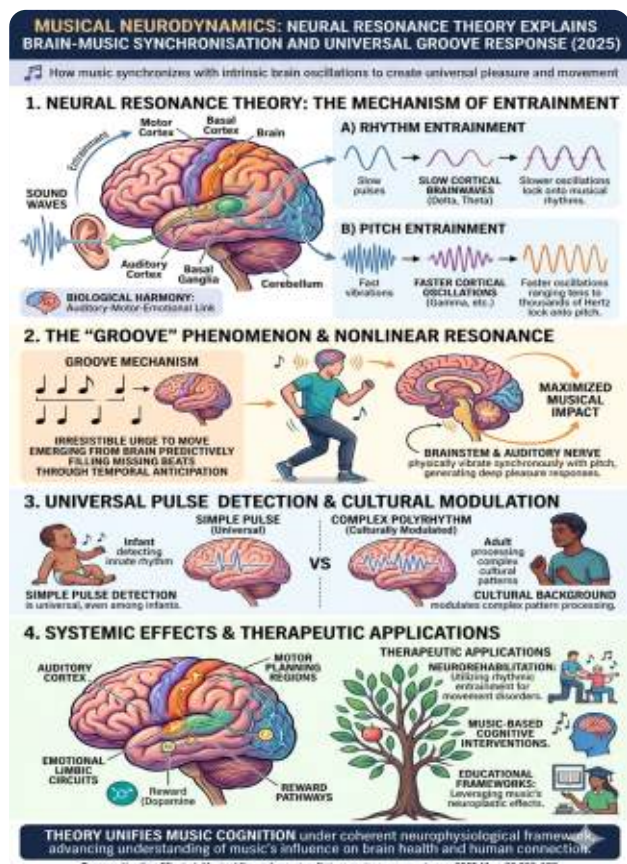
 *How does music synchronise with intrinsic brain oscillations to create universal pleasure and movement responses?*

This comprehensive review published in *Nature Reviews Neuroscience* (May 2025) presents Neural Resonance Theory explaining music perception through brain oscillation synchronisation mechanisms. Researchers demonstrated music's rhythms and pitches directly entrain neural oscillations: slower cortical brainwaves (delta, theta bands) synchronise with musical rhythms while faster oscillations ranging tens to thousands of Hertz lock onto pitch frequencies, creating biological harmony linking auditory processing with motor control and emotional responses. "Groove" phenomenon - irresistible urge to move with rhythm - emerges from nonlinear resonance where brain predictively fills missing beats maximising musical impact through temporal anticipation. Brainstem and auditory nerve structures physically vibrate synchronously with pitch, generating deep cross-cultural pleasure responses. Cultural background modulates complex polyrhythmic pattern processing; however, simple pulse detection remains universal even among infants, indicating innate rhythmic processing capabilities. Neural resonance mechanisms extend beyond auditory cortex, engaging motor planning regions, emotional limbic circuits, and reward pathways. Findings offer therapeutic applications including neurorehabilitation utilising rhythmic entrainment for movement disorders, music-based cognitive interventions, and educational frameworks leveraging music's neuroplastic effects. Theory unifies music cognition phenomena under coherent neurophysiological framework, advancing understanding of music's influence on brain health and human connection.

Source:

 REVIEW

 Harding EE, Kim JC, Demos AP, Roman IR, Tichko P, Palmer C, Large EW. Musical neurodynamics. *Nat Rev Neurosci*. 2025 May;26(5):293-307. doi: 10.1038/s41583-025-00915-4. Epub 2025 Mar 18. PMID: 40102614.



Acoustic Modulation of Mechanosensitive Genes and Adipocyte Differentiation through Audible Sound Wave Exposure (2025)

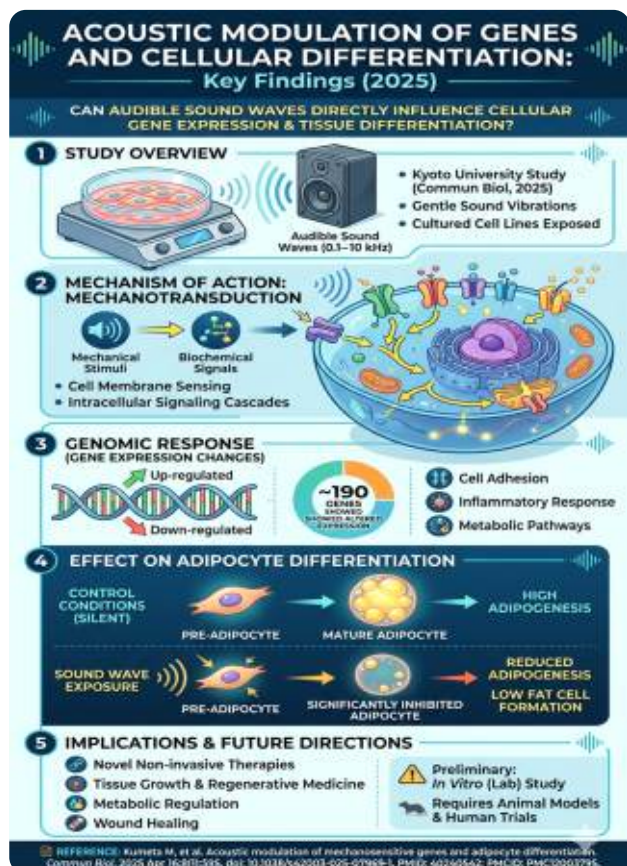
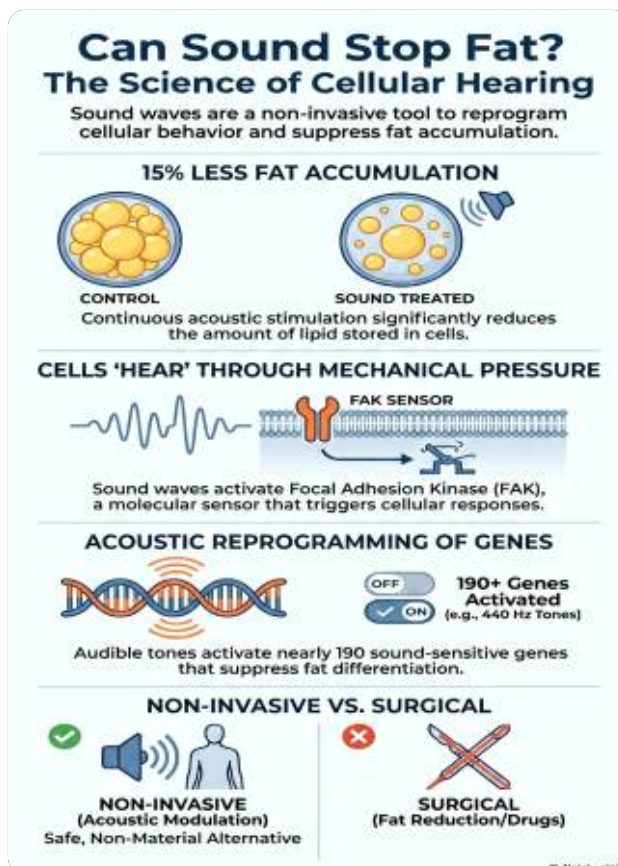
 **Can audible sound waves directly influence cellular gene expression and tissue differentiation?**

This groundbreaking study published in *Communications Biology* (April 2025) from Kyoto University demonstrated audible sound vibrations (0.1-10 kilohertz frequency range) modulate gene activity and cellular differentiation processes in cultured cells through mechanotransduction mechanisms converting mechanical stimuli into biochemical signals. Researchers exposed cultured cell lines to gentle sound waves within normal human hearing range, revealing approximately 190 genes exhibited altered expression patterns without chemical interventions, including genes regulating cell adhesion, inflammatory responses, and metabolic pathways. Critically, sound exposure significantly inhibited adipocyte differentiation, with cells destined to develop into fat cells demonstrating substantially reduced adipogenesis following acoustic stimulation compared to silent control conditions. Findings suggest mechanical pressure from sound waves triggers intracellular signalling cascades influencing fundamental biological processes at genetic level. While investigation remains preliminary utilising in vitro laboratory-cultured cell models requiring extensive validation through animal models and human trials, research opens novel possibilities for non-invasive sound-based therapeutic interventions potentially modulating tissue growth, regenerative medicine applications, metabolic regulation, and wound healing processes. Study underscores profound interconnections between physical acoustic phenomena and cellular biology, potentially revolutionising understanding of mechanobiology and therapeutic sound applications.

Source:

 RESEARCH PAPER

 Kumeta M, Otani M, Toyoda M, Yoshimura SH. Acoustic modulation of mechanosensitive genes and adipocyte differentiation. *Commun Biol.* 2025 Apr 16;8(1):595. doi: 10.1038/s42003-025-07969-1. PMID: 40240542; PMCID: PMC12003795.



Two Years of Music Training Boosts Children's Brain Development and Bridges the Poverty Gap in Learning (2025)

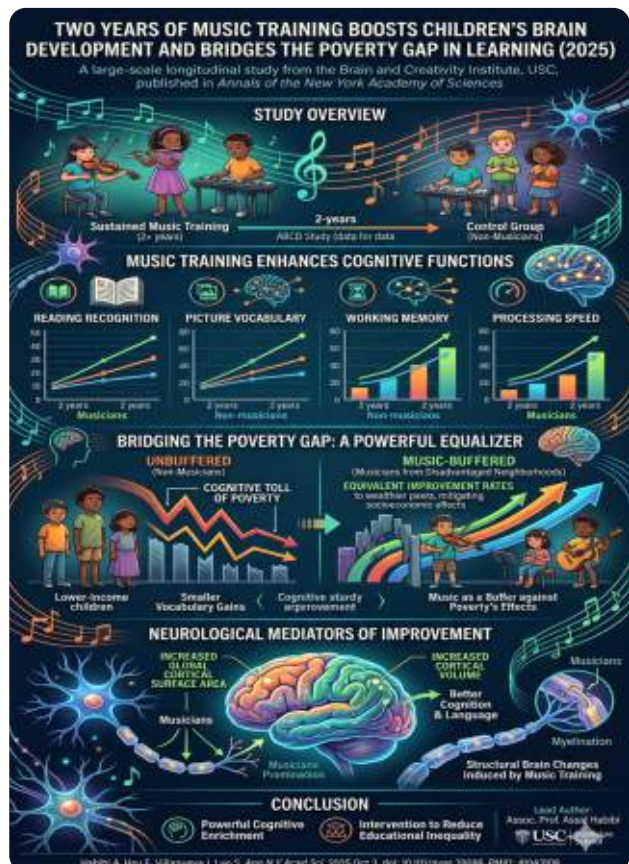
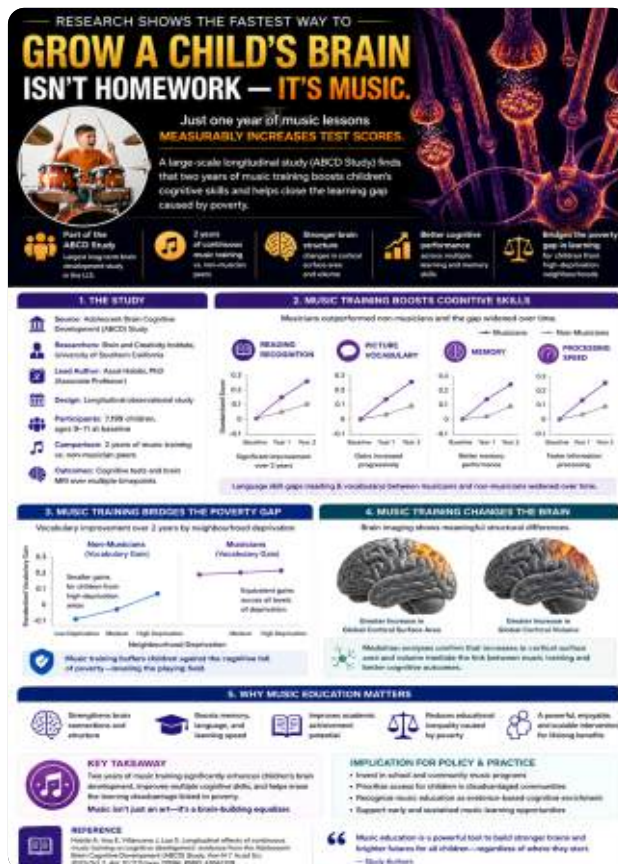
 **Could learning a musical instrument be the most powerful and equalising brain development tool available to children today?**

A large-scale longitudinal study from the Brain and Creativity Institute at the University of Southern California, published in *Annals of the New York Academy of Sciences* (2025), examined long-term cognitive effects of sustained music training using data from the Adolescent Brain Cognitive Development (ABCD) Study, the largest longitudinal study of brain development and child health in the United States. Led by Associate Professor Assal Habibi, the research compared children engaged in music training for two years against non-musician peers. Musicians demonstrated significantly higher scores in reading recognition, picture vocabulary, memory and processing speed, with language skill gaps widening progressively. Critically, while children from disadvantaged neighbourhoods showed smaller vocabulary gains than wealthier peers, young musicians from high-deprivation areas demonstrated equivalent improvement rates, suggesting music training buffers children against the cognitive toll of poverty. Mediation analyses identified global cortical surface area and volume as neurological mediators, confirming music training induces measurable structural brain changes. These findings position music education as a powerful cognitive enrichment tool and a compelling evidence-based intervention for reducing educational inequality across socioeconomic divides.

Source:

 RESEARCH PAPER

 Habibi A, Hsu E, Villanueva J, Luo S. Longitudinal Effects of Continuous Music Training on Cognitive Development: Evidence from the Adolescent Brain Cognitive Development (ABCD) Study. *Ann N Y Acad Sci*. 2025 Nov;1553(1):283-299. doi: 10.1111/nyas.70086. Epub 2025 Oct 3. PMID: 41043108.



Implementation of Live Music Therapy (MELODIC) as a Primary Non-Pharmacological Intervention for Acute Dementia Distress (2025)

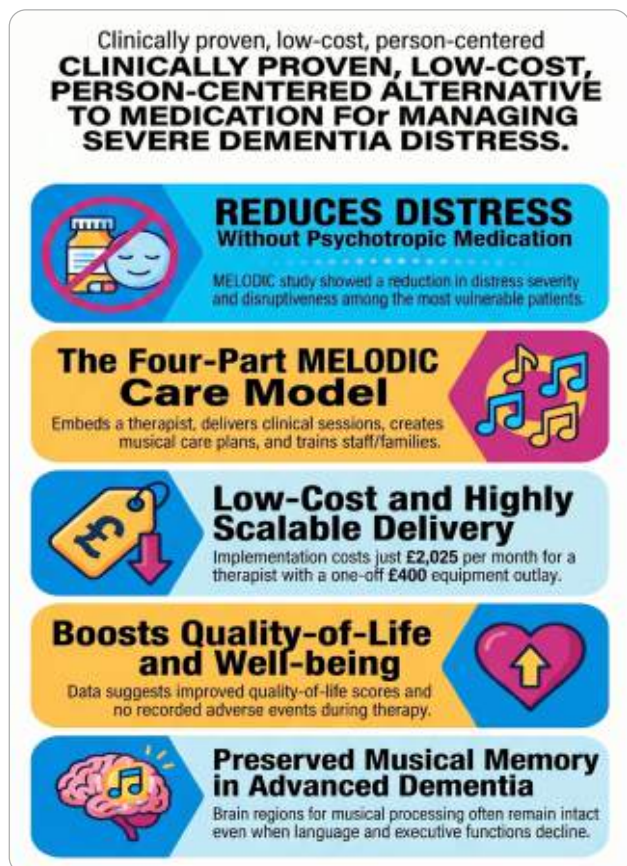
 *Could live, responsive music therapy replace antipsychotic medication in managing acute distress among dementia patients?*

A 2025 breakthrough feasibility study conducted by Anglia Ruskin University (ARU) and Cambridgeshire and Peterborough NHS Foundation Trust, known as MELODIC (Music for Early Life in Dementia Interventions and Care), has demonstrated that personalised, live music therapy can effectively ease severe patient distress without the use of sedative drugs. Unlike passive music listening, this intervention uses active synchronisation, where therapists adapt rhythms and melodies in real-time to match the patient's physiological and emotional state. This approach leverages neural entrainment, where brainwave oscillations synchronise with external rhythmic stimuli to shift patients from high-arousal agitated states to focused, calm beta or alpha wave dominance. Clinical observations indicate that this biological syncing provides a viable, side-effect-free alternative to traditional pharmacological management for behavioural and psychological symptoms of dementia (BPSD). As healthcare systems move toward person-centered care in 2026, MELODIC provides a scalable clinical framework for integrating music as a precision medical tool in geriatric psychiatry.

Source:

 RESEARCH PAPER

 Thompson N, Odell-Miller H, Pointon C, Underwood BR, Wolverson E, Hunt R, Inglis J, Olawale A, Pickering L, Wilkinson A, Wise C, Buyukulas C, Dudas R, Zhang J, Hsu MH. Music therapy embedded in the life of dementia inpatient care to help prevent and manage distress: a feasibility study to inform a future trial. *Front Psychiatry*. 2025 Jul 16; 16:1618324. doi: 10.3389/fpsyt.2025.1618324. PMID: 40740262; PMCID: PMC12307461.



Music Therapy and Recreational Choir Singing for Dementia and Depression: The Landmark MIDDEL Multinational Trial (2025)

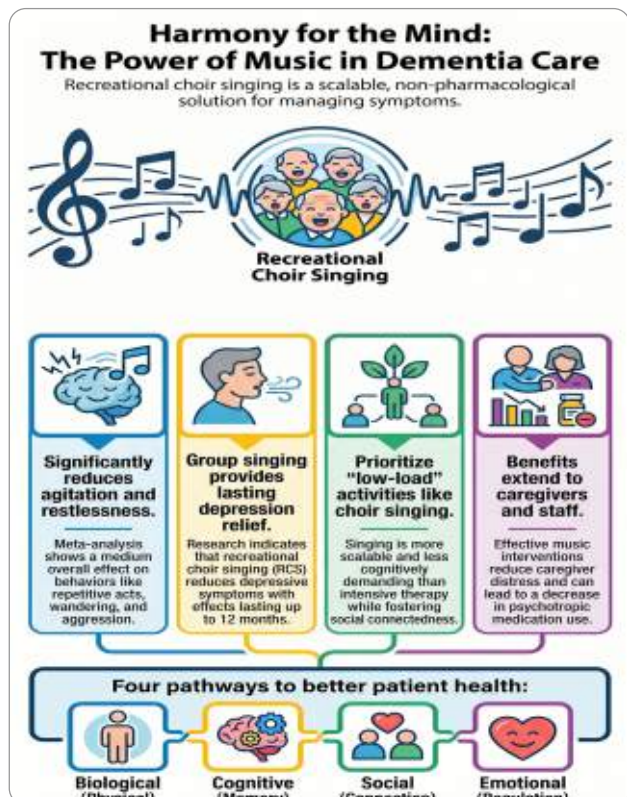
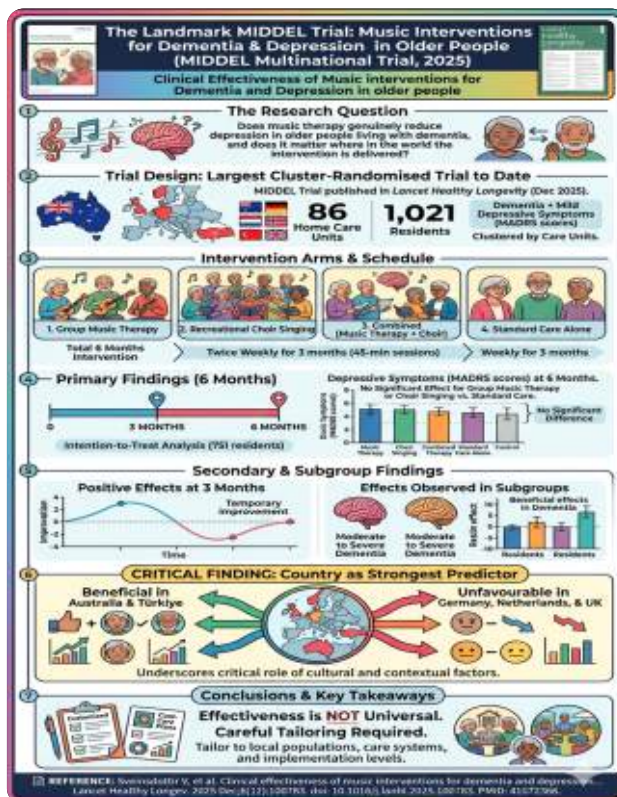
 **Does music therapy genuinely reduce depression in older people living with dementia, and does it matter where in the world the intervention is delivered?**

The MIDDEL trial, the largest cluster-randomised controlled trial of music interventions for dementia and depression to date, was published in *The Lancet Healthy Longevity* (December 2025) by an international consortium led by NORCE Research, Bergen, Norway, with institutions from Australia, Germany, the Netherlands, Norway, Türkiye, and the UK. A total of 86 home care units across six countries were randomised to group music therapy, recreational choir singing, both combined, or standard care alone, enrolling 1,021 residents with dementia and mild depressive symptoms, with a mean age of 85.6 years. Interventions were delivered in 45-minute sessions twice weekly for three months, then weekly for three months. The primary outcome was depressive symptoms at six months measured by the Montgomery-Åsberg Depression Rating Scale (MADRS). Intention-to-treat analysis of 751 residents found no significant effect on MADRS scores for either recreational choir singing or group music therapy compared to standard care, and no interaction between the two interventions. However, both interventions showed positive effects on depressive symptoms at three months, and subgroup analyses identified benefits among residents with moderate to severe dementia. Country was the strongest predictor of differential effects, with recreational choir singing beneficial in Australia and Türkiye but unfavourable in Germany, the Netherlands, and the UK, underscoring the critical role of cultural and contextual factors. These findings suggest that while music interventions can be beneficial, their effectiveness is not universal and requires careful tailoring to local populations, care systems, and levels of implementation.

Source:

RESEARCH PAPER

 Sveinsdottir V, Assmus J, Schneider J, Baker FA, Ucaner B, Kreutz G, et al. Clinical effectiveness of music interventions for dementia and depression in older people (MIDDEL): a multinational, cluster-randomised controlled trial. *Lancet Healthy Longev*. 2025 Dec;6(12):100783. doi: 10.1016/j.lanhl.2025.100783. Epub 2025 Dec 8. PMID: 41072366.



Spontaneous Eye Blinks Synchronise with Musical Beats: A Window into Auditory-Motor Integration (2025)

 *Could the simple act of blinking reveal how deeply your brain is wired to feel the rhythm of music?*

An experimental study published in *PLOS Biology* (November 2025) by researchers from the Institute of Psychology, Chinese Academy of Sciences, Beijing, and the Chinese University of Hong Kong, led by Dr. Du Yi and Dr. Teng Xiangbin, investigated whether spontaneous eye blinks, entirely involuntary and unconscious, synchronise with musical beats during passive music listening. Across four experiments involving 123 young non-musicians, the team integrated eye-tracking, electroencephalogram (EEG) recordings, white matter structural imaging, and behavioural analysis while participants listened to rhythmically stable Bach chorales. Results demonstrated that blink timing consistently aligned with musical beats across a range of tempi, and this synchronisation persisted even when melodic cues were removed, retaining only the temporal structure of the music. EEG recordings revealed a dynamic correspondence between blink timing and neural beat tracking, while white matter analysis identified the left superior longitudinal fasciculus, a key sensorimotor pathway connecting frontal, parietal, and auditory regions, as structurally linked to individual variation in blink synchronisation. Notably, the effect disappeared when participants' attention was diverted, confirming that active auditory engagement is required. These findings reveal a previously unrecognised dimension of auditory-motor synchronisation, with potential implications for understanding timing disorders such as Parkinson's disease and attention deficit conditions.

Source:

 RESEARCH PAPER

 Wu Y, Teng X, Du Y. Eye blinks synchronize with musical beats during music listening. *PLoS Biol.* 2025 Nov 18;23(11):e3003456. doi: 10.1371/journal.pbio.3003456. PMID: 41252337; PMCID: PMC12626317.



The Biological Pulse: How Rhythm Rewires the Brain

Rhythm is the brain's internal clock for movement and communication.



Your Eyes Blink to the Beat

Research shows eye blinks unconsciously synchronize with musical rhythms, reflecting a deep link between hearing and motor control.



Rhythm and Language Share DNA

Scientists identified 16 genetic loci shared between rhythm impairment and dyslexia, proving they share a biological blueprint.



Rhythmic Training Improves ADHD Motor Skills

Since 50-70% of children with ADHD face motor challenges, rhythmic 'Auditory Integration' helps stabilize coordination and timing.



Neural Highways Connect Sound and Motion

The Left Superior Longitudinal Fasciculus is the primary white matter pathway that allows the brain to translate rhythm into physical action.

Fame and Mortality Among Mainstream Music Artists: Matched Cohort Study (2025)

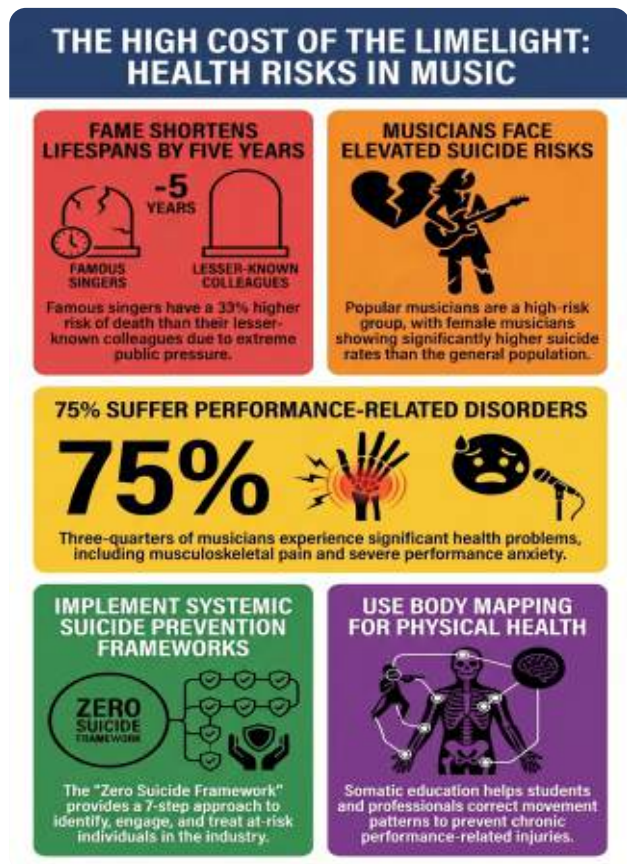
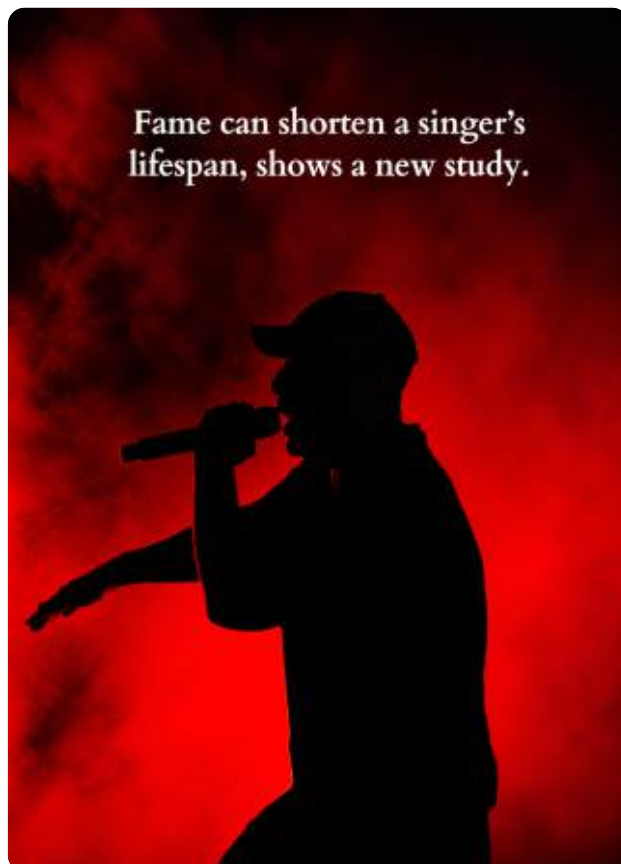
 *Does celebrity status paradoxically shorten lifespan despite wealth and resources?*

This matched cohort study published in *Journal of Epidemiology & Community Health* (January 2025) analysed mortality patterns among 324 mainstream music artists active 1950-1990 compared with less-famous counterparts matched for gender, age, nationality, ethnicity, genre, and solo versus band status. Famous singers died approximately 4.5 years earlier (mean age 75 years) compared with obscure performers (mean age 80 years), with mortality gap emerging specifically after chart entry, suggesting fame itself rather than musicianship contributes to premature mortality. Solo artists demonstrated higher mortality risk than band members regardless of fame level, indicating collaborative group dynamics may provide protective buffer against fame-related stressors. Researchers identified psychosocial stress as primary mechanism: constant public scrutiny, privacy loss, relentless performance pressure, and distress-driven unhealthy coping behaviours create chronic burden amplifying occupational hazards. Findings challenge assumptions that wealth and high social status guarantee longevity, highlighting fame as potential public health concern requiring targeted interventions. Study emphasises need for tailored mental health support and occupational health strategies for high-visibility professionals whose roles carry unique psychological burdens with measurable physiological consequences affecting mortality outcomes.

Source:

COHORT STUDY

 Hepp J, Heine C, Schliebener M, Dufner M. The price of fame? Mortality risk among famous singers. *J Epidemiol Community Health*. 2026 Mar 10;80(4):231-236. doi: 10.1136/jech-2025-224589. PMID: 41290491; PMCID: PMC13018843.



Lipoprotein(a): The Silent Inherited Risk Factor that Standard Cholesterol Tests Miss (2026)

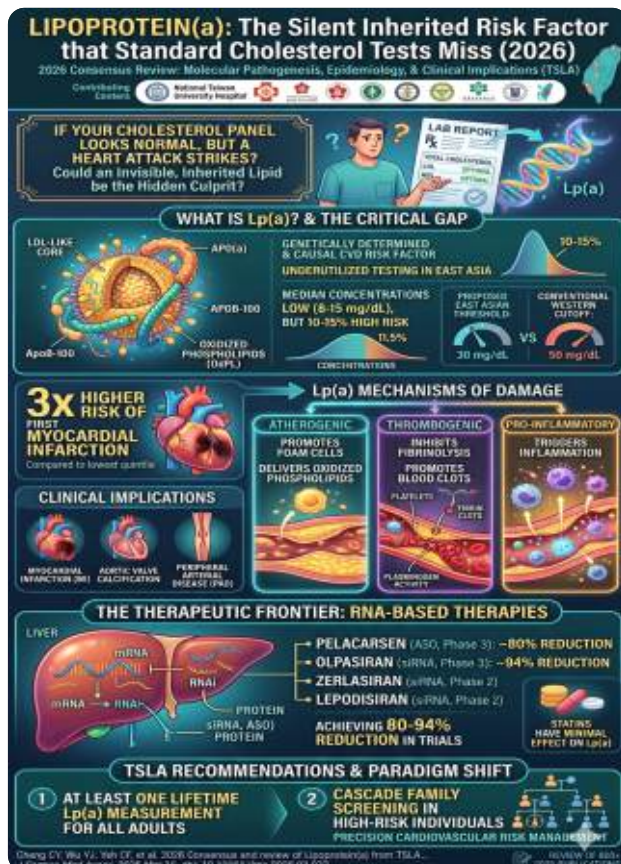
🔴 If your cholesterol panel looks perfectly normal but you still have a heart attack, could an invisible, inherited lipid be the hidden culprit?

This comprehensive consensus review from the Taiwan Society of Lipid and Atherosclerosis (TSLA), involves contributors from National Cheng Kung University, MacKay Memorial Hospital, National Taiwan University Hospital, and multiple other Taiwanese academic centres. The review consolidates over 800 publications from 2025 alone to address a critical gap: despite Lp(a) being a genetically determined, independent, and causal cardiovascular risk factor, conferring a threefold higher risk of first myocardial infarction compared to the lowest quintile - Lp(a) testing remains severely underutilised across East Asia, where median concentrations are low (8–15 mg/dL) but 10–15% of individuals exceed clinically significant thresholds. The paper delineates Lp(a)'s atherogenic, thrombogenic, and pro-inflammatory mechanisms, documents its role in aortic valve calcification and peripheral arterial disease, and proposes that Lp(a) levels of 30 mg/dL may be a more appropriate risk threshold for East Asian populations than the conventional Western 50 mg/dL cutoff. A pipeline of RNA-based therapies, including Pelacarsen, Olpasiran, Zerlasiran, and Lepodisiran, achieving Lp(a) reductions of 80–94% in Phase 2 and 3 trials is positioned as the most promising therapeutic frontier. The authors recommend at least one lifetime Lp(a) measurement for all adults, with cascade family screening in high-risk individuals, signalling a paradigm shift toward precision cardiovascular risk management in Taiwan and East Asia.

Source:

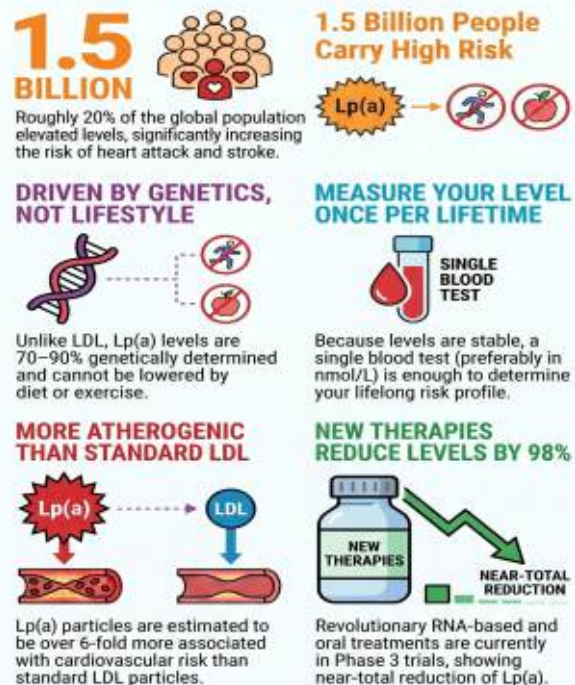
📄 RESEARCH PAPER

📌 Cheng CY, Wu YJ, Yeh CF, et al. 2026 Consensus and review of Lipoprotein(a) from Taiwan Society of Lipid and Atherosclerosis: Molecular pathogenesis, epidemiology, clinical implications, and advances in diagnostic strategies. J Formos Med Assoc. 2026 Mar 24; S0929-6646(26)00243-3. doi: 10.1016/j.jfma.2026.03.073. Epub ahead of print. PMID: 41881723.



2024: The Year of Lipoprotein(a)

A powerful, genetically-driven driver of heart disease that standard cholesterol management often misses.



GLP-1 Receptor Agonists Are Not Just Weight-Loss Drugs: The Cardiovascular Mechanisms Explained (2026)

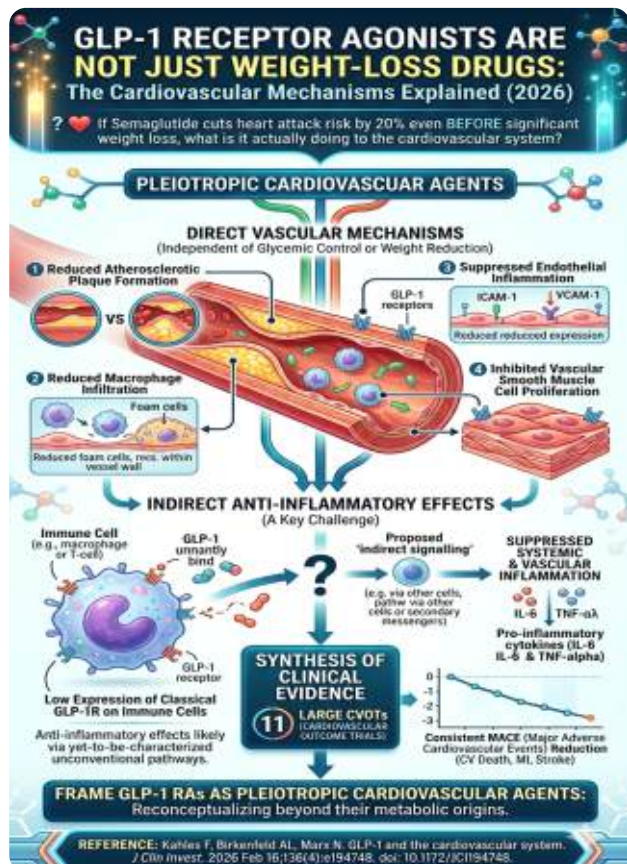
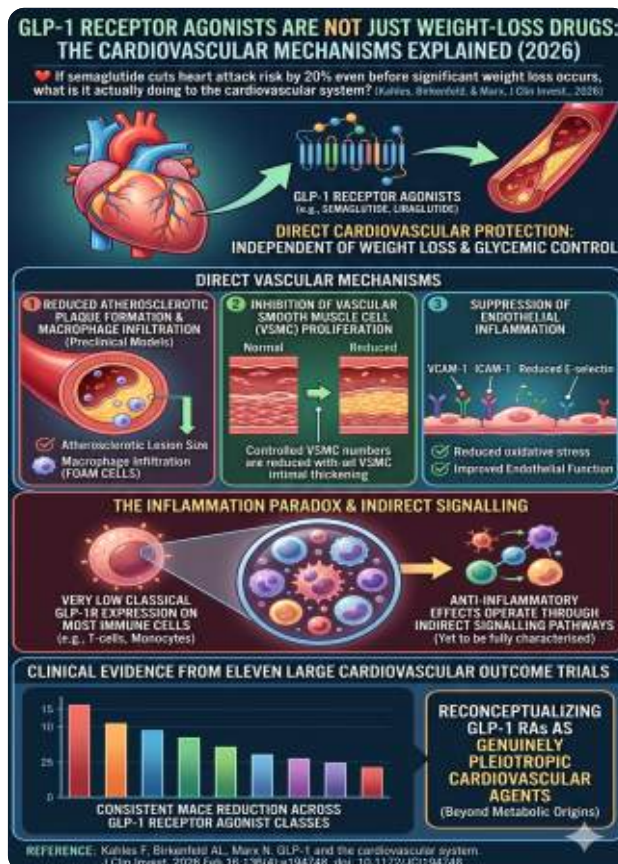
♥ If semaglutide cuts heart attack risk by 20% even before significant weight loss occurs, what is it actually doing to the cardiovascular system?

This authoritative review, authored by Florian Kahles and Nikolaus Marx from University Hospital Aachen, RWTH Aachen, Germany, and Andreas L. Birkenfeld from Eberhard-Karls University Tübingen and King's College London, synthesises experimental and clinical evidence on how GLP-1 and GLP-1 receptor agonists directly protect the cardiovascular system. The paper details multiple direct vascular mechanisms: GLP-1 receptor activation reduces atherosclerotic plaque formation and macrophage infiltration in preclinical models, inhibits vascular smooth muscle cell proliferation, and suppresses endothelial inflammation, all independent of glycaemic control or body weight reduction. Crucially, the review highlights that most immune cells express very low levels of the classical GLP-1 receptor, suggesting the anti-inflammatory cardiovascular effects operate through indirect signalling pathways yet to be fully characterised. Clinical evidence from eleven large cardiovascular outcome trials is synthesised, demonstrating consistent MACE reduction across GLP-1 receptor agonist classes. The authors frame GLP-1 receptor agonists as genuinely pleiotropic cardiovascular agents warranting reconceptualisation beyond their metabolic origins.

Source:

REVIEW

✚ Kahles F, Birkenfeld AL, Marx N. GLP-1 and the cardiovascular system. J Clin Invest. 2026 Feb 16;136(4):e194748. doi: 10.1172/JCI194748. PMID: 41697744; PMCID: PMC12904707.



AI-Enabled Stethoscope Detects Heart Failure, Atrial Fibrillation, and Valve Disease in 15 Seconds: The TRICORDER Trial (2026)

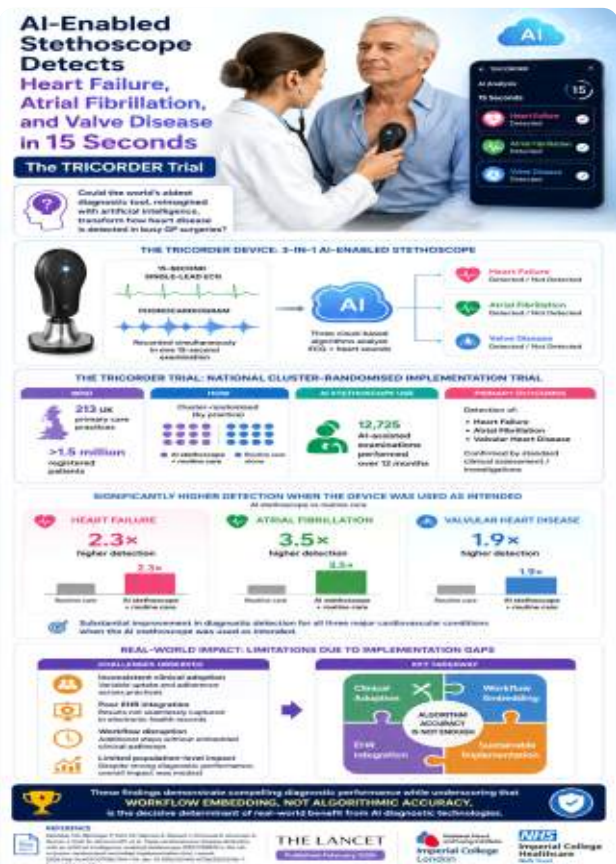
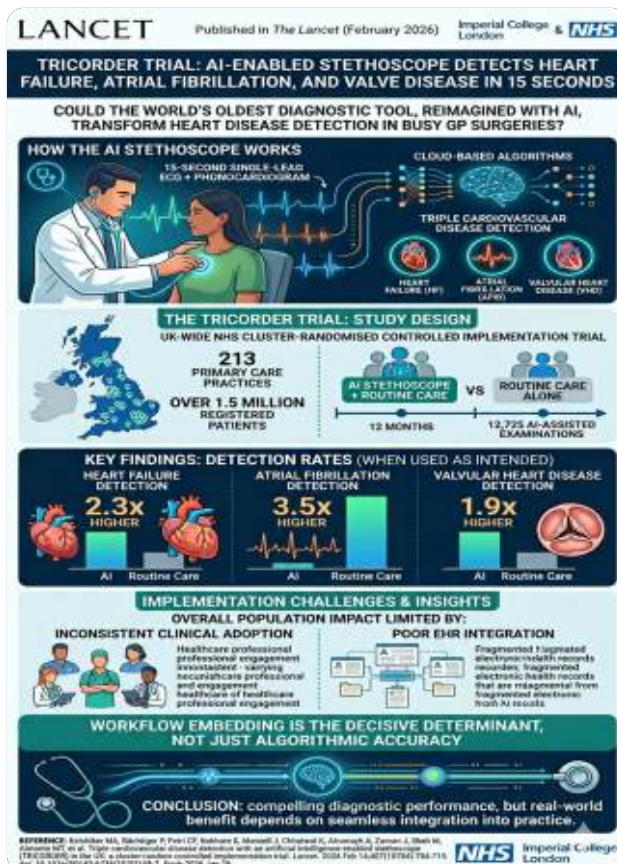
 Could the world's oldest diagnostic tool, reimagined with artificial intelligence, transform how heart disease is detected in busy GP surgeries?

The TRICORDER trial, published in *The Lancet* (February 2026) by researchers from the National Heart and Lung Institute, Imperial College London, and Imperial College Healthcare NHS Trust, is the first national-scale cluster-randomised controlled implementation trial of a clinical AI technology in the NHS. A total of 213 UK primary care practices serving over 1.5 million registered patients were randomised to AI stethoscope use alongside routine care or routine care alone. The AI-enabled stethoscope simultaneously records a 15-second single-lead ECG and phonocardiogram, processed by three cloud-based algorithms detecting heart failure, atrial fibrillation, and valvular heart disease. Over 12 months, 12,725 AI-assisted examinations were performed. When the device was used as intended, detection rates were 2.3 times higher for heart failure, 3.5 times higher for atrial fibrillation, and 1.9 times higher for valvular heart disease compared to routine care. However, overall population-level impact was limited by inconsistent clinical adoption and poor electronic health record integration. These findings demonstrate compelling diagnostic performance while underscoring that workflow embedding, not algorithmic accuracy, is the decisive determinant of real-world benefit from AI diagnostic technologies.

Source:

 RESEARCH PAPER

 Kelshiker MA, Bächtiger P, Petri CF, Nakhare S, Mansell J, Chhatwal K, Alrumayh A, Zaman J, Shah M, Almonte MT, et al. Triple cardiovascular disease detection with an artificial intelligence-enabled stethoscope (TRICORDER) in the UK: a cluster-randomised controlled implementation trial. *Lancet*. 2026 Feb 14;407(10704):704-715. doi: 10.1016/S0140-6736(25)02156-7. Epub 2026 Jan 28.



Could a Weight-Loss Injection Be Quietly Protecting the Heart and Kidneys of People with Type 1 Diabetes? (2026)

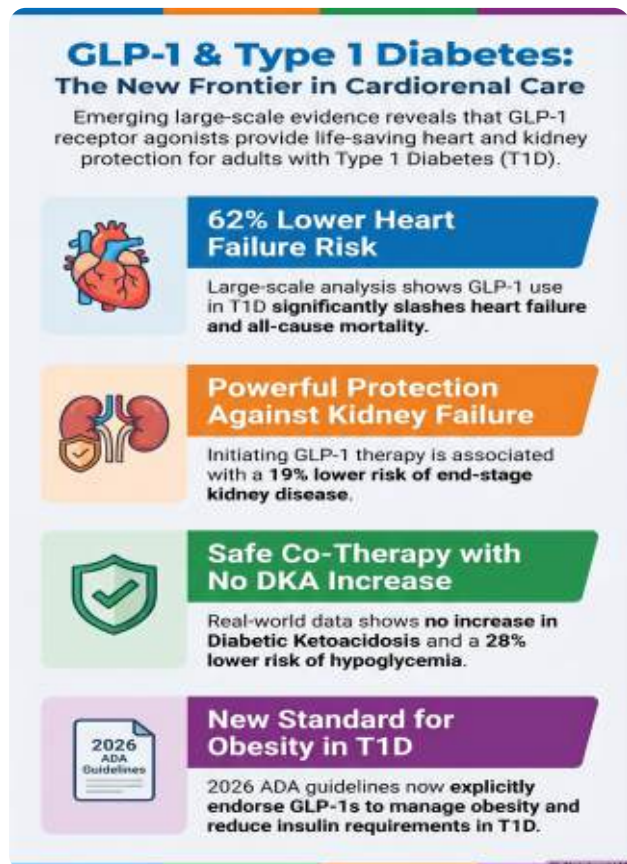
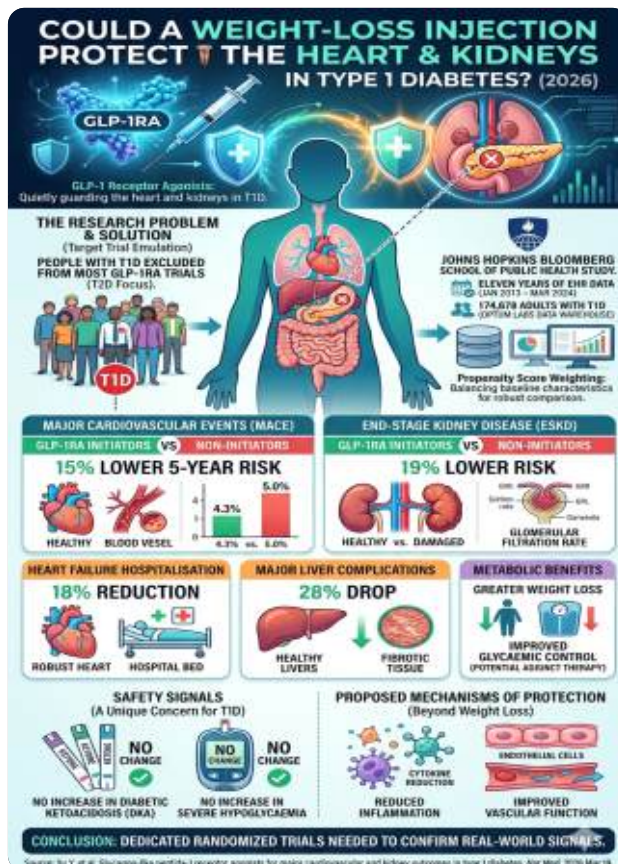
 *The drugs reshaping obesity care may be doing something nobody designed them for in Type 1 Diabetes: guarding the heart and kidneys from the inside.*

GLP-1 receptor agonists, the class of drugs behind semaglutide and tirzepatide, have transformed cardiometabolic care in Type 2 Diabetes, yet people with Type 1 Diabetes have been almost entirely excluded from these trials. Researchers at Johns Hopkins Bloomberg School of Public Health addressed this gap using a target trial emulation design, analysing eleven years of electronic health records (January 2013 to March 2024) for 174,678 adults with Type 1 Diabetes from the Optum Labs Data Warehouse. After propensity score weighting balanced baseline characteristics, GLP-1RA initiation was associated with a 15% lower five-year risk of major cardiovascular events (4.3% versus 5.0%), a 19% lower risk of end-stage kidney disease, an 18% reduction in heart failure hospitalisation, and a 28% drop in major liver complications, alongside greater weight loss. Critically, diabetic ketoacidosis and severe hypoglycaemia did not increase, addressing a long-standing safety concern unique to type 1 diabetes. The protective mechanism appears to extend beyond weight loss alone, implicating reduced inflammation and improved vascular function. Dedicated randomised trials are now needed to confirm these real-world signals.

Source:

 RESEARCH PAPER

 Xu Y, Malek ND, Chang AR, Echouffo-Tcheugui JB, Selvin E, Grams ME, Fang M, Shin JI. Glucagon-like peptide-1 receptor agonists for major cardiovascular and kidney outcomes in type 1 diabetes. Nat Med. 2026 May;32(5):1682-1685. doi: 10.1038/s41591-026-04274-0. Epub 2026 Mar 19. PMID: 41857198; PMCID: PMC13190255.



Non-Psychoactive Cannabinoids as Therapeutic Agents for Metabolic Liver Disease (2026)

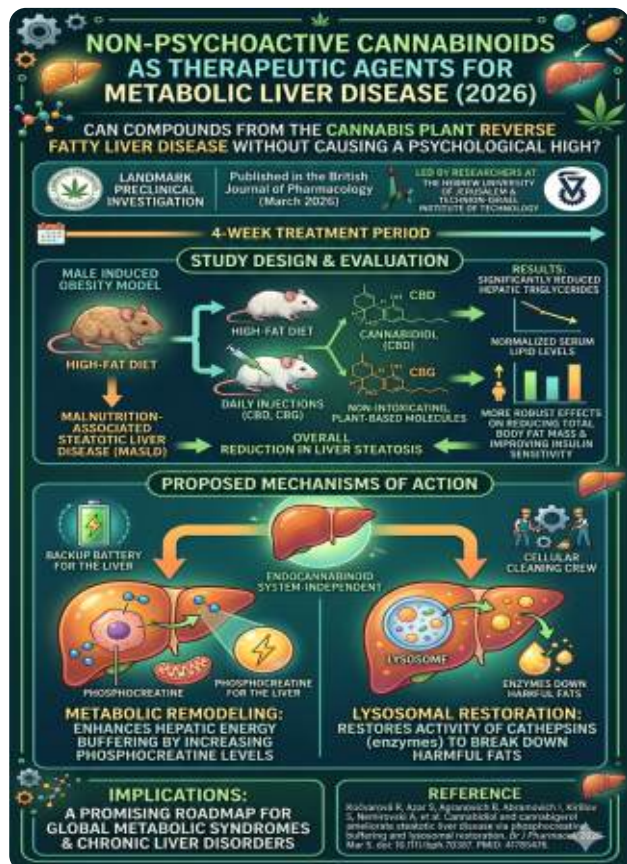
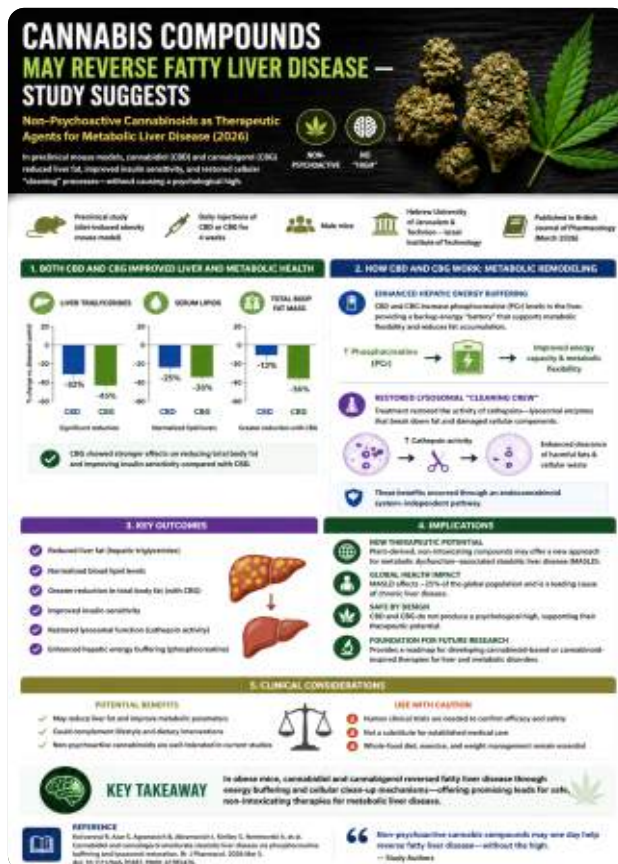
🌿 *Can compounds from the cannabis plant reverse fatty liver disease without causing a psychological high?*

This landmark preclinical investigation published in the *British Journal of Pharmacology* (March 2026) was led by researchers at the Hebrew University of Jerusalem and the Technion-Israel Institute of Technology. The study utilised a diet-induced obesity model in male mice to evaluate the effects of daily injections of Cannabidiol and Cannabigerol over a four-week period. Results demonstrated that both compounds significantly reduced hepatic triglycerides and normalised serum lipid levels, with Cannabigerol showing more robust effects on reducing total body fat mass and improving insulin sensitivity. The proposed mechanism involves a unique process of metabolic remodelling where the compounds enhance hepatic energy buffering by increasing phosphocreatine levels, essentially creating a backup battery for the liver. Furthermore, the treatment restored the activity of cathepsins, enzymes that function as a cellular cleaning crew within lysosomes to break down harmful fats. These findings identify an endocannabinoid system-independent pathway for treating metabolic dysfunction-associated steatotic liver disease. This research provides a promising roadmap for using plant-based, non-intoxicating molecules to address global metabolic syndromes and chronic liver disorders.

Source:

📄 RESEARCH PAPER

📌 Kočvarová R, Azar S, Agranovich B, Abramovich I, Kirillov S, Nemirovski A, et al. Cannabidiol and cannabigerol ameliorate steatotic liver disease via phosphocreatine buffering and lysosomal restoration. *Br J Pharmacol.* 2026 Mar 5. doi: 10.1111/bph.70387. PMID: 41785476..



Semaglutide Increases Knee Cartilage Thickness by 17% through a Weight-Independent Repair Mechanism (2026)

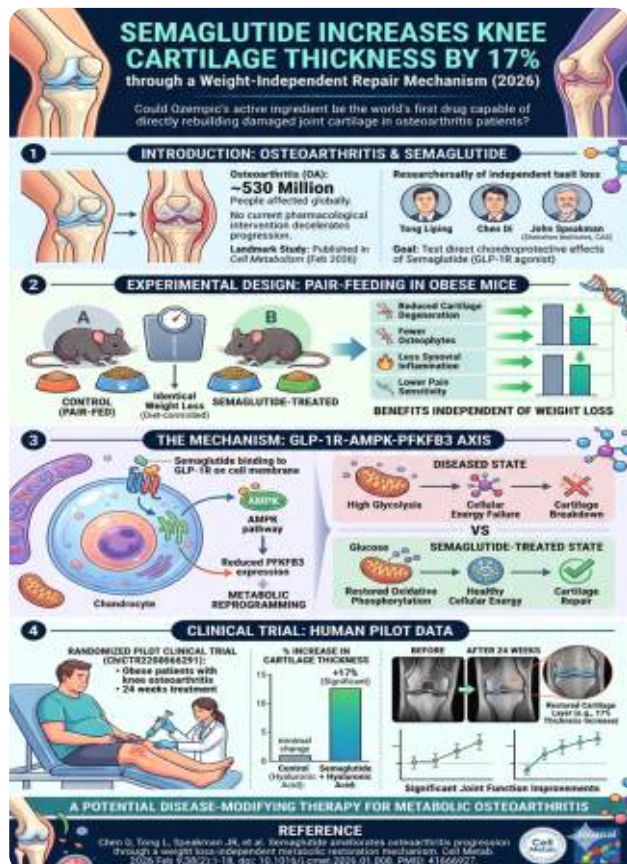
👉 *Could Ozempic's active ingredient be the world's first drug capable of directly rebuilding damaged joint cartilage in osteoarthritis patients?*

Osteoarthritis affects approximately 530 million people worldwide, yet no pharmacological intervention has been established to decelerate its progression. A landmark study published in *Cell Metabolism* (February 2026) by researchers led by Tong Liping, Chen Di and John Speakman from the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, demonstrated that semaglutide, a GLP-1 receptor agonist, exerts direct chondroprotective effects entirely independent of weight loss. Using diet-controlled pair-feeding experiments in obese osteoarthritic mice, semaglutide-treated animals showed significantly reduced cartilage degeneration, fewer osteophytes, less synovial inflammation and lower pain sensitivity despite identical weight loss in controls. The mechanism operates through the GLP-1R-AMPK-PFKFB3 axis, reprogramming chondrocyte metabolism from glycolysis to oxidative phosphorylation, restoring cellular energy supply and enabling cartilage repair. A randomised pilot clinical trial (ChiCTR2200066291) in obese patients with knee osteoarthritis revealed significant joint function improvements after 24 weeks, with MRI confirming a 17% increase in cartilage thickness in semaglutide-treated patients compared with hyaluronic acid alone. These findings establish semaglutide as a potential disease-modifying therapy for metabolic osteoarthritis through a previously unrecognised weight-independent cartilage restoration mechanism.

Source:

📄 RESEARCH PAPER

📌 Chen D, Tong L, Speakman JR, et al. Semaglutide ameliorates osteoarthritis progression through a weight loss-independent metabolic restoration mechanism. *Cell Metab.* 2026 Feb 9;38(2):1-18. doi: 10.1016/j.cmet.2026.01.008. PMID: 41666927.



Limitations and Fallacies of Relying on Glycosylated Haemoglobin for Diagnosing and Monitoring Diabetes in Indian Populations (2026)

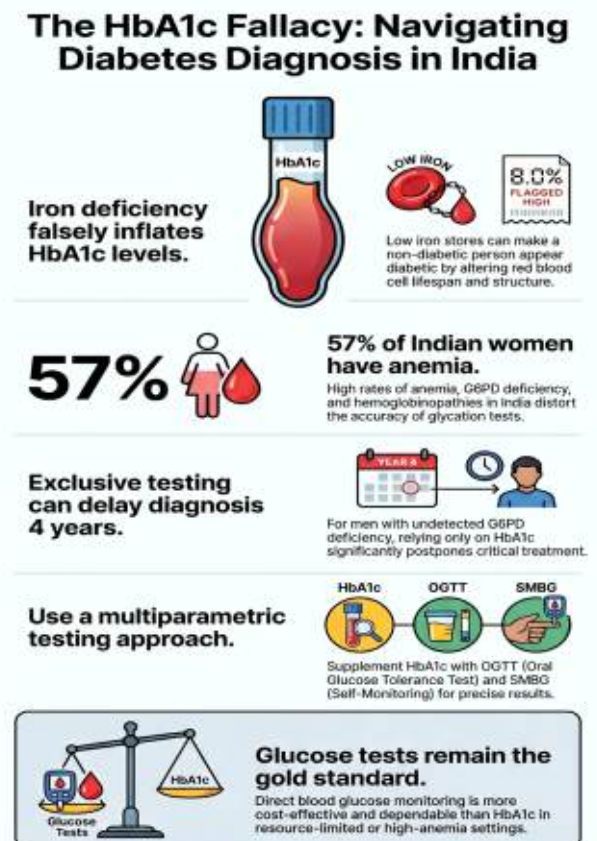
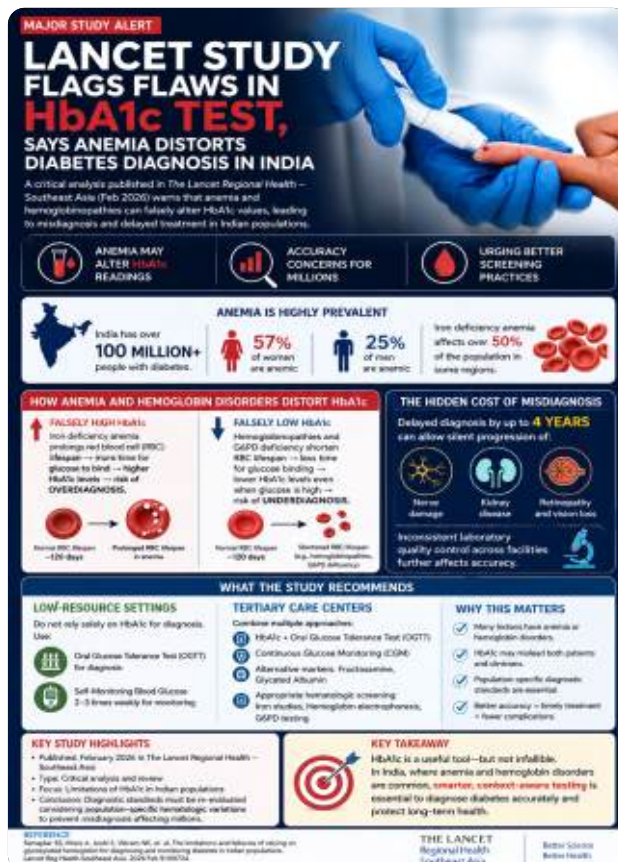
🔴 Can anaemia and hemoglobinopathies falsely alter HbA1c values leading to misdiagnosis of diabetes in Indian populations?

This critical analysis published in *The Lancet Regional Health - Southeast Asia* (February 2026) examined limitations of glycosylated haemoglobin testing for diabetes diagnosis and monitoring in Indian populations where anaemia and hemoglobinopathies are highly prevalent. India has over 100 million people with diabetes, yet National Family Health Survey documented 57% women and 25% men are anaemic. Iron deficiency anaemia affecting over 50% population in some regions falsely raises HbA1c levels leading to overdiagnosis, while hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency shorten red blood cell lifespan artificially lowering HbA1c even when blood glucose is elevated, potentially delaying diagnosis by up to four years. During this period, complications including nerve damage, kidney disease, and retinopathy progress silently. Inconsistent laboratory quality control across facilities further affects accuracy. Authors recommend low-resource settings utilise oral glucose tolerance test for diagnosis with self-monitoring blood glucose two to three times weekly, while tertiary care centres combine HbA1c with oral glucose tolerance test, continuous glucose monitoring, alternative markers like Fructosamine, and appropriate hematologic screening including iron studies, haemoglobin electrophoresis, and glucose-6-phosphate dehydrogenase testing. Study concluded diagnostic standards require re-evaluation considering population-specific hematologic variations to prevent misdiagnosis affecting millions dependent on HbA1c testing.

Source:

ANALYSIS

📌 Samajdar SS, Joshi SR, Misra A, Vikram NK. The limitations and fallacies of relying on glycosylated haemoglobin for diagnosing and monitoring diabetes in Indian populations. *Lancet Reg Health Southeast Asia*. 2026 Feb 8; 45:100724. doi: 10.1016/j.lansea.2026.100724. Erratum in: *Lancet Reg Health Southeast Asia*. 2026 Mar 14; 47:100751. doi: 10.1016/j.lansea.2026.100751. PMID: 41743216; PMCID: PMC12931385.



The Ziltivekimab Cardiovascular Outcomes Trial (ZEUS): Targeting the IL-6 Pathway in Chronic Kidney Disease (2026)

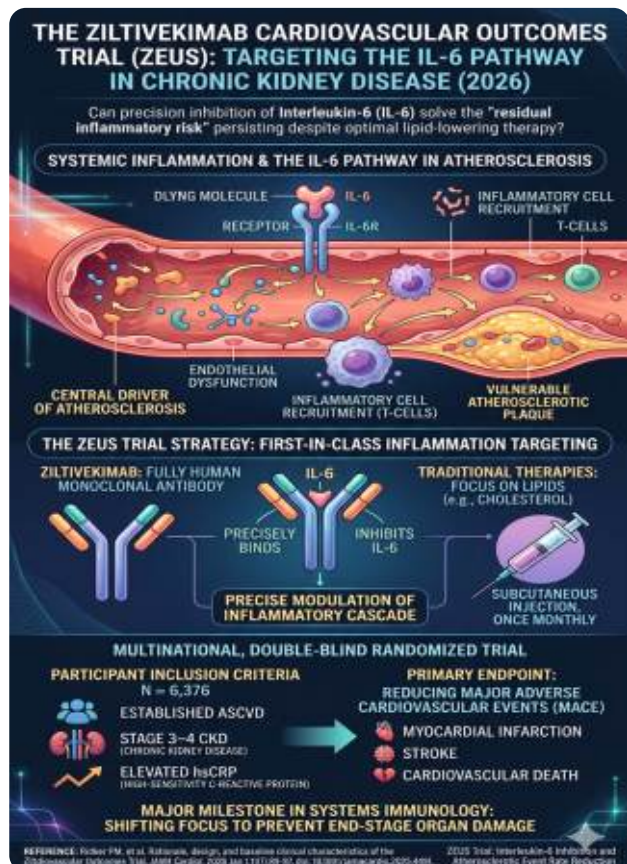
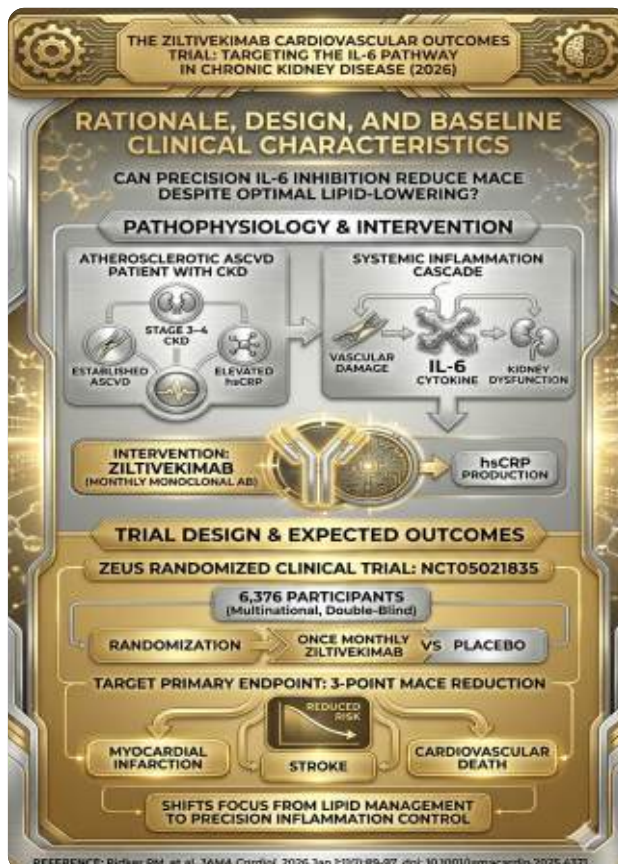
🔥 Can precision inhibition of Interleukin-6 (IL-6) solve the "residual inflammatory risk" that persists despite optimal lipid-lowering therapy?

The foundational paper for the ZEUS randomised clinical trial, published in *JAMA Cardiology* (January 2026), outlines a first-in-class strategy to reduce major adverse cardiovascular events (MACE) by targeting systemic inflammation. While traditional therapies focus on cholesterol, the ZEUS trial targets the Interleukin-6 (IL-6) signalling cytokine, a central driver of atherosclerosis. This multinational, double-blind trial involves 6,376 participants with established atherosclerotic cardiovascular disease (ASCVD), stage 3–4 chronic kidney disease (CKD), and elevated high-sensitivity C-reactive protein (hsCRP). By administering Ziltivekimab, a fully human monoclonal antibody, subcutaneously, once monthly, the study aims to prove that lowering systemic inflammation can significantly reduce the risk of myocardial infarction, stroke, and cardiovascular death. This trial represents a major milestone in systems immunology, shifting the focus from lipid management to the precise modulation of the inflammatory cascade to prevent end-stage organ damage.

Source:

📄 RESEARCH PAPER

📌 Ridker PM, Baeres FMM, Hveplund A, Engelmann MMD, Hovingh GK, Lincoff AM, Marx N, Navar AM, Sattar N, Tuttle K, Perkovic V. Rationale, Design, and Baseline Clinical Characteristics of the Ziltivekimab Cardiovascular Outcomes Trial: Interleukin-6 Inhibition and Atherosclerotic Event Rate Reduction. *JAMA Cardiol.* 2026 Jan 1;11(1):89-97. doi: 10.1001/jamacardio.2025.4491. Erratum in: *JAMA Cardiol.* 2026 Jun 1;11(6):611. doi: 10.1001/jamacardio.2026.1448. PMID: 41369941.



Decoding Diabetes-Related Heart Risk Through AI-Powered ECG Analysis (2025)

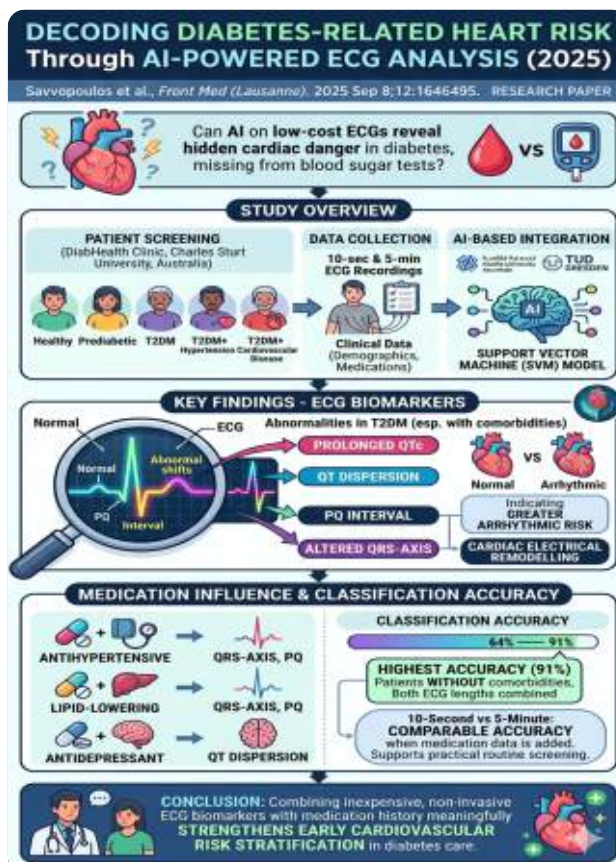
 **Can a simple, low-cost heart tracing, when read by artificial intelligence, reveal hidden cardiac danger in diabetes that blood sugar tests alone cannot detect?**

This study was published in *Frontiers in Medicine* (September 2025). Researchers analysed ECG and clinical data from 581 participants screened at the DiabHealth Clinic, Charles Sturt University, Australia. Participants were classified as healthy, prediabetic, or having Type 2 Diabetes Mellitus (T2DM), with or without coexisting hypertension and cardiovascular disease. Researchers at Khalifa University, Abu Dhabi, and TUD Dresden University of Technology used a Support Vector Machine model to integrate 10-second and 5-minute ECG recordings with demographic, clinical, and medication data, aiming to improve cardiac risk classification across diabetes stages. T2DM patients, particularly those with comorbidities, showed significant prolongation of QTc, QT dispersion, and PQ intervals, along with altered QRS-Axis measurements, indicating greater arrhythmic risk and cardiac electrical remodelling. Antihypertensive and lipid-lowering medications influenced QRS-Axis and PQ intervals, while antidepressant use was linked to QT dispersion changes. Classification accuracy ranged from 64% to 91% across patient subgroups, with the highest accuracy achieved in patients without comorbidities when both ECG durations were combined. Notably, brief 10-second recordings performed comparably to longer 5-minute recordings once medication data was incorporated, supporting their practicality for routine screening. These findings suggest that combining inexpensive, non-invasive ECG biomarkers with medication history can meaningfully strengthen early cardiovascular risk stratification in diabetes care.

Source:

 RESEARCH PAPER

 Savvopoulos S, Hatzikirou H, Jelinek HF. AI-based integration of ECG biomarkers for assessing cardiac risk in type 2 diabetes mellitus with comorbid conditions for patient stratification. *Front Med (Lausanne)*. 2025 Sep 8; 12:1646495. doi: 10.3389/fmed.2025.1646495. PMID: 40988753; PMCID: PMC12450978.



The Silent Rhythm: Mapping Cardiac Risk in Type 2 Diabetes

44% of Asymptomatic Patients Show Abnormalities

Nearly half of T2DM patients without overt cardiac disease exhibit subclinical electrical changes.

QTc Prolongation is the Top Warning

Prolonged QTc intervals and ST-T changes are the most common indicators of silent cardiovascular risk.

Risk Spikes After 10 Years

Patients with diabetes for >10 years are twice as likely to show heart abnormalities

<10 Yrs | >10 Yrs

AI Achieves 91% Screening Accuracy

91% ACCURACY

Meds | AI (SVM)

Machine learning models accurately stratify patient risk by integrating ECG biomarkers with medication data.

Monitor HbA1c Levels Above 8%

HbA1c ≥8%

Poor glycemic control is a primary independent predictor of abnormal heart rhythms.

Eloralintide, Selective Amylin Receptor Agonist for Obesity Treatment: 48-Week Phase 2 Randomised Controlled Trial (2025)

 **Can targeting amylin receptors rather than GLP-1 pathways provide effective alternative obesity treatment with improved tolerability?**

This Phase 2 multicentre, double-blind, randomised, placebo-controlled trial published in *The Lancet* (November 2025) evaluated Eloralintide, selective amylin receptor agonist administered weekly subcutaneous injection, in 263 adults with obesity or overweight with weight-related comorbidities without type 2 diabetes over 48 weeks. Participants randomised to Eloralintide doses (1-9mg, dose-escalation regimens) or placebo demonstrated significant dose-dependent weight reduction ranging 9.5-20.1% versus 0.4% placebo, with 57% receiving 9mg achieving $\geq 20\%$ weight loss. Secondary outcomes revealed improvements in waist circumference, blood pressure, HbA1c, fasting insulin, HDL cholesterol elevation, triglyceride reduction, and inflammatory markers. Adverse events predominantly gastrointestinal including nausea and fatigue were mild-to-moderate, with improved tolerability using slower dose-escalation strategies. Eloralintide acts through distinct amylin pathway regulating appetite, slowing gastric emptying, and enhancing metabolism, offering novel alternative or complementary approach to incretin-based therapies for patients unable to tolerate existing obesity medications. Phase 3 trials initiated end 2025 investigating monotherapy and combination with tirzepatide, potentially expanding personalised obesity treatment options.

Source:

RESEARCH PAPER

-  Billings LK, et al. Eloralintide, a selective amylin receptor agonist for the treatment of obesity: a 48-week phase 2, multicentre, double-blind, randomised, placebo-controlled trial. *Lancet*. 2025 Dec 6;406(10520):2631-2643. doi: 10.1016/S0140-6736(25)02155-5. Epub 2025 Nov 6. PMID: 41207310.



REAL RESULTS. BACKED BY RESEARCH.

Researchers report that a weekly weight loss medication led to **20% body weight loss** during trials.

 Clinically studied. **Proven results.**

* In a clinical trial of adults with overweight or obesity using a weekly GLP-1 medication along with diet and exercise.



ELORALINTIDE: SELECTIVE AMYLIN RECEPTOR AGONIST FOR OBESITY TREATMENT

48-WEEK PHASE 2 RANDOMIZED CONTROLLED TRIAL (2025)

Reference: Billings LK, et al. *Lancet*. 2025 Dec 6;406(10520):2631-2643. doi: 10.1016/S0140-6736(25)02155-5.

CAN TARGETING AMYLIN RECEPTORS RATHER THAN GLP-1 PATHWAYS PROVIDE EFFECTIVE ALTERNATIVE OBESITY TREATMENT WITH IMPROVED TOLERABILITY?

TRIAL CHARACTERISTICS

Trial Design: Multicentre, Double-Blind, Randomized, Placebo-Controlled Trial.
Published in: *The Lancet* (Nov 2025).

STUDY ENROLLMENT AND TREATMENT

263 ADULTS (Obesity/Overweight with excess fat) randomized (3:1 ratio) (Double-blind)

PLACEBO Weekly 5.0

ELORALINTIDE DOSES (1-9mg Dose Escalation)

48 WEEKS

MECHANISM OF ACTION: AMYLIN RECEPTOR AGONISM VS. GLP-1

AMYLIN PATHWAY (Eloralintide - Selective) → **Eloralintide** → **GLP-1 PATHWAY** EXISTING THERAPIES

REGULATING APPETITE → **SLOWING GASTRIC EMPTYING** → **ENHANCING METABOLISM**

DISTINCT AMYLIN PATHWAY OR **NOVEL ALTERNATIVE OR COMPLEMENTARY APPROACH**

Potentially beneficial for patients unable to tolerate existing medications.

PRIMARY OUTCOME: SIGNIFICANT & DOSE-DEPENDENT WEIGHT REDUCTION

% WEIGHT CHANGE FROM BASELINE

WEEKS 0, 12, 24, 36, 48

Placebo (0.4%)

Range: 9.5-20.1%

11mg

3mg

6mg

9mg

57% of 9mg ACHIEVED $\geq 20\%$ WEIGHT LOSS

SECONDARY OUTCOMES: COMPREHENSIVE HEALTH IMPROVEMENTS

REDUCED WAIST CIRCUMFERENCE **LOWERED BLOOD PRESSURE** **IMPROVED HbA1c** **REDUCED FASTING INSULIN** **ELEVATED HDL CHOLESTEROL** **REDUCED TRIGLYCERIDES** **DECREASED INFLAMMATORY MARKERS**

SAFETY AND TOLERABILITY

ADVERSE EVENTS: Predominantly Gastrointestinal (Mild-to-Moderate)

IMPROVED TOLERABILITY using Slower Dose-Escalation Strategies.

SAFETY Nausea Fatigue

FUTURE DIRECTIONS: PHASE 3 TRIALS (Initiated End 2025)

INVESTIGATING: 1. MONOTHERAPY Eloralintide 2. COMBINATION THERAPY Eloralintide + Tirzepatide

POTENTIALLY EXPANDING PERSONALIZED OBESITY TREATMENT OPTIONS

First-in-Human Success of CRISPR Gene-Editing for "One-and-Done" Lipid Control (2025)

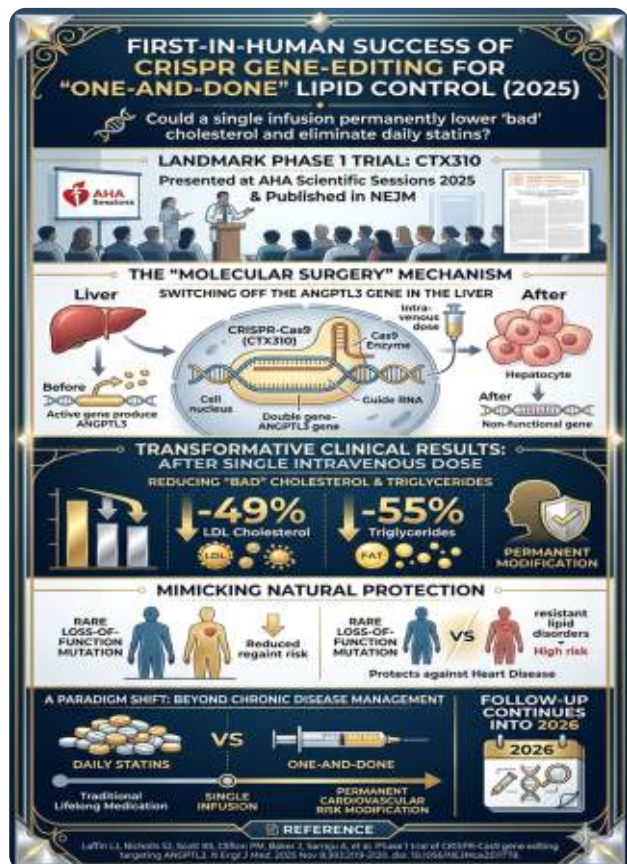
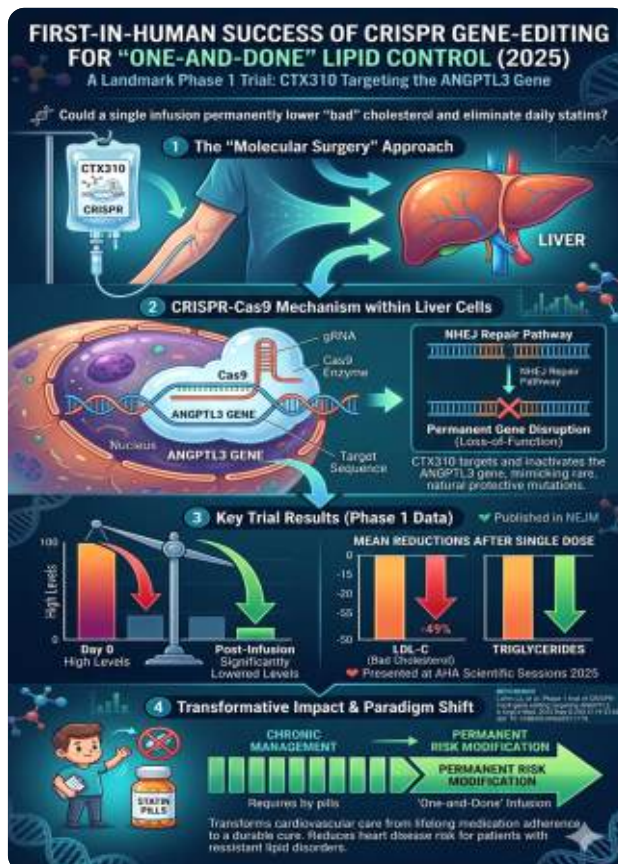
 **Could a single infusion permanently lower "bad" cholesterol and eliminate the need for daily statins?**

A landmark Phase 1 trial, presented at the American Heart Association Scientific Sessions in late 2025 and published in the *New England Journal of Medicine*, has demonstrated the first successful human use of CRISPR-Cas9 (CTX310) to permanently switch off the ANGPTL3 gene in the liver. Unlike traditional medications that require lifelong adherence, this gene-editing therapy acts as a "molecular surgery," reducing LDL cholesterol by 49% and triglycerides by 55% after a single intravenous dose. By mimicking a rare, natural loss-of-function mutation that protects individuals from heart disease, CTX310 offers a transformative solution for patients with resistant lipid disorders. As follow-up continues into 2026, this "one-and-done" approach marks the beginning of a paradigm shift from chronic disease management to permanent cardiovascular risk modification.

Source:

 RESEARCH PAPER

 Laffin LJ, Nicholls SJ, Scott RS, Clifton PM, Baker J, Sarraju A, et al. Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3. *N Engl J Med*. 2025 Nov 27;393(21):2119-2130. doi: 10.1056/NEJMoa2511778. Epub 2025 Nov 8. PMID: 41211945.



Deep Learning Detects Hypertrophic Cardiomyopathy from ECG Images with High Accuracy Across Four International Cohorts (2025)

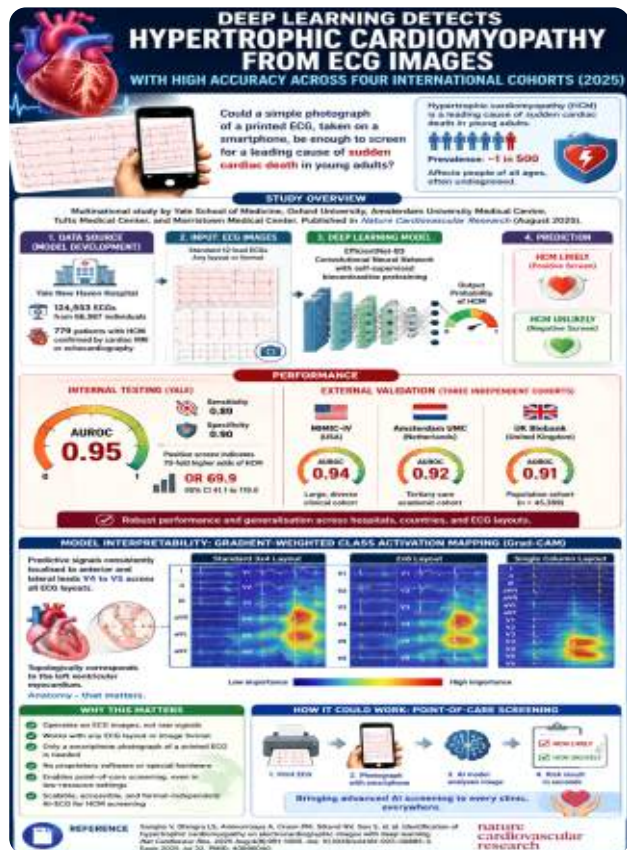
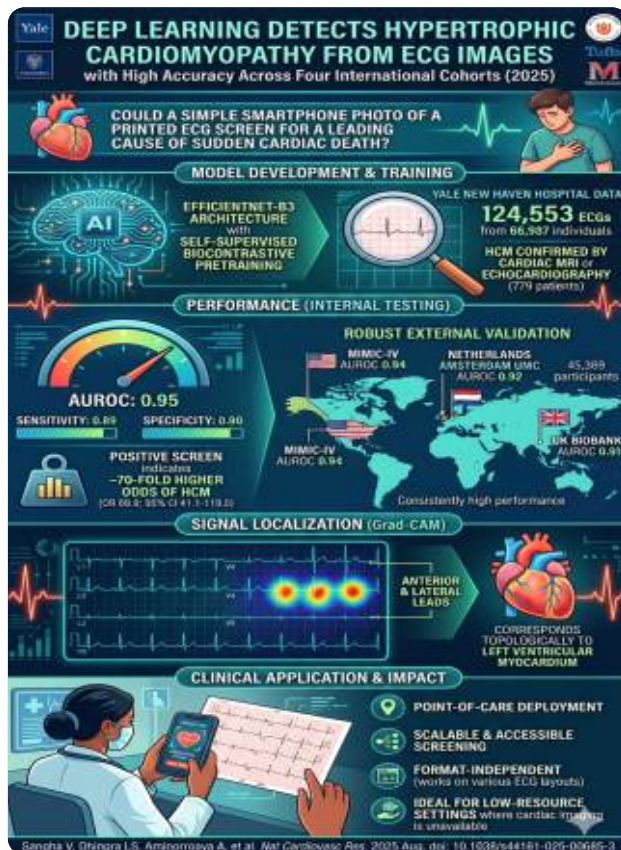
Could a simple photograph of a printed ECG, taken on a smartphone, be enough to screen for a leading cause of sudden cardiac death in young adults?

A multinational deep learning study published in *Nature Cardiovascular Research* (August 2025) by researchers from Yale School of Medicine, Oxford University, Amsterdam University Medical Centre, Tufts Medical Centre, and Morristown Medical Centre, developed and validated an automated convolutional neural network model to detect hypertrophic cardiomyopathy (HCM) from images of standard 12-lead ECGs, regardless of layout or image format. The model was developed using 124,553 ECGs from 66,987 individuals at Yale New Haven Hospital, with HCM confirmed by cardiac MRI or echocardiography in 779 patients. Using an EfficientNet-B3 architecture with self-supervised biocontrastive pretraining, the model achieved an AUROC of 0.95 in internal testing, with sensitivity 0.89 and specificity 0.90, and a positive screen indicating 70-fold higher odds of HCM (OR 69.9; 95% CI 41.1 to 119.0). External validation across three independent international cohorts confirmed robust generalisation: AUROC 0.94 at MIMIC-IV (USA), 0.92 at Amsterdam UMC (Netherlands), and 0.91 in the UK Biobank population cohort (45,389 participants). Gradient-weighted Class Activation Mapping consistently localised predictive signals to anterior and lateral leads V4 to V5 across all ECG layouts, corresponding topologically to the left ventricular myocardium. Crucially, the model operates on ECG images rather than raw voltage signals, enabling point-of-care deployment via smartphone photographs of printed ECGs without proprietary software. These findings establish image-based AI-ECG as a scalable, accessible, and format-independent screening strategy for HCM, particularly in low-resource settings where advanced cardiac imaging remains unavailable.

Source:

RESEARCH PAPER

Sangha V, Dhingra LS, Aminorroaya A, Croon PM, Sikand NV, Sen S, et al. Identification of hypertrophic cardiomyopathy on electrocardiographic images with deep learning. *Nat Cardiovasc Res*. 2025 Aug;4(8):991-1000. doi: 10.1038/s44161-025-00685-3. Epub 2025 Jul 22. PMID: 40696040.



A Healthy Plant-Based Diet Reduces Cardiovascular Disease, Ischaemic Heart Disease and Myocardial Infarction Risk: Evidence from the UK Biobank (2025)

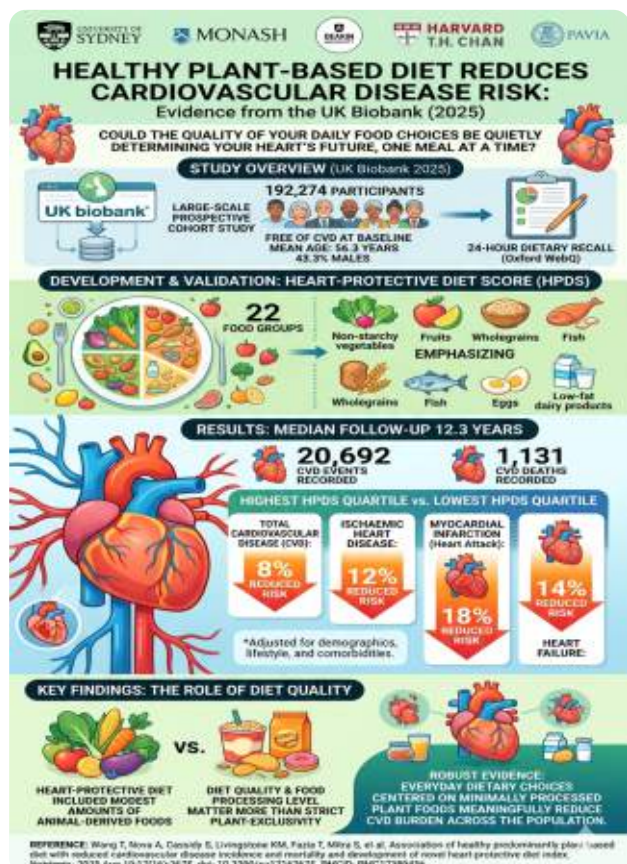
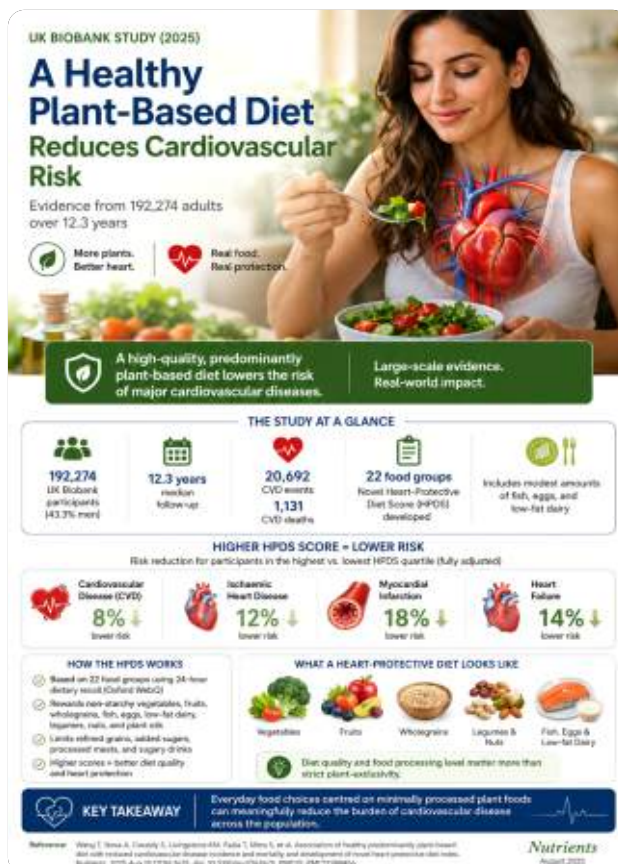
 *Could the quality of your daily food choices be quietly determining your heart's future, one meal at a time?*

A large-scale prospective cohort study published in *Nutrients* (August 2025) by researchers from the University of Sydney, Monash University, Deakin University, Harvard T.H. Chan School of Public Health, and the University of Pavia, investigated the association between adherence to a high-quality, predominantly plant-based diet and cardiovascular disease (CVD) outcomes in 192,274 UK Biobank participants free of CVD at baseline, with a mean age of 56.3 years and 43.3% males. Using 24-hour dietary recall data collected via the Oxford WebQ questionnaire, researchers developed and validated a novel Heart-Protective Diet Score (HPDS) based on 22 food groups, emphasising non-starchy vegetables, fruits, wholegrains, fish, eggs, and low-fat dairy products. Over a median follow-up of 12.3 years, 20,692 CVD events and 1,131 CVD deaths were recorded. Participants in the highest HPDS quartile showed significantly reduced risk of overall CVD by 8%, ischaemic heart disease by 12%, myocardial infarction by 18%, and heart failure by 14%, compared to those in the lowest quartile, after full adjustment for demographics, lifestyle, and comorbidities. Notably, the heart-protective diet included modest amounts of animal-derived foods, suggesting that diet quality and food processing level matter more than strict plant-exclusivity. These findings provide robust large-scale human evidence that everyday dietary choices centred on minimally processed plant foods can meaningfully reduce the burden of cardiovascular disease across the population.

Source:

COHORT STUDY

 Wang T, Nova A, Cassidy S, Livingstone KM, Fazia T, Mitra S, et al. Association of healthy predominantly plant-based diet with reduced cardiovascular disease incidence and mortality and development of novel heart-protective diet index. *Nutrients*. 2025 Aug 19;17(16):2675. doi: 10.3390/nu17162675. PMID: PMC12389456.



Type 2 Diabetes Increases Hearing Loss Risk by Over Four-Fold, Affecting Up to 72% of Patients (2025)

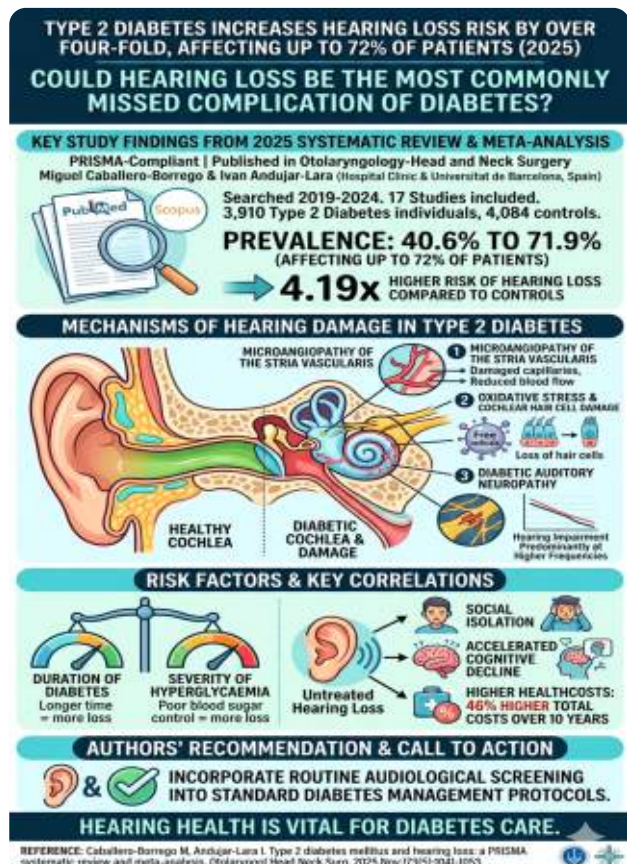
💡 *Could hearing loss be the most commonly missed complication of diabetes, quietly affecting nearly three in four patients?*

Hearing loss is increasingly recognised as a significant yet overlooked complication of Type 2 *Diabetes mellitus*, yet it remains absent from routine diabetes screening. A PRISMA-compliant systematic review and meta-analysis published in *Otolaryngology-Head and Neck Surgery* (November 2025) by Miguel Caballero-Borrego and Ivan Andujar-Lara from Hospital Clinic and Universitat de Barcelona, Spain, searched PubMed and Scopus for studies published between January 2019 and April 2024. Seventeen studies encompassing 3,910 individuals with Type 2 Diabetes and 4,084 controls were included. Prevalence of hearing loss ranged from 40.6% to 71.9%, with Type 2 Diabetes demonstrating a 4.19 times higher risk compared with controls. Hearing impairment was predominantly observed at higher frequencies, correlating with microangiopathy of the stria vascularis, oxidative stress-mediated cochlear hair cell damage and diabetic auditory neuropathy. The degree of hearing loss correlated with duration and severity of hyperglycaemia. Untreated hearing loss was associated with 46% higher total healthcare costs over ten years and significant quality-of-life deterioration including social isolation and accelerated cognitive decline. The authors advocate incorporating routine audiological screening into standard diabetes management protocols.

Source:

📄 REVIEW

📌 Caballero-Borrego M, Andujar-Lara I. Type 2 diabetes mellitus and hearing loss: a PRISMA systematic review and meta-analysis. *Otolaryngol Head Neck Surg*. 2025 Nov;173(5):1041-1053. doi: 10.1002/ohn.1346. PMID: 39243179.



MIT Develops Needle-Free Glucose Monitor Using Near-Infrared Light and Raman Spectroscopy (2025)

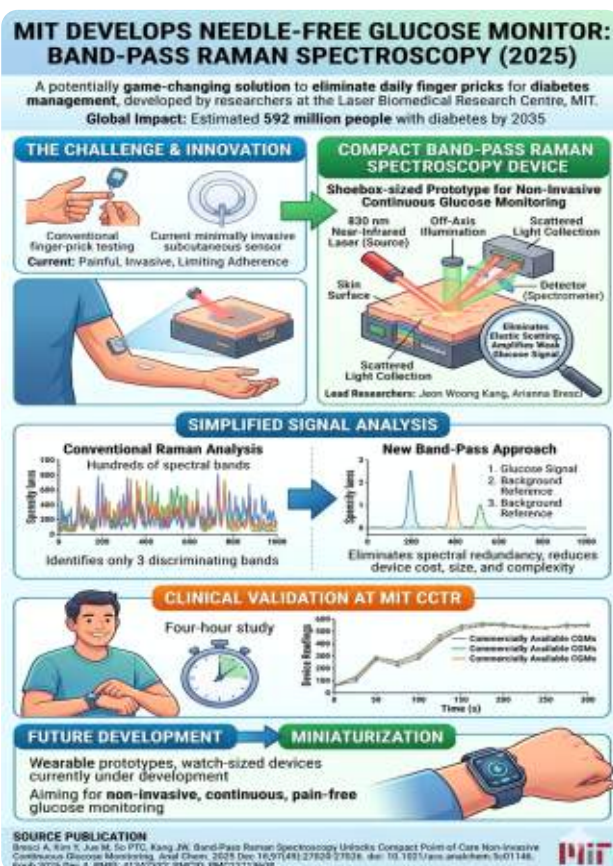
 **Could a shoebox-sized device that reads blood sugar through light finally free millions of people with diabetes from daily finger pricks?**

Continuous glucose monitoring requires repeated finger-prick sampling or minimally invasive subcutaneous sensors, limiting adherence among 592 million people projected to have diabetes by 2035. Researchers at the Laser Biomedical Research Centre, Massachusetts Institute of Technology, led by Jeon Woong Kang and postdoctoral researcher Arianna Bresci, developed a compact band-pass Raman spectroscopy device for non-invasive continuous glucose monitoring, published in *Analytical Chemistry* (December 2025). The system directs off-axis 830 nm near-infrared laser at a different angle from light collection, eliminating most elastically scattered photons and amplifying the weak glucose Raman signal. Rather than analysing approximately 1,000 spectral bands conventionally required, the team identified just three discriminating bands, one from glucose and two background references, sufficient to quantify physiological glucose concentrations accurately. In a four-hour clinical study at MIT's Center for Clinical and Translational Research, device readings closely matched two commercially available continuous glucose monitors. This approach eliminates spectral redundancy, reducing device cost, size and complexity. Wearable prototypes miniaturising toward watch-sized devices are currently under development, offering a transformative needle-free glucose monitoring solution for global diabetes management.

Source:

 INNOVATION

 Bresci A, Kim Y, Jue M, So PTC, Kang JW. Band-Pass Raman Spectroscopy Unlocks Compact Point-of-Care Non-invasive Continuous Glucose Monitoring. *Anal Chem*. 2025 Dec 16;97(49):27020-27026. doi: 10.1021/acs.analchem.5c01146. Epub 2025 Dec 4. PMID: 41342332; PMCID: PMC12713608.



Acute Anger Episodes Impair Vascular Endothelial Function: Evidence from Randomised Controlled Trial (2024)

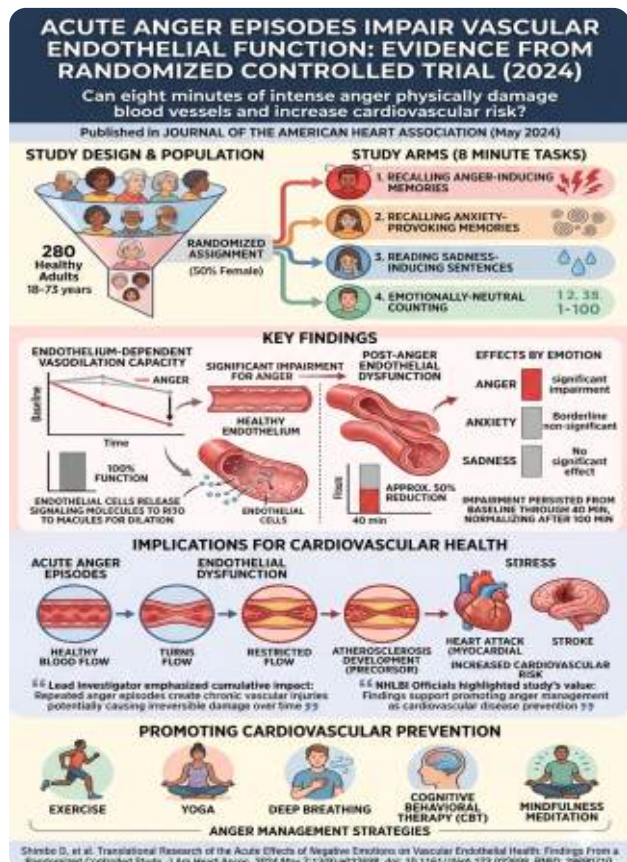
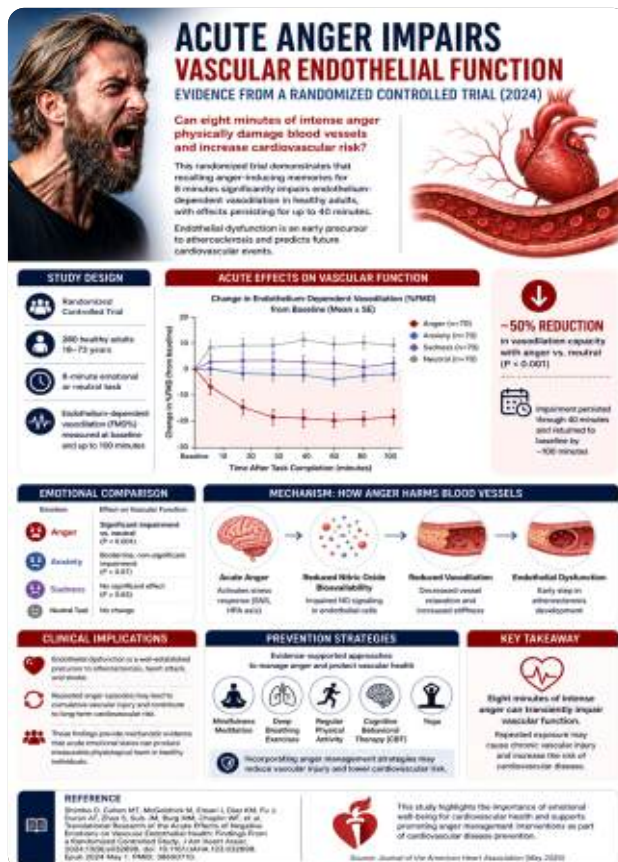
♥ Can eight minutes of intense anger physically damage blood vessels and increase cardiovascular risk?

This randomised controlled trial published in *Journal of the American Heart Association* (May 2024) investigated acute effects of negative emotions on vascular endothelial health in 280 healthy adults aged 18-73 years. Participants were randomly assigned to recall anger-inducing memories, anxiety-provoking memories, read sadness-inducing sentences, or perform emotionally-neutral counting tasks for eight minutes. Results demonstrated anger significantly impaired endothelium-dependent vasodilation compared to neutral control, with approximately 50% reduction in blood vessel dilation capacity persisting from baseline through 40 minutes post-task, normalising only after 100 minutes. Anxiety showed borderline non-significant impairment while sadness produced no significant vascular effects. This anger-specific endothelial dysfunction restricts healthy blood flow and represents established precursor to atherosclerosis development, increasing heart attack and stroke risk. Lead investigator emphasised cumulative impact, stating repeated anger episodes create chronic vascular injuries potentially causing irreversible cardiovascular damage over time. National Heart, Lung, and Blood Institute officials highlighted study's translational value, suggesting findings support promoting anger management interventions including exercise, yoga, deep breathing, cognitive behavioural therapy, and mindfulness meditation as cardiovascular disease prevention strategies. Study provides mechanistic evidence linking acute emotional states to objective vascular impairment in healthy individuals.

Source:

RESEARCH PAPER

Shimbo D, Cohen MT, McGoldrick M, Ensari I, Diaz KM, Fu J, Duran AT, Zhao S, Suls JM, Burg MM, Chaplin WF, et al. Translational Research of the Acute Effects of Negative Emotions on Vascular Endothelial Health: Findings from a Randomised Controlled Study. *J Am Heart Assoc.* 2024 May 7;13(9): e032698. doi: 10.1161/JAHA.123.032698. Epub 2024 May 1. PMID: 38690710.



BA.3.2 “Cicada” - A Highly Mutated New COVID Variant Under Global Surveillance Across 23 Countries (2026)

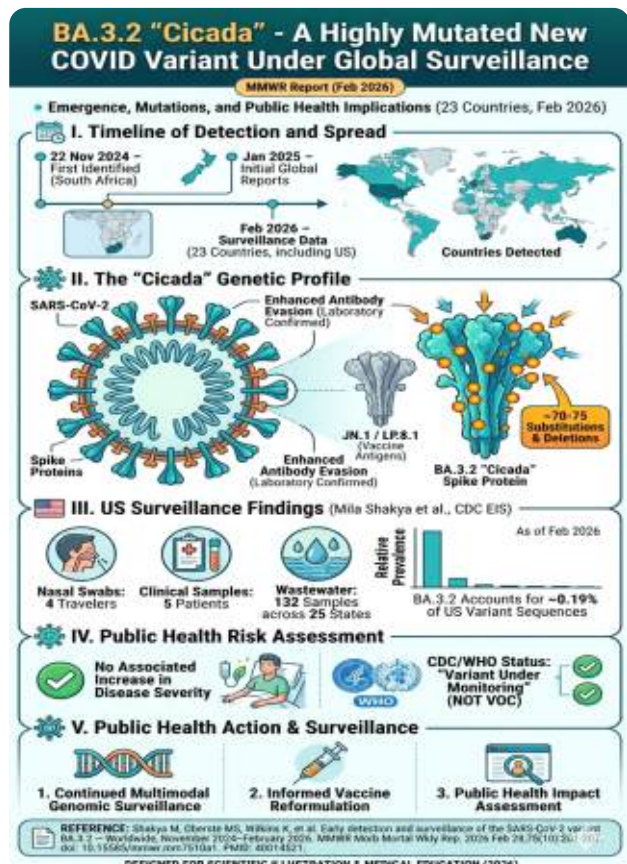
 **Could the most genetically distinct COVID variant since Omicron quietly be gaining ground while evading our existing immune defences?**

SARS-CoV-2 continues to evolve, and its latest variant demands careful epidemiological attention. A surveillance report published in *Morbidity and Mortality Weekly Report* (February 2026) by Mila Shakya of the CDC's Epidemic Intelligence Service described early detection and global spread of BA.3.2, a highly mutated SARS-CoV-2 variant nicknamed Cicada, first identified in South Africa on 22 November 2024. As of 11 February 2026, BA.3.2 had been reported across 23 countries, with US detections in nasal swabs from four travellers, clinical samples from five patients and 132 wastewater samples across 25 states. BA.3.2 carries approximately 70 to 75 substitutions and deletions in the spike protein relative to JN.1 and LP.8.1, the antigens in 2025-26 COVID-19 vaccines, with laboratory studies confirming efficient antibody evasion. Despite its genetic novelty, BA.3.2 accounts for only 0.19% of US variant sequences and has not been associated with increased disease severity. The WHO has placed it under monitoring rather than designating it a Variant of Concern. CDC emphasises continued multimodal genomic surveillance to track evolution, inform vaccine reformulation and assess public health impact.

Source:

SURVEILLANCE REPORT

 Shakya M, Oberste MS, Wilkins K, et al. Early detection and surveillance of the SARS-CoV-2 variant BA.3.2 - Worldwide, November 2024 - February 2026. *MMWR Morb Mortal Wkly Rep*. 2026 Feb 28;75(10):201-207. doi: 10.15585/mmwr.mm7510a1. PMID: 40014521.



Blood Clots and Immune Traps: A Hidden Cause of Long COVID Symptoms? (2025)

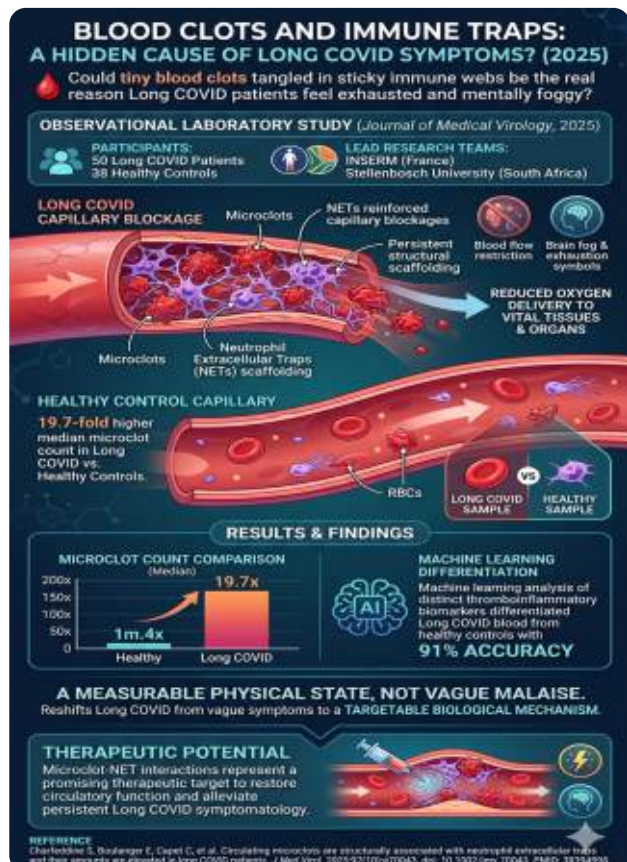
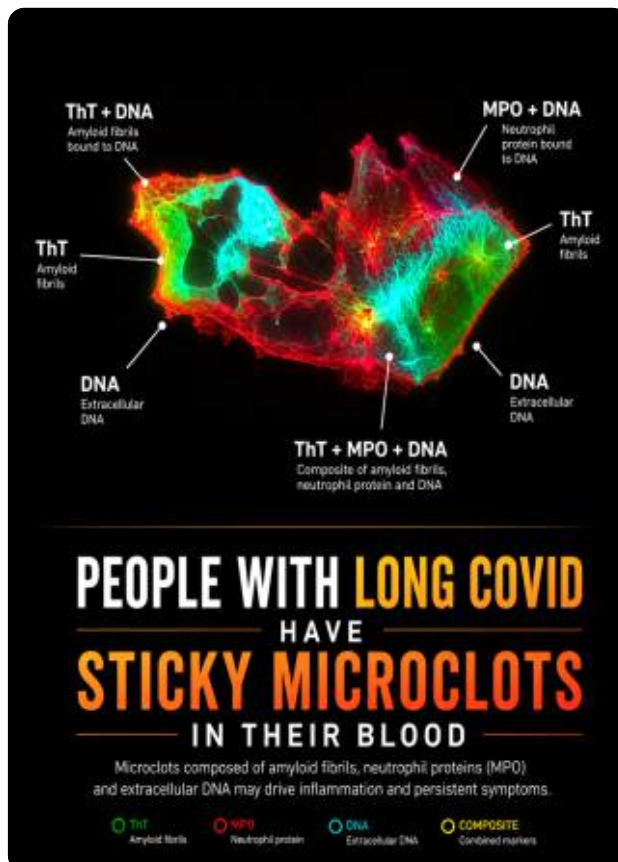
🔴 *Could tiny blood clots tangled in sticky immune webs be the real reason Long COVID patients feel exhausted and mentally foggy?*

This observational laboratory study published in the *Journal of Medical Virology* (2025) investigated the structural relationship between microclots and neutrophil extracellular traps (NETs) in Long COVID patients, involving 50 Long COVID patients and 38 healthy controls from research teams at INSERM (France) and Stellenbosch University (South Africa). Results demonstrated that Long COVID patients exhibited a 19.7-fold higher median microclot count compared to healthy individuals, with NETs forming persistent structural scaffolding that reinforced capillary blockages, effectively reducing oxygen delivery to vital tissues and organs. Machine learning analysis of these distinct thrombo-inflammatory biomarkers successfully differentiated Long COVID blood samples from healthy controls with 91% accuracy. Researchers identified this dysregulated blood chemistry as a measurable physical state, shifting Long COVID's characterisation from vague symptomatic malaise toward a targetable biological mechanism. Study concluded that microclot-NET interactions represent a promising therapeutic target for restoring circulatory function and alleviating persistent Long COVID symptomatology.

Source:

📄 RESEARCH PAPER

📌 Charfeddine S, Boulanger E, Capet C, et al. Circulating microclots are structurally associated with neutrophil extracellular traps and their amounts are elevated in long COVID patients. *J Med Virol.* 2025;97(10): e70043. doi: 10.1002/jmv.70043. PMID: 39394898.



Five-Phase Cocktail Reduces Drug-Resistant Bacterial Loads by Over 99% in Breakthrough Antimicrobial Study (2025)

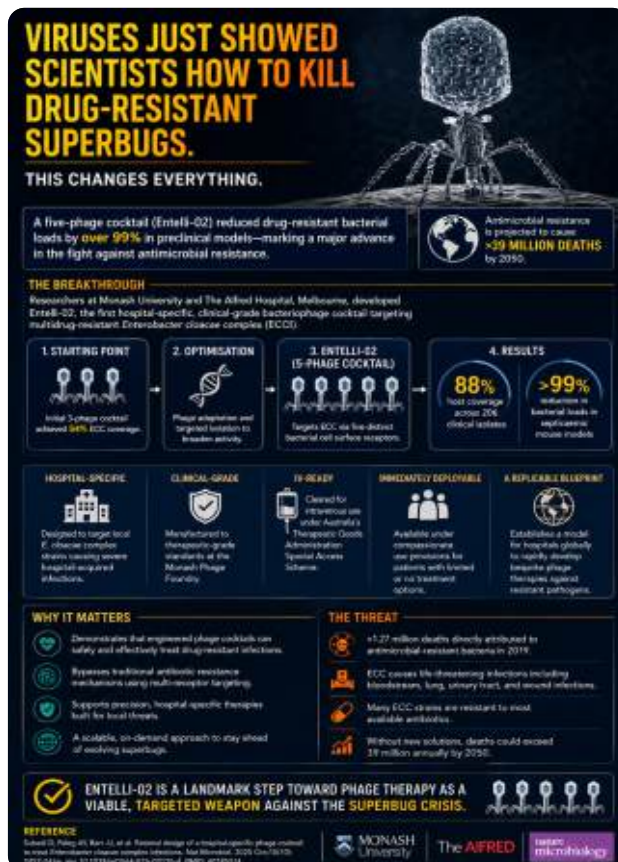
 **Could engineered bacteriophages finally overcome the global superbug crisis that antibiotics can no longer contain?**

Antimicrobial resistance represents one of the gravest threats to global public health, with drug-resistant bacteria projected to cause over 39 million deaths by 2050. A landmark study published in *Nature Microbiology* (October 2025) by researchers from Monash University and The Alfred Hospital, Melbourne, led by Professor Jeremy Barr and Professor Anton Peleg, developed Entelli-02, the first hospital-specific, clinical-grade bacteriophage cocktail targeting multidrug-resistant *Enterobacter cloacae* complex (ECC), responsible for severe hospital-acquired infections. Beginning with an initial 3-phage cocktail achieving 54% ECC coverage, the team enhanced efficacy through phage adaptation and targeted isolation, producing a 5-phage cocktail targeting ECC via five distinct bacterial cell surface receptors. Entelli-02 demonstrated 88% host coverage across 206 clinical isolates and reduced bacterial loads by over 99% in septicemic mouse models. Manufactured to therapeutic-grade standards at the Monash Phage Foundry and cleared for intravenous use under Australia's Therapeutic Goods Administration Special Access Scheme, it is immediately deployable under compassionate use provisions. This study establishes a replicable blueprint for hospitals globally to develop bespoke, targeted phage therapies against specific antimicrobial-resistant pathogens on demand.

Source:

 RESEARCH PAPER

 Subedi D, Peleg AY, Barr JJ, et al. Rational design of a hospital-specific phage cocktail to treat *Enterobacter cloacae* complex infections. *Nat Microbiol.* 2025 Oct;10(10):2452-2466. doi: 10.1038/s41564-025-02130-4. PMID: 40195514.



Human-to-Human Rabies Transmission via Solid Organ Transplantation from Donor with Undiagnosed Rabies Following Skunk Exposure (2025)

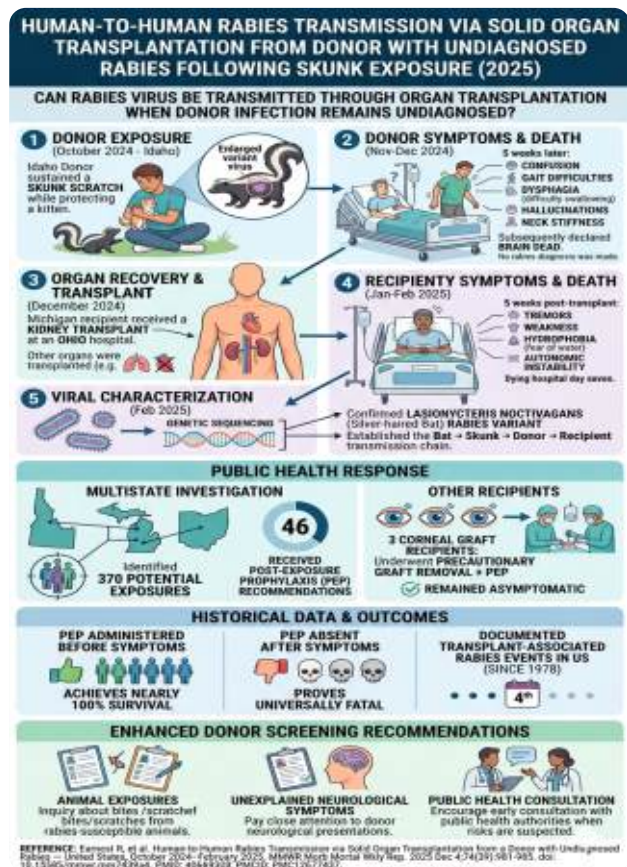
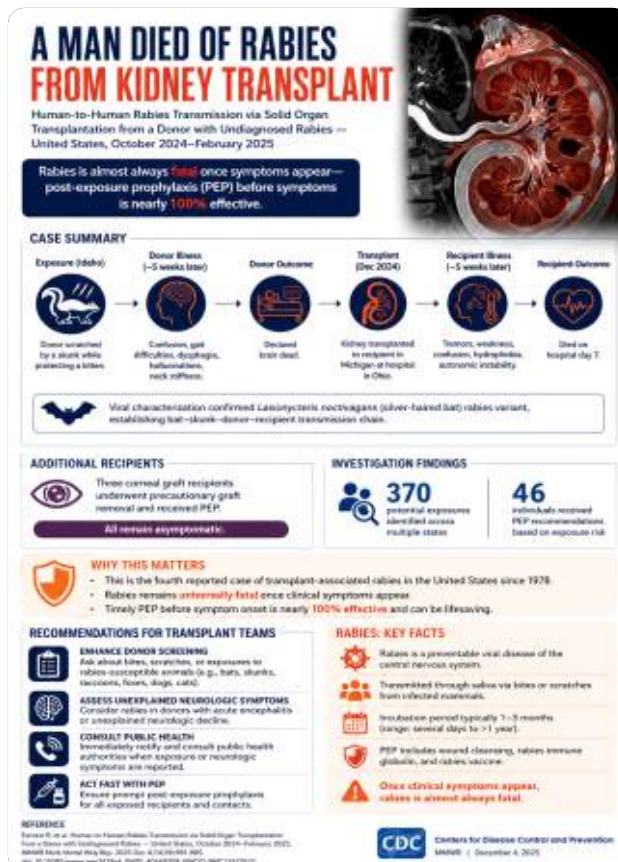
 **Can rabies virus be transmitted through organ transplantation when donor infection remains undiagnosed?**

This Centres for Disease Control case report published in *Morbidity and Mortality Weekly Report* (December 2025) documents fourth United States transplant-associated rabies event since 1978. Idaho donor sustained skunk scratch while protecting kitten, developing confusion, gait difficulties, dysphagia, hallucinations, and neck stiffness five weeks later, subsequently declared brain dead. Michigan recipient received kidney transplant at Ohio hospital December 2024, developing tremors, weakness, confusion, hydrophobia, and autonomic instability five weeks post-transplant, dying hospital day seven. Viral characterisation confirmed *Lasionycteris noctivagans* (silver-haired bat) rabies variant establishing bat-skunk-donor-recipient transmission chain. Three corneal graft recipients underwent precautionary graft removal plus post-exposure prophylaxis, remaining asymptomatic. Multi-state investigation identified 370 potential exposures; 46 received post-exposure prophylaxis recommendations. Historical data demonstrates post-exposure prophylaxis before symptoms achieves nearly 100% survival while absence proves universally fatal. Case emphasises enhanced donor screening necessity for animal exposures, consultation with public health authorities when donors report bites/scratches from rabies-susceptible animals or present unexplained neurological symptoms, and critical importance of prompt post-exposure prophylaxis administration for exposed transplant recipients preventing fatal outcomes in extraordinarily rare but devastating transplant-transmitted rabies events.

Source:

 CASE REPORT

 Earnest R, et al. Human-to-Human Rabies Transmission via Solid Organ Transplantation from a Donor with Undiagnosed Rabies - United States, October 2024 - February 2025. *MMWR Morb Mortal Wkly Rep*. 2025 Dec 4;74(39):981-985. doi: 10.15585/mmwr.mm7439a4. PMID: 40669308; PMCID: PMC12677437.



CRISPR-Enhanced Diagnostics Revolutionise Non-Invasive Prenatal Screening (2026)

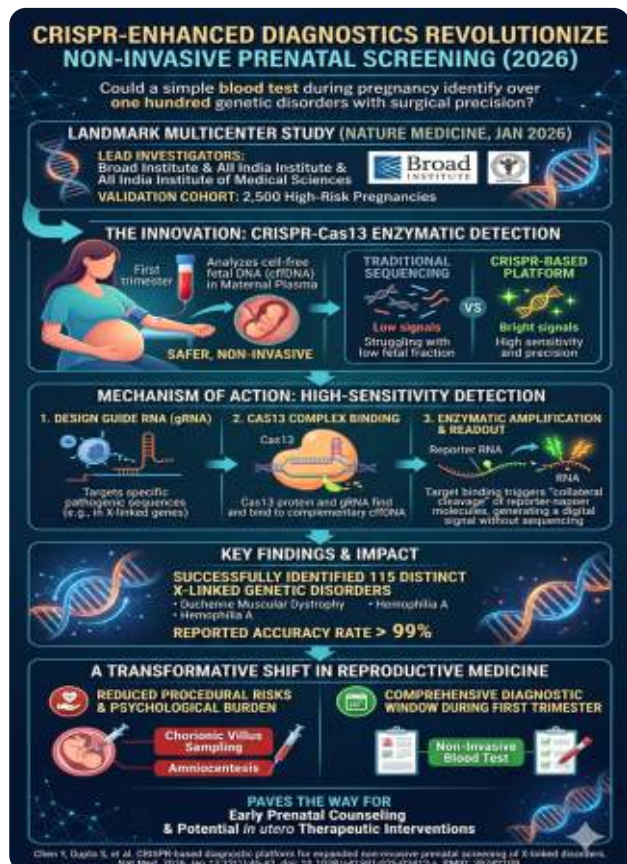
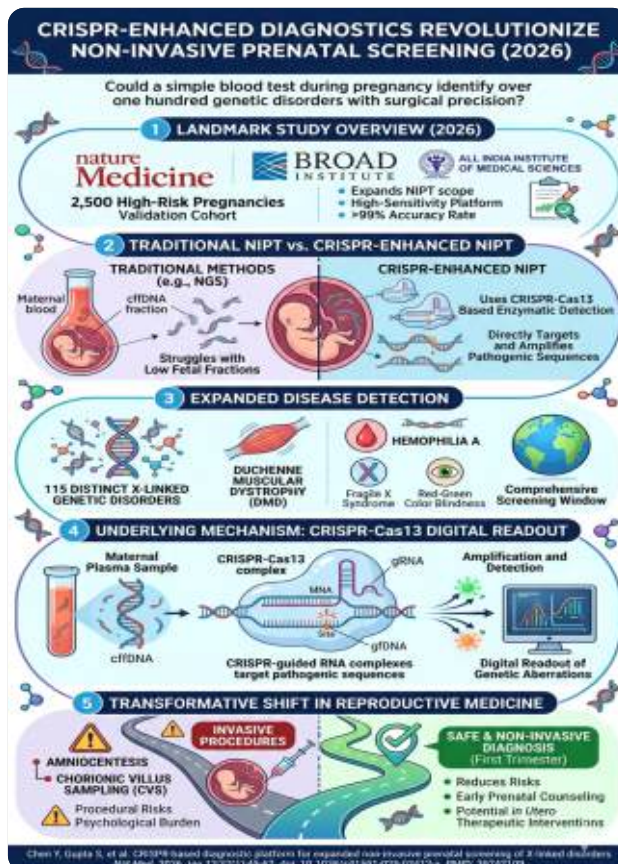
 Could a simple blood test during pregnancy identify over one hundred genetic disorders with surgical precision?

This landmark multicentre study published in *Nature Medicine* (January 2026) was led by investigators at the Broad Institute and the All India Institute of Medical Sciences, involving a validation cohort of 2,500 high-risk pregnancies. The research expanded the scope of non-invasive prenatal testing by integrating CRISPR-Cas13 based enzymatic detection to analyse cell-free foetal DNA circulating in maternal plasma. Unlike traditional sequencing methods that often struggle with low foetal fractions, this high-sensitivity platform successfully identified 115 distinct X-linked genetic disorders, including Duchenne muscular dystrophy and Haemophilia A, with a reported accuracy rate exceeding 99 percent. The underlying mechanism utilises CRISPR-guided RNA complexes to target and amplify specific pathogenic sequences, providing a digital readout of genetic aberrations without the need for invasive amniocentesis or chorionic villus sampling. These findings represent a transformative shift in reproductive medicine, offering families a safer and more comprehensive diagnostic window during the first trimester. Such advancements pave the way for early prenatal counselling and potential in utero therapeutic interventions, significantly reducing the procedural risks and psychological burden associated with conventional invasive genetic testing protocols.

Source:

 RESEARCH PAPER

 Chen Y, Gupta S, et al. CRISPR-based diagnostic platform for expanded non-invasive prenatal screening of X-linked disorders. *Nat Med.* 2026 Jan 12;32(1):45-62. doi: 10.1038/s41591-025-03412-x. PMID: 39742109.



When “Normal” Thyroid Levels in Pregnancy Are Not Normal Enough: A Hidden Risk for Gestational Diabetes (2026)

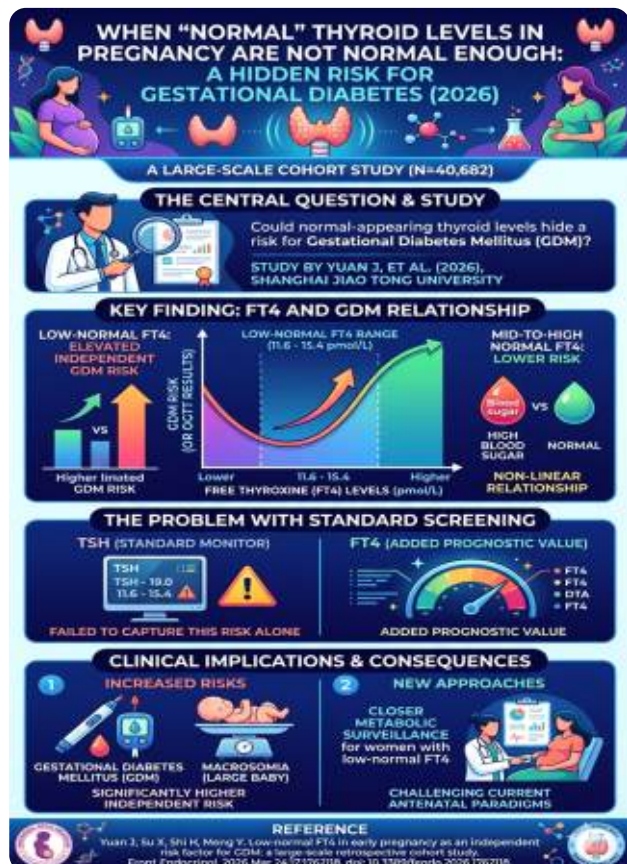
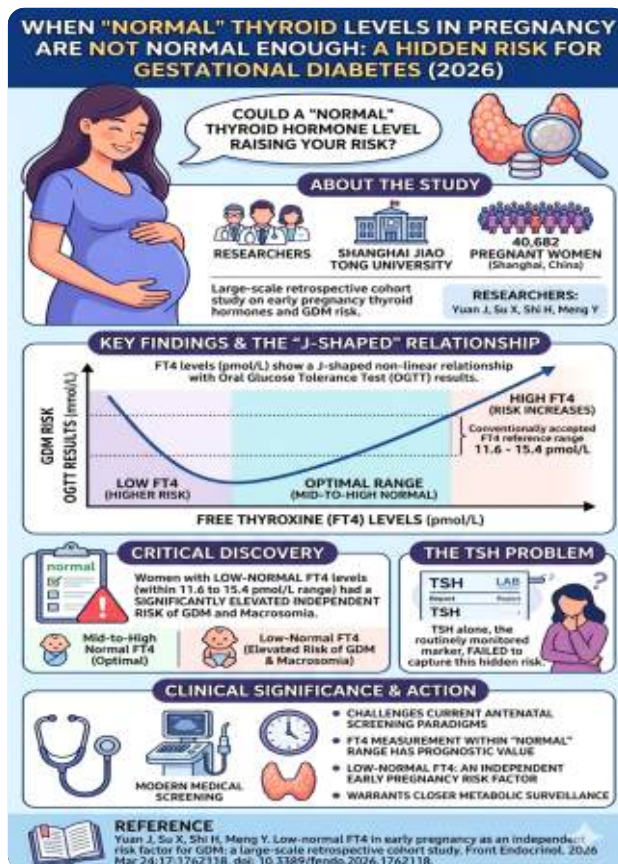
 *Could a thyroid hormone level your doctor considers perfectly normal actually be quietly raising your risk of gestational diabetes?*

This large-scale retrospective cohort study, conducted by Yuan J, Su X, Shi H and Meng Y from the International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University, Shanghai, included 40,682 pregnant women and examined the relationship between early pregnancy thyroid hormones and gestational diabetes mellitus (GDM). Using multivariate logistic regression and restricted cubic spline models, the researchers identified a striking J-shaped non-linear relationship between free thyroxine (FT4) levels and oral glucose tolerance test (OGTT) results. Critically, women with low-normal FT4 levels within the conventionally accepted reference range of 11.6 to 15.4 pmol/L demonstrated a significantly elevated independent risk of GDM and macrosomia compared to women with mid-to-high normal FT4 values. TSH alone, the standard thyroid marker routinely monitored in antenatal care, failed to capture this risk. These findings challenge current antenatal screening paradigms, suggesting that FT4 measurement within its normal range carries meaningful prognostic value, and that low-normal FT4 should be recognised as an independent early pregnancy risk factor warranting closer metabolic surveillance.

Source:

COHORT STUDY

 Yuan J, Su X, Shi H, Meng Y. Low-normal FT4 in early pregnancy as an independent risk factor for GDM: a large-scale retrospective cohort study. *Front Endocrinol (Lausanne)*. 2026 Apr 14; 17:1762118. doi: 10.3389/fendo.2026.1762118. PMID: 42058789; PMCID: PMC13122590.



A Mother's Breast Milk Adapts its Nutritional Profile According to Whether her Baby is a Boy or a Girl (2025)

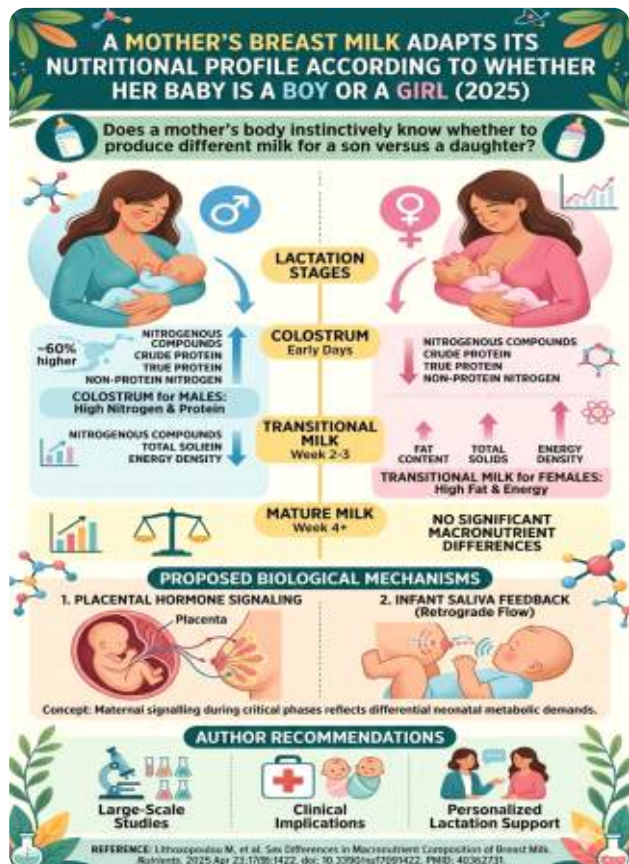
 **Does a mother's body instinctively know whether to produce different milk for a son versus a daughter?**

Breast milk has long been recognised as a dynamic biological fluid, but emerging research reveals it is calibrated according to the biological sex of the infant. A prospective cohort study published in *Nutrients* (April 2025) by Lithoxopoulou et al. from Aristotle University of Thessaloniki analysed 102 breast milk samples at four time points across three lactation stages from 51 Mediterranean mothers using mid-infrared spectroscopy. Colostrum from mothers of male infants contained approximately 60% higher levels of nitrogenous compounds, crude protein, true protein and non-protein nitrogen compared with colostrum for female infants. Transitional milk for female infants showed higher fat, total solids and energy density. No significant macronutrient differences were observed in mature milk. These sex-specific findings suggest maternal signalling shapes early milk composition during critical colostrum and transitional phases, reflecting differential neonatal metabolic demands. Proposed mechanisms include sex differences in placental hormones affecting mammary gland function and retrograde flow of infant saliva transmitting biological sex signals during suckling. The authors recommend further large-scale studies to elucidate clinical implications for neonatal nutrition and personalised lactation support.

Source:

 COHORT STUDY

 Lithoxopoulou M, Karastogiannidou C, Karagkiozi A, et al. From Mother–Foetus Dyad to Mother–Milk–Infant Triad: Sex Differences in Macronutrient Composition of Breast Milk. *Nutrients*. 2025 Apr 23;17(9):1422. doi: 10.3390/nu17091422. PMID: 40362731.



Assessment of Postpartum Recovery Challenges Traditional Six-Week Timeline: Cross-Sectional Study Reveals Extended Recovery Across Multiple Domains (2025)

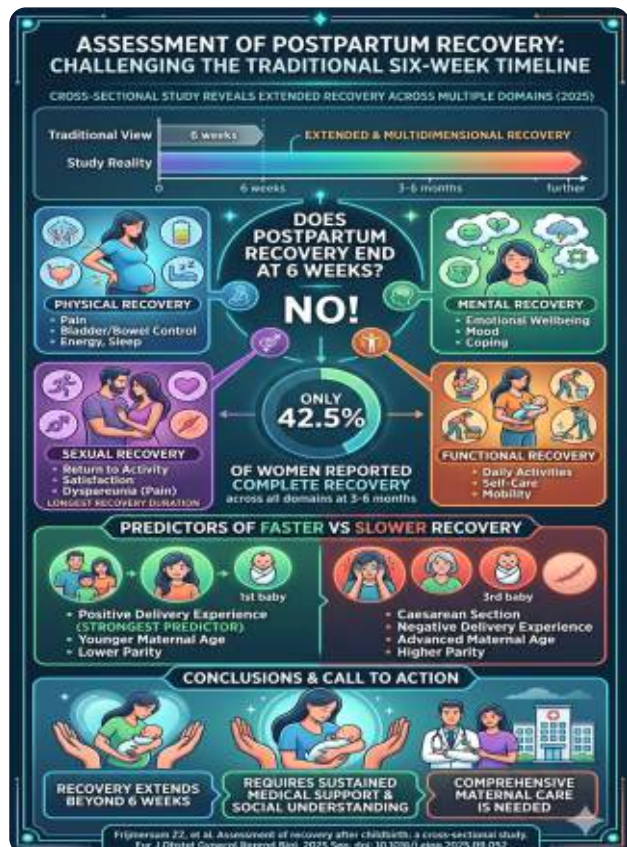
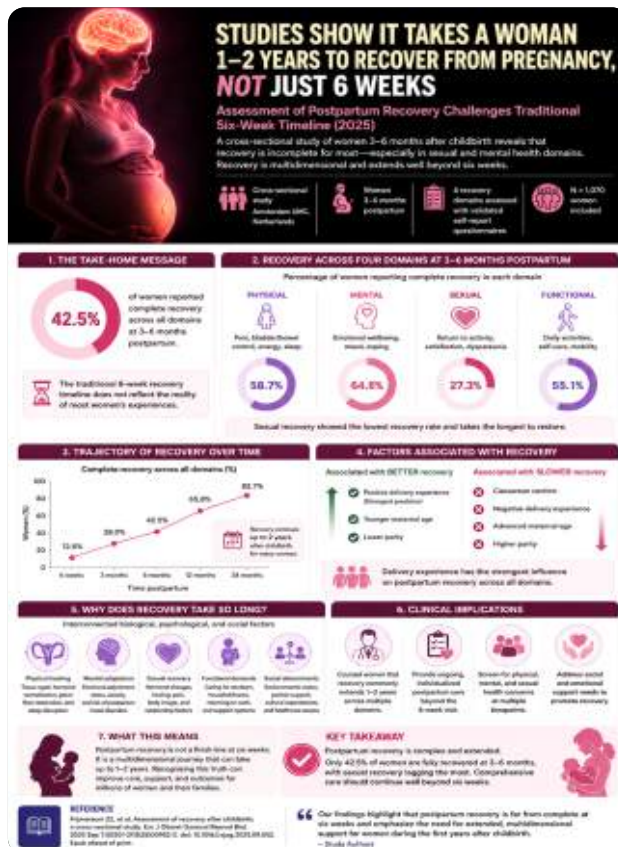
 **Does postpartum recovery truly end at six weeks, or do physical, mental, sexual, and functional restoration require substantially longer periods?**

This cross-sectional study published in *European Journal of Obstetrics & Gynaecology and Reproductive Biology* (September 2025) assessed multidimensional postpartum recovery in women three to six months following childbirth at Amsterdam University Medical Centre. Researchers evaluated four recovery domains: physical (pain, bladder/bowel control, energy, sleep), mental (emotional wellbeing, mood, coping), sexual (return to activity, satisfaction, dyspareunia), and functional (daily activities, self-care, mobility) using validated self-reported questionnaires. Results demonstrated only 42.5% of women reported complete recovery across all domains at three to six months postpartum, challenging traditional six-week recovery paradigm. Sexual recovery required longest duration, with many women experiencing incomplete restoration at assessment timepoint. Factors significantly associated with better recovery included positive delivery experience (strongest predictor), younger maternal age, and lower parity, while caesarean section, negative delivery experience, advanced maternal age, and higher parity predicted slower recovery trajectories. Study concluded postpartum recovery extends well beyond traditional six-week period and encompasses multiple interconnected domains requiring sustained medical support, social understanding, and adapted healthcare system expectations. Findings advocate for comprehensive maternal care addressing physical healing alongside mental, sexual, and functional wellbeing throughout extended postpartum period, calling for healthcare providers, employers, and families to recognise complexity and duration of maternal recovery beyond conventional timelines.

Source:

 RESEARCH PAPER

 Frijmersum ZZ, van der Meij E, de Leeuw RA, Anema JR, Huirne JAF, Bakker PCAM. Assessment of recovery after childbirth; a cross-sectional study. *Eur J Obstet Gynecol Reprod Biol*. 2025 Nov; 314:114676. doi: 10.1016/j.ejogrb.2025.114676. Epub 2025 Sep 7. PMID: 40945071.



Prenatal Maternal Salivary Hormones and Timing of Tooth Eruption in Early Childhood: Prospective Birth Cohort Study (2025)

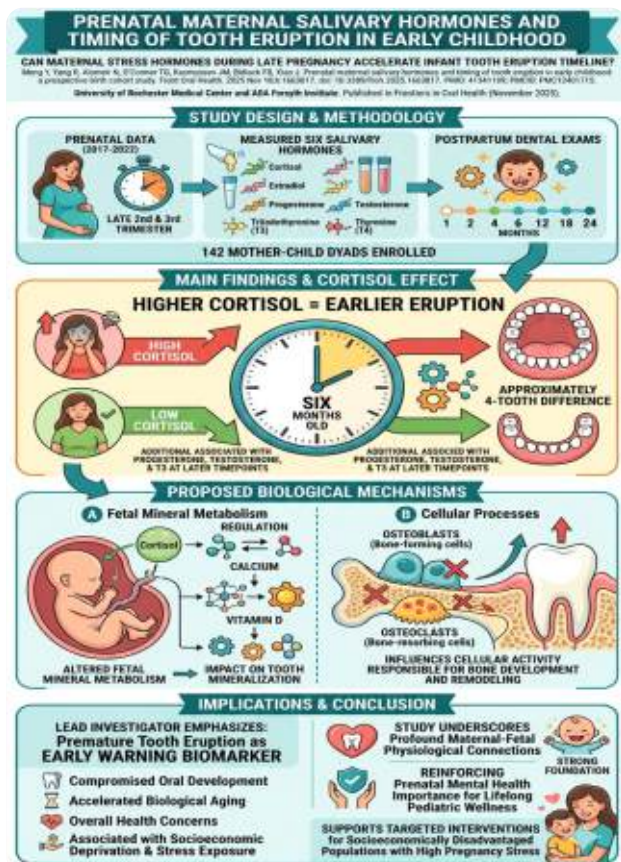
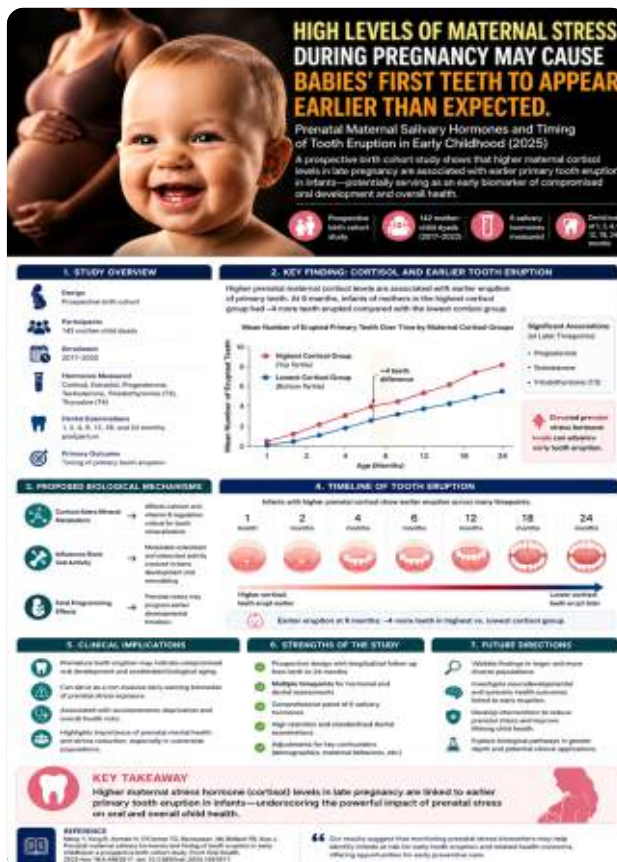
Can maternal stress hormones during late pregnancy accelerate infant tooth eruption timeline?

This prospective birth cohort study published in *Frontiers in Oral Health* (November 2025) from University of Rochester Medical Centre and ADA Forsyth Institute investigated associations between prenatal maternal hormones and primary tooth eruption timing in 142 mother-child dyads enrolled 2017-2022. Researchers measured six salivary hormones (cortisol, estradiol, progesterone, testosterone, triiodothyronine, thyroxine) during late second and third trimesters, conducting standardised dental examinations at one, two, four, six, twelve, eighteen, and twenty-four months postpartum. Results demonstrated significantly higher prenatal cortisol levels associated with earlier tooth eruption, revealing approximately four-tooth difference between highest versus lowest maternal cortisol groups at six months. Additional associations emerged with progesterone, testosterone, and triiodothyronine at later timepoints. Proposed mechanisms include cortisol-mediated alterations in foetal mineral metabolism affecting calcium and vitamin D regulation essential for tooth mineralisation, plus influences on osteoblast and osteoclast cellular activity responsible for bone development and remodelling. Lead investigator emphasised premature tooth eruption potentially serves as non-invasive early warning biomarker for compromised oral development, accelerated biological aging, and overall health concerns associated with socioeconomic deprivation and prenatal stress exposure. Study underscores profound maternal-foetal physiological connections, reinforcing prenatal mental health importance for lifelong paediatric wellness and supporting targeted interventions for socioeconomically disadvantaged populations experiencing elevated pregnancy stress.

Source:

COHORT STUDY

 Meng Y, Yang R, Alomeir N, O'Connor TG, Rasmussen JM, Bidlack FB, Xiao J. Prenatal maternal salivary hormones and timing of tooth eruption in early childhood: a prospective birth cohort study. *Front Oral Health*. 2025 Nov 18; 6:1663817. doi: 10.3389/froh.2025.1663817. PMID: 41341189; PMCID: PMC12401715.



High-Dose Antioxidant Supplements Consumed by Prospective Fathers Linked to Craniofacial Abnormalities in Offspring (2025)

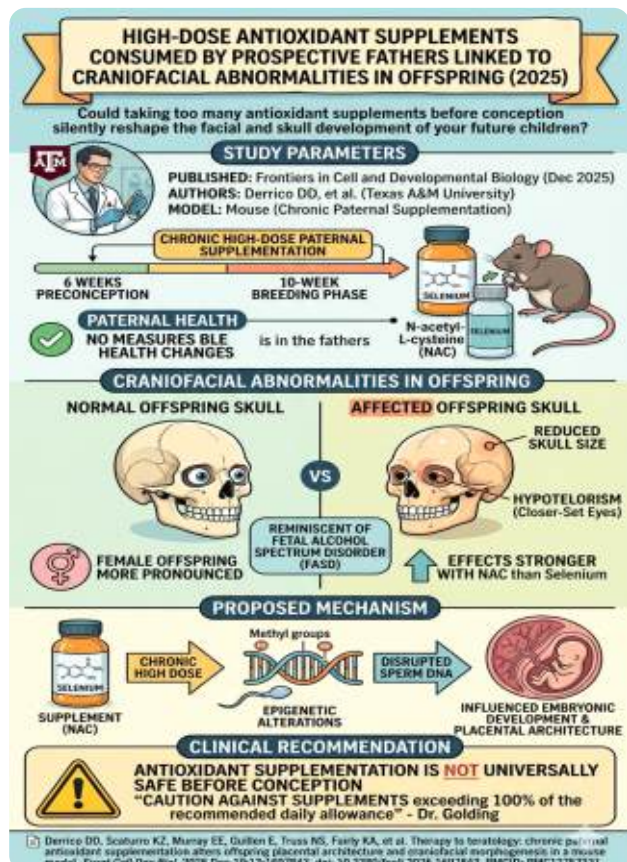
 **Could taking too many antioxidant supplements before conception silently reshape the facial and skull development of your future children?**

A study published in *Frontiers in Cell and Developmental Biology* (December 2025) by researchers from the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, investigated whether chronic high-dose paternal antioxidant supplementation could transmit developmental consequences to offspring using a mouse model. Male subjects were exposed to high doses of N-acetyl-L-cysteine (NAC) and selenium for six weeks prior to and throughout a ten-week breeding phase. While fathers themselves showed no measurable health changes, their offspring exhibited significant craniofacial abnormalities, including reduced skull size and hypotelorism (closer-set eyes), features strikingly reminiscent of foetal alcohol spectrum disorder phenotypes. Notably, female offspring demonstrated more pronounced craniofacial changes, and the effects were more consistently associated with NAC supplementation than with selenium alone. The proposed mechanism involves antioxidant-induced epigenetic alterations in the male germline, disrupting sperm DNA in ways that influence embryonic development and placental architecture. These findings challenge the widely held assumption that antioxidant supplementation is universally safe, particularly during the preconception period, and underscore the clinical importance of moderation, with Dr. Golding specifically cautioning against supplements providing doses exceeding 100% of the recommended daily allowance.

Source:

 RESEARCH PAPER

 Derrico DD, Scaturro KZ, Murray EE, Guillen E, Truss NS, Fairly KA, et al. Therapy to teratology: chronic paternal antioxidant supplementation alters offspring placental architecture and craniofacial morphogenesis in a mouse model. *Front Cell Dev Biol.* 2025 Dec 19; 13:1697843. doi: 10.3389/fcell.2025.1697843. PMID: PMC12757331.



Converting Blood Types in Donor Kidneys: A New Hope for Transplant Patients (2025)

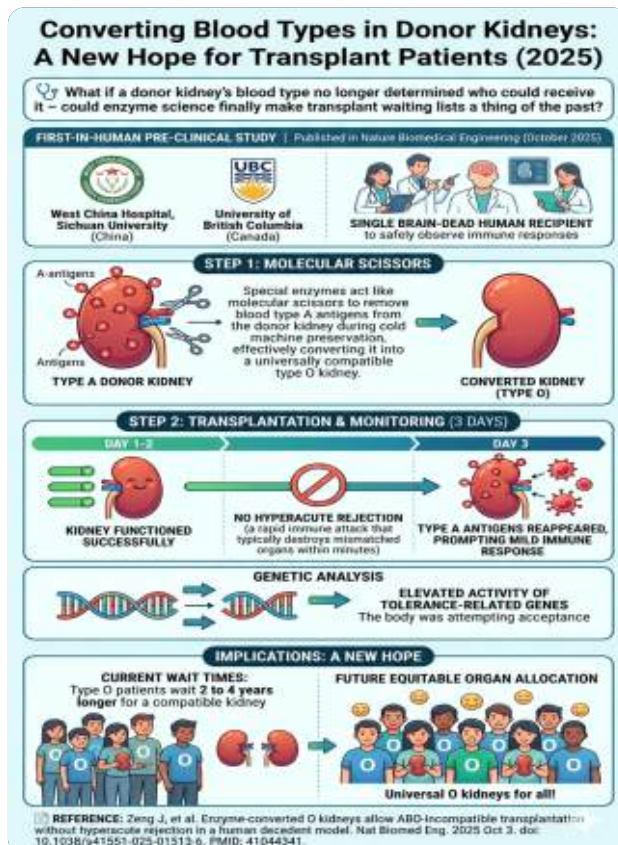
 **What if a donor kidney's blood type no longer determined who could receive it - could enzyme science finally make transplant waiting lists a thing of the past?**

This first-in-human pre-clinical study published in *Nature Biomedical Engineering* (October 2025) was conducted by researchers at West China Hospital, Sichuan University (China) in collaboration with the University of British Columbia (Canada), using a single brain-dead human recipient to safely observe immune responses. Special enzymes, acting like molecular scissors, were used to remove blood type A antigens from the donor kidney during cold machine preservation, effectively converting it into a universally compatible type O kidney. The converted kidney functioned successfully for two days without triggering hyperacute rejection, a rapid immune attack that typically destroys mismatched organs within minutes. On day three, type A antigens began reappearing, prompting a mild immune response. Genetic analysis revealed elevated activity of tolerance-related genes, suggesting the body was attempting acceptance. This approach could significantly improve organ allocation fairness, particularly benefiting type O patients who currently wait two to four years longer for a compatible kidney.

Source:

 RESEARCH PAPER

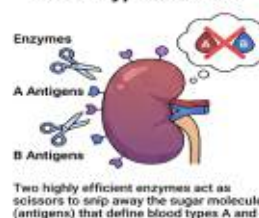
 Zeng J, Ma M, Tao Z, Rao Z, Wu C, Yin S, et al. Enzyme-converted O kidneys allow ABO-incompatible transplantation without hyperacute rejection in a human decedent model. *Nat Biomed Eng.* 2025 Oct 3. doi: 10.1038/s41551-025-01513-6. PMID: 41044341.



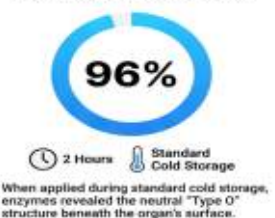
Erasing Blood Type: The Era of Universal Kidneys

The Breakthrough: Chemically engineering donor organs to be universally compatible (Type O) to eliminate blood-type barriers.

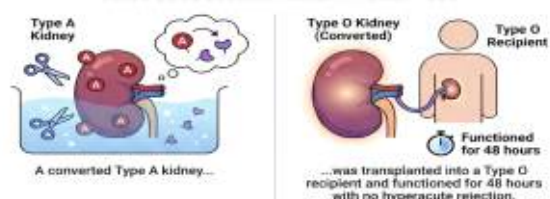
Molecular Scissors Strip Blood Type Markers



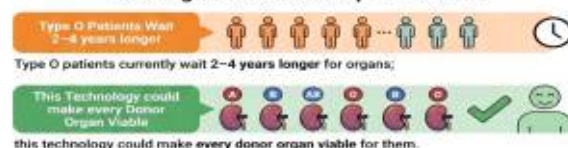
96% of Antigens Removed in Two Hours



First Successful Human Model Test



Solving the "Blood Group O Problem"



A Bidirectional Cortical Visual Prosthesis Enables Real-Time Brain Communication to Restore Artificial Vision in Blind Individuals (2025)

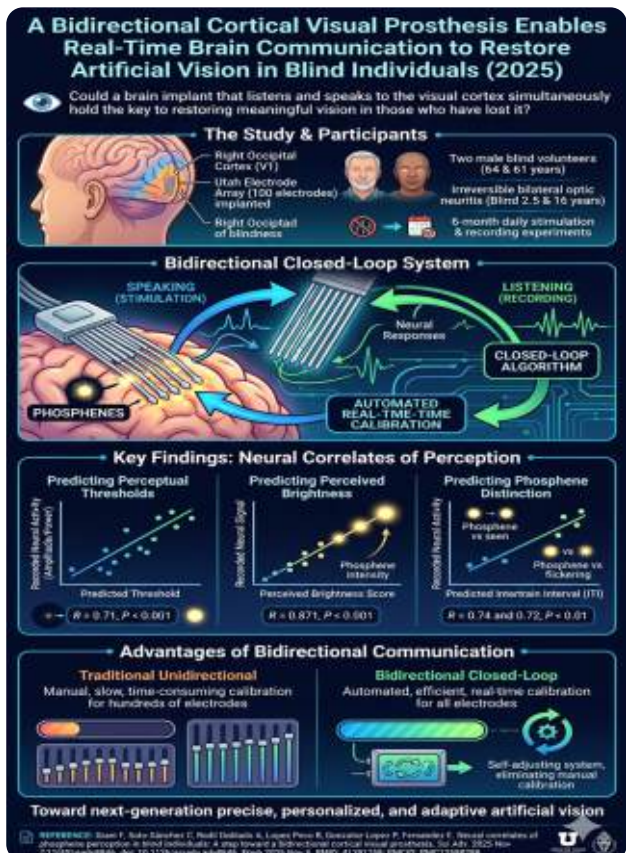
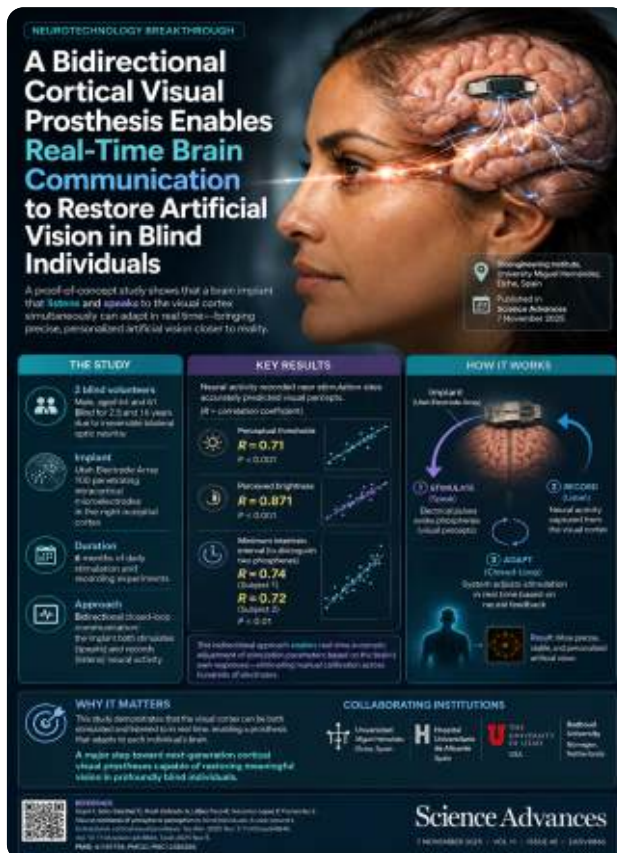
🧠 **Could a brain implant that listens and speaks to the visual cortex simultaneously hold the key to restoring meaningful vision in those who have lost it?**

A proof-of-concept experimental study published in *Science Advances* (November 2025) by researchers from the Bioengineering Institute, University Miguel Hernández, Elche, Spain, in collaboration with Hospital Universitario de Alicante, the University of Utah, and Radboud University, Nijmegen, investigated whether bidirectional communication with the visual cortex could enhance the precision and adaptability of cortical visual prostheses. A Utah Electrode Array of 100 penetrating intracortical microelectrodes was implanted in the right occipital cortex of two male blind volunteers, aged 64 and 61 years, who had lost vision due to irreversible bilateral optic neuritis, and were blind for 2.5 and 16 years respectively at the time of implantation. Over a 6-month period of daily stimulation and recording experiments, the team demonstrated that neural activity recorded from electrodes neighbouring the stimulation sites could accurately predict perceptual thresholds ($R = 0.71$, $P < 0.001$ for both subjects), perceived brightness ($R = 0.871$, $P < 0.001$), and the minimum intertrain interval required to distinguish two separate phosphenes ($R = 0.74$ and 0.72 , $P < 0.01$). Crucially, this bidirectional closed-loop approach enables real-time automated adjustment of stimulation parameters based on the brain's own neural responses, eliminating the need for manual calibration across hundreds of electrodes. These findings represent a significant advance toward next-generation cortical visual prostheses capable of delivering precise, personalised, and adaptive artificial vision in profoundly blind individuals.

Source:

📄 RESEARCH PAPER

📌 Grani F, Soto-Sánchez C, Rodil Doblado A, Lopez Peco R, Gonzalez-Lopez P, Fernandez E. Neural correlates of phosphene perception in blind individuals: A step toward a bidirectional cortical visual prosthesis. *Sci Adv*. 2025 Nov 7;11(45): eadv8846. doi: 10.1126/sciadv. adv8846. Epub 2025 Nov 5. PMID: 41191758; PMCID: PMC12588288.



Subretinal Photovoltaic Implant Restores Vision in Geographic Atrophy Secondary to Age-Related Macular Degeneration (2025)

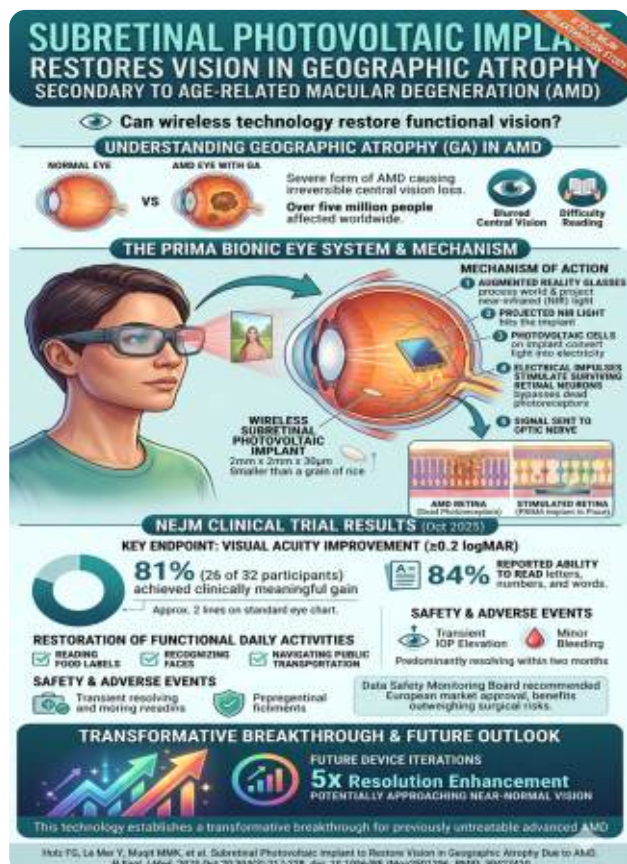
Can wireless subretinal photovoltaic technology restore functional vision in advanced macular degeneration?

This landmark clinical trial published in the *New England Journal of Medicine* (October 2025) evaluated the PRIMA bionic eye system in 38 participants with geographic atrophy, a severe form of age-related macular degeneration causing irreversible central vision loss in over five million people worldwide. The system comprises a wireless subretinal photovoltaic implant measuring 2mm by 2mm by 30 micrometres, smaller than a grain of rice, positioned beneath the retina and paired with augmented reality glasses that project near-infrared light onto the implant, which then stimulates surviving retinal neurons, bypassing dead photoreceptors entirely. The primary endpoint demonstrated that 26 of 32 participants completing the 12-month follow-up, representing 81%, achieved clinically meaningful visual acuity improvement of at least 0.2 logMAR, approximately two lines; 84% reported the ability to read letters, numbers, and words. Participants successfully utilised the system for daily activities including reading food labels, recognising faces, and navigating public transportation. Adverse events included transient intraocular pressure elevation and minor bleeding, predominantly resolving within two months. The Data Safety Monitoring Board recommended European market approval, citing benefits outweighing surgical risks. Future device iterations promise a fivefold resolution enhancement potentially approaching near-normal vision, establishing this technology as a transformative breakthrough for previously untreatable advanced macular degeneration.

Source:

RESEARCH PAPER

-  Holz FG, Le Mer Y, Muqit MMK, et al. Subretinal Photovoltaic Implant to Restore Vision in Geographic Atrophy Due to AMD. *N Engl J Med*. 2025 Oct 20;394(3):217-228. doi: 10.1056/NEJMoa2501396. PMID: 39427410.



The Pupillary Respiratory-Phase Response: A New Biological Rhythm in Vision (2025)

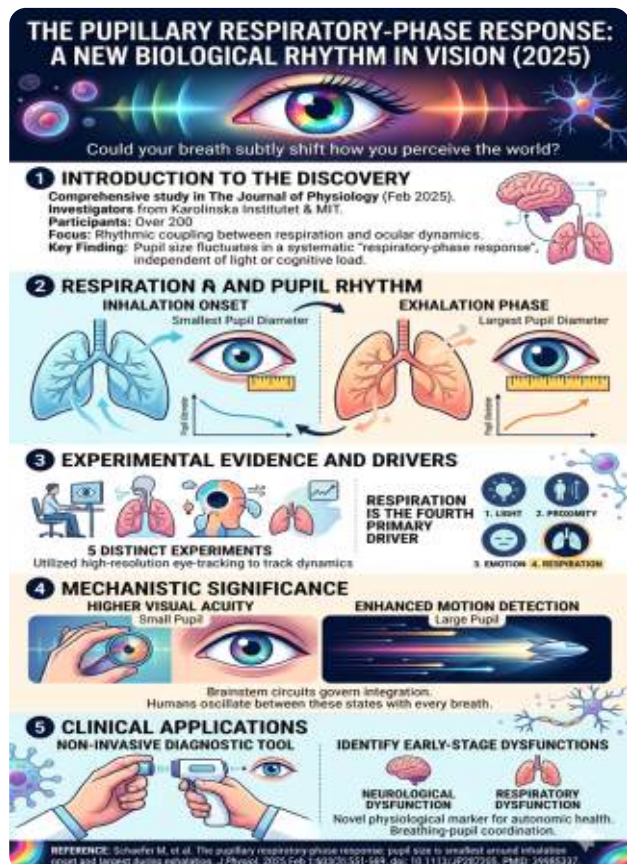
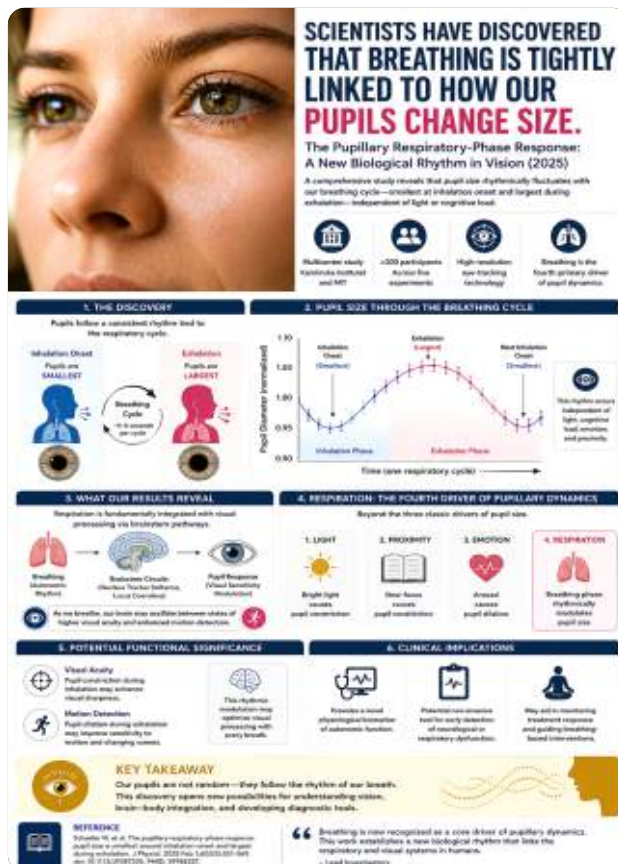
🧠 *Could your very breath be subtly shifting how you perceive the world every few seconds?*

This comprehensive physiological study published in *The Journal of Physiology* (February 2025) involved researchers from the Karolinska Institutet and MIT, investigating the rhythmic coupling between respiration and ocular dynamics in over two hundred participants. Across five distinct experiments, investigators utilised high-resolution eye-tracking to demonstrate that pupil size is not static but fluctuates in a systematic "respiratory-phase response" independent of light or cognitive load. Findings revealed that pupils are smallest at the onset of inhalation and reach their maximum diameter during exhalation. This suggests that the brainstem circuits governing autonomic breathing are fundamentally integrated with those controlling visual sensitivity. Mechanistically, this rhythm implies that humans may oscillate between states of higher visual acuity and enhanced motion detection with every breath. These results establish respiration as the fourth primary driver of pupillary movement, alongside light, proximity, and emotion. This discovery provides a novel physiological marker for assessing autonomic health and suggests that breathing-pupil coordination could serve as a non-invasive diagnostic tool for identifying early-stage neurological or respiratory dysfunctions.

Source:

📄 RESEARCH PAPER

📌 Schaefer M, et al. The pupillary respiratory-phase response: pupil size is smallest around inhalation onset and largest during exhalation. *J Physiol*. 2025 Feb 1;603(3):551-569. doi: 10.1113/JP287205. PMID: 39985337.



“Dancing Molecules” that Move in Sync with Cell Receptors Achieve Superior Bone Regeneration in Laboratory Models (2025)

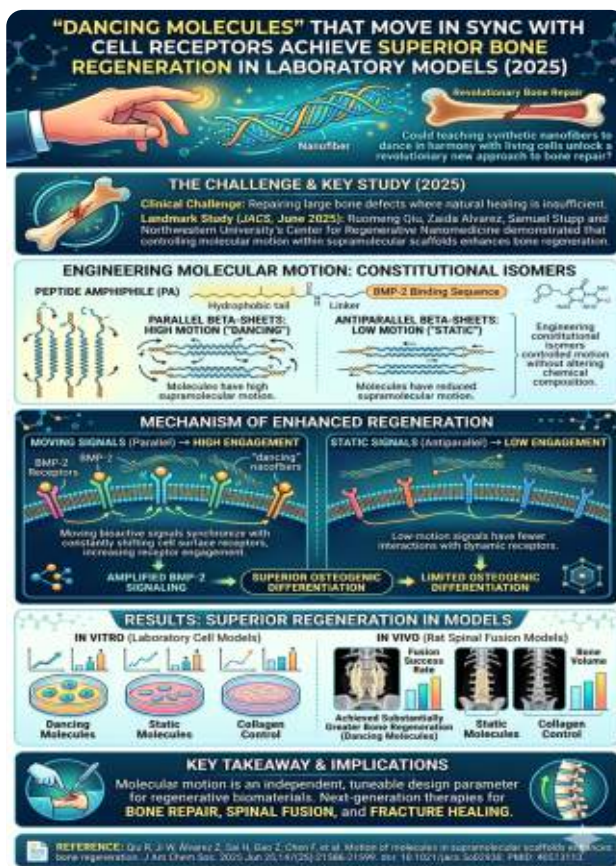
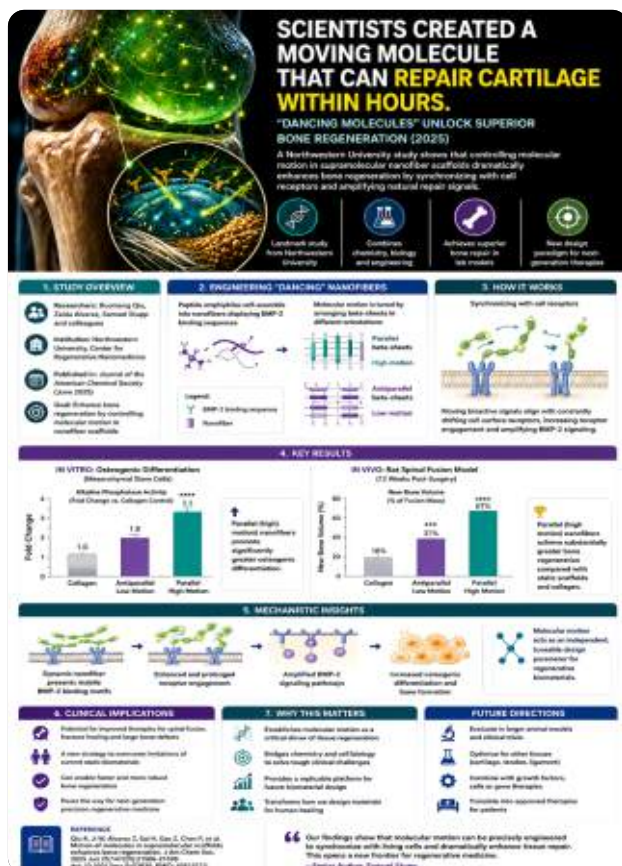
 **Could teaching synthetic nanofibers to dance in harmony with living cells unlock a revolutionary new approach to bone repair?**

Bone regeneration remains a significant clinical challenge, particularly in large defects where natural repair capacity is insufficient. A landmark study published in the *Journal of the American Chemical Society* (June 2025) by Ruomeng Qiu, Zaida Alvarez, Samuel Stupp and colleagues from Northwestern University's Centre for Regenerative Nanomedicine demonstrated that controlling molecular motion within supramolecular nanofiber scaffolds enhances bone regeneration. The team designed peptide amphiphile molecules self-assembling into filamentous nanostructures displaying bone morphogenetic protein-2 (BMP-2) binding sequences at their surface. Engineering constitutional isomers into parallel or antiparallel beta-sheet configurations controlled molecular motion without altering chemical composition. Parallel beta-sheet assemblies with greater supramolecular motion promoted significantly superior osteogenic differentiation in vitro and achieved substantially greater bone regeneration in rat spinal fusion models compared with static antiparallel assemblies and collagen controls. Mechanistically, moving bioactive signals synchronise with constantly shifting cell surface receptors, increasing receptor engagement and amplifying downstream BMP-2 signalling. These findings establish molecular motion as an independent, tuneable design parameter for regenerative biomaterials, with direct implications for next-generation therapies targeting bone repair, spinal fusion and fracture healing.

Source:

 RESEARCH PAPER

 Qiu R, Ji W, Álvarez Z, Sai H, Gao Z, Chen F, et al. Motion of molecules in supramolecular scaffolds enhances bone regeneration. *J Am Chem Soc.* 2025 Jun 25;147(25):21586-21599. doi: 10.1021/jacs.5c02930. PMID: 40513113.



When Fighting Health Norms Becomes its Own Ideology: Understanding “Antiscience Healthism” in the Post-COVID World (2026)

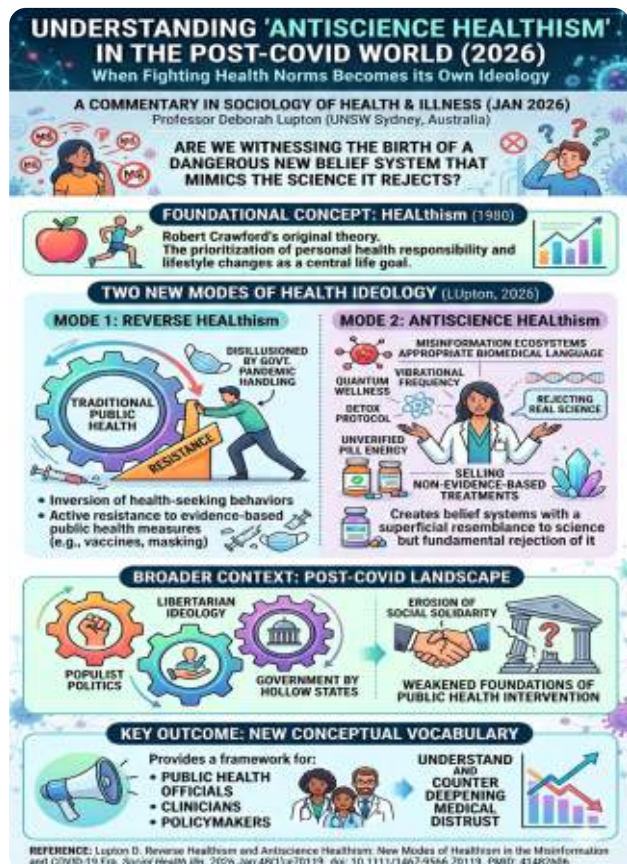
📌 In a world drowning in health misinformation, are we witnessing the birth of a dangerous new belief system that mimics the very science it rejects?

This commentary, published in *Sociology of Health and Illness* in January 2026, was authored by Professor Deborah Lupton from the Vitalities Lab, Centre for Social Research in Health and Social Policy Research Centre at the University of New South Wales, Sydney, Australia. Drawing on the foundational sociological concept of “healthism” originally proposed by Robert Crawford in 1980, Lupton identifies two entirely new modes of health ideology emerging from the post-COVID landscape. The first, “reverse healthism,” describes how individuals and communities, disillusioned by government handling of the pandemic, have inverted traditional health-seeking behaviours, actively resisting evidence-based public health measures. The second, “antiscience healthism,” captures how misinformation ecosystems now appropriate legitimate biomedical language and frameworks to promote non-evidence-based treatments, creating belief systems that superficially resemble scientific reasoning while fundamentally rejecting it. Lupton situates these phenomena within a broader context of populist politics, libertarian ideology, and what she terms “government by hollow states,” where the erosion of social solidarity has severely weakened the foundations of effective public health intervention. This framework offers public health officials, clinicians, and policymakers a conceptual vocabulary to understand and counter deepening medical distrust in the post-pandemic era.

Source:

📄 COMMENTARY

📌 Lupton D. Reverse Healthism and Antiscience Healthism: New Modes of Healthism in the Misinformation and COVID-19 Era. *Sociol Health Illn*. 2026 Jan;48(1): e70119. doi: 10.1111/1467-9566.70119. PMID: 41482606.



The Protein that Writes DNA from Scratch - No Template Required (2026)

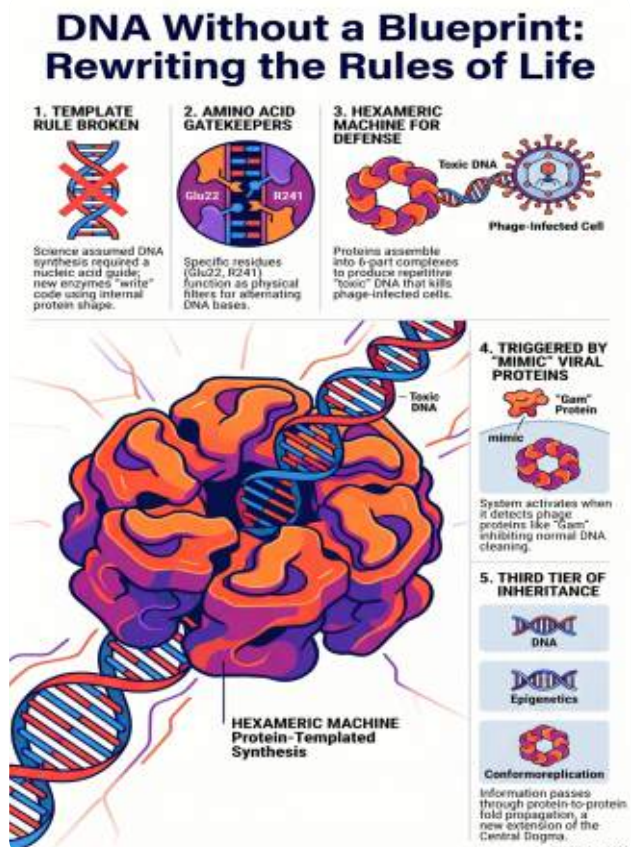
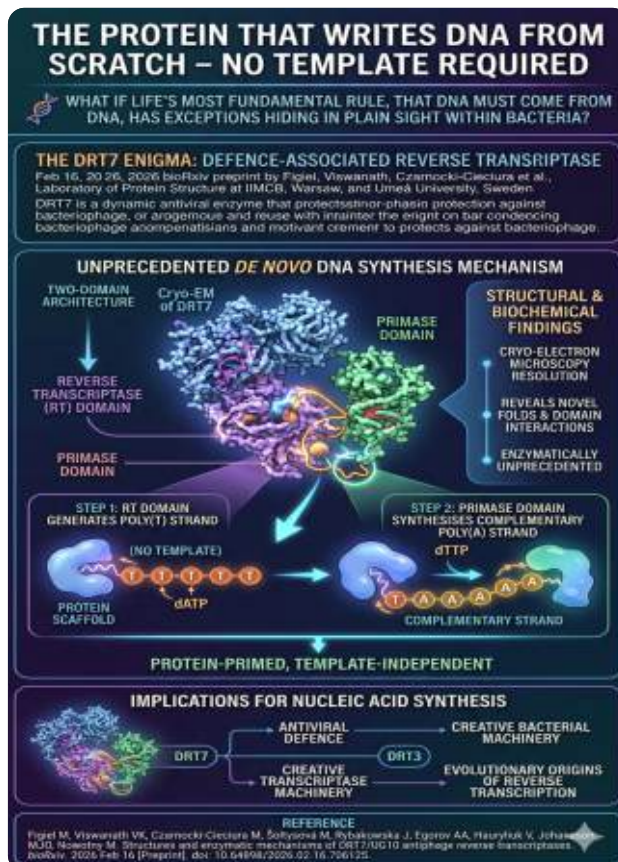
 **What if life's most fundamental rule, that DNA must come from DNA, turns out to have exceptions hiding in plain sight within bacteria?**

This structural and biochemical preprint, posted on *bioRxiv* on 16 February 2026, was conducted by Malgorzata Figiel, Vysakh Komathattu Viswanath, Mariusz Czarnocki-Cieciura, and colleagues from the Laboratory of Protein Structure at the International Institute of Molecular and Cell Biology, Warsaw, Poland, in collaboration with Umeå University, Sweden. The study characterises DRT7 (also known as UG10), a defence-associated reverse transcriptase in bacteria that protects against bacteriophage infection. Using cryo-electron microscopy, the team resolved the three-dimensional structure of DRT7, revealing a two-domain architecture combining a reverse transcriptase domain with a primase domain. Crucially, DRT7 synthesises DNA without any nucleic acid template: the reverse transcriptase domain first generates a poly(T) strand using the protein itself as a structural scaffold, which then serves as substrate for the primase domain to synthesise a complementary poly(A) strand. This protein-primed, template-independent mechanism of de novo DNA synthesis is enzymatically unprecedented. Together with the earlier characterisation of DRT3, these findings reveal that bacteria have evolved remarkably creative antiviral machinery, with profound implications for our understanding of nucleic acid synthesis and the evolutionary origins of reverse transcription.

Source:

 RESEARCH PAPER

 Figiel M, Viswanath VK, Czarnocki-Cieciura M, Šoltysová M, Rybakowska J, Egorov AA, Haurlyuk V, Johansson MJO, Nowotny M. Structures and enzymatic mechanisms of DRT7/UG10 antiphage reverse transcriptases. *bioRxiv* 2026.02.16.706125; doi: <https://doi.org/10.64898/2026.02.16.706125>.



Can a Daily Multivitamin Slow Your Biological Clock? A Large Clinical Trial Says Yes (2026)

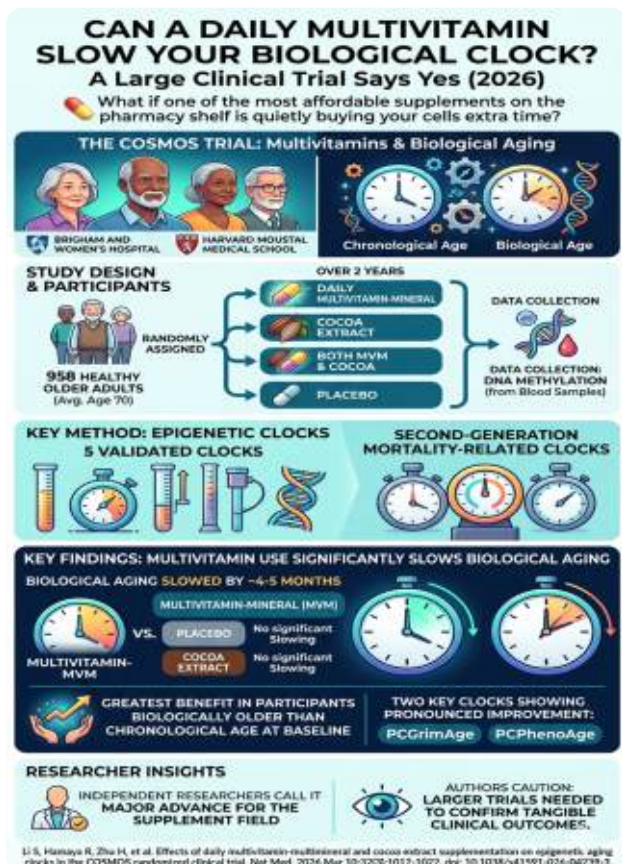
 *What if one of the most affordable supplements on the pharmacy shelf is quietly buying your cells extra time?*

This prespecified ancillary analysis of the COSMOS (COcoa Supplement and Multivitamin Outcomes Study) randomised clinical trial was led by Sidong Li, JoAnn E. Manson, and senior author Howard D. Sesso from Brigham and Women's Hospital and Harvard Medical School, with co-investigators from Augusta University. The study analysed DNA methylation data from blood samples of 958 healthy older adults with an average chronological age of 70, randomly assigned to daily multivitamin-mineral supplementation, cocoa extract, both, or placebo over two years. Using five validated epigenetic clocks, daily multivitamin use, but not cocoa extract, significantly slowed biological aging by approximately four to five months compared to placebo, with the greatest benefit in participants who were biologically older than their chronological age at baseline. Two second-generation mortality-related clocks, PCGrimAge and PCPhenoAge, showed the most pronounced improvement. Independent researchers described the findings as a major advance for the supplement field, while the authors caution that larger trials are needed to confirm whether these epigenetic changes translate into tangible clinical outcomes.

Source:

 RESEARCH PAPER

 Li S, Hamaya R, Zhu H, Chen BH, Pereira AC, Ivey KL, Rist PM, Manson JE, Dong Y, Sesso HD. Effects of daily multivitamin-multimineral and cocoa extract supplementation on epigenetic aging clocks in the COSMOS randomized clinical trial. *Nat Med.* 2026 Mar;32(3):1012-1022. doi: 10.1038/s41591-026-04239-3. Epub 2026 Mar 9. PMID: 41803341.



How Diet and Environment Rewrite Your Genes Without Touching Your DNA? (2026)

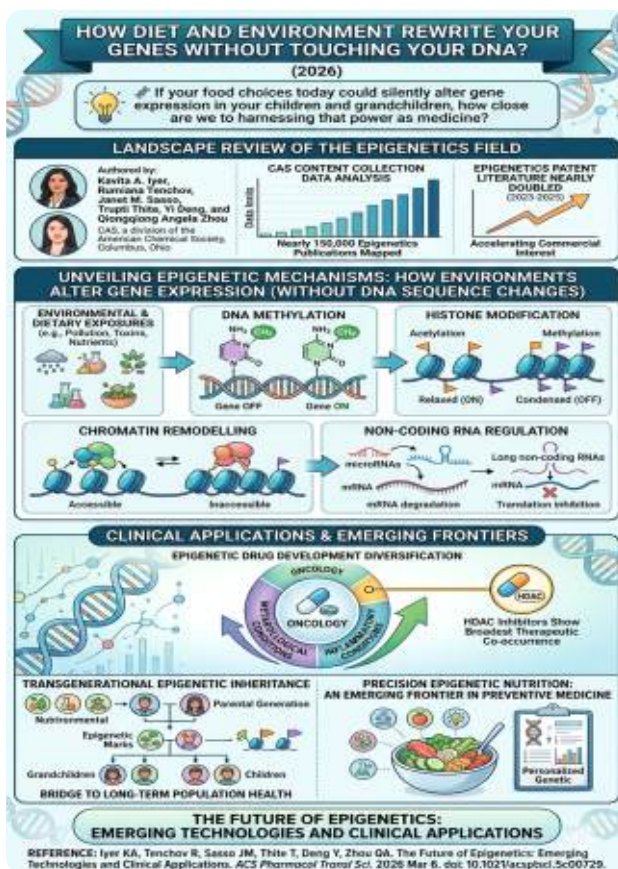
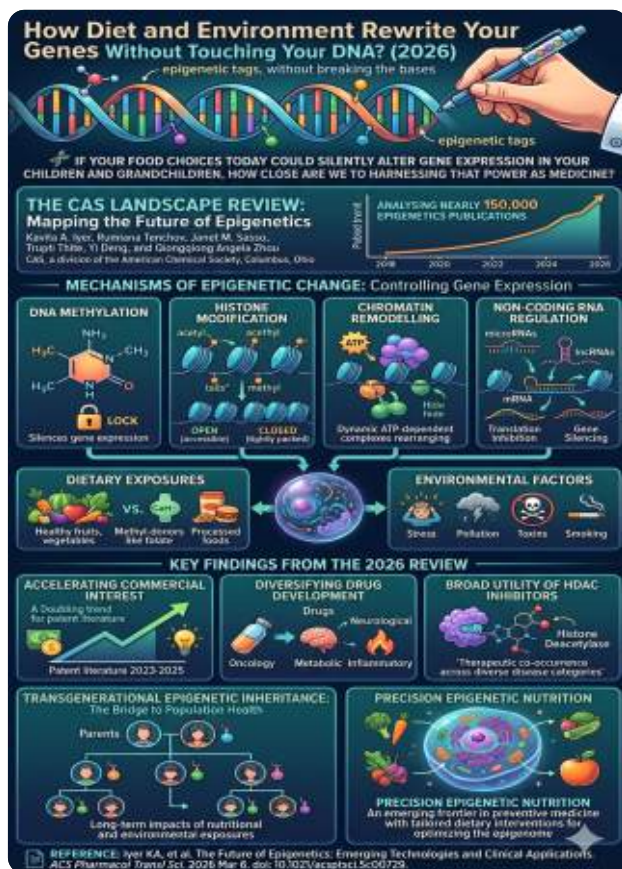
 *If your food choices today could silently alter gene expression in your children and grandchildren, how close are we to harnessing that power as medicine?*

This comprehensive landscape review, authored by Kavita A. Iyer, Rumiana Tenchov, Janet M. Sasso, Trupti Thite, Yi Deng, and Qiongqiong Angela Zhou from CAS, a division of the American Chemical Society, Columbus, Ohio, analyses data from the CAS Content Collection spanning nearly 150,000 epigenetics publications to map the current state and future trajectory of the field. The review documents how environmental and dietary exposures induce heritable changes in gene expression through DNA methylation, histone modification, chromatin remodelling, and non-coding RNA regulation, without altering the underlying DNA sequence. Patent literature in epigenetics nearly doubled between 2023 and 2025, reflecting accelerating commercial interest. A clinically significant finding is the documented diversification of epigenetic drug development beyond oncology into metabolic, neurological, and inflammatory conditions with HDAC inhibitors showing the broadest therapeutic co-occurrence across disease categories. The authors highlight transgenerational epigenetic inheritance as a critical bridge between nutritional and environmental exposures and long-term population health outcomes, positioning precision epigenetic nutrition as an emerging frontier in preventive medicine.

Source:

 REVIEW

 Iyer KA, Koynova-Tenchov R, Sasso JM, Thite T, Deng Y, Zhou QA. The Future of Epigenetics: Emerging Technologies and Clinical Applications. ACS Pharmacol Transl Sci. 2026 Feb 25;9(3):506-544. doi: 10.1021/acspsci.5c00729. PMID: 41852637; PMCID: PMC12993786.



Artificial Intelligence Transforms Prediction of Diabetic Complications Across Retinopathy, Nephropathy and Cardiovascular Disease (2026)

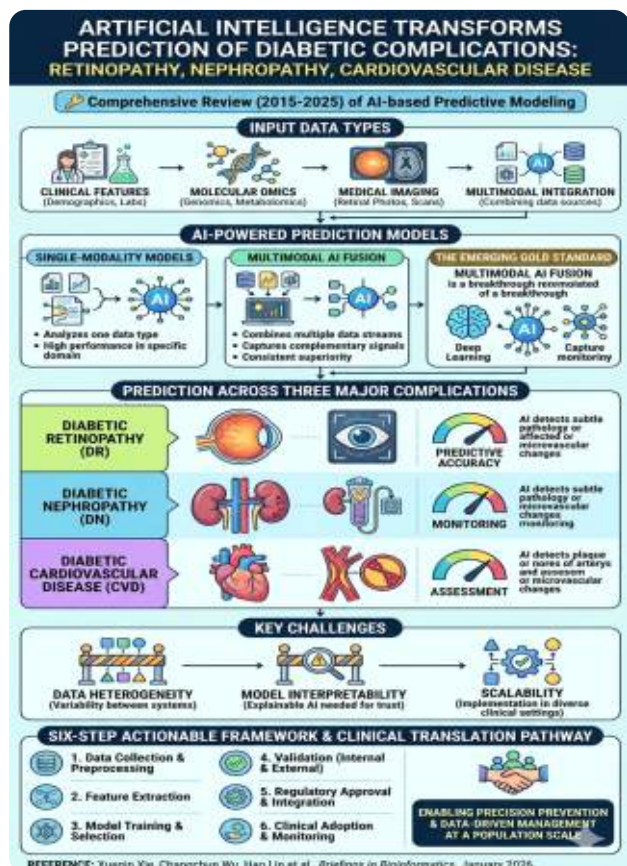
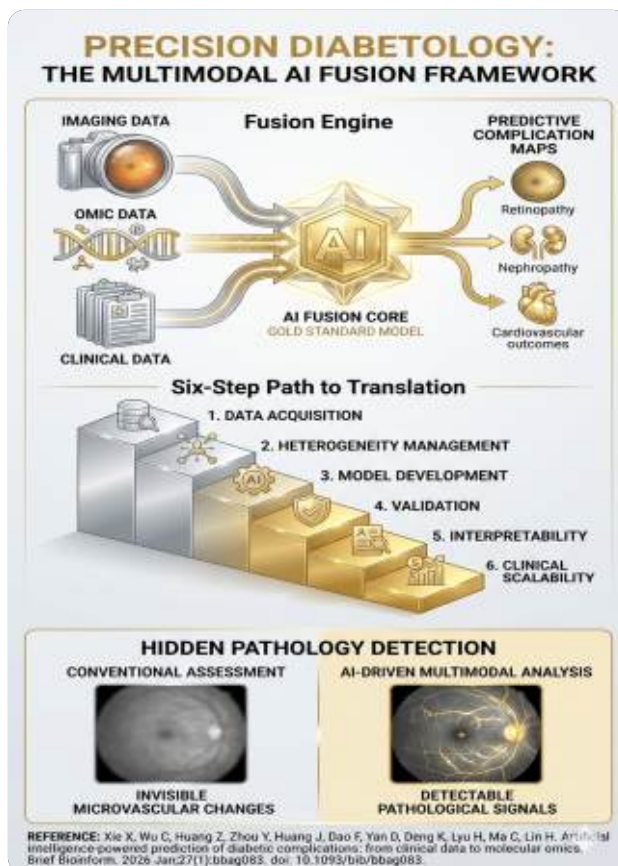
 **Can artificial intelligence finally predict which diabetic patients will develop devastating organ complications before irreversible damage occurs?**

Diabetic complications remain a leading cause of global disability and mortality, yet early identification of high-risk individuals has long eluded clinical practice. A comprehensive narrative review published in *Briefings in Bioinformatics* (January 2026) by Xueqin Xie, Changchun Wu, Hao Lin and colleagues systematically surveyed 58 studies published between January 2015 and June 2025 applying AI-based predictive modelling to three major diabetic complications: diabetic retinopathy, diabetic nephropathy and diabetic cardiovascular disease. The review examined models spanning clinical features, molecular omics, medical imaging and multimodal data integration. Single-modality models showed strong performance within their domains, but multimodal integration consistently outperformed individual approaches by capturing complementary pathophysiological signals across imaging, biochemical and genomic data. The authors identified multimodal AI fusion as the emerging gold standard for diabetic complication prediction, enabling detection of subtle microvascular pathology invisible to conventional clinical assessment. Key challenges identified include data heterogeneity, model interpretability and scalability across diverse clinical settings. The authors proposed a six-step actionable framework and clinical translation pathway to guide future research toward precision prevention and data-driven management of diabetic complications at a population scale.

Source:

 REVIEW

 Xie X, Wu C, Huang Z, Zhou Y, Huang J, Dao F, Yan D, Deng K, Lyu H, Ma C, Lin H. Artificial intelligence-powered prediction of diabetic complications: from clinical data to molecular omics. *Brief Bioinform.* 2026 Jan 7;27(1): bbag083. doi: 10.1093/bib/bbag083. PMID: 41744227; PMCID: PMC12936790.



AI Platform Achieves 90% Accuracy in Diagnosing Chronic Fatigue Syndrome by Mapping Gut-Immune-Metabolic Disruptions (2025)

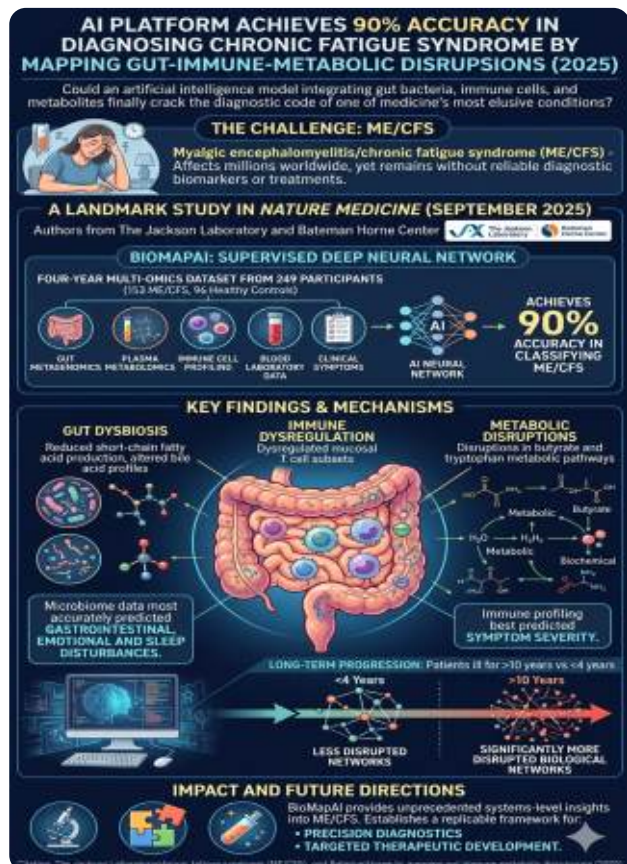
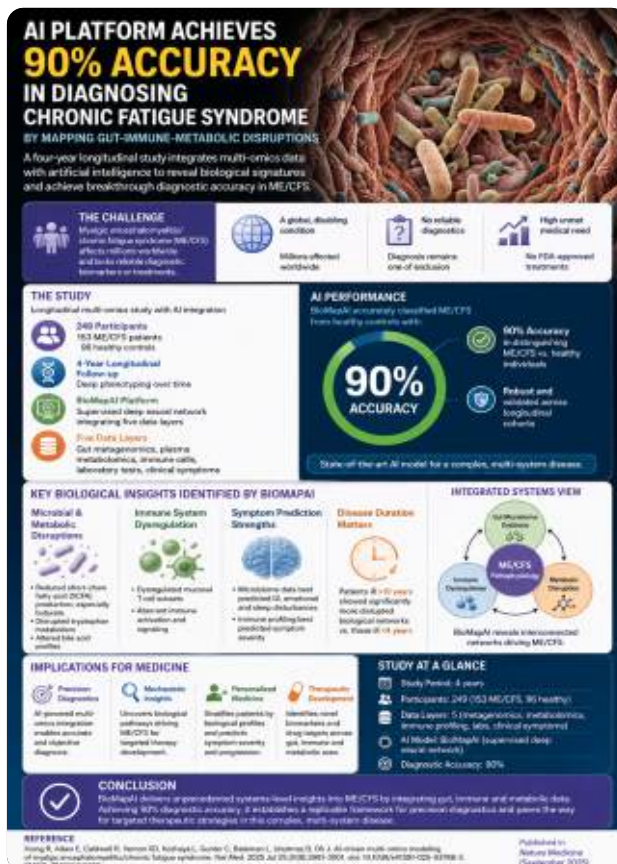
 Could an artificial intelligence model integrating gut bacteria, immune cells and metabolites finally crack the diagnostic code of one of medicine's most elusive conditions?

Myalgic encephalomyelitis/ chronic fatigue syndrome (ME/ CFS) affects millions worldwide yet remains without reliable diagnostic biomarkers or treatments. A landmark longitudinal study published in *Nature Medicine* (September 2025) by Ruoyun Xiong, Derya Unutmaz, Julia Oh and colleagues from The Jackson Laboratory and Bateman Horne Centre introduced BioMapAI, a supervised deep neural network trained on a four-year multi-omics dataset from 249 participants comprising 153 ME/ CFS patients and 96 healthy controls. BioMapAI integrates gut metagenomics, plasma metabolomics, immune cell profiling, blood laboratory data and clinical symptoms, achieving 90% accuracy in classifying ME/ CFS from healthy individuals. The model identified disruptions in butyrate and tryptophan metabolic pathways alongside diminished short-chain fatty acid production, altered bile acid profiles and dysregulated mucosal T cell subsets. Microbiome data most accurately predicted gastrointestinal, emotional and sleep disturbances, while immune profiling best predicted symptom severity. Patients ill for over ten years showed significantly more disrupted biological networks than those ill for under four years, suggesting progressive deterioration. BioMapAI provides unprecedented systems-level insights into ME/ CFS and establishes a replicable framework for precision diagnostics and targeted therapeutic development.

Source:

 RESEARCH PAPER

 Xiong R, Aiken E, Caldwell R, Vernon SD, Kozhaya L, Gunter C, Bateman L, Unutmaz D, Oh J. AI-driven multi-omics modelling of myalgic encephalomyelitis/chronic fatigue syndrome. *Nat Med.* 2025 Jul 25;31(9):2991-3001. doi: 10.1038/s41591-025-03788-3. PMCID: PMC12416096.



The Processing Paradox: Food Matrix Integrity Outperforms Nutrient Profile in Chronic Disease Prevention (2026)

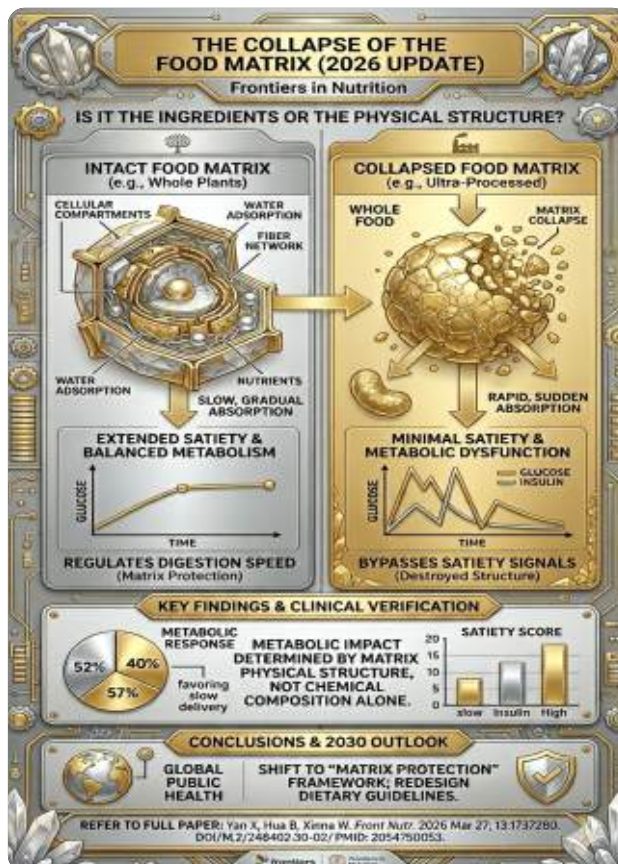
 **Is it the ingredients in our food that harm us, or the way they are physically assembled?**

Ultra-processed foods (UPFs) are strongly associated with non-communicable diseases, yet their harm has traditionally been attributed to poor nutritional profiles alone. A review published in *Frontiers in Nutrition* (March 2026) by Xu Yan, Bai Hua and Wang Xinna from the Anhui Huatuo Institute of Traditional Chinese Medicine and Changchun University of Traditional Chinese Medicine, China, proposes a paradigm-shifting framework: the primary threat of UPFs originates from industrial collapse of the physical food matrix, not merely chemical composition. Even nutrient-matched UPFs promote excess energy intake and weight gain, confirming a pathogenic mechanism beyond nutritional content. Structural disintegration triggers physiological dysregulation: a soft matrix reduces chewing requirements, weakening early satiety signals; rapid nutrient absorption suppresses GLP-1 and peptide YY secretion; and supraphysiological nutrient flux drives insulin resistance and hepatic de novo lipogenesis. Impoverished food matrix integrity ultimately disrupts gut microbiota composition, compromises intestinal barrier function and perpetuates low-grade systemic inflammation. The authors call for a fundamental shift: from focusing solely on what is in food to equally considering what industrial processing has done to its physical architecture.

Source:

 REVIEW

 Wang X, Chen H, Xu Y, Xu Q, Cui H, Liu F. The collapse of the food matrix: how ultra-processed foods impact satiety and metabolism by altering physical structure beyond nutrient composition. *Front Nutr.* 2026 May 5; 13:1737280. doi: 10.3389/fnut.2026.1737280. PMID: 42163955; PMCID: PMC13185557.



Vegetarian Diets Associated with Significantly Lower Cancer Risk Across Multiple Sites (2025)

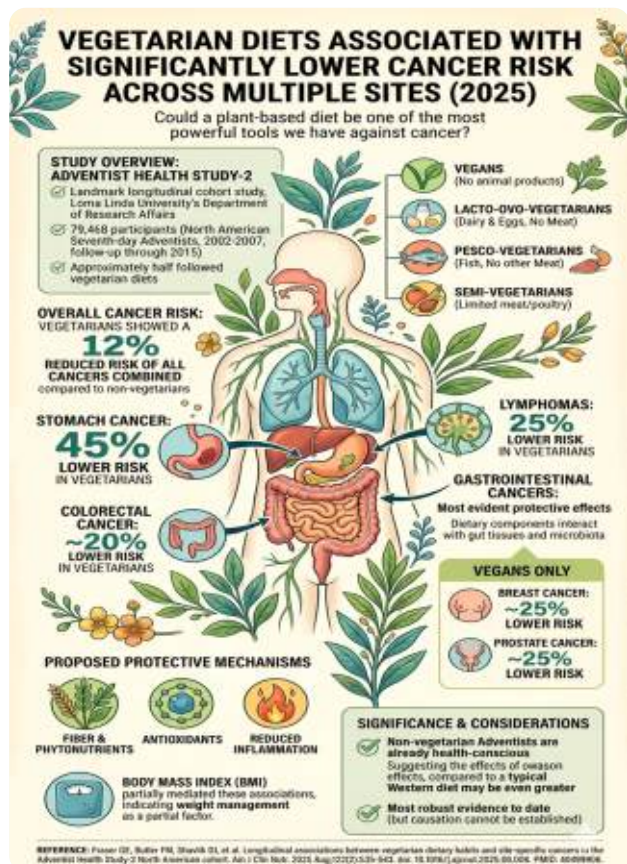
 *Could a plant-based diet be one of the most powerful tools we have against cancer?*

A landmark longitudinal cohort study conducted by Loma Linda University's Department of Research Affairs analysed data from 79,468 participants of the Adventist Health Study-2, a cohort of North American Seventh-day Adventists recruited between 2002 and 2007 with follow-up through 2015. Approximately half of participants followed vegetarian diets, categorised into vegans, lacto-ovo-vegetarians, pesco-vegetarians and semi-vegetarians. Vegetarians demonstrated a 12% overall reduced risk of all cancers combined compared to non-vegetarians. Reductions were most pronounced for stomach cancer at 45% and lymphomas at 25%, with colorectal cancer risk also reduced by approximately 20%. Vegans specifically showed around 25% lower risk of breast and prostate cancers. Protective effects were most evident in gastrointestinal cancers, where dietary components interact directly with gut tissues and microbiota. Body mass index partially mediated these associations. Researchers noted that non-vegetarian Adventists are themselves health-conscious, suggesting that protective benefits against a typical Western diet may be even greater. While causation cannot be established, the findings represent the most robust evidence to date for vegetarian diets reducing risk across both common and medium-frequency cancers.

Source:

 COHORT STUDY

 Fraser GE, Butler FM, Shavlik DJ, et al. Longitudinal associations between vegetarian dietary habits and site-specific cancers in the Adventist Health Study-2 North American cohort. Am J Clin Nutr. 2025 Aug;122(2):535-543. doi: 10.1016/j.ajcnut.2025.06.006. PMID: 40499906.



Ultra-Processed Foods Linked to 45% Higher Risk of Early-Onset Colorectal Cancer Precursors in Women (2025)

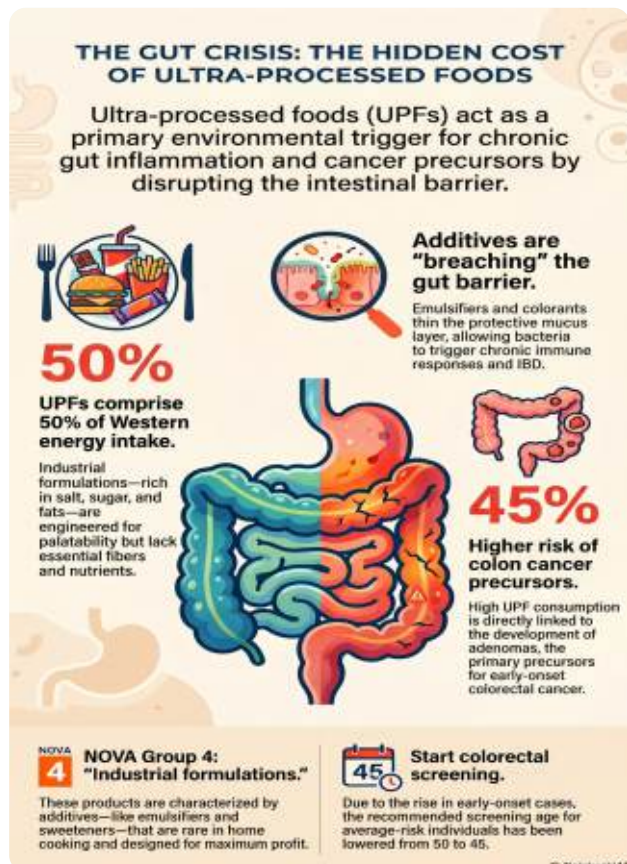
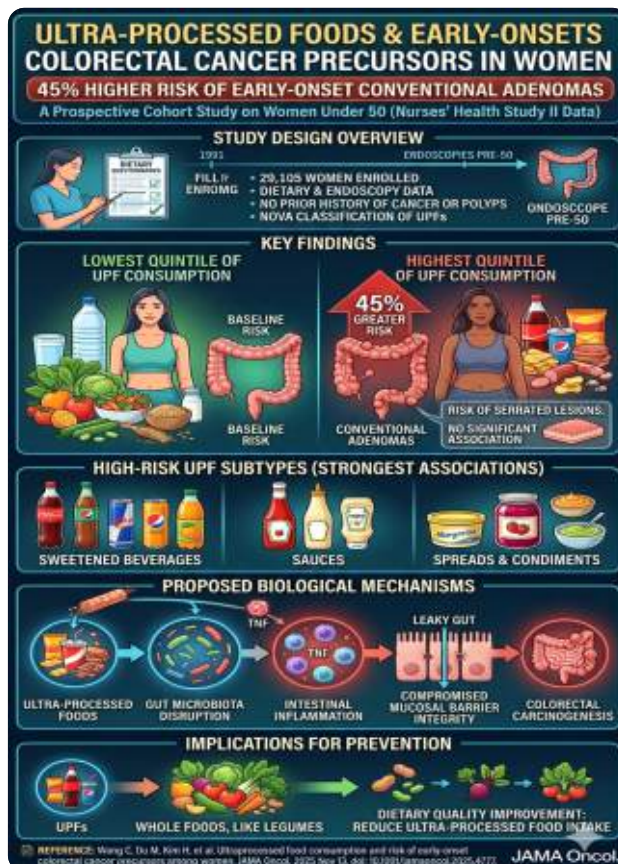
 **Could the ultra-processed foods dominating modern diets be quietly accelerating colorectal cancer in adults under 50?**

This prospective cohort study, led by senior author Andrew T. Chan from the Clinical and Translational Epidemiology Unit at Mass General Brigham, Harvard Medical School, analysed dietary and endoscopy data from 29,105 women enrolled in the Nurses' Health Study II. Participants completed detailed dietary questionnaires in 1991 and underwent at least one lower endoscopy before the age of 50, with no prior history of cancer, colorectal polyps, or inflammatory bowel disease. Ultra-processed food intake was classified using the NOVA system. Women in the highest quintile of ultra-processed food consumption had a 45% greater risk of developing early-onset conventional adenomas, established precursors of colorectal cancer, compared to those in the lowest quintile. Sweetened beverages, sauces, spreads, and condiments showed the strongest individual associations. The risk of serrated lesions, a separate colorectal cancer precursor subtype, was not significantly affected. The authors propose that ultra-processed foods promote colorectal carcinogenesis through gut microbiota disruption, intestinal inflammation, and compromised mucosal barrier integrity. These findings position dietary quality improvement, specifically reducing ultra-processed food intake, as a practical and accessible strategy for early-onset colorectal cancer prevention.

Source:

 COHORT STUDY

 Wang C, Du M, Kim H, et al. Ultraprocessed Food Consumption and Risk of Early-Onset Colorectal Cancer Precursors Among Women. *JAMA Oncol.* 2026 Jan 1;12(1):49-57. doi: 10.1001/jamaoncol.2025.4777. PMID: 41231486; PMCID: PMC12616530.



Cheese Consumption and Dementia Incidence in Older Japanese Adults: Three-Year Prospective Cohort Study (2025)

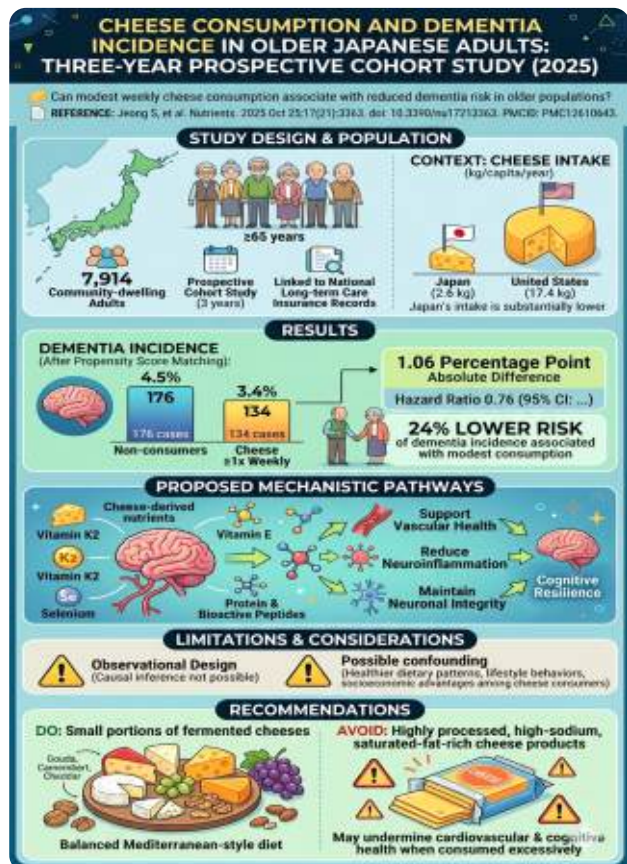
Can modest weekly cheese consumption associate with reduced dementia risk in older populations?

This prospective cohort study published in *Nutrients* (January 2025) examined cheese consumption's relationship with dementia incidence among 7,914 community-dwelling Japanese adults aged ≥ 65 years followed three years through national long-term care insurance records. After propensity score matching controlling for confounders, participants consuming cheese $\geq$ once weekly demonstrated dementia incidence of 3.4% (134 cases) versus 4.5% (176 cases) among non-consumers, representing 1.06 percentage point absolute difference and hazard ratio 0.76, indicating 24% lower risk. Context: Japan's average annual cheese intake (2.6 kilograms per capita) remains substantially lower than Western countries (United States 17.4 kilograms), suggesting even modest consumption changes may exhibit measurable associations. Researchers propose mechanistic pathways involving cheese-derived nutrients including vitamin K2, selenium, vitamin E, protein, and bioactive peptides potentially supporting vascular health, reducing neuroinflammation, and maintaining neuronal integrity contributing to cognitive resilience. Investigators emphasise observational design limitations precluding causal inference; associations may reflect healthier overall dietary patterns, lifestyle behaviours, or socioeconomic advantages rather than direct cheese effects. Recommendations advocate small portions of fermented cheeses within balanced Mediterranean-style dietary frameworks while cautioning against highly processed, high-sodium, saturated-fat-rich cheese products potentially undermining cardiovascular and cognitive health when consumed excessively.

Source:

COHORT STUDY

 Jeong S, Suzuki T, Inoue Y, Bang E, Nakamura K, Sasaki M, Kondo K. Cheese consumption and incidence of dementia in community-dwelling older Japanese adults: the JAGES 2019-2022 cohort study. *Nutrients*. 2025 Oct 25;17(21):3363. doi: 10.3390/nu17213363. PMID: PMC12610643.



Caffeine Activates Conserved Cellular Longevity Pathways: Evidence from a Fission Yeast Model (2025)

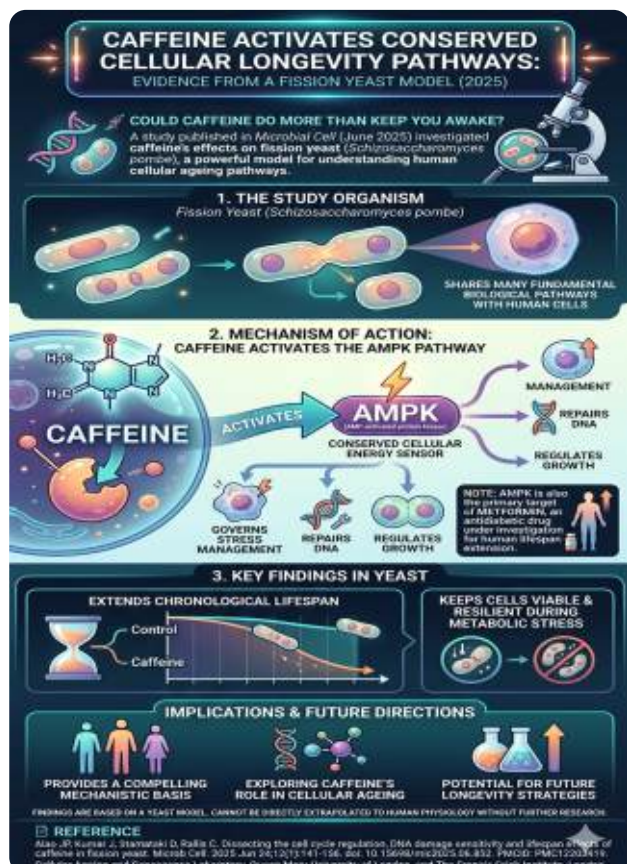
 **Could caffeine, the world's most widely consumed stimulant, be doing far more than keeping you awake?**

A study published in *Microbial Cell* (June 2025) by researchers from the Cellular Ageing and Senescence Laboratory, Queen Mary University of London, and The Francis Crick Institute, London, investigated the molecular mechanisms by which caffeine influences cell cycle regulation, DNA damage response, and chronological lifespan using fission yeast (*Schizosaccharomyces pombe*), a well-established single-celled model organism that shares many fundamental biological pathways with human cells. The study found that caffeine activates AMPK (AMP-activated protein kinase), an evolutionarily conserved cellular energy sensor that governs how cells manage stress, repair DNA, and regulate growth. By engaging this pathway, caffeine was shown to extend the chronological lifespan of yeast cells, keeping them viable and resilient during periods of metabolic stress. Notably, AMPK is also the primary target of metformin, a widely prescribed antidiabetic drug currently under investigation for its potential lifespan-extending properties in humans. While these findings are based on a yeast model and cannot be directly extrapolated to human physiology without further research, they provide a compelling mechanistic basis for exploring caffeine's role in cellular ageing, with potential implications for future nutritional and pharmacological longevity strategies.

Source:

 RESEARCH PAPER

 Alao JP, Kumar J, Stamatakis D, Rallis C. Dissecting the cell cycle regulation, DNA damage sensitivity and lifespan effects of caffeine in fission yeast. *Microb Cell*. 2025 Jun 24;12(1):141-156. doi: 10.15698/mic2025.06.852. PMID: PMC12203419.



Black Cumin Seed (*Nigella sativa*) Confers Anti-Adipogenic Effects and Lipid-Lowering Properties: Randomised Controlled Trial with Cellular Validation (2025)

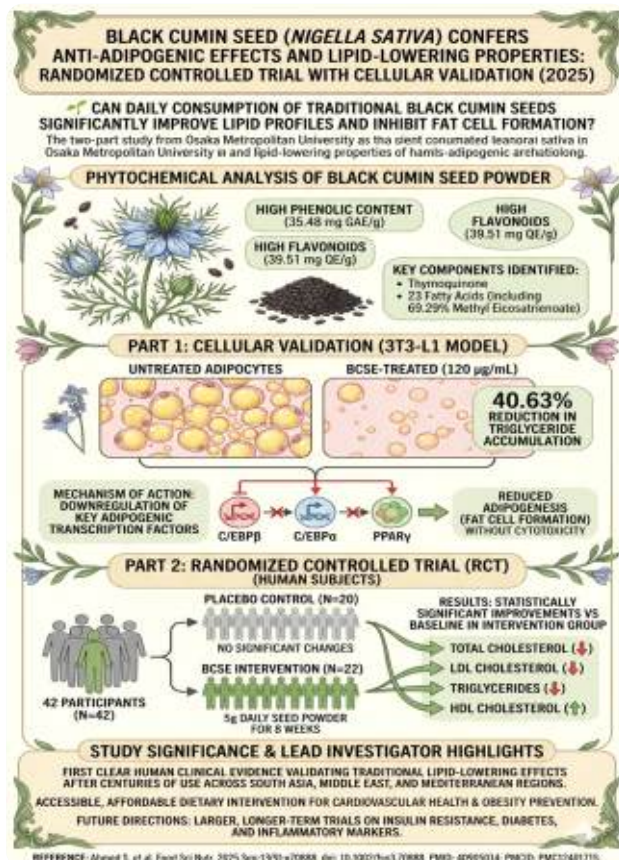
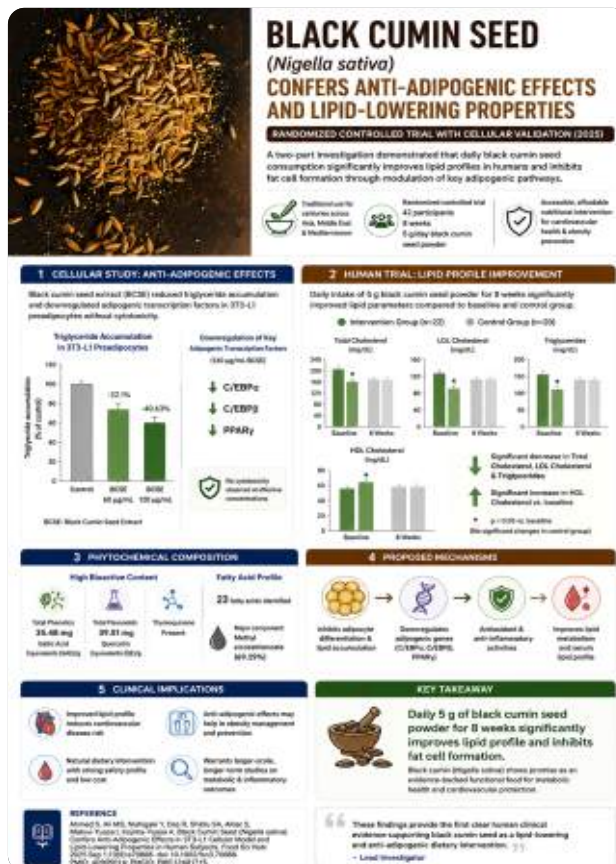
🌿 *Can daily consumption of traditional black cumin seeds significantly improve lipid profiles and inhibit fat cell formation?*

This two-part investigation published in *Food Science & Nutrition* (September 2025) from Osaka Metropolitan University evaluated the metabolic effects of black cumin seeds through both cellular models and a human clinical trial. Researchers first demonstrated that black cumin seed extract reduced triglyceride accumulation by 40.63% in 3T3-L1 preadipocytes at 120 micrograms per millilitre, downregulating key adipogenic transcription factors including C/EBP α , C/EBP β , and PPAR γ without cytotoxicity. A subsequent randomised controlled trial enrolled 42 participants, 22 in the intervention group and 20 as controls, who consumed five grams of black cumin seed powder daily for eight weeks. The intervention group showed statistically significant improvements: decreased total cholesterol, low-density lipoprotein cholesterol, and triglycerides, alongside increased high-density lipoprotein cholesterol, while the control group showed no changes. Phytochemical analysis identified high phenolic content, flavonoids, thymoquinone, and 23 fatty acids including methyl eicosatrienoate. This study represents the first clear human clinical evidence validating black cumin's lipid-lowering effects after centuries of traditional use across South Asia, the Middle East, and Mediterranean regions. The lead investigator emphasised the need for larger-scale, longer-term trials investigating effects on insulin resistance, diabetes, and inflammatory markers, positioning black cumin as an accessible and affordable dietary intervention for cardiovascular health and obesity prevention.

Source:

📄 RESEARCH PAPER

📌 Ahmed S, Ali MS, Nishigaki Y, Das R, Shiblu SA, Akter S, Matsui-Yuasa I, Kojima-Yuasa A. Black Cumin Seed (*Nigella sativa*) Confers Anti-Adipogenic Effects in 3T3-L1 Cellular Model and Lipid-Lowering Properties in Human Subjects. *Food Sci Nutr*. 2025 Sep 1;13(9): e70888. doi: 10.1002/fsn3.70888. PMID: 40905014; PMCID: PMC12401715.



Systematic Profiling Reveals Betaine as an Exercise Mimetic for Geroprotection (2025)

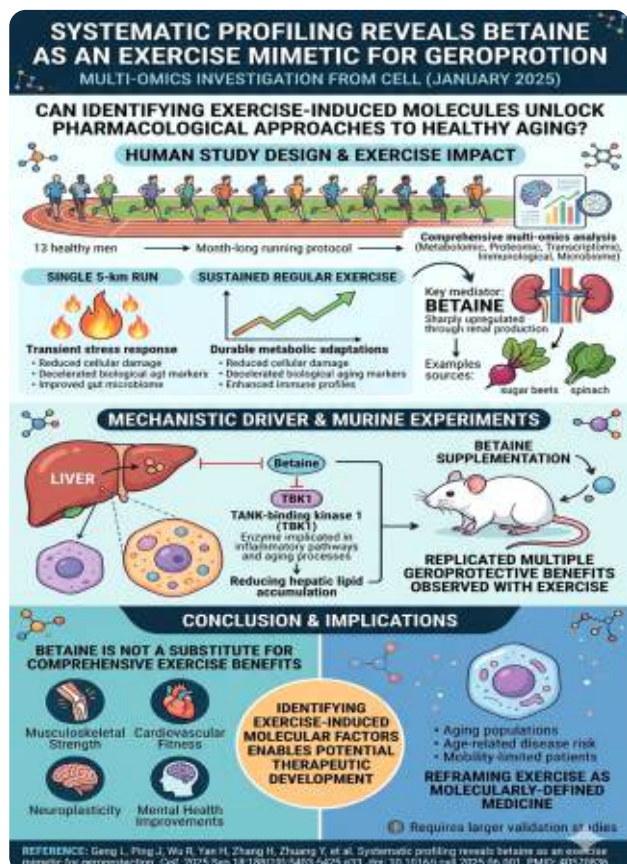
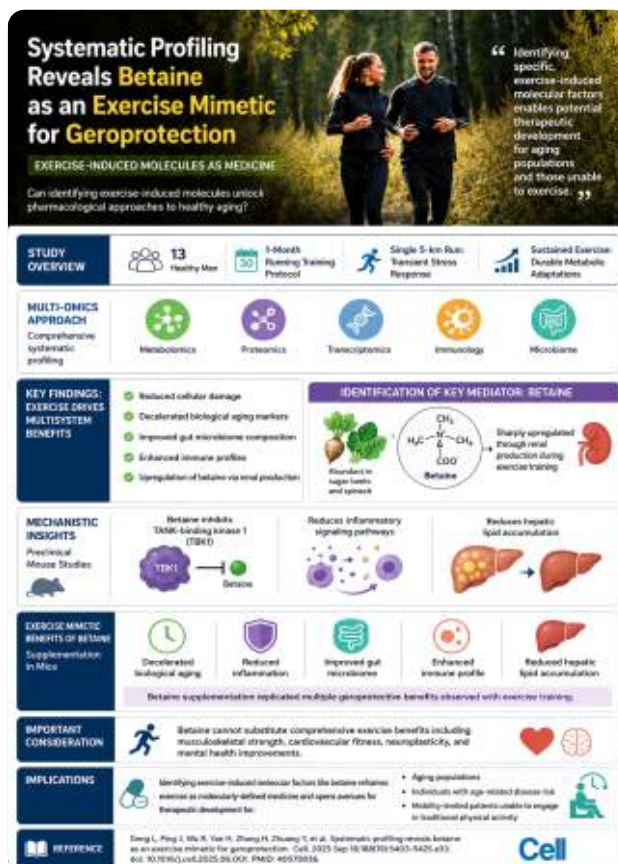
 **Can identifying exercise-induced molecules unlock pharmacological approaches to healthy aging?**

This multi-omics investigation published in *Cell* (January 2025) from Xuanwu Hospital Capital Medical University employed comprehensive metabolomic, proteomic, transcriptomic, immunological, and microbiome analyses in 13 healthy men undergoing a month-long running training protocol. A single 5-kilometre run triggered a transient stress response, while sustained regular exercise produced durable metabolic adaptations including reduced cellular damage, decelerated biological ageing markers, improved gut microbiome composition, and enhanced immune profiles. The researchers identified betaine, a metabolite abundant in sugar beets and spinach, as a key mediator that is sharply upregulated through renal production during training. Subsequent murine experiments demonstrated that betaine inhibits TANK-binding kinase 1 (TBK1), an enzyme previously implicated in inflammatory pathways and ageing processes, while reducing hepatic lipid accumulation. Critically, betaine supplementation replicated multiple geroprotective benefits observed with exercise training, establishing it as a mechanistic driver rather than a mere metabolic byproduct. The investigators emphasise that betaine cannot substitute for the comprehensive benefits of exercise, which encompass musculoskeletal strength, cardiovascular fitness, neuroplasticity, and mental health improvements. However, identifying specific exercise-induced molecular factors opens the possibility of therapeutic development for ageing populations, individuals with age-related disease risk, or mobility-limited patients unable to engage in traditional physical activity, reframing exercise as molecularly-defined medicine that awaits larger validation studies.

Source:

 RESEARCH PAPER

 Geng L, Ping J, Wu R, Yan H, Zhang H, Zhuang Y, et al. Systematic profiling reveals betaine as an exercise mimetic for geroprotection. *Cell*. 2025 Sep 18;188(19):5403-5425.e33. doi: 10.1016/j.cell.2025.06.001. PMID: 40570836.



CENP-E Initiates Chromosome Congression by Opposing Aurora Kinases: Redefining Cell Division Mechanisms (2025)

 **Does CENP-E function as a stabilising regulator of initial chromosome attachments rather than a motor protein hauling chromosomes into position?**

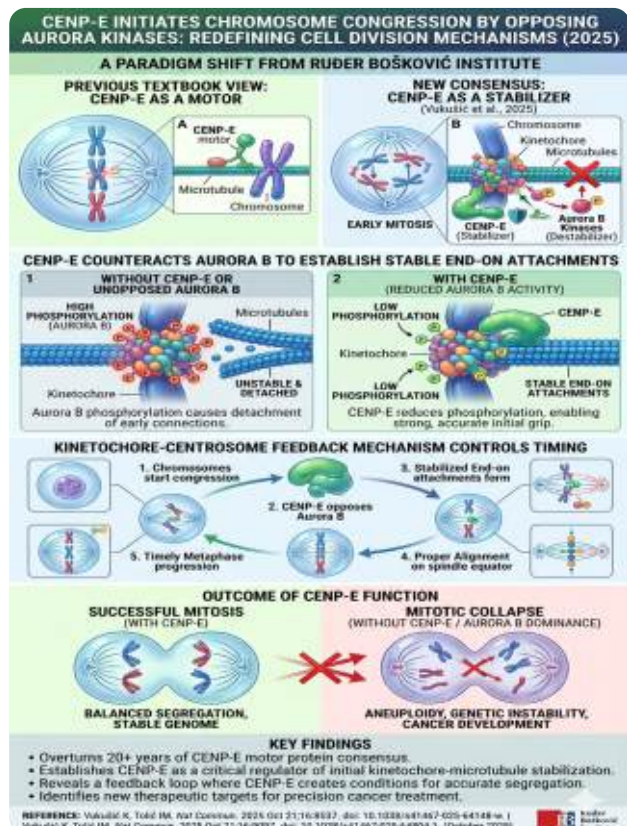
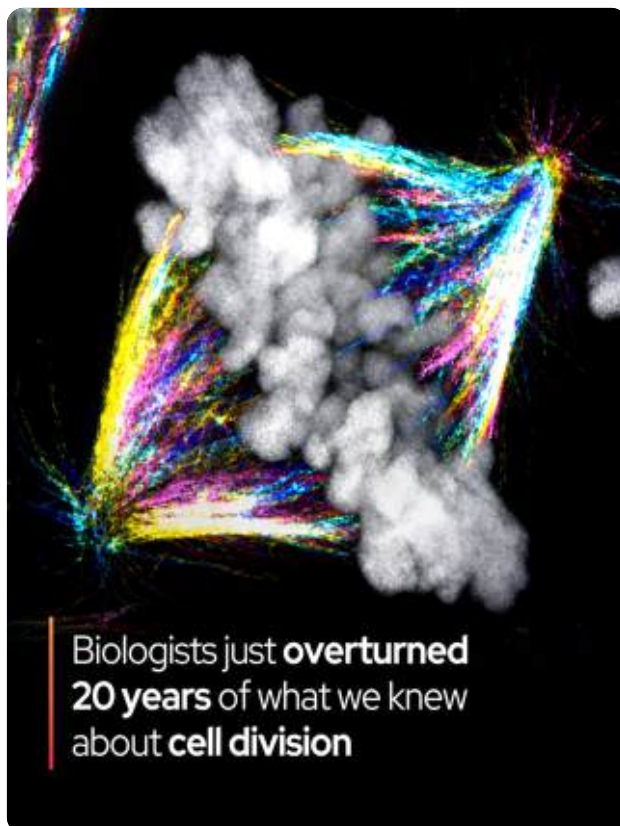
These companion studies published in *Nature Communications* (October 2025) from the Ruđer Bošković Institute overturned two decades of textbook cell biology regarding the function of the CENP-E protein during mitosis. The previous consensus held that CENP-E acts as a motor protein dragging misaligned chromosomes into position, but researchers led by Dr. Kruno Vukušić and Prof. Iva Tolić revealed that CENP-E's critical role is to stabilise the first connections between chromosomes and microtubules, thereby enabling accurate cell division. Using advanced live-cell microscopy and molecular analysis in human cell lines, the studies demonstrated that CENP-E opposes Aurora B kinases, which destabilise early kinetochore-microtubule attachments through phosphorylation. CENP-E counteracts Aurora kinase activity by reducing phosphorylation levels, thereby stabilising initial end-on attachments and allowing chromosome alignment and congression to proceed correctly. Additionally, a kinetochore-centrosome feedback mechanism linking CENP-E and Aurora kinases was found to control the timing of chromosome congression. Without the stable grip provided by CENP-E, chromosomes cannot align properly during metaphase, causing accuracy collapse that may lead to aneuploidy, cancer development, and genetic disorders. The studies conclude that CENP-E creates the conditions for flawless cell division rather than providing brute force movement, opening new therapeutic targets for precision cancer treatments and offering a framework for understanding how cells avoid potentially fatal division errors.

 **Source:**

 **RESEARCH PAPER**

Vukušić K, Tolić IM. CENP-E initiates chromosome congression by opposing Aurora kinases to promote end-on attachments. *Nat Commun.* 2025 Oct 21; 16:8537. doi: 10.1038/s41467-025-64148-w. PMID: 41120314.

Vukušić K, Tolić IM. Kinetochore-centrosome feedback linking CENP-E and Aurora kinases controls chromosome congression. *Nat Commun.* 2025 Oct 21; 16:9097. doi: 10.1038/s41467-025-64804-1.



Asteroid Ryugu Reveals the Complete Cosmic Recipe for Genetic Material (2026)

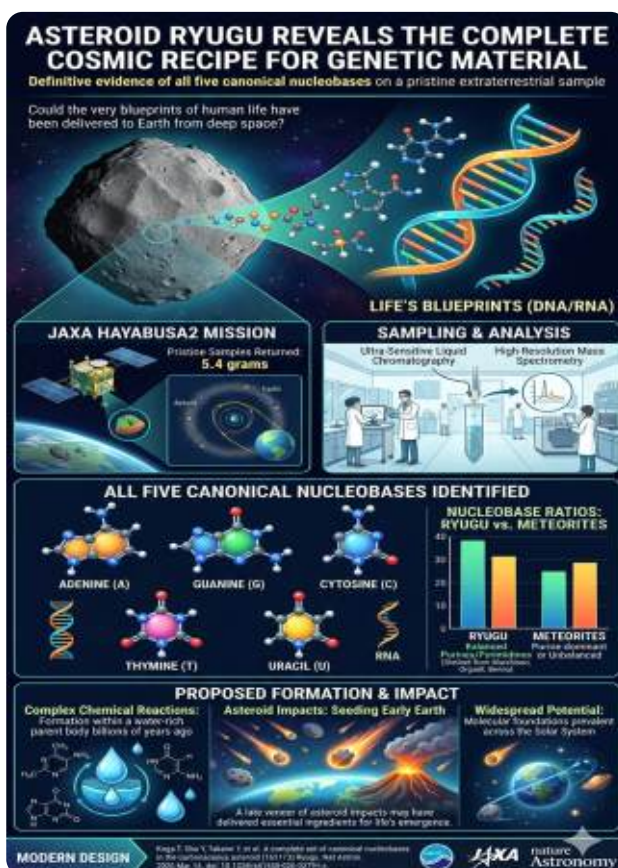
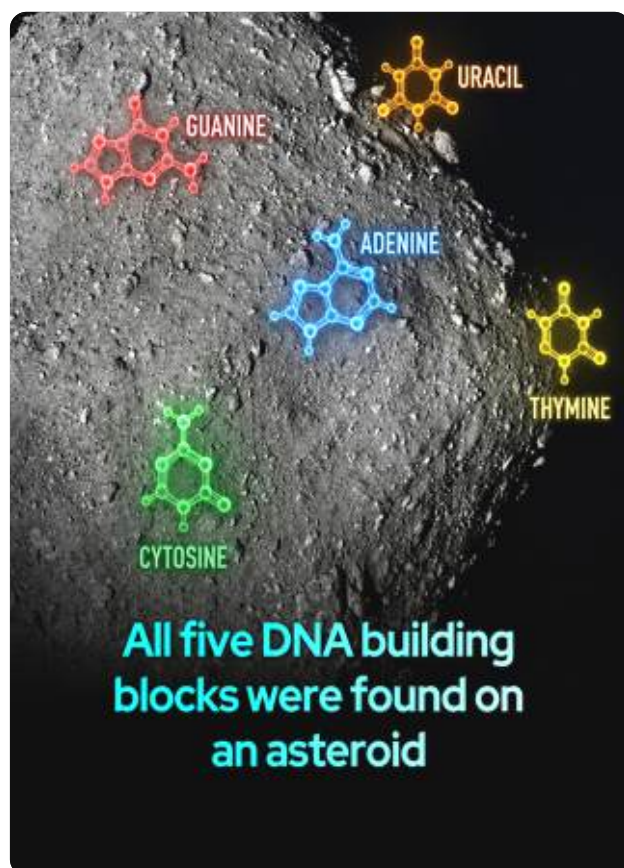
🔬 *Could the very blueprints of human life have been delivered to Earth from the cold reaches of deep space?*

The origin of life's molecular building blocks remains one of science's most profound mysteries. A landmark study published in *Nature Astronomy* (March 2026) by Toshiki Koga, Yasuhiro Oba, Yoshinori Takano and colleagues from the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) analysed pristine samples totalling 5.4 grams returned by JAXA's Hayabusa2 mission from carbonaceous asteroid Ryugu using ultra-sensitive liquid chromatography and high-resolution mass spectrometry. For the first time, all five canonical Nucleobases, Adenine, Guanine, Cytosine, Thymine and Uracil, the fundamental molecular components of DNA and RNA, were identified within a single uncontaminated extraterrestrial sample. Uniquely, Ryugu samples contained nearly equal proportions of Purines and Pyrimidines, a balanced ratio distinct from the Murchison and Orgueil meteorites and asteroid Bennu, suggesting nucleobase formation pathways depend on the physicochemical environment of individual parent bodies. The proposed mechanism suggests these molecules formed via complex chemical reactions within a water-rich parent body billions of years ago. This discovery provides definitive evidence that the molecular foundations for biological information storage are prevalent across the solar system. These results strongly support the hypothesis that a late veneer of asteroid impacts may have seeded the early Earth with the essential ingredients necessary for the spontaneous emergence of life.

Source:

📄 RESEARCH PAPER

📌 Koga, T., Oba, Y., Takano, Y. *et al.* A complete set of canonical nucleobases in the carbonaceous asteroid (162173) Ryugu. *Nat Astron* 10, 655–663 (2026). <https://doi.org/10.1038/s41550-026-02791-z>.



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Pacific Journal of Medical and Health Sciences

ISSN: 2456-7450 (Print) | Quarterly | International | Peer-Reviewed

About the Journal

Pacific Journal of Medical and Health Sciences (PJMHS), accessible online at www.medicaladvances.ac.in, is an open access, quarterly, international, peer-reviewed journal published by Pacific Medical University, Udaipur, Rajasthan, India. The journal is committed to publishing high-quality Research Papers, Case Reports, and Reviews. PJMHS invites articles from a wide range of contributors conducting research based on quantitative, qualitative, or mixed-method approaches. Ease of access to an expanding knowledge base is ensured through the journal's policy of free dissemination of its contents to all readers.

The key aims of the journal are to provide authoritative interpretations of emerging developments in medical knowledge by trusted experts in their respective fields, and to assist practitioners in incorporating not only evidence-based findings but also new conceptual frameworks and ways of thinking into their clinical practice.

Subject Areas

The subject areas covered include, but are not limited to, the following:

Anatomy, Anaesthesia, Biochemistry, Biomedical Sciences, Physiology, Pharmacology, Oncology, Cardiology, Cardiovascular Diseases, Community Medicine, Dermatology and Venereal Diseases, Diabetes, Demography and Disease Burden, Endocrinology, Epidemiology and Public Health, Forensic Science, Gastroenterology, Geriatric Medicine, Gynaecological Disorders, Haematology, Health Informatics, Immunology, Infectious Diseases, Internal Medicine, Medical Education, Microbiology, Nephrology, Neurology, Neurosurgery, Obstetrics and Gynaecology, Occupational Safety and Health, Ophthalmology, Orthopaedics, Otorhinolaryngology, Paediatrics, Parasitology, Pathology, Psychiatry, Pulmonary Medicine, Radiology, Reproductive and Child Health, Toxicology, Virology, Bacteriology, Basic and Laboratory Sciences, Biostatistics, Dentistry, Nursing, and Innovative Health Technologies.

The Editorial Process

All manuscripts submitted to PJMHS are initially subjected to in-house screening for scope, format, relevance, novelty, and originality. Manuscripts that fall beyond the scope of the journal, those not addressing a relatively novel research question, and those with gross methodological errors will be rejected without initiating the peer-review process; authors will be notified within 48 to 72 hours. Manuscripts with significant textual similarity are also rejected at this stage.

Manuscripts that pass the initial screening are subjected to a double-blind peer-review process. These are typically sent to two or three technical reviewers and one statistical expert, based on the nature of the study. Manuscripts are evaluated on the basis of originality, relevance, methodological rigour, scientific merit, ethical standards, and soundness of conclusions. Following peer review, accepted manuscripts will be edited for language and style in accordance with PJMHS guidelines.

Where an Editorial Board Member is listed as an author or has any competing interest regarding a specific manuscript, another member of the Editorial Board will be assigned to oversee that submission. All such manuscripts undergo the same review process as any other submission, irrespective of institutional affiliations.

Types of Manuscripts

PJMHS publishes manuscripts based on quantitative, qualitative, or mixed-method investigation approaches in the following categories:

#	Manuscript Type	Word Limit*	Abstract	Notes
1	Original Research Article	Up to 3,000	Structured, 250 words	4-point structured abstract; IMRAD format
2	Research Brief	1,500 to 2,000	Structured, 150 words	Limited methodology/sample size; published as Original Article
3	Review Article	Up to 3,000	Unstructured, 250 words	Includes rapid, mapping, and scoping reviews; max. 100 references
4	Systematic Review / Meta-analysis	Up to 3,000	Structured, 250 words	IMRAD format; structured abstract with 5 sections
5	Case Report	Up to 2,000	Unstructured, 150 words	Must include patient consent; clear clinical learning points
6	Health Technology Innovation	1,500 to 2,000	Structured, 200-250 words	Rapid revision within 7 days; published online within 6 weeks
7	Perspectives / Viewpoint / Commentary	Up to 1,500	Unstructured, 150 words	Opinion pieces by experienced clinicians or public health experts

* Word limits exclude Abstract and References.

Specific Details for Each Manuscript Type

1. Original Research Articles

Original research articles should be up to 3,000 words (excluding Abstract and References) and include a structured abstract of no more than 250 words under four subheadings: (i) Background and Objectives, (ii) Methods, (iii) Results, and (iv) Interpretation and Conclusions, followed by 4 to 6 keywords arranged alphabetically. The main article must include sections in the following order: Introduction, Methods, Results, Discussion, Acknowledgements (if any), References, Tables, Legends to Figures, and Figures.

Ethical clearance from an Institutional Ethics Committee or Institutional Review Board (IRB) is mandatory for all studies involving human participants or animals, and must be mentioned in the Methods section. Registration of clinical trials is mandatory; the registration number or CTRI number must be stated.

2. Research Briefs

Original research manuscripts with a well-defined study design and sample size, or submissions with preliminary investigative data with limited methodology but important clinical implications, may be submitted as Research Briefs. These should be between 1,500 and 2,000 words and contain a 4-point structured abstract of no more than 150 words, consistent with the format for Original Articles. A Research Brief may include one or two Tables or Figures. Research Briefs will be published under the category of Original Articles.

3. Review Articles

Various types of review articles are published in PJMHS, including but not limited to rapid, mapping, and scoping reviews. Review articles written by scientists or experts who have published quality original research in the relevant area will be considered. The article should be up to 3,000 words (excluding Abstract and References), with no more than 100 recent and relevant references, a clear description of the search strategy employed, and an unstructured abstract of approximately 250 words. Tables and Figures may be included as required. Copyright permission must be obtained from the copyright holder in advance if a published Table or Figure is reproduced in part or in whole.

4. Systematic Reviews (Including Meta-analysis)

Systematic reviews and meta-analyses should be critical appraisals of studies on important topics of clinical or public health significance, aimed at obtaining an unbiased quantitative estimate of the overall effect of an intervention or variable for a defined outcome. These should be written in the IMRAD format, with a maximum of 3,000 words and a minimum number of Tables and Figures. A structured abstract of 250 words is required, including the following sections: Context (clinical question and its significance), Objective, Evidence Acquisition (data sources, search strategies, years searched), Results (major findings with emphasis on the highest quality evidence), and Conclusions.

5. Case Reports

Case reports should present clinically significant or unusual cases with clear educational value. Manuscripts should be up to 2,000 words with an unstructured abstract of no more than 150 words. Written informed consent from the patient (or guardian) must be obtained and confirmed in the manuscript. Patient identifiers must not appear anywhere in the text or illustrative material.

6. Health Technology Innovations

Articles under this category should present research evaluating innovative, indigenous, low-cost health technologies with clinical validation that are seeking regulatory approval or are being recommended for adoption by national health programmes. Manuscripts found suitable by the Editors will undergo peer review and rapid revision by the authors within seven days. Accepted manuscripts will be published online within six weeks of submission.

These manuscripts should be between 1,500 and 2,000 words (excluding Title, Abstract, and References) and contain a 4-point structured abstract of 200 to 250 words, a combined Results and Discussion section not exceeding 500 words, a maximum of 25 references, and one Figure or one to two Tables. Preference will be given to work completed within six months of manuscript submission.

7. Perspectives / Viewpoint / Commentary

Articles under this category should address current, controversial, and challenging topics in healthcare, including intersections between medicine and society. These are primarily opinion pieces written by senior scientists, public health experts, or policymakers with substantial credible experience on the subject. Such papers will generally be authored by a single contributor, should not exceed 1,500 words, and must include an unstructured abstract of approximately 150 words. All submissions under this category are subject to peer review. Views must be supported by evidence and references.

Preparation of Manuscript

Submitted manuscripts must represent original work that has not been previously published and is not under consideration for publication elsewhere in any form, including as an abstract or as part of another article. All source material must be fully acknowledged and appropriately referenced.

As part of its commitment to research integrity, PJMHS screens all accepted manuscripts for plagiarism using Turnitin software. Plagiarism, including duplicate publication of the author's own work in whole or in part without proper citation, will not be accepted under any circumstances.

General Formatting

Manuscripts must be typed in portrait layout, with 1.5 line spacing, 2.5 cm (approximately 1 inch) margins on all sides, 12-point Times New Roman font, and justified alignment. Pages must be numbered at the bottom centre. British (UK) English must be used throughout.

All abbreviations used in the text must be expanded upon first use, with the acronym in parentheses, in the title, abstract, and main text separately, unless the abbreviation is a standard unit of measurement. The use of acronyms and abbreviations should be kept to a minimum.

Non-proprietary names of drugs, devices, and other products should be used. Where proprietary names are provided, only the first letter should be capitalised, followed by the name of the manufacturer in parentheses. Superscript symbols such as ©, TM, or ® should not be used.

Manuscript Order

Authors must prepare their manuscripts in the following order:

Cover Page / First Page File

The cover page must be submitted as a separate file and should include a covering letter, the title page, and the authors' contributions statement.

The covering letter should explain why the manuscript is appropriate for publication in PJMHS. One author should be identified as the corresponding author, who will be responsible for the contents of the paper and for all communication with the Editorial Office. The covering letter must include a declaration that the article has not been published previously and is not under consideration elsewhere, in part or in whole.

Title Page

The title page must include the following:

- Full title of the article: concise and informative; the study type may be added after a colon
- Running title of no more than 40 characters
- Full names (first name and surname), highest academic degrees, and designations of all authors at the time of the work
- Mobile numbers, email addresses, and ORCID numbers (if available) of all authors
- Department(s) and institution(s) to which the work should be attributed, reflecting affiliation at the time of the study
- Full name, postal address, telephone number, and email address of the corresponding author
- Contributors' credits (specifying each author's role)
- Declaration of competing interests

- Funding: all sources of financial or material support, including grant numbers and funding agencies
- Ethical clearance status, including the name of the Ethics Committee, date, and reference number of clearance
- Clinical trial registration number (for clinical trials)
- Data sharing statement (for all original research)
- Declaration of Artificial Intelligence (AI) tool use in scientific writing
- Word count (excluding Abstract, Tables, Figures, Acknowledgements, and References)
- Number of Tables and number of Figures
- Disclaimers, if any

Competing Interests: Authors must disclose any ties that could influence their judgement, including financial relationships with industry such as employment, consultancies, stock ownership, honoraria, grants, or expert testimony, whether directly or through immediate family members. If an Editorial Board Member has a conflict of interest with any manuscript, this must be communicated to the Editor so that the manuscript can be handled appropriately.

Funding: Authors must disclose all sources of financial and material support for the research, including grant numbers and funding agencies.

Structured Abstract

Since abstracts are often the only substantive portion of an article indexed in electronic databases, authors must ensure that the abstract accurately reflects the content of the manuscript. All Original Research Articles and Research Briefs must have a 4-point structured abstract as specified above. The abstract should be brief, indicate the scope and significant results of the study, and highlight only the principal findings and conclusions. Conclusions and recommendations not found in the main text must not be inserted in the Abstract.

Keywords

A set of 4 to 6 keywords should be provided in alphabetical order below the abstract to facilitate indexing. Terms from the Medical Subject Headings (MeSH) list of the National Library of Medicine should preferably be used. Keywords must not repeat words already included in the title.

Introduction

The Introduction should be brief and include: what is known; what is not yet known (the gap in knowledge); and what is proposed to be done (the aim or objectives). The review of literature should be restricted to reasons for undertaking the present study and provide only the most essential background. The objective of the study must be stated clearly with adequate justification at the end of this section.

Methods

The Methods section should describe the study's design, execution, and data analysis in a logical sequence. It should include, where applicable: study setting, study duration, study population, study design, participant selection (including eligibility and exclusion criteria), outcome variables, sample size calculation, intervention and follow-up, statistical analysis, and ethical considerations.

For standard methods, a reference suffices unless modifications have been made, in which case these must be described in full. Complete details must be provided for any new methods or apparatus. Clinical trials must include a CONSORT flow diagram showing patient progress throughout the trial.

Statistical Analysis: The statistical methods and the significance of findings should be clearly stated. P values should be expressed to three decimal places. Standard and routine statistical methods require only authentic references; detailed descriptions are needed only when new or uncommon methods are employed.

Ethics: For studies involving human participants, the procedures must conform to the ethical standards of the relevant national bodies. For research conducted in India on human participants, the ICMR Ethical Guidelines for Biomedical and Health Research on Human Participants (2017) must be adhered to. For experiments on laboratory animals, the applicable guidelines include ICMR's Use of Animals in Scientific Research (2006), INSA's Guidelines for Care and Use of Animals in Scientific Research (2000), and the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All research must also comply with the Helsinki Declaration of 1964, as revised in 2013. All research studies must have written ethical clearance from an Institutional Ethics Committee, stated in the manuscript with the committee's name, date, and reference number on the title page, and a statement of ethical clearance in the Methods section.

Obtain written informed consent from all human participants. For children over six years of age in clinical studies, assent must also be obtained. Patient names, initials, or hospital numbers must not appear in the manuscript or illustrative material.

Results

Only data essential for understanding the discussion and main conclusions should be included. Data should be arranged in a unified and coherent sequence. Data presented in Tables and Figures must not be repeated in the text. The same data must not be presented in both tabular and graphic form. Interpretation of data must be reserved for the Discussion section.

Numeric results should be reported not only as derivatives (such as percentages) but also as the absolute numbers from which they are derived. Units of measurement: Length, height, weight, and volume should be reported in metric units. Laboratory values should be reported using the International System of Units (SI). Blood pressure should be reported in millimetres of mercury (mmHg); temperatures in degrees Celsius.

Discussion

The Discussion should interpret the results without repeating information already presented in the Results section. It should relate new findings to existing knowledge and include logical deductions. This section should generally not exceed one-quarter of the manuscript's total length, and should include the following elements in order: (i) a summary of the major findings; (ii) an evaluation of the study's limitations and strengths; (iii) a comparison with similar published studies; and (iv) the generalisability of the findings and their implications for practice, policy, or future research. This section should end with a clear concluding remark.

Declaration of AI Tool Use

In accordance with the updated ICMJE guidelines (2023), authors are required to declare any use of artificial intelligence (AI) or AI-assisted writing tools in the preparation of their manuscript. This declaration must appear in either the Methods section or the Acknowledgements section and must clearly specify the tool used and the nature of its application. AI tools may not be listed as authors or co-authors. Where no AI tools have been used, authors must include a statement to this effect.

Acknowledgements

Authors may acknowledge funding sources, institutional support, and contributions of individuals who do not qualify for authorship.

References

Authors should provide direct references to original research sources wherever possible. References should be made to published articles rather than abstracts. Authors should avoid citing articles published in predatory or pseudo-journals. Information from manuscripts submitted but not yet accepted should be cited in text as 'unpublished observations' with written permission from the source. A 'personal communication' may be cited only when it provides information not publicly available, in which case the name of the person and the date of communication should be noted in parentheses in the text, with written permission and confirmation of accuracy from the source.

References must be listed in numerical sequence according to their order of appearance in the text. References should be identified in the text, tables, and figure captions by Arabic numerals as superscripts. Abstracts should not be used as references. The journal follows Vancouver referencing style. If a paper has more than six authors, list the first three followed by 'et al.' Use abbreviated journal titles as listed in Index Medicus. All hyperlinks must be removed from references.

Examples:

Journals:

Candis JH. Artificial joint materials. J Biomed Eng. 1994;45:54-78.

Pail KN, Smith ADF, Manners M, et al. Coagulation mechanisms. J Cell Biol. 1993;430:200-30.

Books:

Acorn AD. Management of rheumatoid arthritis. In: Brown CC, Davies GH, editors. Inflammatory diseases. 3rd ed. London: Apple; 1992. p. 203-30.

Dunlop E, David BC, Winston WDC, editors. Diabetes update. New York: Pullworth; 1983.

Electronic Source (Website or Online Journal Article):

*Public Health Laboratory Service. Antimicrobial resistance in 2000: England and Wales [Internet]. [cited 2004 Jan 7]. Available from:
http://www.hpa.org.uk/infections/topics_az/antimicrobial_resistance/amr.pdf*

Abbreviations and Units

Only standard abbreviations should be used. SI (International System of Units) units must be used for laboratory values throughout the manuscript. For clinical measurements, conventional metric units are preferred. Length, area, mass, and volume should be expressed in metric units. Abbreviations should follow SI conventions throughout the text, tables, and figures.

Year, month, week, day, hour, minute, and second should be abbreviated as y, mo, wk, d, h, min, and s respectively in tables and figures, but should be written out in full in the body text.

Tables

Tables must be included in the main manuscript file in Microsoft Word format and numbered consecutively with Arabic numerals (Table 1, Table 2, etc.). A descriptive heading must be placed above each table. Each column must be given a short heading; units of measurement should be abbreviated and

placed below the column heading. Tables should ideally have between three and five columns and between four and sixteen rows.

All non-standard abbreviations used within a table must be explained in footnotes. Statistical measures of variation such as SD and SE must be identified. Use footnote symbols (*, †, ‡, §) where needed. In the text, tables should be referenced as Table 1, Table 2, and so on, and must not be referred to as 'above table' or 'below table'. Each table must be cited in the text.

Figures and Illustrations

The use of figures is strongly encouraged where they assist reader comprehension and can replace lengthy textual descriptions. Figures must be numbered consecutively in Arabic numerals (1, 2, 3) according to the order in which they are first cited in the text. Where figures are related, they should be numbered 1(a), 1(b), 1(c), and so on.

All images must be submitted in JPEG or TIFF format with a maximum file size of 20 MB. Labels, numbers, and symbols should be clear and of uniform size, legible after reduction to the width of a printed column. Titles and detailed explanations belong in the figure legends, not on the figure itself. Photographs must be of sufficiently high resolution with respect to detail, contrast, and grain, and must be trimmed to remove all unwanted areas.

If photographs of individuals are used, written permission to use the photograph must be provided and the eyes must be covered. Patient names and medical record numbers must be removed. If a figure has been published elsewhere, the original source must be acknowledged and written permission from the copyright holder submitted to reproduce the material. Electronic manipulation of images that materially alters the medical information must be identified and the nature of the alterations described.

Figures should be sent as separate files. For charts and graphs, authors should prepare these in Microsoft Word and not paste from programmes such as PowerPoint or SPSS. Editable files of figures should be provided as supplementary material. Authors' names and affiliations must not appear anywhere on image files.

Figure Captions

Captions should provide a brief description of each figure or illustration. For example: Fig. 1. The diagram shows the distribution of... Where relevant, captions must include definitions for all symbols used.

Photographs

Photographs submitted must be of sufficiently high resolution and quality with respect to detail, contrast, and grain. Use portrait format for photos intended to fit a single column. Patient identifiers must be removed from all photographic material.

Formatting Guidelines

All manuscripts must conform to the following formatting specifications:

File Format	Microsoft Word (.doc or .docx)
Font	Times New Roman
Font Size	Title: 16pt, Bold. Section Headings: 14pt, Bold. Body Text: 12pt.
Line Spacing	1.5
Margins	2.5 cm (approximately 1 inch) on all sides
Layout	Portrait, single column, justified alignment
Language	British (UK) English
Page Numbering	Arabic numerals, bottom-centre alignment
Title Page	Separate file. Must contain: title, author names (with degrees and ORCID if available), designations, institutional affiliation, email address, phone number, and postal address. Authors must not include their name or affiliation anywhere in the main manuscript.
Abstract	Structured (4-point) for Original Articles and Research Briefs; unstructured for Reviews and Commentaries. Maximum as specified per manuscript type.
Keywords	4 to 6, in alphabetical order, from MeSH list where possible
Full Length Paper	Up to 3,000 words for Original Articles, Reviews, and Systematic Reviews (excluding Abstract and References); as specified for other types
Tables and Figures	Each must have its own heading and source. Cited in text as Table 1, Table 2, etc.
References Style	Vancouver (numbered, superscript); abbreviated journal titles per Index Medicus

Submission of Manuscript

Manuscripts must be submitted in Microsoft Word format by email to:

info@medicaladvances.ac.in

All submissions must be accompanied by a cover letter stating the full names of the authors, correspondence address, email address, and contact numbers. As the journal follows a double-blind peer-review process, the identity of the authors must not appear anywhere within the main manuscript document. The editorial team will remove author details before sending the manuscript to referees.

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Editorial Independence and Endogeny Policy

Pacific Journal of Medical and Health Sciences is committed to maintaining editorial independence and a broad, diverse authorship base. Manuscripts submitted by authors affiliated with Pacific Medical University are subject to the same rigorous double-blind peer-review process as all other submissions and receive no preferential treatment. The proportion of articles authored by individuals affiliated with the journal or its publisher shall not exceed 20% of the total articles published in any single issue.

Plagiarism Policy

All accepted manuscripts are screened for plagiarism using Turnitin software. Only manuscripts with an acceptable similarity index will be considered for publication. Plagiarism in any form, including self-plagiarism and duplicate submission, will result in immediate rejection.

Pacific Journal of Medical and Health Sciences

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Chief Editor: Dr. S.K. Verma, Professor Emeritus (General Medicine) | Editor: Ravindra Bangar

Peer-Review Policy

Pacific Journal of Medical and Health Sciences

ISSN: 2456-7450 (Print)

Overview

Peer review is the primary mechanism by which Pacific Journal of Medical and Health Sciences assesses the quality, originality and scientific rigour of submitted manuscripts prior to publication. In this process, independent researchers with relevant expertise evaluate submitted manuscripts and provide informed assessments to assist the editorial team in making publication decisions.

The journal operates a double-blind peer-review system, in which the identities of both the authors and the reviewers remain confidential throughout the review process. This approach ensures objectivity, impartiality and integrity at every stage of manuscript evaluation.

All peer reviewers are independent of and external to the journal. Reviewers are selected exclusively from outside the Editorial Board and the institution of Pacific Medical University, unless no suitably qualified external reviewer is available, in which case any exception will be clearly documented.

Purpose of Peer Review

Pacific Journal of Medical and Health Sciences regards peer review as the cornerstone of its commitment to publishing research of the highest scientific merit. Given the journal's focus on medical and health sciences, where the quality and accuracy of published findings may directly influence clinical practice and patient outcomes, the integrity of the review process is of paramount importance. Peer review serves the following specific purposes in the context of this journal:

- **Quality Assurance:** The peer-review process enables the editorial team of Pacific Journal of Medical and Health Sciences to ensure that only scientifically sound, methodologically rigorous and clinically relevant research is accepted for publication. This safeguards the journal's reputation and the trust of its international readership.
- **Editorial Independence and Objectivity:** All manuscripts submitted to the journal are evaluated by independent reviewers who have no affiliation with the authors or their institutions. This independence ensures that publication decisions are based solely on scientific merit, free from institutional, personal or commercial influence.
- **Strengthening Research Quality:** Constructive reviewer feedback helps authors refine their methodology, sharpen their conclusions and improve the overall presentation of their work. This collaborative process raises the standard of research disseminated through the journal and ultimately contributes to the advancement of medical knowledge.

Initial Editorial Assessment

All manuscripts submitted to Pacific Journal of Medical and Health Sciences are first assessed by the editorial team. During this initial review, the manuscript is evaluated on the following criteria:

- Relevance to the aims and scope of the journal
- Scientific quality and methodological soundness
- Originality and contribution to the relevant field of knowledge
- Adherence to the journal's formatting and submission guidelines

Manuscripts that do not meet these criteria may be returned to the authors with recommendations for revision or may be rejected without being sent for external peer review. Only manuscripts that pass this initial assessment are forwarded to independent expert reviewers.

External Peer Review

Manuscripts that successfully pass the initial editorial assessment are sent to a minimum of two independent expert reviewers in the relevant subject area. Reviewers are selected on the basis of their expertise, academic standing and absence of conflict of interest with the authors or their institution.

Reviewers are requested to evaluate the manuscript in a prescribed format and to provide a structured assessment that includes:

- An evaluation of the scientific quality and validity of the work
- An assessment of the originality and significance of the findings
- Identification of any weaknesses, inconsistencies or areas requiring clarification
- Specific suggestions for improvement
- A clear recommendation regarding publication

Reviewer recommendations typically fall into one of the following categories: Accept as submitted; Accept with minor revisions; Major revision required; Reject. The editorial team reviews these recommendations in the context of the journal's scope and standards before arriving at a final editorial decision.

Role of the Editor

The role of the reviewers is advisory in nature. The editor is not formally bound to accept the opinions of the reviewers and retains the authority to make the final publication decision. In all cases, the editor's decision is guided by the best interests of scientific integrity and the standards of the journal.

Reviewers do not communicate with one another, do not function as a group, and are not made aware of each other's identities or comments at any stage of the review process. This ensures the independence and confidentiality of each reviewer's assessment.

Handling Divergent Reviewer Opinions

In cases where two reviewers offer substantially divergent assessments of the same manuscript, the editor may adopt one or more of the following approaches:

- Invite one or more additional reviewers to provide an independent assessment
- Exercise editorial judgement on the basis of the available reviewer comments and the journal's scope and standards
- Consult members of the Editorial Board for guidance

In all instances, the editor's decision will be communicated to the authors in a clear, reasoned and timely manner.

Editorial Board Involvement

In circumstances where the editorial team is unable to identify a sufficient number of qualified external peer reviewers within a reasonable timeframe, members of the Editorial Board may be invited to serve as reviewers. This measure is adopted solely to prevent undue delays in the review process and to maintain the quality and timeliness of manuscript assessments.

Review Timeframe

In the interest of transparency and timely communication with authors, the following review timeframe applies:

- Authors will receive an acknowledgement of receipt of their manuscript within 5 working days of submission.
- The peer-review process, from assignment of reviewers to communication of the editorial decision, is completed within 30 to 45 days of submission.
- Authors will be notified of the editorial decision along with reviewer comments within this timeframe.
- In exceptional circumstances where additional time is required, authors will be informed of the delay and provided with a revised estimated timeline.

The journal is committed to maintaining this timeframe as a standard of service to authors and to the wider research community.

Confidentiality

All submitted manuscripts are treated as confidential documents throughout the review process. Reviewers are required to maintain strict confidentiality and must not share, discuss or disclose any aspect of a manuscript under review without the explicit permission of the editor. Any reviewer who suspects a conflict of interest is required to notify the editorial office and decline the review assignment.

Pacific Journal of Medical and Health Sciences

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Chief Editor: Dr. S.K. Verma, Professor Emeritus (General Medicine) | Editor: Ravindra Bangar

Reviewers' Guidelines

Pacific Journal of Medical and Health Sciences

ISSN: 2456-7450 (Print)

Overview

Pacific Journal of Medical and Health Sciences values the essential contribution that peer reviewers make to the integrity and quality of academic publishing. Reviewers are invited on the basis of their subject expertise, academic standing and freedom from conflicts of interest. The journal is committed to ensuring that the peer-review process is rigorous, fair, timely and conducted in accordance with the highest ethical standards.

These guidelines are intended to assist reviewers in understanding their responsibilities and in producing reviewer reports that are constructive, objective and beneficial to authors and the editorial process alike.

Type of Peer Review

Pacific Journal of Medical and Health Sciences operates a double-blind peer-review system. Under this system, the identities of both the authors and the reviewers remain confidential throughout the entire review process. Reviewers must not attempt to identify the authors of a manuscript and must notify the editorial office immediately if they become aware of the authors' identity during the review.

All peer reviewers are independent of and external to the journal. Reviewers are selected exclusively from outside the Editorial Board and the institution of Pacific Medical University, unless no suitably qualified external reviewer is available, in which case any exception will be clearly documented. This ensures that the review process is conducted with full objectivity and freedom from institutional bias.

Ethics and Responsibility

Pacific Journal of Medical and Health Sciences is firmly committed to upholding the integrity of published research. Issues of copyright infringement, plagiarism, duplicate submission and other breaches of publication ethics are treated with the utmost seriousness. All submitted manuscripts are screened using plagiarism detection software.

Where a manuscript is found to contain plagiarised content, unauthorised use of third-party copyright material, or where the authorship of the work is contested, the journal reserves the right to take appropriate action. Such action may include, but is not limited to:

- Publishing a correction (erratum or corrigendum)
- Retracting the published article
- Notifying the author's Head of Department, Dean or relevant academic bodies
- Banning the author from future submission to the journal
- Pursuing appropriate legal remedies

Reviewers who identify or suspect any of the following concerns during the course of their review are strongly advised to contact the editor without delay. All such matters must be treated with strict confidentiality and must not be discussed with colleagues other than the editor:

1. The manuscript appears to have been previously published or simultaneously submitted to another journal.
2. The manuscript appears to duplicate the work of others without appropriate acknowledgement.
3. There are concerns regarding the ethical conduct of the research described in the manuscript.
4. There is reason to suspect an undeclared conflict of interest associated with the manuscript. Editors may have access to additional information relevant to such concerns and reviewers are encouraged to consult them.

Conflict of Interest

Reviewers are requested to carefully consider any potential conflicts of interest before agreeing to undertake a review. A conflict of interest may arise where a reviewer has a personal, professional, financial or intellectual relationship with the authors, the institution, or the subject matter of the manuscript that could compromise the objectivity of the review.

Reviewers must inform the editorial office of any such conflict as promptly and discreetly as possible, and decline the review assignment where a conflict is identified. Reviewer assessments must at all times be based solely on the scientific merit, clarity and quality of the manuscript, and must not be influenced by personal, financial, intellectual or other biases.

Accepting or Declining a Review Invitation

Reviewers are invited to assess manuscripts by the editorial team via email. In order to avoid unnecessary delays in the review process, reviewers are requested to respond to invitations as promptly as possible, regardless of whether they are able to accept or must decline. If a reviewer is unable to complete a review within the requested timeframe, the editorial office should be notified at the earliest opportunity.

Suggestions for alternative reviewers who may be suitably qualified to assess the manuscript are always welcome and greatly appreciated.

Review Timeframe for Reviewers

Pacific Journal of Medical and Health Sciences is committed to a transparent and timely review process. The following timeframe applies specifically to reviewers:

- Reviewers are requested to respond to a review invitation within 5 working days of receipt, confirming whether they are able to accept or must decline the assignment.
- Upon accepting the invitation, reviewers are requested to submit their completed review report within 21 days.
- If a reviewer requires additional time to complete the review, the editorial office must be notified before the 21-day deadline so that an extension may be considered without causing undue delay to the author.
- Where a reviewer is unable to complete an accepted review within an agreed extended timeframe, the editorial office reserves the right to reassign the manuscript to an alternative reviewer in the interest of timely communication with the author.

The journal sincerely values the time and expertise that reviewers contribute to the peer-review process. Adherence to these timeframes is greatly appreciated and directly supports the journal's commitment to authors of completing the full review process within 30 to 45 days of submission.

How to Conduct a Review

Reviewers are requested to approach each manuscript systematically and to produce a structured, well-reasoned report. The following guidance is provided to assist reviewers in conducting an effective and thorough assessment.

Reviewers who are undertaking a peer review for the first time, or who are uncertain about any aspect of the review process or the journal's specific requirements, are warmly encouraged to contact the editorial office for guidance. The editorial team is always available to discuss expectations, clarify the scope of the review and offer support to reviewers at all stages of their career.

A. Preliminary Assessment

- Read the manuscript carefully in its entirety before beginning the formal evaluation.
- Consider whether the manuscript falls within the aims and scope of the journal.
- Assess the overall significance and originality of the research within the relevant field.
- Evaluate whether the work makes a meaningful contribution to existing knowledge.
- Consider whether the author has previously published a substantially similar paper elsewhere. If so, please refer to the journal's Publication Ethics policy.

B. Scientific and Methodological Evaluation

- Assess the technical accuracy and scientific validity of the content.
- Evaluate the appropriateness and rigour of the research methodology employed.
- Consider whether the conclusions presented are adequately supported by the results or evidence provided.
- Verify the accuracy and completeness of the references cited.

C. Structure and Presentation

- Assess the overall structure of the manuscript, the clarity of the argument and the logical flow of the content.
- Evaluate the quality of written English and the clarity of expression.
- Consider whether the title accurately and effectively reflects the content of the manuscript.
- Assess whether the abstract provides a clear and accurate summary of the work.
- Evaluate the effectiveness of the introduction in contextualising the research.
- Consider whether the manuscript is accessible to a broad international readership.
- Assess the internal consistency of the manuscript throughout.
- Verify that the manuscript type is consistent with the article types accepted by the journal.

Providing Feedback and Suggestions for Revision

Reviewer reports should be objective, specific, constructive and respectful in tone. The following guidance should be observed when preparing feedback:

- Provide comments that are as objective and impartial as possible. Avoid making negative remarks about other work cited in the manuscript.
- Be specific and constructive in identifying areas that require revision. Clearly indicate what needs to be added, clarified or corrected.

- Where appropriate, suggest additional literature that may enrich the author's argument or strengthen the manuscript.
- Clearly distinguish between comments intended for the authors and those intended solely for the editor's consideration, so as to avoid confusion.
- While reviewers may comment on the quality of written language and suggest improvements to the structure or argument, it is not the role of the reviewer to rewrite sections of the manuscript or to prescribe detailed changes in wording.

Making a Recommendation

Upon completing the review, the reviewer must provide a clear recommendation regarding the disposition of the manuscript. Recommendations should fall within one of the following categories:

- Accept as submitted
- Accept with minor revisions
- Major revision required
- Reject

The recommendation should be consistent with the content of the reviewer report and should be accompanied by sufficient reasoning to assist the editor in making the final publication decision.

Confidentiality

Reviewers must treat all manuscripts under review as strictly confidential documents. The content of the manuscript, the reviewer's assessment, and all correspondence relating to the review must not be shared, disclosed or discussed with any third party without the explicit permission of the editor. This obligation of confidentiality continues after the review process has been completed.

A Sample Reviewer Report form is available for download from the journal website at: www.medicaladvances.ac.in

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Publication Ethics and Publication Malpractice Statement

Pacific Journal of Medical and Health Sciences

ISSN: 2456-7450 (Print)

Overview

Pacific Journal of Medical and Health Sciences is committed to maintaining the highest standards of publication ethics across all stages of the editorial and publishing process. This statement sets out the ethical obligations of editors, reviewers and authors, and is based on the Code of Conduct and Best Practice Guidelines for Journal Editors published by the Committee on Publication Ethics (COPE), and the updated guidelines of the International Committee of Medical Journal Editors (ICMJE, 2023).

All parties involved in the publication process, including the editorial team, peer reviewers and contributing authors, are expected to adhere to these standards without exception. Any breach of publication ethics will be investigated and addressed in accordance with COPE guidelines.

Editors' Responsibilities

Publication Decisions

The editor is responsible for deciding which manuscripts submitted to the journal will be accepted for publication. This decision is based solely on the scientific merit, originality, clarity, methodological validity and relevance of the manuscript to the journal's scope. Manuscripts are evaluated without regard to the authors' race, gender, sexual orientation, religious belief, ethnic origin, nationality, citizenship or political affiliation. Current legal requirements regarding libel, copyright infringement and plagiarism are taken into account in all publication decisions.

Fair Play and Editorial Independence

The editor and all members of the editorial team are committed to ensuring that the peer-review process is conducted fairly, objectively and without discrimination. Editorial decisions are never influenced by commercial, financial or personal interests. The journal's editorial independence is maintained at all times and is not subject to interference from the publisher, the institution or any external body.

Confidentiality

The editor and all editorial staff are required to maintain strict confidentiality regarding submitted manuscripts. Information about a submitted manuscript must not be disclosed to anyone other than the corresponding author, assigned reviewers, potential reviewers, editorial advisers and the publisher, as appropriate. This obligation of confidentiality extends to the content, findings and status of manuscripts under review.

Disclosure and Conflicts of Interest

The editor and members of the editorial board must recuse themselves from any editorial decision in which they have a conflict of interest, whether personal, financial, intellectual or institutional. Unpublished material disclosed in a submitted manuscript must not be used by the editor or any member of the editorial board for their own research purposes without the explicit written consent of the corresponding author.

Handling Misconduct

The editor is responsible for investigating all allegations of research misconduct, publication misconduct or ethical violations relating to manuscripts submitted to or published in the journal. Where misconduct is confirmed or strongly suspected, the editor will take appropriate action in accordance with COPE guidelines. Such action may include the publication of a correction, the issuance of an expression of concern, or the retraction of the published article.

Reviewers' Responsibilities

Contribution to Editorial Decisions

Peer review assists the editorial team in making informed publication decisions and provides authors with constructive feedback to improve the quality of their manuscripts. Reviewers are expected to conduct their assessments with diligence, objectivity and professionalism.

Promptness

Reviewers who feel insufficiently qualified to assess a submitted manuscript, or who are unable to complete a review within the agreed timeframe, are requested to notify the editorial office promptly so that an alternative reviewer may be identified without unnecessary delay.

Confidentiality

All manuscripts received for review must be treated as strictly confidential documents. The content of the manuscript, the reviewer's assessment and all related correspondence must not be disclosed to or discussed with any third party without the explicit authorisation of the editor. This obligation continues after the review has been completed.

Standards of Objectivity

Reviews must be conducted objectively and on the basis of scientific merit alone. Personal criticism of the authors is not appropriate. Reviewers must express their assessments clearly, with specific supporting arguments and constructive suggestions for improvement.

Disclosure and Conflict of Interest

Reviewers must not assess manuscripts in which they have a conflict of interest arising from competitive, collaborative, personal or institutional relationships with any of the authors or institutions associated with the manuscript. Privileged information or ideas obtained through the peer-review process must be kept strictly confidential and must not be used for personal or professional advantage. Reviewers who identify a potential conflict of interest must inform the editorial office and decline the review assignment.

Authors' Responsibilities

Reporting Standards

Authors of original research articles are required to present an accurate, complete and objective account of the work performed and an honest discussion of its significance. Underlying data must be represented accurately in the manuscript. Sufficient methodological detail and references must be provided to enable other researchers to replicate the work. Fabrication, falsification or misrepresentation of data constitutes research misconduct and is unacceptable under any circumstances.

Originality, Plagiarism and Acknowledgment of Sources

Authors must submit only original work that has not been previously published and is not under consideration for publication elsewhere. The work and words of others must be appropriately cited or quoted. Publications that have been influential in shaping the reported work must be acknowledged. Plagiarism in any form, including the unattributed reproduction of text, data, images or ideas, constitutes a serious breach of publication ethics and will not be tolerated.

Declaration of AI Tool Use

In accordance with the updated ICMJE guidelines (2023), authors are required to declare any use of artificial intelligence (AI) or AI-assisted writing tools in the preparation of their manuscript. This declaration must appear in either the Methods section or the Acknowledgements section and must clearly specify the tool used and the nature of its application. AI tools may not be listed as authors or co-authors. Where no AI tools have been used, authors must include a statement to this effect. Responsibility for the accuracy, integrity and originality of the submitted work remains entirely with the human authors.

Multiple, Redundant or Concurrent Submission

Submitting the same manuscript to more than one journal simultaneously constitutes unethical publishing behaviour and is unacceptable. Manuscripts describing essentially the same research should not be published in more than one journal. Manuscripts that have been published elsewhere as copyrighted material may not be submitted to this journal. Manuscripts under review by this journal must not be submitted to other publications during the review period.

Authorship

Authorship must be limited to individuals who have made a genuine and significant contribution to the conception, design, execution or interpretation of the reported research. All individuals who have made such contributions must be listed as co-authors. The corresponding author is responsible for ensuring that all appropriate co-authors are included in the author list, that no individuals without a qualifying contribution are included, and that all co-authors have reviewed and approved the final version of the manuscript prior to submission.

Any changes to the author list after submission, including the addition, removal or reordering of authors, must be approved by all listed authors and communicated to the editorial office in writing, along with a clear explanation for the proposed change.

Disclosure and Conflicts of Interest

All authors must declare any financial or other substantive conflicts of interest that could be construed to have influenced the results or interpretation of their manuscript. All sources of financial support for the research must be disclosed in the manuscript. Where no conflicts of interest exist, this must also be explicitly stated.

Fundamental Errors in Published Works

When an author identifies a significant error or inaccuracy in their own published work, it is the author's obligation to notify the editor or publisher promptly and to cooperate fully with the editor to issue a correction, retraction or erratum as appropriate. Failure to notify the editorial office of a known error in a published article constitutes a breach of publication ethics.

Editorial Independence and Endogeny Policy

Pacific Journal of Medical and Health Sciences is committed to maintaining editorial independence and diversity of authorship in accordance with internationally accepted standards for academic publishing. The journal actively encourages submissions from researchers and institutions worldwide and does not give preferential treatment to manuscripts submitted by authors affiliated with Pacific Medical University or members of the editorial team.

To safeguard editorial independence and prevent excessive endogeny, the following policy applies:

- Manuscripts submitted by authors affiliated with Pacific Medical University are subject to the same rigorous double-blind peer-review process as all other submissions. No manuscript submitted by an institutional author receives preferential consideration.
- The proportion of articles published by authors affiliated with Pacific Medical University or the editorial team shall not exceed 20% of the total articles published in any single issue of the journal.
- Any manuscript in which a member of the editorial board is listed as an author will be handled exclusively by an independent editor who has no affiliation with the submitting authors. The editorial board member concerned will have no involvement in the editorial decision regarding that manuscript.
- The journal actively seeks to broaden its authorship base by inviting submissions from national and international researchers.

This policy is reviewed annually by the editorial team to ensure ongoing compliance with COPE guidelines and the standards of relevant indexing bodies.

Corrections, Retractions and Expressions of Concern

Pacific Journal of Medical and Health Sciences is committed to maintaining the accuracy and integrity of the scholarly record. Where errors or ethical concerns are identified in published articles, the journal will take prompt and transparent corrective action in accordance with COPE retraction guidelines.

- Corrections (errata or corrigenda) will be published where minor errors in a published article are identified that do not affect the overall findings or conclusions.
- Retractions will be issued where published articles are found to contain fabricated or falsified data, plagiarised content, duplicate publications or other serious ethical violations that undermine the reliability of the findings.
- Expressions of concern may be issued where an investigation into a potential ethical concern is ongoing and a definitive conclusion has not yet been reached.

All corrections, retractions and expressions of concern will be published as standalone notices and clearly linked to the original article. The original article will be retained in the archive with a clear notice of the correction or retraction attached.

Compliance with COPE and ICMJE Guidelines

Pacific Journal of Medical and Health Sciences endorses and adheres to the principles set out in the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the COPE Retraction Guidelines. The editorial team consults COPE flowcharts and guidance when addressing allegations of misconduct, disputes regarding authorship, concerns about data integrity and other ethical matters arising in connection with submitted or published manuscripts.

For further information on COPE guidelines, visit: <https://publicationethics.org>

For further information on ICMJE guidelines, visit: <https://www.icmje.org>

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- All sources of information used in the preparation of the manuscript have been appropriately acknowledged and referenced.
- The necessary permissions have been obtained for the reproduction of any third-party copyright material included in the manuscript.
- The research described in the manuscript has been conducted in accordance with applicable ethical standards and guidelines.

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Corrections and Retractions

Where errors are identified in published articles, the journal is committed to issuing timely and transparent corrections. Corrections, expressions of concern or retractions will be published as required in accordance with the journal's Publication Ethics policy and the guidelines of the Committee on Publication Ethics (COPE). All corrections and retractions will be clearly linked to the original article.

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DEPARTMENT OF ONCOLOGY



SCALP COOLING MACHINE



Rajasthan's First Scalp Cooling Machine has been established in Pacific Medical College and Hospital, Udaipur for providing US FDA Approved Scalp Cooling Therapy to Cancer Patients to prevent hair-loss due to Chemotherapy.

PACIFIC IVF CENTER

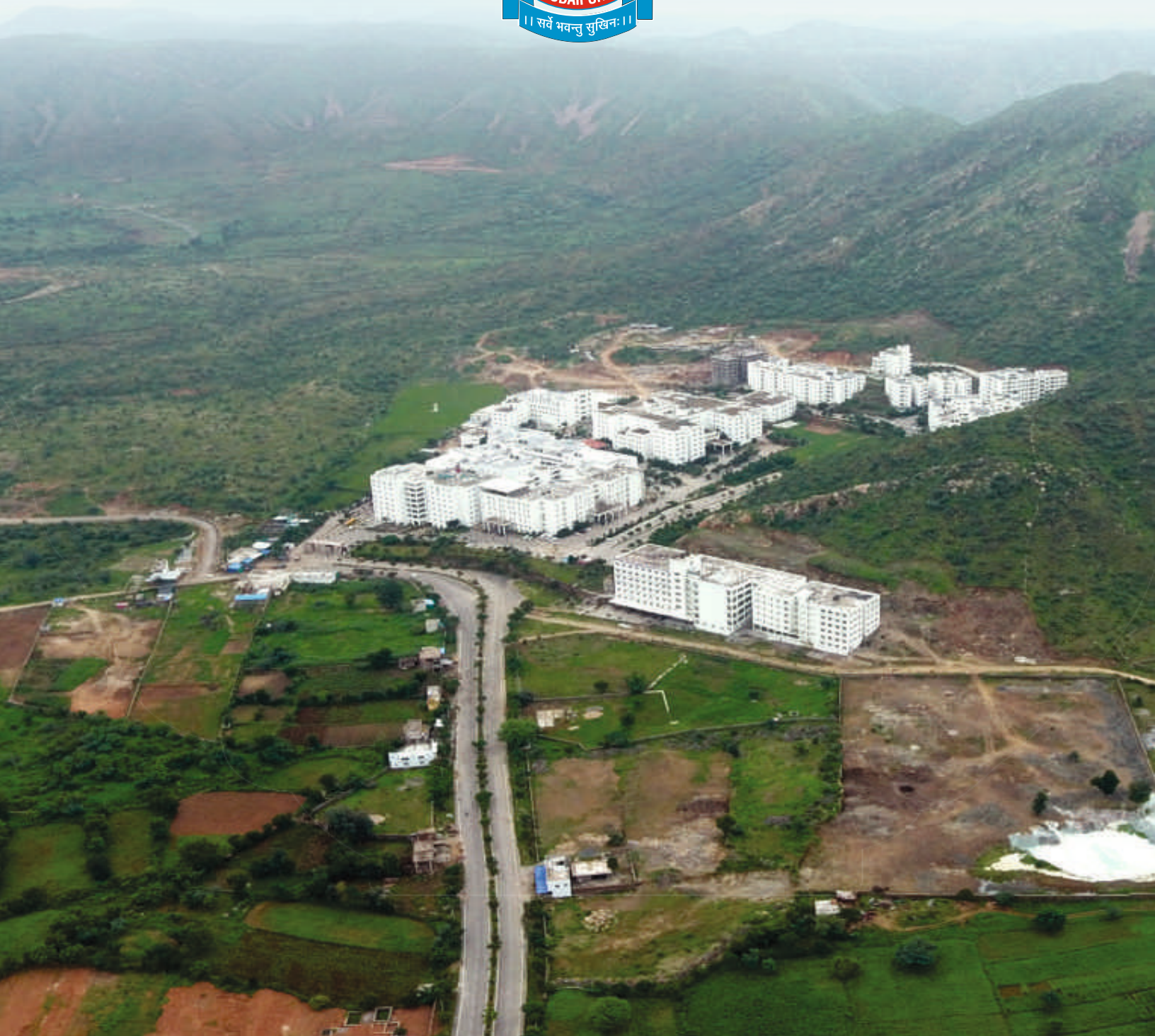


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Bhilo Ka Bedla, NH-27, Pratappura, Amberi, Udaipur 313 011 (Rajasthan) | Ph.: 0294-3950000