# African Journal of Tropical Medicine and Biomedical Research (AJTMBR)

The Journal is the Official Publication of the College of Health Sciences, Delta State University, Abraka, Nigeria.





**ISSN: 2141-6397**

**Vol. 9, No. 1, July 2026**

**Focus and Scope**

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

**Editorial Notices**

The journal will be published biannually in the months of March and September. Annual subscription fee in Nigeria is two thousand naira (N2,000) per volume (2issues); One-thousand-naira single copy (N1000). The annual subscription rate for other parts of the world is as follows: United Kingdom £60 (post free). West Africa $60 (post free). The rest of the World and the United States of America $120 (post free). A charge of $60 is made for reprints inclusive of postage. Cheques should made payable to the African Journal of Tropical Medicine and Biomedical Research and addressed to the Editor-in-Chief.

**Journal Contact**

All correspondence, including manuscripts for publication (in triplicate) should be addressed to:

**Professor P.S. Igbigbi**
The Editor-in-Chief,
Department of Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Delta State University, Abraka,
Delta State, Nigeria.

Or:
**Professor Lawrence Omo-Aghoja**
Editor
Department of Obstetrics and Gynecology,
Faculty of Clinical Medicine,
Delta State University, Abraka, Nigeria.
Email: journalajtmbr@yahoo.com
Cc: all email to
eguono_2000@yahoo.com
Tel: 08039377043

All authors are advised to submit an electronic copy in CD-ROM along with a hard copy of their manuscript, as this will spare remarkable time in the reviewing and typesetting processes.

In the alternative, authors can submit their articles and covering letter by email attachments. A covering letter (signed by all authors) accompanying the manuscript should certify that the article has not been previously published and is not being considered for publication elsewhere.

**Information for Authors**

All manuscript are peer-reviewed and accepted with the understanding that the work has not been published or being considered for publication elsewhere. Indeed, the authors would be requested

to sign a copyright form transferring the ownership of the paper to the African Journal of Tropical Medicine and Biomedical Research. All articles must include the correct names and addresses of author(s) including e-mail addresses and telephone numbers. Articles will be subjected to a thorough peer review process before any decision is made to publish or not. Authors should note that the African Journal of Tropical Medicine and Biomedical Research is not under any obligation to publish articles submitted, as decision to publish will be based on recommendations of reviewers and the editorial advisory board.

**Manuscripts**

Articles submitted for publication should be typed double-spaced with 2.5cm margins with accompanying CD-ROM in Microsoft Word format for easy and quick peer review and typesetting. Each of the following sections should begin in a new page: title page, abstract, introduction, materials and methods, results, discussion, acknowledgment (s), references, tables, legends to figures and illustrations. The manuscript should include:

**Title Page**

The title page should include the following information: 1. the title and sub-title; 2. the name(s) of the author(s); 3. the affiliation(s) of the author(s); 4. name and address of the corresponding author and 5. three to six key words for indexing and retrieval purposes.

**Abstract**

The abstract should be structured and not more than 250 words. It should carry the following headings: Introduction, Materials and Methods, Results and Conclusion.

**Original Research**- The journal welcomes articles reporting on original research, including both quantitative and qualitative studies. Full-length articles should generally not exceed 3000 words, excluding abstract, tables, figures, and references. The subject matter should be organised under appropriate headings and sub-headings as itemized above.

**Review Articles-** Comprehensive review articles on all aspects of tropical medicine and biomedical sciences will also be considered for publication in the journal. Reviews should provide a thorough overview of the topic and should incorporate the most current research. The length of review articles must not exceed 3,000 words and the organisational headings and sub-headings used are at the author's discretion.

**Short Reports -** Brief descriptions of preliminary research findings or interesting case studies will be considered for publication as short reports. The length of the abstract and article should be restricted to 150 and 2,000 words respectively and organisation of short reports are left to the author's discretion.

**Commentaries or Editorials-** Commentaries or editorials on any aspect of tropical medicine and biomedical sciences in Africa will be considered for publication in the journal. Opinion pieces need not reference previous research, but rather reflect the opinions of the author(s). The length should not exceed 2,000 words.

**Tables and Figures**

All tables and figures should be submitted on separate sheets of paper and should be clearly labelled. Coloured tables and figures may be reprinted in black and white. Authors should especially take care that all tables are clear and understandable by themselves, independent of

the text. A reader should be able to read only the tables and easily grasp all information without the text.

**Acknowledgments**

Acknowledgments should be included on a separate sheet of paper and should not exceed 100words. Funding sources should be noted here.

**References**

References should be in the Vancouver style and numbered consecutively in the order in which they are mentioned in the text. Titles of journals should be abbreviated according to the Index Medicus style. Authors must cross-check and make sure that all information provided in the reference list is complete and correctly written. Reference numbers should be inserted above the line on each occasion a reference is cited in the text, e.g., … as 1-3 reported in other studies. Numbered references should appear at the end of the article and should include the names and initials of all authors. The format of references should be as published by the International Committee of Medical Journal Editors in the British Medical Journal 1988, volume 296, pages 401-405. The following are sample references for an article published in a journal and for a book: Ahmed Y, Mwaba P, Chintu C, Grange JM, Ustianowski A, Zumla A. A study of maternal mortality at the University Teaching Hospital, Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. Int J Tuberc Lung Dis 1999; 3: 675-680. Whitby LG, Smith AF, Beckett GJ. Enzyme Tests in Diagnosis. In: Lecture Notes on Clinical Chemistry. Whitby LG, Smith AF & Beckett GJth (eds). 4 editions. Blackwell Scientific Publications. 1988. 103-127.

**Units of Measurement**

All measurements should be expressed in SI (Systeme International) Units.

**Galley proofs**

Corrections of galley proofs should be strictly restricted to Printer's error only. Orders for offprints should be made when the corrected proofs are being returned by the authors. Articles accepted for publication remain the property of the journal and can only be reproduced elsewhere in line with section 5 of the copyright agreement.

"For those journals with their own websites, and a very high degree of in-house technical competency, it is possible to get code snippets from the Creative Commons website for the license type chosen, which can be embedded in your online content, but please note that that is definitely not a requirement, just a **"nice to have".**

# Table of Contents

*Editorial Commentary*

# Traumatic Brain Injury Management in Nigeria: A Critical Review of Systemic Challenges and Integrated, Context-Specific Solutions

[1,2]Campbell FC

**ABSTRACT**

Traumatic brain injury (TBI) is a leading cause of death and disability in Nigeria, driven overwhelmingly by road traffic accidents and affecting the young, economically productive demographic. Yet the health system is poorly equipped to meet this burden. This editorial reviews evidence on challenges spanning the entire continuum of TBI care, from very poor prehospital systems and severe diagnostic bottlenecks to an extreme shortage of neurosurgeons and limited rehabilitation services. It also attempts to integrate practical, evidence-based solutions, some of which are already being piloted within the country. The review will draw on recent Nigerian epidemiological studies, workforce analyses, and nascent national guideline initiatives to propose a coordinated strategy-driven approach. The analysis demonstrates that the necessary tools and local evidence exist; what is absent is the political will and sustained investment to unite fragmented ideas into a coherent national system. This Nigerian case study offers transferable lessons for low-resource settings globally.

**Keywords:** Traumatic Brain Injury; Nigeria; Health Systems Strengthening; Neurosurgery; Prehospital Emergency Care; Secondary Brain Injury

[1]*Delta State University Teaching Hospital*
[2]*Delta State University, Abraka*

**Corresponding author:** Campbell Francis Chukwuebuka, Department of Surgery, Delta State University Teaching Hospital, Oghara, Delta State, Nigeria, drcampbellfrancis4@yahoo.com

## INTRODUCTION

Traumatic brain injury (TBI) imposes a disproportionate burden on low- and middle-income countries (LMICs), where more than three-quarters of global trauma deaths occur.[1,2] Nigeria, with a population exceeding 200 million, exemplifies these problems, characterised by a high incidence of road traffic accidents and a health care system that is fragmented, inequitably distributed, and marked by a shortfall in comprehensive emergency neurosurgical care. This editorial critically examines how systemic barriers can convert a survivable head injury into mortality or a lifetime of severe disability. It clearly elucidates the deficiencies within each domain of the care pathway for TBI patients and highlights practical recommendations anchored in local, sustainable innovations and emerging evidence. By doing so, it offers a clear pathway for strengthening neurosurgical health systems that is both context-specific and scalable to other resource-limited settings, while acknowledging the implementation and financing challenges that must be overcome.

### Epidemiology and Aetiology

A 2024 systematic review and pooled analysis of 45,763 patients across 254 Nigerian studies provides the most comprehensive epidemiological profile to date.[2] Neurotrauma accounted for the overwhelming majority of

neurosurgical presentations, with TBI alone representing nearly 90% of cases. The mean age was 32.5 years, and the male-to-female ratio was approximately 3:1, reflecting a concentration of injury among the highly productive section of the population. Road traffic accidents were the dominant mechanism, responsible for 68.6% of all TBIs.[2] This pattern is consistent with global estimates, which identify road traffic injuries as the leading cause of TBI worldwide and a rapidly growing burden in sub-Saharan Africa.[3,4]

The paediatric burden mirrors this pattern. A systematic review of 2,234 children with TBI found that road traffic accidents (52.2%) and falls from height (32.4%) were the leading causes, with a pooled mortality of 10.2%.[5] A two-centre prospective study from South-East Nigeria reported road traffic accidents in 69.2% of paediatric head injuries.[6] Across all age groups, there is a high burden of severe cases at presentation, with altered consciousness documented in nearly half of patients.[2] While these data are broadly consistent, most originate from tertiary hospitals, potentially overestimating severity and underestimating milder injuries that never reach a formal neurosurgical service. The overwhelming role of road traffic accidents underscores the importance of primary prevention through road safety legislation and enforcement, but it also means that the health system must be configured to receive and manage a large volume of severe head injuries.

**The Prehospital Care Gap**

In well-functioning trauma systems, the prehospital phase is where the greatest survival gains are made by preventing secondary brain injury from hypoxia, hypotension, and raised intracranial pressure. In Nigeria, this phase is almost entirely absent or exists as pockets of activity in a few urban areas. A 3-year retrospective review of 23537 trauma patients at a major centre in Lagos, Nigeria, showed that only 2.3% received formal prehospital care.[7] Basic first aid knowledge, including airway and cervical spine control, is poor among Nigerians (Figure 1), and it is estimated that more than half of all deaths can be prevented by effective prehospital and emergency care delivery.[8,9] A systematic analysis of the neurosurgical capacity reinforces this, documenting the near-absence of formal prehospital coordination for neurotrauma across the country.[10] Globally, the absence of prehospital systems is recognised as one of the most significant contributors to preventable death after injury in LMICs.[8,11,12]

The solution does not require an unaffordable fleet of advanced ambulances as seen in industrialised nations. Instead, a national programme to train lay first responders, including commercial drivers, motorcycle and tricycle operators, teachers in primary and secondary schools, and community volunteers, in basic airway management, cervical spine and bleeding control, and safe transportation can significantly reduce preventable deaths. Such programmes have proven effective in other low-resource settings, and the growing body of evidence on task-sharing in neurosurgery supports the principle of extending life-saving competencies to non-specialist cadres.[13] Simultaneously, the National Emergency Medical Service and Ambulance System (NEMSAS) must be expanded beyond its current limited operations and linked to a single, functioning national emergency number.

Another novel recommendation is mandatory first-aid certification for commercial driving licence renewal, covering commercial bus, motorcycle, and motorbike riders and teaching them safe transportation protocols. In addition, designated hospitals within each region can serve

as the catchment area where the first responder can call before transporting the patient. Although this is a far cry from what is available in developed countries, it will lay the groundwork for further development of Nigeria's prehospital system.

**Diagnostic Bottlenecks in Management of Traumatic Brain Injury**

Computed tomography is the indispensable tool for triaging TBI, yet its availability in Nigeria is grossly inadequate. The geospatial analysis of neurosurgical infrastructure documented that only a minority of the nation's CT scanners are located in public hospitals, and many of these are non-functional due to maintenance failures and erratic power supply.[10] The financial barrier is equally formidable. Nigeria has the largest population living in multidimensional poverty in Africa, with over 133 million people; that's over 63% of the population lacking access to essential services, including healthcare.[14] Compounding this, the National Health Insurance Authority (NHIA) covers fewer than 10% of Nigerians, and over 70% of total health expenditure is out-of-pocket,[15] meaning that the cost of a CT scan, typically ranging from ₦30,000 to ₦80,000 (approximately US$20–50), represents a significant expense for the majority of households.[10]

The clinical consequences are severe. A systematic review of paediatric TBI in Nigeria reported that at Lagos University Teaching Hospital, a leading tertiary centre, only 57.7% of children with TBI were able to obtain a CT scan, with the inability to pay being the primary barrier.[16] Patients are frequently required to travel over 100 km to access a functioning scanner, and the resultant delays may prove fatal for time-sensitive intracranial haematomas.

Addressing this requires a pragmatic, multi-pronged strategy involving both private and public partnerships. An immediate national audit of all CT and MRI equipment, documenting location, ownership, functional status, and maintenance needs, must be conducted to create a transparent inventory and inform procurement planning. The second and most impactful intervention would be a government policy directive subsidising the cost of CT for all emergency trauma patients in public and private hospitals, in addition to ensuring that health insurance providers cover the service.

A medium-term target should be the installation of at least one functional CT scanner in each federal and state teaching hospital, federal medical centre, and even general hospital, to expand coverage for underserved rural populations. The broader neurosurgical literature confirms that diagnostic delays due to financial barriers are a leading cause of preventable mortality in LMIC settings, and implementing these strategies will result in early diagnosis and enable appropriate care to be instituted.[2,10,17] Innovative approaches such as smartphone-based tele-radiology and portable CT technology are emerging as potential solutions in hard-to-reach and underserved areas.[18]

**The Neurosurgical Workforce Deficit in Nigeria**

Nigeria's neurosurgeon-to-population ratio is among the lowest globally. A 2024 geospatial analysis identified approximately 132 neurosurgeons serving 218 million people, a ratio of 1:1.65 million, against the World Health Organisation's recommendation of 1:100,000.[10] This deficit is compounded by severe brain drain. Nigerian neurosurgeons are emigrating at an accelerating rate, citing poor remuneration, lack of equipment, unsafe working conditions, and national insecurity; Nigeria is the largest exporter of health workers in Africa, and specialists are

disproportionately represented.[19,20]

Services that do exist are profoundly maldistributed. Of 86 neurosurgery-capable health facilities nationwide, only 17.4% are accredited for residency training, dedicated neurosurgery beds account for just 4.0% of total hospital beds, and dedicated neurosurgery operating rooms account for only 15.4% of total operating rooms.[10]

The response must centre on expanding specialist training and deliberately restructuring retention incentives. More residency training centres should be established, and a dedicated budgetary allocation should be set aside to increase equipment procurement, infrastructure development, and incentives for trainers within the facilities. Scholarships for postgraduate fellowships in the neurosurgical subspecialty of need should be encouraged, enabling fellows to acquire more skills in centres abroad with advanced neurosurgical programs and to develop competencies that will enable them to commence similar programs in Nigeria on their return.

Retention of specialists is another critical determinant of success. Evidence consistently demonstrates that remuneration is the single most powerful predictor of specialist retention in Africa.[21,22] A dedicated Neurosurgery specialist allowance, separate from the civil service salary structure, should be established to provide internationally competitive consultant salaries, performance-based bonuses, and non-monetary incentives, including subsidised housing and children's education allowances. Equally important is transforming the working environment: functional operating rooms with reliable power, modern instruments, consistent availability of consumables, and dedicated neurosurgery theatres and intensive care beds in every teaching hospital are non-negotiable.

**Preventing Secondary Brain Injury: The Priority of Basic Physiological Monitoring**

While advanced neuromonitoring remains unavailable in many Nigerian public hospitals, the most powerful predictors of death after severe TBI, hypoxia and hypotension, can be detected with simple tools. Landmark studies demonstrate that a single episode of systolic hypotension below 110 mmHg or oxygen saturation below 90% doubles mortality.[23,24] These modifiable insults occur frequently where basic monitoring is absent.[25] In many Nigerian hospitals, automated blood pressure cuffs and pulse oximeters are not applied continuously to TBI patients outside the theatre.

Equipping every emergency department and neurosurgical ward with functioning monitors is immediately achievable. The devices cost US$50–100 per unit and are locally procurable. The greater challenge is standardisation. A simple nurse-driven TBI Early Warning Score that triggers intervention when SpO falls below 94%, systolic pressure drops below 110 mmHg, or the Glasgow Coma Scale declines by 2 points can be tested. A pilot could be established within six months and, if effective, integrated into national guidelines (NANS, 2022)[26,27] and scaled to other centres. Correcting hypoxia and hypotension is the single most cost-effective intervention to reduce mortality.

**Challenges with Rehabilitation Post TBI**

Survival from severe TBI is only the beginning of a long journey to recovery. An estimated 3.2 million people sustain TBI annually across sub-Saharan Africa, a figure projected to reach 14 million by 2050, yet early neuro-rehabilitation guidelines remain undeveloped in most countries in the region.[28,29] In Nigeria, multidisciplinary rehabilitation services involving

neuropsychology, physiotherapy, occupational therapy, and speech-language pathology are scarce, concentrated in a few urban facilities, and inaccessible to the majority of poor survivors.

The shortage of rehabilitation professionals is significant, leading to a care pathway that invests heavily in acute management while inadequately supporting the post-discharge journey, thereby significantly hampering recovery.[28] Globally, fewer than 5% of those needing rehabilitation in LMICs access even basic services.[29]

The immediate priority is a tiered rehabilitation system leveraging community-based care. At tertiary hospitals, a basic multidisciplinary team comprising at least a physiotherapist, a nurse with neuro-rehabilitation training, and a visiting or telelinked psychologist can be established, while community health extension workers provide home-based follow-up after discharge, using principles of the WHO Rehabilitation Competency Framework (WHO, 2020).[30] In addition, post-acute rehabilitation services for TBI should be included in the NHIA benefit package, and coverage should be expanded toward universal health coverage for emergency and rehabilitative care.

**From Guidelines to Implementation**

The absence of standardised, context-appropriate clinical guidelines has long contributed to variability in TBI care across Nigeria. In response, the Nigerian Academy of Neurological Surgeons (NANS 2022), an umbrella body for Neurosurgeons in Nigeria, developed the Proposed Standard of Practice and Guidelines on the Management of Traumatic Brain Injury, Spinal Cord Injury, and Peripheral Nerve Injury.[26,27] However, these guidelines remain in draft form; nationwide adoption requires formal endorsement by the Federal Ministry of Health and incorporation into hospital policies, which has not yet occurred.

The path from guidelines to implementation in Nigeria is achievable through an organised and pragmatic approach. First, the Federal Ministry of Health should formally adopt the NANS guidelines and issue a circular mandating their use in all federal and state teaching hospitals. Second, the guidelines should be embedded in undergraduate and postgraduate medical curricula and reinforced through continuous professional development. Third, adherence must be audited through a functional national neurotrauma registry, enabling tracking of every patient from injury through discharge and follow-up. The value of trauma registries in LMICs is well documented; hospitals with functional registries experience a significantly lower injury burden and improve surveillance and clinical governance.[31] Establishing such a registry in Nigeria requires phased investment in IT infrastructure, dedicated data clerks, and strategies to minimise loss to follow-up, beginning first at tertiary centres with accredited neurosurgery centres and subsequently involving other private and public health care facilities. To achieve a good outcome, strategies based on active policy implementation, regular audit cycles, and feedback must be in place, with execution steady and sustained, resulting in a change in clinical practice.

**Framework for Financing and Implementation**

The proposed interventions require sustainable financing. Nigeria already has mechanisms that can be leveraged. The Basic Health Care Provision Fund, which allocates 1% of the Consolidated Revenue Fund to health, can be partially utilised to provide emergency neurosurgical services, including CT scanners and basic monitors. In addition, the National Health Insurance Authority must expand coverage to the

informal sector and include TBI rehabilitation in the minimum benefit package.

Furthermore, road safety funds, generated from fines and vehicle registration fees, can be legislatively redirected to support prehospital care for accident victims. Pooled procurement of hospital equipment can be explored, tied to service contracts by oil companies and telecommunication giants, to reduce costs and provide a sustainable supply chain.

Implementation should be overseen by a national TBI steering committee co-chaired by the Federal Ministry of Health and the Nigerian Academy of Neurological Surgeons, with a mandate to set priorities, track progress, and report publicly. The first concrete steps should be a national equipment audit and lay-first-responder training, which can begin within existing budgets. What is needed is not a new system, but the coordinated activation of existing structures.

**Figure 1: Pictures showing a road traffic accident and the evacuation of victims**


Figure 1(A-C) shows a road traffic accident (A) Multiple-vehicle crash site with first responders, including Federal Road Safety Corps (FRSC) marshals. (B &C) Victims were transported in the back of the FRSC truck to the hospital without any form of prehospital care or cervical spine immobilisation: Courtesy of Nigerian Top Secret Blog.[32]

## CONCLUSION

While substantial challenges exist in Nigeria in the management of TBI, these include poor prehospital response, a dearth of acute diagnostic capacity, a scarce neurosurgical workforce, and limited rehabilitation services. This review explored various strategies that can be implemented to improve the trauma health care systems and improve the outcomes of patients with TBI. The imperative is not to construct an entirely new system, but to integrate, fund, and scale existing innovations within a coordinated national framework. Achieving this will demand sustained political commitment, transparent resource allocation, and institutional accountability, led jointly by the Federal Ministry of Health and the neurosurgical professional community. The evidence is sufficient, the interventions are defined, and the economic and human costs are achievable. What remains is the decision to act.

## REFERENCES

1 Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung Y-C, Punchak M *et al.* Estimating the global incidence of traumatic brain injury. *J Neurosurg* 2018; **130:** 1080–1097.

2 Ukachukwu AEK, Nischal SA, Trillo-Ordonez Y, Nwaribe EE, Abu-Bonsrah N, Malomo TA *et al.* Epidemiological Burden of

Neurotrauma in Nigeria: A Systematic Review and Pooled Analysis of 45,763 Patients. *World Neurosurg* 2024; **185**: e99–e142.

3 Wang K, Li Z. Global, regional, and national burdens of road injuries from 1990 to 2021: Findings from the 2021 Global Burden of Disease Study. *Injury* 2025; **56**: 112221.

4 Gu D, Ou S, Liu G. Global burden of road injuries and their attributable risk factors from 1990 to 2021: A systematic analysis for the global burden of disease study 2021. *Prev Med Rep* 2025; **53**: 103051.

5 Woo J, Kendall NJ, Petitt Z, Ayantayo TO, Atilola O, Ayodele OA *et al.* Management and outcomes of pediatric traumatic brain injury in Nigeria: a systematic review. *J Neurosurg Pediatr* 2025; **35**: 627–636.

6 Ozor II, Nkwerem SP, Nduagubam OC, Emerue -Iroegbu LU, Nkwerem GI. Clinico-Demographic Profile of Paediatric Head Injuries in South East Nigeria: A Two-Centre Study. *Orient J Med* 2025;37(3-4).65-72.

7 Ibrahim NA, Ajani AWO, Mustafa IA, Balogun RA, Oludara MA, Idowu OE *et al.* Road Traffic Injury in Lagos, Nigeria: Assessing Prehospital Care. *Prehosp Disaster Med* 2017; **32**: 424–430.

8 Reynolds TA, Sawe H, Rubiano AM, Shin S Do, Wallis L, Mock CN. Strengthening Health Systems to Provide Emergency Care. *Disease Control Priorities, Third Edition (Volume 9): Improving Health and Reducing Poverty* 2017; : 247–265.

9 Olaomi OO, Okoye GO, Balarabe-Musa B. Current Trends in Emergency Medicine in Nigeria. *Niger J Clin Pract* 2024; **27**: 1345–1350.

10 Ukachukwu AEK, Njeru PN, Ayodele OA, Ahmad MH, Onyia CU, Morgan E *et al.* A Geospatial Analysis of the Availability, Distribution, and Accessibility of Neurosurgical Facilities, Workforce, and Infrastructure in Nigeria; and Projection Towards 2050. *World Neurosurg* 2024; **185**: e16–e29.

11 Reynolds TA, Stewart B, Drewett I, Salerno S, Sawe HR, Toroyan T *et al.* The Impact of Trauma Care Systems in Low- and Middle-Income Countries. *Annu Rev Public Health* 2017; **38**: 507–532.

12 Mock C, Joshipura M, Arreola-Risa C, Quansah R. An estimate of the Number of Lives that Could be Saved through Improvements in Trauma Care Globally. *World J Surg* 2012; **36**: 959–963.

13 Robertson FC, Esene IN, Kolias AG, Khan T, Rosseau G, Gormley WB *et al.* Global Perspectives on Task Shifting and Task Sharing in Neurosurgery. *World Neurosurg X* 2019; **6**: 100060–100060.

14 Reports | National Bureau of Statistics. https://nigerianstat.gov.ng/elibrary/read/1241254 (accessed 28 Jun2026).

15 Enrollee Details – National Health Insurance Authority. https://www.nhia.gov.ng/1596-2/ (accessed 28 Jun2026).

16 Okei J, Ojo OA, Adaramola OG, Arekhandia B, Shakiru O, Akubueze C. Pediatric Traumatic Brain Injury Patients in Southwestern Nigeria: Presentation Patterns and Outcome. *J West Afr Coll Surg* 2025; **16**: 18-22.

17 Kihunyu AM, Pouwels S, Adeyeye A, Abbas GH, Oldenbeuving AW, Ramnarain D. Navigating neurosurgical care in low- and middle-income countries from Africa: a narrative review of barriers and facilitators. *Annals of Medicine and Surgery* 2025; **87**: 7261-7268.

18 Rubiano AM, Carney N, Chesnut R, Puyana JC. Global neurotrauma research challenges and opportunities. *Nature 2015 527:7578* 2015; **527**: S193–S197.

19 Tankwanchi ABS, Özden Ç, Vermund SH.

Physician Emigration from Sub-Saharan Africa to the United States: Analysis of the 2011 AMA Physician Masterfile. *PLoS Med* 2013; **10**: e1001513.

20 Anand A, Ajayi AO, Ansari A, Mustapha MJ, Verma A, Adinoyi SA *et al.* Academic Neurosurgery in Nigeria- Past, Present, and Future: A Review. *World Neurosurg* 2024; **189**: 108–117.

21 Falola HO, Ojebola OO, Falola HO, Ojebola OO. Assessing the Challenges of Medical Practitioners' Retention and Its Implications in a Developing Economy. *The Changing Landscape of Workplace and Workforce*. IntechOpen publications; 2024.

22 Naicker S, Plange-Rhule J, Tutt RC, Eastwood JB. Shortage of healthcare workers in developing countries Africa. *Ethn Dis* 2009;19: s61-64.

23 Marmarou A, Anderson RL, Ward JD, Choi SC, Young HF, Eisenberg HM *et al.* Impact of ICP instability and hypotension on outcome in patients with severe head trauma. *J Neurosurg* 1991; **75**: S59–S66.

24 McHugh GS, Engel DC, Butcher I, Steyerberg EW, Lu J, Mushkudiani N *et al.* Prognostic value of secondary insults in traumatic brain injury: results from the IMPACT study. *J Neurotrauma* 2007; **24**: 287–293.

25 Chesnut RM, Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N *et al.* The role of secondary brain injury in determining outcome from severe head injury. *J Trauma* 1993; **34**: 216–222.

26 NANS Trauma Committee Document. Available at https://nansorg.com/publications/ (accessed 28 June 2026).

27 Emejulu JK, Malomo A, Oremakinde A, Onyia C, Nwaribe E, Ekweogwu O *et al.* Developing a Guideline for Neurotrauma in Nigeria. *World Neurosurg* 2020; **138**: e705–e711.

28 Buh FC, Hutchinson PJA, Anwar F. Early neuro-rehabilitation in traumatic brain injury: the need for an African perspective. *BMC Med* 2023; **21**: 290.

29 Cieza A, Causey K, Kamenov K, Hanson SW, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* 2020; **396**: 2006–2017.

30 Rehabilitation Competency Framework. https://www.who.int/teams/noncommunicable-diseases/sensory-functions-disability-and-rehabilitation/rehabilitation-competency-framework (accessed 24 Jun2026).

31 Rosenkrantz L, Schuurman N, Arenas C, Nicol A, Hameed MS. Maximizing the potential of trauma registries in low-income and middle-income countries. *Trauma Surg Acute Care Open* 2020; **5**: e000469.

32 NIGERIAN TOP SECRET: Graphic photos from the accident along Sagamu Expressway. https://nigeriantopsecret.blogspot.com/2015/06/graphic-photos-from-accident-along.html (accessed 28 Jun2026).

Campbell FC, Traumatic Brain Injury Management in Nigeria: A Critical Review of Systemic Challenges and Integrated, Context Specific Solutions. Afr J Trop Med & Biomed Res 2026; 9(1) 7-14
https://dx.doi.org/ 10.4314/ ajtmbr.v9i1.1

# Antihyperglycaemic and Hepatorenal Protective Effects of Combined Aqueous Extracts of *Piper guineense* and *Vernonia amygdalina* in Alloxan-Induced Diabetic Rat model

[1]Odinga-Israel T, [1]Amechi PAC, [1]George MS, [1]Nodi CC, [1]Ikoro NM.

**Abstract**

**Introduction:** Diabetes mellitus is a chronic metabolic disorder associated with hepatic and renal complications due to persistent hyperglycemia and oxidative stress. Given limitations of conventional drugs, plant-based therapies are being explored. This study evaluated the hepatoprotective and nephroprotective effects of mixed aqueous extracts of Piper guineense and Vernonia amygdalina in alloxan-induced diabetic rats.

**Materials and Methods:** Thirty male Wistar rats were randomized into five groups (n=6): Normal control, Diabetic control, Positive control [metformin 150 mg/kg], and two treatment groups receiving 200 mg/kg and 400 mg/kg of 1:1 mixed extract orally for 28 days. Fasting blood glucose [FBG] and body weight were measured at days 0, 14, and 28. Serum liver and kidney function markers were analyzed, and liver/kidney tissues were processed for histology. Data were analyzed using One-way ANOVA in GraphPad Prism 10.2.

**Results:** Diabetic controls had elevated FBG, reduced %BWD, and deranged liver and kidney markers. Treatment with the extract significantly reduced final FBG [P=0.002] and increased %BWD in a dose-dependent manner. Liver enzymes [AST][ALT][ALP][GGT] were significantly lowered [P=0.002] while total protein and albumin were restored [P=0.033]. BUN and creatinine were significantly reduced [P=0.002] and electrolyte imbalance corrected [P=0.033]. Histology showed preserved hepatic and renal architecture at 400 mg/kg, comparable to metformin.

**Conclusion:** Mixed aqueous extracts of P. guineense and V. amygdalina exhibited significant hepatorenal protective effects in diabetic rats, supporting their ethnomedicinal use and potential as adjunct therapy for diabetes-related organ damage.

**Keywords:** *Piper guineense*, *Vernonia amygdalina*, Hepatorenal protection, Diabetes mellitus, Medicinal plants.

[1]*Department of Biochemistry, Faculty of Science, Rivers State University, Nigeria.*

**Corresponding author:** Tamuno-Boma Odinga-Israel, Department of Biochemistry, Faculty of Science, Rivers State University, Nigeria. Email: tamuno-boma.odinga@ust.edu.ng,

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes mellitus has been escalating, posing significant health challenges worldwide.[1] Chronic hyperglycemia in diabetes is associated with long-

term damage and dysfunction of various organs, particularly the liver and kidneys, leading to complications such as diabetic hepatopathy and nephropathy.[2]

Oxidative stress and inflammation are key contributors to the pathogenesis of diabetic complications. Excessive production of reactive oxygen species (ROS) in hyperglycemic conditions leads to cellular damage in hepatic and renal tissues.[2] Consequently, there is growing interest in natural antioxidants as potential therapeutic agents to mitigate oxidative damage in diabetes.

*Vernonia amygdalina*, commonly known as bitter leaf, is a widely used medicinal plant in Africa, renowned for its antidiabetic, antioxidant, and hepatoprotective properties. Studies have demonstrated that extracts of *V. amygdalina* can significantly reduce elevated liver enzymes and improve histological architecture in diabetic models, indicating its potential in managing diabetic hepatopathy.[3,4] Additionally, *V. amygdalina* has shown nephroprotective effects by reducing serum creatinine and urea levels in diabetic rats.[5,6]

*Piper guineense*, known as West African black pepper, is another indigenous plant with reported antidiabetic and antioxidant activities. While its individual effects have been studied, the combined impact of *P. guineense* and *V. amygdalina* on hepatic and renal functions in diabetic conditions remains underexplored.

This study evaluated the hepatorenal protective effects of combined aqueous extracts of *Piper guineense* and *Vernonia amygdalina* in alloxan-induced diabetic rats. In addition to assessing liver and kidney function biomarkers, this research also monitored changes in fasting blood glucose (FBG) and percentage body weight difference (%BWD) to provide a comprehensive understanding of the therapeutic potential of the plant combination in mitigating metabolic and organ-related complications of diabetes.

## MATERIALS AND METHODS

### Plant Collection, Preparation, and Extraction

Fresh leaves of *Piper guineense* and *Vernonia amygdalina* with herbarium reference no: UPH/P/042 and RSUPb320 from University of Port Harcourt and Rivers State University for *Piper guineense* and *Vernonia amygdalina* respectively were obtained from Mile 3 Market in Port Harcourt, Rivers State, Nigeria. Botanical authentication was conducted by Professor Green, Department of Plant Science and Biotechnology, Rivers State University. The plant samples were thoroughly washed, air-dried for two weeks, and then pulverized using an electric blender. The powdered leaves were mixed in a 1:1 ratio and extracted using hot aqueous maceration, following the methods previously described.[7,8]

### Experimental Animals and Design

Thirty (30) male Wistar albino rats weighing between 200–250g were procured and housed in the animal facility of the Department of Pharmacology, Rivers State University. The animals were allowed to acclimatize for 14 days under standard conditions (12-hour light/dark cycle), with free access to food and water. The study conformed to the university's ethical committee procedure; all procedures conformed to CPCSEA guidelines.

Rats were randomly divided into five groups (n = 6 per group):

- **Group 1**: Normal control (Feed + water only)
- **Group 2**: Negative control (Alloxan, 120 mg/kg)
- **Group 3**: Positive control (120mg/kg

Alloxan + Metformin 150 mg/kg)
- **Group 4**: 120mg/kg Alloxan + 200 mg/kg mixed extract
- **Group 5**: 120mg/kg Alloxan + 400 mg/kg mixed extract

Hyperglycemia was induced in Groups 2 to 5 via a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) (OECD, 2001). Fasting blood glucose (FBG) levels were monitored at three specific time points: initial (before alloxan administration), intermediate (72 hours post-induction to confirm hyperglycemia), and final (on day 28, after treatment). Blood glucose measurements were taken using a glucometer via tail vein puncture.

The body weight of each rat was measured and recorded at weekly intervals, and the percentage body weight difference (%BWD) was calculated using the formula:

$$\%BWD = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

This was used to assess the effect of diabetes and treatment on body mass regulation. Treatment with metformin and plant extracts commenced after hyperglycemia was confirmed and continued daily for 21 days. This procedure was carried out as previously described.[9]

**Blood Sample Collection and Biochemical Assays**

At the end of the treatment period, animals were fasted overnight, anaesthetized, and sacrificed. Blood samples were collected via cardiac puncture into EDTA-containing and plain tubes for biochemical assays. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis.

Liver function biomarkers—AST, ALT, ALP, GGT, total protein (TP), albumin (ALB), total bilirubin (TB), and conjugated bilirubin (CB)—and kidney function indices—blood urea nitrogen (BUN), creatinine, sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$), bicarbonate ($HCO_3^-$), and calcium ($Ca^{2+}$)—were analyzed using standardized colorimetric and enzymatic protocols, as described by Odinga et al. (2023).

**Histopathological Examination of the tissues (Odinga et al., 2023).**

The liver and kidney tissue biopsies of the rats were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were then cut into 2- to 3-mm-thick sections by a microtome, fixed on the slides and stained with hematoxylin-eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan), and photomicrographs were taken with a Leica DM 750 camera at ×100 magnification.

**Data Analysis**

Data were analyzed using GraphPad Prism® version 10.2 (San Diego, CA, USA). Results were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values with $p < 0.05$ were considered statistically significant.

**Results and Discussion**

**Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Percentage Body Weight Difference in Alloxan-Induced Diabetic Male Wistar Rats**

Alloxan-induced diabetes led to a significant reduction in the percentage body weight difference (%BWD) in the negative control group compared to the normal control as seen in figure 1, indicating catabolic weight loss typically seen in uncontrolled diabetes. This weight reduction is likely due to insulin deficiency, increased muscle proteolysis, and lipid mobilization.[10]

Treatment with the combined extracts significantly improved %BWD, with the 400 mg/kg group showing a near-normal percentage gain comparable to the metformin group. This suggests a reversal of diabetic cachexia and improved metabolic status in treated animals.[11] Restoration of body weight in diabetic rats treated with *V. amygdalina* and *P. guineense* has been previously reported and attributed to improved insulin activity and nutrient utilization.[12,13]

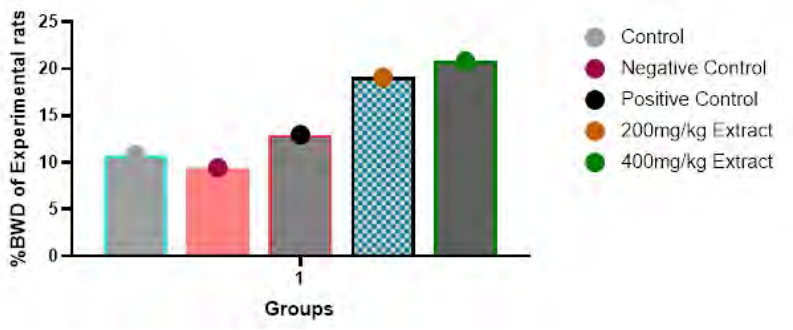



**Figure 1: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Percentage Body Weight Difference in Alloxan-Induced Diabetic Male Wistar Rats**

**Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Fasting Blood Glucose in Alloxan-Induced Diabetic Male Wistar Rats**

Induction of diabetes with alloxan monohydrate resulted in a marked elevation in intermediate and final fasting blood glucose (FBG) levels in the diabetic control group compared to the normal control as shown in Figure 2. This hyperglycemic response reflects pancreatic β-cell destruction and insulin deficiency associated with alloxan toxicity. Treatment with the mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* at both 200 mg/kg and 400 mg/kg significantly reduced final FBG levels compared to the diabetic control group ($p = 0.002$), producing results comparable to the metformin-treated group.

This hypoglycemic effect indicates the potential of the extract combination in improving glycemic control in diabetic conditions. The observed activity may be attributed to the phytochemicals in *V. amygdalina* and *P. guineense*, which have been reported to enhance insulin secretion, improve peripheral glucose uptake, and inhibit glucose absorption in the gut.[14,15] Previous studies have demonstrated that *V. amygdalina* can significantly lower blood glucose in alloxan and streptozotocin-induced diabetic models,[4,16] while *P. guineense* has shown similar effects through its rich content of alkaloids, flavonoids, and phenols.[17]

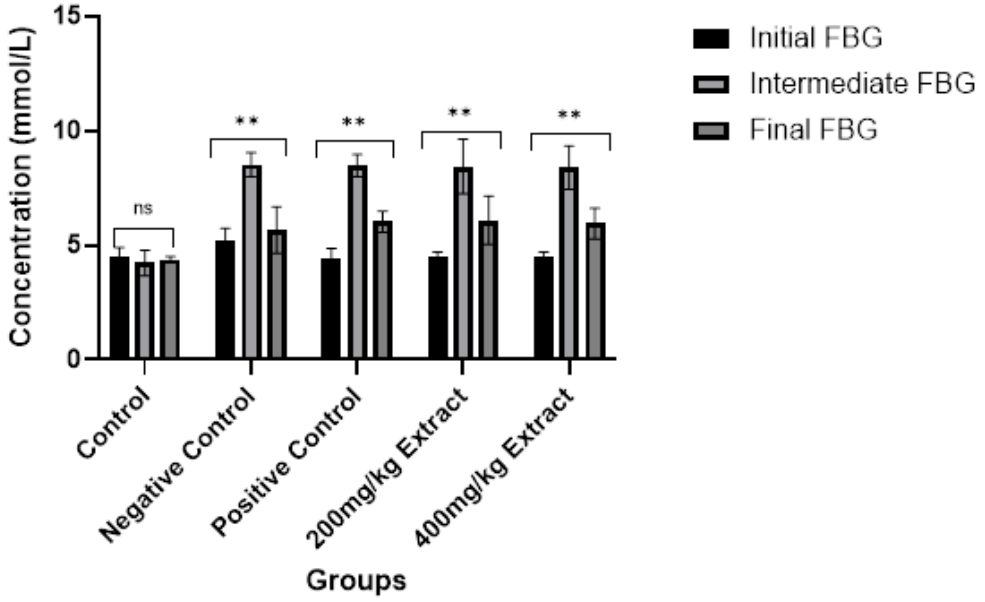



**Figure 2: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Fasting Blood Glucose in Alloxan-Induced Diabetic Male Wistar Rats**

*Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001 (***).*

**Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Liver Function Biomarkers in Alloxan-Induced Diabetic Male Wistar Rats.**

The induction of diabetes using alloxan monohydrate significantly elevated liver enzymes—AST, ALT, ALP, and GGT—in the negative control group, indicating hepatic dysfunction. Treatment with mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* at both 200 mg/kg and 400 mg/kg doses as shown in figure 3 resulted in a significant reduction in these enzyme levels ($p = 0.002$), comparable to the metformin-treated group. This finding suggests hepatoprotective effects and aligns with previous studies demonstrating the hepatoprotective and antioxidant activities of *V. amygdalina*, which mitigates hepatic damage through its antioxidant properties.[18]

Furthermore, total protein and albumin levels, which were decreased in the diabetic control group, were significantly restored in the extract-treated groups ($p = 0.033$), indicating improved hepatic synthetic function. Total and conjugated bilirubin levels, elevated in the diabetic control group, were significantly reduced in the extract-treated groups ($p = 0.033$), suggesting enhanced bilirubin clearance and liver function. These observations are consistent with the hepatoprotective effects of *V. amygdalina* and *P. guineense*, which have been reported to restore liver function markers in diabetic models.[19-21]

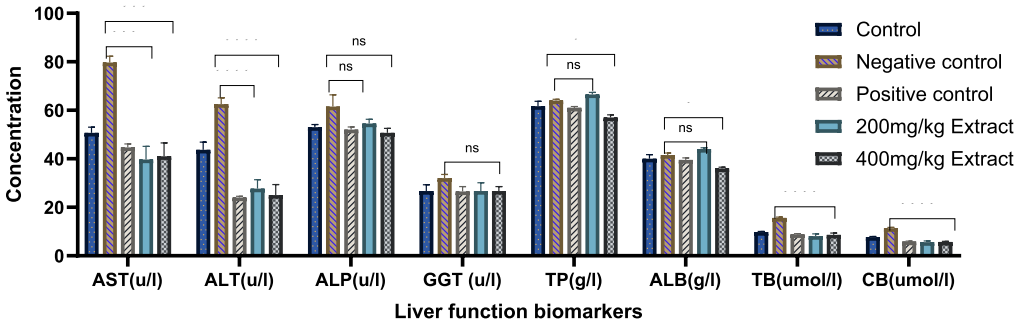



**Figure 3: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Liver Function Biomarkers in Alloxan-Induced Diabetic Male Wistar Rats.**
*Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(****).*

**Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Kidney Function Biomarkers and Serum Electrolytes in Alloxan-Induced Diabetic Male Wistar Rats is as shown in Figure 4.**

Alloxan-induced diabetes led to significant increases in renal biomarkers, with BUN and creatinine levels elevated in the negative control group as shown in figure 4. Treatment with the mixed extracts resulted in a dose-dependent decrease in these parameters ($p = 0.002$), comparable to the metformin group, indicating nephroprotective effects. These findings are supported by studies showing that *V. amygdalina* and *P. guineense* possess nephroprotective properties, reducing oxidative stress and improving renal function in diabetic models.[22,23]

Electrolyte imbalances observed in the diabetic control group, such as hyponatremia, hyperkalemia, and hypochloremia, were significantly corrected in the extract-treated groups ($p = 0.033$), indicating improved renal function and electrolyte balance. The ability of *V. amygdalina* and *P. guineense* to restore electrolyte balance has been documented in previous studies, highlighting their role in maintaining renal homeostasis in diabetic conditions.[21,22]

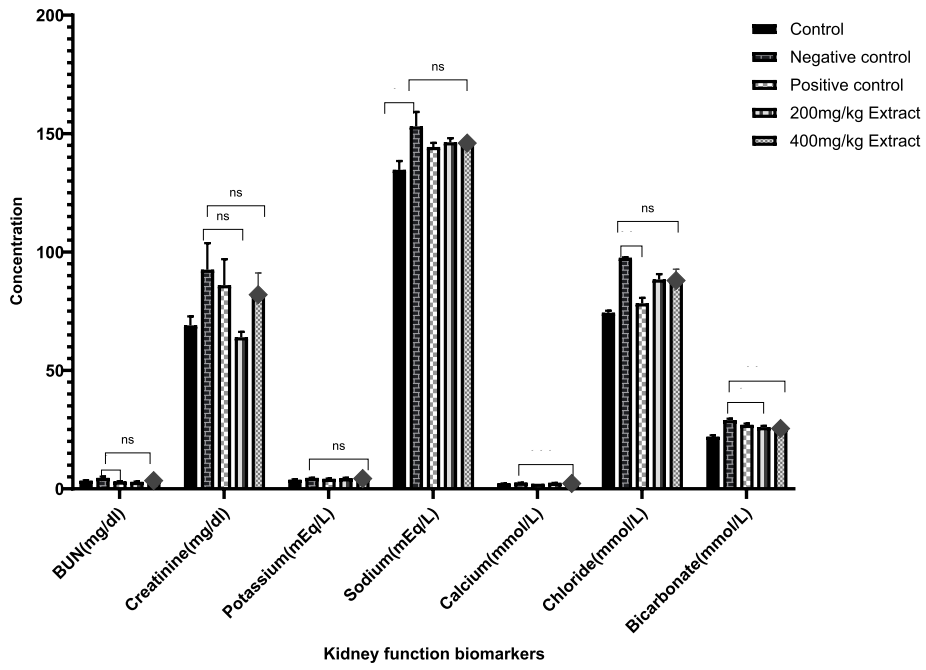
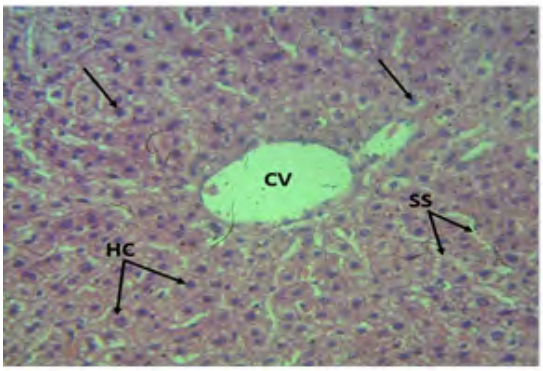


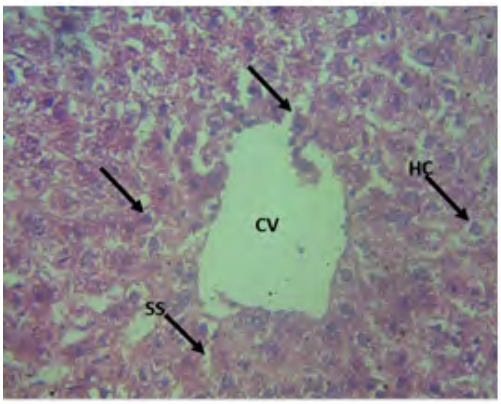

**Figure 4: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Kidney Function Biomarkers and Serum Electrolytes in Alloxan-Induced Diabetic Male Wistar Rats.**
*Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(***).*

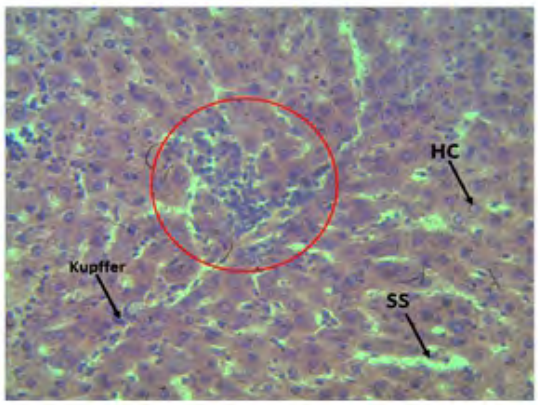
**Histopathological Examination of the Liver and Kidneys of the Experimental rats**

Photomicrograph 1 of the liver of the experimental rats showed a normal appearance of the liver tissues for the group 1 (Normal control) with normal central veins, adjacent sinusoids with hepatocytes. The negative control group photomicrographs (2a and 2b) revealed a disrupted morphology of the central venules and hepatocyte degeneration implying inflammation and second-degree distortion of the liver tissues. Previous studies have reported a distortion of the liver cells when exposed to hyperglycemic conditions without treatment.[24] The Positive control group treated with metformin showed an ameliorating state of the inflamed and distorted liver as shown in Photomicrograph 3a and 3b. The liver tissues showed mild distortion of the liver tissues while the groups treated with 200mg/kg and 400mg/kg extract revealed moderate distortion of the liver tissues as shown in photomicrographs 4 and 5. The amelioration of the inflamed liver tissues by the mixed extract of *Piper guineense* and *Vernonia amygdalina* is comparable to that of the metformin treated groups. The potency of the mixed extract could be associated with the phytochemicals present in the plants extract with greater efficiency when combined.[24] These phytochemicals have been associated with their ability to inhibit or modulate the MAPK and NF-kB pathways, thereby reducing inflammation of tissues.[26]



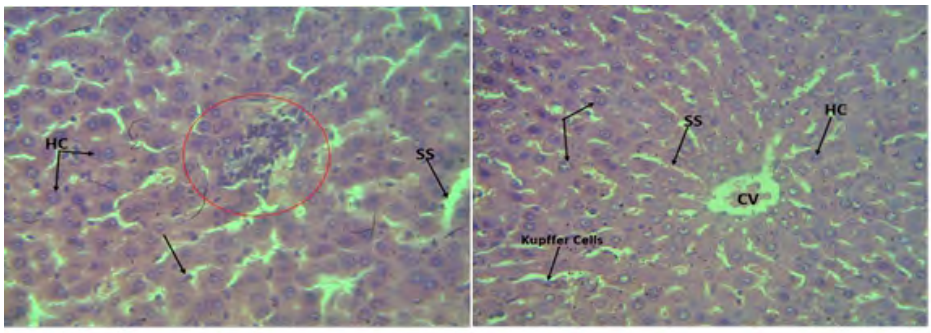

**Photomicrograph 1:** (H&E X400) of the liver outlining a well normal Central vein (CV) with adjacent sinusoids (SS) also with the hepatocytes (HC) (arrows). **Diagnosis:** Normal appearance of the liver tissue



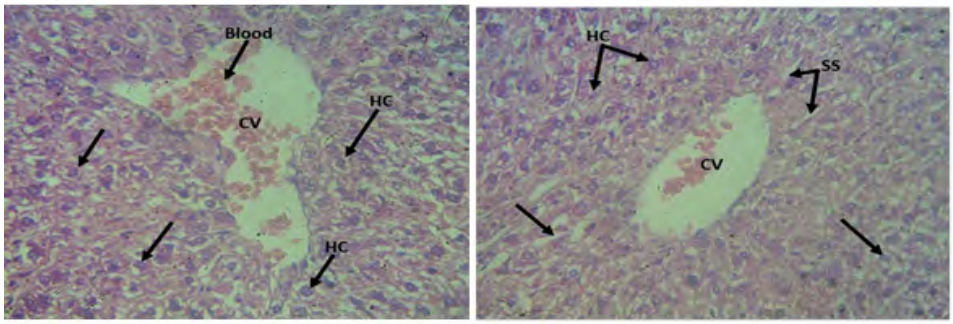

**Photomicrograph 2a:** (H&E X400) of the liver showing disrupted morphology of the central venules (CV); hepatocytes (HC), and sinusoids (SS) within zone 3 of the liver parenchymal (arrows). Diagnosis: Second degree distortion of the liver tissue

**Photomicrograph 2b:** (X400 H&E stain) of the liver showing lymphocytic activities (red circle) with hepatocytes degeneration (HC) and sinusoidal dilatation (arrows). **Diagnosis:** Severe Inflammation and distortion of the liver tissue.




**Photomicrograph 3a:** (X400 H&E stain) of the liver showing reduced lymphocytic infiltration (red circle). **Diagnosis:** Moderate Inflammation of the liver tissue

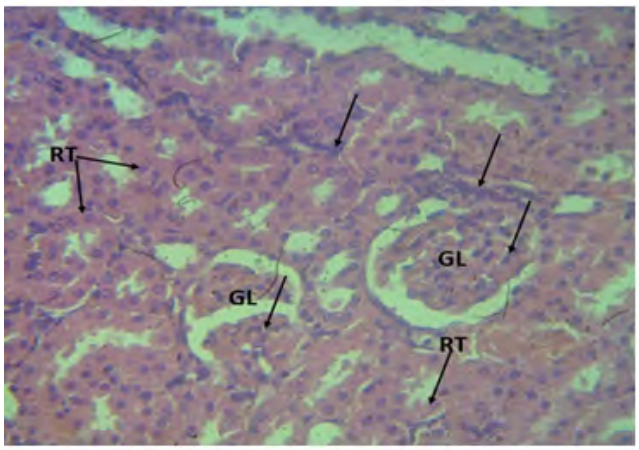
**Photomicrograph 3b:** (X400 H&E stain) of the liver showing the sinusoid (SS); congestion of the central vein (CV) (arrows). **Diagnosis:** Mild distortion of the liver tissue.



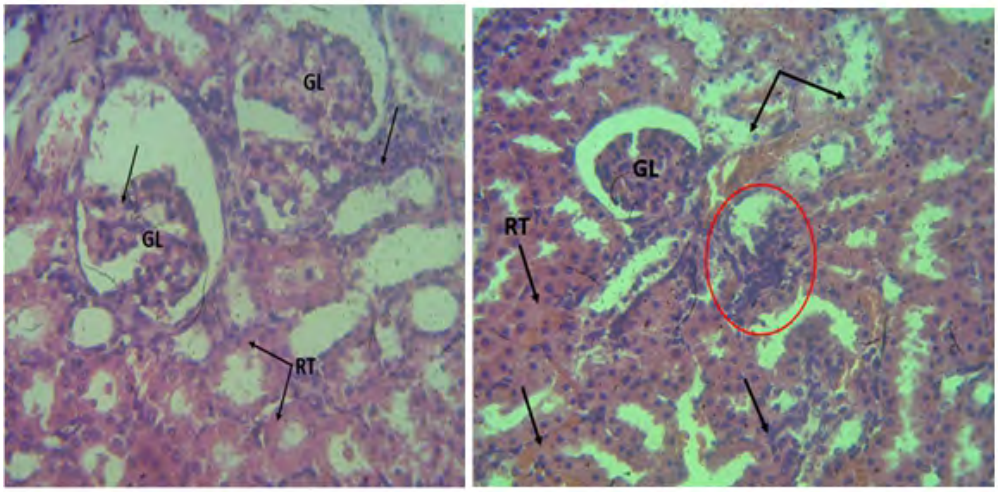

**Photomicrograph 4**: (H&E X400) of the liver showing hepatocyte hypertrophy and fatty degeneration (arrows). **Diagnosis:** Moderate Distortion of the liver tissue.
**Photomicrograph 5:** (H&E X400) of the liver showing reduced hepatocyte hypertrophy and fatty degeneration (arrows). **Diagnosis:** Moderate Distortion of the liver tissue.

**Histopathological examination of the kidney tissues of experimental rats**

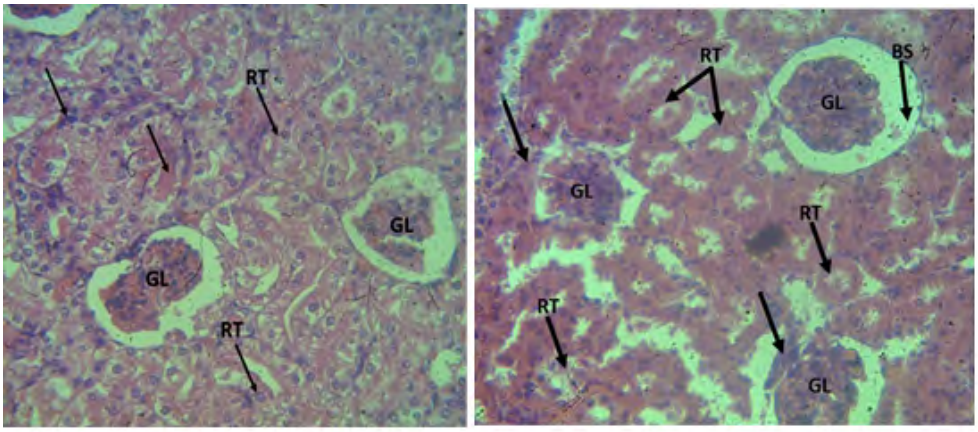
The kidney tissues of the experimental rats as shown in photomicrograph 1a for the normal control group had a normal appearance of the kidney tissue evidenced by the presence of the glomerulus, reduced podocytes and mild lymphoctytic activities around the renal tubules. Severe distortion, inflammation and degeneration of the kidney tissues were observed in the negative control group as shown in Photomicrographs 2a&b. These effects could be associated with the inflammation caused by the inducement of hyperglycemia in the experimental rats in this group using alloxan and were not subjected to any form of treatment.[2] Alloxan monohydrate in experimental rat models has been reported to cause inflammation and damage to the liver and kidney tissues.[27] Treatment with metformin and the mixed extracts of *Piper guineense* and *Vernonia amygdalina* at 200mg/kg and 400mg/kg caused a reduced inflammatory state of the kidney as seen in Photomicrographs 3a&b, 4a&b, 5a&b for metformin (Positive control), 200mg/kg and 400mg/kg mixed extract of *Piper guineense* and *Vernonia amygdalina* Respectively. The use of plant extract as therapeutics has been reported to be comparable to the conventional antidiabetic medications with even less side effects due to their role in the metabolism of inflammation markers such as TNF-alpha, IL-6, IL-1B, and mcp-1.[28]



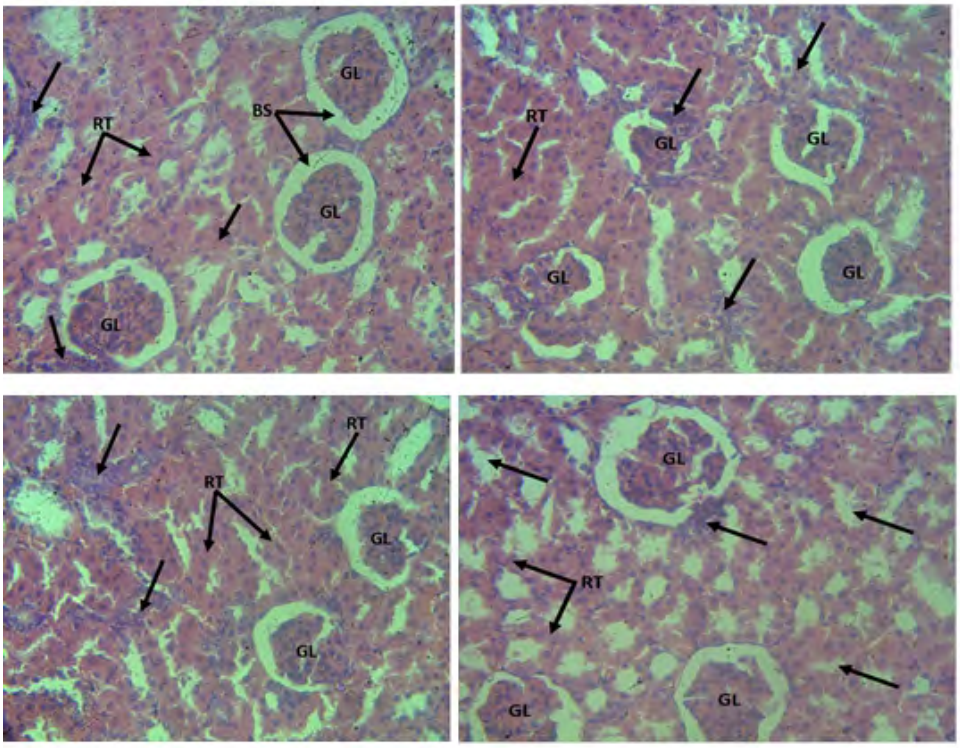

**Photomicrograph 1a**: (X400 H&E stain) of the kidney showing the glomerulus (GL) with reduced podocytes (red) and mild lymphoctytic activities surrounding the renal tubules (RT) (blue arrows) **Diagnosis:** Normal appearance of the kidney tissue.




**Photomicrograph 2a.** (X400 H&E stain) of the kidney showing focal lymphocytic activities; glomerular atrophy with reduced podocyte and interstitial oedema (arrows). **Diagnosis**: Severe distortion of the kidney tissue. **Photomicrograph 2b**. (X400 H&E stain) of the kidney showing lymphocytic infiltration (red circle) along the renal tubules and glomerular atrophy with reduced podocytes and hemosiderin deposit (arrow). **Diagnosis:** Inflammation and degeneration of the kidney tissue.




**Photomicrograph 3a.** (X400 H&E stain) of the kidney showing well delineated parietal epithelia cells lining the renal tubules; glomerular atrophy. **Diagnosis:** Mild glomerular distortion of the kidney tissue. **Photomicrograph 3b.** (H&E X400) of the Kidney showing renal tubular distention. **Diagnosis**: Moderate distortion of the kidney tissue.




**Photomicrograph 4a.** (H&E X400) of the Kidney showing reduced lymphocytic activities within the renal tubules with interstitial oedema(arrows). **Diagnosis**: Minimal Inflammation of the kidney tissue. **Photomicrograph 4b.** (H&E X400) of the Kidney showing reduced lymphocytic activities within the renal tubules (arrows). **Diagnosis**: mild Inflammation of the kidney tissue. **Photomicrograph 5a&b.** (H&E X400) of the Kidney showing renal tubules associated with interstitial oedema(arrows). **Diagnosis**: Mild Inflammation of the kidney tissue.

**Conclusion**

The present study demonstrates that mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* exert significant antidiabetic, hepatoprotective, and nephroprotective effects in an alloxan-induced diabetic rat model. In addition to normalizing elevated liver enzymes and improving renal biomarkers and electrolyte balance, the extracts significantly reduced fasting blood glucose levels and improved percentage body weight difference. These findings indicate that the plant extracts not only mitigate diabetes-induced organ dysfunction but also support systemic metabolic recovery. The therapeutic effects are likely attributable to the phytochemical constituents with antioxidant, anti-inflammatory, insulin-modulating, and cytoprotective properties. Collectively, these results validate the traditional use of *P. guineense* and *V. amygdalina* in managing diabetes and its complications, and provide a scientific foundation for their further pharmacological development.

**Recommendation**

Further research is recommended to validate the efficacy and safety of *P. guineense* and *V. amygdalina* extracts through comprehensive toxicity studies, mechanistic evaluations, and clinical trials. Investigations into their synergistic mechanisms and formulation as standardized antidiabetic herbal products could support their integration into evidence-based complementary therapy for diabetes mellitus and its systemic complications.

**REFERENCES**

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2022;45(Suppl 1):S17-S38. https://doi.org/10.2337/dc22-S002
2. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. J Diabetes Res. 2015;2015:494578. https://doi.org/10.1155/2015/494578
3. Nwahiri JD, Ozoemena CC, Okolonkwo BN, Nwosu DC, Nwanjo HU. Effect of Vernonia amygdalina and Moringa oleifera on some liver parameters in alloxan-induced diabetic rats. Int Res J Gastroenterol Hepatol. 2023;6(1):1-9.
4. Adekemi FE, Folake JK, Omowumi FP. Antidiabetic effects of aqueous leaf extract of Vernonia amygdalina on serum liver markers in streptozotocin-induced diabetic albino rats: A new data to support its anti-diabetic effect. Clin Phytoscience. 2024;10:13. https://doi.org/10.1186/s40816-024-00376-9
5. Yazid F, Hasanah NB, Rosmalena, Hanafi M, Prasasty VD. Antidiabetic and antioxidant potential of Vernonia amygdalina leaf extract in alloxan-induced Sprague-Dawley rats. Online J Biol Sci. 2020;20(4):190-200. https://doi.org/10.3844/ojbsci.2020.190.200[Astereaceae]
6. Adeoye TA, Oyagbemi AA, Omobowale TO, Adedapo AD, Ayodele AE, Yakubu MA, et al. Nephroprotective effects of Vernonia amygdalina in alloxan-induced diabetes in rats. Int J Biochem Res Rev. 2018;21(1):1-15. https://doi.org/10.9734/IJBCRR/2018/40100
7. Frimpong G, Adotey J, Ofori-Kwakye K, Kipo SL, Dwomo-Fokuo Y. Potential of aqueous extract of Hibiscus sabdariffa calyces as colouring agent in three paediatric oral pharmaceutical formulations. J Appl Pharm Sci. 2014;4(12):001-007. https://doi.org/10.7324/JAPS.2014.41201
8. Omeodu SI, Okwakpam FN, Odinga T, Ijeoma SN. Effect of aqueous extract of Tapinathus bangwensis on some diagnostic

enzymes in alloxan-induced diabetic wistar rats. EPRA Int J Multidiscip Res. 2023;9(3).Organisation for Economic Co-operation and Development. OECD Guideline for Testing of Chemicals: Acute Oral Toxicity – Acute Toxic Class Method. OECD; 2001. https://www.oecd.org/content/dam/oecd/en/publications/reports/2002/02/test-no-423-acute-oral-toxicity-acute-toxic-class-method_g1gh294f/9789264071001-en.pdf

9. Odinga-Israel TB, Amechi PAC, Efekemo O, Lemii BC, Nodi CC. Lipid-lowering and antioxidant potentials of mixed aqueous extracts of Piper guineense and Vernonia amygdalina in Alloxan-induced diabetic rat model. Int J Adv Biochem Res. 2025;9(4):908-914. https://doi.org/10.33545/26174693.2025.v9.i4k.4234
10. Meex RC, Blaak EE, van Loon LJ. Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes. Obes Rev. 2019;20(9):1205-1217. https://doi.org/10.1111/obr.12862
11. Naccache DD, Nseir WB, Herskovitz MZ, Khamaisi MH. Diabetic neuropathic cachexia: a case report. J Med Case Rep. 2014;8:20. https://doi.org/10.1186/1752-1947-8-20
12. Nnam NM, Okoye OI. Biochemical Effect of Aqueous Extracts of Turmeric [Curcuma longa], Ginger [Zingiber officinale] and African Black Pepper [Piper guineense] on Blood Glucose Level of Alloxan Induced Diabetic Albino Wistar Rat. J Home Econ Res. 2023;30(2).
13. Mustapha BO, Ademoyegun OT, Ahmed RS. Horticultural crops as natural therapeutic plants for the therapy of diabetes mellitus. Egypt J Basic Appl Sci. 2023;10(1):380-395. https://doi.org/10.1080/2314808X.2023.2217387

164 Erukainure OL, Oyebode OA, Ibeji CU, Koorbanally NA, Islam MS. Vernonia Amygdalina Del. stimulated glucose uptake in brain tissues enhances antioxidative activities; and modulates functional chemistry and dysregulated metabolic pathways. Metab Brain Dis. 2019;34:721-732. https://doi.org/10.1007/s11011-018-0363-7

15. Talwar A, Chakraborty N, Zahera M, Anand S, Ahmad I, Siddiqui S, et al. Antidiabetic potential of phytochemicals found in Vernonia amygdalina. J Chem. 2024;2024:6111603. https://doi.org/10.1155/2024/6111603
16. Ayodele A, Adeoye AT, Adedapo AD, Omobowale TO, Adedapo AA, Oyagbemi AA. Antidiabetic and antioxidant activities of the methanol leaf extract of Vernonia amygdalina in alloxan-induced diabetes in Wistar rats. J Med Plants Econ Dev. 2017;1(1):1-12. https://hdl.handle.net/10520/EJC-df29f617e
17. Amadi G, Iwuji SC, Azeez TO, Nwaokoro CJ, Wodu CO. Biochemical effects of Piper Guineense [African Black Pepper] in female diabetics: opportunities for diabetes treatment. Int J Transl Med Res Public Health. 2019;3(1):59-66. https://doi.org/10.21106/ijtmrph.76
18. Son HL, Tu HNA, Minh TV. Ameliorative Potentials of Vernonia amygdalina on Acetaminophen-induced Acute Liver Injury in Mice. Asian J Biotechnol Bioresour Technol. 2023;9(2):22-35. https://doi.org/10.9734/ajb2t/2023/v9i2180
19. Lukong CB, Ifemeje JC, Chukwuka DT, Onah C. Effects of aqueous extracts of Gongronema latifolium leaves and Piper guineense seeds on liver and kidney biomarkers of albino rats. Trop J Appl Nat Sci. 2018;2(2):26-33. https://doi.org/10.25240/TJANS.2018.2.2.04
20. Tokofai BM, Idoh K, Oke OE, Agbonon A.

Hepatoprotective effects of Vernonia amygdalina extract on CCl4-induced liver injury in broiler chickens. Animals. 2021;11(12):3371. https://doi.org/ 10.3390 /ani11123371

21. Asante DB, Wiafe GA. Therapeutic Benefit of Vernonia amygdalina in the Treatment of Diabetes and Its Associated Complications in Preclinical Studies. J Diabetes Res. 2023;2023:3159352. https://doi.org/ 10.1155/2023/3159352
22. Atangwho IJ, Ebong PE, Eteng MU, Eyong EU, Obi AU. Effects of Vernonia amygdalina Del leaf on kidney function of diabetic rats. Int J Pharmacol. 2007;3(2):143-148. https://doi.org/ 10.3923/ijp.2007.143.148
23. Ugbaja RN, Akinhanmi TF, Onunkwor BO, Ugwor EI, James AS, Babalola AA, et al. Flavonoid-rich fractions of C. volubile and V. amygdalina alleviates arsenic-induced neurotoxicity by improving neurosignaling and antioxidant capacity in rats' brain. Brain Disord. 2022;7:100050. https://doi.org /10.1016/j.dscb.2022.100050
24. Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. Sultan Qaboos Univ Med J. 2016;16(2):e132-e141. https://doi.org/10.18295/squmj.2016.16.0 2.002
25. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 2017;6(4):42. https://doi.org/ 10.3390 /plants6040042
26. Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, et al. Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. Front Pharmacol. 2023;14:1177050. https://doi.org/10.3389/ fphar.2023 .1177050
27. Shah NA, Khan MR. Antidiabetic effect of Sida cordata in alloxan induced diabetic rats. Biomed Res Int. 2014;2014:671294. https://doi.org/10.1155/2014/671294
28. Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA, Chaity NI, et al. Antidiabetic Phytochemicals From Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. Front Endocrinol. 2022;13:800714. https://doi.org/10.3389/fendo.2022.800714

Odinga-Israel T, Amechi PAC, George MS, Nodi CC, Ikoro NM. Antihyperglycaemic and Hepatorenal Protective Effects of Combined Aqueous Extracts of Piper guineense and Vernonia amygdalina in Alloxan-Induced Diabetic Rat model. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 15-28
https://dx.doi.org/ 10.4314/ ajtmbr.v9i1.2

# Subchronic toxicity of hydroethanolic extract of *Azanza garckeana* fruit in Wistar rats

Akinsola AO[1,2], Ojezele MO[1], Eduviere AT[1], and Akpovwre CO[1]

**Abstract**

**Introduction:** The fruit of *Azanza garckeana* is widely consumed in tropical Africa and is traditionally used to treat conditions such as dyslipidemia, oxidative stress, and infertility. Despite its established ethnomedicinal value and promising pharmacological properties, the long-term safety profile of its hydroethanolic extract (AZG-HE) remains insufficiently studied.

**Materials and Methods:** This study evaluated the safety of repeated oral administration of AZG-HE in albino Wistar rats (n=84) across multiple dose levels over extended treatment periods. Hematological, serum biochemical, and antioxidant parameters were assessed, including lipid profiles, renal and hepatic function markers, and oxidative stress indicators in selected organs. A recovery phase followed treatment to evaluate the reversibility of any observed effects.

**Results:** Administration of AZG-HE did not result in clinical signs of toxicity, even at the highest tested dose over the longest treatment period. While minor and transient reductions in weight gain and feed intake occurred at higher doses, these normalized by the end of the study. The extract significantly improved lipid profiles in a dose-dependent manner and markedly enhanced antioxidant status, increasing hepatic and renal glutathione and superoxide dismutase activity while reducing lipid peroxidation. All biochemical and physiological changes were fully reversible upon discontinuation of treatment.

**Conclusion:** Long-term oral administration of *Azanza garckeana* hydroethanolic extract (AZG-HE) demonstrated a favorable safety profile, with no evidence of cumulative toxicity and complete reversibility of transient effects. Its hypolipidemic and antioxidant activities support its traditional use and potential as a nutraceutical for managing metabolic and oxidative stress-related disorders. Further histopathological and clinical investigations are recommended.

**Keywords:** Subchronic, chronic toxicity, *Azanza garckeana*, hypolipidemic, antioxidant, reversibility

[1]*Department of Pharmacology, Faculty of Allied Health Sciences, College of Health Sciences, Delta State University, Abraka, Nigeria*
[2]*Department of Physiology (Pharmacology Unit), Faculty of Basic Medical Sciences, Redeemer's University, Ede, Osun State*

**Corresponding author:** Akinsola AO, Department of Pharmacology, Faculty of Allied Health Sciences, College of Health Sciences, Delta State University, Abraka, Nigeria. Email: akinsolaao@run.edu.ng

## INTRODUCTION

The plant *Azanza garckeana*, also called Goron Tula (in Hausa, Nigeria) and snot apple[1], is a semi-deciduous shrub distributed across tropical and southern Africa, including Nigeria, Botswana, Kenya, Malawi, Mozambique, South Africa, Sudan, Zambia, and Zimbabwe. [2,3] The plant thrives in semi-arid woodlands and its edible fruit pulp[4], valued in rural communities, is consumed fresh, dried, or processed due to its high nutritional content.[5]

The extensive traditional use of *A. garckeana* parts for ailments ranging from inflammation to sexual dysfunction is underpinned by its rich phytochemical profile including flavonoids,

phenols, and alkaloids[9,10] which confers antioxidant, antimicrobial, anti-inflammatory, and hypolipidemic properties.[11,12]

Various animal studies have validated several traditional claims about *Azanza garckeana* uses, demonstrating excellent *in-vitro* and in vivo activities, including radical scavenging, ferric reducing power, antibacterial effects against respiratory pathogens, analgesia, wound healing, and the amelioration of diabetes-associated dyslipidemia, nephropathy and hepatopathy.[13,14,15] Notably, hypolipidemic properties of AZG has been observed in diabetic models and healthy animals, alongside enhancements in antioxidant enzymes like glutathione peroxidase, superoxide dismutase and reduced glutathione in various tissue homogenates.[16]

Despite the widespread ethnomedicinal claim and favorable therapeutic potential exhibited by *Azanza garckeana* fruit pulp, comprehensive toxicological data on prolonged exposure remain limited. Prior acute and short-term subacute studies on fruit pulp, seed, or bark extracts have generally indicated low toxicity (Absence of any noticeable harmful effects even at doses reaching 5000 mg/kg when administered acutely).[17,18,19], but extended repeated-dose evaluations are essential to validate safety for ethnomedicinal uses or, as a potential nutraceutical[20], particularly given emerging interests in the cardioprotective and metabolic benefits of AZG fruit pulp.[21]

This study therefore investigated the sub-chronic oral toxicity and reversibility of toxicity of *Azanza garckeana* hydroethanolic fruit extract (AZG-HE) in albino Wistar rats.

**Methods**

Plant Authentication

*Azanza garckeana* fruit was obtained from local markets in Gombe State, Latitude 10.2894° N, Longitude 11.1677° E Northeastern Nigeria. The plant material was authenticated at the Herbarium, Department of Botany, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria. A voucher specimen was deposited under the reference number DELSUH279.

**Plant Preparation**

Fruits of *A. garkeana* were air-dried (28–33 °C) to constant weight. The air-dried fruits were ground to a uniform powder using a milling machine. One kilogram of the powdered material was macerated in 20% distilled water + 80% ethanol over a period of 72 hours, accompanied by periodic agitation. Subsequently, the mixture was passed through a layer of cotton wool for filtration. followed by Whatman No. 1 filter paper (110 mm). The filtrate was concentrated under reduced pressure using a rotary evaporator (BÜCHI Rotavapor® R-215, Switzerland) at 37 °C and 70 rpm, then further dried on a water bath at 40 °C. The resulting extract residue was weighed, transferred to an airtight container, and stored at 4 °C until use.

**Ethical Approval**

The ethical approval for the study was obtained from the Ethics and Research Committee of Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (Reference No.: RBC/FBMC/DELSU/25/971).

**Experimental Animals**

Eighty-four (84) Wistar rats, aged seven (7) weeks and weighing between 150–160 grams, were used for this study. The rats were obtained from and housed in the animal house of the Faculty of Allied Health Sciences, Delta State University, Abraka, Delta State. They were acclimatized for 14 days, cared for, and handled in accordance with international best practices as outlined by the National Institutes of Health Guide for the Care

and Use of Laboratory Animals.

**Treatment Groups**

Experimental rats were divided into groups and subgroups. Treatments were applied to each subgroup as stated;

- Group $A_I$, $A_{II}$ and $A_{III}$: Distilled water orally for 60, 90 and 105 days respectively, while
- Group $B_I$ and $B_{II}$: 200 mg/kg/day orally AZG-HE for 60 and 90 days respectively. While, $B_{III}$ received distilled water orally for 14 days after termination of AZG-HE treatments on the 90th day (reversibility study),
- Group $C_I$ and CII: 400 mg/kg/day orally AZG-HE for 60 and 90 days, respectively. While, $C_{III}$ received Distill water orally for 14 days after termination of AZG-HE treatments on the 90th day (reversibility study),
- Group $D_I$ and $D_{II}$: 800 mg/kg/day orally AZG-HE for 60 and 90 days respectively. While, $D_{III}$ received Distill water *orally* for 14 days after termination of AZG-HE treatments on the 90th day (reversibility study).

Parameters evaluated in this study included body weight changes, feed/fluid intake, serum lipid profiles, haematology, hepatic and renal function biomarkers, and tissue antioxidant/pro-oxidant status, in alignment with Organisation for Economic Cooperation and Development guidelines for subchronic toxicity testing. This study evaluates not only potential toxicity effects but also the persistence or reversibility of any observed changes, providing critical data on safety margin of this ethnomedicinally important plant.

**Specimen Collection**

Twenty-four (24) hours after AZG-HE administration, the rats were euthanized and blood taken via cardiac puncture for haematological and biochemical assays.

**Haematological assays**

Blood dispensed into the EDTA-coated tubes was carefully inverted to ensure adequate mixing with anticoagulation and was then subjected to a full blood count, including RBC, RDW-CV, RDW-SD, Hb concentration, HCT, MCV, MCHC, MCH, platelet count, PDW, plateletcrit, and MPV, using a haematology automated analyzing machine (Sysmex KX-21N).

**Serological tests**

Serum was obtained from the clots by centrifuging at 5000 revolutions per minute for 10 minutes. The separated serum was used for subsequent biochemical assays (liver function tests, renal function tests, lipid profile and markers of oxidative stress),

**Statistical Analysis**

GraphPad Prism (version 10.0) was used for statistical software. Results are presented as mean ± SEM. Data were considered statistically significant at $p< 0.05$ using one-way ANOVA alongside Tukey's post-hoc test.

## RESULTS

**Effect of AZG-HE on body weight in rats after 60 days oral treatment.**

Figure 1 illustrates a significant variation in the percentage weight across treatment groups. Daily AZG-HE administration at varied doses of 800 and 400 mg/kg over a 60-day period elicited a statistically significant response ($p< 0.05$) relative to untreated group.

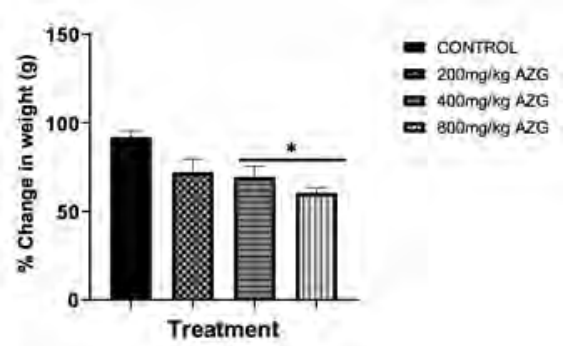



*Figure 1:* Effect of AZG-HE on animal weight in rats after 60 days oral treatment

*p <0.05 in comparison to untreated group

**Effect of AZG-HE on feed intake in rats after 60 days oral treatment**

Figure 2 illustrates a significant variation in the percentage feed intake across treatment groups. Daily AZG-HE administration at varied doses of 800 and 400 mg/kg over a 60 days period elicited a statistically significant variation ($p<0.05$) relative to untreated group,

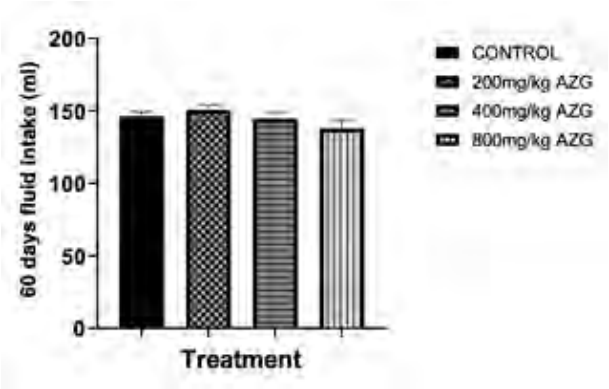



*Figure 3:* Effect of AZG-HE on fluid intake in rats after 60 days oral treatment

p 0.05 in comparison to untreated group

**Effect of AZG-HE on hematological profiles in rats after 60 days oral treatment**

Figure 4(a-e) illustrates no significant variation in RBC, RDW-CV, HB, MCH and plateletcrit level across treatment groups. AZG-HE at varied doses of 800, 400 and 200 mg/kg over a 60 days period elicited no statistically significant variation ($p>0.05$) relative to untreated group.

Figure 4(f-h) illustrates a significant variation in hematocrit, MCV and MCHC across treatment groups. AZG-HE at varied doses of 800, 400 and 200mg/kg mg/kg over a 60 days days period elicited a statistically significant decrease ($p<0.05$) in hematocrit, MCV level, but increase in MCHC relative to untreated group.

Figure 4(i-j) illustrates a significant variation in RDW-SD and PLT across treatment groups. AZG-HE at varied doses of 800 and 400 mg/kg over a 60-day period elicited a statistically significant decrease ($p<0.05$) in RDW-SD, but increase in PLT count relative to the untreated group.

Figure 4(k-l) illustrates a significant variation in PDW and MPV across treatment groups. AZG-HE at a varied dose of 800 mg/kg over a 60-day period elicited a statistically significant decrease ($p<0.05$) in both mean PDW and MPV relative to the untreated group.

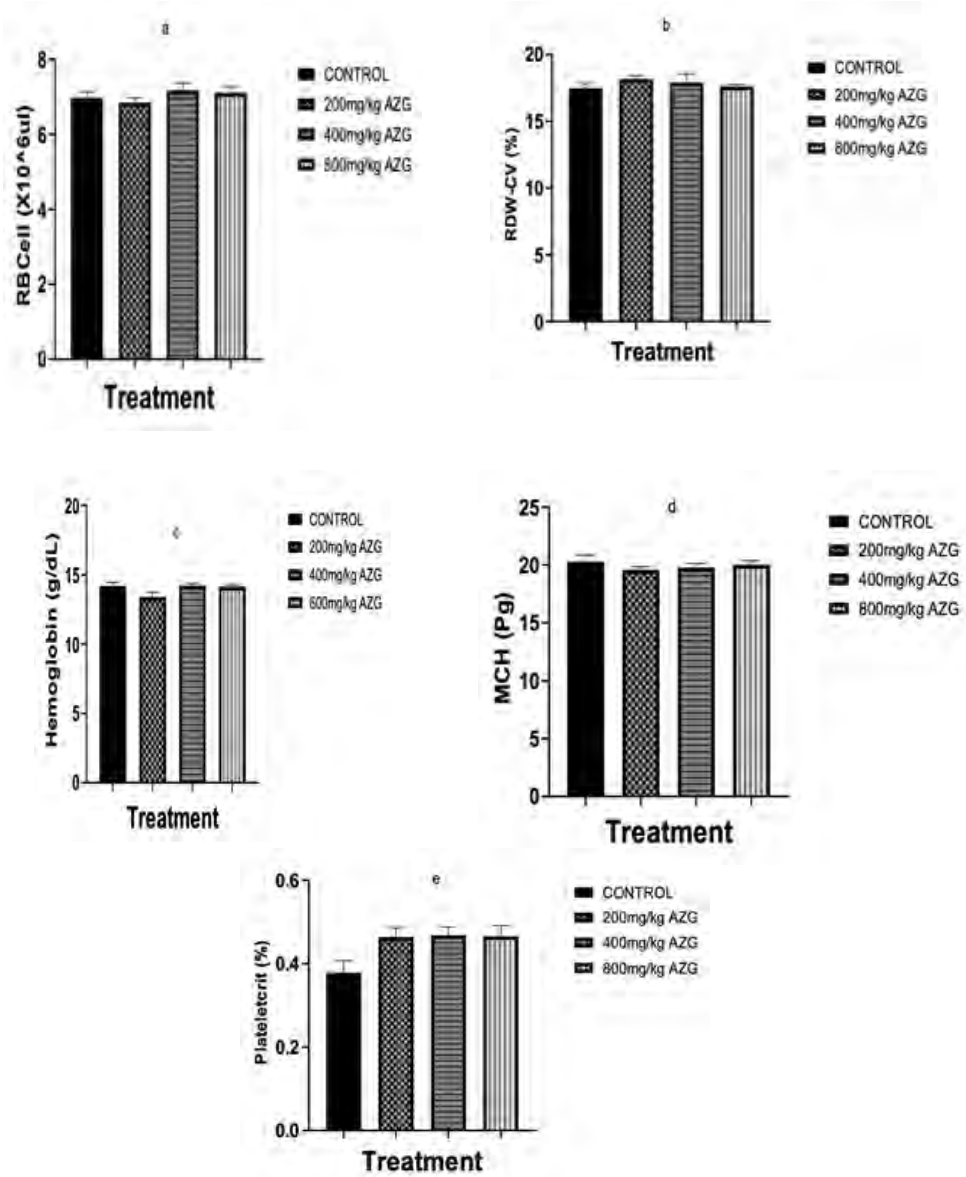



Figure 4: Effect AZG-HE on (a) RBC, (b) RDW-CV, (c) HB, (d) MCH and (e) PCT in rats after 60 days oral treatment
P >0.05 in comparison to untreated group

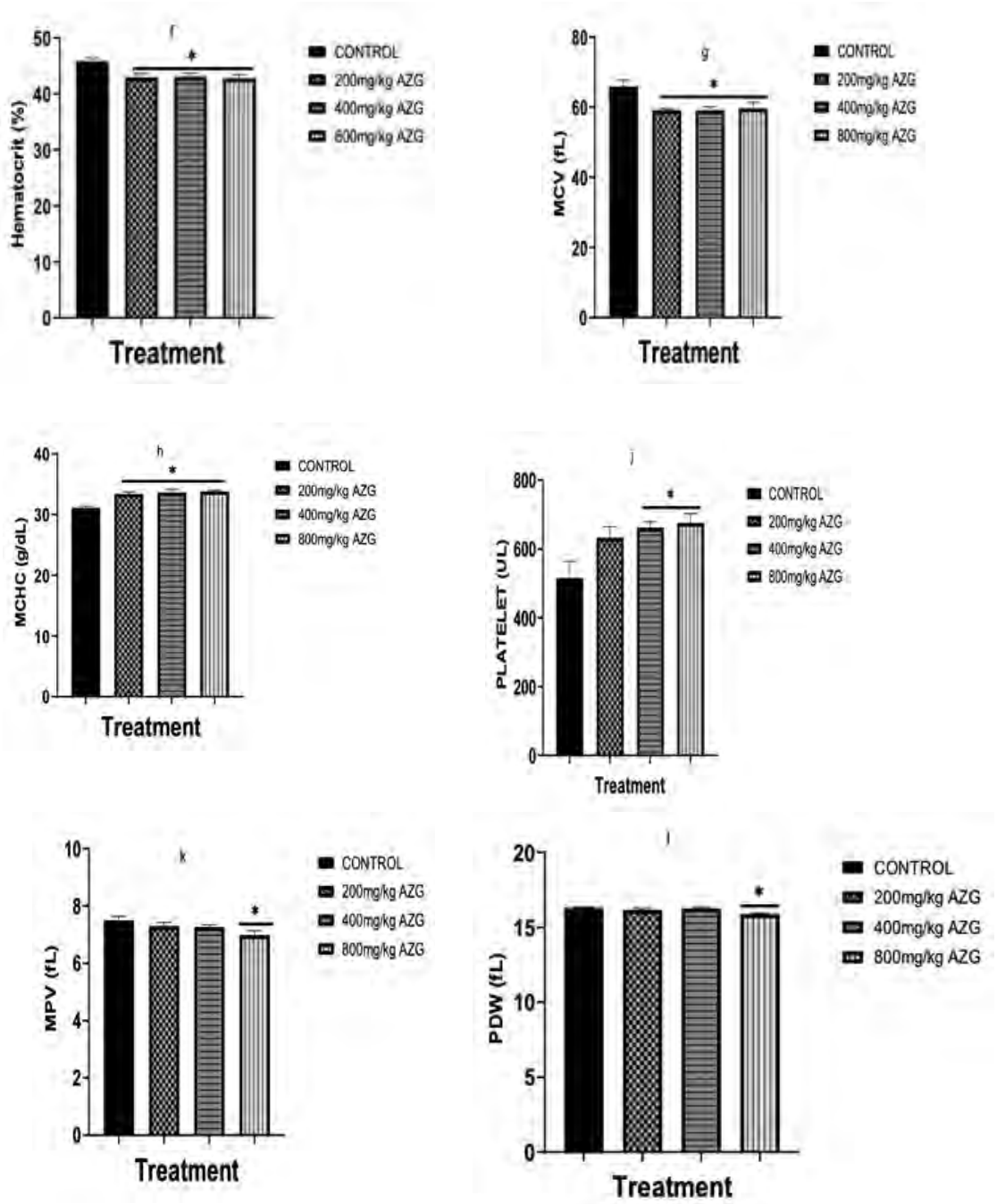



*Figure 4:* Effect of AZG-HE on (f) hematocrit, (g) MCV (h) MCHC, (i) RDW-SD, (j) PLT, (k) MPV and (l) PDW in rats after 60 days oral administration
$p < 0.05$ in comparison to untreated group

**Effect of AZG-HE on serum lipid profiles in rats after 60 days oral treatment**

Figure 5(a-c) illustrates a significant variation in triglycerides, VLDL and LDL levels across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over a 60 days period elicited a statistically significant decrease ($p<0.05$) relative to untreated group,

Figure 5d illustrates a significant variation in HDL across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over 60 days period elicited a statistically significant increase ($p<0.05$) relative to untreated group,

Figure 5e illustrates a significant variation in TC levels across treatment groups. AZG-HE 800, and 400 mg/kg administered over 60 days period elicited a statistically significant decrease ($p<0.05$) relative to untreated group,

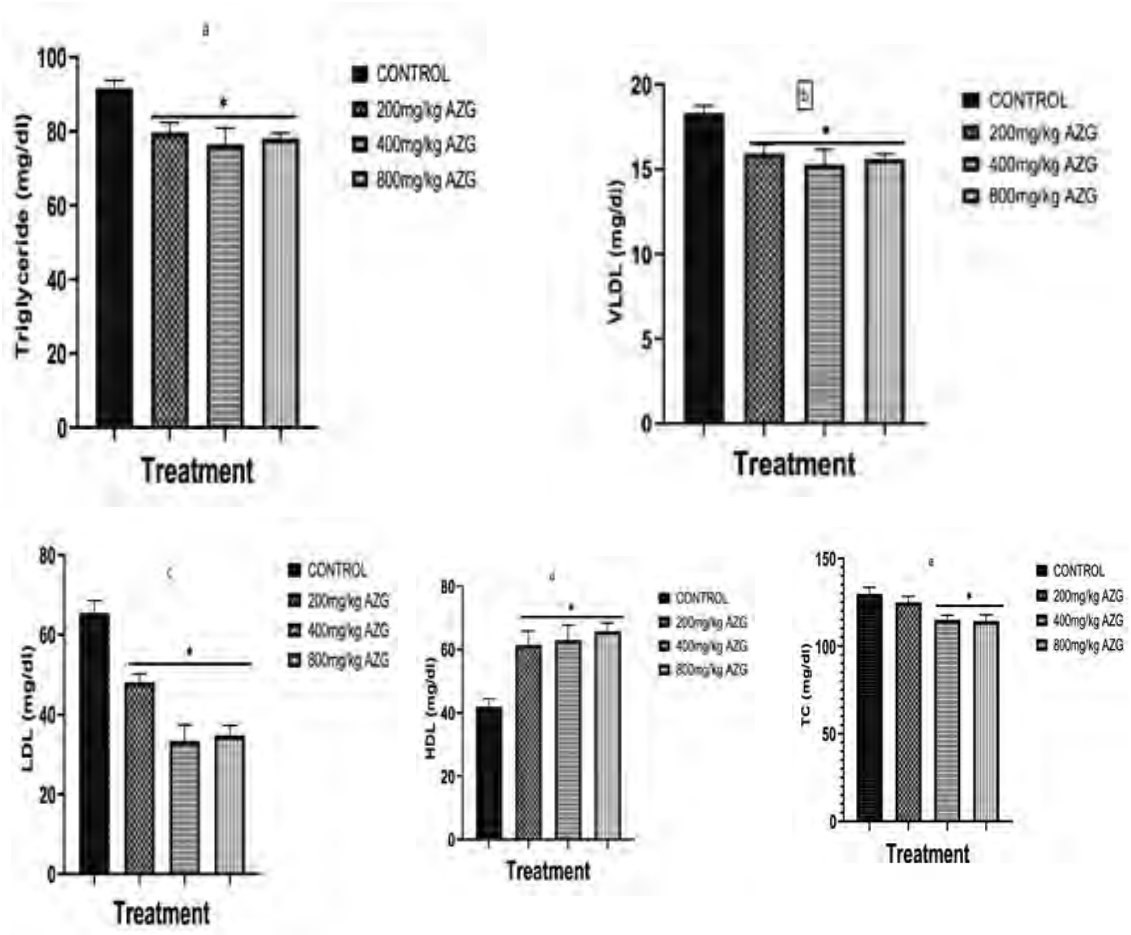


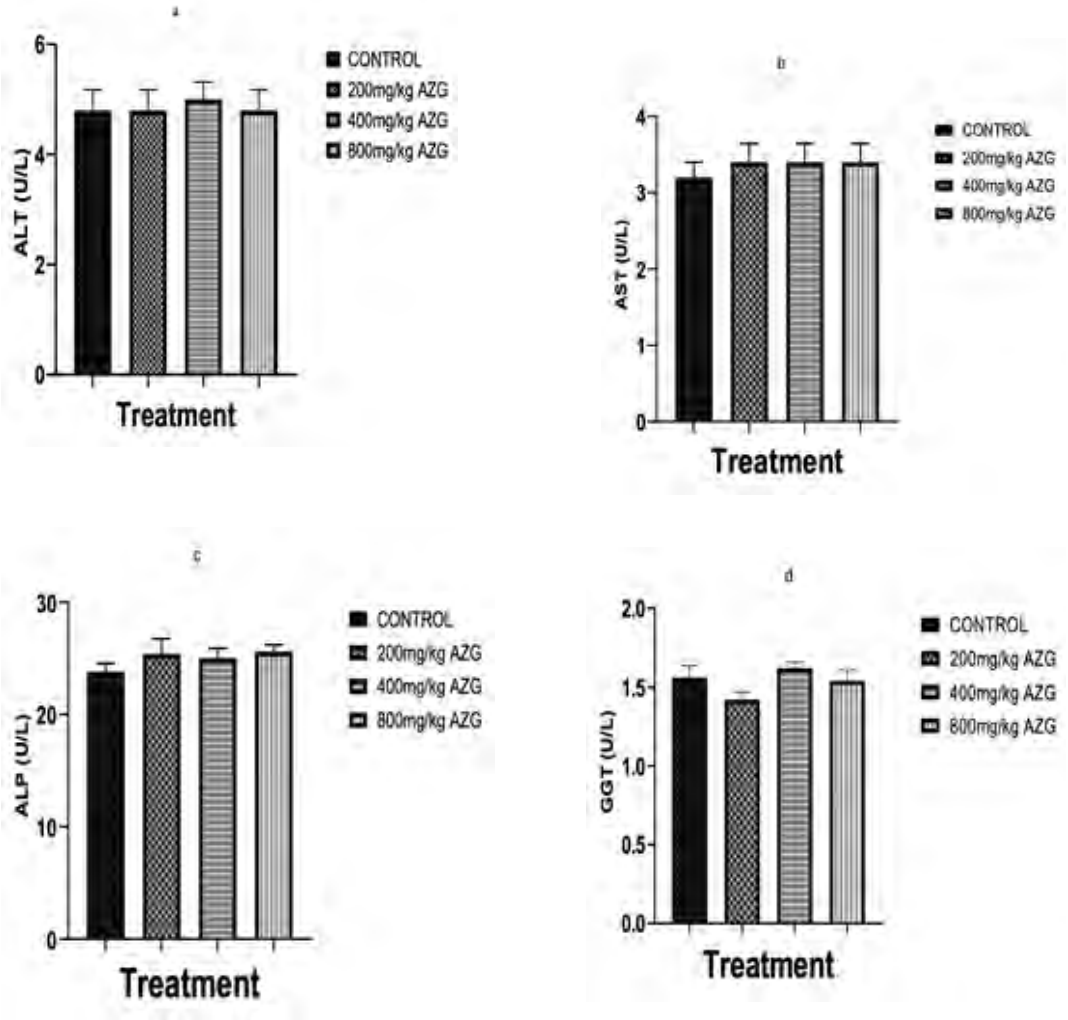

*Figure 5:* Effect of AZG-HE on (a) TG, (b) TC, (c) HDL, (d) LDL, and (e) VLDL level in rats after 60 days oral administration

* $p<0.05$ in comparison to untreated group

**Effect of AZG-HE on liver function indices in rats after 60 days oral treatment**

Figure 6(a-g) illustrates no significant variation in AST, ALT, GGT, ALP, T.protein, T.bil, and D.bil levels

across treatment groups. AZG-HE 800, 400 and 200 mg/kg administration over 60 days period elicited no statistically significant variation (p 0.05) relative to untreated group,



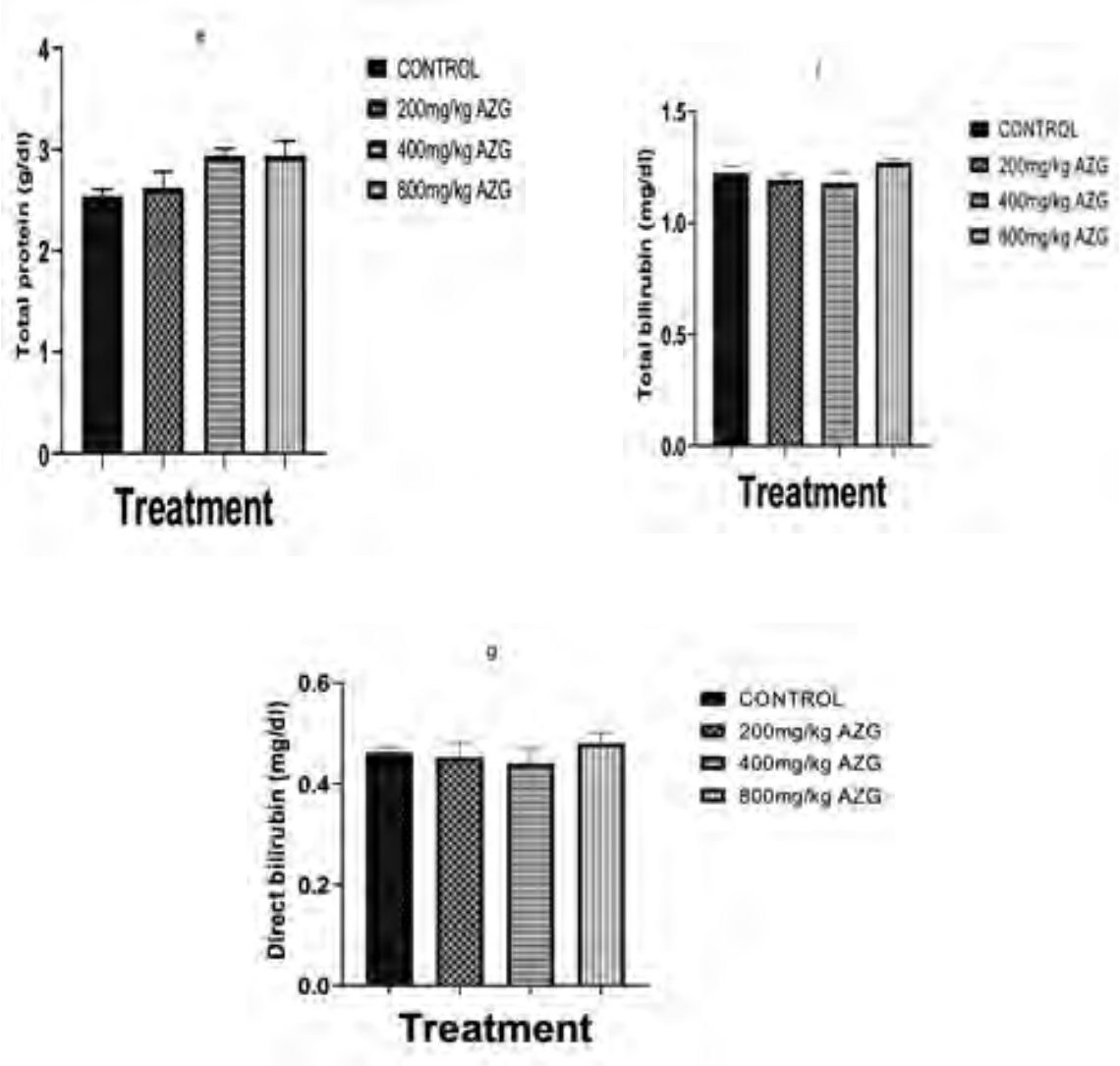



*Figure 6:* Effect of AZG-HE on (a) ALT level, (b) AST level, (c) ALP level, (d) GGT level, (e) T. protein level, (f) T.bil level, (g) D.bil level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on liver antioxidant activity in rats after 60 days oral treatment**
Figures 7(a and b) illustrates no significant variation in SOD and CAT levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 60 days elicited no statistically significant variation ($p>0.05$) relative to untreated group,

However, Figure 7(c and d) illustrates a significant variation in GSH and GPx levels across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over 60 days elicited a statistically significant increase ($p<0.05$) relative to untreated group.

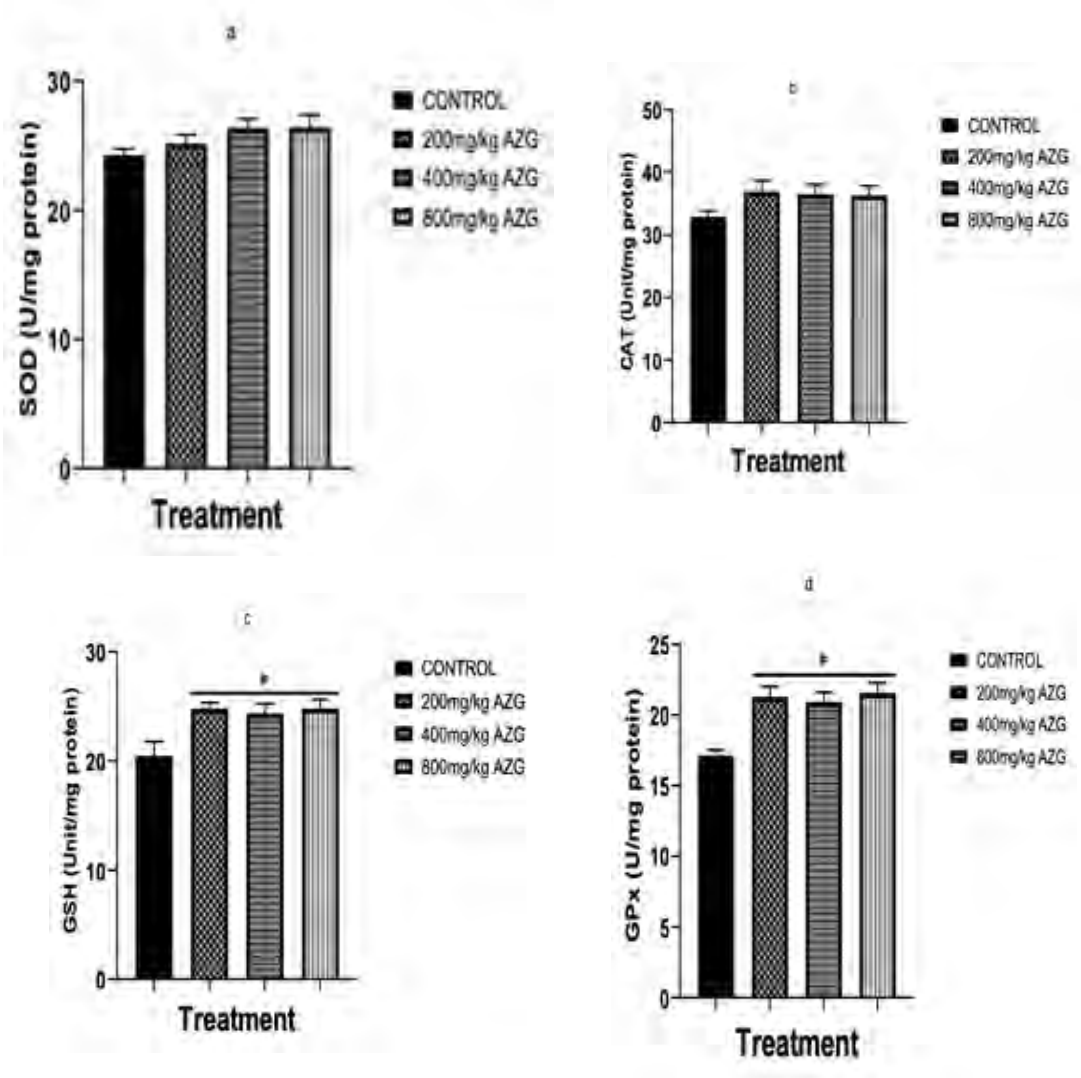



*Figure 7:* Effect of AZG-HE on (a) SOD level (b) CAT level (c) GSH level, and (d) GPx level in rats after 60 days oral administration
* $p < 0.05$ in comparison to untreated group

**Effect of AZG-HE on liver pro-oxidant activity in rats after 60 days oral treatment**

Figures 8 (a and b) illustrates no significant variation in NO and MDA levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg) administered over 60 days elicited no statistically significant variation in NO and MDA levels ($p>0.05$) relative to untreated group,

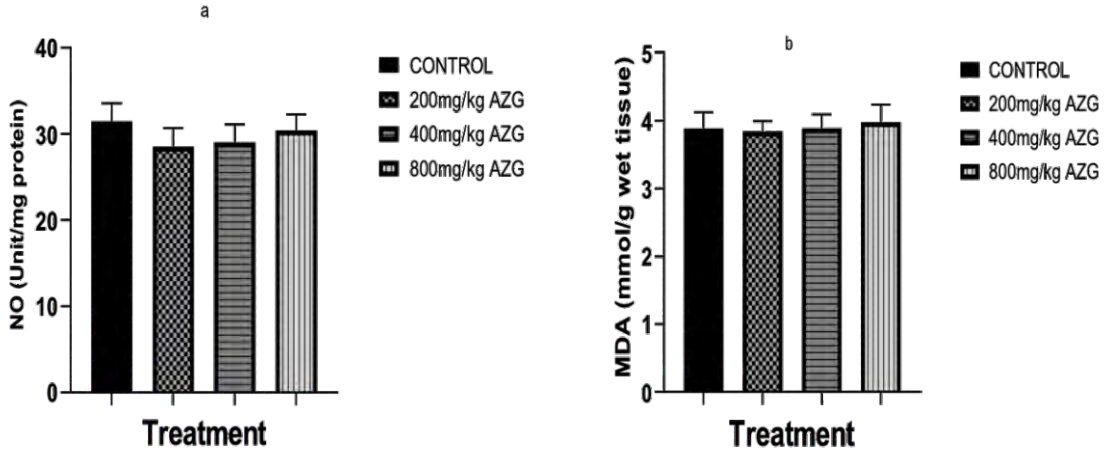



*Figure 8:* Effect of AZG-HE on (a) liver NO level and (b) liver MDA in rats after 60 days oral administration
p >0.05 compared to untreated group

**Effect of AZG-HE on kidney excretory function in rats after 60 days oral treatment**
Figures 9(a and b) illustrates no significant variation in creatinine and BUN levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a 60 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

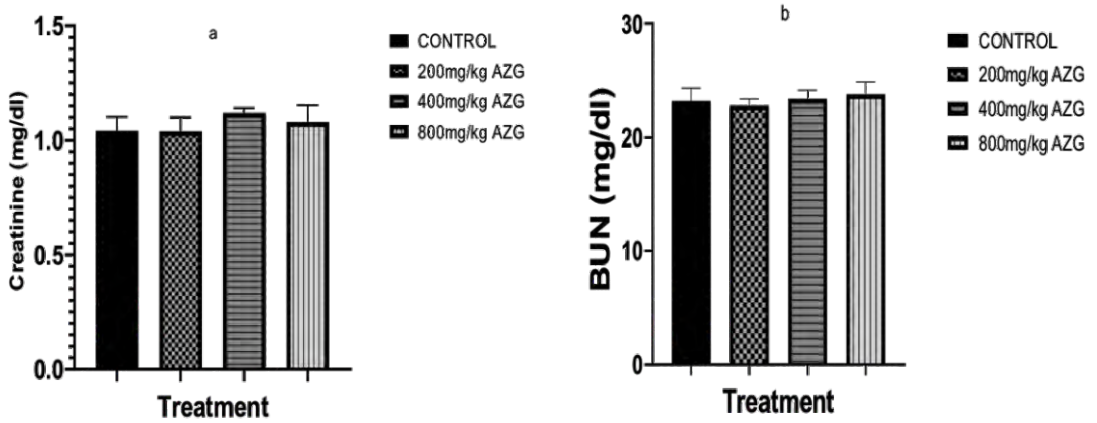



*Figure 9:* Effect of AZG-HE on (a) serum creatinine level and (b) BUN level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on kidney homeostatic function in rats after 60 days oral treatment**
Figures 10(a-e) elicited no significant variation calcium bicarbonate, phosphate, sodium and potassium levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 60 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

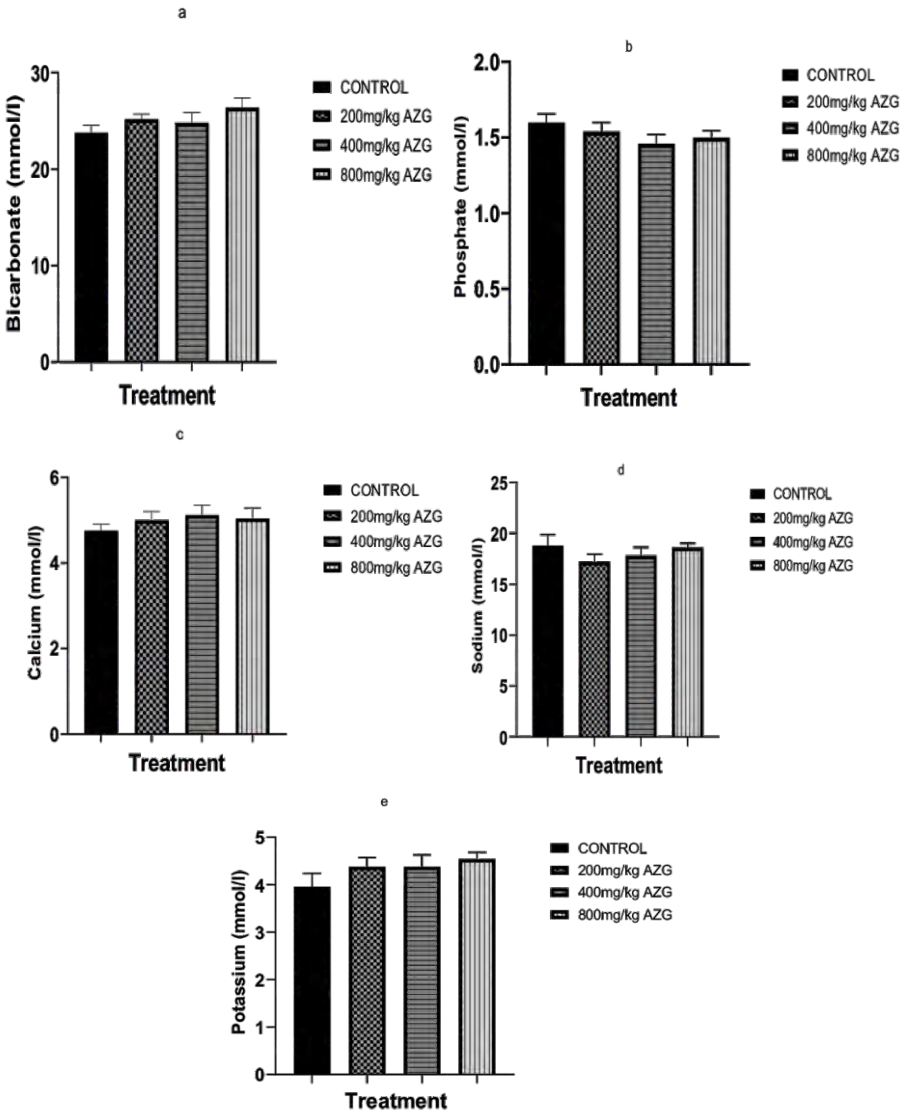



*Figure* 10*:* Effect of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum sodium level, and (e) serum potassium level in rats after 60 days oral administration
p>0.05 in comparison to untreated group

**Effect of AZG-HE on kidney antioxidant parameters in rats after 60 days oral treatment**
Figures 11(a-d) illustrates no significant variation in SOD, CAT, GSH, and GPx antioxidant across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a period of 60 days elicited statistically significant variation (p>0.05) relative to untreated group,

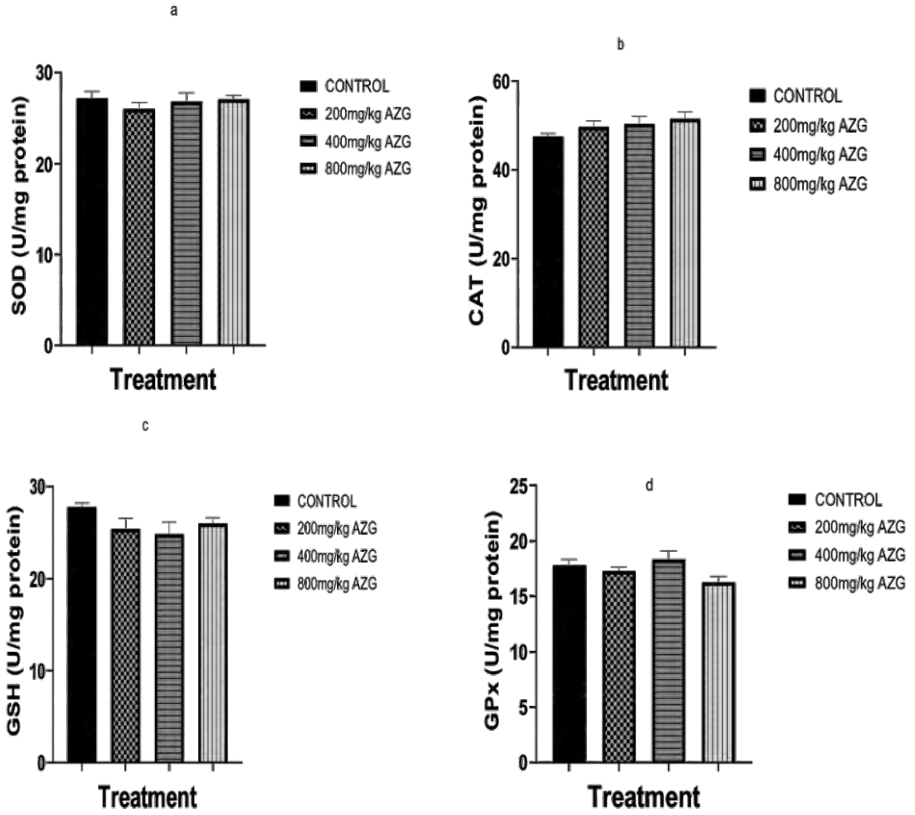



*Figure 11:* Effect of AZG-HE on (a) kidney SOD level, (b) on kidney CAT level, (c) kidney GSH level, and (d) kidney GPx level in rats after 60 days oral administration
p>0.05 in comparison to untreated group

**Effect of AZG-HE on kidney pro-oxidant parameters in rats after 60 days oral treatment**
Figures 12(a and b) illustrates no significant variation in NO and MDA levels across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over a period of 60 days elicited no statistically significant variation (p>0.05) relative to untreated group,

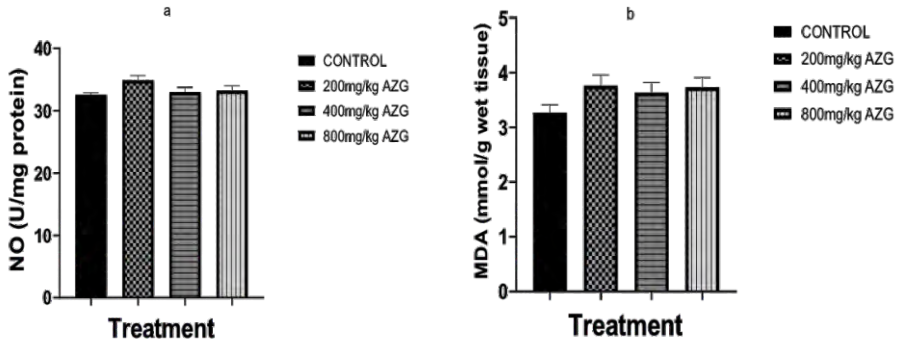



*Figure 12:* Effect of AZG-HE on kidney (a) nitric oxide level and (b) Malondialdehyde level in rats after 60 days oral administration

p>0.05 in comparison to untreated group

**Effect of AZG-HE on heart antioxidant in rats after 60 days oral treatment**

Figures 13(a-d) illustrates no significant variation in SOD, CAT, GSH AND GPx antioxidant across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over a period of 60 days elicited produced no statistically significant variation (p>0.05) relative to untreated group,

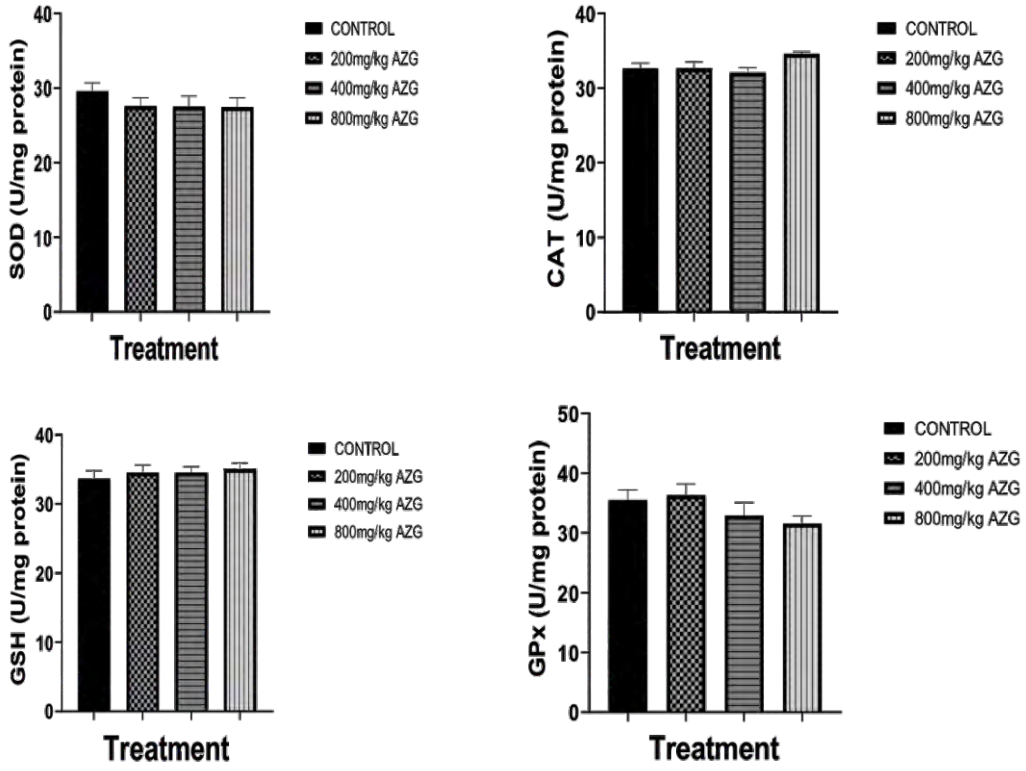



*Figure 13:* Effect of AZG-HE on (a) heart SOD level, (b) heart CAT level, (c) heart GSH level, and (d) heart GPx level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on heart pro-oxidant parameters in rats after 60 days oral treatment**

Figures 14(a and b) illustrate no significant variation in NO and MDA prooxidant across treatment groups. AZG-HE 800, 400, and 200 mg/kg administered over a period of 60 days elicited no statistically significant variation (p>0.05) relative to untreated group,

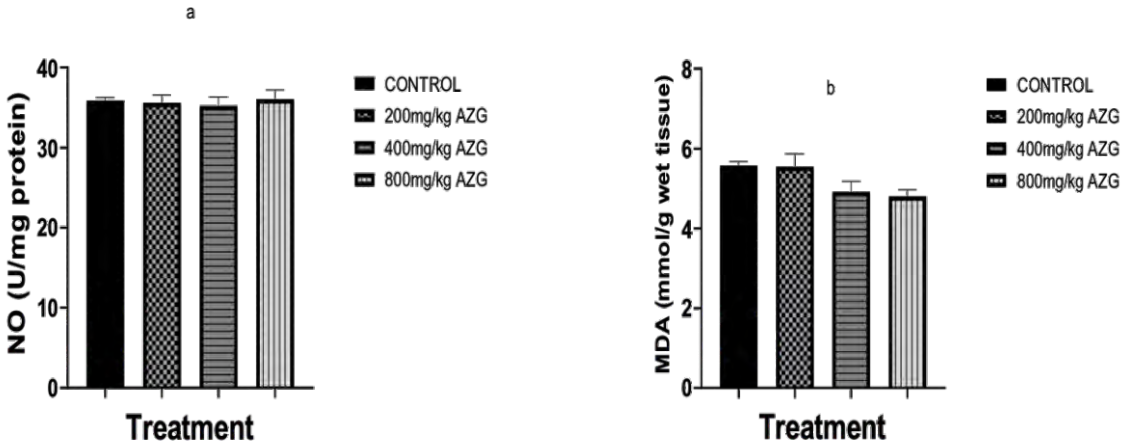



*Figure* 14*:* Effect of AZG-HE on heart homogenate (a) NO level, and (b) MDA level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on body weight in rats after 90 days oral treatment**.
Figure 15 illustrates a non-significant variation in the percentage change in weight across treatment groups. Daily AZG-HE administration at varied doses of 800, 400 and 200 mg/kg over a 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

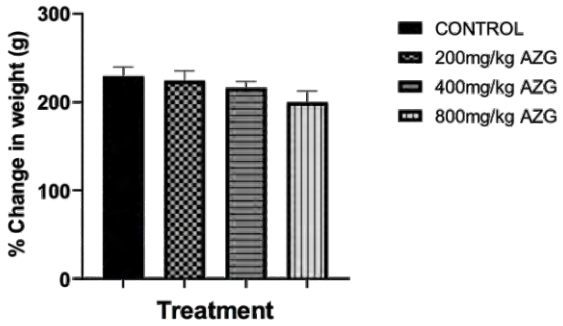



*Figure 15:* Effect of AZG-HE on body in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on feed intake in Wistar rats after 90 days**
Figure 16 illustrates a non-significant variation in feed intake across treatment groups. Daily AZG-HE administration at varied doses of 800, 400 and 200 mg/kg over a 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

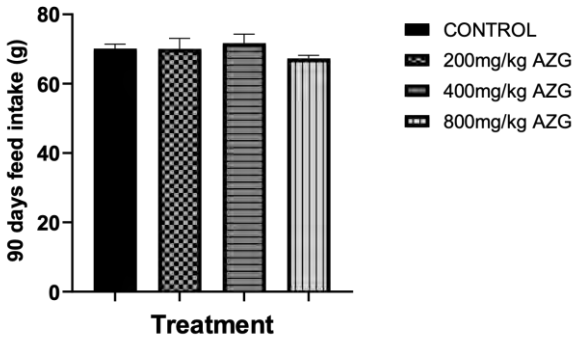



*Figure 16*: Effect of AZG-HE on feed intake in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on fluid intake in rats after 90 days oral treatment**
Figure 17 illustrates a non-significant variation in fluid intake across treatment groups. Daily AZG-HE administration at varied doses of 800, 400 and 200 over a 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

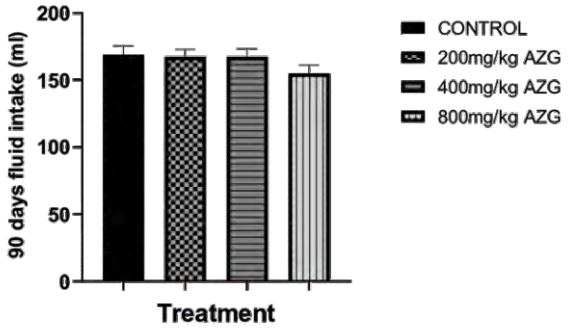



*Figure 17:* Effect of AZG-HE on fluid intake in Wistar rats after 90 days
p >0.05 in comparison to untreated group

**Effect of AZG-HE on haematological indices in rats after 90 days oral treatment**
Figure 18(a-g) illustrates no significant variation in RBC, RDW-SD, hematocrit, MCV, PLT, PDW and MPV across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a period of 90 days elicited no statistically significant (p>0.05) difference relative to untreated group, while

Figure 18(h-k) illustrate a significant variation in RDW-CV, HB, MCH, and MCHC across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a period of 90 days elicited a statistically significant increase (p<0.05) relative to untreated group and,

Figure 18l illustrate a significant variation in plateletcrit across treatment groups. AZG-HE 800, and 400 mg/kg administered over a period of 90 days elicited statistically significant increase (p<0.05) in plateletcrit relative to untreated group,

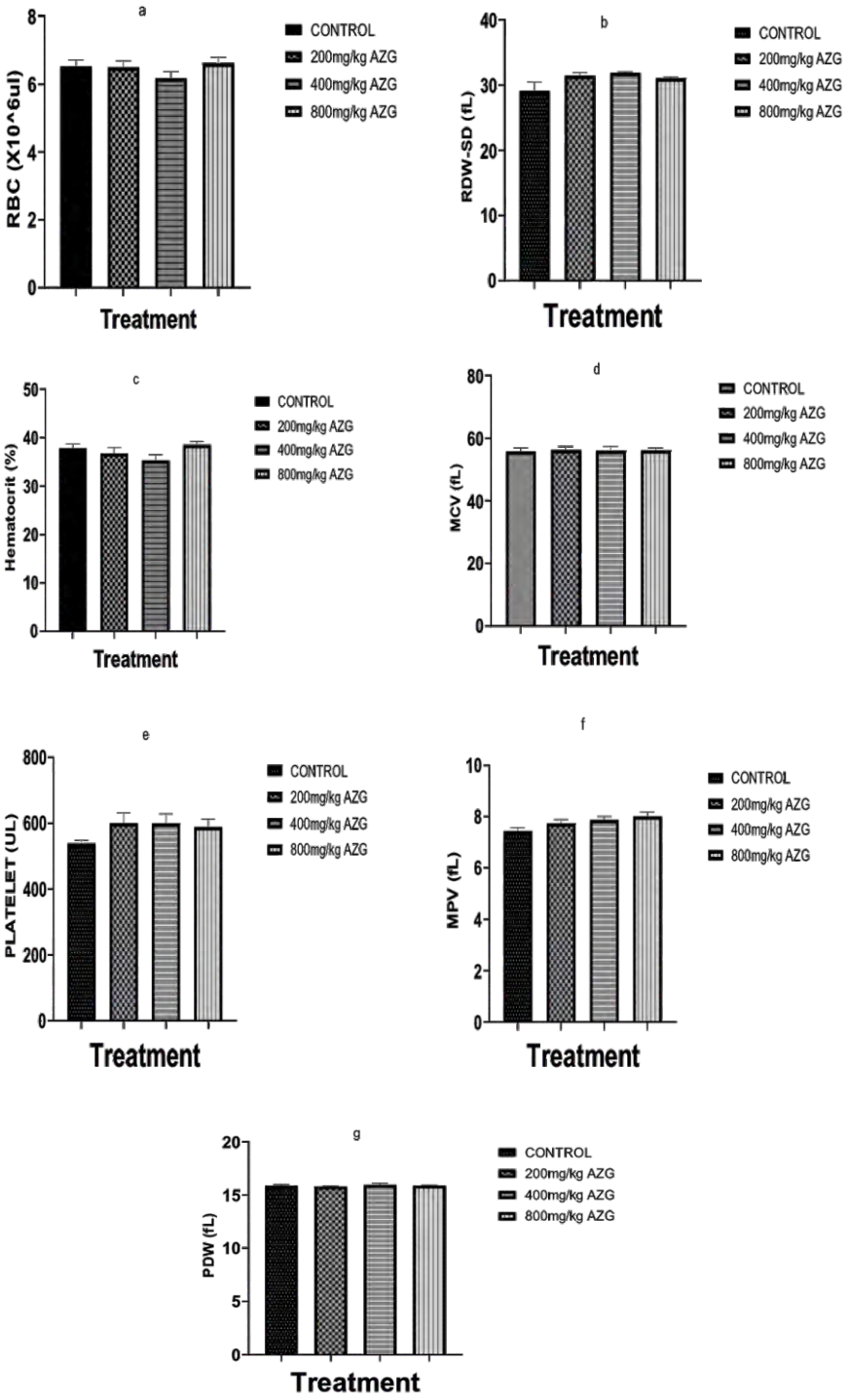



*Figure* 18*:* Effect of AZG-HE on (a) RBC count, (b) RDW-SD, (c) hematocrit, (d) MCV, (e) PLT count, (f) MPV, (g) PDW in rats after 90 days oral administration
p >0.05 in comparison to untreated group

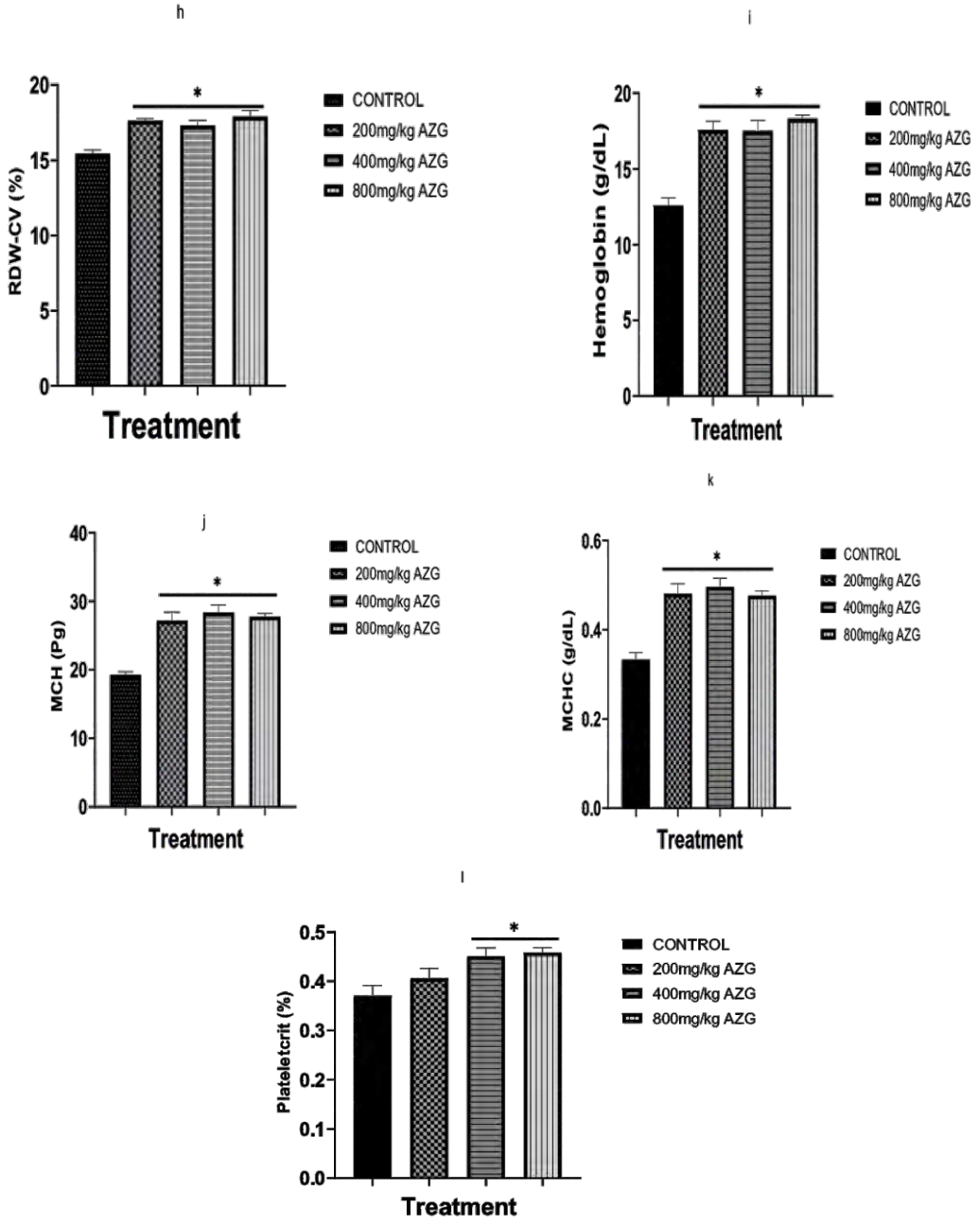



*Figure* 18*:* Effect of AZG-HE on (h) RDW-CV, (i) HB level, (j) MCH levels, (k) MCHC and (l) PCT in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

**Effect of AZG-HE on serum lipid profiles in rats after 90 days oral treatment**
Figure 19(a and b) illustrates a significant variation in TG and LDL levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a period of 90 days elicited a statistically significant

decrease ($p<0.05$) relative to untreated group, while

Figure 19(c and d) illustrates a significant variation in TC and VLDL across treatment groups. AZG-HE 800 and 400 mg/kg administered over a period of 90 days elicited statistically significant variation ($p<0.05$) decrease relative to untreated group and,

Figure 19e illustrates a significant variation in HDL level across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a period of 90 days elicited statistically significant increase ($p<0.05$) relative to untreated group,

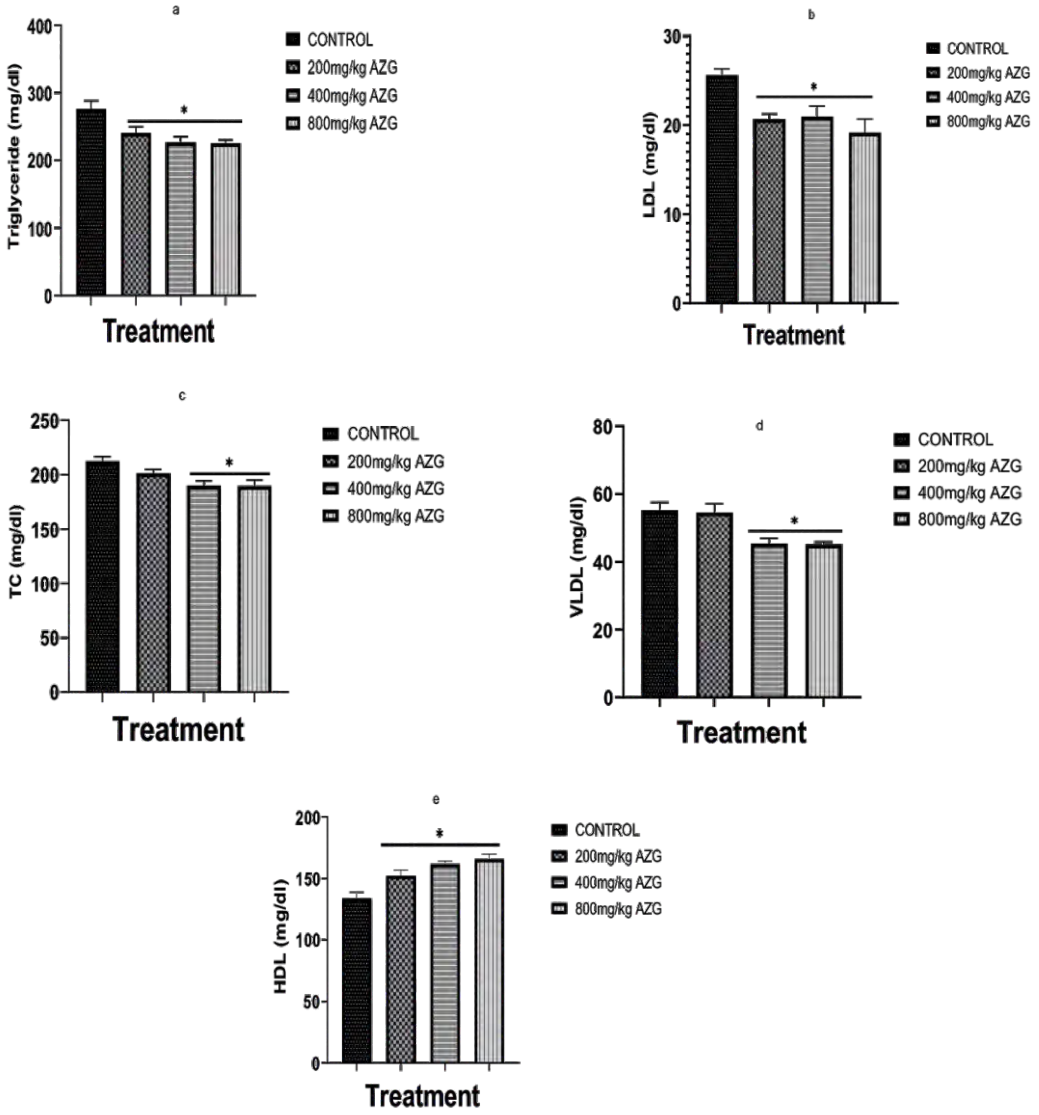



*Figure* 19*:* Effect of AZG-HE on (a) TG level, (b) TC level, (c) HDL level, (d) LDL level, (e) VLDL in rats after 90 days oral administration
* $p<0.05$ in comparison to untreated group

**Effect of AZG-HE on liver function indices in rats after 90 days oral treatment**.
Figure 20(a-e) illustrates no significant variation AST, ALT, ALP, T.bil, and D.bil levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over a 90 days period elicited no statistically significant variation (p 0.05) relative to untreated group, while

Figures 20(f and g) illustrates a significant variation in Total protein and GGT levels. AZG-HE 800 mg/kg administered over 90 days period elicited a statistically significant increase ($p<0.05$) in GGT and total protein relative to untreated group,

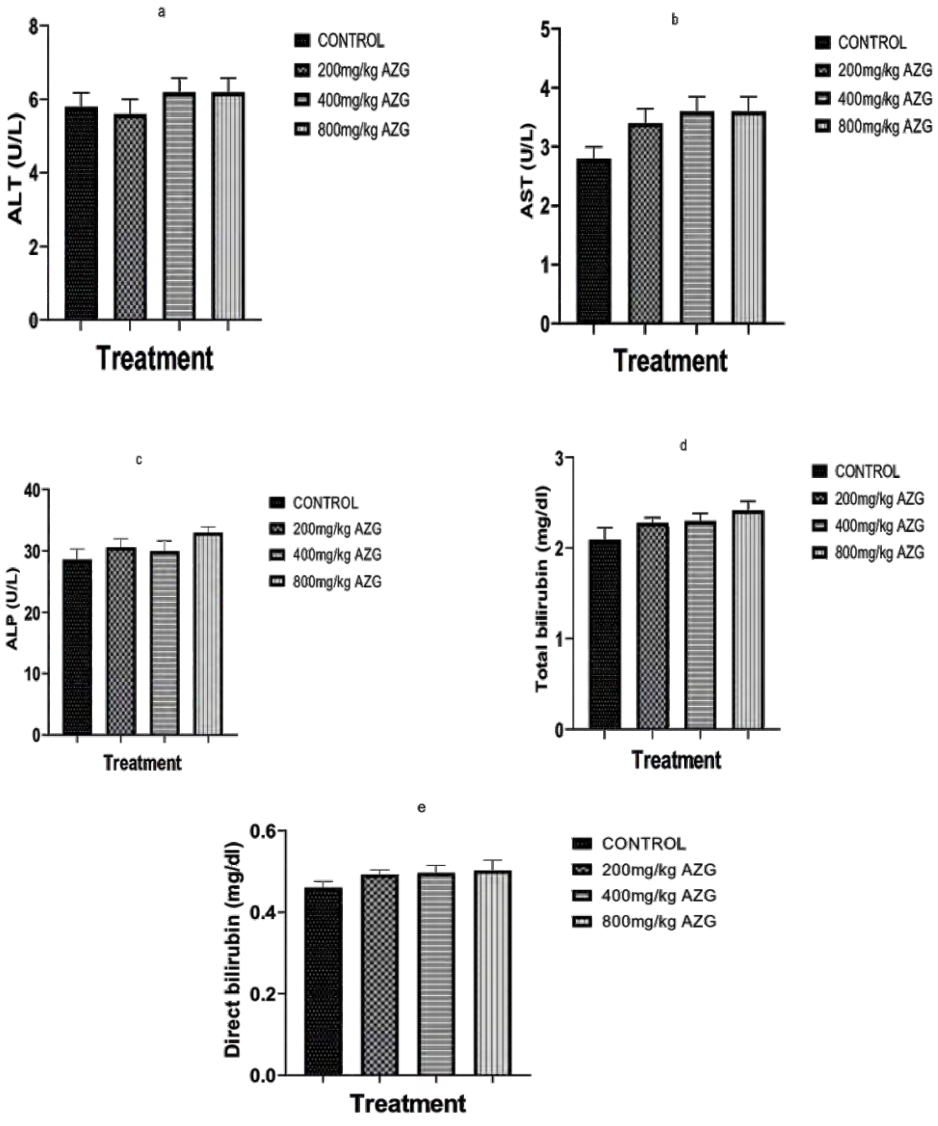



*Figure* 20*:* Effect of AZG-HE on (a) ALT, (b) AST, (c) ALP, (d) T.bil, and (e) D.bil levels in rats after 90 days oral
p >0.05 in comparison to untreated group

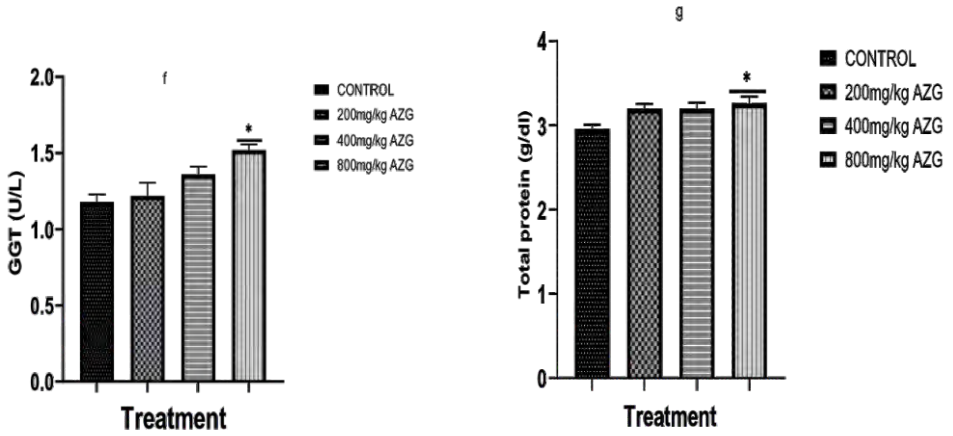



*Figure* 20*:* Effect of AZG-HE on (f) gamma-glutamyl transferase level and (g) total protein level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

**Effect of AZG-HE on liver antioxidant activity in rats after 90 days oral treatment**.
Figures 21(a and b) illustrates no significant variation SOD and CAT antioxidant levels across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

However, from figure 21(c and d), illustrates a statistically significant variation in GSH and GPx antioxidant activity across treatment group. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited a statistically significant increase (p<0.05) relative to untreated group,

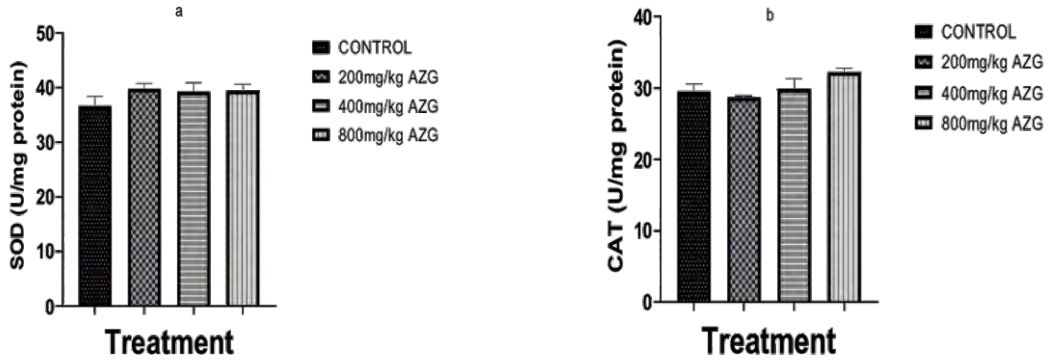



*Figure* 21*:* Effect of AZG-HE on (a) liver SOD level, (b) liver CAT level in rats after 90 days oral administration
p>0.05 in comparison to untreated group

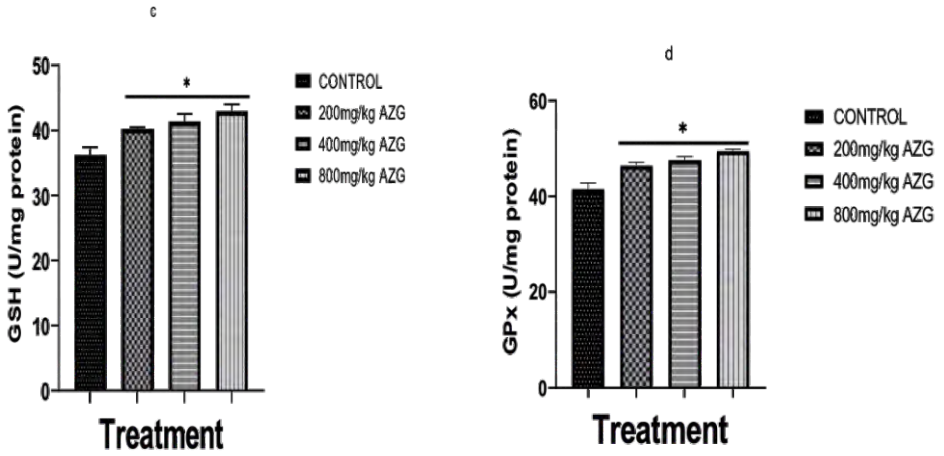



*Figure* 21*:* Effect of AZG-HE on (c) liver glutathione level, (d) liver glutathione peroxidase level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

**Effect of AZG-HE on liver pro-oxidant activity in rats after 90 days oral treatment**.
Figures 22(a and b) illustrates a significant variation in prooxidant levels across treatment groups. AZG-HE (800 mg/kg) administered over 90 days period elicited a statistically significant increase ($p<0.05$) in NO levels relative to untreated group. However, AZG-HE 800, 400, and 200 mg/kg administered over 90 days period elicited a statistically significant decrease ($p<0.05$) in MDA levels relative to untreated group,

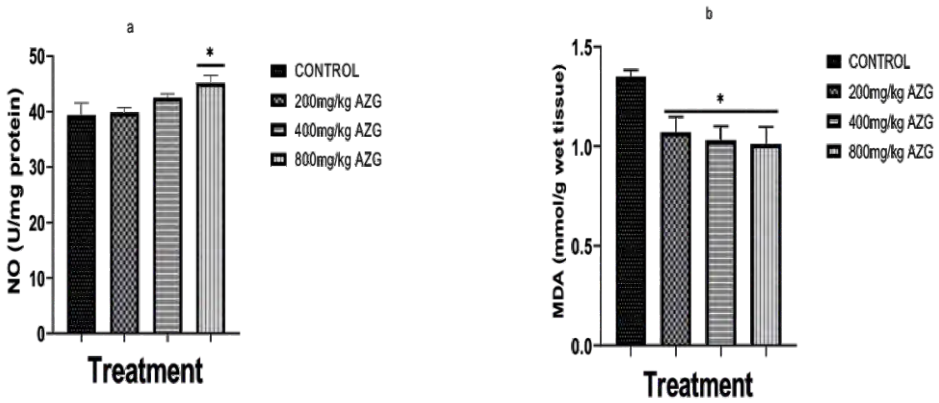



*Figure* 22*:* Effect of AZG-HE on (a) liver NO level, (b) liver MDA level in rats after 90 days oral administration
* p <0.05 in comparison untreated group

**Effect of AZG-HE on kidney excretory indices in rats after 90 days oral treatment**.
Figures 22(a and b) illustrates no significant variation in creatinine and BUN levels across treatment groups. AZG-HE 800 and 400 mg/kg administered over 90 days period elicited no statistically significant variation ($p>0.05$) in creatinine and BUN levels relative to untreated group,

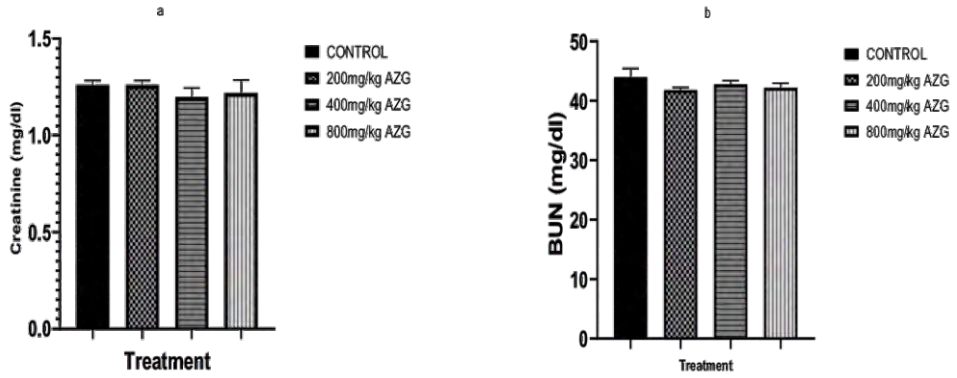
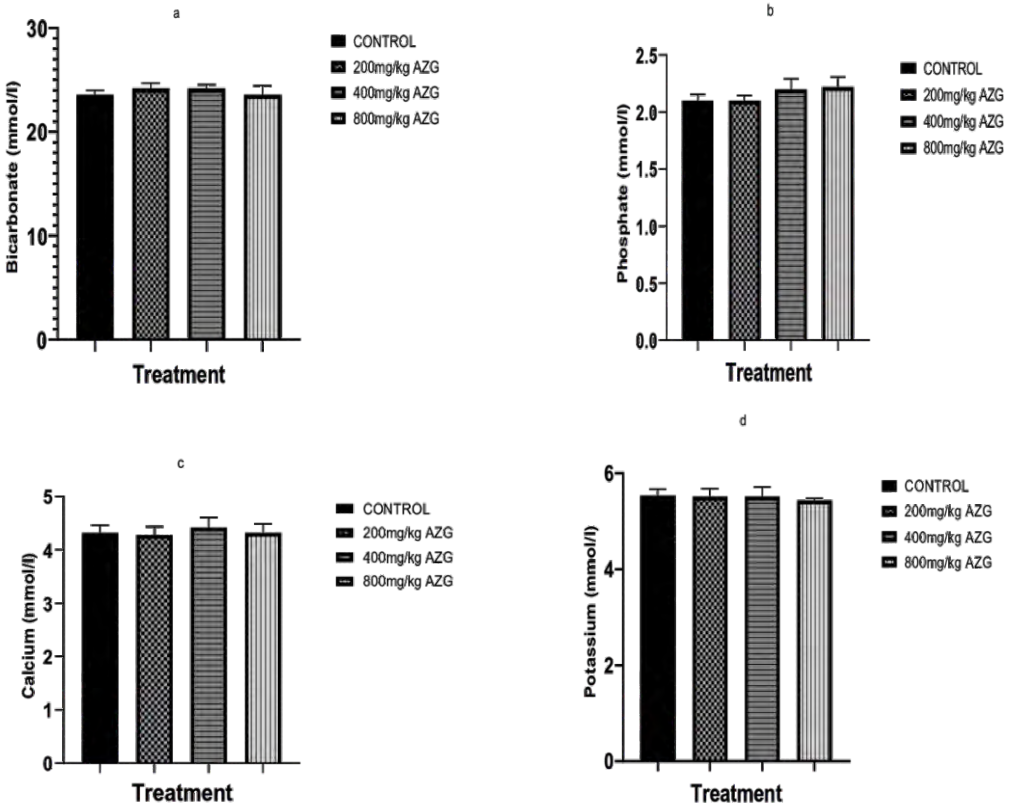



*Figure* 22*:* Effect of AZG-HE on (a) serum creatinine, (b) BUN level in rats after 90 days oral administration
p >0.05 compared to untreated group

**Effect of AZG-HE on kidney homeostatic function in rats after 90 days oral treatment**.
Figures 23(a-e) illustrate no significant variation in bicarbonate, sodium, phosphate, calcium, and potassium level across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,



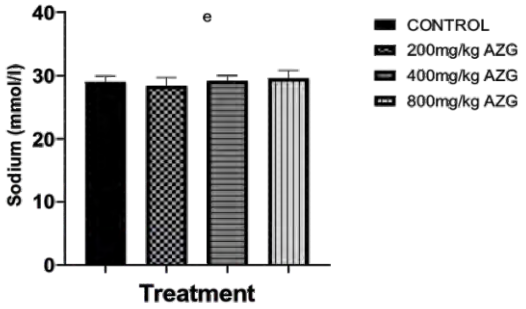



*Figure* 23*:* Effect of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum potassium level, (e) serum sodium level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on kidney antioxidant parameters in rats after 90 days oral treatment**.
Figure 24a illustrate a significant variation in SOD antioxidant across treatment groups. AZG-HE 800 mg/kg administered over 90 days period elicited a statistically significant increase ($p<0.05$) relative to untreated group, while

Figure 24(b-d) illustrate no significant variation CAT, GSH and GPx antioxidant biomarkers across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited no statistically significant variation ($p>0.05$) relative to untreated group,

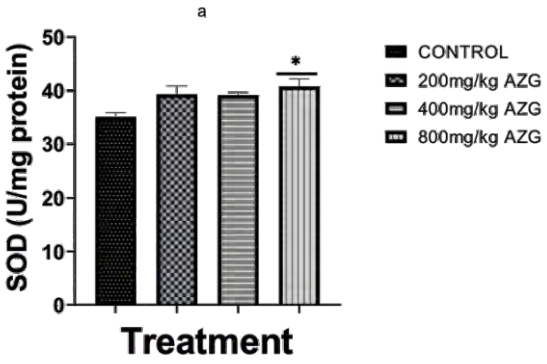



*Figure* 24a*:* Effect of AZG-HE on kidney SOD level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

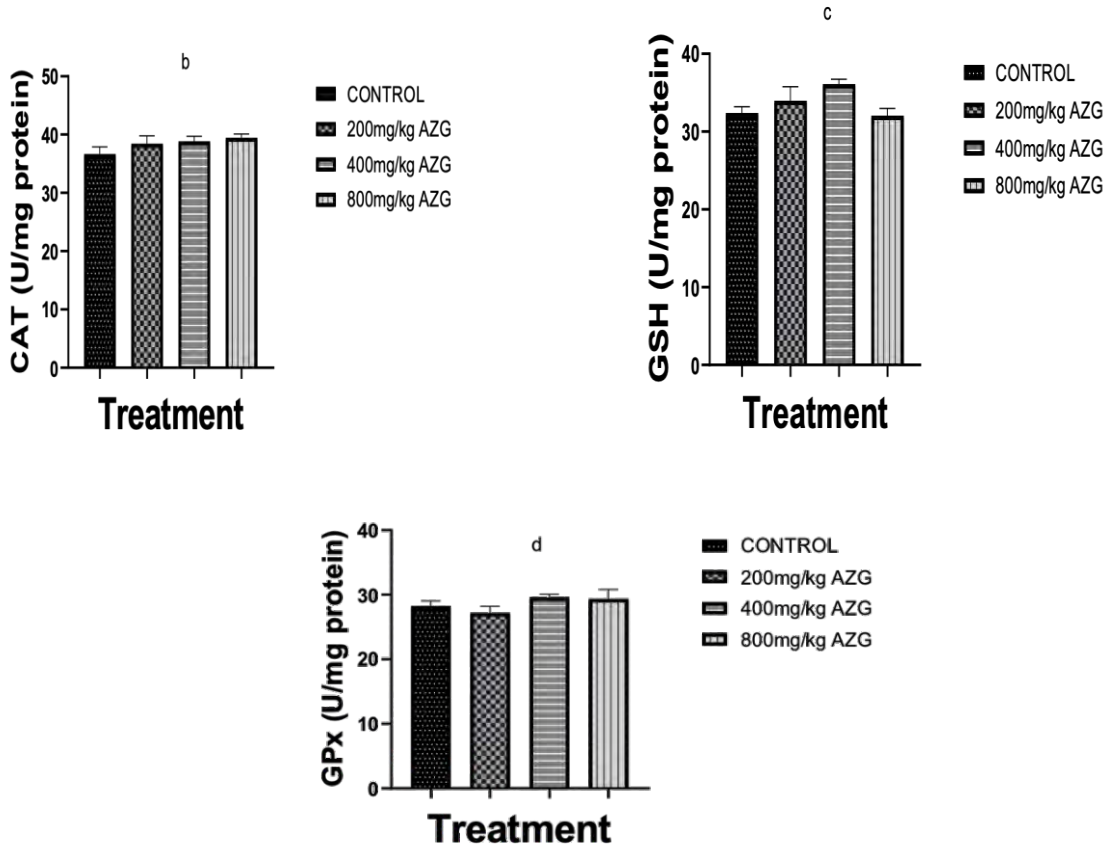



*Figure* 24*:* Effect of AZG-HE on (b) kidney catalase level, (c) kidney reduced glutathione level, (d) kidney glutathione peroxidase level in rats after 90 days oral administration
p >0.05 compared to untreated group

**Effect of AZG-HE on kidney pro-oxidant activity in rats after 90 days oral treatment**.
Figures 25a illustrates a significant variation in NO level across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days elicited a statistically significant increase ($p<0.05$) in No level relative to untreated group,

Figures 25b illustrates no significant variation in MDA level across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days elicited no significant variation ($p>0.05$) in MDA level relative to untreated group,

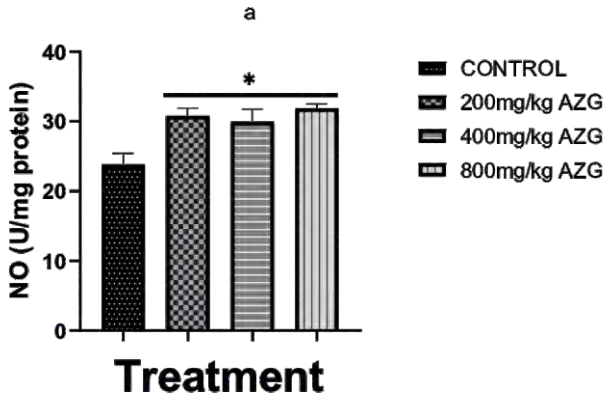



*Figure* 25a*:* Effect of AZG-HE on kidney NO level in rats after 90 days oral administration
*p <0.05 in comparison to untreated group

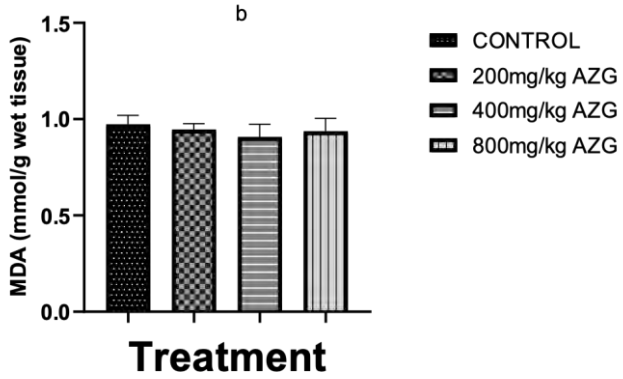



*Figure* 25b*:* Effect of AZG-HE on kidney MDA level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on heart antioxidant parameters in rats after 90 days oral treatment**.
Figures 26(a and b) illustrates no significant variation in SOD, CAT AND GPx level across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited no statistically significant variation (p>0.05) relative to untreated group,

Figures 26d illustrates a significant variation in GSH antioxidant across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited statistically significant increase (p<0.05) in GSH levels relative to untreated group,

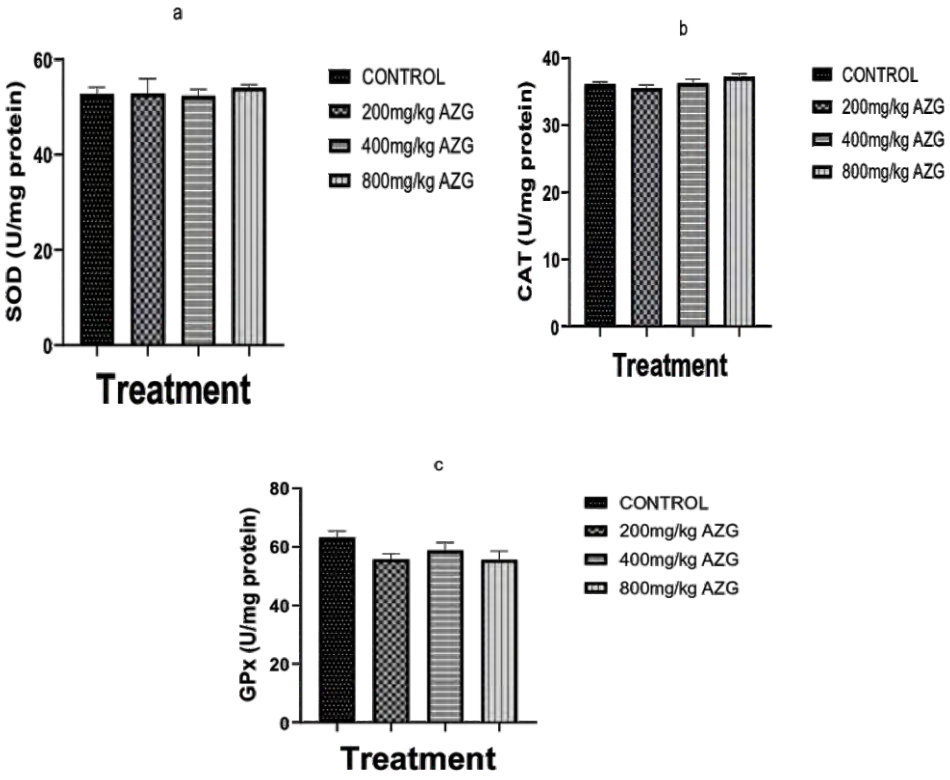



*Figure* 26*:* Effect of AZG-HE on (a) heart SOD level, (b) heart CAT level, (c) heart GPx level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

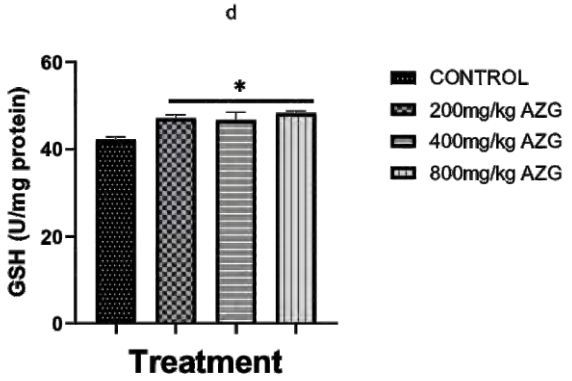



*Figure* 26d*:* Effect of AZG-HE on heart GSH level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of AZG-HE on heart pro-oxidant activity in rats after 90 days oral treatment**.
Figures 27(a and b) illustrates no statistically significant variation in NO and MDA prooxidant biomarkers across treatment groups. AZG-HE 800, 400 and 200 mg/kg administered over 90 days period elicited no statistically significant ($p<0.05$) difference relative to untreated group,

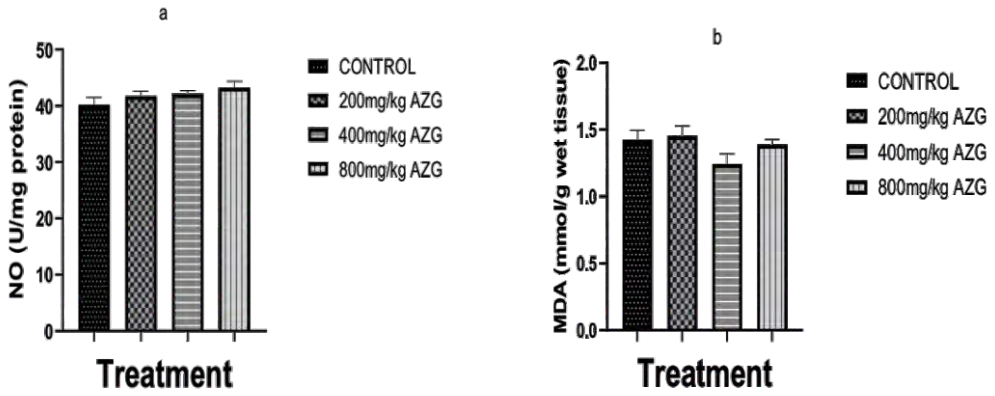



*Figure* 27*:* Effect of AZG-HE on (a) heart NO level and (b) heart MDA level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on body weight changes in rats**

Figure 28 illustrates no significant variation in the percentage change in body weight across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,

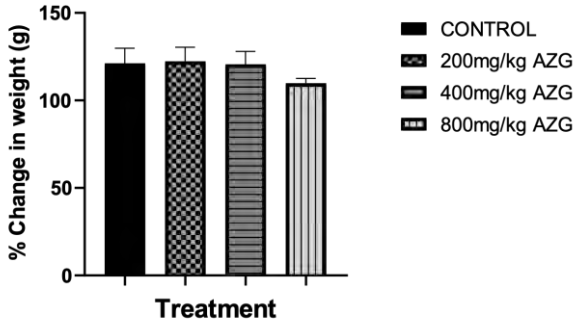



*Figure 28:* Effect of 14 days of toxicity reversibility of AZG-HE on body weight changes in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on feed intake in rats**

Figure 29 illustrates no significant variation in feed intake across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,

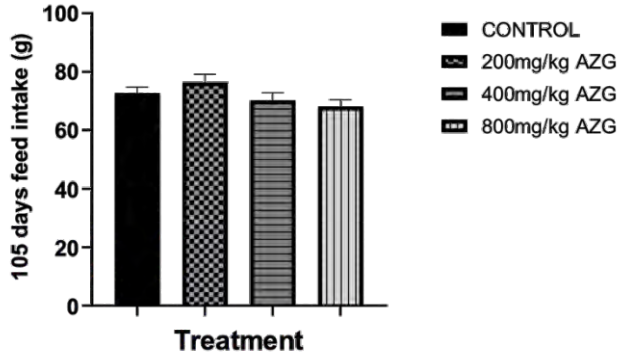



*Figure 29:* **Effect of 14 days of toxicity reversibility of AZG-HE on feed intake in rats**
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on fluid intake in rats**

Figure 30 illustrates no significant variation in fluid intake across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation (p > 0.05) relative to untreated group,

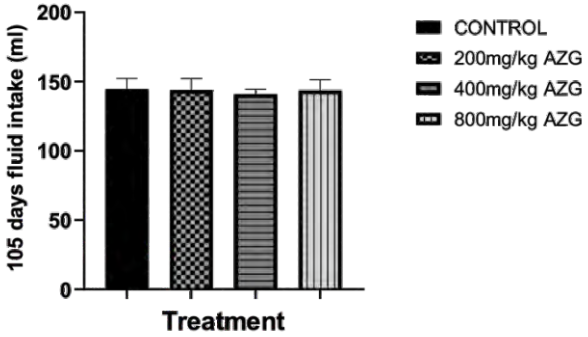



*Figure 30:* Effect of 14 days of toxicity reversibility of AZG-HE on fluid intake in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on hematological parameters in rats**

Figure 31a illustrates a significant variation in RBC count across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800 and 400 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant increase (p <0.05) relative to untreated group,

Figure 31b illustrates a significant variation in RDW-CV across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400 and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant decrease (p <0.05) relative to untreated group.

Figure 31c illustrates a significant variation in RDW-SD across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800 and 400 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant decrease ($p < 0.05$) relative to untreated group,

Figure 31d illustrates a significant variation in hematocrit across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400 and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant increase ($p < 0.05$) relative to untreated group,

Figure 31(e-l) illustrates no significant variation in MCV, HB, MCH, MCHC, PLT count, MPV, PDW, and PCT across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400 and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,

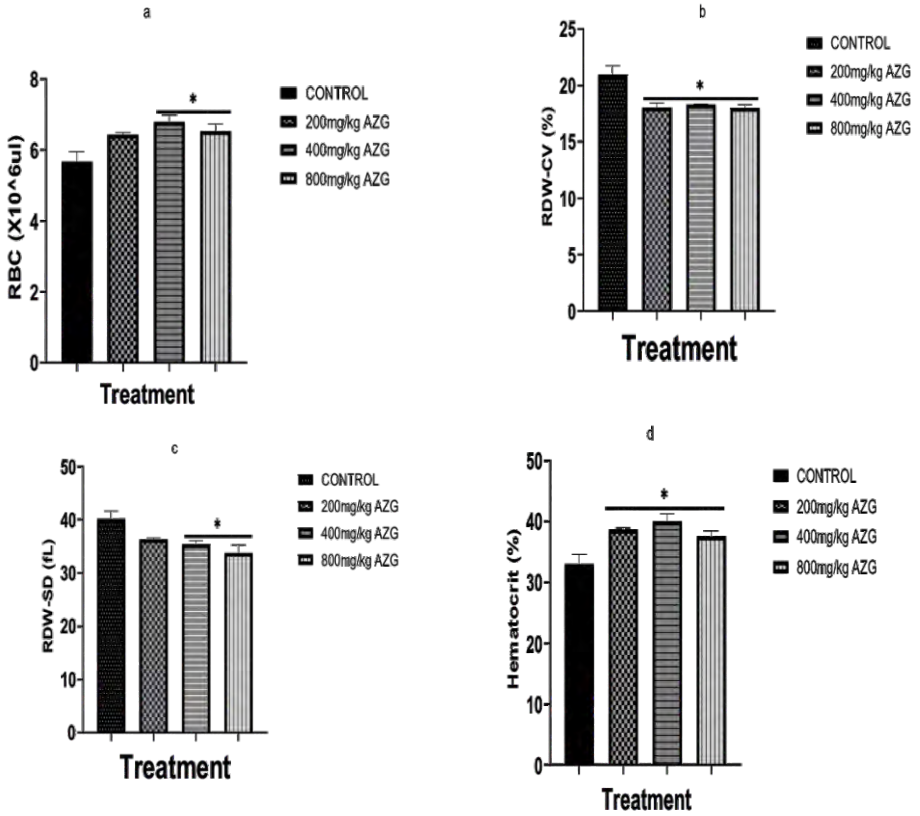
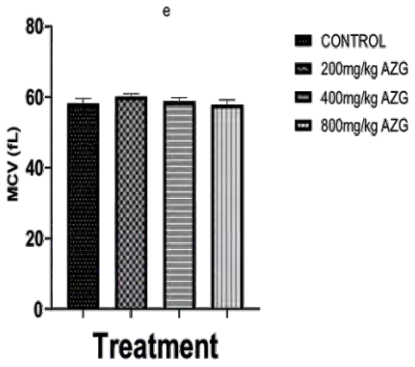
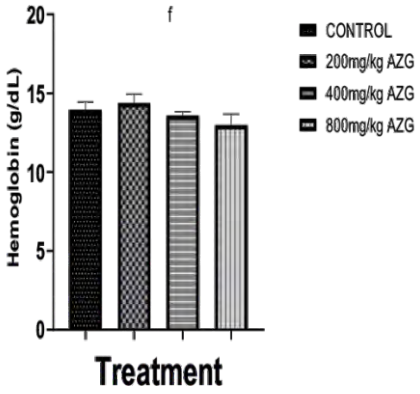
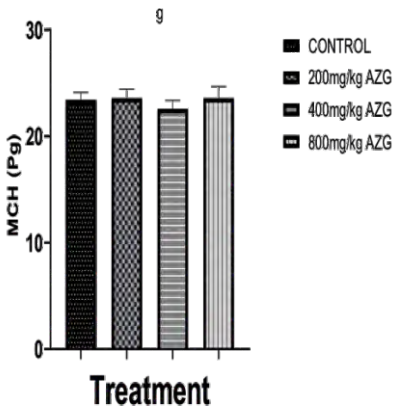
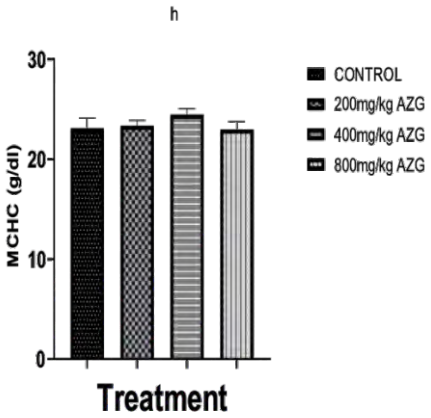


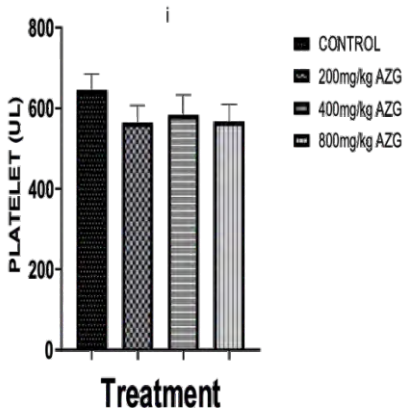

*Figure* 31*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) RBC count, (b) RDW-CV, (c) RDW-SD, (d) hematocrit in rats
* $p<0.05$ in comparison to untreated group

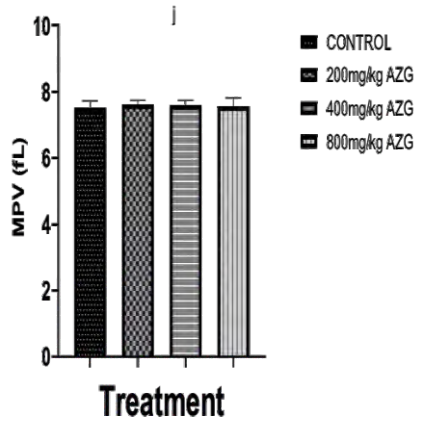

e
MCV (fL)
80
60
40
20
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


f
Hemoglobin (g/dL)
20
15
10
5
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


g
MCH (Pg)
30
20
10
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


h
MCHC (g/dl)
30
20
10
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


i
PLATELET (UL)
800
600
400
200
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


j
MPV (fL)
10
8
6
4
2
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG

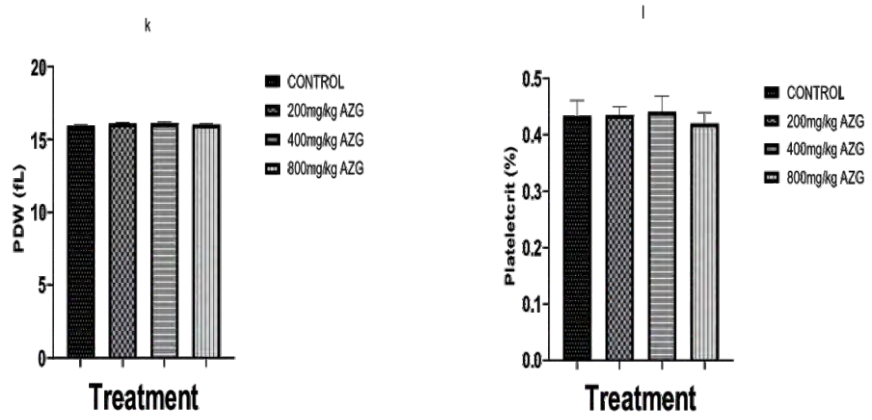



*Figure* 31*:* Effect of 14 days of toxicity reversibility of AZG-HE on (e) MCV, (f) HB level, (g) MCH, (h) MCHC, (i) PLT count, (j) MPV, (k) PDW, and (l) PCT in rats
p>0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on serum lipid profiles in rats**

Figure 32a illustrates a significant variation in triglycerides level across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, and 400 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant decrease (p <0.05) relative to untreated group.

Figure 32b illustrates a significant variation in LDL level across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, and 400 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant decrease (p <0.05) relative to untreated group.

Figure 32(c-e) illustrates no significant variation in TC, HDL and VLDL level across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400 and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced a statistically significant variation (p >0.05) relative to untreated group,

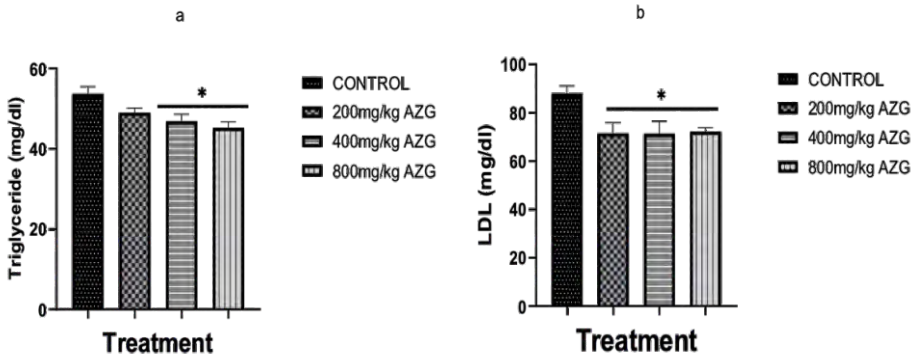



Figure 32: Effect of 14 days of toxicity reversibility of AZG-HE on (a) TG levels and (b) LDL in rats
*p <0.05 in comparison to untreated group

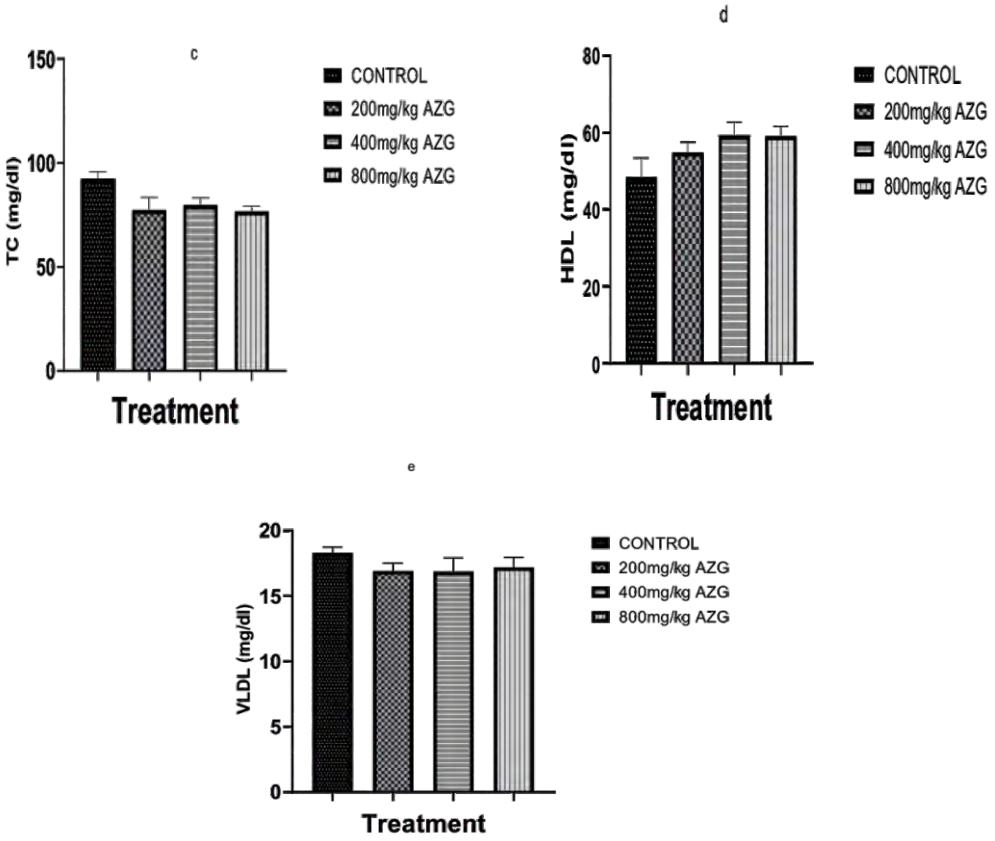
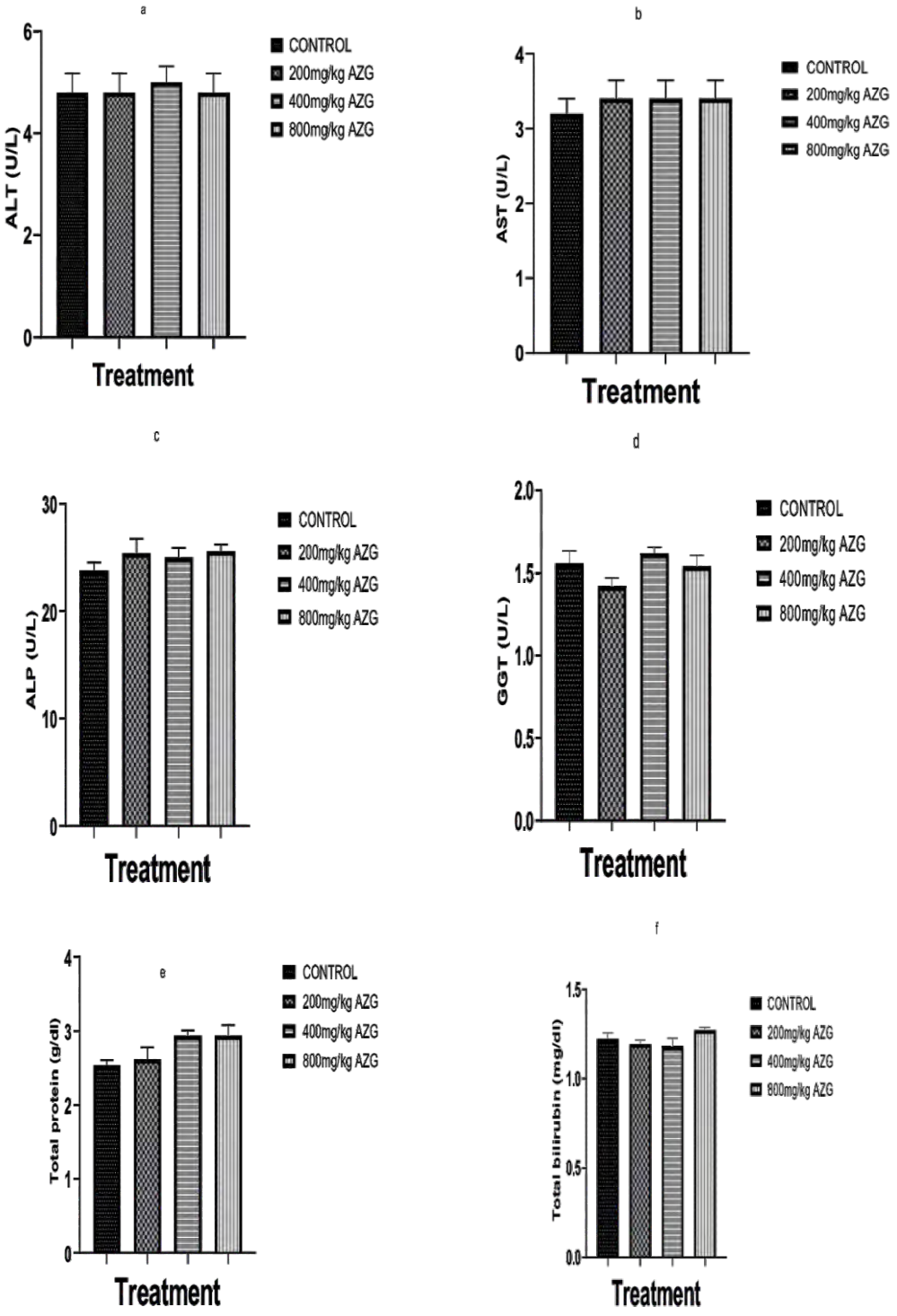



Figure 32*:* Effect of 14 days of toxicity reversibility of AZG-HE on (c) TC levels, (d) HDL levels, and (e) VLDL level in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on liver function parameters in rats**

Figure 33(a-g) illustrates no significant variation in ALP, ALT, GGT, AST, T.protein, T.bil, and D.bil across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,


a
ALT (U/L)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG
b
AST (U/L)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG
c
ALP (U/L)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG
d
GGT (U/L)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG
e
Total protein (g/dl)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG
f
Total bilirubin (mg/dl)
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG

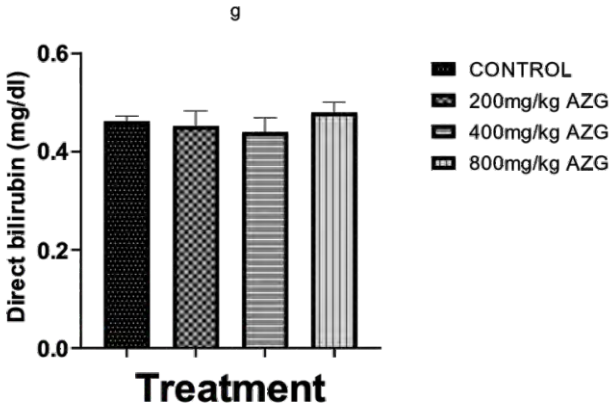
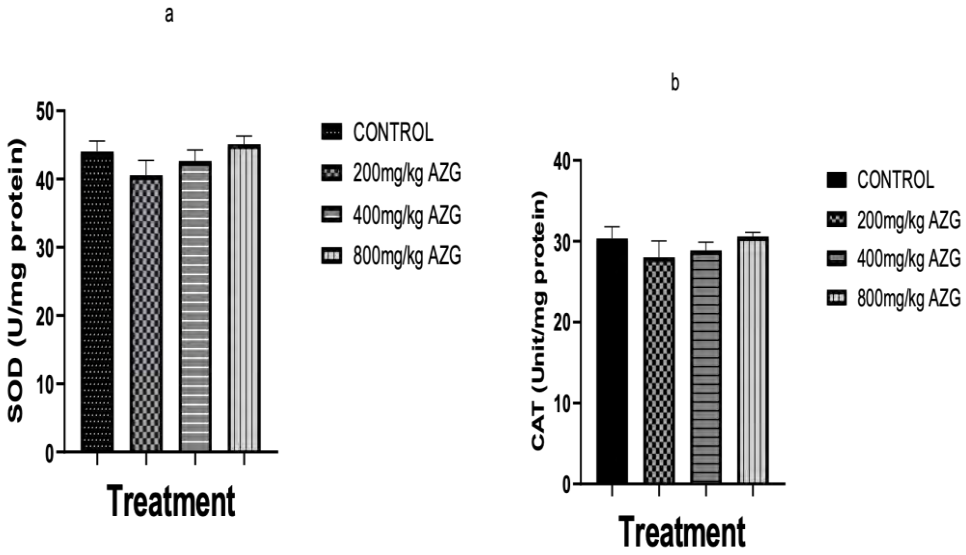



*Figure 33:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) T.protein, (f) T.bil, and (g) D.bil levels in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on liver antioxidant activity in rats**
Figure 34(a-d) illustrates no significant variation in SOD, CAT, GSH, and GPx antioxidant levels across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation (p > 0.05) relative to untreated group,



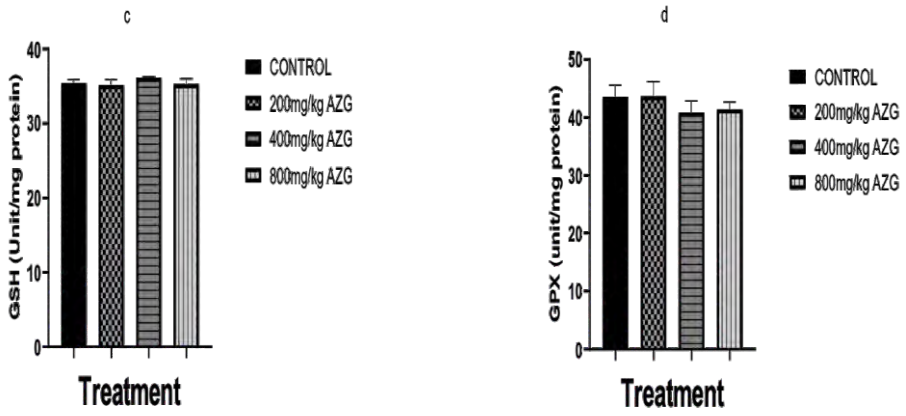



Figure 34*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) liver SOD level, (b) liver catalase level, (c) liver GHS level, (d) liver GPx level in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on liver pro-oxidant activity in rats**

Figure 35(a and b) illustrates no significant variation in NO and MDA prooxidant levels across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation (p > 0.05) relative to untreated group,

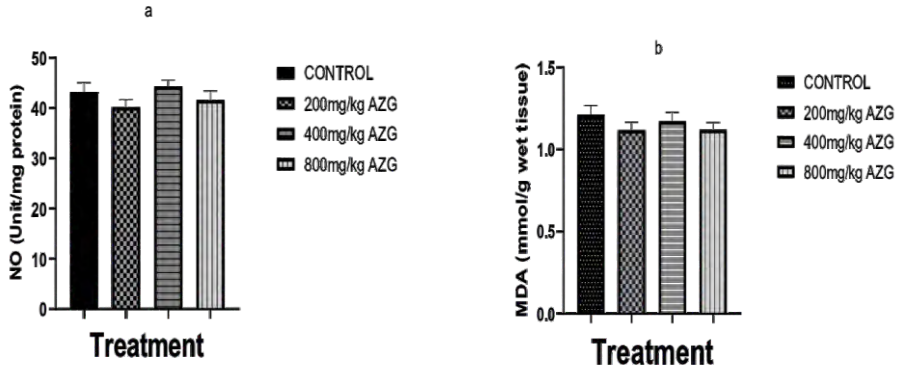



*Figure* 35*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) liver NO level and (b) liver MDA in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on kidney excretory function parameters in rats**

Figure 36(a and b) illustrates no significant variation in creatinine and BUN levels across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation (p > 0.05) relative to untreated group,

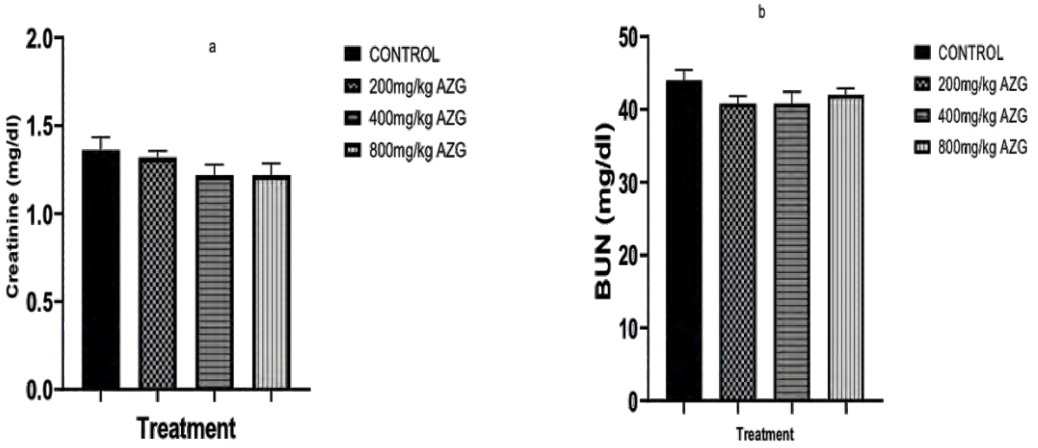
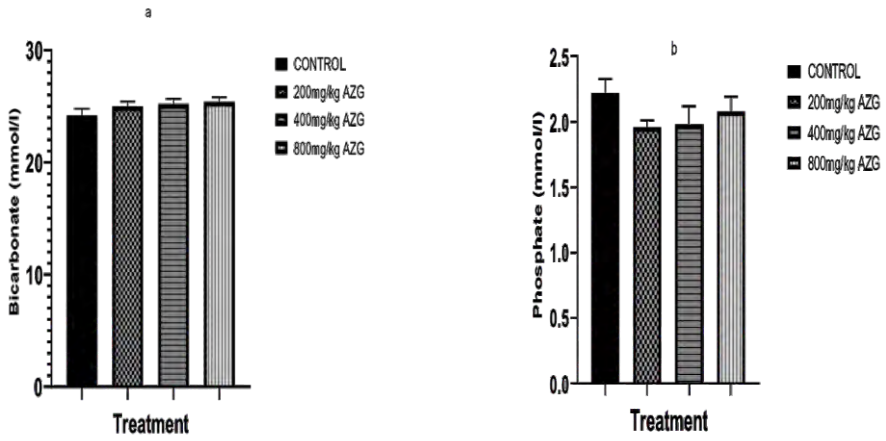



Figure 36: Effect of 14 days of toxicity reversibility of AZG-HE on (a) serum creatinine level and (b) BUN level in rats
p >0.05 in compared to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of** Azanza garckeana **on kidney regulatory (homeostatic) function in rats**

Following 90 days of AZG-HE treatment (200, 400, and 800 mg/kg/day), a 14-day recovery period demonstrated full reversibility, with no statistically significant alterations ($p > 0.05$) in key serum electrolytes (bicarbonate, phosphate, calcium, potassium, sodium) compared to the untreated group (Figure 37a–e)



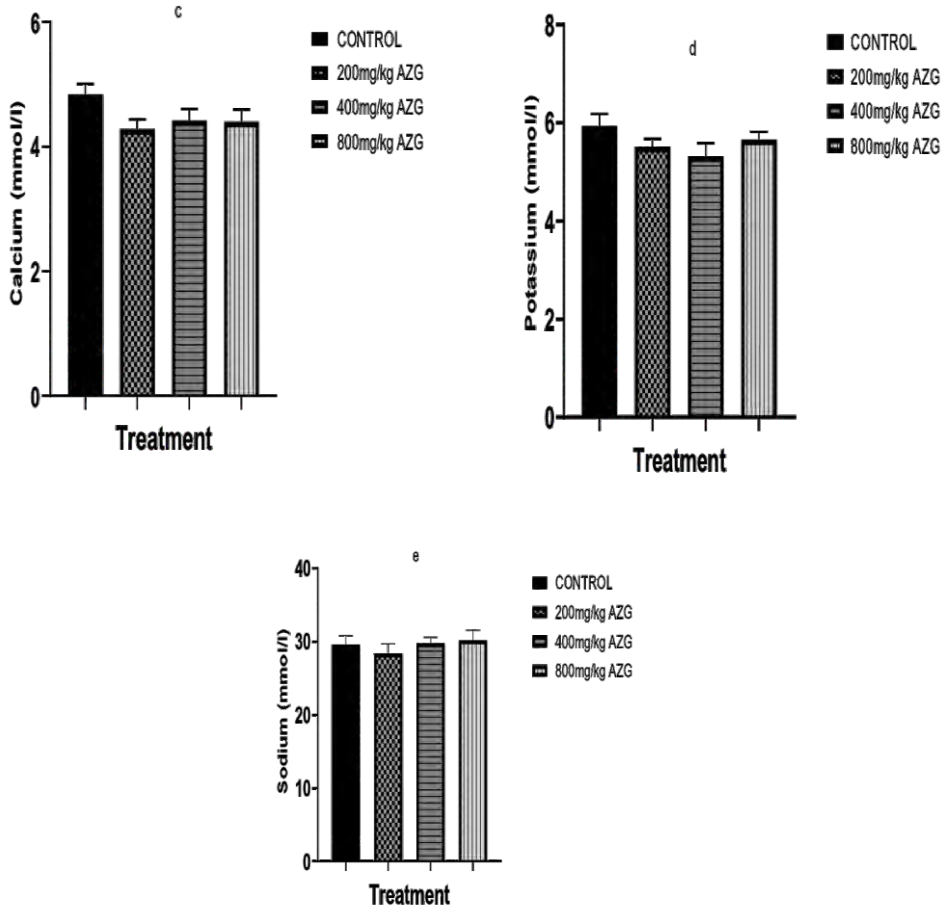



Figure 37: Effect of 14 days of toxicity reversibility of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum potassium level, and (e) serum sodium level in Wistar rats after 105 days
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on kidney antioxidant parameters in rats**

Figure 38(a-d) illustrates no significant variation in SOD, CAT, GSH and GPx level across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,

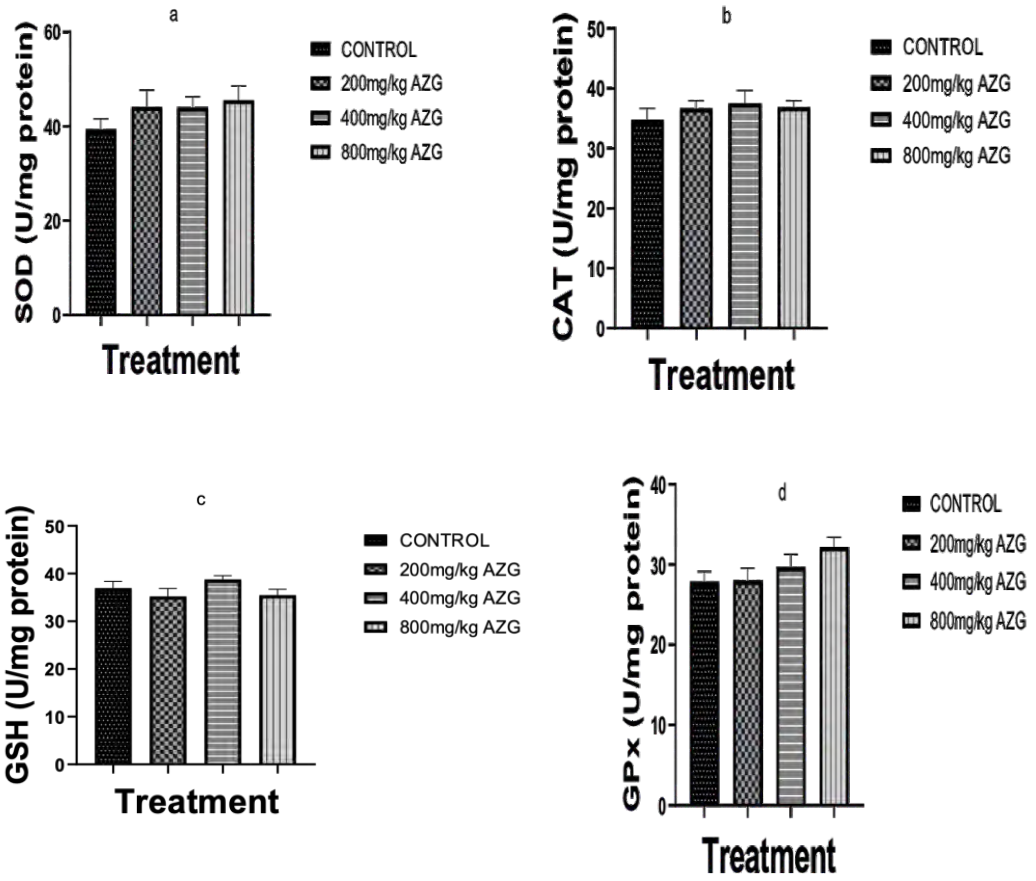



Figure 38: Effect of 14 days of toxicity reversibility of AZG-HE on (a) kidney SOD level, (b) kidney CAT level, (c) GSH level, and (d) kidney GPx level in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on kidney pro-oxidant parameters in rats**

Figure 39(a and b) illustrates no significant variation in NO and MDA levels across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,

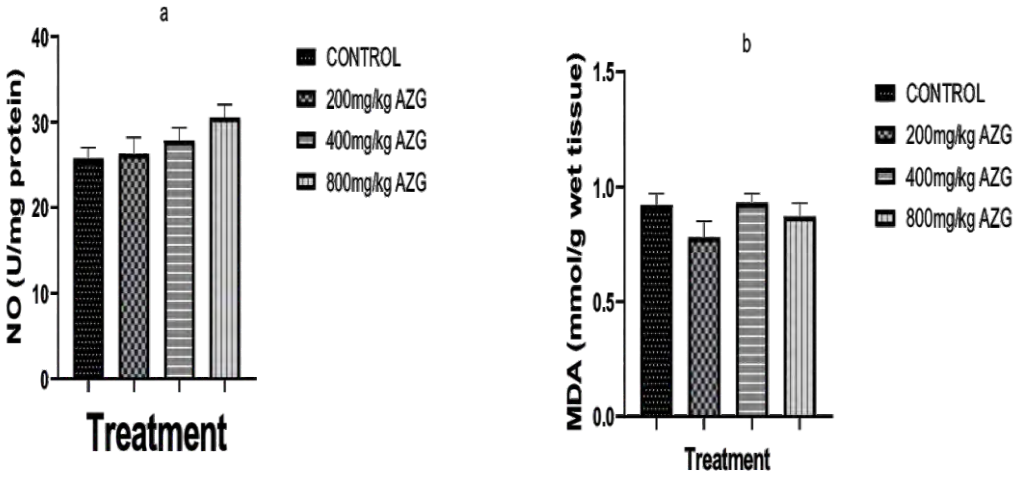
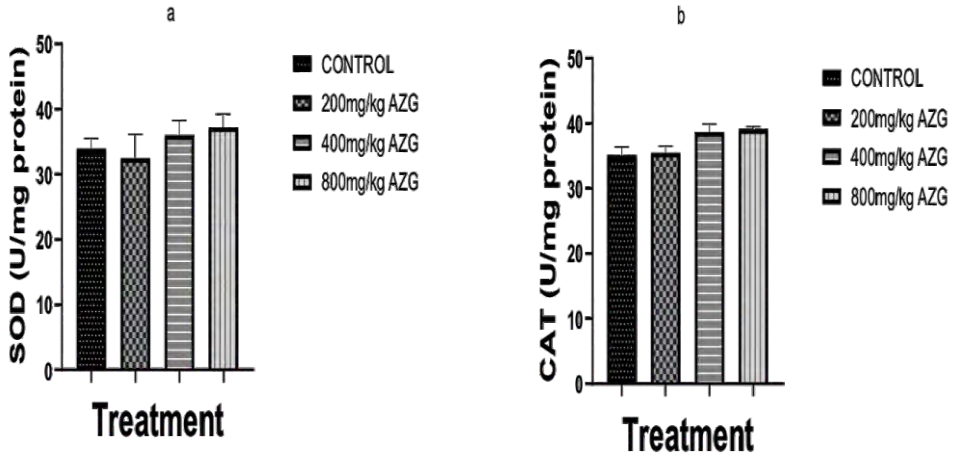



Figure 39*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) kidney NO level and (b) kidney MDA level in rats
p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on Heart antioxidant parameters in rats**

Figure 40(a-d) illustrates no significant variation in SOD, CAT, GSH, and GPx across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation ($p > 0.05$) relative to untreated group,



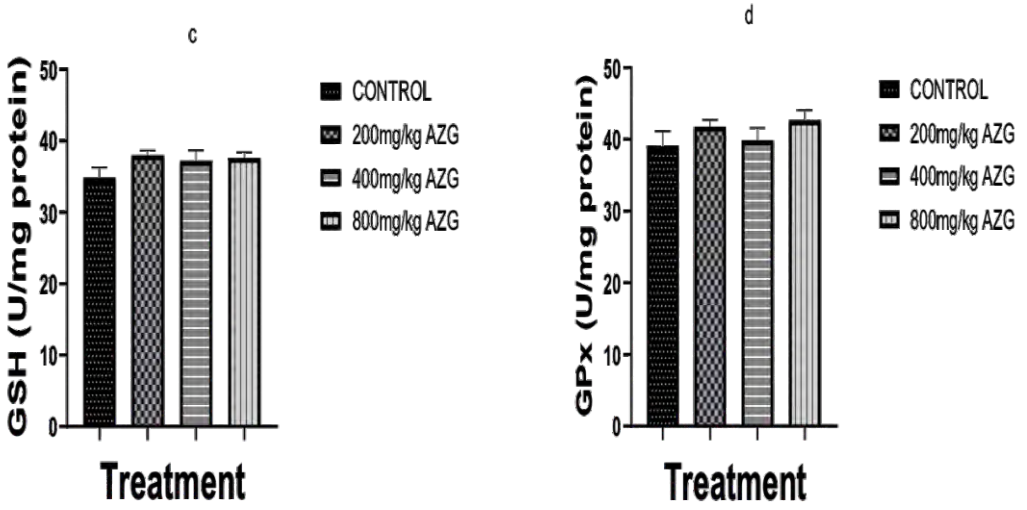



Figure 40: Effect of 14 days of toxicity reversibility of AZG-HE (a) heart SOD level, (b) heart CAT level, (c) heart GSH level, and (d) heart GPx level in rats

p >0.05 in comparison to untreated group

**Effect of 14 days of toxicity reversibility of Hydroethanolic Fruit Extract of Azanza garckeana on heart pro-oxidant parameters in rats**

Figure 41(a and b) illustrates no significant variation in NO and MDA level across treatment groups during a 14 days recovery period following 90 days oral administration. AZG-HE at varied doses of 800, 400, and 200 mg/kg daily for 90 days, followed by a 14-day post-treatment observation period, produced no statistically significant variation (p > 0.05) relative to untreated group,

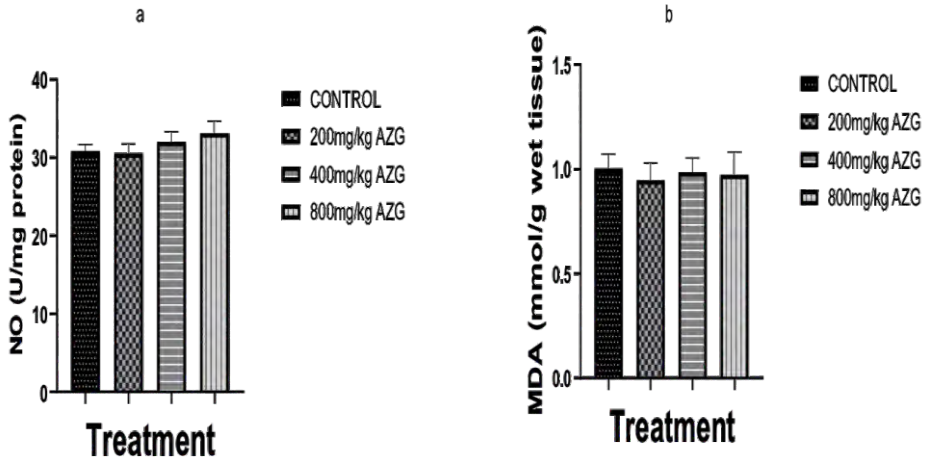



*Figure 41:* Effect of AZG-HE on (a) heart N0 level and (b) heart MDA in Wistar rats 15 days post administration

p >0.05 in comparison to untreated group

## DISCUSSION

In many countries around the world, especially in developing countries across Africa (Ghana, Nigeria, Kenya, Ethiopia, South Africa, Zambia) and Asia (India, China, Sri Lanka, Indonesia), herbal medicine is often the primary or first-line therapy due to affordability, accessibility, and cultural trust. [22] Generally, Herbal remedies commonly applied in conventional ethnomedicine contain phyto-chemical compounds that may have therapeutic effects. [23,24] However, studies have revealed that some of these plants also contains toxic components. [25,26] This study therefore examined the sub-chronic oral doses of *Azanza garckeana* hydroethanolic fruit extract in Wistar rats over 60 and 90 days at doses of 800, 400, and 200 mg/kg body weight, vis-à-vis the reversibility of the toxicity 14 days post-administration.

Body weight, fluid consumption and food intake are key indicators of overall health in toxicity testing. [27] At 60 days, higher doses (800 and 400 mg/kg) of AZG-HE caused noticeable changes in weight gain and food intake compared to controls, possibly indicating mild appetite suppression or metabolic adjustments. However, these effects did not persist at 90 days and 105 days (15 days post administration), as no significant differences were observed. This transient response aligns with typical adaptive responses in toxicity studies, indicating no ongoing impairment of growth or nutrition.[28] While certain phytochemicals can influence appetite and weight, tannins and flavonoids may suppress feed intake or promote weight loss through various mechanisms.[29,30] The absence of significant alteration in these parameters aligns with the established phytochemical profile of *Azanza garckeana*, which is rich in flavonoids, tannins, saponins, phenols, alkaloids, and other secondary metabolites such as carotenoids and vitamin C.[31,32]

Haematological parameters showed variable changes, none of which were indicative of toxicity. At 60 days, AZG-HE caused decreases in haematocrit and MCV, along with increases in MCHC and platelet-related parameters at higher doses. By 90 days (end of dosing), the profile showed signs of erythrocytic enhancement characterized by significant increases in Hb, MCH, and MCHC across doses; decreased RDW-CV; normalized HCT and MCV; and increased PCT) at higher doses, with no changes in PLT, MPV, or PDW. [33] While, in the 15-days recovery period (105 days), many treatment-related changes observed in 60 and 90 days of AZG-HE administration were reversed or stabilized toward beneficial outcomes. That is, increases in RBC (400 and 800mg/kg) and HCT (800, 400 and 200mg/kg); persistent decreases in RDW-CV and RDW-SD (indicating reduced anisocytosis); and normalization of Hb, MCH, MCHC, and all platelet parameters to control levels. This ascertain that AZG-HE demonstrates reversibility upon discontinuation, a hallmark of non-adverse, adaptive responses rather than persistent toxicity.[34,35] These observations align with published toxicity studies on different solvent extract of various parts of *Azanza garckeana*. Acute and subacute evaluations of methanol or aqueous fruit pulp/seed extracts of *Azanza garckeana* reported no adverse haematological changes, with parameters often remaining within normal ranges even at doses up to 1600–5000 mg/kg.[36,37] Liver function assay (ALT, ALP, GGT, AST, total protein, bilirubin) remained mostly unchanged throughout the study, except for a temporary increase in GGT and total protein levels in rats receiving 800 mg/kg for 90 days. This increase was reversed within 14days after stopping administration (reversibility study), indicating no liver toxicity even at the highest dose and longest exposure. Alanine aminotransferase (ALT), predominantly localized in the hepatic

cytoplasm, and alkaline phosphatase (ALP), primarily expressed in the cells lining the hepatic biliary ducts, serve as critical biomarkers for assessing liver injury.[38] Elevations in these enzymes are commonly observed in conditions of hepatotoxicity, reflecting hepatocellular damage (ALT), cholestatic or biliary impairment (ALP and AST).[39,40] The non-significant alterations in serum levels of ALT, ALP, and AST underscore the hepatic safety of *Azanza garckeana* hydroethanolic fruit extract. This finding is especially reassuring, given the liver role in metabolizing foreign substances.[41,42] Minor increases in GGT and total protein at 90 days with 800 mg/kg alone is usually not considered sufficient to indicate clinically relevant liver injury.[43]

Creatinine and BUN are established indicators of glomerular filtration rate and overall kidney function[44,45], with elevations typically signaling tubular damage, or nephrotoxicity.[46,47] Similarly, disruptions in electrolytes (e.g., sodium, potassium, chloride) reflect impaired tubular reabsorption or acid-base balance.[48] The absence of significant alterations in key renal function markers such as serum creatinine, blood urea nitrogen (BUN), and electrolytes following 60 and 90 days of administering different doses of AZG-HE strongly indicates a favourable renal safety profile. This finding contradicts other short-term safety studies of *Azanza garckeana*. For example, aqueous extracts and methanolic extracts of *Azanza garckeana* administered at doses up to 500 mg/kg for 14 to 28 days showed significant differences in kidney function indices (urea, creatinine, electrolytes) compared to controls[49,50] with emphasis on increasing dose as a link to increasing toxicity. In protective models (e.g., alloxan-induced diabetic nephropathy), solvent fractions from *Azanza garckeana* preserved renal integrity by normalizing elevated urea, creatinine, and electrolytes[51,52]. These observations are consistent with the current results, reinforcing that mild or absent changes likely reflect physiological tolerance rather than toxicity, especially in healthy models over extended periods.

The safety profile of AZG-HE on vital organs such as the kidney, liver, and heart are further supported by its strong antioxidant properties observed in this study. Administration of AZG-HE over 60 and 90 days resulted in significant increases in hepatic levels of GSH and GPx at both time points (60 and 90 days) across all doses. Additionally, delayed (90 days study) but noticeable increases in SOD and GSH levels were observed in heart and kidney tissues. Meanwhile, markers of oxidative damage, like malondialdehyde (MDA) remained stable or decreased, indicating effective reduction of lipid peroxidation.[53,54] These findings highlight AZG-HE ability to enhance natural antioxidant systems while reducing pro-oxidant activity, thus helping to protect organs during long-term exposure. Oxidative stress plays a crucial role in the development of organ problems, especially in the liver, heart, and kidney, where reactive oxygen species (ROS) can cause cellular damage through lipid peroxidation, protein damage, and DNA injury.[55,56] The observed increase in GSH (neutralizes ROS and helps regenerate other antioxidants) and GPx, which specifically breaks down hydrogen peroxide, supports a liver-protective mechanism that maintains redox balance.[57,58] Similarly, increased SOD activity in heart and kidney tissues indicates improved breakdown of superoxide radicals, a type of ROS involved in blood vessel and kidney cell dysfunction.[59] The stability or reduction of MDA and NO levels further suggests that AZG-HE does not induce pro-oxidant effects but instead decreases existing oxidative stress[60], potentially preventing hidden damage during prolonged use.
The absence of mortality, clinical signs of toxicity,

or significant organ dysfunction at doses up to 800 mg/kg over 90 days indicates a substantial safety margin for AZG-HE. Furthermore, the 15-day post-administration recovery phase revealed no evidence of delayed or persistent toxicity in any examined organ, with previously observed transient changes fully reversible and oxidative stress parameters normalized to control levels. These findings from an extended repeated-dose study align with OECD guidelines for sub-chronic oral toxicity testing (OECD 408) and provide robust preclinical evidence supporting the safety of *Azanza garckeana*. The results reinforce its long-standing traditional use across Africa as a safe edible and medicinal plant for managing dyslipidemia, oxidative stress-related conditions, and general health promotion, while highlighting its potential for further development as a therapeutic agent.

**Conclusion**

In summary, repeated oral doses of AZG-HE appear safe in Wistar rats under the tested conditions, with notable lipid-lowering and antioxidant effects that merit further research for therapeutic use, including in metabolic syndrome and heart health. Future investigations should include tissue examinations, longer-term toxicity studies, and clinical trials to better understand its safety and benefits in humans.

**REFERENCES**

1. Gadzama US, Muhammad MJ, Ali M. Assessment of nutritional value of Azanza garckeana (Goron Tula/Apple Snot) in Maiduguri Metropolis. Int. J. Converg. Inform. Sci. Res. 2024;6(9).123-128 https://doi.org/10.70382/hijcisr.v06i9.011
2. Mwove J. Potential for development of novel food products from Azanza garckeana tree fruit: a review. Int. J. Food Stud. 2021;10:SI121-32
3. Zakawa NN, Yusuf CS, Oyebanji EO, Timon D, Isa A, Bala U, et al. Anti-microbial activities and phytochemical screening of aqueous fruit pulp extracts of Azanza garckeana (F.Hoffm.). Niger. J. Bot. 2021;34(1). https://doi:/10.4314/njbot.v34i1.8.
4. Bioltif YE, Edward NB, Tyeng TD. A chemical overview of Azanza garckeana. Biol. Med. Nat. Prod. Chem. 2020;9(2):91-5. https://doi:10.14421/biomedich.2020.92.91-95.
5. Ochokwu IJ, Dasuki A, Oshoke JO. Azanza garckeana (Goron Tula) as an edible indigenous fruit in north eastern part of Nigeria. J. Biol. Agric. Healthc. 2015; 5(15):26-31.
6. Lawal B, Sani S, Onikanni AS, Ibrahim YO, Agboola AR, Lukman HY, et al. Preclinical anti-inflammatory and antioxidant effects of Azanza garckeana in STZ-induced glycemic-impaired rats, and pharmacoinformatics of its major phytoconstituents. Biomed. Pharmacother. 2022;152:113196. https://doi:10.1016/j.biopha.2022.113196.
7. Maroyi A. Azanza garckeana fruit tree: phytochemistry, pharmacology, nutritional and primary healthcare applications as herbal medicine: a review. Res. J. Med. Plants. 2017;11:115-123.
8. Laz-Okenwa JOA, Amah-Tariah FS, Ojeka SO. Changes in reproductive hormones levels following administration of hydroethanolic extract of Azanza garckeana fruit in cadmium chloride exposed female Wistar rats. Arch. Curr. Res. Int. 2024;24(5):280-7. https://doi:10.9734/acri/2024/v24i5703.
9. Michael KG, Onyia L, Jidauna SB. Evaluation of phytochemicals in Azanza garckeana (Gorontula) seed. IOSR J. Agric. Vet. Sci. 2015;10(8):71-4. https://doi:10.9790/2380-

08517174.
10. Nkwocha CC, Ogah FJ, Lomi OM, Ale BA. Phytochemical screening and GC-FID identification of bioactive compounds in n-hexane, ethylacetate and methanol fractions of methanolic leaves extract of Azanza garckeana. Food Chem. Adv. 2024;4:100712. https://doi:10.1016/j.focha.2024.100712.
11. Nechchadi H, Nadir Y, Benhssaine K, Alem C, Sellam K, Boulbaroud S, et al. Hypolipidemic activity of phytochemical combinations: a mechanistic review of preclinical and clinical studies. Food Chem. 2024;459:140264. https://doi:10.1016/j.foodchem.2024.140264.
12. Olorundare O, Adeneye A, Akinsola A, Kolo P, Agede O, Soyemi S, et al. Irvingia gabonensis seed extract: an effective attenuator of doxorubicin-mediated cardiotoxicity in Wistar rats. Oxid. Med. Cell. Longev. 2020;2020:1602816. https://doi:10.1155/2020/1602816.
13. Williams ET, Ambrose S, Timothy N. Assessment of nutritional composition of Azanza garckeana (Goron Tula) seed in Gombe, Nigeria. Int. J. Innov. Sci. Eng. Technol. 2020;7(11):213-222
14. Sirajo K, Shehu Z, Binanci MU, Mas'ud I, Diri AA. Study of proximate composition of seed and peel of (Azanza garckeana) goron tula. Asian J. Food Res. Nutr. 2022;1(2):76-81.
15. Japhet CP, Philip C, D CLuka, Ishaya YL. Exploring the antidiabetic potential of Azanza garckeana parts on streptozotocin induced diabetic rats. Asian J. Biochem. Genet. Mol. Biol. 2025;17(5):26-36. https://doi:10.9734/ajbgmb/2025/v17i5465.
16. Ibrahim P, Abdurahman EM, Bisallah M. Pharmacognostic evaluation of the leaves of Azanza garckeana (F.Hoffm.) Exell et Hillc. (Malvaceae). J. Pharm. Bioresources. 2019;16(2):125-32. https://doi:10.4314/jpb.v16i2.5.
17. Mshelia EH, Watirahyel EM, Maigari AU, Yohanna C, Ismail F. Cytotoxicity and antioxidant activity of stem bark extracts of Azanza garckeana (kola of Tula). Eur. J. Pure Appl. Chem. 2016;3(2):16-24
18. Hassan AI, Aminu AI. Evaluation of the Antimicrobial Activity and Toxicity of Local and Foreign Seeds of Azanza garckeana (Goron Tula) Extracts. UMYU J. Microbiol. Res. 2024;9(1):1-14. https://doi:10.47430/ujmr.2491.001.
19. Ibrahim M, Idoko AS, Ganiyu AI. Evaluation of the acute and sub-acute toxicity of Azanza garckeana aqueous leaves extract in Wistar rats. Niger. J. Biochem. Mol. Biol. 2024;39(1):8-15. https://doi:10.4314/njbmb.v39i1.2.
20. Jităreanu A, Trifan A, Vieriu M, Caba IC, Mâr u I, Agoroaei L. Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review. Processes. 2023;11(1):83. https://doi:10.3390/pr11010083.
21. Iloabuchi GC, Idoko AS, Ganiyu AI, Hannafi AM, Umar S. Effects of Azanza garckeana Fruit Pulp on Metabolic Syndrome in Wistar Rats Fed on High Fructose Diet. UMYU J. Microbiol. Res. 2024;9(1):55-64. https://doi:10.47430/ujmr.2491.006.
22. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016;31(8):984-991. https://doi:10.1093/heapol/czw022.
23. Agunbiade OS, Ojezele OM, Ojezele JO, Ajayi AY. Hypoglycaemic activity of Commelina africana and Ageratum conyzoides in relation to their mineral composition. Afr. Health Sci. 2012;12(2):198-203. https://doi:10.4314

/ahs.v12i2.19.
24. Olorundare OE, Adeneye AA, Akinsola AO, Sanni DA, Koketsu M, Mukhtar H. Clerodendrum volubile Ethanol Leaf Extract: A Potential Antidote to Doxorubicin-Induced Cardiotoxicity in Rats. J. Toxicol. 2020;2020:8859716. https://doi:10.1155/2020/8859716.
25. Okaiyeto K, Oguntibeju OO. African herbal medicines: adverse effects and cytotoxic potentials with different therapeutic applications. Int. J. Environ. Res. Public Health. 2021;18(11):5988. https://doi:10.3390/ijerph18115988.
26. Ahmad MH, Zezi AU, Anafi SB, Alshargi OY, Mohammed M, Mustapha S, et al. Sub-acute toxicity study on hydromethanolic leaves extract of Combretum hypopilinum (Combretaceae) Diels in Wistar rats. Toxicol. Res. 2022;38(4):487-502. https://doi:10.1007/s43188-022-00133-5.
27. Hoffman WP, Ness DK, van Lier RB. Analysis of rodent growth data in toxicology studies. Toxicol. Sci. 2002;66 (2):313-9. https://doi:10.1093/toxsci/66.2.313.
28. Flanagan EW, Spann R, Berry SE, Berthoud HR, Broyles S, Foster GD, et al. New insights in the mechanisms of weight-loss maintenance: summary from a Pennington symposium. Obesity (Silver Spring). 2023;31(12):2895-2908. https://doi:10.1002/oby.23905.
29. Tucci SA. Phytochemicals in the control of human appetite and body weight. Pharmaceuticals (Basel). 2010;3(3):748-763. https://doi:10.3390/ph3030748.
30. Cosme F, Aires A, Pinto T, Oliveira I, Vilela A, Gonçalves B. A comprehensive review of bioactive tannins in foods and beverages: functional properties, health benefits, and sensory qualities. Molecules. 2025;30(4):800. https://doi:10.3390/molecules30040800.
31. Mohammed AH, Mshelia EH, Watirahyel EM, Christopher Y. Phytochemicals Screening and Antimicrobial Activity of the Stem Bark Extracts of Azanza garckeana (Kola of Tula). Sch. Int. J. Tradit. Complement. Med. 2021;4(6):122-127. https://doi:10.36348/sijtcm.2021.v04i06.010.
32. Ushie OA, Iyen IS, Aasegh TJ, Ama SO, Aikhoje EF, Shamaki HA. Phytochemical screening and antimicrobial activities of hexane extract of Azanza garckeana. Res. J. Pharm. Chem. Biol. Sci. 2022;3(2):7-12. https://doi:10.5281/zenodo.6667091.
33. Cui MT, Liang ZW, Sun YZ, Wu J, Lu H, Wang WJ, et al. The prognostic roles of red blood cell-associated indicators in patients with resectable gastric cancers. Transl. Cancer Res. 2020;9(4):2300-2311. https://doi:10.21037/tcr.2020.03.46.
34. Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F. Recognition of adverse and non-adverse effects in toxicity studies. Toxicol. Pathol. 2002;30(1):66-74. https://doi:10.1080/01926230252824725.
35. Baldrick P, Cosenza ME, Alapatt T, Bolon B, Rhodes M, Waterson I. Toxicology paradise: sorting out adverse and non-adverse findings in animal toxicity studies. Int. J. Toxicol. 2020;39(5):365-378. https://doi:10.1177/1091581820935089.
36. Ugwu GN, Ugorji CO, et al. Phytoconstituents, acute toxicity and the effect of methanol extract of Azanza garckeana (Malvaceae) on the haematological indices of adult Wistar rats. Afr. J. Pharm. Res. Dev. 2024;16(3):117-129. https://doi:10.59493/ajopred/2024.3.13.
37. Kanta E, Musalwa-Muyangwa S, Rehana O, Prashar L. Acute and sub-acute toxicity of methanol root extract of Azanza garckeana (Malvaceae) in albino rats. Med. J. Zambia.

2025;52(4):497-510.
38. Hall P, Cash J. What is the real function of the liver 'function' tests? Ulster Med. J. 2012;81(1):30-6.
39. Afolabi SO, Olorundare O, Akanbi OB, Bello MK, Njan A, Agede OA, et al. GC/MS analysis and a 30-day toxicological evaluation of a Nigerian immunomodulatory polyherbal supplement (PHS). Pharmacol. Toxicol. Nat. Med. 2023;3(2):1-14. https://doi:10.52406/ptnm.v3i2.70.
40. Umana IK, Tochuckwu TA, Ehigiator BE, Ikem CJ, Udom GJ, Akinsola AO. Toxicity and antioxidant assessment of ethanol leaf extract of Hibiscus surattensis in Wistar rats: hepatotoxicity evaluation. Afr. J. Pharm. Res. Dev. 2024;16(3):25-31.
41. Alamri ZZ. The role of liver in metabolism: an updated review with physiological emphasis. Int. J. Basic Clin. Pharmacol. 2018;7(11):2271-2276. https://doi:10.18203/2319-2003.ijbcp20184211.
42. Vaja R, Rana M. Drugs and the liver. Anaesth. Intensive Care Med. 2020;21(10):517-523. https://doi:10.1016/j.mpaic.2020.07.001.
43. Weber S, Allgeier J, Denk G, Gerbes AL. Marked increase of gamma-glutamyltransferase as an indicator of drug-induced liver injury in patients without conventional diagnostic criteria of acute liver injury. Visc. Med. 2022;38(3):223-8. https://doi:10.1159/000519752.
44. Salazar JH. Overview of urea and creatinine. Lab. Med. 2014;45(1):19-20. https://doi:10.1309/LM920SBNZPJRJGUT.
45. Xu X, Zeng T, Chen S, Tian N, Zhang C, Chen Y, et al. Acute kidney injury: pathogenesis and therapeutic interventions. Mol. Biomed. 2025;6(1):61. https://doi:10.1186/s43556-025-00293-4.
46. Lin W. Clinical significance of serum creatinine, urea nitrogen and uric acid levels in patients with chronic renal failure. Int. J. Biol. Life Sci. 2023;3(3):19-26. https://doi:10.54097/ijbls.v3i3.04.
47. Madhavan H, Nobel MM, Rajalakshmi M, Kulkarni S, Thomas TRA, Shalini B, et al. Sociodemographic features, serum urea, creatinine and blood urea nitrogen/creatinine ratio in chronic kidney disease patients – a record based retrospective study. Glob. J. Health Sci. Res. 2024;2:82-5. https://doi:10.25259/GJHSR_8_2024.
48. Shrimanker I, Bhattarai S. Electrolytes. Treasure Island (FL): StatPearls Publishing; 2025.
49. Nurudeen QO, Asinmi MR, Falana MB, Dikwa MA, Yusuf ZM, Lambe MO. Antioxidant activity and toxicological implications of the aqueous extract of Azanza garckeana fruit pulp in female Wistar rats. Niger. J. Biochem. Mol. Biol. 2024;39(1):1-7.
50. Yusuf AA, Mohammed BA, et al. Alterations in serum urea, creatinine and electrolytes concentrations in Wistar rats following repeated administration of methanol extracts of Azanza garckeana pulp. Sch. Int. J. Biochem. 2020;3(6):127-131. https://doi:10.36348/sijb.2020.v03i06.002.
51. Alozieuwa UB, Lawal D, Sani S, Onikanni AS, Osuji O, Ibrahim YO, et al. Luteolin-rich extract of Thespesia garckeana (snot apple) contains potential drug-like candidates and modulates glycemic and oxidoinflammatory aberrations in experimental animals. Oxid. Med. Cell. Longev. 2022;2022:1215097. https://doi:10.1155/2022/1215097.
52. Lawal B, Kuo YC, et al. Computational identification of novel signature of T2DM-induced nephropathy and therapeutic bioactive compounds from Azanza garckeana. Am. J. Transl. Res. 2023;15 (7): 4504-4520

53. Ojezele OJ, Ojezele MO, Adeosun AM. Comparative phytochemistry and antioxidant activities of water and ethanol extract of Annona muricata Linn leaf, seed and fruit. Adv. Biol. Res. 2016;10(4):230-5. https://doi:10.5829/idosi.abr.2016.10.4.10514.
54. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 2018;54(4):287-93. https://doi:10.1016/j.ajme.2017.09.001.
55. Ozbek E. Induction of oxidative stress in kidney. Int. J. Nephrol. 2012;2012:465897. https://doi:10.1155/2012/465897.
56. Chandimali N, Bak SG, Park EH, Lim HJ, Won YS, Kim EK, et al. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discov. 2025;11:19. https://doi:10.1038/s41420-024-02278-8.
57. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative stress in liver pathophysiology and disease. Antioxidants (Basel). 2023;12(9):1653. https://doi:10.3390/antiox12091653.
58. Manful CF, Fordjour E, Subramaniam D, Sey AA, Abbey L, Thomas R. Antioxidants and reactive oxygen species: shaping human health and disease outcomes. Int. J. Mol. Sci. 2025;26(15):7520. https://doi:10.3390/ijms26157520.
59. Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics. Antioxidants (Basel). 2023;12(9):1675. https://doi:10.3390/antiox12091675.
60. Maurya RP, Prajapat MK, Singh VP, Roy M, Todi R, Bosak S, et al. Serum malondialdehyde as a biomarker of oxidative stress in patients with primary ocular carcinoma: impact on response to chemotherapy. Clin. Ophthalmol. 2021; 15:871-9. https://doi:10.2147/OPTH.S287747.

Akinsola AO, Ojezele MO, Eduviere AT and Akpovwre CO. Subchronic toxicity of Hydroethanolic extract of Azanza garckeana fruit in Wistar Rats. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 29-76
https://dx.doi.org/10.4314/ajtmbr.v9i1.3

# Beliefs And Perceptions Of Hypertensive Patients Regarding Socio-cultural factors On Blood Pressure Control In Two Rural Communities Of Ekiti State: An Exploratory Qualitative Study

[1] Ibrahim AO, [2]Udonwa N, [3]Oku AO, [4]Archibong F, [5]Adewoye KR, [6]Musbau OO, [7]Oguntoye OA, [8]Alabi A K, [9]bello IS, [10]Ajani GO, [11] Erinomo O, [12] Afolayan OF

**ABSTRACT**

**Introduction**: Achieving optimal blood pressure control remains a challenge, especially in rural setting and could be due to sociocultural factors influencing patient's beliefs and biomedical understanding of blood pressure control. Hence, this study explored hypertensive patient's beliefs and perceptions regarding blood pressure control in two rural communities of Ekiti State, Southwest Nigeria.

**Materials and Methods:** This cross-sectional qualitative study explored four focus group discussions with open ended questions on 16 hypertensive patients who were purposively recruited. All the conversations were audio-recorded, transcribed verbatim and thematically analyzed.

**Results:** The only theme was participants' perceptions of sociocultural factors influencing their blood pressure and five sub-themes including access to healthcare services, health literacy, dietary and lifestyle behaviors, medication adherence, family and social support. Majority of participants claimed they have unlimited access to healthcare facility, could read the information on hypertension, and were aware of dietary food for optimal blood pressure but cost, long waiting time, unavailability of drugs at healthcare facility, lack of information and certain cultural practices and traditional food during social events were barriers to achieving optimal blood pressure.

**Conclusion**: Misconceptions of certain sociocultural factors were influencing management of hypertension in rural communities of Ekiti State. Therefore, it is important to institute measures to overcome these misconceptions and barriers and facilitate consistent adherence to biomedical treatment to improve blood pressure control.

**Key words**: Qualitative research, Perceptions, Focus Groups, High Blood Pressure, Rural Nigeria

[1]*Department of Family Medicine, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria.*
[2]*Department of Family Medicine, University of Calabar, Cross River State, Nigeria.*
[3]*Department of Community Medicine, University of Calabar, Cross River State, Nigeria.*
[4]*Department of Family Medicine, University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria.*
[5]*Department of Community Medicine, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria.*
[6]*Department of Community Medicine, Federal University, Oye-Ekiti, Nigeria.*
[7]*Department of Medicine, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria.*
[8]*Department of Community Medicine, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria.*
[9]*Department of Family Medicine, Obafemi Awolowo University Teaching Hospital Complex, Ilesa Road , Ile-Ife , Osun state, Nigeria.*
[10]*Department of Medicine, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.*
[11]*Department of Pathology, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*
[12]*Department of Family Medicine, Federal Medical Center, Ikole Ekiti, Ekiti State, Nigeria.*

**Corresponding author:** Azeez Oyemomi Ibrahim, Department of Family Medicine, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria. Email: ibrahimao@abuad.edu.ng.

## INTRODUCTION

Hypertension or high blood (BP) pressure is the most prevalent and modifiable risk factors for cardiovascular diseases such as strokes and heart attacks.[1,2] It accounts for a global population of one billion and predicted to rise to 1.56 billion by 2025.[1,3] Previous reports suggest that over the past decade, the prevalence of hypertension has increased globally with higher burden recorded in low- and middle income countries.[2,4] In Nigeria, a recent study estimated the crude prevalence of hypertension to be between 2.1 to 47.2% depending on the BP cut-off point, setting, sex and ethnic group being assessed.[5] However, despite the increased advancement in hypertension diagnosis and availability of antihypertensive medications, achieving optimal BP remains a challenge, especially in rural setting.

A possible reason for this could be difference between patient's beliefs and biomedical understanding of sociocultural factors contributing to BP control.[6] Kleinman in his lay explanatory model proposes that people's understanding of their illness often differs from biomedical understanding of diseases and these differences could influence treatment outcomes.[7] Patients' perceptions of blood pressure control have been different due to differences in sociocultural factors such access to healthcare, low health literacy, [8] unhealthy dietary and lifestyle behaviors,[4] poor medication adherence,[9] and low availability of social support.[10]

Health literacy helps patient to obtain the necessary information about high BP, and empowering them to make a better decision on best way to manage their BP [11, 12]Improving health literacy is one of the most cost- effective interventions to reduce the incidence of non-communicable diseases.[13]

Previous studies on perceptions of hypertensive patients towards healthy dietary consumptions to improve BP control have recently been documented.[4, 14] A recent study targeting dietary changes for BP control has focused on providing advice on diet that is usually not affordable or not acceptable by the rural dwellers.[15]

Medication adherence is another important component of BP control.[16] However, perception of side effects of medications often lead to poor adherence resulting in poor BP control.[6]

In addition to perceptions of medication usage for BP control, family and social support has also been identified as an important sociocultural factor in BP management.[9, 10] Strong family and social networks and support systems can encourage medication adherence, promote healthy lifestyle behaviors, and provide emotional and practical assistance to individuals with hypertension.[9,10,17]

Previous studies on sociocultural factors influencing BP control in sub-Sahara Africa (SSA) are either quantitative approach or conducted in urban settings. To the best of our knowledge, this is the first qualitative study on hypertensive patients' beliefs and perceptions regarding sociocultural factors on BP control in rural communities of Ekiti State. Given the high burden of hypertension, low rate of BP control in SSA, this study is crucial to provide targeted interventions that could influence individuals' actions towards their BP control.

## MATERIALS AND METHODS

**Study area**: The study areas were Ilogbo and Igbole rural communities which are located in Ido-Osi local government area of the Northern Senatorial district of Ekiti State, Nigeria.[18] Igbole and Ilogbo rural communities were purposively selected because of their rich cultural and socio-

economic characteristics.

**Study population**: This was the population of individuals diagnosed with hypertension who were permanent resident of Ilogbo and Igbole rural communities.

**Study design:** The study involved a qualitative approach using an explanatory model design. The explanatory model was Kleinman's Explanatory Model.[11]

**Selection criteria:** Participants for this study were recruited from November 2024 to February 2025. Inclusion criteria were patients diagnosed with hypertension, aged 18 years and above, who were permanent resident of the communities, and being on antihypertensive medication for at least six months. Exclusion criteria were patients with secondary hypertension, with other co-morbidities, pregnant and lactating women and those with known glaucoma on treatment.

**Instrument and procedure for qualitative data collection**

A semi structured interview and Focus Group Discussion (FGDs) were the research instrument while the principal investigator served as the interviewer and coordinator of the discussion. A trained research assistant served as recorder using a tape recorder, note pads, and a camera.[19]

**Ethical Consideration**:

Ethical approval was obtained from the Institution Review Board of Federal Teaching Hospital Ido-Ekiti with approval number (ERC/2024/10/30/1207A) before the study was conducted.

Permissions for the study was obtained from the traditional leaders of the autonomous communities.

**Operational definition**

**Focus group discussions (FGDs)**: This is a valuable tool used to generate insights into people's experiences, attitudes, perceptions, values, and opinions about a defined area of interest.

**Selection of participants, validation and reliability of Research Instrument and procedure for data collection:**

Prior to the study, the records of hypertensive patients attending the comprehensive health center (CHC) in the two rural communities were assessed and 18 participants identified with uncontrolled blood pressure were selected using a purposive sampling technique and were invited to participate in the survey after their consent has been taken.[15] In order to achieve the objectives of FGD, the content of semi-structured interview was developed based on the English literatures which have highlighted the global hypertension management.[9, 21, 22] The questionnaires were developed in English and translated into Yoruba and later backward translation to English language by a competent translator to ensure quality control. The questions were prepared in an opened ended format and were pre-tested for content validity, face validity, and clarity by two cardiologists and one community health physician with vast experience in hypertension management research and were further adjusted after pilot study with six hypertensive patients in another rural community who were not part of the study target population. Initially, 18 participants were invited to participate in the FGD, but two participants did not attend due to their time constraint. Eventually a total of 16 participants attended which were divided into four groups (with two groups each from Ilogbo and Igbole communities respectively) which followed the standard guideline.[23] A FGD of 4-8 people are usually recommended as group more than eight participants are difficult to control.[6, 9, 22]

FGDs were grouped by sex because previous research suggests that due to the socio-cultural context, women may not actively participate in discussions in the presence of men.[6,9,24]

Prior to the FGD, participants were told to be seated for at least five minutes before their BP were being measured. A BP monitoring apparatus (Brand: Omron® with model HEM-7080) was used to monitor participants' BP level. The participants were enjoyed to interact freely with each other, exchange ideas based on their experiences, and consider no individual contribution as right or wrong.[6] Information sheet was explained and informed written, thumb-printed and verbal consent was obtained from each participant. The researcher assumed the role of moderator and was assisted by local community extension officer. Prior to the FGD, moderator introduced himself, his background and qualification. He then briefed the participants about the general objectives of the study. He encouraged them to freely participate in the discussion by giving several probes and the discussion was conducted in their local language. The conversations and discussions were audio-recorded and field notes were utilized when necessary during FGD. Four FGDs were conducted until saturation of the content which ranged from one hour to one hour and 30 minutes. When there were no new theme identified, a conclusion was made at this saturation point. The study was in accordance with strengthening the COnsolidated criteria for REporting Qualitative research (COREQ).[25]

**Data Analysis:**

After the FGDs, all the conversations and interviews were transcribed verbatim from Local Language directly to English language by two appointed translators from the local communities while listening to the audio recording played by the research assistant in the presence of the researcher who also counterchecked the data for quality. Because some of the participants could not read, write, or speak English, transcripts were not returned to the participants for cross-checking. Using thematic approach to data analysis, textual data were explored and read several times by the researcher to identify themes and sub-themes (categories). The coding framework generated by the researcher involved initial pre-identified codes derived from previous studies on hypertension in Ghana.[6, 9] Open ended inductive codes were generated based on socio-cultural context of the study areas.[6,9] Codes were reviewed and active data coding of transcripts begins. Checking was done by the researcher where the transcription was compared to ensure correctness and the text data were coded thematically and analyzed deductively and inductively using Nvivo* version 12 pro which is a qualitative software for data storage, coding, and theme development. The researcher analysed all transcripts in constant comparison in QRS NVivo 12 pro.

**RESULTS**

In this study, 16 hypertensive patients were recruited. The age of participants ranged from 21-72 years, and most of the participants were traders (31.7%) and 6.3% had no formal education and majority of the participants were Christians (75.0%). In terms of their blood pressure, only one- quarter (25.0%) of the participants had their BP control. The demographics and clinical findings of the participants are shown in Table 1.

**Table 1: The demographics and clinical findings of the participants**

| Variables | Frequency | Percentage |
|---|---|---|
| **Age in years** | | |
| 21-39 | 3 | 18.8 |
| 40-59 | 8 | 50.0 |
| 60 & above | 5 | 31.2 |
| **Gender** | | |
| Male | 8 | 50.0 |
| Female | 8 | 50.0 |
| **Occupation** | | |
| Farmer | 4 | 25.0 |
| Trader | 5 | 31.3 |
| Artisan | 3 | 18.7 |
| Civil Servant | 2 | 12.5 |
| Housewife/unemployed | 2 | 12.5 |
| **Education** | | |
| None | 1 | 6.3 |
| Primary | 5 | 31.2 |
| Secondary | 9 | 56.2 |
| Tertiary | 1 | 6.3 |
| **Religion** | | |
| Christianity | 10 | 62.5 |
| Islam | 4 | 25.0 |
| Traditional | 2 | 12.5 |
| Blood Pressure Level | | |
| Controlled BP | 6 | 37.5% |
| Uncontrolled BP | 10 | 62.5% |

One theme was developed via the thematic content analysis namely, sociocultural factors influencing BP control and five subthemes as shown in Table 2.

**Theme and sub-themes of sociocultural factors influencing BP control**
**Theme: Sociocultural beliefs and practices influencing BP control.**
Five subthemes were identified which were; (1) accessibility, affordability, and availability of hypertension care services, (2) Health literacy, (3) diets and lifestyle behaviors, (4) medication adherence and (5) family support.

**Table 2: Sociocultural factors influencing blood pressure control**

| Theme: | Sub-themes: | Categories/quotes: |
|---|---|---|
| Sociocultural factors influencing blood pressure control | 2.1: Accessibility and affordability of healthcare services | 2.1: "My house is not too far from government facility, so, I visit government facility anytime I want" (n=14)<br>"My house is very far from government facilities but I have a motorcycle that I used to ride whenever I want to go and treat myself" (n=3)<br>"My problem is not about going to government facilities but the cost of drugs is too expensive for me" (n=17)<br>"At times, I will get to government facility and the nurse will tell me that the type of drugs that is good for my BP has finished" (n=5)<br>"The last time I went to the government facility, the y told me that doctor was not around" (n=4)<br>"I prefer going to Pharmacy shop close to my house because pharmacists are drug expert" (n=11) |
| | 2.2: Participants' health literacy | 2.2: "Emm…. For me, I have read the flyer pasted on the clinic wall before, but I couldn't understand the content written there" (n=10)<br>"Am a pastor, I can read, write, and speak English very well"(n=18)<br>"Emm…We are Ekitis, the Fountain of knowledge" (with chorus "yesoooo" and "claps" from majority of participants).n=6) |
| | 2.3: Diets and Lifestyle behaviors/practices | 2.3: "I have since stopped adding salt to m y food for over a year now because doctor told me that it is risk for my life" (n=4)<br>"Emm… for me I still use salt but very small" (n=2)<br>"Why would I not add salt to my food? food without salt is tasteless" (n=2)<br>"I have taken alcohol before but since doct or told me that my BP was high, have been running away from alcohol" (n=5)<br>"Emm… for me, I take alcohol but not much to intoxicate me" (n=1). One man nodded his head in agreement.<br>"I was told in the clinic that I should take bitter kola instead of cigarette smoking" (n=1)<br>"Hmm…doctor (referring to the moderator) you live in city. Car takes you to all places" (n=1)<br>Another participant chipped in" from air conditional car to air conditional office and air conditional room" there was laughter.<br>"Going up and do wn in the house, trekking to farm, church and market in this town is even more than any exercise" (n=2).<br>"Yeah, I do not need any other exercise" (n=12) |

| Theme: | Sub-themes: | Categories/quotes: |
|---|---|---|
| | 2.4: Medication adherence | "I go with my drugs anywhere I travel to" (n=5)<br>"I take my drugs everyday" (n=12)<br>"Em.. as for me, I didn't like taking the drug every day. So, at times, i missed them for 2-3 days" (n=4)<br>"I stopped taken the drug when my BP is normal" (n=3)<br>"I skipped taking the drug when I have no money to buy them" (n=2)<br>"Am bored of taking too much medications every day" (n=3)<br>"I stopped taken the drugs because it affects my sexual performance" laughter (n=1)<br>"I only take the drug when I felt uncomfortable with serious headache or poor sleep" (n=1) |
| | **2.5:** Family and Social Support | "I didn't enjoy the support of my family in the management of my high" ( n=6)<br>"Anytime I asked my brother to send me money for my BP, he would say that I should stop thinking too much (laughter)" (n=2)<br>"My husband is really supporting me. He provides the money and would not allow me to sleep at night until I take my dr ugs" (n=2)<br><br>"I don't know what our government is doing for us? Please doctor, help us tell our governor to subsidize the cost of these drugs for us" (n=6) |

## DISCUSSION

This qualitative study provides in-depth insights on participants' perceptions regarding BP and it explores the sociocultural beliefs and practices influencing BP control in two rural communities of Ekiti State, Nigeria, a relative grey area of research. This study is the first of its kind in Ekiti State, Southwest Nigeria, highlighting the sociocultural factors influencing BP control.

In this study, despite participants' recognition of easy access to health care facility, barriers such as cost, unavailable of medications and long waiting time due to inadequate physicians were the contributing socio-economic factors to achieving better BP control. This findings was consistent with previous study where financial constraint is the major reason for not adherence to antihypertensive medications.[26]

The health literacy of majority of hypertensive patients in this study is inadequate, making opinions on the information regarding high BP to be low. This is consistent with recent research in Indonesia which reported that health literacy is not limited to measuring patient skill in reading health-related words or numbers, but instead focuses more on how written materials can changes patients' beliefs and actions, and how patients understand medical instruction.[8] Inadequate health literacy which was explored in

this study can cause patients to be misinformed and to have difficulty following medical instructions, and is associated with BP control. However, despite the inadequate health literacy explored in this study, eagerness for controlling BP was portrayed through the concerns raised by hypertensive patients as they perceived high BP as a dangerous and fast killer. This finding is consistent with the study by Mgbahurike *et al*,[27] where participants in agreed that hypertension is a serious health concern.[27] Tailored intervention strategies to improve understanding of health information relating to BP control is advocated in rural setting to improving their health literacy.

Furthermore, with regards to lifestyle practices of the participants, proven biomedical risk factors such as smoking, alcohol consumption, and inadequate exercise were believed by the participants as contributing to uncontrolled BP. This is comparable with several other studies in Nigeria.[4, 5, 28] Also, other studies in SSA were in agreement with finding in this study.[4,6,9] The idea that -household and workplace chores are equally regarded as adequate exercise has not been reported in other studies and may give false impression to the patients that they are compliance with recommended activities leading to poor BP control. Dietary approaches to stop hypertension (DASH) is a globally recommended strategy for patients with high BP which has been implemented for over three decades.[28] The DASH approach emphasized varieties of fruits and vegetables, however, the daily diet consumed by the participants in this study indicates lack of different types of food in their local market. This barrier of availability of variety of fruits and vegetables highlights the significant of considering the local market context when designing culturally acceptable dietary interventions for the control of high BP in rural areas. Charlton and colleagues in their study posited that sometimes the DASH diet is not feasible and sustainable to people living in rural settings due to high poverty rate and poor knowledge of complications of uncontrolled BP.[29] They recommend that in order to control BP in the rural area, people may not need to change what they eat, but instead be making small adjustments to current daily salt intake.[29] This recommendation is important to adopt as it has proven to be effective in other similar settings.[4]

With regards to medication adherence, a significant numbers of participants were not adherence to their medications and this is not unique to this study. Non adherence to antihypertensive medications has been the most important contribution to poor BP control particularly in rural setting and has been reported by several studies.[4, 6] While several reasons may contribute to poor adherence to antihypertensive medications, the reasons explore in this study include high cost of drugs and taken too many drugs on daily basis, which are similar to findings of other studies on hypertension and other chronic diseases.[6,9] Another barrier to medication adherence and subsequently poor BP control was the fear of sexual dysfunction as experienced by few participants in this study. This finding is in line with previous studies by Tan et al, [9] and Kretchy et al, [30] which have reported side effects of antihypertensive medications as contributing to poor BP control. [9, 30] Health interventions therefore need to take these perceptions into consideration in designing appropriate and culturally sustained methods of addressing these challenges.

Furthermore, it appears that while many participants in this study recognized the role of family and social support to improving their compliance to medication adherence and subsequently BP control, there are sociocultural and economic barriers that impede family and

other social support groups' inability to adequately support people with high BP. Barriers explored in this study include failure of participants to disclose their diagnosis to their relations, perceptions of family members on the etiology of hypertension, poverty within family members and lack of support from government. Studies have shown that social support contributes significantly to improving health outcomes.[6, 10] Therefore, targeted interventions involving stakeholders from communities and government circles are needed to promote family and social support for patients with high BP.

## CONCLUSIONS

It is evident that hypertensive patients experienced several sociocultural factors and barriers that influence their management of BP in the study areas. These socio-cultural barriers often delayed or discouraged the use of biomedical treatments which are essential in ensuring adequate control of BP. It is important to institute measures to overcome these barriers and facilitate consistent adherence to biomedical treatment and lifestyle behaviors especially in rural areas to improve rate of BP control.

### Limitations:

The dominant voice from the vocal participants would override other voices in the method of FGDs.[30] Also, limiting the FGD size to four participants at a time would reduce the diverge range of information compared to when there is increased numbers of participants in the FGDs.

## REFERENCES


1. World Health Organization: Global action plan for the prevention and control of non-communicable diseases 2013-2020. WHO; 2013
2. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020; 16(4): 223-237. https://doi.org/10.1038/s41581-019-0244-2.
3. Egan BM. Adherence in Hypertension. Circ Res. 2019; 124 (7):1124-1140. https://doi:10.1161/CIRCRESAHA.118.313220.
4. Sekome K, Gsomez-Olive FX, Sherar LB, Eslger D, Myezwa H. Sociocultural perceptions of physical activity and dietary habits for hypertension control: voices from adults in a rural sub-district of South Africa. BMC Public Health.2024; 24:2194. https://doi.org/10.1186/s12889-024-19320-0.
5. Akinlua JT, Meakin R, Bashir I, Freemantle N. Beliefs about hypertension among primary health care workers and clients in Nigeria: A qualitative study. PLoS ONE.2018; 13(12):e0209334. https://doi.org/10.1371/journal.pone.0209334.
6. Nyaaba GN, Masana L, Aikins Ama de-Graft, Stronks K, Agyermang C. Lay community perceptions and treatment options for hypertension in rural northern Ghana: a qualitative analysis. BMJ Open. 2018; 8(11):e023451. https://doi.org/10.1136/bmjopen-2018-023451.
7. Kleinman A. Patients and healers in the context of culture: An exploration of the borderland between anthropology, medicine, and psychiatry. 3rd ed. Berkeley: University of California Press; 1980.
8. Rahmawati R, Sastiarini JA, Hakim AF. Health Literacy Among Patients With Stage 2 Hypertension: A Survey in Rural Yogyakarta, Indonesia. Advances in Health Sciences Research. 2020; 3: 223-227
9. Tan CS, Hassali MA, Nech CF, Saleem F. A qualitative exploration of hypertensive patients' perception towards quality use of medication and hypertension management at the community level. Pharm Pract 2017; 15 (4): 1074. https://doi.org/10.18549

/Pharmpract.2017.04.1074.
10. Oseni TIA, Emonriken A, Ahmad SD, Dic-Ijiwere M. Determinants of blood pressure control among hypertensive patients attending a rural Teaching Hospital in Southern Nigeria. Niger J Clin Pract.2023;26 (3):260-266. https://doi.org/10.4103/njcp_1678_21.
11. Isa DM, Shahar S, He FJ, Majid HA. Associations of health literacy with blood pressure and dietary salt intake among adults: A systematic Review. Nutrients.2021; 13(12):4534. https://doi.org/10.3390/nu13124534.
12. Yilmazel G, Cetinkaya F. Relationship between health literacy levels, hypertension awareness and control among primary-secondary school teachers in Turkey. AIMS Public Health. 2017; 4 (4):314-325. https://doi.org/10.3934/publichealth.2017.4.314.
13. Du S, Zhou Y, Fu C, Wang Y, Du X, Xie R. Health Literacy and Health outcomes in Hypertension: An Integrative Review. Int. J. Nurs. Sci. 2018; 5(3):301-309. https://doi.org/10.1016/j.ijnss.2018.06.001.
14. Lloyd-Sherlock P, Gomez-Olive FX, Ngobeni S, Wagner RG,Tollman S. Pensions and low sodium salt: a qualitative evaluation of a new for managing hypertension in rural South Africa. Curr Aging Sci.2018; 11(2):140-146. https://doi.org/10.2174/1874609811666180718114250.
15 Temple NJ, Steyn NP, Fourie J, De Villiers A. Price and availability of healthy food: a study in rural South Africa. Nutrition.2011; 27 (1):55-8. https://doi.org/10.1016/j.nut.2009.12.004.
16. Vrijens B, DeGeest S, Hughes DA, Przemyslaw K, Demonceaus J. A netaxonomy for describing and defining adherence to medications. Br J Clin Pharmacol.2012; 73 (5):691-705. https://doi.org/10.1111/j.1365-2125. 2012.04167.x.
17. Ojo OS, Malomo SO, Sogunle PT. Blood pressure control and perceived family support in patients with essential hypertension seen at a primary care clinic in Western Nigeria. J Fam Med Prim Care.2016; 5(3):569-575. https://doi.org/10.4103/2249-4863.197284.
18. Ekiti State Government-About Ido-Osi. Available from: ekitistate.gov.ng. Accessed April 20, 2025.
19. Ahmed A.K. Ojo, OY, Ahmed A., Akande TM, Osagbemi GK. A Comparative Study of Predictors of Health Service Utilization Among Rural And Urban Areas In Ilorin East Local Government Area of Kwara State. BUMJ. 2021;4(2):120-132. https://doi.org/10.38029/bumj.v4i.88.
20. Teshome DF, Balcha SA, Ayele TA, Atnafu A, Sisay M, Asfaw MG, Mitike G, Gelaye KA. High burden of hypertension amongst adult population in rural districts of Northwest Ethiopia: A call for Community based intervention. PLOS ONE. 2022; 17 (10): e0275830. https://doi.org/10.1371/journal.pone.0275830.
21. Jena S, Sahoo KC,Samal M. Rural community attitude towards mental healthcare: a mixed –method study in Khurda district of Odisha, India. Middle East Curr Psychiatry. 2020; 27:48. https://doi.org/10.1186/s43045-020-00057-6.
22. Jolles EP, Padwal RS, Clark AM, Braam B. A qualitative study of patient perspectives about hypertension. ISRN Hypertension. 2013; 2013(1):671691. https://doi.org/10.5402/2013/671691.
23. Dawson S, Manderson L, Tallo VL. A manual for the use of focus groups. 1st ed. Boston: international Nutrition foundation for developing countries; 1993.
24. Asobayire A, Barley R. Women's cultural perceptions and attitudes towards breast

cancer: Northern Ghana. Health Promot Int. 2015; 30 (3): 647-57. https://doi.org/10.1093/heapro/dat087.

25. Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): A 32-item checklist for interviews and focus groups. Int J Qual Health Care.2007; 6: 349-57. https://doi:10.1093/intqhc/mzm042.
26. Adeyemo A, Tayo BO, Luke A, Ogedegbe O, Durazo-Arvizu R, Cooper RS. The Nigeria Anti-hypertensive Adherence Trial (NAHAT): a community-based randomized trial. J Hypertens. 2013; 31(1):201-207. https://doi.org/10.1097/HJH.0b013e32835b0842.
27. Mgbahurike AA, Lelesi TN. Knowledge, attitude and perception of hypertension among staff of a tertiary institution in Nigeria. Univ J Pharm Res. 2022; 7: 1. https://doi.org/10.22270/ujpr.v7i1.719.
28. Steinberg D, Bennett GG, Svetkey L. The DASH diet, 20 years later. JAMA. 2017; 317 (15): 1529-30. https://doi.org/10.1001/jama.2017.1628.
29. Charlton KE, Steyn K, Levitt NS, Peer N, Jonathan D, Gogela T, et al. A food based dietary strategy lowers blood pressure in a low socio-economic setting: a randomized study in South Africa. Public Health Nutr. 2008; 11 (12): 1397-40. https://doi.org/10.1017/S136898000800342X.
30. Kretchy IA, Owusu-Daaku FT, Danquah SA,Asampong E. A psychosocial perspective of medication side-effects, experiences, coping approaches and implications for adherence in hypertension management. Clin Hypertens. 2015; 21:19. https://doi.org/10.1186/s40885-015-0028-3.

Ibrahim AO, Udonwa N, Oku AO, Archibong F, Adewoye KR, Musbau OO, Oguntoye OA, Alabi AK, Bello IS, Ajani GO, Erinomo O, Afolayan OF. Beliefs And Perceptions Of Hypertensive Patients Regarding Socio-cultural Factors On Blood Pressure Control In Two Rural Communities Of Ekiti State: An Exploratory Qualitative Study. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 77-87
https://dx.doi.org/10.4314/ajtmbr.v9i1.4

# Prevalence, Perception And Management Practices of Dysmenorrhea Among Students of Delta State University, Abraka, South-south Nigeria

Omogbiya IA[1],Orovwigho O[1], Moke EG[1], Demaki W[1], Oboh M[1,2], Emudainohwo JOT[1], Okoro OE[3], Jewo AO[4], Chidebe E[1], Otah OJ[1]

**Abstract**

**Introduction:** Dysmenorrhea is a common gynaecological disorder among women of reproductive age worldwide. Undergraduate students may be particularly affected, as dysmenorrhea may negatively affect academic engagement and quality of life. This study assessed the prevalence and management of dysmenorrhea among DELSU undergraduates, and explored its self-reported association with examination stress and academic performance.

**Materials and Methods:** A descriptive cross-sectional survey design was conducted among 400 female undergraduate students recruited from all faculties of DELSU using a convenience sampling technique. Data were collected using a structured questionnaire and summarised using frequency/percentage distribution and Spearman rho to determine association with academic performance. Statistical significance was set at $p<0.05$.

**Results:** The prevalence rate of dysmenorrhea was 78.9%. Onset occurred at menarche in 53.6%, and 54.9% reported a family history. Lower abdomen was the commonest pain site (67.2%). Common associated symptoms were headache (30.1%), loss of appetite (19.8%), and vomiting (13.5%). Rest (40.9%) and drug use (22.6%) were the main management approaches, with piroxicam and paracetamol mostly used; 72.6% of drug users self-medicated. No significant correlation was found between dysmenorrhea and examination stress ($r=-0.013$, $p=0.804$) or self-reported academic performance ($r=-0.061$, $p=0.254$).

**Conclusion:** Dysmenorrhea is highly prevalent among DELSU undergraduates, and management remains largely non-pharmacological or informal, with substantial self-medication. No significant correlation was found between dysmenorrhea and self-reported examination stress or academic performance in this sample, although a considerable proportion of respondents reported dysmenorrhea-related absenteeism and reading difficulty.

**Keywords:** Dysmenorrhea; Prevalence, Management; Undergraduate students; Academic Performance

[1]Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria
[2]Delsu Joint Canadian-Isreal Neuroscience Laboratory, Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria.
[3]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria
[4]Department of Microbiology, Faculty of Sciences, Delta State University, Abraka, Nigeria

**Corresponding author:** Itivere Adrian Omogbiya, Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria, Email: adrianomotive@gmail.com

## INTRODUCTION

Painful menstruation, or dysmenorrhea, has been described as a gynecologic problem characterized by cramping pain experienced around the lower abdomen at the onset or during menstrual bleeding, constituting one of the most commonly observed gynecological problems in reproductive age women.[1,2] This medical term has been categorized into two broad classes of primary dysmenorrhea and secondary dysmenorrhea. The former is caused by higher levels of prostaglandin production and not related to any underlying pelvic pathologies while the latter is caused by pelvic disorders, such as endometriosis and adenomyosis.[3,4]

Internationally, the prevalence rate of dysmenorrhea among adolescents and young women is estimated between 45% and 90%, and this has earned its name as the most common gynecological problem for this demographic group. The health condition is the leading cause of school and work absenteeism among women of reproductive age in this population. Apart from causing physical discomfort, menstrual pain has been associated with poor concentration and performance in academics, poor sleep, social isolation, and reduced health-related quality of life.[5,6] One particular study conducted in Nigeria among female students corroborated the effect of dysmenorrhea on academic performance.[7]

Prevailing prevalence statistics for Nigeria include varying levels of prevalence rates which are between 25% and 83%. The variation results from differences in methodology, sample population and criteria used for diagnosing the condition.[8,9] This is further corroborated by findings from south-western Nigeria and from Ethopia which reported increased prevalence of dysmenorrhea and strong association with an increased proportion of student's absenteeism from classes due to the pain associated with menstruation.[3,10] With all these problems associated with dysmenorrhea among female students, Nigerians inclusive, many lack knowledge about treatment options and use self-medication techniques that are harmful to them.[5,11,12]

The burden of dysmenorrhea, its perception and management strategies among students in the Delta State University, Abraka, a major public institution of higher learning in South-South Nigeria, was never explored before this project. No literature had identified the association between dysmenorrhea prevalence and academic pressure, which is known as a causative factor of menstrual irregularities.[13,14] It is worth noting that there is a considerable level of coincidence between dysmenorrhea peak prevalence period and period when females study in higher institutions of learning.

The study was therefore geared towards: identifying the prevalence rate of dysmenorrhea among students of DELSU, investigating the perception and knowledge of students on the disease, describing the present management measures and finding out the connection between examination stress, dysmenorrhea, and academic achievement.

## MATERIALS AND METHODS

### Study Design and Setting

This was a descriptive cross-sectional study conducted at Delta State University (DELSU), Abraka, Delta State, Nigeria (approximately 5.79°N, 6.10°E). DELSU is a public tertiary institution comprising of the following faculties: Faculty of Arts, Social sciences, Basic Medical sciences, Management sciences, Agriculture, Engineering, Science, Education, Law and

Pharmacy.

**Study Population and Sampling**

All female undergraduate students of DELSU constituted the target population. Convenience (quota) sampling technique was adopted. Forty questionnaires were distributed randomly to female students in each faculty of the institution giving a target sample size of 400 participants. Students from 100 level to final year were eligible. Of the 400 questionnaires distributed, 399 were retrieved and analysed (99.75% response rate). This is a non-probability sampling approach rather than random or stratified random sampling, and self-selection/ convenience bias is acknowledged as a limitation; findings may not be fully generalisable to the entire DELSU female student population.

Sample size was determined using the Cochran's formula as previously described [15] and expressed herein:

$$n = Z^2pq/e^2$$

*Where Z = 1.96 (95% confidence level), p = 0.5 (assumed prevalence, used for maximum variability in the absence of a prior DELSU-specific estimate), q = 1-p = 0.5, and e = 0.05 (5% margin of error).*

This gives a minimum required sample size of n ≈ 384, which was rounded up to 400 to allow for non-response, in proportion to DELSU's estimated undergraduate population of over 20,000 students across the different faculties.

**Inclusion and Exclusion Criteria**

Inclusion criteria included; (i) Female undergraduate student of DELSU, (ii) Female Undergraduate of adult age (18 years and above), and (iii) Willingness to participate voluntarily. Male students and those unwilling to participate were excluded.

**Instrument for Data Collection**

An instrument comprising a structured, self-administered questionnaire containing 37 questions (36 closed-ended and 1 open-ended) was developed by the researchers after an extensive literature review, and was reviewed for face and content validity by the study supervisor. The questionnaire was not adapted from a previously published, externally validated instrument, and no reliability coefficient (e.g. Cronbach's alpha) was computed prior to administration; this is acknowledged as a methodological limitation. Dysmenorrhea itself was assessed as a self-reported binary experience ("Do you experience painful menstruation? Yes/No") rather than through a validated pain-severity scale such as the Visual Analogue Scale (VAS) or Numeric Rating Scale (NRS); consequently, prevalence estimates in this study reflect self-reported occurrence of menstrual pain rather than a clinically graded diagnosis, and severity was not systematically scored. The questionnaire also did not include items to exclude secondary dysmenorrhea (e.g. endometriosis, adenomyosis, pelvic inflammatory disease); reported family history and menarche-onset patterns are therefore suggestive of, but cannot confirm, a primary dysmenorrhea aetiology in this sample. The questionnaire had five sections as follows:

*Section A:* Demographic information (age, marital status, religion, and faculty).

*Section B:* Incidence and characteristics of dysmenorrhea (nature of pain, when it started, how often it occurred, where it was felt, what other symptoms it was accompanied by, and menstrual history).

*Section C:* Perceptions about dysmenorrhea (knowledge, awareness, perception, attitude, and whether anyone in their family suffers from

dysmenorrhea).

*Section D:* Dysmenorrhea management (treatment used, drug type, pharmacological and non-pharmacological methods, and seeking medical care).

*Section E:* Influence of stress on dysmenorrhea and academics (ability to read, absenteeism in classes, examination results).

The questionnaires were distributed to students face-to-face after being informed about the study.

**Ethical Approval and Reporting Guideline:**

The study was conducted in compliance with regulations approved by the Ethical Committee on Human and Animal Research of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State (Approval No: RBC/FBMC/DELSU/18/0974). Written/verbal informed consent was obtained from all participants prior to administration of the questionnaire. Participants were informed of the study's purpose, that participation was voluntary, that responses would be anonymised, and that they could withdraw at any point without penalty. Only students who provided consent were included in the study. Moreover, the study is reported in line with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist for cross-sectional studies.

**Data Analysis**

The data were coded, inputted, and analyzed statistically using SPSS v.23.0 and Microsoft Excel. Frequency and percentage distributions were employed to characterize participants' demographics and the outcome variables. Spearman's rho was used to investigate the hypotheses on the correlations between (i) exam stress and dysmenorrhea; and (ii) dysmenorrhea and academic achievement. The significance level was considered at $p < 0.05$.

## RESULTS

### Demographic Characteristics of Study Participants

The demographics characteristics of the study participants is presented in Table 1. From a total of 399 questionnaires analysed, a large proportion of participants (58.9%) were within the age range of 21-25 years, while 30.8% were within the age range of 15-20 years. The vast majority of participants were single (96.0%) and Christian (99.5%). The sample was highly homogeneous with respect to religion (99.5% Christian) and marital status (96.0% single), which likely reflects the demographic composition of the university's undergraduate population and/or the convenience sampling method used, rather than deliberate stratification by these variables. This limits the generalisability of findings to more religiously or maritally diverse student populations, and the possibility of non-response bias among under-represented subgroups (e.g. Muslim or married students) cannot be excluded.

**Table 1. Demographic characteristics of study participants (N = 399)**

| Variable | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Age (years) | 15–20 | 123 | 30.8 |
| | 21–25 | 235 | 58.9 |
| | 26–30 | 36 | 9.0 |
| | 31–35 | 4 | 1.0 |
| | 36–40 | 1 | 0.3 |
| Marital Status | Single | 383 | 96.0 |
| | Married | 13 | 3.3 |
| | Divorced | 3 | 0.8 |
| Religion | Christian | 397 | 99.5 |
| | Muslim | 2 | 0.5 |

**Prevalence and Pain Characteristics in Patients with Dysmenorrhea**

Table 2 shows details on prevalence and pain characteristics in students experiencing dysmenorrhea. The prevalence rate of dysmenorrhea among the subjects was 78.9% (n = 315). The onset of dysmenorrheal pain occurred during menarche in 53.6% of patients whereas 41.6% experienced dysmenorrhea during later stages of their lives. Among those, 30.3% of respondents experience it regularly whereas 50.6% experience it occasionally. Abdomen was the most common site for pain in 67.2%, followed by lower back (8.8%), hips (3.0%), and thighs (1.8%). In total, 54.9% of respondents had a family history of dysmenorrhea.

**Table 2. Prevalence and pain characteristics of dysmenorrhea (N = 399)**

| Characteristic | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Experience painful menstruation | Yes | 315 | 78.9 |
| | No | 84 | 21.1 |
| Age at menarche (years) | 10–15 | 214 | 53.6 |
| | 16–20 | 132 | 33.1 |
| Onset of pain | At first period (menarche) | 156 | 39.1 |
| | Later in life | 166 | 41.6 |
| Frequency of pain | Always | 121 | 30.3 |
| | Sometimes | 202 | 50.6 |
| Pain location | Lower abdomen | 268 | 67.2 |
| | Lower back | 35 | 8.8 |
| | Hip | 12 | 3.0 |
| | Thigh | 7 | 1.8 |
| Family history of dysmenorrhea | Yes (not the only one in family) | 219 | 54.9 |
| Heavy menstrual flow | Yes | 183 | 45.9 |

**Symptoms associated with dysmenorrhea**

As shown in Table 3, headache (30.1%) was the most frequently reported symptom associated with dysmenorrhea, followed by loss of appetite (19.8%), vomiting (13.5%), other symptoms (11.0%), and nausea (5.8%). This table reflects the 320 respondents who reported at least one associated symptom (a subset of the 315 respondents who experienced dysmenorrhea, as some respondents skipped this item or the item allowed limited multiple responses); percentages are calculated on valid responses to this item.

**Table 3. Symptoms associated with dysmenorrhea (N = 320)**

| Symptom | Frequency (n) | Percentage (%) |
|---|---|---|
| Headache | 120 | 30.1 |
| Loss of appetite | 79 | 19.8 |
| Vomiting | 54 | 13.5 |
| Other symptoms | 44 | 11.0 |
| Nausea | 23 | 5.8 |

**Perception of Dysmenorrhea among Undergraduate Students of DELSU**

As shown in **Table 4,** Familiarity with the term 'dysmenorrhea' was indicated by 45.9% of participants, with 39.6% having never heard about dysmenorrhea. Of those familiar with dysmenorrhea, the main sources of their knowledge included classroom learning (35%), social networking sites (30%), various sources (25%), and friends (10%). The majority of respondents (60.7%) were comfortable speaking freely about the topic, whereas 23.6% did not feel at ease. With regard to perceived causes of dysmenorrhea, the highest number mentioned excessive sugar consumption (45.4%), followed by stress (18.0%), infection (7.3%), genetic predisposition (6.8%), prostaglandins (5.0%), and nutrition (1.5%). Majority (59.9%) thought dysmenorrhea can be treated.

**Table 4. Perception and awareness of dysmenorrhea (N = 335)***

| Variable | Frequency (n) | Percentage (%) |
|---|---|---|
| Heard of dysmenorrhea: Yes | 183 | 45.9 |
| Comfortable discussing: Yes | 242 | 60.7 |
| Perceived cause: Excess sugar | 181 | 45.4 |
| Perceived cause: Stress | 72 | 18.0 |
| Perceived cause: Infection | 29 | 7.3 |
| Perceived cause: Genetics | 27 | 6.8 |
| Perceived cause: Prostaglandins | 20 | 5.0 |
| Believes dysmenorrhea is treatable | 239 | 59.9 |
| Believes diet plays a role | 199 | 49.9 |
| Positive family history | 219 | 54.9 |
| Feel anxious before period | 210 | 52.6 |

**Table reflects 335 valid responses to perception items; N differs from the total sample of 399 due to item non-response*

**Management Practices of Dysmenorrhea among Undergraduate Students of DELSU**

Tables 5 and 6 respectively, depicts the self-reported responses by study participants as regards the different methods employed in managing their episodes of dysmenorrhea. Table 5 shows the different self-reported management practices employed by undergraduate students of DELSU against dysmenorrhea (N=348 valid responses to this item; N differs from the total sample due to item non-response or skip logic for students who reported no management practice). As shown, resting was the most frequently employed method in dealing with menstrual pain (40.9%), followed by drug-taking (22.6%), warm bath (13.5%), massage (6.5%), and exercise (3.8%). Additionally, Table 6 shows the specific drugs used among the 208 respondents who reported taking medication; piroxicam (brand name: Felvin; 17.5%) and paracetamol (17.0%) were the most frequently used, followed by ibuprofen (10.0%), diclofenac (3.3%), other drugs (2.5%), and herbal preparations (1.8%). Piroxicam and paracetamol are two distinct medications with similar (but not identical) usage rates in this sample; this is not a double-count. The largest proportion of drug users (34.3%) took medication during menstrual bleeding, while 17.8% took medication prior to onset of the period. Self-medication was practiced by 72.6% of drug-taking respondents

**Table 5. Management practices for dysmenorrhea (N = 348)***

| Management Method | Frequency (n) | Percentage (%) |
|---|---|---|
| Rest | 163 | 40.9 |
| Drugs | 90 | 22.6 |
| Warm bath | 54 | 13.5 |
| Massage | 26 | 6.5 |
| Exercise | 15 | 3.8 |

**N=348 valid responses to this item; N differs from the total sample due to item non-response or skip logic for students who reported no management practice*

**Table 6. Drugs used for dysmenorrhea management (N = 208)***

| Drug | Frequency (n) | Percentage (%) |
|---|---|---|
| Felbinac (Felvin) | 70 | 17.5 |
| Paracetamol | 68 | 17.0 |
| Ibuprofen | 40 | 10.0 |
| Diclofenac | 13 | 3.3 |
| Other drugs | 10 | 2.5 |
| Herbal preparations | 7 | 1.8 |

**N=208 valid responses to this item; N differs from the total sample due to item non-response or skip logic for students who reported specific drug used to manage pain during dysmenorrhea*

**Associative Effect of Stress and Dysmenorrhea on Academic Performance**

These findings are shown in Table 7. In terms of self-reported effects on academics, 30.6% of participants stated that they could not read while menstruating, 31.8% reported that they could read, and 23.8% could sometimes read. Of those who could read, 21.6% rated their assimilation as low, 43.4% average, and 4.8% high. School absence due to dysmenorrhea was reported as always occurring by 14.5% of respondents, sometimes by 22.3%, while 49.9% reported no absenteeism. 9.8% believed dysmenorrhea definitely affects their academic performance, 63.2% said it does not, and 13.8% said it sometimes does. Nearly half of respondents (47.4%) felt they performed well in examinations despite dysmenorrheic pain. All academic-performance items in this section were self-reported perceptions; no objective measure such as GPA/CGPA or examination scores was collected.

The perceived effect of stress on dysmenorrhea was reported as "yes" by 46.4% of participants (sometimes: 25.6%; no: 17.5%). Spearman's correlation between perceived examination stress and frequency of dysmenorrhea produced a negligible, non-significant coefficient ($r = -0.013$, $p = 0.804$), indicating essentially no linear association rather than merely a lack of statistical significance. Similarly, the correlation between dysmenorrhea and self-reported academic performance was negligible and non-significant ($r = -0.061$, $p = 0.254$). It should be noted that Spearman's rho is not the ideal test for these largely categorical/ordinal variables; a chi-square test of association or ordinal logistic regression may have been more appropriate and is recommended for future analyses of this dataset.

**Table 7. Spearman's correlation between stress, dysmenorrhea, and academic performance**

| Correlation | Rho (r) | p-value | Significance |
|---|---|---|---|
| Exam stress vs. dysmenorrhea | -0.013 | 0.804 | Not significant |
| Dysmenorrhea vs. academic performance | -0.061 | 0.254 | Not significant |

## DISCUSSION

**Prevalence of Dysmenorrhea**

In the current study, the prevalence of dysmenorrhea was 78.9%, reflecting the high prevalence that is reported amongst Nigerian and African universities. In Nigeria, Esan et al. (2024) recorded prevalence of 76.4% amongst female university undergraduate students[16], whereas in Ethiopia, a prevalence of 74.7% was reported among students of Haramaya University in 2022[10], while a 2023 study reported prevalence up to 80.2% in Ghana.[11] These results corroborate the world prevalence range of 60-93%.[17]

The fact that 53.6% of participants started suffering from dysmenorrhea at menarche and 54.9% of participants have a family history of the condition corroborate the current scientific literature on the association between early onset of menstrual cramps and the genetic factor, as well as prostaglandins.[3,4] In this way, these results further emphasise the contribution of genetics to the etiology of the problem and prove the possibility of using family health education for its prevention. The dominance of the lower abdominal pain site (67.2%) coincides with the findings in Iran[12], Ethiopia[10], and South East Nigeria.[5]

The most frequently associated symptom among participants (30.1%) was headache, which is an observation different from what is found in the larger literature wherein fatigue and irritability are more frequent.[5,12] Nevertheless, such an observation is particularly relevant considering the established link between prostaglandin-induced vasospasm and the development of headaches.[2] Other symptoms frequently observed were lack of appetite (19.8%) and vomiting (13.5%), which align with the established role of prostaglandins in the digestive tract.[11] Lastly, 52.6% of the respondents felt anxious prior to the commencement of menstruation, which is consistent with studies that have qualitatively analysed the psychological impact experienced by university students in Ethiopia[10] and Australia.[18]

**Perception and Awareness**

Awareness of the term "dysmenorrhea" was found to be relatively low at only 45.9%. The lack of knowledge about the condition that nearly all females suffer from corresponds to results reported by researchers in Nigeria and sub-Saharan Africa.[11,19] Moreover, excess sugar intake was perceived as the reason for the development of this problem by 45.4% of the participants. Such an opinion contradicts medical literature where the increased production of prostaglandins during the late luteal phase is recognised to be the principal mechanism of dysmenorrhea.[4,20] At the same time, 18.0% mentioned stress and only 5.0% correctly identified prostaglandins, indicating a limited understanding of the underlying hormonal mechanism. This knowledge gap plausibly links to the high rate of self-medication observed in this study (72.6% of drug users): students who misattribute dysmenorrhea to diet rather than to a treatable, prostaglandin-mediated process may be less likely to seek evidence-based pharmacological management or professional advice, and more likely to rely on informal remedies. Lack of accurate information on the condition may lead students toward inappropriate self-management rather than evidence-based care. However, a relatively large number of respondents (60.7%) felt that they could comfortably discuss their condition compared to respondents from other studies conducted among public universities in southern Ethiopia and Ireland, who felt that stigma prevented them from seeking help.[5,21]

**Management Practices**

Non-pharmacological strategies formed the majority of management practices, with rest being the most common (40.9%). This is similar to findings from previous studies in Nigeria, Ghana, Turkey, and Ireland[5,8,11,22,23,24], where rest, heat, and bathing were popular self-management approaches. Overall, 67.7% of respondents reported using some form of management (rest and/or drugs), indicating that utilisation of management strategies was not itself low; rather, the appropriateness of the strategies used is of concern, since rest alone only temporarily relieves pain and does not address the prostaglandin-mediated pathway underlying primary dysmenorrhea. Furthermore, repeated reliance on rest across multiple cycles may itself contribute to increased absenteeism among students.

Regarding medication use, NSAIDs used by respondents included ibuprofen (10.0%), diclofenac (3.3%), and piroxicam (brand name: Felvin; 17.5%), alongside paracetamol (17.0%), a non-NSAID analgesic/antipyretic. NSAIDs remain a first-line pharmacological option for dysmenorrhea because of their dual mechanism of prostaglandin-synthesis inhibition and analgesia.[23,24,25]. This is supported by the Cochrane review by Marjoribanks et al., as well as a study

from Ireland which found NSAIDs more effective than placebo for dysmenorrhea-related pain.[19,24] The relatively high combined use of paracetamol and piroxicam relative to other NSAIDs such as ibuprofen may reflect availability, cost, and habit rather than adherence to a specific evidence-based first-line recommendation, and suggests a need for pharmacist- or clinician-led counselling on optimal NSAID choice, dosing, and timing (started before or at the onset of pain) for dysmenorrhea management.

Crucially, 72.6% of drug users self-medicated, while only 9.5% sought medical advice. This high prevalence of self-medicating among drug users is consistent with a systematic trend seen among African university communities[6,11,26], which can be explained by factors such as lack of accessibility to health facilities at universities, economic concerns, stigma around menstruation, as well as the idea that dysmenorrhea is a 'normal' phenomenon that does not require medical assistance.[3] The 2024 study conducted in Nigeria by Esan et al. came to very similar conclusions regarding over-the-counter painkiller use and lack of healthcare seeking behaviors.[16] The persistent focus of the WHO on menstrual health care services which are accessible and evidence-based in educational institutions[27] highlights the importance of tackling such structural issues.

**Academic Performance and Exams Stress**

Unlike several previous studies, there were no statistically significant correlations observed between dysmenorrhea and exam stress ($r = -0.013$, $p = 0.804$) or self-reported academic performance ($r = -0.061$, $p = 0.254$) in this sample; both coefficients are close to zero, indicating negligible linear association rather than a borderline non-significant one. The relationship between dysmenorrhea and academic performance is not uniformly reported across student populations, with variation across study designs and institutional contexts.[28] Individual coping mechanisms, resilience, and study practices may play a role, and the near-zero correlation may also partly reflect a mismatch between the largely dichotomous/ordinal outcome measures used here and the continuous assumptions of Spearman correlation, rather than a true absence of any relationship. This is therefore acknowledged as a key limitation of the current study null hypothesis interpretation.

Nevertheless, the descriptive data tell a different story altogether: 30.6% could not read when on their period; 14.5% would always miss class because of pain; and 22.3% would do so sometimes. Furthermore, 9.8% reported perceiving dysmenorrhea as having a negative impact on their school work, while 13.8% experienced such a situation sometimes. Collectively, all these data show that while there may be no statistical significance when it comes to overall impact, especially given the diversity in pain intensity and coping abilities among individuals, there remains a significant number of students affected academically.

The findings have mostly been confirmed through multiple-site studies carried out in Nigeria[5,28] Ethiopia[10,29], Turkey[23], and Ireland[24], where the challenges related to dysmenorrhea involved absenteeism, inability to focus, and decreased academic involvement. A study by Bolado et al. using the qualitative approach on university students enrolled at publicly funded institutions in southern Ethiopia in the year 2025 revealed that dysmenorrhea posed several challenges for the participants, which were portrayed through a first-person account where the participants had trouble concentrating on classwork, missing practical sessions, and test anxiety due to their menstrual period.[30]

## CONCLUSION AND RECOMMENDATIONS

Dysmenorrhea is highly prevalent (78.9%) among female undergraduate students of Delta State University, Abraka, but awareness of its underlying mechanism remains limited, and management relies predominantly on rest and informal or self-medicated drug use rather than evidence-based pharmacological care. Most respondents did not correctly identify prostaglandins as the principal cause of primary dysmenorrhea. No statistically significant correlation was found between dysmenorrhea and self-reported examination stress or self-reported academic performance in this sample; however, descriptive findings indicate that a substantial proportion of students experience dysmenorrhea-related absenteeism, reading difficulty, and perceived academic impact, suggesting this remains an area of concern despite the null correlation. These findings should be interpreted in light of the study's cross-sectional, non-probability-sampled design and self-developed, non-validated instrument.

On this basis, the following recommendations are proposed:

1. Dysmenorrhea-related health education programmes which discuss its etiopathogenesis, evidence-based management and treatment options need to be introduced in university orientation programmes as well as promoted through social media platforms due to their widespread use as a source of information.
2. Gynaecological health care must be provided at universities where it is affordable and accessible. It will also be necessary to facilitate the accessibility of NSAIDs.
3. Accommodating academic policies such as extended deadlines for assignments during menstruation should be considered.
4. Further research must use longitudinal studies along with pain assessment tools to evaluate the differential effect of dysmenorrhea among Nigerian university students.
5. Education regarding menstrual problems needs to be included in high school science programmes.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1 Carlson K, Nagy H, Mikes BA. Dysmenorrhea. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2026. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560675/ PMID: 32809669 accessed on May 30th, 2026

2 Ezebialu IU, Ezenyeaku CC, Umeobika JC. Prevalence of dysmenorrhea and its contribution to school absenteeism among Nigerian undergraduate students. *Ann Health Res*. 2021;7(1):59–66. https://doi.org/10.4103/ahr.ahr_2_21.

3 Bello YO, Adeyeye OV, Adeyeye MM, et al. Association between common mental disorders and the severity of dysmenorrhea among female medical students at the University of Ibadan, Nigeria. *PLOS Glob Public Health*. 2025;5(4):e0004492. https://doi.org/10.1371/journal.pgph.0004492.

4 Irozulike FC, Asiwe N, Ekokodje JW, et al. Prevalence of dysmenorrhea and its management among undergraduate students of the University of Port Harcourt, Nigeria. *Asian J Adv Res Rep*. 2023;17(12):19–28. https://doi.org/10.9734/ajarr/2023/v17i12582.

5 Njelita IA, Nwachukwu CC, Eyisi IG, et al. Dysmenorrhea: prevalence, impacts and coping mechanisms among medical students in South East Nigeria. *Int J Heal Medi Res*. 2023;2(3):49–57

6 Ore TO, Ogundeko CA. Knowledge and self-management of dysmenorrhea among

female adolescents in selected secondary schools in Ogun state, Nigeria. *Commonw J Acad Res*. 2021;2(5):60–70.

7 Amu E., Bamidele J. (2014). Prevalence of menstrual disorders among adolescent girls in Osogbo, South Western Nigeria. *Int J Adolesc Med Health* 26:101-106.

8 Maity S, Wray J, Coffin T, et al. Academic and social impact of menstrual disturbances in female medical students: a systematic review and meta-analysis. *Front Med*. 2022;(9):e821908. https://doi.org/10.3389/fmed.2022.821908.

9 Rennie V, Rashid A & Emmanuel F. The Impact of Traditional Practices and Customs on Pregnant Women and Newborn Mothers' Healthcare-Seeking Behaviors at Primary, Secondary, and Tertiary Health Levels. Afr. J. Trop. Med. & Biomed. Res. 2025; 8(1) 9-36 ttps://doi.org/10.4314/ajtmbr.v8i1.2

10 Mesele TT, Ayalew HG, Syoum AT, Antehneh TA. Impact of dysmenorrhea on academic performance among Haramaya University undergraduate regular students, East Ethiopia. *Front Reprod Health*. 2022;4:939035. https://doi.org/10.3389/frph.2022.939035.

11 Wuni A, Nyarko BA, Ibrahim MM, et al. Prevalence, management, and impact of dysmenorrhea on the lives of nurse and midwife trainees in northern Ghana. *Obstet Gynecol Int*. 2023;2023:8823525. https://doi.org/ 10.1155/2023/8823525.

12 Adib-Rad H, Kheirkhah F, Faramarzi M, Omidvar S, Basirat Z, Haji Ahmadi M. Primary dysmenorrhea associated with psychological distress in medical sciences students in the north of Iran: a cross-sectional study. *Int J Fertil Steril*. 2022;16(3):224–229. https://doi.org/10.22074/ijfs.2022.542056.1216.

13 Ferries-Rowe E, Corey E, Archer JS. Primary dysmenorrhea: diagnosis and therapy. *Obstet Gynecol*. 2020;136(5):1047–1058. https://doi.org/10.1097/AOG.0000000000004096.

14 Omogbiya AI, Anachuna KK, Umukoro EK, Moke EG, Nzei A. Academic-related stress and prevalence of migraine and tension-type headaches amongst undergraduates of Delta State University, Abraka, Nigeria. *Res J Health Sci*. 2020;8(2):133–145. https://doi.org/10.4314/rejhs.v8i2.9.

15 Munro AK, Hunter EC, Hossain SZ, Keep M. A systematic review of the menstrual experiences of university students and the impacts on their education: a global perspective. *PLoS One*. 2021;16(9):e0257333. https://doi.org/10.1371/journal.pone.0257333.

16 Esan DT, Ariyo SA, Akinlolu EF, et al. Prevalence of dysmenorrhea and its effect on the quality of life of female undergraduate students in Nigeria. *J Endometriosis Uter Disord*. 2024;5:100059. https://doi.org/10.1016/j.jeud.2024.100059.

17 Karabulutlu Ö, Yıldız M. Coping methods of women aged 15–49 with dysmenorrhea. *Konuralp Med J*. 2024;14(1):37–47. *(Corrected: "MJ Yildiz" → "M Yıldız")*

18 Non-pharmacological interventions and coping mechanisms during dysmenorrhea among female undergraduates in a tertiary institution in Nigeria. *Urogynaecologia*. 2024;36. https://doi.org/10.4081/uij.2024.310.

19 Marjoribanks J, Ayeleke RO, Farquhar C, Proctor M. Nonsteroidal anti-inflammatory drugs for dysmenorrhoea. *Cochrane Database Syst Rev*. 2015;(4):eCD001751. https://doi.org/10.1002/14651858.CD001751.

20 Kirsch E, Rahman S, Kerolus K, et al. Dysmenorrhea: a narrative review of

therapeutic options. *J Pain Res*. 2024;17:2657–2666. https://doi.org/10.2147/JPR.S459584.

21 Getahun SB, Berhe S, Mekonnen B, Melaku G. Reasons for not seeking healthcare among students with dysmenorrhea: a qualitative study. *Int J Womens Health*. 2023;15:1689–1700. https://doi.org/10.2147/IJWH.S432943.

22 World Health Organization and United Nations Children's Fund. Progress on drinking water, sanitation and hygiene in schools 2015–2023: special focus on menstrual health. New York: UNICEF and WHO; 2024. Available at: https://www.who.int/publications/i/item/9789240097490

23 Bilir E, Yıldız Ş, Yakın K, Ata B. The impact of dysmenorrhea and premenstrual syndrome on academic performance of college students, and their willingness to seek help. *Turk J Obstet Gynecol*. 2020;17(3):196–201. https://doi.org/10.4274/tjod.galenos.2020.97266.

24 Durand H, Monahan K, McGuire BE. Prevalence and impact of dysmenorrhea among university students in Ireland. *Pain Med*. 2021;22(12):2835–2845. https://doi.org/10.1093/pm/pnab122.

25 Burnett M; SOGC Clinical Practice Gynaecology Committee. Guideline No. 345: Primary Dysmenorrhea. *J Obstet Gynaecol Can*. 2025;47(5):102840. https://doi.org/10.1016/j.jogc.2025.102840.

26 Ní Chéileachair F, McGuire BE, Durand H. Coping with dysmenorrhea: a qualitative analysis of period pain management among students who menstruate. *BMC Womens Health*. 2022;22:407. https://doi.org/10.1186/s12905-022-01988-4.

27 de Arruda GT, Barbosa-Silva J, Driusso P, et al. Worldwide prevalence of dysmenorrhea: a systematic review and meta-analysis across 70 countries. *Pain*. 2026;167(1):41–55. https://doi.org/ 10.1097/j.pain.0000000000003768.

28 Uzoechi CA, Parsa AD, Mahmud I, Alasqah I, Kabir R. Menstruation among in-school adolescent girls and its literacy and practices in Nigeria: a systematic review. *Medicina*. 2023;59(12):2073. https://doi.org/ 10.3390/medicina59122073.

29 Belayneh W, Kassaye Z, Arusi T, Abera N, Hantalo A, Melkamu B, Gutulo M. Prevalence of dysmenorrhea and associated factors and its effect on daily academic activities among female undergraduate students of Hawassa University College of Medicine and Health Sciences, Hawassa, Ethiopia. *Front Reprod Health*. 2023;(5):e1244540. https://doi.org/10.3389/frph.2023.1244540.

30 Bolado GN, Ataro BA, Endrias EE, et al. The impact of dysmenorrhea on academic performance and coping strategies: lived experiences of female students in Ethiopian public universities. *BMC Womens Health*. 2025;25:379. https://doi.org/10.1186/s12905-025-03924-8.

Omogbiya IA, Orovwigho O, Moke EG, Demaki W, Oboh M, Emudainohwo JOT, Okoro OE, Jewo AO, Chidebe E, Otah OJ. Prevalence, Perception And Management Practices of Dysmenorrhea Among Students of Delta State University, Abraka, South-south Nigeria. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 88-100
https://dx.doi.org/10.4314/ajtmbr.v9i1.5

# Determination of $LD_{50}$ of *Naja ashei* Venom and its Effects on the Heart and Serum Electrolytes in BALB/c Mice through Intraperitoneal, Subcutaneous and Intramuscular Routes

[1]Wafula E, [1]Gikunju J, [2]Kamanja I, [3]Shiundu BM.

**Abstract**

**Introduction:** *Naja ashei* envenomation causes severe local and systemic toxicity, yet experimental evidence on route-dependent lethality, electrolyte disturbances, and cardiotoxicity remains limited. This study determined the median lethal dose ($LD_{50}$) of *Naja ashei* venom in BALB/c mice after intraperitoneal, intramuscular, and subcutaneous administration and evaluated electrolyte imbalance and cardiac injury.

**Materials and methods:** Fresh *Naja ashei* venom was collected, lyophilized, and characterized. One hundred BALB/c mice received venom doses through intraperitoneal, intramuscular, or subcutaneous routes. $LD_{50}$ values were calculated using the Reed-Muench method and confirmed by probit and dose-response analyses. Cardiac injury was assessed using creatine kinase-MB and troponin assays, while histopathological examination of cardiac tissue confirmed venom-induced myocardial damage.

**Results:** Venom profiling showed predominance of three-finger toxins (~69%) and phospholipase $A_2$ (~27%), consistent with cytotoxic and systemic activity. Toxicity was route dependent, with intraperitoneal administration producing the lowest $LD_{50}$ (0.70 mg/kg), followed by intramuscular (2.36 mg/kg) and subcutaneous (2.69 mg/kg) routes. Envenomation caused severe hyperkalemia (mean 7.8 mmol/L) with hyponatremia. Mean troponin-I reached 11.7 ± 5.6 ng mL¯1, while creatine kinase-MB averaged 917 ± 122.2 U L¯1, indicating myocardial injury. Histology revealed myocardial necrosis, interstitial edema, and vascular congestion.

**Conclusion:** *Naja ashei* venom exhibits high lethality and produces electrolyte imbalance and myocardial injury in a route-dependent manner. These findings support antivenom administration, electrolyte correction, intensive cardiac monitoring, and inclusion of *Naja ashei* venom in antivenom development to improve clinical outcomes.

**Keywords:** *Naja ashei; Snake-venom; $LD_{50}$ -toxicity; Cardiotoxicity; mice*

[1]*Department of Medical Laboratory Sciences, School of Biomedical Sciences, Jomo Kenyatta University Agriculture and Technology, Kenya*
[2]*Dept of Vet. Public Health, Pharmacology & Toxicology Faculty of Veterinary Medicine and Surgery, Jomo Kenyatta University Agriculture and Technology, Kenya*
[3]*Department of Medical Laboratory Sciences, Egerton University, Kenya*

**Corresponding author:** Wafula E, Department of Medical Laboratory Sciences, School of Biomedical Sciences, Jomo Kenyatta University Agriculture and Technology, Kenya. Email: elias.wafula@jkuat.ac.ke

## INTRODUCTION

Snakebite envenoming remains a major but persistently neglected public health challenge, particularly in low- and middle-income countries where environmental exposure, agricultural livelihoods, and limited access to healthcare increase the risk of human-snake interactions. Globally, snakebite envenoming is responsible for an estimated 2.7 million cases each year, resulting in approximately 81,000-138,000 deaths and a substantial number of survivors living with permanent disability and

socioeconomic consequences.[1] In recognition of this significant burden, the World Health Organization classified snakebite envenoming as a Category A neglected tropical disease in 2017 and subsequently launched a global strategy aimed at reducing snakebite-related mortality and morbidity by 50% by the year 2030.[2]

Sub-Saharan Africa bears a substantial proportion of the global snakebite burden, with an estimated 435,000 to 580,000 snakebite envenoming cases and approximately 20,000-32,000 deaths occurring annually across the region.[3,4] In Kenya, snakebite envenoming remains a significant public health concern, particularly in rural and arid regions where access to antivenom and specialized healthcare services is limited. Current estimates suggest that Kenya records between 15,000 and 20,000 snakebite cases annually, resulting in considerable mortality, disability, and socioeconomic burden.[5,6] These figures likely underestimate the true burden due to underreporting, poor surveillance systems, and reliance on traditional remedies in many affected communities

One of the medically important snake species implicated in cobra envenomation in Kenya is *Naja ashei*, commonly known as the large brown spitting cobra. This species is widely distributed in open, arid, and semi-arid regions of East Africa and is responsible for a substantial proportion of spitting cobra bites.[6] African spitting cobras are classically associated with severe local cytotoxic effects, including tissue necrosis, blistering, and permanent disfigurement, largely mediated by cytotoxins and phospholipase $A_2$ enzymes.[7] Although neurotoxicity is less prominent compared to non-spitting cobras, emerging evidence indicates that spitting cobra venoms may also induce clinically significant systemic effects including cardiotoxicity, electrolyte imbalance, and inflammatory injury that have not been adequately characterized.[12,13]

The primary intervention for snakebite envenoming is the administration of antivenom, which neutralizes circulating venom toxins and reduces the risk of mortality and severe complications when given promptly.[2] Preclinical evaluation of venom toxicity and antivenom efficacy remains central to improving clinical outcomes and guiding antivenom development. Such evaluations typically rely on standardized animal models, with mice being the most commonly used experimental species due to their genetic uniformity, reproducibility, and well-characterized physiological responses.[8]

Determination of the median lethal dose ($LD_{50}$) is a cornerstone of venom toxicology, providing quantitative estimates of venom potency and enabling comparisons across different routes of exposure.[10] Prior investigations into *N. ashei* venom toxicity have primarily focused on the intraperitoneal route, leaving important gaps regarding route-dependent variations in lethality that are relevant to real-world envenomation scenarios, where venom is typically delivered via subcutaneous or intramuscular deposition.[11] In the present study, $LD_{50}$ values were determined for *N. ashei* venom using intraperitoneal, subcutaneous, and intramuscular routes in mice, thereby providing a more comprehensive toxicological profile of the venom.[11]

In addition to lethality, snake venoms are known to cause systemic biochemical and physiological disturbances, particularly involving electrolyte homeostasis and cardiovascular function. Alterations in serum electrolytes may contribute to neuromuscular dysfunction and cardiac instability, while cardiotoxic venom components

can lead to myocardial injury, arrhythmias, and circulatory compromise.[7] In this study, serum electrolyte levels and cardiac biomarkers were measured following envenomation, and histopathological examination of cardiac tissue was performed to evaluate venom-induced myocardial damage.

Furthermore, concerns remain regarding the effectiveness of currently available polyvalent antivenoms against *Naja ashei* venom. In Kenya, the currently available antivenoms include AFRIVEN® and PANAF-Premium®, which are primarily developed using immunizing venom mixtures from selected African snake species and may provide limited specific coverage against *Naja ashei* venom.[11] Previous studies have demonstrated partial neutralization of certain venom components using heterologous antivenoms; however, data regarding their effectiveness in counteracting lethality, cardiotoxicity, and systemic toxicity in mammalian models remain limited.[11]

This study therefore presents a comprehensive toxicological evaluation of *Naja ashei* venom based on experimentally generated data. By characterizing route-specific $LD_{50}$ values and documenting associated electrolyte disturbances and cardiac effects, the study provides evidence that is directly relevant to clinical management, antivenom selection, and future antivenom development. The findings contribute to closing critical knowledge gaps and offer a scientific basis for improving outcomes of *N. ashei* envenomation in Kenya and across sub-Saharan Africa.

## MATERIALS AND METHODS

### Study Site

This study was conducted at the Small Animal Facility for Research and Innovation (SAFARI) at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja Town, Kenya. The facility is equipped with standardized animal housing units, biosafety laboratories, and analytical infrastructure suitable for toxicological and biomedical research involving laboratory animals.

### Study Design

A controlled laboratory-based experimental study was conducted in two sequential phases. Phase I involved determination of Chemical and physical properties of lethal dose ($LD_{50}$) of *Naja ashei* venom in mice using three routes of administration: intraperitoneal (I.P.), intramuscular (I.M.), and subcutaneous (S.C.). The Reed and Muench interpolation method (1938) was employed for $LD_{50}$ estimation. Phase II assessed sub-lethal systemic effects of the venom, including electrolyte disturbances, cardiac injury biomarkers, and myocardial histopathological changes, following administration of $0.5\times LD_{50}$ doses via each route. The study design incorporated random allocation of animals, blinded outcome assessment, and predefined humane endpoints in accordance with ethical guidelines.

### Animal Model

Ninety male and female BALB/c mice (45 male and 45 female) aged 2-3 months and weighing between 18 and 40 g were used. Animals were acclimatized for five days prior to experimentation under controlled environmental conditions (22 ± 2 °C, 55 ± 10% humidity, 12-hour light/dark cycle). Mice were housed in polycarbonate cages with wood-chip bedding, provided with nesting materials for environmental enrichment, and had *ad libitum* access to commercial pelleted feed and water. Bedding was changed twice weekly. Only healthy mice within the target weight range were included in the study, while animals showing signs of

illness, pregnancy, or prior experimental exposure were excluded. All procedures were approved by the JKUAT Institutional Animal Care and Use Committee and complied with international guidelines for laboratory animal welfare.

**Venom Source, Handling, and Protein Standardization**

Crude venom was obtained from six adult *Naja ashei* specimens maintained at Bio-Ken Snake Farm, Watamu, Kenya, and milked by accredited herpetologists. The snakes were housed in secure species-specific enclosures under controlled environmental conditions with regulated temperature, humidity, feeding schedules, hydration, and routine veterinary monitoring in accordance with institutional animal welfare and biosafety guidelines.[12] Bio-Ken Snake Farm is a recognized snake conservation and venom research facility licensed by the Kenya Wildlife Service (KWS) for handling venomous reptiles and venom extraction activities.

Venom extraction was performed using standardized manual milking procedures as previously described for venomous snakes.[12] Briefly, individual snakes were gently restrained using snake hooks and secure handling techniques, after which venom was collected by allowing the snake to bite onto a sterile parafilm-covered collection vessel. The extracted venom was immediately transferred into sterile containers maintained on ice to preserve protein integrity. Venom samples from all snakes were pooled to minimize inter-individual venom variability, flash-frozen in liquid nitrogen, lyophilized, transported on dry ice, and stored at -20 °C until use.

Prior to experimentation, venom was reconstituted in sterile phosphate-buffered saline (PBS, pH 7.4), vortexed, centrifuged to remove particulates, and serially diluted. Protein concentration was quantified using a bicinchoninic acid (BCA) assay with bovine serum albumin as the standard according to the manufacturer's instructions (Thermo Fisher Scientific, USA). All dosing calculations were normalized to the measured protein concentration. Venom-contaminated materials were chemically inactivated and disposed of through incineration according to institutional biosafety guidelines[1].

**Precautions While working with venomous Snakes and Venom Samples**

All snake specimens were taxonomically verified and handled exclusively by experienced snake handlers and licensed reptile veterinarians. Laboratory personnel received training on venom handling and biosafety procedures. Personal protective equipment, including laboratory coats, eye protection, and double gloves, was always used, and venom preparation was conducted within a biosafety cabinet. Standard operating procedures for accidental exposure were strictly followed.

**$LD_{50}$ Determination**

Ninety BALB/c mice were randomly assigned to three groups corresponding to intraperitoneal (I.P.), intramuscular (I.M.), and subcutaneous (S.C.) administration routes (n = 30 per group). The sample size was determined based on the World Health Organization guidelines for preclinical venom toxicity testing and previously published $LD_{50}$ studies utilizing the Reed–Muench method, which recommend a minimum of five dose levels with multiple animals per dose group to ensure reliable mortality estimation and statistical validity.[9,13] Each group was subdivided into five dose subgroups (n = 6 per dose). Venom doses were selected based on preliminary range-finding

studies and administered in fixed volumes appropriate for each route. Animals were observed for 24 hours post-injection, and mortality was recorded at predefined intervals. $LD_{50}$ values were calculated using the Reed–Muench method as previously described by Reed and Muench and recommended for venom lethality studies.[9,13], while probit analysis was employed to confirm estimates and generate 95% confidence intervals.

**Sub-lethal Dosing and Clinical Observation**

Separate cohorts comprising a total of eighteen BALB/c mice were used for sub-lethal toxicity assessment, with six mice assigned to each administration route (intraperitoneal, intramuscular, and subcutaneous). Animals received $0.5\times LD_{50}$ doses to assess sub-lethal systemic effects, as previously described in experimental venom toxicology studies evaluating biochemical and cardiotoxic alterations in murine models. [10,11] Animals were monitored for clinical signs including body weight changes, temperature, posture, locomotion, piloerection, respiratory distress, and responsiveness at regular intervals up to 72 hours post-envenomation. Humane endpoints triggered immediate euthanasia using isoflurane overdose followed by cervical dislocation in accordance with international laboratory animal welfare guidelines.[12]

**Blood and Serum Collection**

Blood samples were collected from the lateral tail vein under brief anesthesia at 6-, 24-, and 48-hour post-envenomation. Samples were processed to obtain serum, which was either analyzed immediately or stored at -80 °C pending analysis.

**Serum Electrolyte and Cardiac Biomarker Assays**

Serum sodium and potassium concentrations were measured using an automated clinical chemistry analyzer (Humastar 200, HUMAN Diagnostics, Wiesbaden, Germany) following the manufacturer's protocols and standard laboratory quality control procedures. Cardiac injury biomarkers, including troponin I and creatine kinase-MB (CK-MB), were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Inc., Houston, TX, USA) according to standardized immunoassay procedures previously applied in venom cardiotoxicity studies.[10,11] All analyses were performed in accordance with manufacturer instructions, and internal quality control samples were included in each analytical run to ensure assay accuracy and reliability.

**Gross Necropsy and Cardiac Histopathology**

Following the final observation period, animals were perfused with cold phosphate-buffered saline (PBS), and the hearts were carefully excised, examined grossly, weighed, and fixed in 10% neutral-buffered formalin. Histopathological processing and hematoxylin and eosin (H&E) staining were performed according to standard procedures previously described in experimental venom cardiotoxicity studies.[10] Briefly, fixed cardiac tissues were dehydrated through graded alcohol concentrations, embedded in paraffin wax, sectioned at 5 μm thickness using a rotary microtome, and stained with hematoxylin and eosin for microscopic evaluation. Tissue sections were examined by a blinded veterinary pathologist

**Protein Electrophoresis**

Venom protein composition was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions as previously described in venom proteomic studies.[14,15] Briefly, lyophilized *Naja ashei* venom was reconstituted in phosphate-

buffered saline (PBS, pH 7.4), and protein concentration was determined using a bicinchoninic acid (BCA) assay prior to electrophoresis. A total of 20 μg of venom protein mixed with Laemmli sample buffer containing 5% β-mercaptoethanol was loaded into each gel well. Samples were heated at 95 °C for 5 minutes to ensure complete protein denaturation before loading.

Electrophoresis was performed using 12% polyacrylamide resolving gels with a 4% stacking gel at a constant voltage of 120 V for approximately 90 minutes alongside a pre-stained molecular weight protein marker (Thermo Fisher Scientific, USA). Following separation, gels were stained with Coomassie Brilliant Blue R-250 for 1 hour and subsequently destained using a methanol-acetic acid solution until clear protein band visualization was achieved. Gel images were captured using a Gel Doc imaging system (Bio-Rad Laboratories, Hercules, CA, USA), and densitometric analysis was performed using ImageJ software (National Institutes of Health, USA) to estimate the relative abundance of major toxin protein families based on molecular weight distribution patterns.[14,15]

**Data Handling and Statistical Analysis**

Data were analyzed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA) and Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD). Data was presented using tables, graphs, and descriptive summaries to illustrate variations in venom toxicity, electrolyte disturbances, and cardiac biomarker levels across the different administration routes. Group comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

**Biosafety and Waste Disposal**

All experimental waste was handled according to Jomo Kenyatta University of Agriculture and Technology biosafety policies and National Environment Management Authority (NEMA) regulations. Personal protective equipment was used throughout, and hazardous waste was disposed of through approved institutional channels.

**Ethical approval**

Ethical approval of this study was granted by Jomo Kenyatta University of Agriculture and Technology (JKUAT) Institutional Scientific and Ethics Review committee (Approval number: *JKU/ISERC/0216/1712*) and data collection permit sought from National Commission for Science, Technology and Innovation - NACOSTI- (License No: *NACOSTI/P/25/4174280*). The study data privacy and confidentiality were adhered to.

## RESULTS

*Table 1: Physical attributes of crude Naja ashei venom measured in this study and contextualized against published data for African Naja species*

| Parameter | This study | Comparative data in *Naja* spp. |
|---|---|---|
| Appearance and colour | Clear, pale-yellow, viscous fluid | Similar description reported for other African spitting cobras |
| pH (25 °C) | 6.3 ± 0.1 (fresh); 6.8 ± 0.1 (after lyophilization) | Mean 5.77 (range: 5.49–6.02) across 10 *Naja* venoms (Avella et al., 2021). |
| Total protein (mg $mL^{-1}$) | 63.4 ± 2.7 (n = 4; Bradford assay) | 51–159 mg $mL^{-1}$ (mean ≈133 mg $mL^{-1}$) in African *Naja* spp. (Avella et al., 2021). |
| Dynamic viscosity (mPa·s, 100 $s^{-1}$, 25 °C) | 46 ± 5 | 44–151 mPa·s for *Naja pallida* venom, representative of African spitting cobras (Avella et al., 2021). |
| Density (specific gravity) | 1.03 ± 0.01 g $mL^{-1}$ | 1.084 g $mL^{-1}$ reported for *Naja pallida* venom (Avella et al., 2021). |

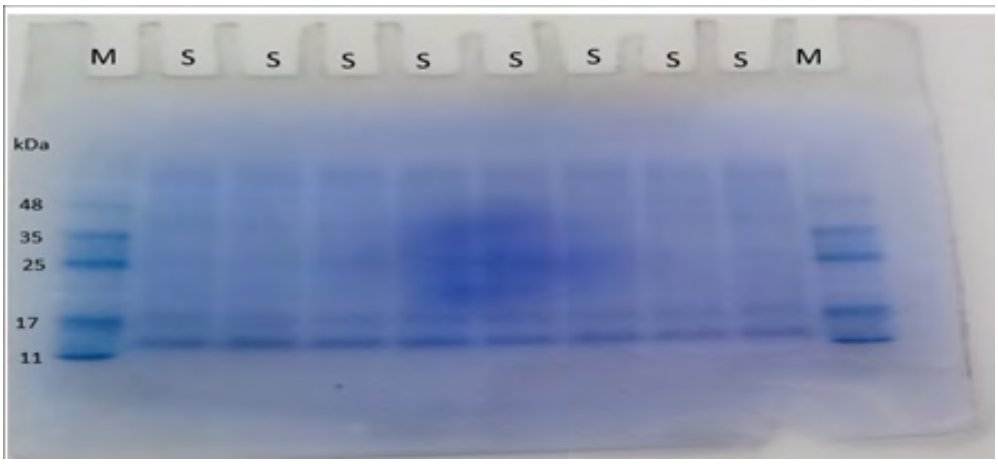



*Figure 1: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of Naja ashei venom proteins*

**Table 2 :** demonstrates that *Naja ashei* venom is predominantly composed of three-finger toxins and phospholipase $A_2$ enzymes

| Protein family | Typical MW range (kDa) | Relative abundance (% of total protein) | Principal biological activity |
|---|---|---|---|
| Three-finger toxins (3FTx) | 6–9 and 12–24 | 69.0 | Cytolytic activity and postsynaptic neurotoxicity |
| Phospholipase $A_2$ ($PLA_2$) | 13–15 | 27.0 | Phospholipid hydrolysis; myotoxic and cytotoxic synergy |
| PIII snake venom metalloproteinases (SVMP) | 60–90 | 2.1 | Extracellular matrix degradation and local haemorrhage |
| Venom nerve growth factor (VNGF) | ≈13 | 1.0 | Neurotrophic signalling and metalloproteinase inhibition |
| Cysteine-rich secretory proteins (CRISP) | 20–25 | 0.7 | Ion-channel modulation |
| Cobra venom factor (CVF) | 150–200 | 0.12 | Complement C3 depletion |
| 5'-Nucleotidase | 55–60 | 0.014 | Adenosine generation and hypotension |

*Table 3: Mortality profile and $LD_{50}$ estimation of Naja ashei venom following intraperitoneal route administration in BALB/c mice.*

| Dose (mg $kg^{-1}$) | Dead | Alive | Mortality (%) | Cumulative % Dead (↓) | Cumulative % Survival (↑) |
|---|---|---|---|---|---|
| 0.50 | 0 | 6 | 0.0 | 50.0 | 100.0 |
| 0.70 | 1 | 5 | 16.7 | 62.5 | 91.7 |
| 0.90 | 3 | 3 | 50.0 | 77.8 | 77.8 |
| 1.10 | 5 | 1 | 83.3 | 91.7 | 62.5 |
| 1.30 | 6 | 0 | 100.0 | 100.0 | 50.0 |

*Table 4: Mortality profile and $LD_{50}$ estimation of Naja ashei venom following intramuscular administration in BALB/c mice.*

| Dose (mg $kg^{-1}$) | Dead | Alive | Mortality (%) | Cumulative % Dead (↓) | Cumulative % Survival (↑) |
|---|---|---|---|---|---|
| 0.50 | 0 | 6 | 0.0 | 53.3 | 100.0 |
| 1.00 | 1 | 5 | 16.7 | 66.7 | 91.7 |
| 1.50 | 4 | 2 | 66.7 | 83.3 | 72.2 |
| 2.00 | 5 | 1 | 83.3 | 91.7 | 58.3 |
| 2.50 | 6 | 0 | 100.0 | 100.0 | 46.7 |

*Table 5: Mortality profile and $LD_{50}$ estimation of Naja ashei venom following subcutaneous administration in BALB/c mice.*

| Dose (mg $kg^{-1}$) | Dead | Alive | Mortality (%) | Cumulative % Dead (↓) | Cumulative % Survival (↑) |
|---|---|---|---|---|---|
| 1.00 | 0 | 6 | 0.0 | 56.7 | 100.0 |
| 1.50 | 2 | 4 | 33.3 | 70.8 | 83.3 |
| 2.00 | 4 | 2 | 66.7 | 83.3 | 66.7 |
| 2.50 | 5 | 1 | 83.3 | 91.7 | 54.2 |
| 3.00 | 6 | 0 | 100.0 | 100.0 | 43.3 |

*Table 6: Comparative $LD_{50}$ values of Naja ashei venom across different administration routes in BALB/c mice.*

| *Route* | *$Ld_{50}$ (mg $kg^{-1}$)* | *Relative to Intraperitoneal (I.P.)* |
|---|---|---|
| *I.P.* | *0.70* | *1×* |
| *I.M.* | *2.36* | *3.4× higher* |
| *S.C.* | *2.69* | *3.8× higher* |

*Table 7: Serum electrolytes and cardiac biomarkers (n = 20 BALB/c mice, 0.5×$LD_{50}$)*

| Analyte (mouse reference range) | Mean ± SD | Range | Abnormal results (%) |
|---|---|---|---|
| $Na^+$ (135–155 mmol $L^{-1}$) | 144 ± 9.4 | 133–167 | 20% >155 |
| $K^+$ (3.5–6.0 mmol $L^{-1}$) | 7.77 ± 2.2 | 3.9–10.0 | 65% >6 |
| Troponin-I (<0.05 ng $mL^{-1}$) | 11.7 ± 5.6 | 2.0–23.0 | 100% elevated |
| CK-MB (100–400 U $L^{-1}$) | 917 ± 122.2 | 568–>1000 | 100% elevated |

*Table 8: Integrated toxicological, biochemical, and histopathological findings following sub-lethal Naja ashei envenomation in BALB/c mice*

| Variable | Intraperitoneal $LD_{50}$ Benchmark | Sub-lethal Exposure |
|---|---|---|
| Cytotoxin/$PLA_2$ load | 0.70 mg $kg^{-1}$ kills 50% of mice (rapid uptake) | 0.25–0.35 mg $kg^{-1}$ used in this study |
| Serum $K^+$ | – | 7.8 ± 2.3 mmol $L^{-1}$ |
| Troponin-I | – | 11.7 ± 6.1 ng $mL^{-1}$ |
| Heart (cardiac tissue) | – | Myocardial necrosis and interstitial oedema |

## DISCUSSION

This study elucidates the toxicological profile of *Naja ashei* venom by integrating biochemical characterization, proteomic profiling, route-dependent lethality, and systemic cardiotoxic effects in a mouse model. The evidence positions *N. ashei* within the wider context of elapid toxins and provides comparative insights with other

cobra species.[14,15,16,17] The physical properties observed for *N. ashei* venom clear, pale-yellow fluid with mildly acidic pH and moderate viscosity agree with proteomic reports for African spitting cobras and other *Naja* species in which low-molecular-weight toxins predominate.[14] The measured venom pH in this study is similar to previously reported profiles, and slight increases after lyophilization are consistent with loss of labile acidic components rather than substantive chemical changes. Protein concentration (~63 mg/mL) is modest relative to some *Naja* venoms but falls within reported ranges for African spitting cobras, where proteomic studies show dominance of three-finger toxins and phospholipase $A_2$ enzymes [14,15]. Similar rheological measures, including viscosity and density, have been observed in *Naja pallida* venom, suggesting shared physical properties among African spitting cobras that may facilitate rapid tissue invasion and toxin dissemination.[15]

Proteomic profiling revealed that low-molecular-weight three-finger toxins account for the vast majority of venom proteins, consistent with *N. ashei* and related species.[14,15] This pattern aligns with other African spitting cobra venoms in which three-finger toxins typically constitute 65–75% of the proteome, with phospholipase $A_2$ as the second most abundant toxin family[16]. The predominance of three-finger toxins explains the potent cytotoxicity and membrane-disrupting effects observed experimentally and clinically, as these toxins form pores in cellular membranes leading to lysis and necrosis.[14] Phospholipase $A_2$ enzymes, though less abundant, act synergistically by destabilizing phospholipid bilayers and facilitating deeper toxin penetration.[15] The comparatively low abundance of snake venom metalloproteinases aligns with clinical observations that *N. ashei* envenomation typically results in marked cytotoxicity with limited hemorrhagic effects compared with viperid envenomation.[16,23]

A key finding is the significant variation in $LD_{50}$ values across administration routes: intraperitoneal (0.70 mg/kg), intramuscular (2.36 mg/kg), and subcutaneous (2.69 mg/kg). This sequence (I.P. < I.M. < S.C.) reflects differences in venom absorption kinetics, where more vascularized compartments facilitate faster systemic access and greater toxicity at lower doses. Similar route-dependent differences have been reported in other snake toxicity studies, including Chinese elapids, where intraperitoneal and intravenous routes consistently yielded lower $LD_{50}$ values than subcutaneous routes[18]. These findings reinforce that $LD_{50}$ is not an intrinsic fixed property of venom but is influenced by administration context and tissue vascularity.[18,19]

Clinically, bites delivering venom into muscle or highly vascular tissues may lead to faster systemic envenoming and more severe early effects compared with superficial dermal bites, corroborating reports that route and depth of envenomation contribute significantly to clinical severity and should be considered in venom pharmacokinetic modelling.[18,19]

Sublethal envenomation resulted in prominent hyperkaliemia in the majority of mice, whereas serum sodium remained largely unaffected. This selective potassium elevation suggests direct membrane disruption and ion efflux rather than dehydration or renal dysregulation. Cobra cytotoxins form non-selective pores in cell membranes, leading to efflux of intracellular cations, including potassium, a mechanism likely underlying the observed hyperkaliemia.[14] Comparable electrolyte imbalances have been described in other toxin-mediated injuries where pore-forming agents induce potassium shifts that

increase arrhythmogenic risk.[12] Such findings are clinically important, as hyperkaliemia exceeding 6.0 mmol/L is associated with cardiac conduction disturbances and potential sudden cardiovascular collapse.[12,13]

This study provides clear evidence of venom-induced cardiotoxicity. Elevated cardiac biomarkers troponin I and creatine kinase-MB were accompanied by histopathological lesions, including myofiber necrosis and interstitial oedema. These findings align with broader venom research showing that elapid venom components, particularly cardiotoxins and phospholipase $A_2$, directly impair cardiomyocyte integrity and function.[12,23] Although cardiotoxicity has been under-recognized in snakebite pathophysiology, clinical reports document electrocardiographic abnormalities following envenomation, including conduction disturbances and arrhythmias, indicating significant cardiovascular involvement in severe cases.[20,22,24] The combination of direct membrane injury and electrolyte imbalance creates a pro-arrhythmic environment that likely contributes to the profound myocardial injury observed in this model.

While this study did not include coagulation assays, existing evidence indicates that $PLA_2$ toxins in cobra venoms can exert anticoagulant effects by interfering with factor Xa and prothrombinase activity, contributing to systemic pathophysiology when venoms reach the circulation. The combined effects of cytotoxicity, cardiotoxicity, and anticoagulation provide a coherent mechanistic framework explaining the patterns of tissue damage and systemic failure observed in severe envenomation's.

## CONCLUSION

This study demonstrates that *Naja ashei* venom possesses a highly potent and complex toxicological profile capable of causing severe local and systemic effects. Proteomic analysis confirmed that the venom is predominantly composed of three-finger toxins and phospholipase $A_2$ enzymes, accounting for its marked cytotoxicity and systemic toxicity. Lethality was shown to be strongly dependent on the route of venom administration, with intraperitoneal exposure producing the lowest LD50, followed by intramuscular and subcutaneous routes, indicating that deeper tissue or internal exposure results in significantly higher toxicity. Envenomation also induced pronounced electrolyte disturbances, particularly hyperkalemia and mild hyponatremia, consistent with extensive muscle damage and cellular disruption. In addition, clear cardiotoxic effects were observed, evidenced by myocardial necrosis, edema, vascular congestion, and elevated cardiac biomarkers, confirming direct venom-induced cardiac injury. Collectively, these findings highlight the potential for rapid cardiovascular compromise following *N. ashei* envenomation and emphasize the need for prompt, aggressive clinical management, even in cases initially presenting with predominantly local tissue involvement.

### Limitations of the study

This study was conducted in a murine model, and while BALB/c mice provide a standardized and reproducible system for venom toxicology, extrapolation of the findings to human envenomation should be made with caution due to interspecies physiological differences. Venom effects were assessed over a limited observation period, which may not fully capture delayed or chronic cardiotoxic and systemic outcomes. Electrocardiographic monitoring and coagulation parameters were not evaluated,

limiting comprehensive cardiovascular and hemostatic assessment. In addition, the study did not assess antivenom neutralization efficacy against *Naja ashei* venom, which would have strengthened translational relevance to clinical management.

**Acknowledgements**

The authors sincerely acknowledge the Small Animal Facility for Research and Innovation (SAFARI), Jomo Kenyatta University of Agriculture and Technology, for providing laboratory and animal research facilities. Appreciation is also extended to Perminus Kimathi and Mary Kimathi who are laboratory technologists at the SAFARI. Appreciation is extended to Bio-Ken Snake Farm, Watamu, Kenya, for provision of venom samples and technical support during venom collection. The authors also thank all laboratory personnel and veterinary staff who contributed to this study.

**REFERENCES**

1. World Health Organization. Snakebite envenoming: A strategy for prevention and control. Geneva: World Health Organization; 2019.
2. Benjamin JM, Chippaux JP, Sampson HA, Warrell DA. Venom composition and clinical effects of African cobra envenomation. Clin Toxicol (Phila). 2020;58(7):641-650.
3. Chippaux JP. Snakebite envenomation turns again into a neglected tropical disease. J Venom Anim Toxins Incl Trop Dis. 2017;23:38.
4. Dyba J, Offor BC, Piater LA, Muller B. Proteomic characteristics of African spitting cobra venom profiles. Sci Rep. 2024;14:69459.
5. Dyba J, Offor BC, Piater LA, Muller B. Three-finger toxin pore-forming dynamics and cytolytic mechanisms in cobra venom. Sci Rep. 2024;14. In press.
6. Dyba J. Venom absorption and systemic toxicokinetics in elapid envenomation. Sci Rep. 2024;14. In press.
7. Farooq U, Rashid MH, Rashid MI, Akram M, Ahmad N. Snakebite envenoming in sub-Saharan Africa: Burden, challenges, and management strategies. Trop Med Infect Dis. 2022;7(10):278.
8. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, et al. Snakebite envenoming. Nat Rev Dis Primers. 2022;8(1):1-23.
9. Halilu S, Iliyasu G, Hamza M, Chippaux JP, Habib AG. Snakebite burden in sub-Saharan Africa: Estimates from available data. Toxicon. 2019;159:109-115.
10. Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG. Snake envenoming: A disease of poverty. PLoS Negl Trop Dis. 2009;3(12):e569.
11. Kalita B, Patra A, Mukherjee AK. Cardiotoxic and biochemical alterations induced by cobra venoms: Experimental evidence from murine models. Toxicol Rep. 2022;9:1120-1129.
12. Khourcha S, El Hattimy F, Chgoury F. Cardiovascular complications of snakebite envenomation: Mechanisms and clinical implications. Front Cardiovasc Med. 2023;10:1154321.
13. Longbottom J, Shearer FM, Devine M, Alcoba G, Chappuis F, et al. Vulnerability to snakebite envenoming: A global mapping of hotspots. Lancet. 2018;392(10148):673-684.
14. Manson P, Wüster W, Broadley DG, Malhotra A. Phylogeography and venom variation in African spitting cobras. Toxins (Basel). 2022;14(2):118.
15. Ochola FO, Okumu MO, Muchemi GM, Mbaria JM. Epidemiology of snakebite envenomation in Kenya: Current status and future perspectives. Toxicon X. 2021;

11:100074.
16. Offor BC, Piater LA, Muller B. The proteome of African spitting and non-spitting cobra venoms and cytotoxicity against pancreatic cancer cells. J Appl Toxicol. 2025;45(10):2055-2067.
17. Okumu MO, Nyongesa AW, Wamola BM. Toxicity profiling and antivenom neutralization of Naja ashei venom. Toxicon. 2020;181:84-92.
18. Otieno MO, Nyongesa AW, Wamola BM. Toxicity and proteome analysis of Naja ashei venom: Evidence of major three-finger toxins and phospholipase A2 influence. Toxicon X. 2022;11:100077.
19. Patra A, Mukherjee AK, Chatterjee U. Determination of LD50 and ED50 of snake venoms and antivenoms: Statistical approaches and applications. J Venom Anim Toxins Incl Trop Dis. 2021; 27:e20200155.
20. Potet J, Smith J, McIver L. Reviewing evidence of the clinical effectiveness of antivenoms in snakebite management. Toxicon X. 2021;11:100077.
21. Pouokam I, Akoachere MB. Cardiac sequelae and troponin elevations in snakebite patients. Pan Afr Med J. 2020;36. In press.
22. Pouokam I, Akoachere MB. Electrocardiographic and biochemical effects of snake envenomation: Clinical enlargements. Pan Afr Med J. 2020;36. In press.
23. Saaiman Engelbrecht A, Piater LA, Offor BC. Comparative proteomics of African spitting cobra venoms highlights cytotoxic and enzymatic toxin families. J Appl Toxicol. 2024;44. In press.
24. Theakston RDG, Warrell DA, Griffiths E. Report of a WHO workshop on the standardization and control of antivenoms. Toxicon. 2003;41(5):541-557.
25. Zhao LX, et al. Administration route influences venom lethality: Comparative toxicokinetics in murine models of snakebite. Toxins (Basel). 2025;17(3). In press.

Wafula E, Gikunju J, Kamanja I, Shiundu BM. Determination of LD50 of Naja Ashei Venom and its Effects on the Heart and Serum Electrolytes in Balb C Mice Through I.P, S.C, and I.M Routes. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 101-113
https://dx.doi.org/ 10.4314/ ajtmbr.v9i1.6

# D-Ribose-L-Cysteine Mitigates Hepato-Renal Dysfunction in a STZ + HFD Rat Model of Diabetes.

[1]Isibor H, [1]Eduviere AT, [1,2]Akpovwre CO, [1]Ben-Azu B.

**Abstract**

**Introduction:** Diabetes mellitus is a major global health concern often associated with oxidative stress–induced organ complications, particularly in the liver and kidneys. D-ribose-L-cysteine (DRLC), a cysteine prodrug and glutathione precursor, has been suggested as a potential antioxidant therapy. This study investigated the ameliorative effects of DRLC on hepato-renal dysfunction in HFD-fed, and Streptozotocin (STZ)-induced diabetic rats.

**Materials and Methods:** Male Wistar rats were randomly assigned to five groups. For 28 days, the experimental groups were treated orally with either HFD+STZ, metformin (100 mg/kg), or DRLC at doses of 150 mg/kg and 300 mg/kg. Biochemical parameters, including oxidative stress biomarkers, liver function indices like Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Gamma-Glutamyl Transferase (GGT), renal markers, proteins (albumin, total protein, globulin), and electrolytes were evaluated.

**Results:** HFD+STZ significantly increased MDA, MPO, NO, liver enzymes, creatinine, urea, and bilirubin, while markedly reducing SOD, CAT, GSH, GST, GPx, albumin, globulin, total protein, and electrolytes ($p < 0.05$), indicating hepato-renal dysfunction. These alterations were significantly mitigated ($p<0.05$) following DRLC treatment, with effects comparable to metformin. DRLC decreased pro-oxidant biomarkers, enhanced antioxidant defense, normalized serum proteins, and restored electrolyte balance.

**Conclusion:** DRLC effectively attenuated oxidative stress and improved liver and kidney function in HFD+STZ-induced diabetic rats, suggesting its therapeutic potential as an antioxidant-based intervention against diabetes-associated organ complications.

**Keywords:** D-Ribose-L-Cysteine, Diabetes Mellitus, High-Fat Diet, Streptozotocin

*1.Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka.*
*2.Department of Pharmacology, School of Basic Medical Sciences, Igbinedion University, Okada*

**Corresponding author:** Isibor Happy, Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka. Email: happy-isibor@delsu.edu.ng.

## INTRODUCTION

Metabolic diseases, such as diabetes, represent a major public health challenge. The combination of a high-fat diet (HFD) and low-dose streptozotocin (STZ) in rodents serves as a validated model for type 2 diabetes, effectively mimicking key clinical features including hyperglycemia, insulin resistance, dyslipidemia, and oxidative stress.[1,2]. This model posits oxidative stress as a key mediator of hepato-renal impairment in diabetes. An overproduction of reactive oxygen species (ROS) depletes endogenous antioxidant capacity, triggering lipid peroxidation and resulting in organ dysfunction[3,4].

The liver, central to metabolic regulation and the kidneys which both manifest and exacerbate metabolic disturbances via glycosuria, oxidative stress, and advanced glycation end-product (AGE) formation are critically impacted[5,6]. In diabetes, both the liver and kidneys undergo profound metabolic disturbances that contribute to disease progression and complications[7-12].

The sustained metabolic and hydraulic stress of diabetes inflicts severe structural damage to the glomerulus. This includes thickening of the glomerular basement membrane (GBM), podocyte injury and loss, and mesangial matrix expansion, which collectively lead to glomerulosclerosis and capillary loss. This structural decline manifests clinically as progressive diabetic nephropathy, beginning with microalbuminuria and advancing through proteinuria to a falling glomerular filtration rate (GFR), culminating in end-stage renal disease (ESRD)[13-19].

D-Ribose-L-cysteine (DRLC), a cysteine prodrug and glutathione precursor, has recently garnered attention as a potent antioxidant intervention. Unlike N-acetylcysteine, DRLC provides cysteine in a stable, bioavailable form while simultaneously supplying ribose to support cellular metabolism[20]. Preclinical studies demonstrate that DRLC elevates intracellular GSH levels, enhances antioxidant enzyme activities, and reduces oxidative stress in models of heavy metal toxicity, high-fat feeding, and neurotoxicity[21,22,23]. Moreover, DRLC admini--stration has been shown to improve liver histology, mitigate renal oxidative injury, and normalize serum markers of hepatic and renal function[22,24]. These findings underscore its therapeutic potential in metabolic disease–related organ dysfunction.

Despite this evidence, limited data exists on the integrated effect of DRLC on hepato-renal parameters in a combined STZ and HFD model of type 2 diabetes, particularly encompassing liver oxidative indices, renal oxidative markers, liver function tests, and electrolyte profiles. Addressing this gap is crucial for understanding whether glutathione restoration translates into measurable biochemical and functional protection. Therefore, this study investigates the effect of D-ribose-L-cysteine on a comprehensive panel of hepato-renal biomarkers including oxidative parameters, functional indices, and electrolyte balance in male Wistar rats exposed to STZ and HFD.

## MATERIALS AND METHODS

### Drugs and Chemicals

DRLC and STZ (Sigma-Aldrich, St. Louis, MO, USA), Antioxidant enzyme assays were conducted using the following kits: Superoxide Dismutase (SOD) Activity Assay Kit from Sigma-Aldrich (catalog no. 19160; St. Louis, MO, USA), Catalase (CAT) Activity Assay Kit from Abcam (catalog no. ab83464; Cambridge, UK), Glutathione (GSH) Assay Kit from Sigma-Aldrich (catalog no. CS0260; St. Louis, MO, USA), Glutathione S-Transferase (GST) Activity Assay Kit from Abcam (catalog no. ab65326; Cambridge, UK), and Glutathione Peroxidase (GPx) Activity Assay Kit from Sigma-Aldrich (catalog no. CGP1; St. Louis, MO, USA). Malondialdehyde (MDA) assay reagents, including thiobarbituric acid (TBA) and trichloroacetic acid (TCA), were sourced from Sigma-Aldrich (St. Louis, MO, USA; catalog no. MAK085). Myeloperoxidase (MPO) activity was measured using a Myeloperoxidase Activity Assay Kit from Abcam (Cambridge, UK; catalog no. ab105136).

**Animal care**

Experimental animals were obtained from the Laboratory Animal Centre, College of Health Science, Delta State University, Abraka, Nigeria. The animals were maintained under standard laboratory conditions and acclimatized for a period of ten days before experimentation. Throughout the study, they were provided with unrestricted access to standard feed and clean water. Ethical approval (RBC/FBMC/ DELSU /25/655) for the study was granted by the Animal Care and Use Research Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

**Experimental Diet**

**Purchase of Animal Feeds**

Groundnut seeds were obtained from a local market in Abraka Latitude: 5.7917° N Longitude: 6.1043° E Delta State, while commercial pelleted feed was sourced from Rainbow Top Feed Company, Amukpe, Latitude: 5.5099° N Longitude: 5.7508° E Sapele, Nigeria.

**Identification**

Groundnut seeds were identified and authenticated in the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria, by Mr. Michael O.E

**Preparation of High-Fat Diet**

The groundnut diet was prepared according to the method of Ossai et al. (2024)[25]. Seeds were cleaned, air-dried, and milled using a manual grinder. The feed was stored under hygienic conditions to prevent pest and mold contamination. To preserve nutrient integrity, fresh diets were prepared weekly.

**Composition of High-Fat Diet**

Eighty grams (80 g) of the milled Groundnut were added to 20 g of the normal commercial animal pellet to make a High Fat Diet.

**Nutritional composition**

Eighty grams (80 g) of the milled Groundnut were added to 20 g of the normal commercial animal pellet to make a high-fat diet of nutrient composition below

| Nutrients | Composition of groundnut in 80 g | Composition of groundnut in 20 g |
|---|---|---|
| Fat | 39.1 | 15.9 |
| Carbohydrate | 19.0 | 2.1 |
| Protein | 21.9 | 2.0 |

**Experimental Design**

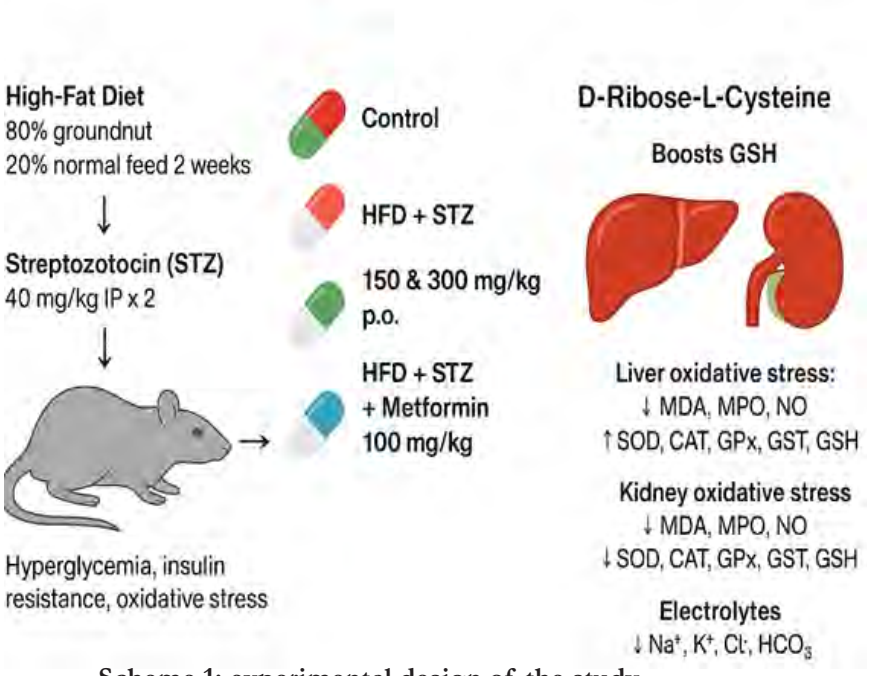
Animals were maintained on a high-fat diet (HFD) for four weeks. On the 14th day of the protocol, they received an intraperitoneal injection (i.p) of streptozotocin (STZ) at 40 mg/kg, followed by a second dose on day 28. The onset of DM was confirmed by measuring fasting blood glucose, with rats classified as diabetic when postprandial glucose levels were ≥288 mg/dL. Male Wistar rats (200–250 g) were randomly assigned to five groups (n = 6). The control group received a standard rodent diet, while the second group was maintained on HFD-STZ only. Groups 3 and 4 were given HFD-STZ, along with oral doses of D-ribose-L-cysteine (DRLC) at 150 mg/kg and 300 mg/kg, respectively. Group five was treated with HFD-STZ and the standard drug, metformin (100

mg/kg body weight)[26]. DRLC was prepared by dissolving 125 mg in 12.5 mL of distilled water to yield a stock solution of 10 mg/mL. The doses of STZ and HFD composition were adapted from an earlier study[25]. Treatments were continued for four weeks, and DRLC was administered orally.

**Sample Collection**

At the end of the experimental protocol, animals were euthanasia under ketamine anesthesia. Blood and serum were collected and stored. Liver and kidney were also isolated on an ice trail, homogenized, centrifuged (10000 rpm for 10 min at 4°C in a phosphate buffer, PBS), and supernatants were stored at -20 °C for analysis of biochemical levels as previously described by Akpovwre et al. (2024)[27].

Liver oxidative parameters (GSH, SOD, CAT, GPx, GST, MDA, MPO, NO ), liver function test parameters (ALT, ALP, AST, and GGT), renal oxidative stress parameters (GSH, SOD, CAT, GPx, GST, MDA, MPO, NO ) renal function markers (creatinine, urea, bilirubin, albumin, and globulin) and serum electrolyte ($Na^+$, $K^+$, $Cl^-$, $HCO^{3-}$) were all assayed for.




**Scheme 1: experimental design of the study**

**Determination of reduced glutathione (GSH), glutathione-s-transferase (GST), glutathione peroxidase (GPx) concentration**

Aliquots of liver and kidney supernatants of rat in the respective treatment groups were taken, and reduced glutathione (GSH), glutathione-s-transferase (GST), and glutathione peroxidase (GPx) concentration was determined using the Moron' method[28].

**Determination of superoxide dismutase (SOD) activity**

The level of SOD activity was determined liver

and kidney supernatants of rat in the respective treatment groups by the method of Misra and Fridovich[29].

**Estimation of catalase activity**

Catalase activity in liver and kidney tissues was determined according to the method previously described by Sinha[30].

**Estimation of brain level of malondialdehyde (MDA)**

The liver and kidney levels of MDA, a biomarker of lipid peroxidation, was estimated according to the method of Adam-Vizi and Seregi[31].

**Determination of brain myeloperoxidase (MPO) activity**

The myeloperoxidase activity in liver and kidney tissues of rat in the respective treatment groups was determined according to the method previously described by Bradley[32].

**Estimation of brain nitrite level**

Liver and kidney nitrite concentration was estimated using Greiss reagent, which serves as an indicator of nitric oxide production.

**Determination of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)**

ALT and AST activities are commonly determined using the Reitman and Frankel colorimetric method[34].

**Determination of Alkaline Phosphatase (ALP)**

ALP was assayed using a colorimetric method based on its ability to hydrolyze p-nitrophenyl phosphate into p-nitrophenol and phosphate in an alkaline medium. The liberated p-nitrophenol produces a yellow color, measured spectrophotometrically at 405 nm. Enzyme activity is proportional to the rate of color formation[35].

**Determination of Gamma-Glutamyl Transferase (GGT)**

GGT activity is determined by its transfer of the γ-glutamyl group from the substrate γ-glutamyl-p-nitroanilide to glycylglycine, releasing p-nitroaniline. The formation of p-nitroaniline is monitored spectrophotometrically at 405 nm, with activity expressed in IU/L. This assay is highly sensitive for hepatobiliary dysfunction[36].

**Determination of Creatinine, Urea, Bilirubin, Albumin, Total protein, And Globulin**

The biochemical parameters were determined using standard spectrophotometric and enzymatic methods. Serum creatinine was assayed based on the Jaffe reaction[37]. Urea concentration was determined by the urease–Berthelot method[38]. Serum bilirubin was estimated by the diazo method[39]. Albumin concentration was measured by the bromocresol green (BCG) dye-binding method, and globulin concentration was calculated as the difference between total serum protein and albumin levels[40].

**Determination of serum electrolyte ($Na^+$, $K^+$, $Cl^-$, $HCO^{3-}$)**

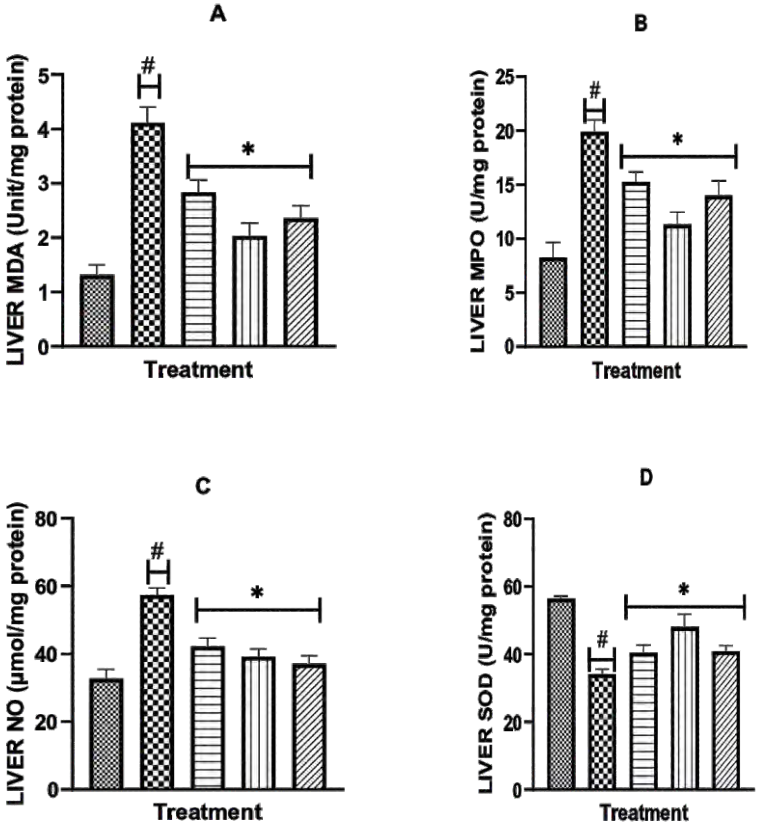
Electrolyte estimation was performed using an ion-selective electrode method. Sodium, potassium, and chloride ions were measured directly by their respective electrodes and reported in mmol/L. Serum bicarbonate ($HCO^{3-}$) was estimated as total $CO^2$ by enzymatic methods, with values reflecting the principal buffer base in plasma[41].

## RESULTS

### Effect of D-ribose-L-cysteine on Liver oxidative biomarkers in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on liver oxidative biomarkers is shown in **Figures 1(A-H), respectively**. One-way ANOVA from figures 1A-C showed that there was significant difference between treatment groups. Post hoc analysis using Tukey's post hoc test revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) increase in prooxidant biomarkers (MDA, MPO and NO) when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) decrease in prooxidant biomarkers (MDA, MPO and NO) when compared with the (HFD+ STZ) group only.

Post hoc analysis using Tukey's post hoc test from figure 1D-H revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) decrease in antioxidant biomarkers (SOD, CAT, GSH, GST, GPx) when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) increase in prooxidant biomarkers (SOD, CAT, GSH, GST, GPx) when compared with the (HFD+ STZ) group only.



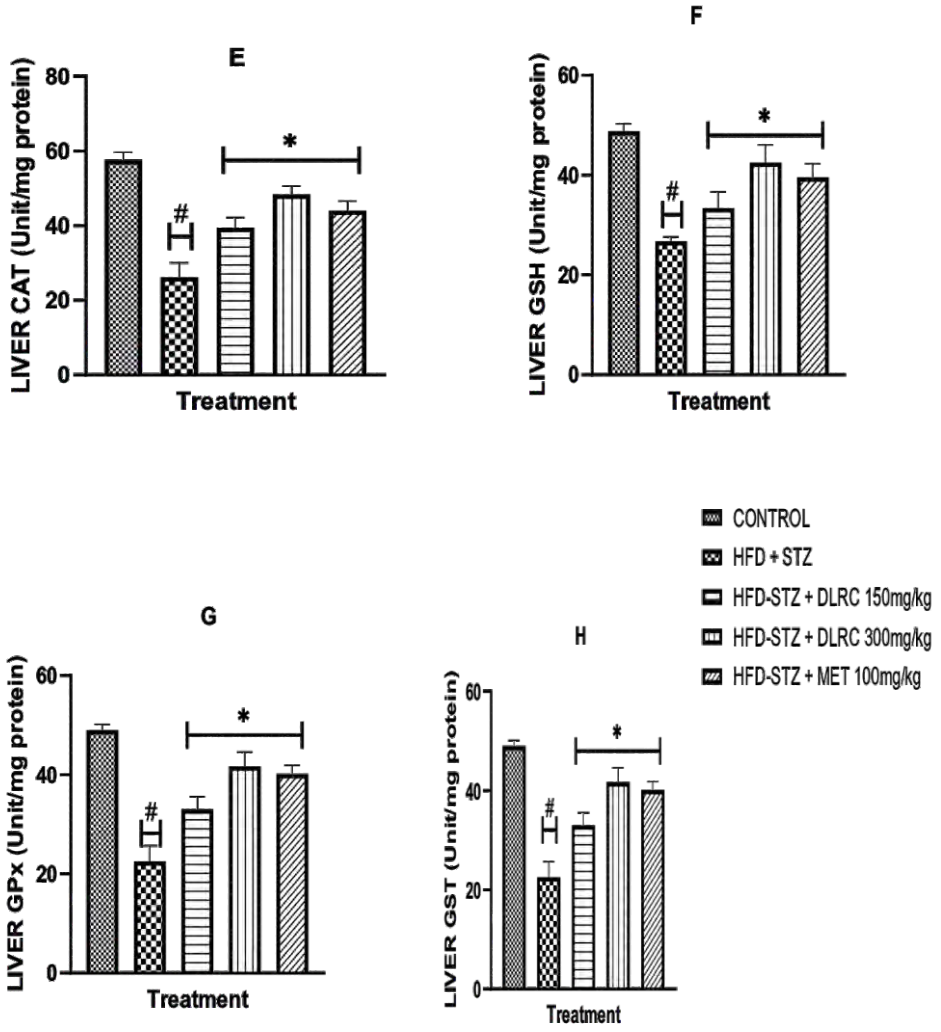



**Figure 1: Effect of D-ribose-L-cysteine on liver (A) malondialdehyde (MDA), (B) myeloperoxidase (MPO), (C) nitric oxide (NO), (D) superoxide dismutase (SOD), (E) catalase (CAT), (F) reduced glutathione (GSH), (G) glutathione peroxidase (GPx), (H) glutathione-S-transferase (GST) in rats exposed to STZ and HFD**

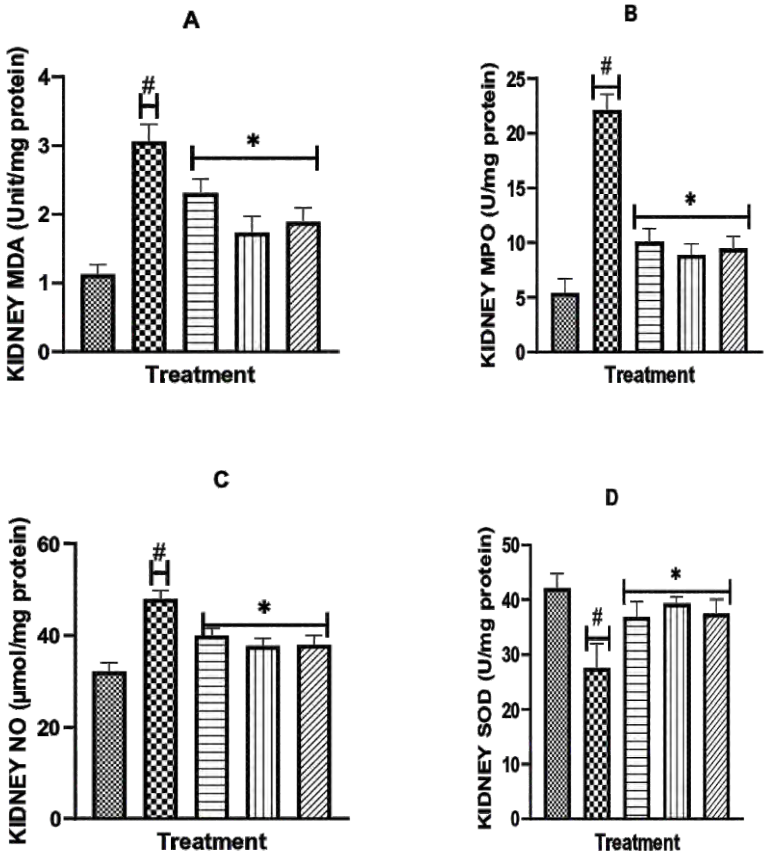
Values represent the mean ± S.E.M for 5 animals per group
# $p<0.05$ compared to the control group (ANOVA followed by Tukey's *post hoc* test).
* $p<0.05$ compared to the pathologic group (ANOVA followed by Tukey's *post hoc* test).

**Effect of D-ribose-L-cysteine on Renal oxidative biomarkers in rats exposed to STZ and HFD**

The effect of D-Ribose-L-Cysteine given daily for 28 days on renal oxidative biomarkers is shown in **Figures 2A-H, respectively**. One-way ANOVA from figures 2A-C showed that there was significant difference between treatment groups. Post hoc analysis using Tukey's post hoc test revealed that the high fat diet and

streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) increase in prooxidant biomarkers (MDA, MPO and NO) when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) decrease in prooxidant biomarkers (MDA, MPO and NO) when compared with the (HFD+ STZ) group only.

Post hoc analysis using Tukey's post hoc test from figure 2D-H revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) decrease in antioxidant biomarkers (SOD, CAT, GSH, GST, GPx) when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) increase in prooxidant biomarkers (SOD, CAT, GSH, GST, GPx) when compared with the (HFD+ STZ) group only.



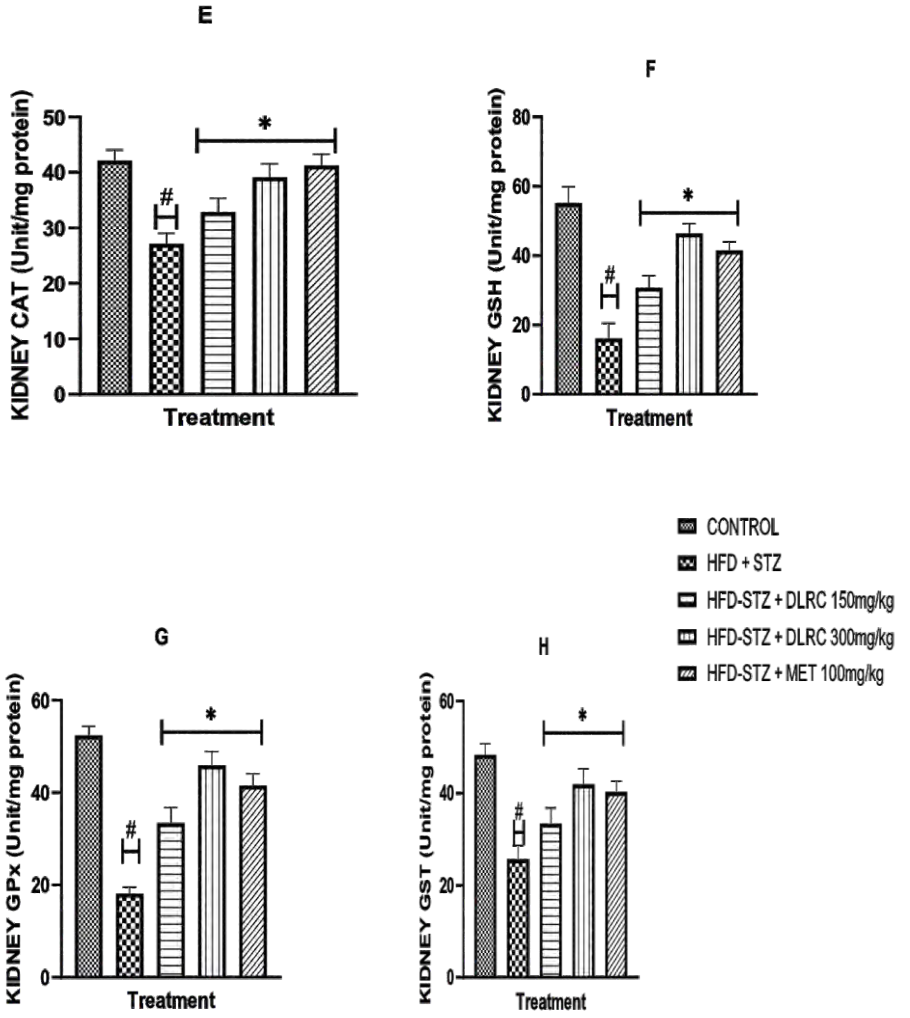



**Figure 2: Effect of D-ribose-L-cysteine on kidney (A) malondialdehyde (MDA), (B) myeloperoxidase (MPO), (C) nitric oxide (NO), (D) superoxide dismutase (SOD), (E) catalase (CAT), (F) reduced glutathione (GSH), (G) glutathione peroxidase (GPx), (H) glutathione-S-transferase (GST) in rats exposed to STZ and HFD**

Values represent the mean ± S.E.M for 5 animals per group

# $p<0.05$ compared to the control group (ANOVA followed by Tukey's *post hoc* test).

* $p<0.05$ compared to the pathologic group (ANOVA followed by Tukey's *post hoc* test).

**Effect of D-ribose-L-cysteine on liver function test in rats exposed to STZ and HFD**

The effect of D-Ribose-L-Cysteine given daily for 28 days liver function test parameters was shown in **Figure 3A-D respectively**. One-way ANOVA from figure 3A-D showed that there was significant difference between treatment groups. Post hoc analysis using Tukey's post hoc test revealed that the high fat diet and streptozotocin

(HFD + STZ) group showed a significant ($p<0.05$) increase in liver function parameters (ALT, ALP, GGT, and AST) when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) decrease in liver function parameters ALT, ALP, GGT, and AST) when compared with the (HFD+ STZ) group only.

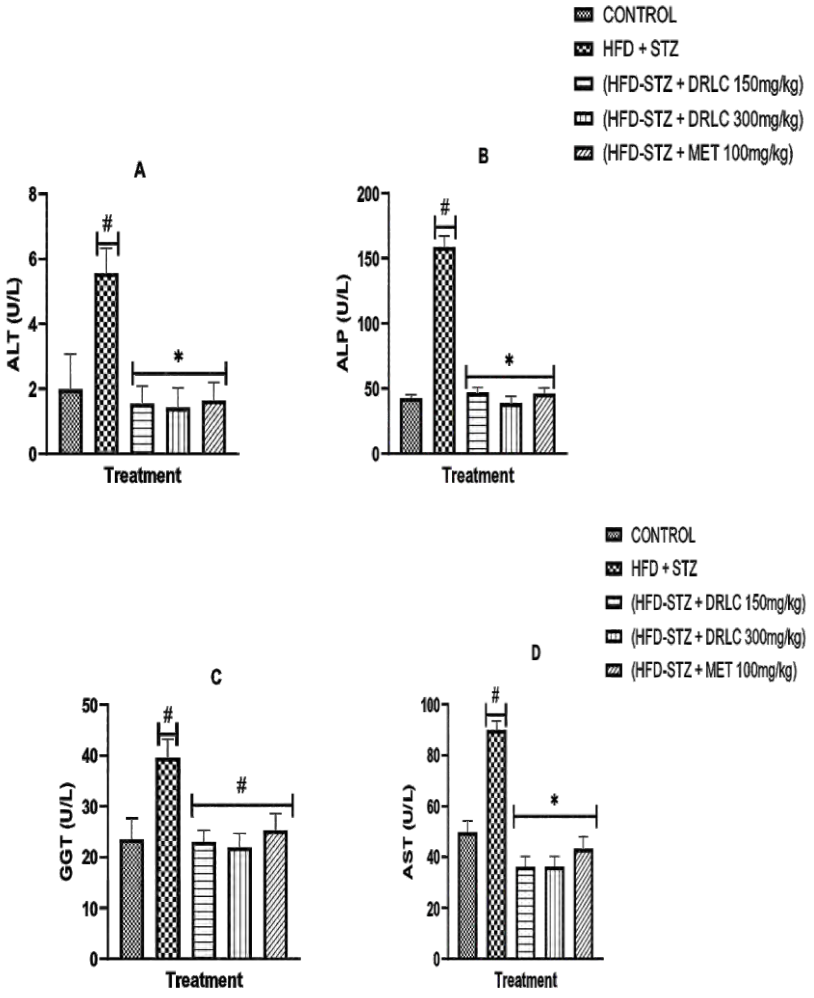



**Figure 3: Effect of D-ribose-L-cysteine on (A) Alanine Aminotransferase, (B) Alkaline Phosphatase, (C) Gamma-Glutamyl Transferase, (D) Aspartate Aminotransferase in rats exposed to STZ and HFD**

Values represent the mean ± S.E.M for 5 animals per group

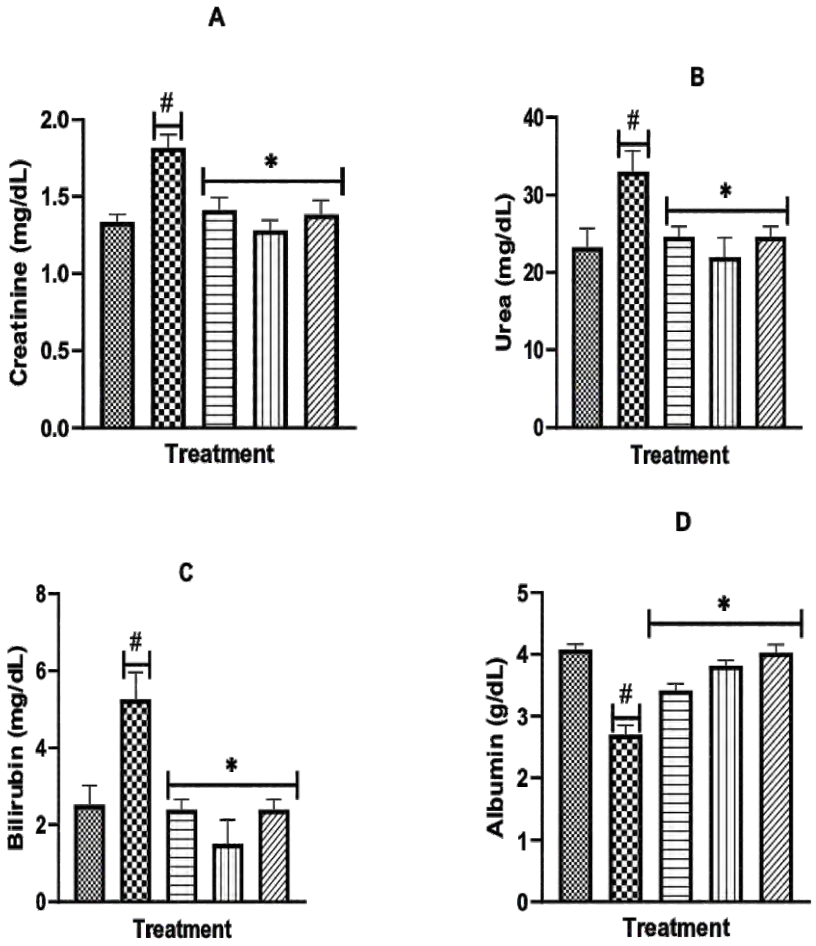
# $p<0.05$ compared to the control group (ANOVA followed by Tukey's *post hoc* test).

* $p<0.05$ compared to the pathologic group (ANOVA followed by Tukey's *post hoc* test).

**Effect of D-ribose-L-cysteine on Creatinine, Urea, Bilirubin, Albumin, Total protein And Globulin in rats exposed to STZ and HFD**

The effect of D-Ribose-L-Cysteine given daily for 28 days on liver function test parameters was shown in **Figures 4A-F, respectively**. One-way ANOVA from figures 4A-C showed that there was significant difference between treatment groups. Post hoc analysis using Tukey's post hoc test revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) increase in Creatinine, Urea, Bilirubin when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) decrease in Creatinine, Urea, Bilirubin when compared with the (HFD+ STZ) group only.

Post hoc analysis using Tukey's post hoc test from 4D-F revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant ($p<0.05$) decrease in Albumin, Total protein And Globulin when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant ($p<0.05$) increase in Albumin, Total protein And Globulin when compared with the (HFD+ STZ) group only.



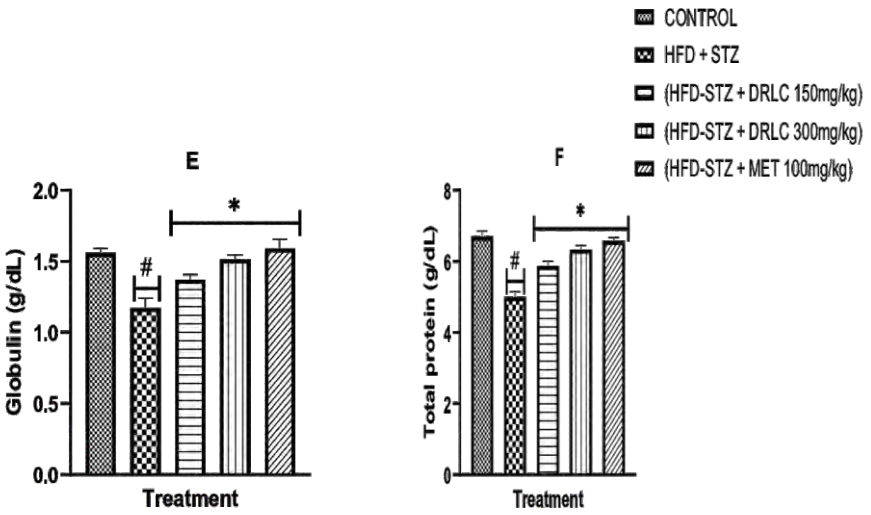
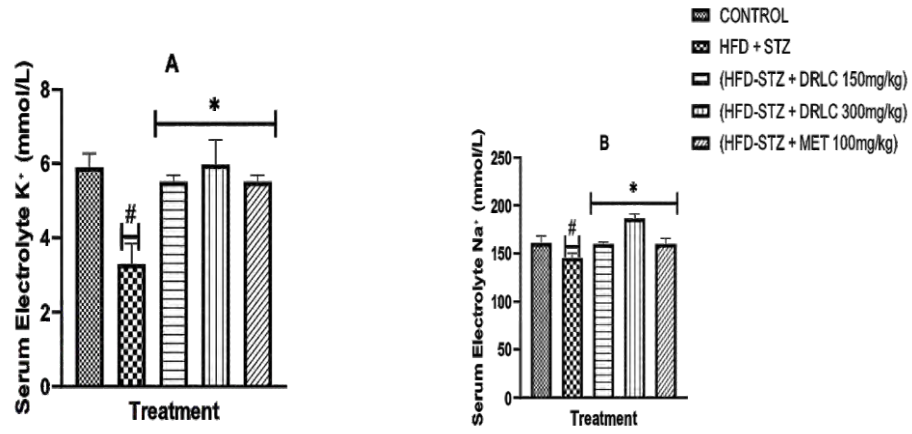



**Figure 4: Effect of D-ribose-L-cysteine on (A) creatinine, (B) urea, (C) bilirubin, (D) Albumin, (E) globulin, (F) total protein in rats exposed to STZ and HFD**
Values represent the mean ± S.E.M for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's *post hoc* test).
* p<0.05 compared to the pathologic group (ANOVA followed by Tukey's *post hoc* test).

**Effect of D-ribose-L-cysteine on Serum Electrolytes in rats exposed to STZ and HFD**

The effect of D-Ribose-L-Cysteine given daily for 28 days on liver function test parameters is shown in **Figures 5A-D, respectively**. One-way ANOVA from figures 5 A-D showed that there was significant difference between treatment groups. Post hoc analysis using Tukey's post hoc test revealed that the high fat diet and streptozotocin (HFD + STZ) group showed a significant (p<0.05) decrease in **serum electrolyte** when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant (p<0.05) increase in **serum electrolytes** when compared with the (HFD+ STZ) group only.



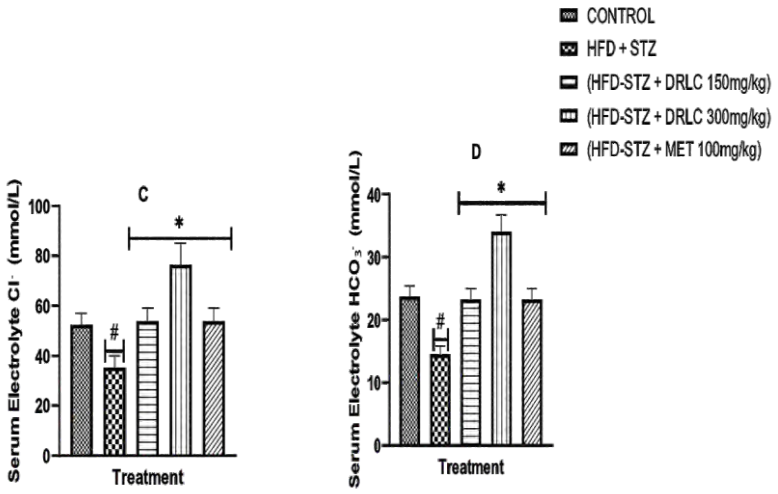



**Figure 5: Effect of D-ribose-L-cysteine on (A) potassium ion, (B) sodium ion, (C) chloride ion, (D) biocarbonate ion in rats exposed to STZ and HFD**
Values represent the mean ± S.E.M for 5 animals per group
# $p<0.05$ compared to the control group (ANOVA followed by Tukey's *post hoc* test).
* $p<0.05$ compared to the pathologic group (ANOVA followed by Tukey's *post hoc* test).

## DISCUSSIONS

The present findings show that combined High-Fat Diet (HFD) and Streptozotocin (STZ) produced a clear hepato–renal oxidative and functional derangement characterized by increased lipid peroxidation and pro-oxidant activity (MDA, MPO, NO), suppression of enzymatic and non-enzymatic antioxidants (SOD, CAT, GSH, GST, GPx), and concurrent impairment of liver and kidney function (elevated ALT, AST, ALP, GGT, creatinine, urea, bilirubin; reduced albumin, total protein, and globulin, and disturbed electrolytes). These changes are consistent with an imbalance between reactive oxygen/nitrogen species generation and endogenous antioxidant defenses induced by metabolic stress and β-cell toxin exposure, and they mirror what has been reported in a diet- and toxin-induced model of metabolic and oxidative injury[24]. Administration of D-ribose-L-cysteine (DRLC) at both 150 and 300 mg/kg for 28 days, and metformin (100 mg/kg), substantially reversed these pathological alterations: DRLC lowered pro-oxidant markers, restored antioxidant enzyme activities in liver and kidney, reduced markers of hepatocellular and renal injury, and improved serum protein and electrolyte profiles. The effect of DRLC on pro-oxidant and antioxidant markers is similar to the effect seen in Ejiro et al. (2025)[21], where coadministration of lead with DRLC led to significantly ($p<0.05$) reduced MDA levels when compared to the negative control group, and significantly ($p<0.05$) improved antioxidant defense in the kidneys of the mice exposed to lead for a period of 21 days. Taken together, these data indicate that DRLC exerts a multifaceted protective effect on hepato-renal systems in the HFD+STZ model.

Mechanistically, the protective profile observed here is biologically plausible because DRLC is a

cysteine-donor pro-drug that supports intracellular glutathione (GSH) synthesis[22], thereby replenishing the major cellular thiol buffer and enabling recovery of downstream antioxidant enzyme activities and redox-sensitive pathways. Several recent preclinical reports show comparable patterns[24,42] DRLC or riboceine supplementation increases tissue GSH, reduces MDA and nitrosative stress, and attenuates pro-inflammatory cytokines in models of heavy-metal[22], xenobiotic or diet-induced oxidative injury[43], supporting the fact that enhanced GSH biosynthesis underpins the observed normalization of antioxidant indices and reduced lipid peroxidation. The parallel benefit of metformin in the current experiment provides a useful comparator: metformin's protective effects in metabolic disease models have been attributed in part to reductions in mitochondrial ROS generation and indirect enhancement of cellular antioxidant responses[44], so the similar direction of effect seen with DRLC suggests complementary, perhaps synergistic, mechanisms — metformin improving metabolic flux and mitochondrial redox tone, and DRLC directly increasing the glutathione pool and thiol-dependent detoxification. This idea is consistent with broader recent hypotheses arguing that bolstering glutathione and its precursors is a rational strategy to prevent or reduce tissue damage in conditions of metabolic stress and early diabetes[45,46]. Also, restoration of serum proteins and reduction in transaminases after DRLC indicate not only reduced oxidative injury but also partial recovery of synthetic and metabolic liver function — an important translational signal when considering hepato-renal protection[47]. Thirdly, the renal improvements (electrolyte normalization, lower creatinine/urea) suggest DRLC protects not just by systemic antioxidant replenishment but also by preserving tubular and glomerular integrity in the context of metabolic-toxic stress.

In summary, the data supports that DRLC ameliorates HFD+STZ-induced oxidative stress and associated hepato-renal dysfunction, most likely through augmentation of glutathione-dependent antioxidant defenses and suppression of pro-oxidant and pro-inflammatory cascades. These findings align with recent preclinical work showing DRLC/riboceine raises tissue GSH, lowers lipid peroxidation and inflammatory mediators, and improves organ-specific functional endpoints, and they fit within a growing conceptual framework that glutathione precursors can be protective in early metabolic disease. Taken together, DRLC merits further mechanistic and translational study as a candidate adjunctive strategy to reduce oxidative injury and preserve liver and kidney function in metabolic and toxin-related disease models.

## REFERENCE

1. Phillips S, Feola A, Solomon J, Cardelle L, Douglass A, Bales KL, et al. Retinal and metabolic changes in a high-fat diet (HFD)+STZ model of Type II diabetes. Mol Vis. 2024;30:e239.
2. Brito AK, Mendes AV, Timah-Acha B, Santos-Oliveira AS, Lopes-Macedo J, Suzuki-Cruzio A, et al. Experimental Models of Type 2 Diabetes Mellitus Induced by Hyperlipidemic Diet (HFD) and Streptozotocin Administration in Rats: An Integrative Review. Biomedicines. 2025; 13(5):1158.
3. Abozaid OA, Moawed FS, Gabr HF, Esmat MA. Combined Effects of Metformin, Quercetin, and Fractionated Gamma Irradiation on MiR-107-Mediated Brain Injury in HFD/STZ-Induced Diabetic Rats. Dose-Response. 2025;23(3):e1559 3258251367627.
4. Ha E, Alhakami A, Alqahtani S, Alqahtani Y, Shati A, Almohaimeed H. Mesenchymal Stem Cells Attenuate Renal Microscopic Alterations in Induced Diabetic

Nephropathy in Rats through Suppression of Oxidative Stress, Inflammation, Apoptosis and Upregulation of Nrf2/PPAR-γ Inflammatory Signaling Pathway. Int J Morphol. 2025;43(1):226-236.
5. Maiorana A, Tagliaferri F, Dionisi-Vici C. Current understanding on pathogenesis and effective treatment of glycogen storage disease type Ib with empagliflozin: new insights coming from diabetes for its potential implications in other metabolic disorders. Front Endocrinol. 2023;14: e1145111.
6. Kumar M, Dev S, Khalid MU, Siddenthi SM, Noman M, John C, et al. The bidirectional link between diabetes and kidney disease: mechanisms and management. Cureus. 2023;15(9):e45615.
7. Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. Nat Rev Endocrinol. 2022;18(9):525-539.
8. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative stress in liver pathophysiology and disease. Antioxidants. 2023;12(9):e1653.
9. Hanke J, Romejko K, Niemczyk S. Sodium-Glucose Cotransporter-2 Inhibitors in Diabetes and Beyond: Mechanisms, Pleiotropic Benefits, and Clinical Use—Reviewing Protective Effects Exceeding Glycemic Control. Molecules. 2025;30(20):e4125.
10. Ravindran S, Munusamy S. Renoprotective mechanisms of sodium glucose co transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. J Cell Physiol. 2022;237(2):1182-1205.
11. Pardawala N, Al Hassan M, Bahig J, Szaszi K, Doan H, Shoker A, et al. Exploring Glomerular Filtration Mechanisms and Molecular Pathways: Insights for Advancing Hemodialysis Membrane Design. ACS Biomater Sci Eng. 2025. [Epub ahead of print]
12. Yang Y, Xu G. Update on pathogenesis of glomerular hyperfiltration in early diabetic kidney disease. Front Endocrinol. 2022; 13:e872918.
13. Wu T, Ding L, Andoh V, Zhang J, Chen L. The mechanism of hyperglycemia-induced renal cell injury in diabetic nephropathy disease: an update. Life. 2023;13(2):e539.
14. Ebefors K, Lassén E, Anandakrishnan N, Azeloglu EU, Daehn IS. Modeling the glomerular filtration barrier and intercellular crosstalk. Front Physiol. 2021;12:e689083.
15. Thomas HY, Ford Versypt AN. Pathophysiology of mesangial expansion in diabetic nephropathy: mesangial structure, glomerular biomechanics, and biochemical signaling and regulation. J Biol Eng. 2022;16(1):e19.
16. Thomas HY. Computational Modeling of Mesangial Expansion and Glomerular Fibrosis in Diabetes [dissertation]. State University of New York at Buffalo; 2022.
17. Wang N, Zhang C. Recent advances in the management of diabetic kidney disease: slowing progression. Int J Mol Sci. 2024;25(6):e3086.
18. Ahmed K, Dubey MK, Dubey S, Pandey DK. Chronic kidney disease: Causes, treatment, management, and future scope. In: Computational Intelligence for Genomics Data. Academic Press; 2025:99-111.
19. Pottanat N, Friederich A, LaMotte J. End-Stage Kidney Disease, Dialysis, and Transplant Basics. In: Psychosocial Considerations in Pediatric Kidney Conditions: Guidance for Collaborative Practice. Cham: Springer Nature Switzerland; 2024: 67-94.
20. Obukohwo OM, Faith FY, Ejiro OP, Onyebuchi MM, Ajayi AF. Medicinal applications of d-ribose l-cysteine in neuro-endocrinopathy, reproductive dysfunction and cardio-metabolic syndrome: A review of evidence in animal investigations. Eur J Med Chem Rep. 2024;11:e100153.
21. Ejiro A, Ikechukwu AA. Nephroprotective

Effect of D-Ribose-L-Cysteine (DRLC) On Renal Oxidative Stress and Proinflammatory Markers in Lead Acetate-Treated Mice. Trop J Chem. 2025;1(1):26-30.
22. Isibor H, Ajayi AM, Ben-Azu B, Omeiza NA, Ademola AP, Umukoro S. D-ribose-L-cysteine reduces oxidative stress and inflammatory cytokines to mitigate liver damage, and memory decline induced by copper sulfate in mice. J Trace Elem Med Biol. 2022;73:127001.
23. Ramos V, Reis M, Ferreira L, Silva AM, Ferraz R, Vieira M, et al. Stalling the course of neurodegenerative diseases: could cyanobacteria constitute a new approach toward therapy? Biomolecules. 2023;13 (10):e1444.
24. Ojetola AA, Adedeji TG, Fasanmade AA. Changes in antioxidants status, atherogenic index and cardiovascular variables after prolonged doses of D-ribose-L-cysteine in male Wistar rats. Heliyon. 2021;7(2):e06113.
25. Ossai NR, Ojieh AE, Nwangwa EK, Nwogueze BC. Attenuating Potential of Modified Diets on Testicular Inflammatory Biomarkers in Streptozotocin-Induced Diabetic Wistar Rats. Pak Heart J. 2024; 57(01):297-318.
26. Hacioglu C, Kar F, Kara Y, Yucel E, Donmez DB, Sentürk H, et al. Comparative effects of metformin and Cistus laurifolius L. extract in streptozotocin-induced diabetic rat model: oxidative, inflammatory, apoptotic, and histopathological analyzes. Environ Sci Pollut Res. 2021;28(41):57888-57901.
27. Akpovwre CO, Eduviere AT, Isibor H, Edje KE, Ewhre LO, Akinsola AO, et al. Theaflavin Alleviates Memory Deficit and Anxiety-Like Phenotypes in Valproic Acid Murine Model of Autism: Impact on Cholinergic and Oxido-Nitrigic Stress Mechanisms. Trop J Nat Prod Res. 2024; 8(12):9345-9355.
28. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta Gen Subj. 1979;582(1):67-78.
29. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-3175.
30. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-394.
31. Ádám-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na, K-ATPase in rat brain homogenate: Role of lipid peroxidation. Biochem Pharmacol. 1982;31(13):2231-2236.
32. Bradley PP, Christensen RD, Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood. 1982; 60(3):618-622.
33. Green CL. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Infect Immun. 1994;62:e1171.
34. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28(1):56-63.
35. Tang Z, Chen H, He H, Ma C. Assays for alkaline phosphatase activity: Progress and prospects. TrAC Trends Anal Chem. 2019;113:32-43.
36. Goldberg DM, Martin JV. Role of γ-glutamyl transpeptidase activity in the diagnosis of hepatobiliary disease. Digestion. 1975;12(4-6):232-246.
37. Chromý V, Rozkošná K, Sedlak P. Determination of serum creatinine by Jaffe method and how to calibrate to eliminate matrix interference problems. Clin Chem Lab Med. 2008;46(8):1127-1133.
38. Taylor AJ, Vadgama P. Analytical reviews in clinical biochemistry: the estimation of urea. Ann Clin Biochem. 1992;29(3):245-264.
39. Thaler M, Luppa PB, Schlebusch H. Bilirubin measurement-an updated survey.

Laboratoriums Medizin. 2008;32(1):1-9.
40. Adair GS, Robinson ME. The specific refraction increments of serum-albumin and serum-globulin. Biochem J. 1930; 24(4):e993.
41. Burnett RW, Covington AK, Fogh-Andersen N, Külpmann WR, Lewenstam A, Maas AH, et al. Use of ion-selective electrodes for blood-electrolyte analysis. Recommendations for nomenclature, definitions and conventions. J Int Fed Clin Chem. 1994;6(4):154-159.
42. Chidebe EO, Moke EG, Asiwe JN, Ben-Azu B, Demaki WE, Oritsemuelebi B, et al. Therapeutic effect of coenzyme-Q10 pretreatment on isoprenaline-induced cardiogenic hepatorenal complications in rats. Int J Physiol Pathophysiol Pharmacol. 2024;16(6):126-138.
43. Jayanti S, Vitek L, Verde CD, Llido JP, Sukowati C, Tiribelli C, et al. Role of natural compounds modulating Heme catabolic pathway in gut, liver, cardiovascular, and brain diseases. Biomolecules. 2024;14(1):63.
44. Buczyńska A, Sidorkiewicz I, Krętowski AJ, Adamska A. Examining the clinical relevance of metformin as an antioxidant intervention. Front Pharmacol. 2024;15:31330797.
45. Dawi J, Misakyan Y, Affa S, Kades S, Narasimhan A, Hajjar F, et al. Oxidative stress, glutathione insufficiency, and inflammatory pathways in type 2 diabetes mellitus: Implications for therapeutic interventions. Biomedicines. 2024;13(1):e18.
46. Tuell D, Ford G, Los E, Stone W. The Role of glutathione and its precursors in type 2 diabetes. Antioxidants. 2024;13(2):e184.
47. Li Z, Lin D, Miao J, Fan J, Lu F, Zhang X, et al. Effects of transcatheter tricuspid valve replacement on hepatic and renal function in severe TR. IJC Heart Vasc. 2025;59:e101714.

Isibor H, Eduviere AT, Akpovwre CO, Ben-Azu B.. D-Ribose-L-Cysteine Mitigates Hepato-Renal Dysfunction in a STZ + HFD Rat Model of Diabetes. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 114-130
https://dx.doi.org/10.4314/ajtmbr.v9i1.7

*Review Articles*

# Prevalence of Non-Communicable Diseases in Nigeria; a Nine Year Systematic Review

Abdulshahid Sarki[1], Hamza Bello[2], Oluwatomi V. Adara[3], Opeyemi Abolarin[4], Kallah Patience Asa[5], Alabi Taofiqat Ikeoluwa[6], Babangida Fatima Mana[7], Izu Edesiri Godswill[8], Tessy Theodore [9], Samaila Muhammad[10].

**ABSTRACT**

**Introduction**: Globally, Non-Communicable Diseases are responsible for 41 million Deaths each year, which contribute to 74% of all death. Furthermore, 17 million people die before they reach age 70, and 86% of these premature deaths occurs in low to middle income countries like Nigeria. This Systematic review aimed at studying the status of Non-communicable disease in Nigeria, a nine year review, from January 2015 to December 2024.

**Materials and Methods**: A relevant studies via systematic searched of PubMed and Google scholar using the terms prevalence, non-communicable diseases and Nigeria. The search was limited to studies published between January 1st, 2015 and December 1st, 2024.

**Results**: The total sample size of all studies included in the meta-analysis was 138,818 subjects. The overall prevalence of non-communicable disease in the Nigeria was estimated to be 12.9 % (95 % CI: 9.3 % - 17.6 %). The subgroup analysis illustrated that the highest prevalence rate of non-communicable disease in Nigeria was related to the south-south zone with 19.3 % (95% CI: 1.0 – 33.9%), followed by south west 18.3% (95% CI: 1.22 – 26.7%) , north west 11.0%(95% CI: 5.4 – 21.1%), south east 8.4% (95% CI: 3.3 – 19.9%), north central 4.5% (95% CI: 1.9 – 10.2%) and north east 1.1% (95% CI: 1.0 – 1.3%).

**Conclusion:** The pooled prevalence of Non-communicable disease in our study was found to be 12.9%, with South-south having the highest prevalence of 19.3% and while the North-east having the least prevalence of 1.1%, which underscores the surge of Non-communicable diseases in Nigeria.

*1.Department Of Internal Medicine, National Hospital Abuja.*
*2.Department of Obstetrics and Gynecology, University of Maiduguri Teaching Hospital.*
*3.Department of Public Health, Ahmadu Bello University.*
*4.Geospatial Analytics Technology, Abuja.*
*5.Department of Family Medicine, Federal Medical Center Jalingo.*
*6.Department of Ophthalmology, Ahmadu Bello University Teaching Hospital, Zaria.*
*7.Department of Hospital Services, Federal Ministry of Health and Social Welfare, Abuja.*
*8.Department of Public Health, Federal Ministry of Health and Social Welfare.*
*9.KNCV Foundation, Nigeria.*
*10.Department of Nursing services, Usman Danfodiyo University Teaching Hospital.*

**Corresponding author:** Abdulshahid Sarki, Department of Internal Medicine , National Hospital Abuja. Email: sarki442@yahoo.com,

## INTRODUCTION

Non-Communicable Diseases are increasingly becoming major causes of morbidity and mortality in Nigeria. Based on the 1990-1992 national survey, the prevalence of hypertension was 11.2% (4.3 million Nigerians aged 15 years and above) while that of diabetes mellitus was 2.7% (1.05 million Nigerian 15 years and above). However, with the shift in paradigm using a cut-off point of 140/90 mmHg in 1999 by World Health Organization and International Society for Hypertension, the prevalence of hypertension

now exceed 20%.[1]

Non-communicable disease include hypertension, diabetes mellitus, cardiovascular diseases, mental illness, chronic respiratory diseases, chronic kidney diseases, cancers. Globally, NCD are responsible for 41 million Deaths each year, which contribute to 74% of all death. Furthermore, 17 million people die before they reach age 70, and 86% of these premature deaths occurs in low to middle income countries like Nigeria.[2]

Non-communicable diseases pose a threat towards the 2030 Agenda for Sustainable Development, which include targets of reducing probability of death from any of the NCDs between ages 30 and 70 years by one-third by 2030.[3] In low to lower-middle income countries where most of the payment of healthcare is out-of-pocket or by donors.[4] Healthcare cost of Non-communicable disease easily deplete household resources with deleterious consequences. The exorbitant cost of managing NCDs plunge millions of people into poverty.[5]

In Nigeria, NCDs have taken a bigger share of diseases due to numerous reasons including Globalization, urbanization, life style, and socio-economic issues. Nigeria is among the countries that are expected to have a surge of this NCDs as a percentage of disease burden, but they spend less than expected even after including aid from external donors.[6] In Nigeria about 30% of all death are due to NCDs.[7] The risk of premature death from NCDs is quite substantial to 22%.[8] They accounted for 567 deaths/100,000 (age standardized) in 2019[8], overtaking the previous predominant causes: communicable, maternal, neonatal and nutritional. Specifically, CVD as a group account for 10% of death and 3.8% of disability-adjusted life years (DALYs).[9] The DALYs loss to NCDs in general increases tremendously by approximately 21.3% from 24,987 in 2010 to 30,306 in 2019 compared to 6.5% DALYs lost to infectious disease over the same period.[10] This scenario presents an opportunity for intervention at many levels, from the primary healthcare level up to the tertiary centers in the country, with adequate training of healthcare staff the surge of NCDs can be mitigated and its financial burden to the individual and country can be reduce significantly.[11]

Nigeria is undergoing epidemiological transition, with emergences of NCDs, adding to the burden of diseases in the country, people due to urbanization often engage in sedentary life style, consumption of unhealthy diet, use of tobacco products. These risk factors as well as rapidly growing and aging population are increasing the speed of the change from communicable diseases, maternal, neonatal and nutritional diseases to Non-communicable diseases.[12]

The major risk factors of CVDs include physical inactivity, hypertension, obesity, tobacco use, diabetes mellitus, and hyperlipidemia. Hypertension provide unique opportunity for intervention, because of its ease of diagnosis, but yet it prevalence in Nigeria is as high as 30.6% with only 29.0% being aware of their hypertension, 12.0% receiving treatment, and 2.8% at-goal blood pressure in 2020.[13] Nigeria contributed 15% to the estimated 681,000 new cases of cancer that occurred in Africa in 2008.[14] Diabetes has an estimated prevalence of 5.8% in Nigeria.[15] Nigeria has the highest prevalence of asthma in Africa with an estimated 13 million Nigerians suffer from clinical asthma with age adjusted death rate of about 10 per 100,000 population.[16] Chronic kidney disease has as high as 15% prevalence in Nigeria.[17] The pooled crude prevalence of dementia in Nigeria 4.9% in 2019.[18]

Schizophrenias, Manic illness and organic psychosis affect about 1% of the population.[19] Depression, Anxieties and Somatoform disorders are far more prevalent. At least 10% of the population will be suffering from those poorly identifiable disorders. These conditions run a chronic course and are responsible for more morbidity. There is evidence that depression is particularly common among Nigerian elderly, with over 7% reporting major depressive disorder in a 12-month period and over 25% reporting same in the course of a lifetime.[20]

Globalization and urbanization has tremendously affect the life style of Nigerians predisposing them to risk of developing NCDs, the prevalence is soaring and the surge need to be mitigated to prevent premature deaths in Nigeria.

## MATERIALS AND METHODS

### Formulating a Research Question

The group of reviewers (10 participants) had a group discussion to arrive at a research question as the pooled prevalence of Non-Communicable Diseases (NCDs) in Nigeria reported from clearly defined studies where both the numerator and the denominator for computing the prevalence were discernible.

### Study Design

This was systematic review that was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P 2015).[21]

### Study Protocol and Registration

The study was registered with the Pan African Clinical Trials Registry (PACTR). [22,23]

### Search Strategy

We retrieved potentially relevant studies via systematic search of PubMed and Google scholar using the terms 'prevalence, non-communicable diseases and Nigeria' as well as 'hypertension, diabetes, chronic kidney disease, cardiovascular disease, chronic respiratory disease (asthma and chronic obstructive pulmonary disease), neurological disease, cancers, obesity, dementia, depression, schizophrenia, epilepsy, and learning disorders'. Boolean operators 'and' 'or' and 'not' were used to join and construct various search terms. Further review was made from the reference list of the identified articles and related articles from the search results. The review was carried out by nine of the participants to avoid having a tie vote. The search was limited to studies published between Jan 1st, 2015 and December 1st, 2024.

Effort was made to identify not only complete articles but also published abstracts that contained the ingredient data required to compute pooled prevalence of NCDs in Nigeria. However, ongoing studies and studies awaiting publication or studies that do not contained the ingredients data were excluded. Among the studies retrieved in the search, the reviewers remove duplicate studies using Endnote, select studies that meet the inclusion criteria based on the abstracts and then make the final selection of studies based on their full text. In order to maintain transparency and objectivity throughout this process, study selection is conducted independently by at least two investigators. Disagreement was resolved via voting by nine of the reviewers and simple majority was used. The group leader was excluded from voting. For all studies that were excluded, the reason for exclusion were given in the PRISMA-P flow chart. A total of eighty two abstract were identified, only fifty two abstract were included.

**Study Selection and Eligibility Criteria**

Studies of any design that reported the prevalence of any NCD in Nigerian population published in English language were included. Studies must have clear number of cases included, number analyzed, as well as the denominator used for computing the prevalence were included. We excluded letters to the editor, narrative reviews, systematic reviews as well as studies not published in or studies reporting secondary data.

**Assessment of Quality of Publication**

The quality of the included studies was assessed using the Joanna Briggs Institute (JBI) critical assessment checklist for prevalence data.[65] This checklist assesses nine items: (1) appropriate sampling frame, (2) proper sampling techniques, (3) adequate sample size, (4) study subject and setting description, (5) sufficient data analysis (6) use of valid methods for the identified conditions, (7) valid measurement for all participants, (8) using appropriate statistical analysis and (9) adequate response rate. Yes, no, unclear, or not applicable is assigned to each item. The 'yes' response received a 1 score, whereas the 'no' and 'unclear' responses received 0 scores. Finally, the average score for the studies was determined. The quality of studies with scores below and above the average was then classified as bad and good, respectively.[23]

**Data Extraction**

Using a standard pro-forma, one reviewer extracted relevant data from each of the included articles and an independent reviewer for each subheading verify all the data. Discrepancies were discussed and solved by consensus or voting by simple majority in similar manner as above.

We extracted the following data from individual studies: general information about the paper authors, journal and year of publication, type of NCD reported, the prevalence, the numerator and denominator used as well as the 95% confidence interval of each of the prevalence. Region of Nigeria where the study was done and state was also extracted.

**Data Analysis**

The summary of the quality of all the studies included in the review was reported. Data was entered into SPSS version 26. Forest plot was used to determine the pooled prevalence of NCDs in Nigeria while I2 static was used to report the level of heterogeneity of the included studies. P-values of less than 0.05 was considered statistically significant.

## RESULTS

**Data Review**

The total sample size of all studies included in the meta-analysis was 138,818 subjects. The maximum and minimum sample size were related to the study of amanc et al, 2023. With 21,369 subjects and the study of Maarten C et al, 2021. With 40 subjects, respectively. The oldest study was done in 2015 and the most recent study in 2024. A large number of studies were conducted in the south west with twenty six (26) articles. Below table indicates the characteristics and data of studies included in the systematic review and meta-analysis (table 1).

**Statistical analysis and result presentations**

The present study estimated the prevalence rate of non-communicable disease in the Nigeria and the prevalence rate or relative frequency of non-communicable disease in each study was employed to combine the results of different studies. Heterogeneity among studies was checked using $I^2$ index and considering the high heterogeneity between the results of the studies

included in the meta-analysis ($I^2$ > 75 %), the random effects model was used, which calculates the parameter changes between studies. Therefore, the results of random effects model in heterogeneous conditions are more generalizable than those of fixed effect model. Funnel plot were used to assess the publication bias. Further, the meta-regression was applied to examine the relationship between the prevalence rate of stroke in the elderly and sample size, year of publication, and mean age of elderly. The subgroup analysis was performed based on the different political zones in Nigeria (North Central, North East, North West, South-South, South East, South West). The comprehensive meta-analysis software (version 4) was used for meta-analysis and P-value <0.05 was considered as statistically significant.

**Meta-analysis of the overall prevalence rate of non-communicable disease in Nigeria**

Considering that the result of $I^2$ test for the overall prevalence of non-communicable disease in the Nigeria demonstrated a significant heterogeneity among studies ($I^2$ = 99.73), the data were analyzed using a random effects model (table 2). The results of Egger's regression intercept revealed no publication bias in the studies at the level of 0.1 (P = 0.000). After combining the all results of studies included in the *meta*-analysis, the overall prevalence of non-communicable disease in the Nigeria was estimated to be 12.9 % (95 % CI: 9.3 % - 17.6 %) based on the random effects model. The black square illustrates the prevalence rate, the length of the line segment displays the 95 % CI in each study (table 3 &4). The results of sensitivity analysis demonstrated that the pooled estimation did not change significantly by eliminating each of the studies.

**Sub grouped analysis**

Given the high heterogeneity among the studies ($I^2$ = 99.73), subgroup analysis was reported based on the different in to six geopolitical zones (north central, north east, north west, south-south, south east, south west) . The results of the subgroup analysis illustrated that the highest prevalence rate of non-communicable disease in Nigeria was related to the south-south zone with 19.3 % (95% CI: 1.0 – 33.9%), followed by south west 18.3% (95% CI: 1.22 – 26.7%) , north west 11.0%(95% CI: 5.4 – 21.1%), south east 8.4% (95% CI: 3.3 – 19.9%), north central 4.5% (95% CI: 1.9 – 10.2%) and north east 1.1% (95% CI: 1.0 – 1.3%). Respectively (table 5)

**Table 1: Distribution of characteristics of the data studies included in the Systematic review and meta-analysis on Non-communicable disease**

| Author and Year | Political zone | Sample size (n) | Prevalence (%) | NCD types |
|---|---|---|---|---|
| fasanmade et al, 2015 | SW | 416 | 21.4 | Obesity |
| Adetola et al, 2021 | SW | 1059 | 58.9 | Obesity |
| Boma oyan et al, 2023 | SS | 204 | 36.6 | Obesity |
| Nkenditin et al, 2021 | SE | 450 | 38.9 | Obesity |
| fasanmade et al, 2021 | SW | 1059 | 37.4 | HTN |
| Njideku et al, 2019 | SW | 5365 | 55.0 | HTN |
| Ajayi et al, 2016 | SW | 806 | 33.1 | HTN |
| A.U.owo labi et al, 2015 | SW | 324 | 20.1 | HTN |
| Njideku et al, 2021 | SW | 1059 | 18.9 | DM |
| AA sabir et al, 2017 | NW | 280 | 4.3 | DM |
| BO bello ovosi et al, 2018 | NW | 181 | 23.3 | DM |
| Ezeani et al, 2020 | SE | 1680 | 3.3 | DM |
| CO. Eze et al, 2024 | SW | 172 | 15.2 | Stroke |
| Akin O et al, 2020 | SW | 150 | 52.0 | Stroke |
| C.Eze et al, 2019 | SW | 983 | 20.0 | Stroke |
| G.O osagbowo et al, 2015 | NC | 2398 | 4.3 | Stroke |
| amanc et al, 2023 | SW | 21369 | 1.6 | CAD |
| O samuel et al,2023 | SW | 567 | 30.8 | CAD |
| Victor M et al, 2016 | SW | 211 | 1.9 | CAD |
| Dike Ojji et al, 2015 | NC | 2398 | 0.3 | CAD |
| amanc et al, 2023 | SW | 21369 | 36.2 | HF |
| Dike Ojji et al, 2015 | NC | 2398 | 22.6 | HF |
| O Adebayo et al, 2023 | SW | 160 | 83.8 | HF |
| Oloseyi et al, 2018 | SW | 90 | 54.4 | HF |
| Dike Ojji et al,2015 | NC | 2398 | 1.1 | CKD |
| T.Olusegun et al, 2020 | NC | 1353 | 12.0 | CKD |
| I. Ijezie et al, 2019 | SW | 165 | 7.5 | CKD |
| Okwuonu et al,2017 | SE | 328 | 7.8 | CKD |
| Boni M et al,2022 | SS | 4234 | 9.2 | COPD |
| OB Ojuawo et al,2022 | NC | 338 | 24.3 | COPD |

| | | | | |
|---|---|---|---|---|
| Akinrami et al, 2015 | **SW** | **211** | **4.7** | **COPD** |
| Adeniyi B et al,2016 | SW | 914 | 9.0 | COPD |
| O Deselu et al, 2020 | NC | 870 | 2.1 | Asthma |
| O Ozoh et al, 2019 | SW | 20063 | 8.0 | Asthma |
| O Ozoh et al, 2019 | NC | 20063 | 1.1 | Asthma |
| A Oyemomi et al, 2023 | SW | 124 | 4.8 | Asthma |
| Maarten C et al, 2021 | SW | 40 | 8.8 | Cancers |
| CC Nwafor et al, 2018 | SS | 1186 | 11.1 | Cancers |
| yusuf et al, 2017 | NW | 6548 | 10.9 | Cancers |
| Onwusah DO et al,2017 | SS | 782 | 26.3 | Cancers |
| B Adikaibe et al, 2016 | SE | 8228 | 6.0 | Epilepsy |
| West BA et al, 2019 | SS | 298 | 51.0 | Epilepsy |
| P Obemeke et al, 2019 | SE | 1802 | 5.2 | Epilepsy |
| C donald et al, 2019 | SS | 2283 | 6.5 | Epilepsy |
| A Ogunniyi et al, 2016 | SW | 613 | 2.9 | Dementia |
| Akin O et al, 2021 | SW | 150 | 6.2 | Dementia |
| Abolagade et al, 2020 | SW | 532 | 25.4 | Dementia |
| JO yaria et al, 2016 | SW | 142 | 22.1 | Dementia |

**Note: SW= south west, SE= south East, SS=South South, NC= North Central, NE= North East, NW= North West, CKD= Chronic Kidney Disease, HTN= Hypertension, DM= Diabetes Mellitus, COPD= Chronic Obstructive Pulmonary Disease, HF= Heart Failure.**

**Table 2: the result of fixed and random effect model on meta-analysis of prevalence of Non -Communicable disease**

| Model effect | Number Studies | Point estimate | Lower limit | Upper limit | Tau | $Tau^2$ | Q-value | df (Q) | P-value | $I^2$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Fixed** | 48 | 0.224 | 0.221 | 0.227 | 1.286 | 1.654 | 17728.08 | 47 | 0.000 | 99.73488 |
| **Random** | 48 | 0.129 | 0.093 | 0.176 | | | | | | |

**Figure 1: funnel plot of the result of the overall estimate of the prevalence rate of non-communicable disease in Nigeria**

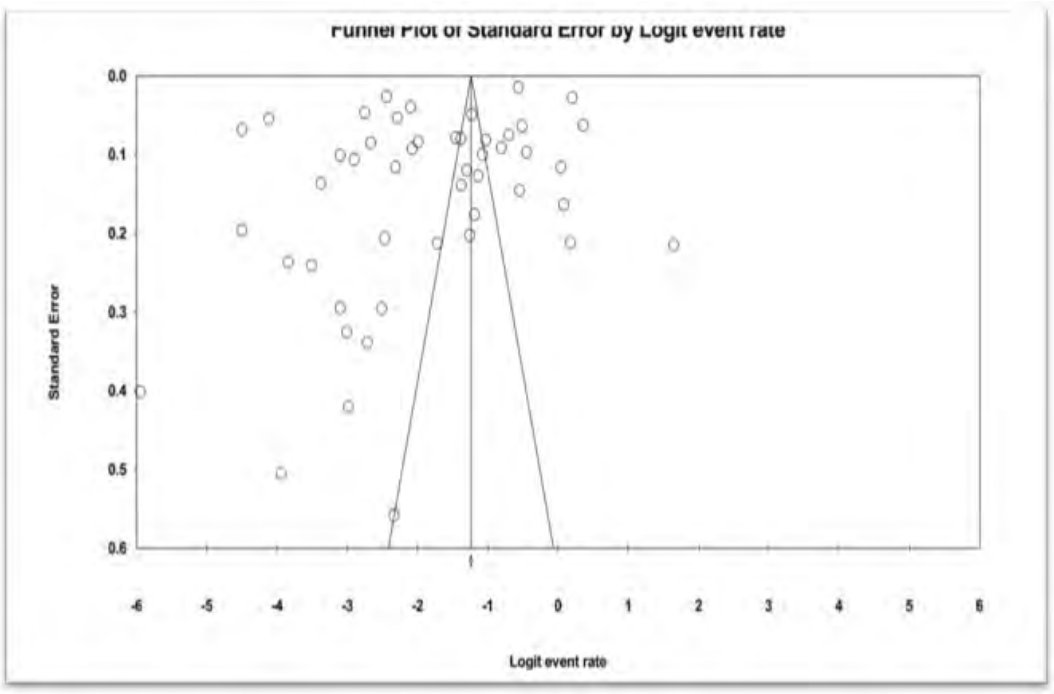


**Table 3: Distribution of the overall estimate of the prevalence rate of Non-communicable disease in Nigeria**

| | Statistics for each study | | | |
|---|---|---|---|---|
| | | 95% Confidence interval | | |
| **Study name** | Rate | Lower limit | Upper limit | P-Value |
| fasanmade et al, 2015 | 0.214 | 0.177 | 0.256 | 0.000 |
| Adetola et al, 2021 | 0.589 | 0.559 | 0.618 | 0.000 |
| Boma oyan et al, 2023 | 0.366 | 0.303 | 0.434 | 0.000 |
| Nkenditin et al, 2021 | 0.389 | 0.345 | 0.435 | 0.000 |
| fasanmade et al, 2021 | 0.374 | 0.345 | 0.404 | 0.000 |
| Njideku et al, 2019 | 0.550 | 0.537 | 0.563 | 0.000 |
| Ajayi et al, 2016 | 0.331 | 0.299 | 0.364 | 0.000 |
| A.U.owolabi et al, 2015 | 0.201 | 0.161 | 0.248 | 0.000 |
| Njideku et al, 2021 | 0.189 | 0.167 | 0.214 | 0.000 |
| AA sabir et al, 2017 | 0.043 | 0.025 | 0.074 | 0.000 |
| BO bello ovosi et al, 2018 | 0.233 | 0.177 | 0.300 | 0.000 |
| Ezeani et al, 2020 | 0.033 | 0.025 | 0.043 | 0.000 |
| CO. Eze et al, 2024 | 0.152 | 0.106 | 0.214 | 0.000 |
| Akin O et al, 2020 | 0.520 | 0.440 | 0.599 | 0.000 |
| C.Eze et al, 2019 | 0.200 | 0.176 | 0.226 | 0.000 |
| G.O osagbowo et al, 2015 | 0.043 | 0.036 | 0.052 | 0.000 |
| amanc et al, 2023 | 0.016 | 0.014 | 0.018 | 0.000 |
| O samuel et al,2023 | 0.308 | 0.271 | 0.347 | 0.000 |
| Victor M et al, 2016 | 0.019 | 0.007 | 0.049 | 0.000 |
| Dike Ojji et al, 2015 | 0.003 | 0.001 | 0.006 | 0.000 |
| amanc et al, 2023 | 0.362 | 0.356 | 0.368 | 0.000 |
| Dike Ojji et al, 2015 | 0.226 | 0.210 | 0.243 | 0.000 |
| O Adebayo et al, 2023 | 0.838 | 0.773 | 0.887 | 0.000 |
| Oloseyi et al, 2018 | 0.544 | 0.441 | 0.644 | 0.000 |
| Dike Ojji et al,2015 | 0.011 | 0.008 | 0.016 | 0.000 |

| | | | | |
|---|---|---|---|---|
| T.Olusegun et al, 2020 | 0.120 | 0.104 | 0.138 | 0.000 |
| I. Ijezie et al, 2019 | 0.075 | 0.043 | 0.126 | 0.000 |
| Okwuonu et al,2017 | 0.078 | 0.053 | 0.112 | 0.000 |
| Boni M et al,2022 | 0.092 | 0.084 | 0.101 | 0.000 |
| OB Ojuawo et al,2022 | **0.243** | **0.200** | **0.292** | **0.000** |
| Akinrami et al, 2015 | 0.047 | 0.025 | 0.085 | 0.000 |
| Adeniyi B et al,2016 | 0.090 | 0.073 | 0.110 | 0.000 |
| O Deselu et al, 2020 | 0.021 | 0.013 | 0.033 | 0.000 |
| O Ozoh et al, 2019 | 0.080 | 0.076 | 0.084 | 0.000 |
| O Ozoh et al, 2019 | 0.011 | 0.010 | 0.013 | 0.000 |
| A Oyemomi et al, 2023 | 0.048 | 0.022 | 0.103 | 0.000 |
| Maarten C et al, 2021 | 0.088 | 0.031 | 0.224 | 0.000 |
| CC Nwafor et al, 2018 | 0.111 | 0.094 | 0.130 | 0.000 |
| yusuf et al, 2017 | 0.109 | 0.102 | 0.117 | 0.000 |
| Onwusah DO et al,2017 | 0.263 | 0.233 | 0.295 | 0.000 |
| B Adikaibe et al, 2016 | 0.060 | 0.055 | 0.065 | 0.000 |
| West BA et al, 2019 | 0.510 | 0.453 | 0.566 | 0.000 |
| P Obemeke et al, 2019 | 0.052 | 0.043 | 0.063 | 0.000 |
| C donald et al, 2019 | 0.065 | 0.056 | 0.076 | 0.000 |
| A Ogunniyi et al, 2016 | 0.029 | 0.018 | 0.046 | 0.000 |
| Akin O et al, 2021 | 0.062 | 0.033 | 0.114 | 0.000 |
| Abolagade et al, 2020 | 0.254 | 0.219 | 0.293 | 0.000 |
| JO yaria et al, 2016 | 0.221 | 0.160 | 0.297 | 0.000 |
| **Overall prevalence** | **0.129** | **0.093** | **0.176** | **0.000** |

**Table 4: Distribution of the overall estimate of the prevalence rate of Non-communicable disease in Nigeria based on the random effects model**

| | Statistics for each study | | | |
|---|---|---|---|---|
| | 95% Confidence interval | | | |
| **Study name** | Point | Lower limit | Upper limit | P-Value |
| fasanmade et al, 2015 | 0.127 | 0.091 | 0.175 | 0.000 |
| Adetola et al, 2021 | 0.123 | 0.088 | 0.169 | 0.000 |
| Boma oyan et al, 2023 | 0.125 | 0.090 | 0.172 | 0.000 |
| Nkenditin et al, 2021 | 0.125 | 0.090 | 0.172 | 0.000 |
| fasanmade et al, 2021 | 0.125 | 0.089 | 0.172 | 0.000 |
| Njideku et al, 2019 | 0.124 | 0.089 | 0.169 | 0.000 |
| Ajayi et al, 2016 | 0.126 | 0.090 | 0.173 | 0.000 |
| A.U.owolabi et al, 2015 | 0.127 | 0.091 | 0.175 | 0.000 |
| Njideku et al, 2021 | 0.127 | 0.091 | 0.175 | 0.000 |
| AA sabir et al, 2017 | 0.131 | 0.094 | 0.180 | 0.000 |
| BO bello ovosi et al, 2018 | 0.127 | 0.091 | 0.174 | 0.000 |
| Ezeani et al, 2020 | 0.132 | 0.095 | 0.181 | 0.000 |
| CO. Eze et al, 2024 | 0.128 | 0.092 | 0.176 | 0.000 |
| Akin O et al, 2020 | 0.124 | 0.089 | 0.170 | 0.000 |
| C.Eze et al, 2019 | 0.127 | 0.091 | 0.175 | 0.000 |
| G.O osagbowo et al, 2015 | 0.132 | 0.095 | 0.180 | 0.000 |
| amanc et al, 2023 | 0.134 | 0.099 | 0.180 | 0.000 |
| O samuel et al,2023 | 0.126 | 0.090 | 0.173 | 0.000 |
| Victor M et al, 2016 | 0.133 | 0.096 | 0.182 | 0.000 |
| Dike Ojji et al, 2015 | 0.138 | 0.099 | 0.188 | 0.000 |
| amanc et al, 2023 | 0.125 | 0.088 | 0.176 | 0.000 |
| Dike Ojji et al, 2015 | 0.127 | 0.090 | 0.175 | 0.000 |
| O Adebayo et al, 2023 | 0.120 | 0.086 | 0.165 | 0.000 |
| Oloseyi et al, 2018 | 0.124 | 0.089 | 0.170 | 0.000 |
| Dike Ojji et al,2015 | 0.135 | 0.097 | 0.184 | 0.000 |
| T.Olusegun et al, 2020 | 0.129 | 0.092 | 0.177 | 0.000 |

| | | | | |
|---|---|---|---|---|
| I. Ijezie et al, 2019 | 0.130 | 0.093 | 0.178 | 0.000 |
| Okwuonu et al,2017 | 0.130 | 0.093 | 0.178 | 0.000 |
| Boni M et al,2022 | 0.129 | 0.093 | 0.178 | 0.000 |
| OB Ojuawo et al,2022 | **0.127** | **0.091** | **0.174** | **0.000** |
| Akinrami et al, 2015 | 0.131 | 0.094 | 0.180 | 0.000 |
| Adeniyi B et al,2016 | 0.130 | 0.093 | 0.178 | 0.000 |
| O Deselu et al, 2020 | 0.133 | 0.096 | 0.182 | 0.000 |
| O Ozoh et al, 2019 | 0.130 | 0.093 | 0.178 | 0.000 |
| O Ozoh et al, 2019 | 0.135 | 0.099 | 0.182 | 0.000 |
| A Oyemomi et al, 2023 | 0.131 | 0.094 | 0.179 | 0.000 |
| Maarten C et al, 2021 | 0.129 | 0.093 | 0.177 | 0.000 |
| CC Nwafor et al, 2018 | 0.129 | 0.092 | 0.177 | 0.000 |
| yusuf et al, 2017 | 0.129 | 0.092 | 0.178 | 0.000 |
| Onwusah DO et al,2017 | 0.126 | 0.090 | 0.174 | 0.000 |
| B Adikaibe et al, 2016 | 0.131 | 0.094 | 0.179 | 0.000 |
| West BA et al, 2019 | 0.124 | 0.089 | 0.170 | 0.000 |
| P Obemeke et al, 2019 | 0.131 | 0.094 | 0.179 | 0.000 |
| C donald et al, 2019 | 0.130 | 0.094 | 0.179 | 0.000 |
| A Ogunniyi et al, 2016 | 0.132 | 0.095 | 0.181 | 0.000 |
| Akin O et al, 2021 | 0.130 | 0.094 | 0.179 | 0.000 |
| Abolagade et al, 2020 | 0.127 | 0.091 | 0.174 | 0.000 |
| JO yaria et al, 2016 | 0.127 | 0.091 | 0.174 | 0.000 |
| **Pooled prevalence rate** | **0.129** | **0.093** | **0.176** | **0.000** |

**Table 5: The subgroup analysis of estimating the prevalence rate of non-communicable disease in Nigerian by political zones.**

| Model | Number Studies | Point estimate | Lower limit | Upper limit | Tau | Tau$^2$ | Q-value | df (Q) | P-value | $I^2$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **North Central** | 7 | 0.045 | 0.019 | 0.102 | 1.184 | 1.401 | 707.480 | 6 | 0.000 | 99.152 |
| **North East** | 1 | 0.011 | 0.010 | 0.013 | 0.000 | 0.000 | 0.000 | 0 | 1.000 | 0.000 |
| **North West** | 3 | 0.110 | 0.054 | 0.211 | 0.657 | 0.431 | 37.849 | 2 | 0.000 | 94.716 |
| **South East** | 5 | 0.084 | 0.033 | 0.199 | 1.131 | 1.279 | 533.377 | 4 | 0.000 | 99.250 |
| **South South** | 6 | 0.193 | 0.100 | 0.339 | 0.954 | 0.910 | 613.769 | 5 | 0.000 | 99.185 |
| **South West** | 26 | 0.183 | 0.122 | 0.267 | 1.241 | 1.539 | 10374.135 | 25 | 0.000 | 99.759 |

## DISCUSSION

The present study aimed at estimating the prevalence of Non-communicable disease in Nigeria using systematic review and meta-analysis. After combining the data collected from 48 articles included in the meta-analysis with a sample size of less than one million people, the overall prevalence of Non-communicable disease in Nigerian was estimated to be 12.9%.

The prevalence of Non-communicable disease was found to be higher is south-south zone of the country and lowest in the North-east with prevalence of 19.3% and 1.1% respectively, this might be attributed to paucity of data, and where the data analyzed from North-east was only one, while the one from south-south six publications were used.

## CONCLUSION

The pooled prevalence of Non-communicable disease in our study was found to be 12.9%, with South-south having the highest prevalence of 19.3% and while the North-east having the least prevalence of 1.1%, which underscores the surge of Non-communicable diseases in Nigeria and also provide room for necessary interventions especially where the prevalence was high to mitigate the soaring prevalence of Non-communicable disease in Nigeria.

### Limitations of the Study

- There was a paucity of prevalence studies in some part of the country.
- There is paucity of recent studies for Non-communicable diseases in Nigeria.

## REFERENCES

1. Federal Ministry of Health. National Demographic and Health Survey Final Report. Abuja: Federal Ministry of Health; 2008.
2. World Health Organization. Non-communicable diseases. WHO. 2017. Available from: https://www.who.int/mediacentre/factsheets/fs355/en/
3. Global Burden of Disease Health Financing Collaborator Network. Evolution and patterns of global health financing 1995-2014: development assistance for health, and government, prepaid private, and out-of-pocket health spending in 184 countries. Lancet. 2017;389(10083):1981-2004.

4. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Results. Seattle: Institute for Health Metrics and Evaluation; 2020. Available from: https://vizhub.healthdata.org/gbd-results/
5. Gomez-Gonzalez JE, Reyes NR. Pattern of global health financing and potential future spending on health. Lancet. 2017; 389 (10083):1955-1956.
6. World Health Organization. Non-communicable diseases country profiles 2018. Geneva: World Health Organization; 2018.
7. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020; 396:1223-1249.
8. World Health Organization. Global health estimates: Leading causes of DALYs. WHO. 2022. Available from: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/global-health-estimates-leading-causes-of-dalys
9. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-1544.
10. Ogah OS, Stewart S, Falase AO, Akinyemi JO, Adegbite GD, Alabi AA, et al. Contemporary profile of acute heart failure in southern Nigeria: data from Abeokuta Heart Failure Clinical Registry. JACC Heart Fail. 2014;2:250-259.
11. Kankeu HT, Saksena P, Xu K, Evans DB. The Financial burden from Non-communicable diseases in low-and middle-income countries: a literature review. Health Res Policy Syst. 2013;11:31.
12. Skirbekk V, Loichinger E, Weber D. Variation in cognitive functioning as a refined approach to comparing aging across countries. Proc Natl Acad Sci U S A. 2012;109(3):770-774.
13. World Economic Forum. From burden to "best buys": reducing the economic impact of non-communicable diseases in low-and middle income countries. Geneva: The Forum; 2011.
14. Uloko AE, Musa BM, Ramalan MA, Gezawa ID, Puepet FH, Uloko AT, et al. Prevalence and risk factors for diabetes mellitus in Nigeria: a systematic review and meta-analysis. Diabetes Ther. 2018;9(3):1307-1316.
15. Ozoh OB, Adebiyi SA, Ayuk AC, Desalu OO, Oridota OE, Olufemi O, et al. The prevalence of asthma and allergic rhinitis in Nigeria: A nationwide survey among children, adolescents and adults. PLoS One. 2019;14(9):e0222281.
16. Kadiri S, Odubanjo MO, Oluwasola AO. The epidemiology of end-stage renal disease in Nigeria: the way forward. Int Urol Nephrol. 2011;43:785-792.
17. Omoleke SA. Chronic non-communicable disease as a new epidemic in Africa: focus on the Gambia. Pan Afr Med J. 2013;14:87.
18. Gureje O, Lasebikan VO. Lifetime and 12-month Prevalence of Mental Disorders In the Nigerian Survey of Mental Health and Well-being. Br J Psychiatry. 2006;188:465-471.
19. Miranda JJ, Patel V. Achieving the MDGs: Does Mental Health Play a Role? PLoS Med. 2005;2(10):e291.
20. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.
21. Ndwandwe DE, Runeyi S, Pienaar E,

Mathebula L, Hohlfeld A, Wiysonge CS. Practices and trends in clinical trial registration in the Pan African Clinical Trials Registry (PACTR): a descriptive analysis of registration data. BMJ Open. 2022; 12(1):e057474.
22. Booth A, Clarke M, Dooley G, Ghersi D, Moher D, Petticrew M, et al. The nuts and bolts of PROSPERO: An international prospective register of systematic reviews. Syst Rev. 2012;1:2.
23. Raimi TH, Odusan O, Fasanmade O. High prevalence of central obesity in rural South-Western Nigeria: Need for targeted prevention. J Diabetes Endocrinol. 2015;6(3):12-18.
24. Adetola AB, Ani I, Matthew JE, Abodunde GB. Prevalence of obesity, hypertension, and diabetes among patients attending outpatient clinic at a tertiary health institution in Southwestern Nigeria. Niger J Cardiol. 2021;18(2):51-56.
25. Oyan B, Ndu-Akinla C. Sociodemographic determinants of overweight and obesity in adults attending a tertiary hospital in Rivers State, Nigeria. Niger J Med. 2023;32(3):280-287.
26. Nkemdilim IU, Ezeala-Adikaibe BA, Donatus OO, Innocent O, Ernest N, Nkeiruka M, et al. Obesity in Patients with Hypertension Attending a Medical Outpatient Clinic in a Tertiary Health Facility in Enugu, Southeast Nigeria. Ann Afr Med. 2021;20(2):84-91.
27. Njideka U, Ogah O, Adebayo R, et al. Prevalence of hypertension and blood pressure profile among urban-dwelling adults in Nigeria: a comparative analysis based on recent guideline recommendation. Cardiovasc J Afr. 2019;25(7).
28. Ajayi I, Dada A, Adebayo A. Prevalence of hypertension and associated factors among residents of Ibadan-North local government area of Nigeria. Niger J Clin Immunol. 2016;3(1):67-75.
29. Owolabi AO, Owolabi MO, OlaOlorun AD, Amole IO. Hypertension prevalence and awareness during health workforce in Nigeria. Internet J Med Update. 2015;10(2):10-19.
30. Sabir AA, Isezuo SA, Fasanmade OA, et al. Prevalence of diabetes mellitus and its risk factors among the Sub-urban population of Northwest Nigeria. Ann Afr Med. 2017;16(4):165-171.
31. Bello-ovosi BJ. Prevalence and correlates of hypertension and diabetes mellitus in urban community in North-western Nigeria. Sahel Med J. 2018;21(3):125-130.
32. Ezeani I, Onyemelukwe G, Oli J. Prevalence and risk factors for Diabetes mellitus in a state in south-east Nigeria: results of population based house to house survey. West Afr J Med. 2020;16(2):181-187.
33. Eze CO, Ozigboegwu O, Ezeala-Adikaibe B. The Prevalence and pattern of admission mortality among acute stroke patients managed at a tertiary hospital in Abaliki, Nigeria. Ann Med Health Sci Res. 2024;14(1):45-52.
34. Akin O, Owolabi M, Ogunniyi A. Prevalence, predictors and prognosis of post-stroke neuropsychiatric symptoms at 3 month post-stroke. J Neurol Sci. 2020;418:117073.
35. Chukwemeka Eze C, Ezeala-Adikaibe B, Ozigboegwu O. Stroke in the young adults: experience at Abakaliki south-east Nigeria. Niger J Clin Pract. 2019;22(8):1075-1080.
36. Amanc B, Sani M, Tijani B, et al. Trends and outcome of cardiovascular disease admission in Lagos Nigeria: a 16 year review. West Afr J Med. 2023;40(7):678-685.
37. Okechukwu S, Okoye I, Akpa M, et al. Cardiovascular disease in Nigeria: current status, threats and opportunities. Glob Heart. 2023;18(1):62.

38. Victor M, Ogedengbe O, Adebayo R. Association between metabolic syndrome and 10 year risk of developing cardiovascular disease in a Nigerian population. Afr Health Sci. 2016;8:354-359.
39. Dike B, Isezuo S, Chijioke A, et al. Risk Factors profile and comorbidities in 2398 patients with newly diagnosed hypertension from the Abuja Heart study. J Clin Hypertens. 2015;17(9):691-697.
40. Adebayo O, Ogah O, Oladapo O, et al. Clinical characteristics, management and six-month outcomes after discharge of patients admitted for acute heart failure in Ibadan, Nigeria. West Afr J Med. 2023;40(5):421-428.
41. Oloseyi A, Adewole O, Akinyemi O. Prevalence and pattern of cardiovascular related causes of out-of-hospital death in Lagos, Nigeria. BMC Public Health. 2018;18:1245.
42. Timothy O, Chijioke A, Isezuo S, et al. Prevalence of chronic kidney disease and risk factors in North Central Nigeria: a Population base study. Niger J Clin Pract. 2020;23(10):1385-1392.
43. Innocent I, Arogundade F, Sanusi A, et al. Prevalence and predictors of chronic kidney disease in a semi-urban community in Lagos. Pan Afr Med J. 2019;32:45.
44. Okwuonu C, Ojimadu N, Oche M. Prevalence of chronic kidney disease and its risk factors among adults in a semi-urban community of south-east Nigeria. Niger Postgrad Med J. 2017;24(2):81-87.
45. Boni M, Desalu O, Onyedum C, et al. Estimating the prevalence of chronic obstructive pulmonary disease in an African country: evidence from southern Nigeria. Int J Tuberc Lung Dis. 2022;26(4):312-318.
46. Ojuawo OB, Desalu O, Salami A, et al. Out-patient burden of adult respiratory disease in university of Ilorin Teaching Hospital, Nigeria. Afr J Respir Med. 2022;18(1):12-18.
47. Akinremi AA, Desalu O, Onyedum C. Prevalence of Lung function impairment among a Nigerian Undergraduate Population. Niger J Physiol Sci. 2015;30(1-2):45-49.
48. Adeniyi A, Desalu O, Adeoti A, et al. Pattern of respiratory diseases among patient seen at the emergency unit of tertiary health facility in south-west Nigeria. West Afr J Med. 2016;35(3):201-206.
49. Desalu O, Bello K, Salami A, et al. Prevalence of Asthma and respiratory symptoms during pregnancy in the middle belt of Nigeria. J Obstet Gynaecol. 2020;40(5):645-649.
50. Obianju O, Erhabor G, Adewole O, et al. The prevalence of Asthma and allergic rhinitis in Nigeria: A nationwide survey among adults. Allergol Immunopathol. 2019;47(3):224-231.
51. Azeez O, Desalu O, Opeyemi C, et al. Acute severe asthma and its predictors of mortality in rural south-western Nigeria: a five year retrospective observational study. J Asthma. 2023;60(5):912-918.
52. Maarten C, Aisuodionoe-Shadrach O, Ogunbiyi J. Prevalence of prostate cancer at autopsy in Nigeria- A preliminary report. Prostate Cancer Prostatic Dis. 2021; 24(2):456-461.
53. Nwafor CC, Ugboko H, Oluwasola O. The pattern and distribution of cancers in Akwa Ibom state, Nigeria. Niger J Clin Pract. 2018;21(5):612-617.
54. Yusuf I, Atanda A, Umar A. Cancer in Kano, northwestern Nigeria. A 10 year update of the Kano Cancer Registry. Niger J Surg Res. 2017;8(2):87-93.
55. Onwusah DO, Ekanem I, Udoh E. Prevalence of cancer in a tertiary Health Facility in south-south Nigeria. Int J Cancer Res. 2017;13(3):145-150.
56. Adikaibe B, Onwuekwe I, Ezeala-Adikaibe B. Prevalence of active convulsive epilepsy in

urban slum in Enugu south-east Nigeria. Seizure. 2016;39:45-49.
57. West BA, Opara P, Eneh A. Pattern and impact of childhood seizure disorders in port Harcourt, Nigeria. Niger J Clin Pract. 2019;22(4):512-517.
58. Paul O, Ogunniyi A, Baiyewu O. Epidemiology of acute symptomatic seizures among adult medical admissions. Afr J Neurol Sci. 2016;35(2):23-28.
59. Chidozie D, Ani C, Nwosu M. Seizure disorders at Madonna University Teaching Hospital prevalence, pattern and co-morbidities. A seven year review. Niger J Med. 2019;28(3):189-194.
60. Ogunniyi A, Baiyewu O, Gureje O, et al. Prevalence estimates of Major neurocognitive disorder in a rural Nigeria community. Dement Geriatr Cogn Disord. 2016;42(3-4):198-205.
61. Akin O, Owolabi M, Arulogun O, et al. Pre-stroke cognitive decline in Africans: Prevalence, predictors and association with post-stroke dementia. Stroke. 2021;52(8): 2567-2574.
62. Amao A, Adebayo R, Oshodi Y, et al. Prevalence and pattern of psychiatric morbidity among community dwelling elderly population in Abeokuta, Nigeria. Afr J Psychiatry. 2020;23(4):145-150.
63. Yaria JO, Ojagbemi A, Akinyemi R, et al. Neurocognitive impairment in Nigerian community: Pattern of cognitive dysfunction and risk factors. Int J Geriatr Psychiatry. 2016;31(12):1345-1352.
64. Munn Z, Barker TH, Moola S, Tufanaru C, Stern C, McArthur A, Stephenson M, Aromataris E. Methodological quality of case series studies: an introduction to the JBI critical appraisal tool. JBI evidence synthesis. 2020; 18(10):2127-2133.

Abdulshahid S, Hamza B, Oluwatomi VA, Opeyemi A, Kallah PA, Alabi TI, Babangida FM, Izu G, Theodore T, Samaila M.Prevalence of noncommunicable diseases in Nigeria; a Nine year systematic review. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 131-147
https://dx.doi.org/10.4314/ajtmbr.v9i1.8

# Pharmacotherapy of Hypertension and Diabetes Co-morbidity in the Elderly: A Narrative Review

Akponah E[1,2], Moke EG[2], Elijah OB[1], Ovili-Odili BZ[3], Ekakitie LI[4], Ben-Azu B[2], Ilevbare F[1], Imolede OI[1]

**Abstract**

**Introduction:** Ageing is a normal physiological process, and with improving healthcare and standard of living, the elderly population is projected to increase substantially over time. Hypertension occurs in up to 80% of type 2 diabetes cases, and co-morbidity between the two conditions is common among the elderly, posing significant challenges in clinical management. If not adequately addressed, this co-morbidity can lead to increased mortality and complications such as retinopathy, neuropathy and nephropathy.

**Materials and Methods:** This narrative review searched Google Scholar, ResearchGate, PubMed and Scopus for literature on the pharmacotherapy of hypertension and diabetes co-morbidity published up to May 2025. A total of 117 articles meeting the inclusion criteria were selected and analysed.

**Results:** The review describes the pathophysiology of and interactions between hypertension and diabetes, the complications arising from their co-morbidity, and the clinical guidelines and pharmacological strategies used in their management. Treating elderly patients with both conditions requires a nuanced, multidisciplinary approach, as age-associated alterations in drug metabolism, polypharmacy, frailty and comorbid conditions must all be considered to avoid adverse outcomes and promote adherence while ensuring optimal efficacy.

**Conclusion:** Effective management of hypertension and diabetes co-morbidity in the elderly requires individualised, multidisciplinary care guided by current clinical guidelines, with emerging approaches such as personalised medicine and telemedicine offering promise for improving outcomes in this population.

**Keywords:** ageing**,** co-morbidity, diabetes, hypertension, pharmacotherapy

[1]*Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Delta State University, Abraka, Nigeria*
[2]*Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria*
[3]*Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria*
[4]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria*

**Corresponding author:** Emuesiri Goodies Moke, Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria. E-mail: hiligoodies@gmail.com; egmoke@delsu.edu.ng

## INTRODUCTION

All people will experience and cope with the ageing process at some point in their life; it is a natural and unavoidable phenomenon. Being thirty years old indicates that a person has experienced all of the stages of life, including newborn, toddler, preschool, adolescent, adult and elderly. These different stages of life begins both biologically and psychologically.[1] In the world, people who aged more than 60 years old was amounted to 13.4% of the whole human population in 2013, and this percentage was estimated to be doubled up to 25.3% in 2050, in which 8% of these elderly people live in Asia.[2] Two alterations in the natural/physiological functioning of the body's systems and various organs will occur gradually in the aged. As a result of biological changes, older people will experience a variety of physical issues, including the occurrence of chronic illnesses, sometimes

known as non-communicable diseases (NCDs). NCD is a long-lasting condition that typically progresses slowly and cannot be spread from person to person. The four main types of NCD are cardiovascular disease (e.g. hypertension), cancer, chronic respiratory disease, and diabetes mellitus (DM). Almost three quarters of NCD deaths (28 million) occur in low- and middle-income countries; 16 million NCD deaths occur before the age of 70; 82% of these "premature" deaths occurred in low- and middle-income countries.[3]

There are several physiological similarities between diabetes and hypertension, including increased body fluid, increased arterial stiffness, and impaired insulin regulation. Similar symptoms, including as increased body mass, excessive fat and processed sugar intake, and decreased physical activity, may be seen in patients. It's likely that either can result in the other.

Hypertension occurs in up to 80% of type 2 diabetics.[4-6] Among the elderly, the probability of exhibiting co-morbidities is common, although rates vary significantly by countries (Netherlands, Sweden, Spain, Australia, US, Canada), where prevalence ranges from 55% to 98%[7]: the highest rates always occur among older people, women and individuals in lower socio-economic classes. Co-morbidity significantly complicates medication management in the aged, as health care practitioners currently lack a systematic method to integrate age-related complexities into standard clinical practice.[8]

A literature search was conducted across major scientific databases, including Google Scholar, ResearchGate, PubMed and Scopus, covering publications up to May 2025. Articles that directly addressed the pharmacological treatment of hypertension and diabetes co-morbidity were identified and analysed for inclusion in this review. Only 117 articles were adapted for this review. Excluded articles included those outside the scope of this study, not in English, or whose full texts were unavailable and not retrievable. The keywords that directed our literature searches included: hypertension, diabetes, ageing, co-morbidities of hypertension and diabetes, and pharmacotherapy.

**Prevalence of hypertension and diabetes co-morbidity in elderly populations**

Several epidemiologic studies have indicated that the prevalence of hypertension in patients with diabetes mellitus is approximately 1.5 to 2.0 times greater than in a suitably matched non-diabetic population.[9] In individuals with insulin-dependent diabetes mellitus (IDDM), hypertension is typically absent at the time of diagnosis.[10] As renal insufficiency progresses, blood pressure increases, potentially accelerating the transition to end-stage renal failure. In non-insulin-dependent diabetes mellitus (NIDDM), numerous patients exhibit hypertension at the time of diagnosis. The prevalence of hypertension in NIDDM correlates with the extent of obesity, advanced age, and significant atherosclerosis commonly observed, likely encompassing many individuals with essential hypertension.

Multiple supplementary pathophysiological mechanisms contribute to the initiation and maintenance of hypertension in individuals with diabetes. Hyperglycaemia and elevated total body exchangeable sodium may result in extracellular fluid buildup and plasma volume expansion. In certain cases, modifications in the functionality of the renin-angiotensin-aldosterone system and vascular responsiveness to vasoactive hormones may also contribute. Recent suggestions indicate that hyperinsulinaemia and insulin resistance may

contribute to sustained increased blood pressure, as insulin is recognised for promoting salt retention and augmenting sympathetic nervous system activity. However, the pathogenetic relationship between these two common disorders remains elusive. The investigation of the complex interaction between hypertension and diabetes has proceeded along two distinct but interrelated lines. Physicians whose primary area of investigation was hypertension observed a high frequency of diabetes or abnormal glucose tolerance among their hypertensive patients and suggested that hypertension was a prediabetic state.[11] Conversely, diabetologists focused on the heightened incidence of hypertension in their patients and its potential adverse effects on the long-term microvascular and macrovascular complications of diabetes.[12] Recently, these two lines of inquiry have merged to discover a common pathophysiologic underpinning for both disorders.

The incidence of hypertension and diabetes markedly escalates with advancing age. The World Health Organization (WHO) reports that around 1.28 billion adults globally are affected by hypertension, with a significant increase in prevalence among individuals aged 60 years and older.[13] Diabetes impacts over 537 million adult individuals worldwide, with the statistics expected to project to 643 million in the next five years, with significant prevalence among the elderly population.[14,15]

In several elderly folks, these two illnesses frequently coexist. Research demonstrates that more than 70% of elderly individuals with diabetes also suffer from hypertension.[4] This co-morbidity is especially concerning because of the cumulative impact these diseases have on the cardiovascular system, significantly increasing the risk of myocardial infarctions, cerebrovascular accidents, and renal failure.[16] Older persons are more likely to have both illnesses due to the cumulative effects of ageing, extended exposure to cardiovascular risk factors, and sedentary lifestyles.[17]

**Physiological changes in ageing and their impact on risk for hypertension and diabetes**

As individuals age, numerous physiological alterations transpire that elevate the risk of obtaining hypertension and diabetes. These will include:

*i.* *Vascular stiffening*: As individuals age, arteries often become rigid, resulting in a loss of elasticity and suppleness. This vascular stiffening enhances peripheral resistance, resulting in higher blood pressure levels. Older persons also have a decrease in the sensitivity of baroreceptors, which regulate blood pressure.[18] This may result in inadequate blood pressure regulation, hence exacerbating hypertension.

*ii.* *Insulin resistance:* Ageing correlates with a decline in insulin sensitivity. The body's cells exhibit diminished sensitivity to insulin, leading in a less efficient removal of glucose from the bloodstream. The reduction in insulin sensitivity, exacerbated by diminished physical activity and alterations in body composition (including increased fat mass and reduced muscle mass), heightens the chance of developing Type 2 diabetes.[19]

*iii.* *Decline in pancreatic function*: The pancreas, responsible for producing insulin, also undergoes functional decline with age.[20] This reduction in pancreatic beta-cell function means that even if insulin is produced, it may not be enough to regulate blood sugar levels effectively, contributing to the onset of diabetes.

*iv.* *Kidney function decline*: Both hypertension and diabetes exert pressure on the kidneys.[4] The

age-related deterioration of renal function renders elderly patients more sensitive to the detrimental effects of hypertension and hyperglycaemia, hence hastening the advancement of both illnesses.

**Importance of managing co-morbidities in the elderly**

The simultaneous presence of hypertension and diabetes in elderly adults poses distinct obstacles regarding treatment and management.[21] The cumulative impact of these disorders hastens the decline of the cardiovascular system, kidneys, and other essential organs.[22] Moreover, geriatric individuals are predisposed to other chronic conditions, including hyperlipidaemia and cardiovascular disease, which exacerbates the management of hypertension and diabetes.[7] One of the primary reasons for handling these co-morbidities is prevention of severe complications.[23] Untreated hypertension can result in stroke, myocardial infarction, and congestive heart failure, whereas inadequately managed diabetes heightens the risk of diabetic retinopathy, neuropathy, nephropathy, and cardiovascular disease. Collectively, these disorders substantially increase the risk of mortality and adversely affect quality of life.[24]

The management of hypertension and diabetes in the elderly is further complicated by polypharmacy, which refers to the administration of many drugs for diverse illnesses.[25] Numerous older adults concurrently administer drugs for hypertension, glycaemia, hyperlipidaemia, and various other conditions. This elevates the possibility of drug interactions and adverse consequences, complicating treatment protocols. Healthcare professionals must implement a holistic, customised strategy that effectively manages both illnesses while mitigating the dangers of over-medication.[26]

**Pathophysiology and interactions between hypertension and diabetes**

**Pathophysiology of essential hypertension in adults**

Blood pressure (BP) is the fundamental mechanism that distributes blood to all bodily organs; therefore, the regulation of BP is crucial for survival. In this background, it is unsurprising that the control of blood pressure involves a very intricate and occasionally redundant interaction among renal, neuronal, cardiac, vascular, and endocrine processes, influenced by hereditary and environmental variables.[27]

*a) Role of kidneys in the pathogenesis of essential hypertension*

The kidney is crucial for regulating blood pressure by managing salt and water excretion, hence preserving extracellular volume homeostasis.[28] Blood pressure and salt homeostasis are closely connected through the pressure natriuresis mechanism, which aids in maintaining blood pressure at a specific fixed point.[29] An augmentation in renal perfusion pressure results in an increase in renal sodium excretion and a decrease in extracellular fluid volume, causing blood pressure to return to its baseline level.[30] This hypothesis asserts that hypertension results from a shift in the blood pressure set point due to a dysfunction in the pressure-natriuresis mechanism.[31] The regulation of sodium excretion in the pressure natriuresis phenomenon primarily occurs in the proximal nephron areas, with changes in medullary blood flow being crucial for the pressure-natriuresis interaction. Furthermore, additional endocrine factors, including as the renin-angiotensin-aldosterone system, nitric oxide, and prostaglandins, might modify the pressure-natriuresis relationship to increased or decreased set points. Significant evidence demonstrates that sodium management by the distal nephron segments is crucial for maintaining sodium equilibrium in reaction to variations in

blood pressure. Moreover, elements including the sympathetic nervous system, localised intrarenal inflammation, and the generation of reactive oxygen species (ROS) can affect the pressure-natriuresis association.[32]

*b) Sympathetic nervous system and renin-angiotensin-aldosterone system*

Multiple neuroendocrine systems also increase blood pressure and maintain the hypertensive condition, partly due to their impact on renal function. The two most critical systems are undoubtedly the renin-angiotensin-aldosterone system and the sympathetic nervous system.[33] The renin-angiotensin-aldosterone system (RAAS), activated by renin secretion from the juxtaglomerular cells of the kidney, plays a significant role in the pathophysiology of hypertension via its vascular, endocrine, central, and renal processes. Recent years have demonstrated that the RAAS operates not only as a circulatory system but also as a localised tissue system.[33] Thus, renal angiotensin II serves as a potent vasoconstrictor and a growth-promoting peptide, modulating blood pressure via affecting renal haemodynamics and glomerular filtration rate, as it alters the tone of both afferent and efferent arterioles.[34] There is substantial evidence that the sympathetic nervous system has a role in blood pressure regulation and the pathogenesis of hypertension. Numerous animal models of hypertension exhibit heightened sympathetic activity.[35,36] Furthermore, in the initial phases of clinical hypertension, a conjunction of sympathetic hyperactivity and parasympathetic dysfunction has been noted in young individuals exhibiting an elevated heart rate.[37] The sympathetic system significantly affects renin secretion, renal haemodynamics, and tubular salt management in the kidney.[31] The involvement of the sympathetic nervous system and baroreflex regulation in the onset of hypertension has prompted the creation of novel interventional approaches for managing resistant hypertension, such as renal denervation and baroreflex sensitisation.[38]

c) *Prostaglandins*

Prostaglandins are a class of molecules that regulate blood pressure and may play a role in the onset of hypertension. Prostaglandins originate from the metabolism of arachidonic acid. Their production involves multiple stages: initially, the release of arachidonic acid from membrane phospholipids, facilitated by phospholipase $A_2$; subsequently, the enzymatic transformation of arachidonic acid by cyclooxygenases (COX-1, COX-2, or COX-3) to yield $PGH_2$; and finally, the synthesis of specific prostaglandins, mediated by prostaglandin synthases, such as prostacyclin synthase, leading to the formation of prostacyclin ($PGI_2$), or thromboxane synthase, which produces thromboxane $A_2$. This cascade leads to the synthesis of several prostaglandins with distinct biological activities, including vascular, renal, and inflammatory actions.[39]

Phospholipase $A_2$ is stimulated by various factors, including angiotensin II, norepinephrine, and bradykinin. Prostaglandins primarily operate around their release sites due of their rapid breakdown by local metabolism into inactive metabolites. Prostaglandins can elicit either vasoconstriction (thromboxane $A_2$, $PGF_2\alpha$) or vasodilation ($PGE_2$, prostacyclin) at the vascular level.[40] A notable feature of vasodilatory prostaglandins is their ability to modulate vasoconstriction induced by potent vasoactive drugs such as angiotensin II. In the average adult kidney, both COX-1 and COX-2 are expressed continuously. COX-1 is located in the glomerulus, afferent arteriole, and tubular cells, whereas COX-2 is found in the afferent and efferent arterioles, podocytes, macula densa, and some tubular and interstitial cells. Intrarenal

prostaglandins are essential in regulating renal perfusion and glomerular filtration rate. They also participate in the maintenance of sodium, potassium, and chloride homeostasis, along with the regulation of renin secretion.[41]

The impact of prostaglandins on vascular function, renal electrolyte balance, and renin secretion may be especially relevant to the onset of hypertension.[42] Data demonstrate that mice devoid of the $PGI_2$ receptor show resistance to the development of renovascular hypertension, a renin-dependent form of hypertension.[43] Individuals with hypertension demonstrate a deficiency in vasodilatory prostaglandins[44], although an increase in thromboxane $A_2$ has been noted in essential hypertension.[45] The data indicate a possible imbalance between anti-hypertensive and pro-hypertensive prostaglandins in hypertension.[46] The three proposed functions of prostaglandins in hypertension are highlighted by the observation that both selective and nonselective COX-1 and COX-2 inhibitors increase blood pressure, encourage salt retention, and may trigger hypertension in some patients.[46] However, the hypertensive effect of non-steroidal anti-inflammatory drugs is more pronounced in patients with hypertension than in normotensive individuals.

**Pathophysiology of diabetes in the elderly**

The aetiology of diabetes entails a disturbance in the feedback mechanisms governing insulin action and production, resulting in increased blood glucose levels.[47] In cases of β-cell failure, insulin synthesis is reduced, limiting the body's capacity to maintain physiological glucose levels. Conversely, insulin resistance (IR) results in increased glucose production in the liver and reduced glucose uptake in muscle, liver, and adipose tissue. While both processes arise early in pathophysiology and facilitate disease progression, β-cell dysfunction is generally more evident than insulin resistance. However, the simultaneous presence of β-cell dysfunction and insulin resistance intensifies hyperglycaemia, promoting the progression of type 2 diabetes mellitus (T2DM).[48]

Pancreatic β-cells are accountable for the generation of insulin, which is generated as pre-proinsulin. During maturation, pre-proinsulin has a structural alteration facilitated by several proteins in the endoplasmic reticulum (ER), resulting in proinsulin.[49] Subsequently, proinsulin is transported from the endoplasmic reticulum to the Golgi apparatus, where it enters immature secretory vesicles and is broken into C-peptide and insulin.[50] Upon maturation, insulin is sequestered in granules until its release is activated. The secretion of insulin is predominantly stimulated by elevated glucose levels. It is important to acknowledge that additional variables, including amino acids, fatty acids, and hormones, can also stimulate insulin release. As circulation glucose levels rise, β-cells absorb glucose mostly via the glucose transporter 2 (GLUT2), a solute carrier protein that functions as a glucose sensor for β-cells. Following glucose entry, glucose catabolism commences, increasing the intracellular ATP/ADP ratio[47], which activates the closure of ATP-sensitive potassium channels in the plasma membrane. This leads to membrane depolarisation and the activation of voltage-gated $Ca^{2+}$ channels, permitting $Ca^{2+}$ influx into the cell. The elevation of intracellular $Ca^{2+}$ concentration triggers the priming and fusion of insulin-containing secretory granules with the plasma membrane, resulting in insulin exocytosis.

β-cell dysfunction has traditionally been associated with β-cell mortality.[51] Recent studies suggest that the dysfunction of β-cells in T2DM may arise from a complex interaction between

environmental variables and many molecular pathways in cell biology.[52] In a condition of overconsumption, similar to obesity, hyperglycaemia and hyperlipidaemia often arise, fostering insulin resistance and chronic inflammation. Beta cells, because to differences in their genetic vulnerability, are subjected to toxic stimuli including inflammation, inflammatory stress, endoplasmic reticulum stress, metabolic/oxidative stress, and amyloid stress, which may ultimately lead to the degradation of islet integrity. Excess free fatty acids and hyperglycaemia lead to β-cell dysfunction by inducing endoplasmic reticulum stress through the activation of apoptotic unfolded protein response pathways.[53] Lipotoxicity, glucotoxicity, and glucolipotoxicity linked to obesity elevate metabolic and oxidative stress, leading to β-cell death.[53]

Insufficient synthesis of insulin precursors or insulin, along with disruptions in the secretion pathway, can lead to insulin secretory dysfunction, which are the primary cause of β-cell failure and a critical component of T2DM. The reduced expression of the GLUT2 glucose transporter may affect the ensuing signalling cascade[54], while misfolding of proinsulin is another frequently noted factor linked to insufficient insulin production and diabetes.[55]

**Interactions between hypertension and diabetes**

The interplay between hypertension and diabetes is intricate, and these two illnesses frequently coexist, especially in older adults. Both exhibit shared risk factors, including obesity, a sedentary lifestyle, and advanced age. The coexistence of hypertension with diabetes produces a synergistic impact, exacerbating the risk of cardiovascular disease, stroke, and renal impairment. Multiple mechanisms elucidate the robust interplay between these two diseases:

*i. Insulin resistance and hypertension*: Insulin resistance, a hallmark of Type 2 diabetes, is closely linked to the development of hypertension. Insulin frequently induces vasodilation, promoting the relaxing of blood vessels and improving blood circulation.[56] In individuals with insulin resistance, the vasodilatory response is impaired, resulting in heightened peripheral resistance and higher blood pressure.[57] Moreover, insulin resistance leads to increased salt retention by the kidneys, so elevating blood volume and further raising blood pressure.[58]

*ii. RAAS activation in both conditions*: The RAAS is excessively active in both hypertension and diabetes. In diabetes, hyperglycaemia and insulin resistance stimulate the RAAS, leading to increased concentrations of angiotensin II and aldosterone. These hormones promote vasoconstriction, sodium retention, and inflammation, all of which contribute to hypertension.[5] The simultaneous dysfunction of the RAAS explains the high incidence of hypertension in diabetic people.[59]

*iii. Endothelial dysfunction*: Endothelial dysfunction is a common pathophysiological mechanism in hypertension and diabetes. In diabetes, chronic hyperglycaemia damages the endothelial cells of blood vessels, leading to reduced nitric oxide production.[60] Inadequate nitric oxide hinders proper vasodilation, leading to increased peripheral resistance and hypertension. Hypertension exacerbates vascular damage, creating a harmful cycle of endothelial injury and elevated blood pressure.[61]

*iv. Inflammation and oxidative stress*: Chronic inflammation and oxidative stress are pivotal contributors to the onset of hypertension and diabetes.[62] Hyperglycaemia in diabetes prompts the production of reactive oxygen species (ROS), leading to oxidative damage to cells, including endothelial and smooth

muscle cells in blood vessels.[63] Oxidative stress causes arterial stiffness and inflammation, both of which lead to hypertension.[64] Hypertension similarly increases oxidative stress in the vascular system, hence accelerating the progression of diabetes-related complications.[65]

v. *Obesity as a common risk factor*: Obesity represents a significant risk factor for hypertension and diabetes, as excess body fat exacerbates the pathophysiology of both conditions.[66] Adipose tissue, particularly visceral fat, secretes pro-inflammatory cytokines that exacerbate insulin resistance and vascular inflammation.[67] Furthermore, obesity increases cardiac workload and hypertension, hence complicating the management of diabetes and hypertension in obese patients.[68]

**Complications arising from hypertension and diabetes co-morbidity**

The simultaneous presence of hypertension and diabetes markedly elevates the risk of serious consequences, predominantly concerning cardiovascular health. These encompass:

i. *Cardiovascular disease (CVD):* Individuals with hypertension and diabetes are at a markedly increased risk of acquiring cardiovascular diseases, such as coronary artery disease, myocardial infarctions, and cerebrovascular accidents.[69] The combined consequences of insulin resistance, hyperglycaemia, and hypertension accelerate the advancement of atherosclerosis, or arterial stiffening. This may lead to plaque buildup, narrowing of blood vessels, and ultimately, blockages that trigger heart attacks or strokes.[69]

ii. *Diabetic nephropathy and hypertension-induced renal damage*: Diabetes and hypertension both contribute to renal impairment, potentially culminating in chronic kidney disease (CKD).[70] In diabetes, elevated blood glucose levels damage the small blood vessels in the kidneys, leading to diabetic nephropathy.[71] Hypertension intensifies this damage by increasing pressure within the kidneys' filtration units, so further diminishing their function.[72] This combination significantly elevates the risk of end-stage renal disease (ESRD), requiring dialysis or kidney transplantation.[73]

iii. *Retinopathy and microvascular complications*: Diabetes is a key factor in microvascular problems, particularly diabetic retinopathy, which affects the retinal blood vessels and can lead to blindness.[74] Hypertension accelerates the progression of retinopathy by increasing pressure in the retinal arteries, leading to rupture or leaking. The combined impact of hyperglycaemia and hypertension leads to increased retinal damage and visual impairment.[75]

iv. *Neuropathy*: Diabetes-induced neuropathy constitutes a substantial complication that may be exacerbated by hypertension.[76] Both conditions hinder circulation, depriving nerves of the oxygen and nutrients essential for proper function.[77] This leads to nerve damage, particularly in the peripheral nervous system, resulting in pain, numbness, and functional deficits in the limbs.[77]

**Clinical guidelines for treatment for hypertension and diabetes co-morbidity**

Managing elderly people with hypertension and diabetes necessitates a sophisticated, interdisciplinary strategy, since both ailments markedly elevate the danger of cardiovascular incidents, renal failure, and other critical complications.[78] Clinical guidelines are formulated based on comprehensive research and expert consensus to guarantee that patients receive optimal care while reducing unfavourable consequences.[78] The primary objectives in

managing hypertension and diabetes are to regulate blood pressure and glucose levels, mitigate the risk of complications, and enhance overall quality of life.[79] In geriatric patients, specific considerations are addressed due to characteristics including frailty, polypharmacy, and the existence of several comorbidities.[80]

The broadly referenced clinical guidelines for managing hypertension and diabetes co-morbidity in the elderly include recommendations from the American Diabetes Association (ADA), the American Heart Association (AHA), the European Society of Hypertension (ESH), and the European Society of Cardiology (ESC).[81] These guidelines emphasise a comprehensive, individualised approach, which includes lifestyle interventions, pharmacological therapy, and regular monitoring of both blood pressure and blood glucose levels.[81]

**Blood pressure targets for elderly patients with diabetes**

Managing hypertension in elderly diabetic patients necessitates meticulous evaluation of blood pressure objectives to mitigate cardiovascular disease risk while avoiding adverse effects.[78] Excessive lowering of blood pressure, especially in elderly individuals, may result in negative consequences including dizziness, falls, and diminished renal function.[82] Clinical guidelines often advocate for a more cautious strategy in managing blood pressure among elderly adults relative to younger demographics. Current recommendations from organisations such the ADA, ESH, and ESC indicate that a goal systolic blood pressure (SBP) below 140 mmHg is suitable for the majority of older adults with diabetes. Certain guidelines advocate for even more personalised aims, especially for old or weak patients.[81]

*Optimal blood pressure target:* For elderly patients with diabetes, most guidelines suggest a blood pressure target of 130-140/80-90 mmHg. This range mitigates the risk of cardiovascular events while minimising the likelihood of unfavourable therapeutic effects.[83]

*Consideration for frail elderly patients*: Guidelines advocate for more lenient blood pressure objectives in frail elderly adults or those with restricted life expectancy.[84] A systolic blood pressure target of 140-150 mmHg may be deemed acceptable, particularly if lower targets induce symptoms like dizziness or exhaustion.[85] The determination of particular blood pressure targets must consider individual patient characteristics, including age, overall health, co-morbidities, and patient preferences.[86] The blood pressure targets must be attained progressively to avert abrupt declines that may result in problems like hypotension and falls.[87]

**Glycaemic control goals for elderly patients with hypertension**

Glycaemic control is critical in the management of Type 2 diabetes in the elderly, as hyperglycaemia contributes to cardiovascular disease, kidney damage, and other complications.[88] However, like blood pressure targets, glycaemic goals should be individualised based on the patient's overall health, risk of hypoglycaemia, and co-existing conditions.[89]

*HbA1c target*: Glycated haemoglobin (HbA1c) is the primary measure used to assess long-term glycaemic control.[90] For most elderly patients with diabetes, guidelines recommend an HbA1c target of 7.0-8.0%.[91] This range helps prevent the development of diabetic complications while reducing the risk of hypoglycaemia, which can be particularly dangerous in older adults.[91]

*Relaxed glycaemic targets for frail patients*: In elderly patients who are frail, have multiple co-morbidities, or a limited life expectancy, a more

relaxed HbA1c target of 8.0-8.5% may be appropriate. This approach reduces the risk of hypoglycaemia and other treatment-related complications that could negatively impact quality of life.[92]

*Avoiding hypoglycaemia*: In elderly patients, hypoglycaemia poses a serious risk, particularly if they are on medications such as insulin or sulphonylureas, which increase the risk of low blood sugar.[93] Clinical guidelines emphasise the importance of avoiding hypoglycaemia by choosing medications with a lower risk of this side effect and by setting appropriate, individualised glycaemic targets.[89]

**Pharmacological management of hypertension and diabetes co-morbidity**

The simultaneous occurrence of hypertension and diabetes, especially in senior individuals, poses a considerable difficulty for healthcare professionals. Both illnesses independently elevate the chance of cardiovascular diseases (CVD), stroke, renal failure, and other consequences.[94] When these conditions coalesce, the risk of negative outcomes escalates, rendering pharmacological intervention essential for alleviating long-term harm and enhancing quality of life.

Managing both conditions simultaneously requires a careful selection of medications that not only control blood pressure and blood glucose but also protect vital organs such as the heart and kidneys.[95] However, the presence of co-morbidity in elderly patients introduces additional complexities. Factors like polypharmacy, age-related decline in organ function, frailty, and drug interactions need to be carefully considered to avoid adverse outcomes, such as hypoglycaemia or hypotension.[17,96]

**Therapeutic goals for managing co-morbid hypertension and diabetes**

The primary goals in pharmacological management of co-morbid hypertension and diabetes are[97]:

i. Reduction of blood pressure to target levels, minimising cardiovascular risk without causing harm (e.g., hypotension, falls).[98]
ii. Achieving optimal glycaemic control to prevent diabetic complications, while avoiding hypoglycaemia, especially in elderly patients.[99]
iii. Minimisation of polypharmacy risks, including drug interactions, side effects, and the burden on elderly patients, who may be dealing with multiple medications for various conditions.[100]

**Antihypertensive medications in diabetic patients**

Choosing antihypertensive agents for diabetic patients requires an understanding of the interplay between hypertension, diabetes, and the patient's overall health status. Specific drug classes are favoured for their ability to manage both blood pressure and complications related to diabetes. (Table 1)

Table 1: Antihypertensive agents for diabetic patients

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| **Angiotensin-Converting Enzyme (ACE) Inhibitors** | ACE inhibitors, such as lisinopril and enalapril, are widely considered first-line therapy for managing hypertension in patients with diabetes. They work by inhibiting the renin-angiotensin-aldosterone system (RAAS), which is often overactive in diabetic patients. This not only lowers blood pressure but also reduces the progression of diabetic nephropathy by decreasing proteinuria (the presence of protein in urine) and slowing kidney damage | ACE inhibitors have been shown to reduce cardiovascular risk and protect the kidneys, making them ideal for elderly diabetic patients who are at increased risk of both cardiovascular and renal complications | ACE inhibitors may cause side effects such as hyperkalemia (high potassium levels) and a persistent cough, and they are contraindicated in patients with advanced renal impairment or those who experience angioedema | (101) |
| **Angiotensin II Receptor Blockers (ARBs)** | ARBs, such as losartan and valsartan, provide similar benefits to ACE inhibitors but are often prescribed when patients cannot tolerate ACE inhibitors due to side effects. | ARBs reduce proteinuria, slow the progression of kidney disease, and are well - tolerated by m ost patients. | Similar to ACE inhibitors, ARBs can cause hyperkalemia and must be used with caution in patients with renal insufficiency | (102, 103) |

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| | They work by blocking the angiotensin II receptor, leading to vasodilation and reduc ed blood pressure. Like ACE inhibitors, ARBs have nephroprotective effects and are crucial for diabetic patients who may be progressing toward diabetic nephropathy | | | |
| **Calcium Channel Blockers (CCBs)** | Calcium channel blockers, like amlodipine and diltiazem, are frequently prescribed for aged people, especially those with isolated systolic hypertension. These drugs facilitate the relaxation of blood vessels, diminishing vascular resistance and decreasing blood pressure. | Calcium channel blockers (CCBs) effectively lower blood pressure without negatively impacting glucose metabolism, rendering them a suitable choice for diabetic individuals. They also mitigate the risk of stroke, a prevalent issue in individuals with both hypertension and diabetes | CCBs may cause peripheral edema, particularly in elderly patients, and should be monitored in patients with heart failure | ([104]) |

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| **Thiazide Diuretics** | Thiazide diuretics, such as hydrochlorothiazide and chlorthalidone, help manage hypertension by reducing fluid retention and decreasing bl ood volume. They are often included as part of combination therapy in diabetic patients to achieve optimal blood pressure control | Thiazides are effective at decreasing blood pressure and have been reported to lower the danger of stroke in hypertensive patients | In diabetic patients, thiazides may increase blood glucose levels and potentially worsen insulin resistance. They should be used cautiously in aged patients with a history of diabetes, and potassium levels must be monitored due to the possibility of hypokalemia | ([105]) |
| **Beta-Blockers** | Beta-blockers, such as metoprolol and carvedilol, are occasionally used in hypertensive patients with diabetes, particularly in those with co-existing coronary artery disease or heart failure. They work by slowing heart rate and reducing myocardial oxygen demand | Beta-blockers provide cardiovascular protection, particularly for patients with a history of myocardial infarction or heart failure | Beta-blockers can mask the symptoms of hypoglycemia, such as tachycardia, making them a less favorable option for patients who are at high risk for low blood sugar episodes. Additionally, non-selective beta-blockers can potentially worsen glucose tolerance, making cardio-selective agents preferable | ([106]) |

**Antidiabetic drugs in Hypertensive Patients**

In patients with diabetes and hypertension, blood glucose control is critical to prevent microvascular complications, such as retinopathy, neuropathy, and nephropathy, as well as macrovascular complications like heart disease and stroke. The pharmacological options for diabetes management must take into account the presence of hypertension to avoid adverse effects and drug interactions.[4] (Table 2)

Table 2: Pharmacological options for diabetes management in hypertensive patients

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| **Metformin** | Metformin is the first-line treatment for Type 2 diabetes in most patients. It works by reducing hepatic glucose production and improving insulin sensitivity. It is particularly useful in hypertensive patients with diabetes because it does not cause weight gain or hypoglycemia, two major concerns in elderly populations | Metformin has cardiovascular benefits and helps with weight management, making it a strong choice for elderly patients who are overweight or at risk for cardiovascular diseases. | Metformin should be used with caution in elderly patients with renal impairment due to the risk of lactic acidosis. Monitoring renal function is essential in this population | [107, 108] |
| **Sulfonylureas** | Sulfonylureas, such as glipizide and glyburide, work by stimulating the pancreas to release insulin. These drugs are effective at lowering blood glucose but have a higher risk of causing hypoglycemia, particularly in elderly patients | Sulfonylureas are effective at controlling blood glucose levels in the short term, and their relatively low cost makes them accessible | The risk of hypoglycemia, particularly in elderly patients with declining kidney function or irregular eating patterns, makes sulfonylureas less ideal. Newer agents with a lower risk of hypoglycemia are generally preferred in elderly populations | [109] |

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| **Dipeptidyl peptidase-4 (DPP-4) Inhibitors** | DPP-4 inhibitors, such as sitagliptin and saxagliptin, are a newer class of drugs that increase insulin release and decrease glucagon levels. These drugs are generally well-tolerated and have a low risk of causing hypoglycemia, making them suitable for elderly patients with co-morbid hypertension and diabetes | DPP-4 inhibitors are weight-neutral, have a low risk of hypoglycemia, and can be safely combined with other antihypertensive and antidiabetic medications | DPP-4 inhibitors, while typically well-tolerated, may pose a somewhat elevated risk of heart failure in certain patients, particularly those with pre-existing cardiovascular problems | (110) |
| **Glucagon-like peptides-1 (GLP-1) Receptor Agonists** | GLP-1 receptor agonists, such as liraglutide and exenatide, are injectable medications that enhance insulin secretion, suppress glucagon release, and promote weight loss. These agents have shown cardiovascular benefits, making them an attractive option for patients with both diabetes and hypertension | GLP-1 receptor agonists not only improve glycemic control but also promote weight loss and provide cardiovascular protection, reducing the risk of adverse outcomes in hypertensive patients with diabetes | The main drawbacks of GLP-1 receptor agonists are gastrointestinal side effects, such as nausea, and the need for injections, which may be less desirable for elderly patients | (111) |

| Drugs | Notes | Therapeutic effects | Adverse effects | References |
|---|---|---|---|---|
| **Sodium-glucose transporter protein-2 (SGLT-2) Inhibitors** | SGLT -2 inhibitors, such as empagliflozin and canagliflozin, lower blood glucose by promoting the excretion of glucose in the urine. These drugs also offer significant cardiovascular and renal benefits, making them a valuable addition to the treatment regimen for patients with both hypertension and diabetes | SGLT -2 inhibitors have demonstrated efficacy in diminishing the chance of heart failure and decelerating the advancement of renal disease in diabetic individuals, rendering them a robust option for aged patients with concomitant hypertension | SGLT -2 inhibitors can cause urinary tract infections and dehydration, particularly in elderly patients. Proper hydration and monitoring of renal function are important when using these medications | ([112]) |

**Special considerations for elderly patients**

Elderly patients present unique challenges in the pharmacological management of hypertension and diabetes.[113] Age-associated alterations in drug metabolism, polypharmacy, frailty, and comorbid conditions must all be considered to avoid adverse outcomes.[113] Strategies for managing these complexities include:

*i. Dose adjustments*: Lower starting doses and slower titration of medications to avoid adverse effects.[114]

*ii. Monitoring for drug interactions:* Given the likelihood of polypharmacy, careful attention must be paid to potential drug-drug interactions that could exacerbate side effects or reduce the effectiveness of therapy.[115]

*iii. Minimising the risk of hypoglycaemia:* Elderly patients, particularly those with declining renal function or irregular meal patterns, are at higher risk of hypoglycaemia.[116] Medications with a lower risk of hypoglycaemia should be prioritised.

*iv. Promoting medication adherence:* Simplifying drug regimens and using combination therapies can reduce pill burden and improve adherence in elderly patients, who may struggle with complex treatment plans.[117]

**Conclusion and Future prospects**

Ageing is a natural physiological process experienced by all individuals, and with advancements in healthcare and living standards, the average old population is anticipated to rise over time. The rise in the older population also correlates with a higher prevalence of non-communicable diseases, such as hypertension and diabetes mellitus. Indeed, there is a correlated rise in co-morbidity between these two disorders. Physiological alterations associated with ageing,

including vascular rigidity, heightened insulin resistance, and diminished pancreatic and renal function, contribute to the elevated susceptibility to diabetic hypertensive co-morbidity. Moreover, the activation of the renin-angiotensin-aldosterone system, endothelial dysfunction, inflammation, oxidative stress, and obesity are pivotal in the pathogenesis of both disorders. This co-morbidity presents considerable hurdles in therapeutic management and, if not properly handled, may result in heightened mortality and the emergence of sequelae such as retinopathy, neuropathy, and nephropathy, among others. Managing older people with concurrent hypertension and diabetes necessitates a sophisticated, interdisciplinary strategy. Age-associated alterations in drug metabolism, polypharmacy, frailty, and comorbid conditions must all be considered to avoid adverse outcomes and promote adherence while ensuring optimal efficacy. Attempts must also be made to shift management patterns towards personalised medicine with genetic profiling and telemedicine with remote monitoring.

As the world's population ages, the prevalence of hypertension and diabetes among the elderly is expected to increase. This necessitates ongoing research into novel techniques to managing these co-morbid conditions, with a particular emphasis on personalised medicine, emerging technology, and more integrated care models.

1. *Personalised Medicine and Genetic Profiling:* Advances in genetic profiling and personalised medicine hold the potential of enabling more targeted therapy in hypertension and diabetes treatment. Understanding an individual's genetic propensity enables healthcare providers to tailor pharmacological treatments and lifestyle suggestions to improve outcomes and avoid complications.
2. *Telemedicine and Remote Monitoring:* Telemedicine and remote monitoring technologies are increasingly vital instruments for controlling chronic diseases in the senior demographic. Remote surveillance of blood pressure, glucose levels, and other health parameters facilitates more proactive interventions, diminishing the necessity for frequent clinic visits while enhancing patient outcomes through early treatment.
3. *Integrated Care Models*: Future care for elderly people with co-morbid hypertension and diabetes is expected to take a more integrated, multidisciplinary approach. Coordination of therapy among physicians, dietitians, physical therapists, mental health professionals, and social workers ensures that all aspects of a patient's health are addressed, leading to better overall outcomes.

**Acknowledgements**
None.

**Funding**
No funding was received for conducting this study.

**Conflict of interest**
The authors declare no competing interests.

**REFERENCES**
References

1. Zhao J, Han Z, Ding L, Wang P, He X, Lin L. The molecular mechanism of aging and the role in neurodegenerative diseases. Heliyon. 2024; 10(2):e24751.
2. Gu D, Andreev K, Dupre ME. Major Trends in Population Growth Around the World. China CDC Wkly. 2021; 3(28):604-613.
3. Martinez R, Lloyd-Sherlock P, Soliz P, Ebrahim S, Vega E, Ordunez P, et al. Trends in premature avertable mortality from non-

communicable diseases for 195 countries and territories, 1990-2017: a population-based study. Lancet Glob Health. 2020; 8(4):e511-e523.

4. de Boer IH, Bangalore S, Benetos A, Davis AM, Michos ED, Muntner P, et al. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. Diabetes Care. 2017; 40(9):1273-1284.
5. Jia G, Sowers JR. Hypertension in Diabetes: An Update of Basic Mechanisms and Clinical Disease. Hypertension. 2021; 78(5):1197-1205.
6. Taheri A, Khezri R, Dehghan A, Rezaeian M, Aune D, Rezaei F. Hypertension among persons with type 2 diabetes and its related demographic, socioeconomic and lifestyle factors in the Fasa cohort study. Sci Rep. 2024; 14(1):e18892.
7. Jobe M, Mactaggart I, Bell S, Kim MJ, Hydara A, Bascaran C, et al. Prevalence of hypertension, diabetes, obesity, multimorbidity, and related risk factors among adult Gambians: a cross-sectional nationwide study. Lancet Glob Health. 2024; 12(1):e55-e65.
8. Al-Musawe L, Torre C, Guerreiro JP, Rodrigues AT, Raposo JF, Mota-Filipe H, et al. Polypharmacy, potentially serious clinically relevant drug-drug interactions, and inappropriate medicines in elderly people with type 2 diabetes and their impact on quality of life. Pharmacol Res Perspect. 2020; 8(4):e00621.
9. Atlas. IDF Diabetes Atlas. 9th ed. International Diabetes Federation, 2019.
10. Katsimardou A, Imprialos K, Stavropoulos K, Sachinidis A, Doumas M, Athyros VG. Treatment strategies for hypertension in patients with type 1 diabetes. Expert Opin Pharmacother. 2020; 21(10):1241-1252.
11. Tuomilehto J, Rastenyte D, Qiao Q, Barengo NC, Matz K. Epidemiology of macrovascular disease and hypertension in diabetes mellitus. Int Textbook of Diabetes Mellitus; 2015:1005-1030.
12. Avogaro A, Fadini GP. Microvascular complications in diabetes: A growing concern for cardiologists. Int J Cardiol 2019; 291:29-35.
13. Lu WL, Yuan JH, Liu ZY, Su ZH, Shen YC, Li SJ, et al. Worldwide trends in mortality for hypertensive heart disease from 1990 to 2019 with projection to 2034: data from the Global Burden of Disease 2019 study. Eur J Prev Cardiol. 2024; 31(1):23-37.
14. International Diabetes Federation (IDF). IDF Diabetes Atlas, 10th edn. Brussels, Belgium: International Diabetes Federation, 2021. Available from: https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdfo.
15. Moke EG, Onyilo PO, Umukoro EK, Awhin EP, Ben-Azu B, Asiwe JN, et al. Efficacy evaluation of contemporary and alternative medicine in managing diabetes mellitus: A review. Orapuh Journal. 2025; 6(1):e1203.
16. Brown TT, Guaraldi G. Multimorbidity and Burden of Disease. Interdiscip Top Gerontol Geriatr. 2017; 42:59-73.
17. Ciumărnean L, Milaciu MV, Negrean V, Ora an OH, Vesa SC, Sălăgean O, et al. Cardiovascular Risk Factors and Physical Activity for the Prevention of Cardiovascular Diseases in the Elderly. Int J Environ Res Public Health. 2021; 19(1):e207.
18. Dai X, Hummel SL, Salazar JB, Taffet GE, Zieman S, Schwartz JB. Cardiovascular physiology in the older adults. J Geriatr Cardiol. 2015; 12(3):196-201.
19. Cleasby ME, Jamieson PM, Atherton PJ. Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. J Endocrinol. 2016;

229(2):R67-81.
20. Kitada M, Koya D. Autophagy in metabolic disease and ageing. Nat Rev Endocrinol. 2021; 17(11):647-661.
21. Sinclair AJ, Abdelhafiz AH. Multimorbidity, Frailty and Diabetes in Older People-Identifying Interrelationships and Outcomes. J Pers Med. 2022; 12(11):1911.
22. Carracedo J, Alique M, Vida C, Bodega G, Ceprián N, Morales E, et al. Mechanisms of Cardiovascular Disorders in Patients With Chronic Kidney Disease: A Process Related to Accelerated Senescence. Front Cell Dev Biol. 2020; 8:185.
23. Akkus G, Sert M. Diabetic foot ulcers: A devastating complication of diabetes mellitus continues non-stop in spite of new medical treatment modalities. World J Diabetes. 2022; 13(12):1106-1121.
24. Zhou D, Xi B, Zhao M, Wang L, Veeranki SP. Uncontrolled hypertension increases risk of all-cause and cardiovascular disease mortality in US adults: the NHANES III Linked Mortality Study. Sci Rep. 2018; 8(1):e9418.
25. Kim J, Parish AL. Polypharmacy and Medication Management in Older Adults. Nurs Clin North Am. 2017; 52(3):457-468.
26. Mardani A, Paal P, Weck C, Jamshed S, Vaismoradi M (2022) Practical Considerations of PRN Medicines Management: An Integrative Systematic Review. Front Pharmacol. 2022; 13:e759998.
27. de Sousa FG, Queiroz FSF, Muzzi RAL, Veado JCC, Beier SL. Systemic Arterial Hypertension and Factors Associated with Blood Pressure Dysregulation in Companion Animals. Vet Sci, 2025; 12(5):e453.
28. Wiig H, Luft FC, Titze JM. The interstitium conducts extrarenal storage of sodium and represents a third compartment essential for extracellular volume and blood pressure homeostasis. Acta Physiol (Oxf). 2018; 222(3).
29. AlMarabeh S, Abdulla MH, O'Halloran KD. Is Aberrant Reno-Renal Reflex Control of Blood Pressure a Contributor to Chronic Intermittent Hypoxia-Induced Hypertension? Front Physiol. 2019; 10:465.
30. Bie P. Mechanisms of sodium balance: total body sodium, surrogate variables, and renal sodium excretion. Am J Physiol Regul Integr Comp Physiol. 2018; 315(5):R945-R962.
31. Díaz-Morales N, Baranda-Alonso EM, Martínez-Salgado C, López-Hernández FJ. Renal sympathetic activity: A key modulator of pressure natriuresis in hypertension. Biochem Pharmacol. 2023; 208:115386.
32. Bernal A, Zafra MA, Simón MJ, Mahía J. Sodium Homeostasis, a Balance Necessary for Life. Nutrients. 2023; 15(2):395.
33. Steckelings UM, Unger T. The Renin-Angiotensin-Aldosterone System. In: Manual of Hypertension of the European Society of Hypertension, 3rd ed., pp. 101-108. CRC Press, 2019.
34. Nishiyama A, Kobori H. Independent regulation of renin-angiotensin-aldosterone system in the kidney. Clin Exp Nephrol. 2018; 22(6):1231-39.
35. Lerman LO, Kurtz TW, Touyz RM, Ellison DH, Chade AR, Crowley SD, et al. Animal Models of Hypertension: A Scientific Statement From the American Heart Association. Hypertension. 2019; 73(6):e87-e120.
36. Jama HA, Muralitharan RR, Xu C, O'Donnell JA, Bertagnolli M, Broughton BRS, et al. Rodent models of hypertension. Br J Pharmacol. 2022; 179(5):918-937.
37. Queiroz MG, Prado AGK, Alves-Santos ÉT, Araújo JA, Damazo AS, Cambri LT. Influence of family history of hypertension on blood pressure and heart rate variability in young adults: a meta-analysis. Blood Press

Monit. 2022; 27(4):259-271.
38. Grassi G. The Sympathetic Nervous System in Hypertension: Roadmap Update of a Long Journey. Am J Hypertens. 2021; 34(12):1247-54.
39. Hanna VS, Hafez EAA. Synopsis of arachidonic acid metabolism: A review. J Adv Res. 2018; 11:23-32.
40. Norel X, Sugimoto Y, Ozen G, Abdelazeem H, Amgoud Y, Bouhadoun A, et al. International Union of Basic and Clinical Pharmacology. CIX. Differences and Similarities between Human and Rodent Prostaglandin E2 Receptors (EP1-4) and Prostacyclin Receptor (IP): Specific Roles in Pathophysiologic Conditions. Pharmacol Rev. 2020; 72(4):910-968.
41. Hoorn EJ, Gritter M, Cuevas CA, Fenton RA. Regulation of the Renal NaCl Cotransporter and Its Role in Potassium Homeostasis. Physiol Rev. 2020; 100(1):321-356.
42. Sequeira-Lopez MLS, Gomez RA. Renin Cells, the Kidney, and Hypertension. Circ Res. 2021; 128(7):887-907.
43. Meurer M, Ebert K, Schweda F, Höcherl K. The renal vasodilatory effect of prostaglandins is ameliorated in isolated-perfused kidneys of endotoxemic mice. Pflugers Arch. 2018; 470(11):1691-1703.
44. Avendaño MS, García-Redondo AB, Zalba G, González-Amor M, Aguado A, Martínez-Revelles S, et al. mPGES-1 (Microsomal Prostaglandin E Synthase-1) Mediates Vascular Dysfunction in Hypertension Through Oxidative Stress. Hypertension. 2018; 72(2):492-502.
45. D'Agostino I, Tacconelli S, Bruno A, Contursi A, Mucci L, Hu X, et al. Low-dose Aspirin prevents hypertension and cardiac fibrosis when thromboxane A2 is unrestrained. Pharmacol Res. 2021; 170:105744.
46. van Dorst DCH, Dobbin SJH, Neves KB, Herrmann J, Herrmann SM, Versmissen J, et al. Hypertension and Prohypertensive Antineoplastic Therapies in Cancer Patients. Circ Res 2021;128(7):1040-1061.
47. Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, et al. Role of Insulin in Health and Disease: An Update. Int J Mol Sci. 2021; 22(12):6403.
48. Dludla PV, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, et al. Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. World J Diabetes. 2023; 14(3):130-146.
49. Liu M, Weiss MA, Arunagiri A, Yong J, Rege N, Sun J, et al. Biosynthesis, structure, and folding of the insulin precursor protein. Diabetes Obes Metab. 2018; 20 Suppl 2(Suppl 2):28-50.
50. Norris N, Yau B, Kebede MA. Isolation and Proteomics of the Insulin Secretory Granule. Metabolites. 2021; 11(5):288.
51. Kawamori D. Exploring the molecular mechanisms underlying α- and β-cell dysfunction in diabetes. Diabetol Int. 2017; 8(3):248-256.
52. Eizirik DL, Pasquali L, Cnop M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat Rev Endocrinol. 2020; 16(7):349-362.
53. Vilas-Boas EA, Almeida DC, Roma LP, Ortis F, Carpinelli AR. Lipotoxicity and β-Cell Failure in Type 2 Diabetes: Oxidative Stress Linked to NADPH Oxidase and ER Stress. Cells. 2021; 10(12):3328.
54. Holman GD. Structure, function and regulation of mammalian glucose transporters of the SLC2 family. Holman Pflugers Arch - Eur J Physiol. 2020; 472:1155-75.
55. Arunagiri A, Haataja L, Cunningham CN, Shrestha N, Tsai B, Qi L, et al. Misfolded proinsulin in the endoplasmic reticulum during development of beta cell failure in

diabetes. Ann N Y Acad Sci. 2018; 1418(1):5-19.
56. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovasc Diabetol. 2018; 17(1):122.
57. Limberg JK, Soares RN, Padilla J. Role of the Autonomic Nervous System in the Hemodynamic Response to Hyperinsulinemia-Implications for Obesity and Insulin Resistance. Curr Diab Rep. 2022; 22(4):169-175.
58. Brosolo G, Da Porto A, Bulfone L, Vacca A, Bertin N, Scandolin L, Catena C, Sechi LA. Insulin Resistance and High Blood Pressure: Mechanistic Insight on the Role of the Kidney. Biomedicines. 2022; 10(10):2374.
59. Libianto R, Batu D, MacIsaac RJ, Cooper ME, Ekinci EI. Pathophysiological Links Between Diabetes and Blood Pressure. Can J Cardiol. 2018; 34(5):585-594.
60. Maruhashi T, Higashi Y. Pathophysiological Association between Diabetes Mellitus and Endothelial Dysfunction. Antioxidants (Basel). 2021; 10(8):1306.
61. Konukoglu D, Uzun H. Endothelial Dysfunction and Hypertension. Adv Exp Med Biol. 2017; 956:511-40.
62. Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. Oxidative Stress and Hypertension. Circ Res. 2021; 128(7):993-1020.
63. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis. 2018; 9(2):119.
64. Guzik TJ, Touyz RM. Oxidative Stress, Inflammation, and Vascular Aging in Hypertension. Hypertension. 2017; 70(4):660-667.
65. Charlton A, Garzarella J, Jandeleit-Dahm KAM, Jha JC. Oxidative Stress and Inflammation in Renal and Cardiovascular Complications of Diabetes. Biology (Basel). 2020; 10(1):18.
66. Piché ME, Tchernof A, Després JP. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. Circ Res. 2020; 126(11):1477-1500.
67. Janochova K, Haluzik M, Buzga M. Visceral fat and insulin resistance - what we know? Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2019; 163(1):19-27.
68. Lavie CJ, Arena R, Alpert MA, Milani RV, Ventura HO. Management of cardiovascular diseases in patients with obesity. Nat Rev Cardiol. 2018; 15(1):45-56.
69. Yen FS, Wei JC, Chiu LT, Hsu CC, Hwu CM. Diabetes, hypertension, and cardiovascular disease development. J Transl Med. 2022; 20(1):9.
70. Kalantar-Zadeh K, Jafar TH, Nitsch D, Neuen BL, Perkovic V. Chronic kidney disease. Lancet. 2021; 398(10302):786-802.
71. Yu SM, Bonventre JV. Acute Kidney Injury and Progression of Diabetic Kidney Disease. Adv Chronic Kidney Dis. 2018; 25(2):166-180.
72. Verbrugge FH, Guazzi M, Testani JM, Borlaug BA. Altered Hemodynamics and End-Organ Damage in Heart Failure: Impact on the Lung and Kidney. Circulation. 2020; 142(10):998-1012.
73. Locke JE, Reed RD, Massie A, MacLennan PA, Sawinski D, Kumar V, et al. Obesity increases the risk of end-stage renal disease among living kidney donors. Kidney Int. 2017; 91(3):699-703.
74. Kropp M, Golubnitschaja O, Mazurakova A, Koklesova L, Sargheini N, Vo TKS, et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications-risks and mitigation. EPMA J. 2023; 14(1):21-42.

75. Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. ISRN Ophthalmol. 2013; 343560.
76. Sethi Y, Uniyal N, Vora V, Agarwal P, Murli H, Joshi A, et al. Hypertension the 'Missed Modifiable Risk Factor' for Diabetic Neuropathy: a Systematic Review. Curr Probl Cardiol. 2023; 48(4):101581.
77. Yachmaneni A Jr, Jajoo S, Mahakalkar C, Kshirsagar S, Dhole S. A Comprehensive Review of the Vascular Consequences of Diabetes in the Lower Extremities: Current Approaches to Management and Evaluation of Clinical Outcomes. Cureus. 2023; 15(10):e47525.
78. Sukumar S, Orkaby AR, Schwartz JB, Marcum Z, Januzzi JL, Vaduganathan M, et al. Polypharmacy in Older Heart Failure Patients: a Multidisciplinary Approach. Curr Heart Fail Rep. 2022; 19(5):290-302.
79. Trento M, Fornengo P, Amione C, Salassa M, Barutta F, Gruden G, et al. Self-management education may improve blood pressure in people with type 2 diabetes. A randomized controlled clinical trial. Nutr Metab Cardiovasc Dis. 2020; 30(11):1973-1979.
80. Umegaki H. Frailty, multimorbidity, and polypharmacy: Proposal of the new concept of the geriatric triangle. Geriatr Gerontol Int. 2025; 25(5):657-662.
81. Morley JE, Abbatecola AM, Woo J. Management of Comorbidities in Older Persons With Type 2 Diabetes. J Am Med Dir Assoc. 2017; 18(8):639-645.
82. Rivasi G, Ceolin L, Capacci M, Matteucci G, Testa GD, Ungar A. Risks associated with intensive blood pressure control in older patients. Kardiol Pol. 2023; 81(5):446-454.
83. Park S. Ideal Target Blood Pressure in Hypertension. Korean Circ J. 2019; 49(11):1002-1009.
84. Bogaerts JMK, von Ballmoos LM, Achterberg WP, Gussekloo J, Streit S, van der Ploeg MA, et al. Do we AGREE on the targets of antihypertensive drug treatment in older adults: a systematic review of guidelines on primary prevention of cardiovascular diseases. Age Ageing. 2022; 51(1):afab192.
85. Qaseem A, Wilt TJ, Rich R, Humphrey LL, Frost J, Forciea MA, et al. Pharmacologic Treatment of Hypertension in Adults Aged 60 Years or Older to Higher Versus Lower Blood Pressure Targets: A Clinical Practice Guideline From the American College of Physicians and the American Academy of Family Physicians. Ann Intern Med. 2017; 166(6):430-437.
86. Aschmann HE, Boyd CM, Robbins CW, Mularski RA, Chan WV, Sheehan OC, et al. Balance of benefits and harms of different blood pressure targets in people with multiple chronic conditions: a quantitative benefit-harm assessment. BMJ Open. 2019; 9(8):e028438.
87. Joseph A, Wanono R, Flamant M, Vidal-Petiot E. Orthostatic hypotension: A review. Nephrol Ther. 2017; 13 Suppl 1:S55-S67.
88. Fasil A, Biadgo B, Abebe M. Glycemic control and diabetes complications among diabetes mellitus patients attending at University of Gondar Hospital, Northwest Ethiopia. Diabetes Metab Syndr Obes. 2018; 12:75-83.
89. Rodríguez-Gutiérrez R, Millan-Alanis JM, Barrera FJ, McCoy RG. Value of Patient-Centered Glycemic Control in Patients with Type 2 Diabetes. Curr Diab Rep. 2021; 21(12):63.
90. Kim KJ, Choi J, Bae JH, Kim KJ, Yoo HJ, Seo JA, et al. Time to Reach Target Glycosylated Hemoglobin Is Associated with Long-Term Durable Glycemic Control and Risk of Diabetic Complications in Patients with Newly Diagnosed Type 2

Diabetes Mellitus: A 6-Year Observational Study. Diabetes Metab J. 2021; 45(3):368-378.
91. Sugimoto T, Noma H, Kuroda Y, Matsumoto N, Uchida K, Kishino Y, et al. Time trends (2012-2020) in glycated hemoglobin and adherence to the glycemic targets recommended for elderly patients by the Japan Diabetes Society/Japan Geriatrics Society Joint Committee among memory clinic patients with diabetes mellitus. J Diabetes Investig. 2022; 13(12):2038-2046.
92. Kezerle L, Shalev L, Barski L. Treating the elderly diabetic patient: special considerations. Diabetes Metab Syndr Obes. 2014; 7:391-400.
93. Hoogwerf BJ. Hypoglycemia in Older Patients. Clin Geriatr Med. 2020; 36(3):395-406.
94. Verma M, Grover S, Tripathy JP, Singh T, Nagaraja SB, Kathirvel S, et al. Co-existing Non-communicable Diseases and Mental Illnesses Amongst the Elderly in Punjab, India. Eur Endocrinol. 2019; 15(2):106-112.
95. Moke EG, Demaki WE, Daubry TME, Ataikiru OM, Agbonifo-Chijiokwu E, Ofulue OO, et al. Coexistence of hypertension with diabetes mellitus and its pharmacotherapy. Scientia Africana. 2023; 22(2):135-154.
96. Kwakye AO, Oppong MB, Kretchy IA. Pharmacist interventions to improve medication adherence in patients with co-morbid hypertension and diabetes: a scoping review and bibliometric analysis. Int J Pharm Pract. 2025; 33(2):134-145.
97. Foguet-Boreu Q, Violán C, López Jiménez T, Pons-Vigués M, Rodríguez-Blanco T, Valderas JM, et al. Pharmacological control of diabetes and hypertension comorbidity in the elderly: A study of "real world" data. Prim Care Diabetes. 2017; 11(4):348-359.
98. Böhm M, Schumacher H, Teo KK, Lonn EM, Mahfoud F, Mann JFE, et al. Achieved blood pressure and cardiovascular outcomes in high-risk patients: results from ONTARGET and TRANSCEND trials. Lancet. 2017; 389(10085):2226-2237.
99. Mathur S, Zammitt NN, Frier BM. Optimal glycaemic control in elderly people with type 2 diabetes: what does the evidence say? Drug Saf. 2015; 38(1):17-32.
100. Hoel RW, Giddings Connolly RM, Takahashi PY. Polypharmacy Management in Older Patients. Mayo Clin Proc. 2021; 96(1):242-256.
101. Banerjee D, Winocour P, Chowdhury TA, De P, Wahba M, Montero R, et al. Management of hypertension and renin-angiotensin-aldosterone system blockade in adults with diabetic kidney disease: Association of British Clinical Diabetologists and the Renal Association UK guideline update 2021. BMC Nephrol. 2022; 23(1):9.
102. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, Xie J, et al. Association of Inpatient Use of Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers With Mortality Among Patients With Hypertension Hospitalized With COVID-19. Circ Res. 2020; 126(12):1671-1681.
103. Moke EG, Omogbai EKI, Osagie-Eweka SE, Uchendu AP, Obayuwana OM, Okoro-Akpandu E, et al. Antihypertensive and antihyperglycemic effects of combinations of losartan with metformin and/or glibenclamide in desoxycorticosterone acetate and streptozotocin-induced hypertensive diabetic rats. Lab Anim Res. 2023; 39(1):7.
104. Jones KE, Hayden SL, Meyer HR, Sandoz JL, Arata WH, Dufrene K, et al. The Evolving Role of Calcium Channel Blockers in Hypertension Management:

Pharmacological and Clinical Considerations. Curr Issues Mol Biol. 2024; 46(7):6315-6327.
105. McNally RJ, Morselli F, Farukh B, Chowienczyk PJ, Faconti L. A review of the prescribing trend of thiazide-type and thiazide-like diuretics in hypertension: A UK perspective. Br J Clin Pharmacol. 2019; 85(12):2707-2713.
106. Pathak A, Mrabeti S. β-Blockade for Patients with Hypertension, Ischemic Heart Disease or Heart Failure: Where are We Now? Vasc Health Risk Manag. 2021; 17:337-348.
107. Grammatiki M, Sagar R, Ajjan RA. Metformin: Is it Still the First Line in Type 2 Diabetes Management Algorithm? Curr Pharm Des. 2021; 27(8):1061-1067.
108. Moke EG, Omogbai EKI, Osagie-Eweka SDE, Uchendu AP, Omogbiya AI, Ben-Azu B, et al. Co-administration of metformin and/or glibenclamide with losartan reverse NG-nitro-l-arginine-methyl ester-streptozotocin-induced hypertensive diabetes and haemodynamic sequelae in rats. Microvasc Res. 2023; 147:104497.
109. Islam N, Reynier P, Douros A, Yu OHY, Filion KB. Sulphonylureas versus metformin and the risk of ventricular arrhythmias among people with type 2 diabetes: A population-based cohort study. Diabetes Obes Metab. 2023; 25(6):1523-1533.
110. Lundkvist P, Pereira MJ, Kamble PG, Katsogiannos P, Langkilde AM, Esterline R, et al. Glucagon Levels During Short-Term SGLT2 Inhibition Are Largely Regulated by Glucose Changes in Patients With Type 2 Diabetes. J Clin Endocrinol Metab. 2019; 104(1):193-201.
111. Lazzaroni E, Ben Nasr M, Loretelli C, Pastore I, Plebani L, Lunati ME, et al. Anti-diabetic drugs and weight loss in patients with type 2 diabetes. Pharmacol Res. 2021; 171:105782.
112. Forycka J, Hajdys J, Krzemińska J, Wilczopolski P, Wronka M, Młynarska E, et al. New Insights into the Use of Empagliflozin-A Comprehensive Review. Biomedicines. 2022; 10(12):3294.
113. Benetos A, Petrovic M, Strandberg T. Hypertension Management in Older and Frail Older Patients. Circ Res. 2019; 124(7):1045-1060.
114. Seiden LG, Connor GS. The importance of drug titration in the management of patients with epilepsy. Epilepsy Behav. 2022; 128:108517.
115. Papotti B, Marchi C, Adorni MP, Potì F. Drug-drug interactions in polypharmacy patients: The impact of renal impairment. Curr Res Pharmacol Drug Discov. 2021; 2:100020.
116. Mordarska K, Godziejewska-Zawada M. Diabetes in the elderly. Prz Menopauzalny. 2017; 16(2):38-43.
117 Marcum ZA, Hanlon JT, Murray MD. Improving Medication Adherence and Health Outcomes in Older Adults: An Evidence-Based Review of Randomized Controlled Trials. Drugs Aging. 2017; 34(3):191-201.

Akponah E, Moke EG, Elijah OB, Ovili-Odili BZ, Ekakitie LI, Ben-Azu B, Ilevbare F, Imolede OI. Pharmacotherapy of Hypertension and Diabetes Co-morbidity in the Elderly: A Narrative Review. Afr. J. Trop. Med. & Biomed. Res. 2026; 9(1) 148-171
https://dx.doi.org/ 10.4314/ ajtmbr.v9i1.9