



Original Article

Health Practices by Primary Caregivers on Clinical Cases of Malaria among Under-Five Children in Rural Health Facilities in Delta State, Nigeria

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Abstract:

Background: Malaria remains a major public health threat for children under five in Nigeria. This study evaluated the health practices of primary caregivers in managing clinical malaria among under-five children in Delta State, with emphasis on prevention strategies, home-based treatment, and healthcare-seeking behaviours.

Methods: A cross-sectional hospital-based study was conducted from June 2023 to May 2024 in three rural secondary hospitals representing Delta State's senatorial districts. A total of 633 primary caregivers of under-five children presenting with fever or malaria-like symptoms were consecutively recruited. Malaria diagnosis was performed using both Rapid Diagnostic Tests (RDTs) and microscopy. Data on preventive practices, home management, and care-seeking timing were collected using a validated structured questionnaire. Associations were examined using chi-square analysis and multivariable logistic regression.

Results: The overall malaria prevalence was high: 71.1% by RDT and 66.3% by microscopy. Children aged 13–36 months were the most affected group. Consistent use of insecticide-treated nets (ITNs) showed a significant protective effect against malaria infection (Adjusted OR = 0.26, 95% CI: 0.15–0.45, $p < 0.001$). Use of herbal remedies showed no statistically significant association with malaria status (Adjusted OR = 0.91, 95% CI: 0.62–1.34, $p = 0.67$). Although delayed care-seeking beyond three days was common, it was not independently associated with malaria positivity in adjusted models.

Conclusion: There is a critical need for targeted public health interventions that enhance ITN utilization, promote timely access to healthcare, and reduce over-reliance on unproven home remedies. Improved caregiver education and community-focused malaria control strategies are essential to reducing the disease burden among under-five children in rural Delta State.

Keywords: Caregiver practices; Insecticide-treated nets (ITNs); Herbal remedies; Rapid Diagnostic Test (RDT); Microscopy; Malaria prevalence; Public health intervention; Healthcare-seeking behaviour.

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1. Introduction

Malaria, an acute febrile illness induced by *Plasmodium* parasites and predominantly transmitted through bites from infected female *Anopheles* mosquitoes remains one of the most persistent infectious diseases globally. In 2023, the World Health Organization (WHO) estimated 249 million cases and 608,000 deaths worldwide, with sub-Saharan Africa shouldering 94% of the burden [1]. Children under five years are also extremely vulnerable, contributing to about 76% of all malaria deaths in the region due to their underdeveloped immune systems, nutritional vulnerabilities, and increased outdoor activity exposure [2]. Nigeria, being the epicenter of this crisis, has also reported over 67 million cases and 132,000 deaths in 2023, with under-fives accounting for nearly 70% of fatalities a figure that has shown only marginal decline despite scaled-up interventions [1, 3].

In Delta State, a Niger Delta region, characterized by humid tropical climates conducive to mosquito proliferation, malaria transmission remains perennial, exacerbated by seasonal flooding and limited sanitation infrastructure [4]. Recent national surveys indicate that malaria prevalence among under-fives in Nigeria hovers at 22.6% (2021 Malaria Indicator Survey), but hospital-based studies in rural enclaves like Delta State reveal rates exceeding 60%, reflecting underreporting and access disparities [5]. Despite global and national commitments to malaria elimination—such as the WHO's Global Technical Strategy for Malaria 2016–2030 and Nigeria's National Malaria Strategic Plan 2021–2025—progress is hampered by multifaceted barriers [1, 6]. These include suboptimal uptake of core interventions like insecticide-treated nets (ITNs) and indoor residual spraying (IRS), alongside entrenched socio-cultural practices that delay effective treatment [7].

Primary caregivers, predominantly mothers or female guardians, serve as the frontline decision-makers in child health management, influencing early symptom recognition, preventive adherence, and treatment initiation [8]. Their practices are shaped by a complex interplay of factors: educational attainment, economic constraints, cultural beliefs favouring traditional remedies, and geographic isolation from formal healthcare [9]. For instance, a 2024 study in Southwest Nigeria found that while 86.4% of caregivers possessed good malaria knowledge, only 43.9% consistently used ITNs, with self-medication and herbal treatments prevalent in 37.4% of cases [10]. Similarly, care-seeking delays—often exceed 48 hours—correlate with severe disease progression, anaemia, and mortality [11]. In Gombe State, recent assessments revealed that 92.5% of caregivers were willing to adopt malaria vaccines, yet barriers like misinformation and access issues persist [12].

Despite extensive national data on malaria burden and caregiver behaviours, a clear knowledge gap remains in rural Delta State, where caregiver-driven prevention and treatment practices have not been sufficiently examined despite high malaria prevalence. While studies across Nigeria outline general trends, the specific behavioural determinants, home-management methods, and their direct associations with laboratory-confirmed malaria outcomes in under-five children are poorly understood in this region. This gap highlights the need for localized, context-specific evidence that reflects the realities of caregivers who serve as the first responders in child health management. Therefore, this study aims to assess caregivers' malaria preventive practices, evaluate home-based treatment behaviours including the use of herbal remedies, examine healthcare-seeking patterns and delays, and determine how these practices relate to malaria prevalence among under-five children in Rural Health Facilities in Delta State, thereby providing actionable insights to strengthen community-focused malaria control efforts.

2. Materials and Methods

2.1 Study Design and Setting:

This study employed a cross-sectional, descriptive design conducted over a 12-month period from June 2023 to May 2024. Data collection occurred in three purposively selected rural secondary healthcare facilities representing Delta State's three senatorial districts: General Hospital Kiagbodo (Delta South), Otor-Udu General Hospital (Delta Central), and Ashaka General Hospital (Delta North). These hospitals were selected based on high paediatric malaria caseload and rural population coverage, and geographic diversity, enabling assessment of varying socio-economic and environmental malaria risks [13]. Within each hospital, consecutive sampling was carried out after purposive site selection to ensure all eligible participants presenting during the study period were included until quotas were met.

2.2 Study Population and Sample Size

The study population comprised primary caregivers (biological mothers or guardians) of under-five children (0–59 months) presenting with fever or malaria-like symptoms at outpatient clinics. Exclusion criteria included non-biological caregivers, children with major comorbidities (e.g., sickle cell disease, HIV), or incomplete diagnostic records. Sample size was determined using the formula for single population proportion: $(n = \frac{Z^2 p(1-p)}{d^2})$ assuming a 50% malaria prevalence ($p=0.5$ for maximum variability), 5% margin of error ($d=0.05$), and 95% confidence level ($Z=1.96$), yielding 384 participants. After adjusting for 10 % non-response and applying a design effect of 1.65 to account for clustering across the three hospitals, the final sample size was 633, with an allocation of 211 participants per hospital [14].

2.3 Malaria Diagnosis

Suspected cases underwent dual confirmation; SD Bioline Malaria Ag Pf RDT (Abbott, USA) for *Plasmodium falciparum* histidine-rich protein 2 (HRP2) detection, following WHO guidelines; [15] and microscopy of Giemsa-stained thick and thin blood smears, with parasitemia quantified as

parasites per 200 white blood cells (asexual forms only) by WHO-certified microscopists[16]. Discordant results were reviewed by a senior parasitologist for final confirmation.

2.4 Data Collection

A structured, pretested questionnaire was administered by trained nurses in English and Local Pidgin. The instrument underwent pilot testing on 50 caregivers (not included in the main study) to assess clarity, internal consistency, and cultural appropriateness. The pilot generated a Cronbach's $\alpha = 0.82$, confirming reliability. The validation process included item clarity assessment and correction of ambiguous questions. While data collectors were not blinded to the child's diagnosis due to workflow constraints, standardized questioning procedures minimized interviewer bias. The questionnaire covered four domains:

- i. Socio-demographics (age, education, occupation, income)
- ii. Preventive practices (ITN ownership/use, IRS within the last 12 months)
- iii. Home management behaviours (use of herbal remedies such as *Phyllanthus amarus*, scarification, self-medication)
- iv. Healthcare-seeking patterns (time from symptom onset to hospital visit, categorized as early ≤ 3 days or delayed > 3 days)
- v. ITN use was verified by recall of sleeping under a net the previous night. Interviews lasted 15–20 minutes and ensured confidentiality and cultural sensitivity.

2.5 Ethical Considerations

Ethical approval was granted by the Delta State Hospital Management Board Ethics Committee (Approval No.: DSMB/EC/2023/045). Written informed consent was obtained from all caregivers, and confidentiality was strictly maintained. Data were anonymized, and all malaria-positive children received WHO recommended artemisinin-based combination therapy treatment following diagnosis.

2.6 Statistical Analysis

Data were analysed using SPSS version 25.0. Descriptive statistics were presented using means and standard deviations for continuous data, and frequencies and percentages for categorical variables. Associations with malaria status were assessed using Pearson's chi-square test. Predictors of malaria positivity were examined using multivariable logistic regression, controlling for child age, caregiver education, household income, district, and season. Significance level was set at $p < 0.05$. Model validity was evaluated using the Hosmer–Lemeshow goodness of fit test.

3. Results

3.1 Demographic Characteristics and Malaria Prevalence

Figure 1 shows the gender proportion of the patients, while Figure 2 presents the prevalence of malaria using RDT and microscopic diagnostic tools. Then, Figure 3 indicates the frequency and percentage distributions of malarial prevalence across age stratification

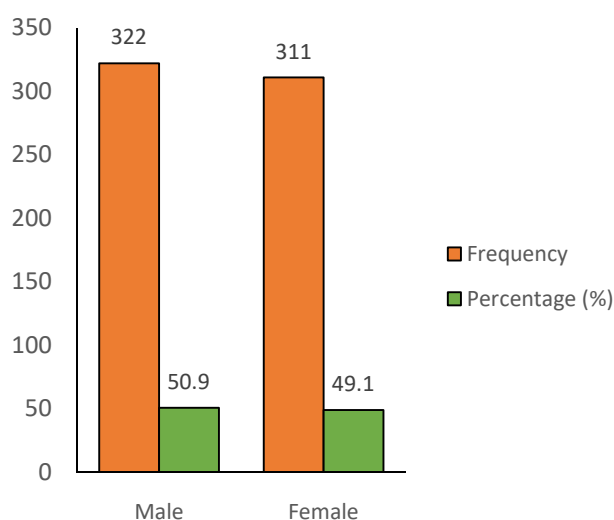


Figure 1: Proportions of patients' gender

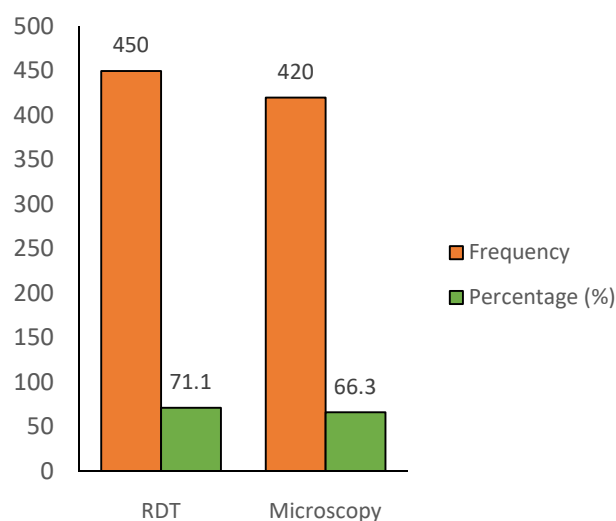


Figure 2: Comparison of malaria prevalence by RDT and microscopic examinations

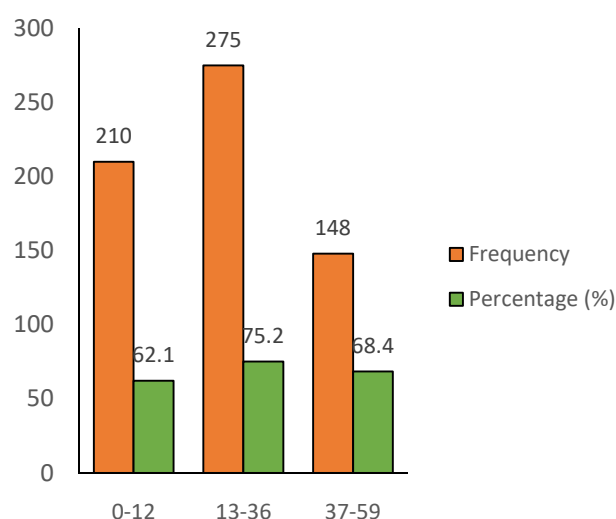


Figure 3: Distribution of malaria prevalence across age groups.

The study cohort comprised 633 caregiver-child dyads, with near-equal gender distribution: 322 (50.9%) males and 311 (49.1%) females (Figure 1). Mean child age was 24.6 ± 14.2 months, with 43.4% ($n=275$) in the 13–36-month bracket—the peak vulnerability window (Figure 3). Caregivers were primarily mothers (92.3%), aged 25–34 years (58.1%), with secondary education or below (71.4%) and low household income ($< \text{₦}50,000/\text{month}$; 64.8%). Malaria prevalence was elevated, with 450 children (71.1%) RDT-positive and 420 (66.3%) microscopy-confirmed ($\kappa=0.82$ for agreement; Figure 2). Age-stratified prevalence peaked at 75.2% for 13–36 months (Figure 3), declining to 62.1% in 0–12 months (maternal immunity effect) and 68.4% in 37–59 months. District variations showed highest rates in Delta South (74.5%), linked to mangrove ecosystems favouring *Anopheles* breeding.

Table 1. Practices of caregivers and relationship with malaria status of children under care

Practice / Category	Malaria positive	Malaria negative	Total
Overall	450	183	633
Used ITN	220 (48.9%)	150 (82.0%)	370 (58.5%)
Did not use ITN	200 (51.1%)	33 (18.0%)*	233 (41.5%)*
Used herbal remedies	240 (53.3%)	130 (71.0%)	370 (58.5%)
Did not use herbal remedies	210 (46.7%)	53 (29.0%)*	263 (41.5%)*
Delayed hospital visit (>3 days)	250 (55.6%)	100 (54.6%)	350 (55.3%)
Early hospital visit (≤3 days)	200 (44.4%)	83 (45.4%)	283 (44.7%)

**Note:* Category counts for “Did not use ITN” and “Did not use herbal remedies” have been aligned to the total column totals (the totals remain as previously reported: 370 used ITN, 263 did not; 370 used herbal remedies, 260 did not). Percentages in parentheses are row percents.

Of 633 children, 370 (58.5%) caregivers reported ITN use, while 263 (41.5%) did not; 370 (58.5%) caregivers used herbal remedies; and 350 (55.3%) caregivers delayed hospital visits >3 days. Malaria positivity by crude proportion was higher among non-ITN users (76.0% vs 59.5% among users) and among delayed visitors (71.4% vs 56.7% for early visitors). Malaria positivity was similar between those who used herbal remedies and those who did not (65.1% vs 69.2%).

Table 2: Data obtained from the Chi-square test on univariate association

Factor	χ^2	df	p-value
Use of ITNs	18.21	1	< 0.0001
Use of Herbal Remedies	1.12	1	0.29
Hospital Visit Timing	14.76	1	< 0.001

ITN use and hospital visit timing were significantly associated with malaria status ($p < 0.001$), while herbal remedy use was not ($p = 0.29$).

Table 3: Values for the determination of multivariable logistic regression

Practice	Malaria Positive (n = 420)	Malaria Negative (n = 213)	p-value (χ^2)	Crude OR (95% CI)	Adjusted OR (95% CI)*
Child slept under ITN previous night	205 (48.8%)	165 (77.5%)	<0.001	0.28 (0.19–0.40)	0.26 (0.15–0.45)
Used any herbal remedy for current illness	248 (59.0%)	122 (57.3%)	0.672	1.07 (0.78–1.48)	0.91 (0.62–1.34)
Hospital visit >3 days after fever onset	238 (56.7%)	112 (52.6%)	0.327	1.18 (0.86–1.62)	1.08 (0.78–1.49)

*Adjusted for child age, maternal education, household income, senatorial district, and season.

4. Discussion

This study revealed high malaria prevalence among under-five children in rural Delta State, highlighting the persistent public health challenge in this region despite ongoing malaria control interventions, where hospital-based surveillance recorded a prevalence of 71.1% by RDT and 66.3% by microscopy. These values reflect hyperendemic transmission patterns typical of the Niger Delta’s humid, swampy ecology that favours year-round *Anopheles* breeding. These findings exceed community-level estimates from Nigeria’s 2021 MIS (22.6%) and recent pooled African under-five prevalence of 26.2% [1, 17]. However, they remain consistent with passive case detection in similar high-transmission settings, including recent Nigerian and West African studies reporting 36–40% prevalence among children aged 6–59 months [18]. As documented in the WHO 2024 report, Nigeria continues to contribute a substantial global malaria burden, with an estimated 67 million cases and 95,000 child deaths annually, largely concentrated among under-fives.

Socio-demographic vulnerabilities strongly shape infection patterns. Low household income (<₦50,000; 64.8%) and limited maternal education mirror 2025 Nigerian analytics, where poverty

and low literacy doubled infection odds in under-fives [19]. Age-specific distribution in this study, with peak prevalence in the 13–36-month group (75.2%, n=275), coincides with the period of waning maternal antibodies and incomplete acquisition of adaptive immunity. Similar concentration in toddlers has been reported in Ethiopian and Asian studies identifying this age group as a key reservoir sustaining transmission [20].

Also, a major protective factor in this population was insecticide-treated net (ITN) use, which reduced odds of infection by nearly fourfold (OR=3.79, 95% CI: 2.2–6.5). This effect size is slightly higher than the 50–70% risk reduction estimated in a 2025 African meta-analysis [20]. However, ITN use in the study population (58.5%) remains below WHO's recommended ≥80% threshold and aligns with other Nigerian surveys showing suboptimal adherence due to heat discomfort, limited bed space, and declining insecticide potency in humid climates [21]. Resistance-mitigating dual-insecticide nets introduced in Nigeria since 2024 demonstrate superior efficacy (20–50% improvement) and may enhance protection in pyrethroid-resistant Delta populations [22]. Determinants of poor ITN adherence mirror findings from Gambian and Nigerian studies, where large household size, low malaria knowledge, and economic constraints reduce uptake [23,24].

Herbal remedy use was widespread (58.5%) but exhibited no significant association with malaria risk (OR=0.79, 95% CI: 0.4–1.5). This neutral effect reflects wide variability in plant types, preparation methods, and dosing, which limits their reliability as preventive or therapeutic interventions. Comparable findings from a 2025 randomized trial in sub-Saharan Africa showed that commonly used herbs—*Artemisia annua*, *Cryptolepis sanguinolenta*, *Morinda lucida*—produced only modest parasite reductions without independent curative benefit [25]. Despite cultural acceptance, delayed formal care due to confidence in herbal treatments has been associated with increased risk of severe disease in Nigerian and regional studies [26,27]. While some reviews support scientifically validated integration of select plant-based therapies, current evidence—as reflected in our findings—supports prioritizing biomedical approaches including ACTs [28].

Care-seeking delays (>3 days) were common (55.3%) and associated with increased risk in univariate analysis ($\chi^2=14.76$, $p<0.001$), although significance declined after adjustment (OR=0.98), suggesting interactions with socioeconomic status and ITN use. Delayed treatment, previously shown to triple severe-malaria hospitalization odds in Nigerian modeling studies, remains driven by cost barriers, distance, reliance on patent medicine vendors, and cultural norms favouring self-medication [29,30]. A meta-analysis from the Horn of Africa estimated 42% delay prevalence, linking it to maternal illiteracy and long travel distances—patterns mirrored within this cohort's predominantly low-education caregivers. Behavioural change campaigns targeting perceived severity and urgency of fever treatment have demonstrated up to 35% improvements in prompt care and may be beneficial in this setting [31].

Emerging interventions offer additional opportunities for under-five protection. Nigeria began deploying RTS,S/AS01 and R21/Matrix-M vaccines in 2024, with high caregiver willingness (92.5% in recent surveys), though hesitancy persists due to concerns about side effects [32]. Seasonal malaria chemoprevention (SMC) uptake in northern Nigeria reached 58.4% for ≥1 dose and reduced incidence by up to 50%, suggesting scalability to southern seasonal peaks where implementation remains limited. Community-engaged delivery systems have shown improved acceptance (≥90% first-dose uptake) and may complement ITN distribution and behaviour-change strategies [33]. Study limitations include possible recall bias for ITN and herbal use, reduced generalizability beyond rural hospital settings, and potential overestimation of burden due to symptomatic case concentration. Nonetheless, dual diagnostics ($\kappa=0.82$), substantial sample size (n=633), and multi-variable adjustments strengthen study reliability. Strengths include ecological diversity of participants, contextual socio-demographic data, and robust statistical modelling.

Overall, the findings underscore the need for integrated, district-level strategies combining ITN intensification (including dual-insecticide nets), rapid care-seeking promotion, improved ACT access, and regulated herbal-use education. Alignment with Nigeria's 2021–2025 Malaria Strategic Plan and scaling of vaccine/SMC programmes may avert substantial under-five morbidity and mortality, contributing meaningfully to SDG 3.3 goals [34,35].

5. Conclusion

This study demonstrates that malaria remains highly prevalent among under-five children in rural Delta State, with caregiver practices playing a critical role in determining child health outcomes. Consistent use of ITNs was the only behaviour independently associated with reduced malaria infection. In contrast, reliance on herbal remedies and delays in seeking medical care did not contribute to malaria prevention and may pose risks if they lead to postponed treatment. Strengthen-

ing evidence-based malaria prevention and ensuring timely access to healthcare are therefore essential for reducing disease burden in this population. Targeted health promotion interventions are required to improve caregiver awareness of the importance of ITN usage and prompt treatment-seeking behaviours. Collaboration with community leaders, media, and health workers should be emphasized to reinforce adoption of effective preventive strategies. Additionally, the government and stakeholders should ensure sustained ITN availability, improved health facility access, and continued integration of malaria education into maternal and child health programmes.

Declarations

Consent for publication: We confirm that all participants provided informed consent for the publication of their data in this article, and all identifiable information has been anonymized to protect participant confidentiality.

Data availability statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to privacy and ethical restrictions, the data are not publicly available.

Competing Interests: The authors declare that there are no conflicts of interest associated with this study.

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Authors' Contributions: ETO conceptualized and designed the study and participated in manuscript drafting. MIO coordinated data collection and preliminary statistical analysis. GNE contributed to laboratory diagnostics and data validation. IO provided supervisory oversight, methodological guidance, and critical manuscript revisions. All authors approved the final version of the manuscript.

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Original Article

Impact of Occupational Exposure to Metallic Welding Fumes and Smoking on Reno-Hepatocellular Homeostasis in Relation to Serum Copper Level

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Abstract:

Background: Metallic welding is a highly skilled process that involves the joining of metals or metallic substances by heating to a specific temperature. Constant exposure to metallic fumes leads to organ and system injuries and deleterious health effects on welders. The study examined the effect of occupational exposure to metallic welding fumes and smoking on reno-hepatocellular homeostasis in relation to serum copper levels of welders.

Methods: Forty (40) subjects were recruited, comprising 10 welders who were smokers, 10 welder non-smokers, 10 non-welders that are smokers and 10 non-welders who were non-smokers. Blood samples were collected to estimate biochemical assays of liver and kidney parameters. The collected data were expressed in mean $\pm$ SEM, and a one-way ANOVA was used for comparison between means.

Results: Findings revealed a significant increase ($p < 0.05$) in the levels of serum copper of welders compared with the control group. Urea levels were significantly increased ($p < 0.05$) in welders who were smokers, whereas, levels of serum creatinine showed a non-significant decrease in welders who were smokers compared with the control group, and other experimental groups. For the liver function assays, the activities of Aspartate aminotransferase (AST) were not significantly different from those of the control. Activities of Alanine aminotransferase (ALT) were significantly increased ($p < 0.05$) in non-welders that were smokers compared to those of the control. Remarkably, ALP activities were significantly decreased ($p < 0.05$) in all experimental groups compared to control levels, the observed reduction was least in welders that are non-smokers. Total bilirubin and direct bilirubin levels were non-significantly increased. The level of total protein was significantly reduced ($p < 0.05$) in smokers that were non-welders compared with the control group.

Conclusion: Exposure to fume particles from metallic welding, particularly copper, affected kidney function parameter values by significantly increasing urea levels and reducing creatinine levels in smoking welders. In summary, this study suggests that changes in serum copper levels among smoker welders may be associated with the severity of changes in kidney and liver functional status following exposure to metallic fumes with possible risk of hepatic dysfunction over time, especially when smoking is involved.

Keywords: *Metallic welding, Creatinine, Urea, Copper, Enzymes, Kidney, Liver, Smoking.*

1. Introduction

The workplace is an important part of the human environment. Occupational exposure to hazards associated with metallic welding cannot be overstated, as workers are routinely exposed to toxic fumes linked to a wide range of severe acute and chronic health effects. As such, the overall health of workers in any organization is greatly affected by the conditions in their work environment, thus

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impacting their productivity, although, evidence suggests that no profession is without risk [1]. Exposure to metallic fumes is complex and varies significantly depending on the welding process, the electrode used and the material being welded. Obviously, metallic welding produces gases and small solid particles, collectively known as metallic fumes, most of this occurs in arc welding [1, 2]. Metallic welding processes generate hazardous substances like; gases, fumes, heat, noise, infrared radiation and ultraviolet which is very harmful to the eyes and deleterious to different organs of the body with risk of attenuating homeostatic processes [3, 4, 5]. Fumes from metallic welding are considered to be the most harmful compared to other welding by-products [6]. Particles contained in fumes from metallic welding are small enough to get airborne and are easily inhaled and potentially cause respiratory problems [1, 7].

Copper (Cu) is an essential trace element that becomes toxic at elevated concentrations. It is mainly used in agriculture as a pesticide and in the leather industry. Copper is required by the body most notably as a cofactor for a number of enzymes such as ceruloplasmin, cytochrome oxidase, dopamine β -hydroxylase, superoxide dismutase and tyrosinase. Cu is present in different bodily tissues, and is primarily stored in the liver, but it is present in smaller amounts in the brain, heart, kidneys and muscles. Human exposure to copper occurs primarily through consumption of food and drinking water or fumes [8]. Occupational exposure to nephrotoxic metals like copper has been associated with tubular and glomerular injuries in welders. As a result of this exposure, welders develop an insidious incipient kidney and liver diseases related to the type of metal, electrodes and duration of exposure [9]. The health risk impact of metallic fumes depends to a large extent on the type of metal, the concentration of fumes, and the duration of exposure. The short-term effect could include; noticeable toxicity symptoms, allergies and irritation to the eyes, throat, nose and lungs, metal fume fever, and irritant-induced asthma, meanwhile, long-term health effects include; damage to the lungs, emphysema, pneumonia, bronchitis, and increased risk of respiratory cancers [10]. The kidneys and liver are not spared from the harmful effects of exposure to metallic fumes. Due to the compensatory ability of the kidneys, and detoxifying potentials of the liver, exposure to metallic fumes does not usually produce early clinical symptoms and becomes evident mainly during chronic exposure [5].

The kidney is a primary retroperitoneal bean shaped organ of the renal system located on the posterior abdominal wall outside of the abdominal cavity that play significant homeostatic role in the removal of metabolic waste products such as creatinine and urea out of the body [9, 11]. Creatinine is a waste product formed from creatinine phosphate. This conversion is spontaneous, non-enzymatic, and dependent on the body's total muscle mass. It is not affected by diet, age or exercise. Serum creatinine levels have been used as a screen for renal function status. Urea is a parameter of kidney function also called carbamide (the diamide form of carbonic acid) which is a nitrogenous waste product from protein breakdown [9, 12]. Another major and very important secretory organ in the human body is the liver. It is a vital organ that makes up around 2% of an adult's body weight and is in charge of numerous processes including metabolism support, digestion, immunity, detoxification, and vitamin storage [13, 14, 15]. Since most fumes from metallic welding are initially stored largely in the liver when inhaled, prolonged exposure to metallic fumes causes pathobiological changes in the liver and other sensitive organs increasing oxidative stress and inflammation [16, 17, 18, 19]. Perhaps, exposure to toxic fumes from metallic welding can result in adverse health outcomes, including damage to the nervous system, liver and kidneys [15]. Some studies have suggested the effects of these fumes on liver is linked to complexity of the metallic fume compositions, variability in the levels of exposure, individual susceptibility levels, and the lack of robust long-term studies with large sample sizes, though the evidence in this area has conflicting results [20, 21, 22].

Smoking is the practice of inhaling smokes prepared from burning plant materials. Smoking negatively impact liver and kidney health by causing kidney damage, increasing the levels of blood urea and contributing to liver condition like; fatty acid disease, reduce blood flow, which is largely due to the contamination of the blood with components of tobacco smoke [23]. Not only can smoking increase the risk of kidney cancer, but it can also contribute to additional kidney damage. Smokers have a significantly higher risk of chronic kidney disease than non-smokers. A history of smoking promotes the progression of diabetic nephropathy. Outbreaks of certain types of diseases and infections have been found to affect smokers' more than regular smokers. It is known that smoking tobacco, due to the toxic effects of the components, damages the liver and kidneys, causes cancer and the degeneration of kidney and liver cells. Smokers have a significantly increased risk of chronic kidney disease than non-smokers [22, 23]. Findings from previous study indicated that elevated blood urea levels above the normal range and higher creatinine and uric acid levels, suggest risk of hepatic dysfunction and buildup of potential renal diseases [24]. Chronic exposure to essential trace element and heavy metals like; lead, chromium, cadmium, and copper can induce an

insidious but progressive tubular interstitial nephropathy that often leads to kidney failure [5, 24, 25]. Therefore, the study examined impact of serum metallic fumes on the functional status of the liver and kidney following exposure to smoking status as a modulator in welders.

2. Materials and Methods

2.1 Study design

The study adopted a cross-sectional analytical study design in collecting important information from among male smokers and non-smokers with a welding experience of more than one (1) year, who satisfactorily met the inclusion criteria. The participants were categorized into four groups as follows; Group 1 served as control; without history of welding job and who were non-smokers. Group 2 were smoker's that are non-welders, Group 3 were non-smokers welders, while Group 4 represents smokers who were welders.

2.2 Place of study

The area of study is Obiaruku and Abraka, with Obiaruku being the headquarters of the Ukwani LGA. It is one of the most important homelands of the Ukwani speaking people. It is made up of parishes and sub-districts, known as quarters, bordering Edo to the east, Abraka to the southwest, Eziopkor to the southeast, and Obinomba to the northeast. Abraka on the other hand shares a common border with Obiaruku, Abraka is at Ethiopie East LGA with its headquarters as Isiokolo, it is home to 24 Urhobo Kingdoms, thus, study setting is popularly known as the university host community and has the main campus of the University.

2.3 Subjects

The study population comprised adult male welders residing in the Abraka (Ethiopie East LGA) and Obiaruku (Ukwani LGA) communities. A total of 40 apparently healthy participants were recruited using a two-stage sampling technique. First, convenience sampling was utilized to identify and locate accessible welding workshops within the study areas. Subsequently, 20 consenting participants from each community were selected through simple random sampling by balloting without replacement. This randomization involved the use of 'Yes' and 'No' ballots, with only those selecting 'Yes' being enrolled in the final study cohort.

2.4 Exclusion and inclusion criteria

Only welders whose shops are located in Abraka and Obiaruku communities, respectively, and are within the ages of 18-64 years constituted the study participants. Only those who have worked at least for one year as welders and must have agreed to fill the informed consent form were recruited. The adults chosen seemed to be in good health and were not employed in the fields that dealt with aluminum or metallic fabrications. Individuals with neurological disorders, heart issues, fractures, arthritis, trauma, and hypertension were excluded from the study.

2.5 Ethical consideration

Ethical permission for this study was secured from the Research, Ethics and Grants Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (RBC/FBMC/DELSU /23/268). Prior to data collection, the proprietors of the welding workshops were contacted and duly informed about the study. In addition, each participant provided verbal informed consent after the study objectives and procedures had been clearly explained in their preferred language, including English, Pidgin, Urhobo, or Ukwuani.

2.6 Sample collection

Using a tourniquet, 5 mL sterile syringe and sterile needle, cotton wool, methylated spirit, 5 mL of venous whole blood samples were obtained from the study from each subject and were dispensed into labeled containers and transported to the laboratory for analysis. Samples were centrifuged using bucket centrifuge at 3000 rpm for 5 minutes. A micropipette was used to separate the plasma from the samples. The materials used for data collection include: syringe (for collecting venous blood), cotton wool for sterilization and stoppage of blood flow from the site of puncture, alcohol for sterilization of the site of puncture, tourniquet: a tool used to partially stop blood flow and make vein at site of puncture visible for easy blood collection. Hand gloves to protect the hands from infection while collecting data, sample containers used to store the blood collected for the experiment. Stadiometer was used for measuring the height of participants; bathroom weighing scale that is calibrated in kilogram for weight measurement; sphygmomanometer (a digital blood pressure monitor was used in measuring the blood pressure of both the systolic and diastolic), bucket centrifuge to spin samples collected for easy decanting and a refrigerator for preservation of blood samples collected.

2.7 Biochemical investigations

2.7.1 Serum copper assay:

Buck Scientific Analytical Methods for Atomic Absorption Spectroscopy (model 210/211 VGP) were used to examine the samples adopting the methods of Makino and Takahara, [26]. Dilution of the plasma was done with deionized water. The viscosity parameters of the diluted samples were approximated by performing the analysis against standards produced in glycerol. The concentration of each metal in each sample was shown on the digital readout after the metals were measured at a certain wavelength. The copper metal has a wavelength of 324.8nm and the metal was read using an acetylene gas to ignite the machine.

2.7.2 Serum creatinine assay:

Creatinine concentration directly relates to the amount of complex that forms. For this serum creatinine levels estimation, a randox test kit was utilized. A colorful complex is formed when creatinine and picric acid combine in an alkaline solution. Perform a new gain calibration in cuvette mode using fresh ddH₂O. Select CREA from the run test screen and run water blank as described in the test manual. The reaction rate and absorption of the reaction product are very sensitive to temperature.

2.7.3 Serum urea assay:

For this serum creatinine levels estimation, a randox test kit was utilized. Ammonia and carbon dioxide are produced when urea is hydrolyzed in the presence of water and urease. In the presence of glutamate dehydrogenase, the ammonia produced in the first step mixes with α -oxoglutarate and NADH. Using new ddH₂O, perform a new gain calibration in flow cell mode. From the perform Test screen, choose UREA, then follow the instructions to perform a water blank.

2.7.4 Alanine aminotransferase estimation:

For *alanine aminotransferase (ALT)* estimation, 0.5 mL of substrate was added into a test tube containing 0.5 mL of blood, the mixture is put in a water bath at 37°C for 30 minutes, 0.5 mL of 2,4-dinitrophenylhydrazine was added to the solution and incubated for 20 mins. Then 5 mL of sodium hydroxide was added to mixture which turned brown and was placed in a spectrophotometer at 540 nm and results were obtained from the calibrated graph. Calculation = ALT (U/L) = change Abs/mm x 1750.

2.7.5 Aspartate aminotransferase estimation:

For *aspartate aminotransferase (AST)* estimation, 0.5 mL of the substrate, sodium azide was added to the test tube plus 0.5 mL of the blood or serum. It was then put in a water bath at 37°C for 30 minutes. After this, 0.5 mL of 2, 4 dinitrophenylhydrazine was added to the mixture, incubated for 20 mins, and 5 mL of sodium hydroxide was added to the mixture which turned brown. It was then placed in a spectrophotometer at 540 nm and the results were read on the calibrated graph.

2.7.6 Alkaline phosphatase estimation:

For *alkaline phosphatase (ALP)* estimation, 1 mL of p-nitrophenyl phosphatase was added to 1 mL of the serum in a test tube, incubated for 30 mins and results were obtained from the spectrophotometer at 410 nm and correlated with values on the calibrated graph to give the results.

2.7.7 Bilirubin estimation:

The levels of bilirubin in the blood or urine are determined by a bilirubin test. A high bilirubin level may be a sign of biliary or liver illness. When making a differential diagnosis of hyperbilirubinemia, the estimation of total and direct bilirubin in the serum is crucial. Using a buck scientific atomic absorption spectrophotometer, model 210/211 variable giant pulse correction and biochemical assay test kits, blood serum was examined for total and direct bilirubin estimation levels.

2.7.8 Estimation of total protein:

The method developed by Fares, [27], was used to estimate the total protein level. The two main categories of total protein techniques are chemical and physical. The physical approaches include UV light absorption, refractive index, and specific gravity measurements. The majority of the chemical techniques are variations on the biuret reaction. The preferred technique for clinical laboratories is thought to be the biuret reaction.

2.8 Data analysis

Data collected were calculated using mean $\pm$ SEM. The one-way ANOVA statistics was used to compare means across groups. Tukey's HSD test was used for post hoc multiple comparison for association between variables. Graph Pad Prism version 8 was used to calculate the data. A $p < 0.05$ was considered statistically significant.

3. Results

Figure 1 presents the levels of serum copper of welders following exposure to fumes and smoking. Data from our study showed that serum copper levels were significantly reduced ($p<0.05$) in non-smokers who were non-smoker welders compared to control group. The reduction in levels of serum copper in smokers' non-welders was not significant in comparison those of the control. In contrast, the serum copper levels were significantly increased ($p<0.05$) in smokers who were welders compared to smokers' non-welders and non-smokers welders. The observed increase was non-significant compared those of the control group.

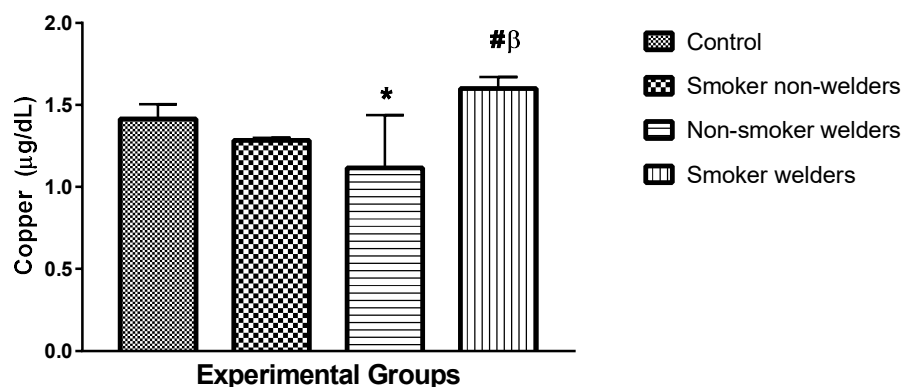


Figure 1: Levels of Serum Copper in Welders exposed to Metallic Fumes and smoking

*: Significant ($p<0.05$) compared to control; #: Significant ($p<0.05$) compared to smoker Non-welders; β: Significant ($p<0.05$) compared to non-smoker welders; +: Significant ($p<0.05$) compared to smoker welders

The result from Figure 2 revealed that the levels of serum urea of welders were elevated following exposure to fumes and smoking. A graded increase in levels of serum urea in the various experimental groups compared those of the control was observed. However, a significant ($p<0.05$) increase in serum urea levels was only noted in smokers who were welders (smoker welders) compared to those of the control. In addition, non-significant difference was observed in serum urea level compared to the other experimental groups.

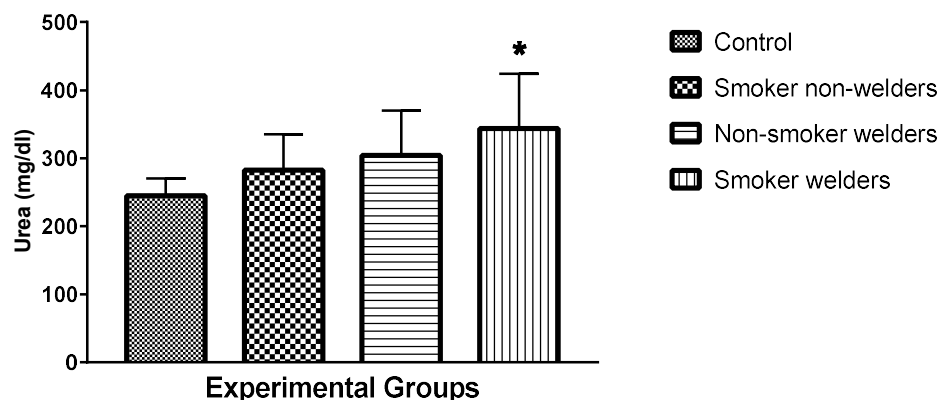


Figure 2: Levels of Serum Urea in Welders exposed to Metallic fumes and smoking

*: Significant ($p<0.05$) compared to control; #: Significant ($p<0.05$) compared to smoker Non-welders; β: Significant ($p<0.05$) compared to non-smoker welders; +: Significant ($p<0.05$) compared to smoker welders

The results presented in Figure 3 evaluated the serum creatinine levels of welders following exposure to fumes and smoking. The serum creatinine level across different experimental groups was not significantly different from those of the control. A significant ($p<0.05$) decrease was observed in the serum creatinine level in smokers who were welders (smoker welders) compared to welders who were non-smokers (non-smoker welders).

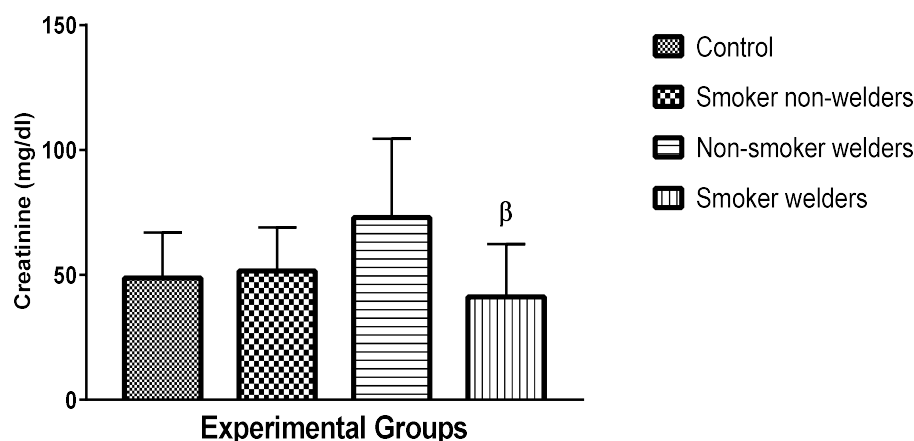


Figure 3: Levels of Serum Creatinine in welders exposed to Metallic fumes and smoking

*: Significant ($p < 0.05$) compared to control; #: Significant ($p < 0.05$) compared to smoker Non-welders, β : Significant ($p < 0.05$) compared to non-smoker welders; +: Significant ($p < 0.05$) compared to smoker welders

Results presented in Figure 4 evaluated aspartate aminotransferase (AST) activities in welders following exposure to fumes and smoking. Serum AST activities were elevated across all experimental groups and the noted increase were not significantly different compared to those of the control. The observed increase in the activities of serum AST was not significant across the experimental groups compared with the control group.

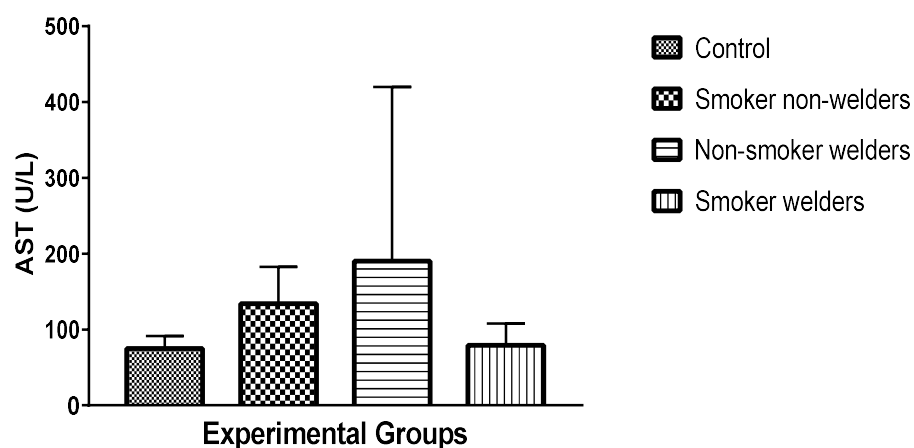


Figure 4: Aspartate aminotransferase (AST) activities in Welders exposed to Metallic fume and smoking

*: Significant ($p < 0.05$) compared to control; #: Significant ($p < 0.05$) compared to smoker Non-welders, β : Significant ($p < 0.05$) compared to non-smoker welders; +: Significant ($p < 0.05$) compared to smoker welders

Results presented in Figure 5 represent the activities of alanine aminotransferase (ALT) in welders following exposure to fumes and smoking. The present study revealed a significant ($p < 0.05$) increased activities of serum ALT in smokers who were non-welders compared to control group. However, there was no significant difference noted in the activities of serum ALT when comparison was made between the different experimental groups.

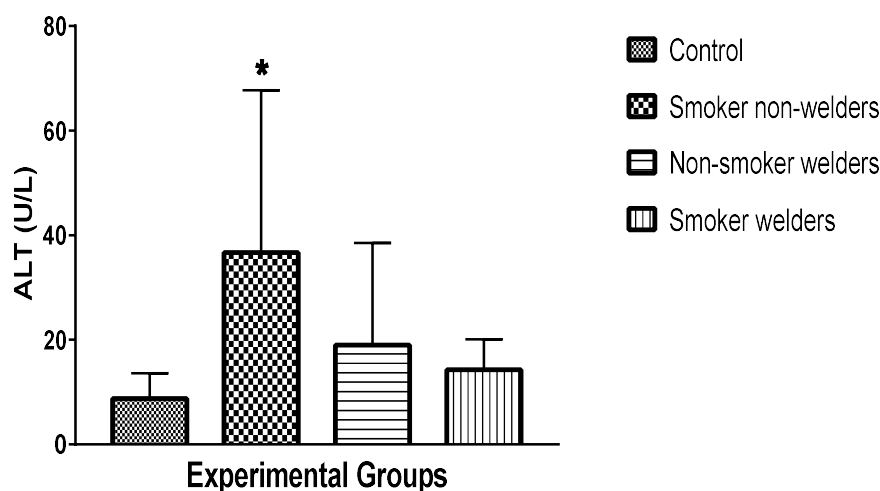


Figure 5: Alanine aminotransferase (ALT) activities in welders exposed to Metallic fume and smoking

*: Significant ($p < 0.05$) compared to control; #: Significant ($p < 0.05$) compared to smoker Non-welders; β : Significant ($p < 0.05$) compared to non-smoker welders; +: Significant ($p < 0.05$) compared to smoker welders

Result presented in Figure 6 illustrates the activities of alkaline phosphatase (ALP) in welders following exposure to fumes and smoking. This study recorded a significant ($p < 0.05$) decreased activities of ALP across all experimental groups (smokers non-welders, non-smokers welders; and smokers welders), when compared to those of the control. The observed reduction in alkaline phosphatase (ALP) activities was least among welders who were non-smokers. However, there was no significant difference observed in the ALP activities when comparison was made between the different experimental groups.

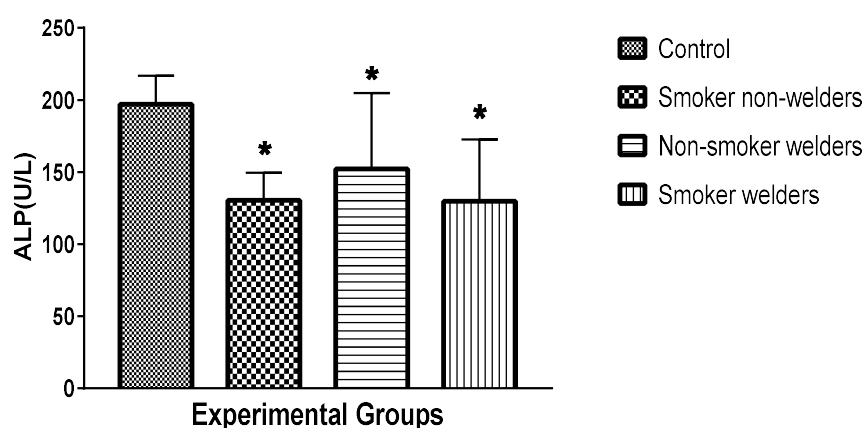


Figure 6: Alkaline Phosphatase (ALP) activities in welders exposed to Metallic fume and smoking

*: Significant ($p < 0.05$) compared to control; #: Significant ($p < 0.05$) compared to smoker Non-welders; β : Significant ($p < 0.05$) compared to non-smoker welders; +: Significant ($p < 0.05$) compared to smoker welders

Results presented in Figure 7, evaluated the levels of total bilirubin (a); direct bilirubin (b); and total protein (c) in welders following exposure to fumes and smoking. This study recorded a graded non-significant increased levels of total bilirubin across all experimental groups (smokers' non-welders, non-smokers welders and smoker welders), compared to control levels. Meanwhile, there was non-significant difference observed in the levels of total bilirubin when comparison was made between the different experimental groups. This study showed a non-significant increased level of direct bilirubin in smokers and non-smokers who were welders compared to those of the control group. However, a significant ($p < 0.05$) increase in direct bilirubin level was observed among smokers who were welders (smoker welders) compared to smokers who were not welder (non-smoker welders). Similarly, the results revealed that total protein level of smoker non-welders was significantly ($p < 0.05$) decreased compared to those of the control. However total protein levels of welders that are both smokers and non-smokers, was significantly ($p < 0.05$) increased when compared to smoker non-welders.

(a)

(b)

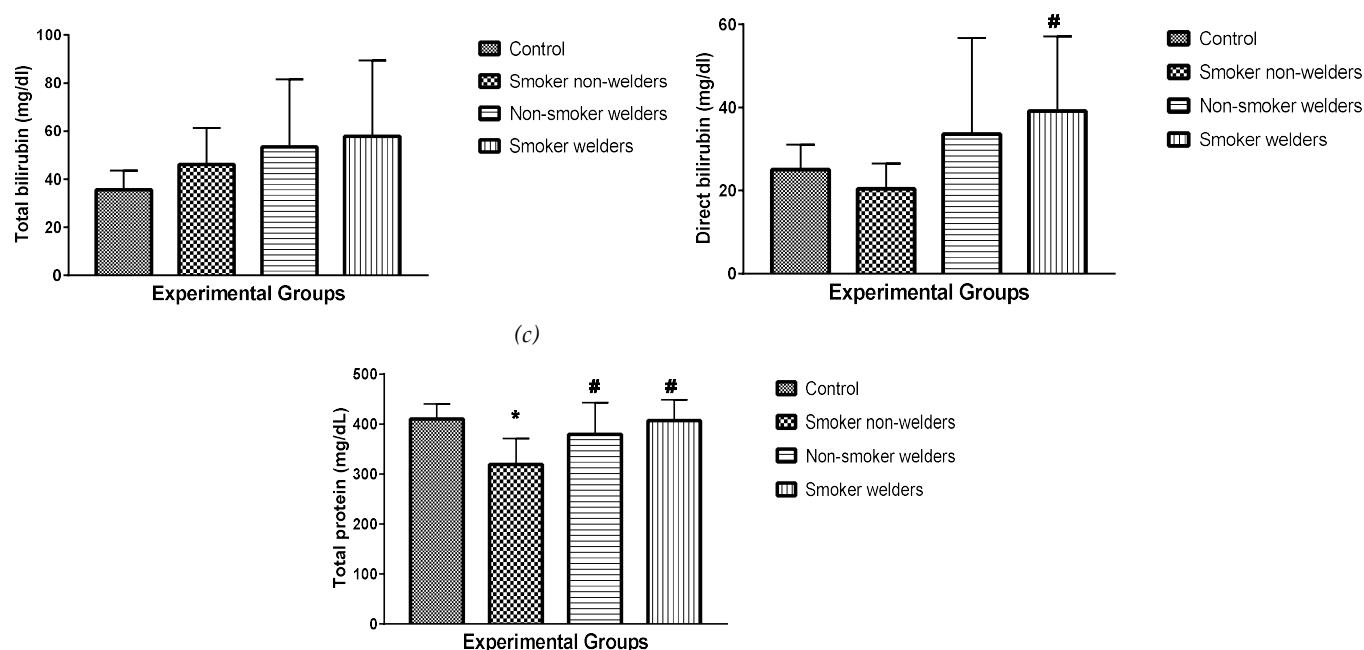


Figure 7^{a-c}: Levels of Bilirubin (Total and Direct) and Total Protein in Welders exposed to Metallic fumes and smoking

*: Significant ($p < 0.05$) compared to control; #: Significant ($p < 0.05$) compared to smoker Non-welders, β: Significant ($p < 0.05$) compared to non-smoker welders; +: Significant ($p < 0.05$) compared to smoker welders

4. Discussion

Fumes from metallic welding consist of a complex mixture of metal oxides, silicates and fluorides. Vapors are formed when a metal is heated above its boiling point and its vapors condense into very fine particles (solid particles). Metallic welding fumes generally contain particles from the electrode and the material being welded [22, 28]. The liver and the kidneys have the critical job of maintaining the body's metabolic homeostasis. This includes the processing of dietary amino acids, carbohydrates, lipids and vitamins: removal of microbes and toxins in splanchnic blood and route to the systemic circulation; synthesis of many plasma proteins; and detoxification and excretion into the bile of endogenous waste products and pollutants xenobiotics [29, 30]. The result in Figure 1 showed that the serum copper levels of welders exposed to metallic fumes. Serum copper levels of non-smoking welders were significantly reduced, why that of smokers were significantly increased compared to control, this is in line with that of El-Maksoud, et al. [31], who conducted a study on biochemical changes in occupational stress between smoking and non-smoking iron and steel workers in Egypt, this study found that exposure to metallic fumes is associated with high concentrations of Fe, Cu, Pb, Mn, MDA, catalase activity, cortisol and immunoglobulins and low activity of Zn, SOD, GR, GPX, GSH and NO. These can all be considered risk factors from exposure to metallic fumes in iron and steel workers [32, 33, 34, 35].

Data from our study showed that serum urea levels were significantly increased in the experimental groups, most significantly in smoking welders compared to those of the control (see Figure 2), this finding agreed with the opinion of Adejumo et al. [36] who conducted a study evaluating renal biomarkers of renal function in industrial auto workers in Benin City, Edo State, Nigeria, and they reported higher levels of some kidney biomarkers; urea and creatinine in autoworkers compared to controls. Specifically, they observed that blood urea levels were significantly elevated above the normal control range. Hence, their results suggest that occupationally exposed automotive workers may be at risk for kidney disease. Similarly, our finding is consistent with that of Duru, et al. [23], who conducted a study to assess the levels of serum cholesterol and urea in smokers and non-smokers, and found that serum total cholesterol levels and urea concentrations were higher in smokers than in non-smokers, suggesting that smokers are predisposed to cardiovascular disease and possible kidney problems [37].

Our results showed that the levels of serum creatinine of smoking welders was significantly decreased compared to non-smoking welders, and significantly increased in non-smoking welders compared to control (see Figure 3), such observe increase was similar with the view of Okpogba et al, [38], who conducted a study to assess renal function status in occupationally exposed individuals working in a metalworking factory in Nnewi, the study showed significantly increased levels of sodium, potassium, urea, and creatinine, and significantly decreased levels of both bicarbonate and of chloride ions due to exposure to heavy metals in the metal processing plant at

Nnewi. This suggests a possible association of exposure to heavy metals at the workplace on kidney function. A previous study examining the association between serum levels of cotinine and renal function in active and passive smokers, and found that kidney and glomerular functions can even be affected by secondhand smoke. In addition, an elevated microalbumin/creatinine ratio in these individuals may be a sign of an increased risk of atherosclerosis [39]. The observed reduction in serum creatinine levels in this study may have been influenced by factors beyond renal function.

Data from this study revealed increased activities of AST in non-smokers welders, followed by smokers' non-welders, smokers' welders and non-smokers and non-welders. The implication of this result is that the activities of AST in the serum of non-smokers, and non-welders were lower than welders that smoke and welders that don't smoke. Surprisingly, AST activity in serum of smokers' non-welder is higher than smokers' welders while it is lower than that of non-smokers welders. Although variations were observed, causal interpretation regarding smoking effects on AST was beyond the scope of this study. The increased activities of AST indicated possible risk of hepatic injury and this is explained by the leakage of enzymes from the tissue to the plasma due to the alteration of the membrane permeability [40]. Such observed increased activities of AST following exposure to metallic fumes among smoker welders is consistent with the views that many serum enzymes have are appropriate stress indicators [22]. Thus, activities of many serum enzymes, which included AST and ALT, have been frequently applied in the diagnosis of some of the cases of diseases, in addition, the discovery of impairment in tissues induced by environmental pollution. Therefore, the increased activity of the enzymes in the serum is considered a sensible indicator of minor cellular impairment subsequently tissue damage which results in stress [41, 42, 43].

The activity of ALT in serum of smokers who were non-welder was higher, followed by non-smokers welders, smokers' who were welders and the least activity of ALT were observed in non-smokers who were non-welders. The observed increase in the serum ALT activities of welders in this study could be attributed to alteration in the cellular membrane of the liver. The toxic materials in smokes caused an increase in cell membrane permeability leading to enzyme leaching or leakage of enzymes from liver to blood or another case the permeability has reduced which directly lead to the accumulation of enzymes in the hepatocytes which may increase the risk of hepatic dysfunction over time [44, 45]. Generally, the increase in activities of AST and ALT may infer deterioration changes and dysfunction of liver because the effect of toxicants on the hepatocytes induced in necrosis of the liver and thus, lead to leakage of these cellular enzymes into blood circulation. This is supported by the study of Rahimikia [46], who indicated that releasing these transaminases into the bloodstream which attribute to impairment of the hepatic tissue, heart, and kidney under stress affected by metal. In addition, he recommended that serum enzymes could be used in biomarkers to environmental toxicity. Thereby, these enzymes activity in serum is mostly resulted from inhaling from metallic fumes component of the cytoplasm of liver cells into the blood circulation due to liver damage via copper metal ions. The current research is in agreement with the finding by Naveed et al. [47] that frequent exposure to heavy metals led to increased activities of both aminotransferases, indicating enhanced transamination reactions.

Activities of ALP in serum of welders exposed to metallic fumes revealed that there was increased serum ALP activities in smokers who were non-welder compared those of the control and other experimental groups. The implication is that such increase observed in the activities of ALP may be due to the contents of what is being inhaled, not necessarily the metallic fumes alone. Previous research has demonstrated that smoking raises serum ALP activities produced by the kidney, liver, and bones [48, 49, 50]. Our results support the claims made by certain researchers that smoking increases ALP activities, other studies maintained that smoking and exposure to heavy metals had no effect on ALP [51, 52]. As a result, smoking's effects on ALP level may be complex due to a variety of extrahepatic pathways, as this study's findings did not consistently link smoking to levels of serum copper in welders exposed to welding smoke.

A graded increase (non-significant) in the serum total bilirubin level of all experiment groups was observed compared with the control group as illustrated in Figure 7^{a-c}. Furthermore, a significant increase in direct bilirubin level was observed among smokers who were welders when compared to non-smokers who were non-welder. It can be deduced from this that the increase is as a result of the combined effects of smoking and metallic fumes. Meanwhile, in this study total protein levels was significantly decreased among smokers who were not welders. Metallic fumes significantly increased levels of total protein in welders (for both smokers and non-smokers) compared to non-welders who were smokers. However, the increase observed was not up those of the control. This result is in agreement with the findings of previous study reported that metallic welding process caused elevation in serum total protein, whereby, the rate of smoking is inversely related to an increased levels of total protein in participants [53].

5. Conclusion

Exposure to fume particles from metallic welding, particularly copper, affected kidney function parameter values by significantly increasing urea levels and reducing creatinine levels in smoking welders. The activities of AST, ALT and ALP varied significantly; such changes can be useful as a pointer of understanding the impact of elevated copper induced by metallic fumes on liver function of welders. Total and direct bilirubin levels revealed a graded non-significant increase, while total protein levels were decreased in relation to exposure to metallic fumes as exacerbated by smoking among welders. Interestingly, exposure to metallic fumes worsens reno-hepatocellular homeostatic status in smokers compared to non-smokers. However, no correlations were established between serum copper levels and renal function parameters, and no causal effect was observed. The elevated serum copper in the exposed groups does not exclude that other metals or substances that were not assessed could be the cause of these effects, hence, the need for further study.

Declarations

Consent for publication: All data generated or analyzed during this study are included in this published article.

Consent to Participate: An informed consent was obtained from all subjects and/or their legal guardian(s) prior to the study.

Data availability statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to privacy and ethical restrictions, the data are not publicly available.

Competing Interests: There is no conflict of interest to this study.

Funding: None was received.

Authors' Contributions: NBC and NOJ-F conceptualized and designed the study and participated in manuscript drafting. ESJ and EEI coordinated data collection and preliminary statistical analysis. NNT and OOB contributed to biochemical analysis and data validation. EMT and OMI provided supervisory oversight and timely manuscript revisions. OPO participated in literature search. All authors approved the final version of the manuscript.

Acknowledgments: Not applicable.

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Original Article

Ameliorative Effects of D-Ribose-L-Cysteine on Hematological Indices and Metabolic Intermediates in Diabetic Male Wistar Rats

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic disorder characterized by hyperglycemia, insulin resistance, oxidative stress, and progressive β -cell dysfunction. Hematological parameters and metabolic intermediates often reflect systemic inflammation, immune competence, mitochondrial activity, and redox balance, all of which are perturbed in diabetes. This study evaluated the effects of D-ribose-L-cysteine (DRLC), a glutathione precursor, on hematological indices and selected intermediates of energy metabolism in male Wistar rats exposed to a High-Fat Diet (HFD) and Streptozotocin (STZ) (HFD+STZ).

Methods: Animals were randomly divided into control, HFD+STZ, HFD+STZ+DRLC (150 and 300 mg/kg), and HFD+STZ+metformin (100 mg/kg) groups. Treatments were administered orally for 28 days. Hematological indices (White Blood Cells (WBC), Lymphocytes (LYM), Hemoglobin (Hb), Red Blood Cells (RBCs), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), and metabolic intermediates (pyruvate, succinate, lactate) together with Malate Dehydrogenase (MDH) activity were assessed.

Results: The HFD+STZ group developed significant leukocytosis, lymphocytosis, anemia, elevated MCV, reduced pyruvate and succinate, increased lactate levels, and altered MDH activity compared to controls ($p < 0.05$). DRLC administration significantly reversed these alterations in a dose-dependent manner, comparable to metformin. DRLC improved red blood cell integrity, enhanced hemoglobin levels, normalized energy metabolites, and modulated MDH activity, suggesting potent antioxidant and hemato-protective actions mediated through glutathione-dependent pathways.

Conclusion: DRLC effectively ameliorated hematological and mitochondrial derangements in diabetic rats, highlighting its therapeutic potential in mitigating diabetes-associated complications.

Keywords: D-Ribose-L-Cysteine, Hematological Indices, Energy Metabolism Intermediates, Streptozotocin -Induced Diabetes, High-Fat Diet

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1. Introduction

Besides disturbances in glucose and lipid metabolism that are the hallmark of type 2 diabetes mellitus (T2DM), there are significant alterations in hematological parameters, reflecting systemic inflammation and oxidative stress associated with chronic hyperglycemia [1]. Hematological parameters often reflect systemic inflammation, immune competence, and overall physiological stress, while metabolic intermediates such as succinate, lactate, and pyruvate serve as indicators of mitochondrial function, glycolytic flux, and shifts in oxidative metabolism [2, 3]. In diabetic pathophysiology, disturbances in these biomarkers may reveal underlying mitochondrial dysfunction, altered redox status, and energy deficits [4]. Persistent high glucose levels promote non-enzymatic glycation of hemoglobin and other blood components, leading to changes in erythrocyte structure, lifespan, and oxygen-carrying capacity [1]. This often results in reduced Hb concentration and may contribute to the development of anemia commonly observed in diabetic individuals, particularly those with renal complications that impair erythropoietin synthesis [1-5].

White Blood Cell count is frequently elevated in diabetes, indicating a state of low-grade chronic inflammation. Hyperglycemia-induced oxidative stress activates inflammatory cytokines such as TNF- α and IL-6, which stimulate leukocytosis and enhance leukocyte adhesion to the endothelium. These immune responses, while initially protective, can exacerbate vascular injury and insulin resistance [6]. Conversely, in poorly controlled diabetes, immune dysfunction may also lead to leukopenia due to impaired bone marrow activity and increased apoptosis of leukocytes [7]. Similarly, lymphocyte count and function are adversely affected by metabolic dysregulation in diabetes. Chronic hyperglycemia alters lymphocyte proliferation, differentiation, and cytokine secretion, leading to immune suppression and increased susceptibility to infections [8]. Some studies have reported lymphopenia in diabetic patients, attributed to oxidative DNA damage and impaired antioxidant defense systems [9-11]. Reduced lymphocyte activity also contributes to the impaired wound healing and increased infection risk observed in diabetic individuals [12]. Among experimental models that replicate key facets of human T2DM, the combined HFD and low-dose STZ regimen has gained prominence for its reproducibility and translational relevance [13]. This model reliably induces metabolic derangements, including dyslipidemia, oxidative stress, and impaired glucose homeostasis, providing an ideal platform for evaluating therapeutic interventions [14, 15].

D-Ribose-L-cysteine (DRLC), a potent glutathione precursor, has emerged as a promising agent in mitigating oxidative and inflammatory insults across diverse disease states [16]. Its antioxidant and anti-inflammatory capacities have been demonstrated in rodents exposed to high-fat/high-fructose diets, where DRLC restored hepatic and systemic oxidative balance by enhancing superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC), while reducing markers like malondialdehyde (MDA), C-reactive protein (CRP), and xanthine oxidase (XO), [17, 18]. Parallel findings underscore its efficacy in preventing cardiometabolic syndrome and improving lipid and glucose homeostasis in rats fed a high-fat/high-fructose diet [19, 20].

In male Wistar rats rendered diabetic via STZ, DRLC normalized lipid profiles, attenuated oxidative stress markers, and enhanced reproductive hormone levels and sperm parameters [21-23]. These effects collectively underscore its multifaceted potential in ameliorating both systemic and organ-specific complications of diabetes. Despite these encouraging outcomes, a critical gap persists as the impact of DRLC on comprehensive hematological indices and intermediates of energy metabolism, including WBCs, LYM, Hb, RBC, MCV, MCHC, inorganic phosphate, succinate, lactate, and pyruvate, remains unexplored, particularly within the HFD-STZ rodent model. Therefore, investigating whether DRLC can exert ameliorative effects on both hematological indices and key metabolic intermediates in HFD-STZ-induced diabetic male Wistar rats is both timely and scientifically compelling. Such a study would deepen understanding of DRLC's mechanistic actions and its potential to restore hematological integrity and metabolic equilibrium in complex diabetic milieus.

2. Method

2.1 Equipment and Apparatus

Plastic cages (42 x 30 x 27 cm), needles, syringes, oral cannula, wash bottles, hand gloves, tissue homogenizer, sensitive electrical weighing balance (model JA 2003), cold centrifuge (AT KE), VIS spectrophotometer (model 722N), refrigerator, pH meter (Shanghai Weiye Instruments, China), micropipettes (REMI) 100-1000 μ l, mortar and pestle, cotton filter, 10 liters can, plastic bucket with cover, plain bottles and racks, test tubes, microplate ELISA reader, Eppendorf tubes, and beaker.

2.2 Animal care

Animals were obtained from the Laboratory Animal Centre of the College of Medicine, Delta State University, Abraka. Animals were acclimatized in the laboratory for ten days under normal standard conditions. All animals were allowed free access to water and feed.

2.3 Drugs and Chemicals

DRLC and STZ (Sigma-Aldrich, St. Louis, MO, USA), Sodium citrate buffer (0.1 M, pH 4.5) for STZ dissolution was prepared using citric acid and sodium citrate from Sigma-Aldrich (St. Louis, MO, USA). EDTA (ethylenediaminetetraacetic acid) as an anticoagulant for blood collection was sourced from Becton Dickinson (Franklin Lakes, NJ, USA). For hematological analysis of paramete-

ters such as WBCs, LYM, Hb, RBCs, MCV, and MCHC, standard diluent and lysing reagents compatible with the Sysmex hematology analyzer were supplied by Sysmex Corporation (Kobe, Japan). The Succinate Colorimetric Assay Kit was obtained from Abcam (catalog no. ab204718; Cambridge, UK). The L-Lactate Assay Kit was sourced from Sigma-Aldrich (catalog no. MAK064; St. Louis, MO, USA). The Pyruvate Assay Kit was procured from Sigma-Aldrich (catalog no. MAK071; St. Louis, MO, USA). All other reagents and solvents were of analytical grade and purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.4 Experimental Diet

2.4.1 Purchase of Animal Feeds

Groundnut seeds were purchased from the local market in Abraka, Ethiopia East Local Government Area, Delta State, Nigeria. Standard commercial pelleted feed was purchased from Rainbow Top Feed Company, Amukpe, Sapele, Delta State, Nigeria.

2.4.2 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

2.4.3 Preparation of High Fat Diet

Groundnut seed diet was prepared by the method described by a previous study [24]. The groundnut seeds were cleaned by removing stones and dirt and air-dried. The dried groundnut seeds were milled with the aid of a local manual grinder. The groundnut feed was properly stored to avoid contamination from pests and moulds. Throughout the course of the experiment, the diet was made twice a week to prevent the degradation of its concentration.

2.4.4 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.

2.4.5 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:

Table 1: Nutritional Composition Groundnut

Nutrient	Groundnut (80g)	Commercial feed (20g)	Total per 100g HFD	Energy contribution (Kcal)
Fat (g)	39.1	3.2	42.3	381 (9 kcal/g)
Carbohydrate (g)	19	10.8	29.8	119 (4 kcal/g)
Protein (g)	21.9	4.0	25.9	104 (4 kcal/g)
Total energy (kcal)	–	–	–	604 kcal/100 g

2.5 Experimental Design

Animals were fed an HFD for four weeks. On day 14 of the protocol, animals were injected with an initial dose of STZ 40 mg/kg intraperitoneally (IP) and a second dose on day 28 of the experimental protocol. Male Wistar rats weighing approximately 200-250 g were randomly divided into five groups (n=5). This sample size was consistent with similar experimental pharmacology studies. The control group was fed with a normal rodent diet. Group two was fed HFD-STZ, and groups three and four were administered HFD-STZ and graded doses of DRLC (150 mg/kg and 300 mg/kg *p.o.*). Group five was administered HFD-STZ and the standard drug (metformin, 100 mg/kg BW) [16]. DRLC was prepared by dissolving 125 mg of DRLC in 12.5 ml of distilled water to obtain a stock solution of 10 mg/ml. The doses of STZ and composition of HFD that were used for this research work are based on a previous study [24]. Animals were treated for four weeks. DRLC was administered orally. At the end of the experimental protocol, animals were sacrificed by euthanasia. Blood samples were collected, and serum was obtained to determine the concentration of white blood cells, lymphocytes, hemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), inorganic phosphate, succinate, lactate, and pyruvate.

2.6 Hematological Analysis

The Sysmex XW-100 analyzer is factory-calibrated and does not require on-site calibration. System performance was verified through weekly instrument care and quality control procedures prompted by the analyzer software. Specimens producing unreliable results were automatically flagged. The analyzer is manufacturer-validated for EDTA-anticoagulated whole blood collected and stored under defined conditions, and only samples within the validated population and pre-analytical criteria were analyzed.

3. Results

3.1 Effect of D-ribose-L-cysteine on white blood cell count (WBC) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on white blood cell count (WBC) is shown in Figure 1. One-way ANOVA 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 WBC count when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant $F(4,25) = 21.17$, $p < 0.05$ decrease in WBC count when compared with the (HFD+ STZ) group only.

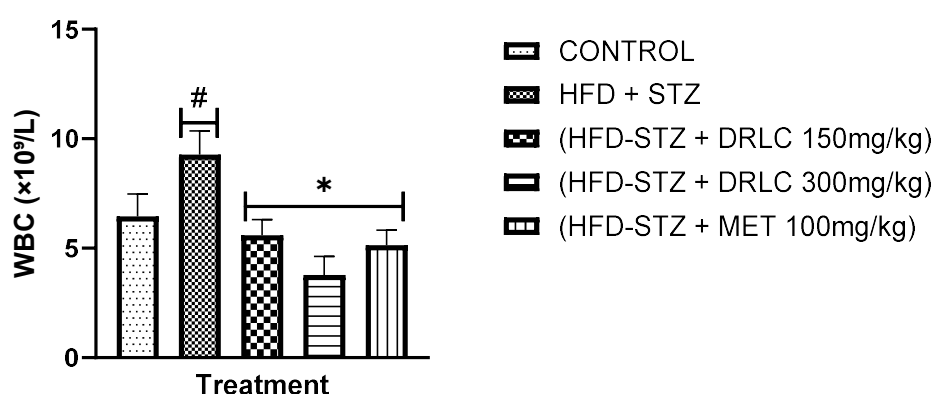


Figure 1: Effect of D-ribose-L-cysteine on white blood cell count (WBC) in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.2 Effect of D-ribose-L-cysteine on lymphocytes (LYM) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on lymphocytes (LYM) is shown in Figure 2. One-way ANOVA 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 $F(4,25) = 25.72$, $p < 0.05$ increase in LYM count 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 LYM count when compared with the (HFD+ STZ) group only.

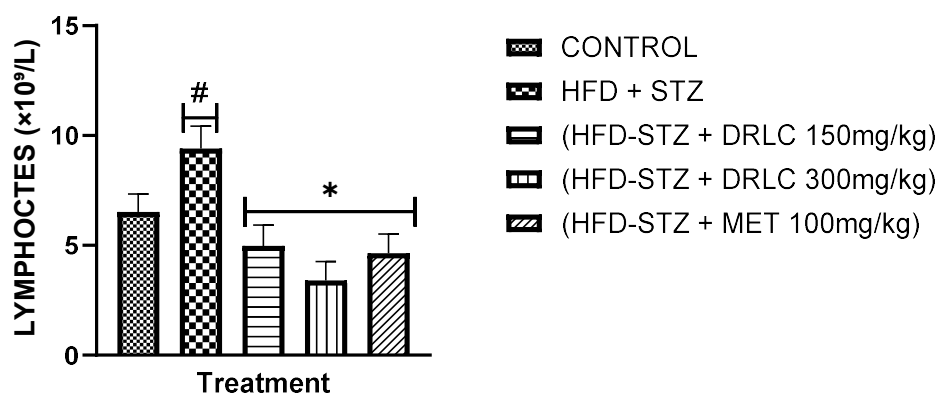


Figure 2: Effect of D-ribose-L-cysteine on lymphocytes (LYM) in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.3 Effect of D-ribose-L-cysteine on Hemoglobin (Hb) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on Hemoglobin (Hb) is shown in Figure 3. One-way ANOVA 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 $F(4, 25) = 43.70, p < 0.05$ decrease in Hb levels 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 Hb levels when compared with the (HFD+ STZ) group only.

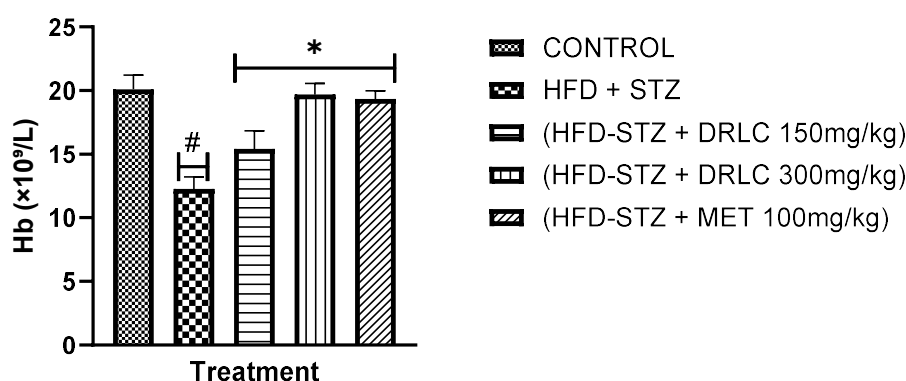


Figure 3: Effect of D-ribose-L-cysteine on Hemoglobin (Hb) in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.4 Effect of D-ribose-L-cysteine on red blood cell (RBC) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on red blood cell (RBC) is shown in Figure 4. One-way ANOVA 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 $F(4,25) = 21.25, p < 0.05$ decrease in RBC count 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 RBC count when compared with the (HFD+ STZ) group only.

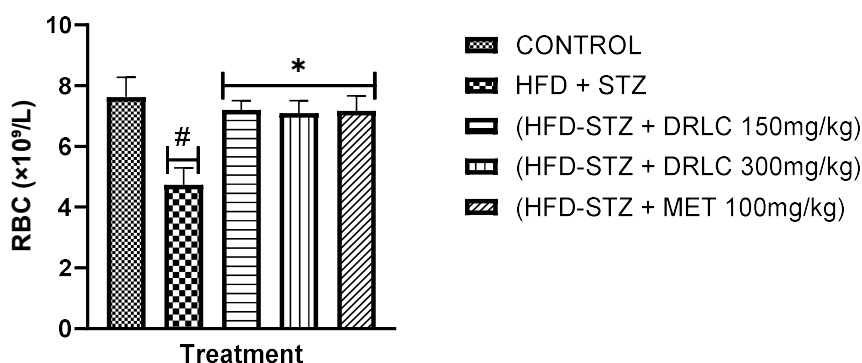


Figure 4: Effect of D-ribose-L-cysteine on red blood cell (RBC) in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.5 Effect of D-ribose-L-cysteine on Mean Corpuscular Volume (MCV) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on mean corpuscular volume (MCV) is shown in Figure 5. One-way ANOVA 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 MCV levels when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant $F(4,25) = 25.16$, $p<0.05$ decrease in MCV levels when compared with the (HFD+ STZ) group only.

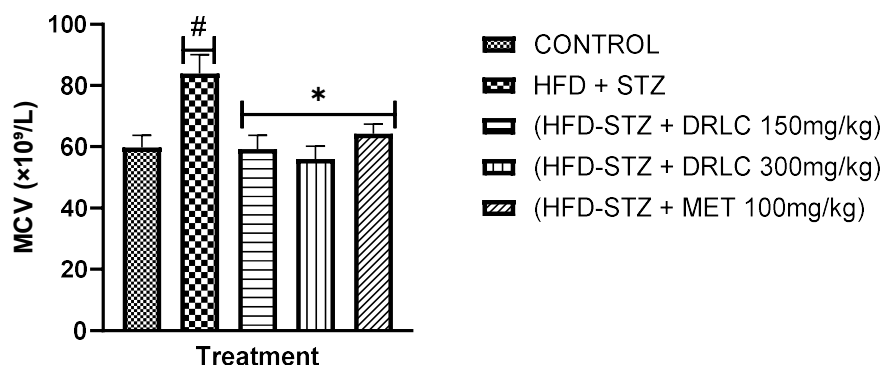


Figure 5: Effect of D-ribose-L-cysteine on Mean Corpuscular Volume in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.6 Effect of D-ribose-L-cysteine on Mean Corpuscular Hemoglobin Concentration (MCHC) in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on mean corpuscular hemoglobin concentration (MCHC) was shown in Figure 6. One-way ANOVA 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 MCHC levels when compared to the control group, while DRLC (150, 300 mg/kg), metformin 100mg/kg, given daily for 28 days produced a significant $F(4,25) = 17.28$, $p<0.05$ increase in MCHC levels when compared with the (HFD+ STZ) group only.

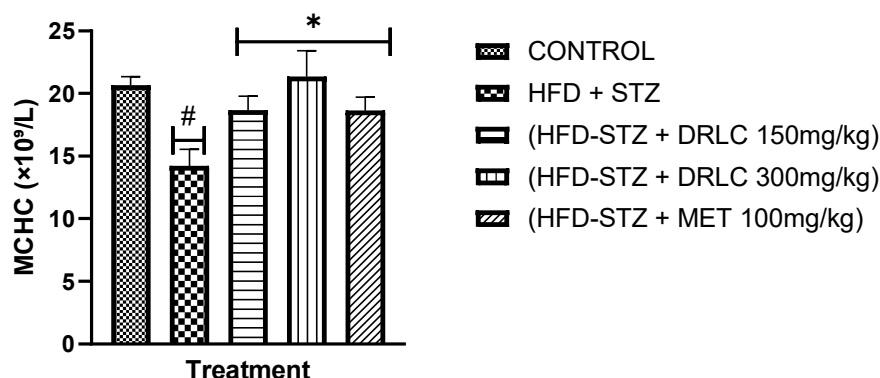


Figure 6: Effect of D-ribose-L-cysteine on Mean Corpuscular Hemoglobin Concentration in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.7 Effect of D-ribose-L-cysteine on pyruvate levels in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on pyruvate levels is shown in Figure 7. One-way ANOVA 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 $F(4,25) = 31.34$, $p<0.05$ decrease in pyruvate levels 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 pyruvate levels when compared with the (HFD+ STZ) group only.

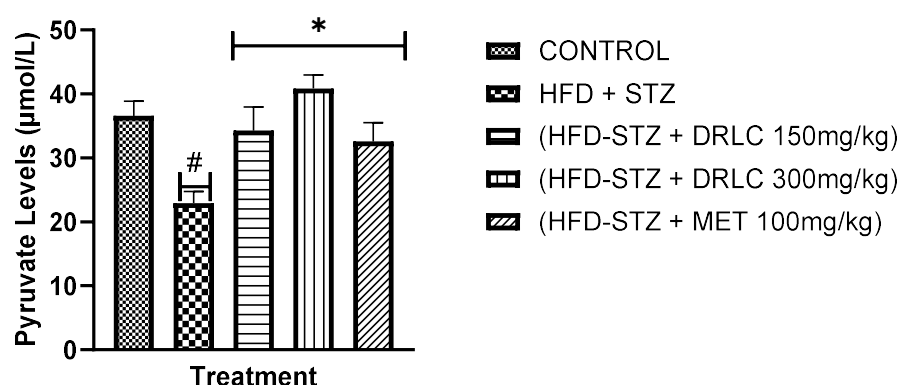


Figure 7: Effect of D-ribose-L-cysteine on pyruvate levels in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.8 Effect of D-ribose-L-cysteine on Succinate levels in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on succinate levels was shown in Figure 8. One-way ANOVA showed that there was a 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 $F(4,25) = 97.82$, $p<0.05$ decrease in succinate levels 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 succinate levels when compared with the (HFD+ STZ) group only.

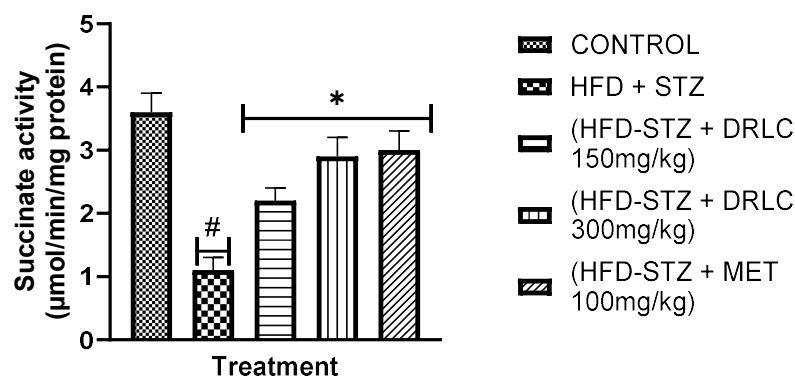


Figure 8: Effect of D-ribose-L-cysteine on Succinate levels in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.9 Effect of D-ribose-L-cysteine on lactate levels in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on lactate levels is shown in Figure 9. One-way ANOVA 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 $F(4,25) = 30.34$, $p<0.05$ increase in lactate levels 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 lactate levels when compared with the (HFD+ STZ) group only.

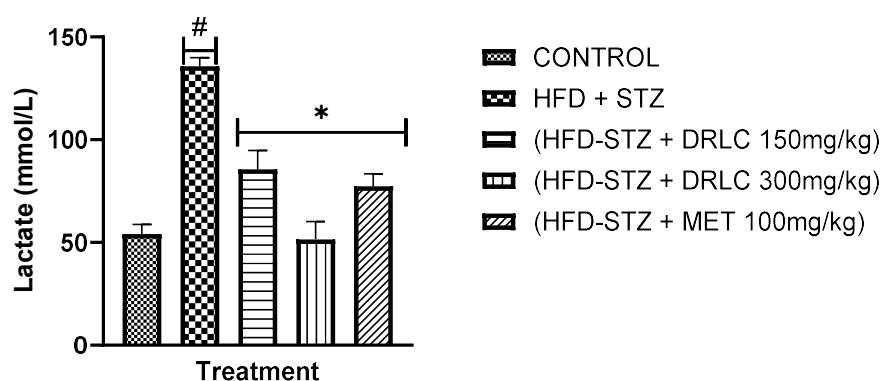


Figure 9: Effect of D-ribose-L-cysteine on lactate levels in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

3.10 Effect of D-ribose-L-cysteine on Malate Dehydrogenase levels in rats exposed to STZ and HFD

The effect of D-Ribose-L-Cysteine given daily for 28 days on malate dehydrogenase levels is shown in Figure 10. One-way ANOVA 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 $F(4,25) = 76.12$, $p < 0.05$ decrease in malate dehydrogenase levels 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 malate dehydrogenase levels when compared with the (HFD+ STZ) group only.

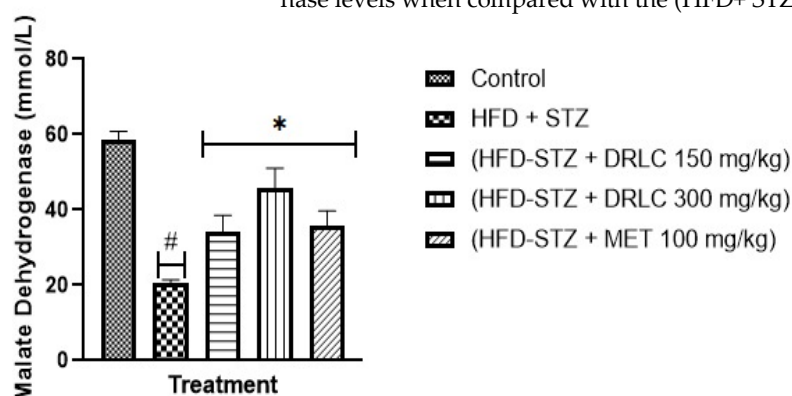


Figure 10: Effect of D-ribose-L-cysteine on malate dehydrogenase levels in rats exposed to STZ and HFD

Values represent the mean $\pm$ 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).

4. Discussion

The provided results demonstrate the multifaceted therapeutic potential of D-Ribose-L-cysteine (DRLC) in ameliorating metabolic and hematological derangements in a rat model of type 2 diabetes (T2DM) induced by a high-fat diet (HFD) and streptozotocin (STZ). The current data specifically highlight DRLC's impact on red blood cell health and core energy metabolic pathways, suggesting a significant role in improving cellular bioenergetics. The study found a significant increase in both WBC count and lymphocyte count in the HFD+STZ group compared to the control. This is a crucial finding, as elevated white blood cell counts, particularly lymphocytes, are widely recognized as markers of systemic inflammation associated with metabolic syndrome, obesity, and type 2 diabetes [26-28]. Hyperglycemia, dyslipidemia, and visceral adiposity in the HFD+STZ model contribute to the activation of immune cells, leading to increased production of pro-inflammatory cytokines and infiltration of immune cells into various tissues [27-30]. Both DRLC (at 150 and 300 mg/kg) and metformin significantly reduced the elevated WBC and LYM counts in the HFD+STZ group. Metformin's anti-inflammatory properties are well-documented, extending beyond its glucose-lowering effects [31].

Furthermore, the HFD+STZ group exhibited a significant decrease in both Hb levels and RBC counts compared to the control group, thus indicating the development of anemia in the diabetic model. Anemia is a common complication in diabetic patients, often multifactorial in origin, involving impaired erythropoiesis, reduced erythrocyte lifespan due to oxidative damage and increased osmotic fragility, chronic inflammation, and sometimes nutritional deficiencies [32-33]. Hyperglycemia significantly contributes to oxidative stress in red blood cells, leading to their premature destruction (hemolysis) and decreased production (erythroid suppression), [34]. Chronic inflammation also suppresses erythropoiesis by altering iron metabolism and inhibiting erythropoietin production or response [35].

Remarkably, DRLC (both doses) and metformin significantly increased Hb levels and RBC counts when compared to the HFD+STZ group, effectively ameliorating the anemia. This protective effect aligns with their potential to reduce systemic inflammation and oxidative stress. By improving the antioxidant status via GSH synthesis, DRLC can protect red blood cells from oxidative damage, thereby prolonging their lifespan and improving erythropoiesis. Regarding mean corpuscular volume (MCV), the HFD+STZ group exhibited a significant increase in MCV compared to the control group. Elevated MCV values typically indicate macrocytic anemia, which can be associated with various conditions, including vitamin deficiencies (B12 and folate), liver disease, hypothyroidism, and occasionally chronic inflammatory states and oxidative stress [36]. In the context of diabetes, increased oxidative stress can lead to swelling and morphological changes in red blood cells, potentially contributing to an elevated MCV [34]. Both DRLC (150, 300 mg/kg) and metformin significantly decreased the elevated MCV levels back toward normal, compared to the HFD+STZ group.

Additionally, the HFD+STZ group exhibited a significant decrease in pyruvate and a corresponding significant increase in lactate levels. This metabolic signature is indicative of a shift toward anaerobic glycolysis and impaired oxidative phosphorylation [37, 38]. The decreased pyruvate suggests either reduced glycolytic flux leading to pyruvate or impaired utilization of pyruvate, while increased lactate points to inefficient aerobic respiration and a compensatory mechanism for ATP generation [39]. Moreover, the significant decrease in succinate levels in the HFD+STZ group provides further evidence of severe mitochondrial dysfunction. Succinate is a crucial intermediate of the tricarboxylic acid (TCA) cycle and also acts as a substrate for Complex II of the electron transport chain (succinate dehydrogenase), [40-41]. Reduced succinate levels directly imply a slowdown or impairment within the TCA cycle, which is central to aerobic energy production in the mitochondria [40-42]. Dysregulation of the TCA cycle and mitochondrial respiration is a hallmark of diabetes pathophysiology, leading to reduced ATP synthesis and accumulation of ROS [43].

5. Conclusion

Overall, these results strongly suggest that DRLC effectively counteracts the metabolic derangements induced by HFD+STZ, particularly by improving hematological parameters (WBC, LYM, Hb, RBC, and MCV), mitochondrial function and, possibly enhancing aerobic energy production. The observed improvements in metabolic intermediates (pyruvate, lactate, succinate, and malate dehydrogenase) further solidify that DRLC's shows promise as a therapeutic agent in mitigating diabetes-associated hematological and mitochondrial dysfunction.

Declarations

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Contribution of authors: IH and EAT, conceptualized and designed the study; IH and ACO did the experimental studies; EAT and ACO did the data analysis; IH, EAT, and BB did the original draft of the manuscript. All authors revised and approved the manuscript for submission.

Ethical approval: Before the commencement of the study, ethical approval (RBC/FBMC/DELSU/25/655) was sought from the Delta State University Animal Care and Use Research Ethics Committee of the Faculty of Basic Medical Sciences according to the directions from the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Availability of data and materials: All data supporting the findings of this research work are included within the manuscript. Additional data related to this study are available from the corresponding author

Use of artificial intelligence (AI): All authors confirm that the writing of this manuscript is an original work and we that the use of artificial intelligence was not employed in the writing process

Use of research reporting tools: The authors affirm the use of Graph Pad prism version 10.0 for this study.

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Original Article

Hematological and Biochemical Evaluation of Acute and Sub-acute Oral Toxicity of *Azanza garckeana* Hydroethanolic Fruit Extract in Male Wistar Rats

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Abstract

Background: *Azanza garckeana* is a widely used African medicinal plant, particularly for reproductive health and various ailments. Despite its ethnomedicinal applications and pharmacological evidence, comprehensive data on the safety of its hydroethanolic fruit extract remain limited. This study evaluated the acute and sub-acute (15 and 30 days) oral toxicity of hydroethanolic fruit extract of *Azanza garckeana* (AZG) in male Wistar rats, focusing on hematological, biochemical, and oxidative stress markers.

Methods: Acute toxicity was assessed using Lorke's method. For sub-acute studies, rats received daily oral doses of hydroethanolic fruit extract of *Azanza garckeana* (200, 400, or 800 mg/kg) or distilled water for 15 or 30 days (n = 5/group). Body weight, food/fluid intake, hematology, serum biochemistry, and tissue oxidative stress markers were evaluated. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test (p<0.05).

Results: No mortality or overt toxicity was observed. The oral LD₅₀ was estimated to be >5000 mg/kg, indicating low acute toxicity. At 15 days, minor changes included decreased platelet distribution width (all doses) and mean platelet volume (800 mg/kg), increased HDL-cholesterol (800 mg/kg), reduced blood urea nitrogen (all doses), and enhanced antioxidant markers (e.g., glutathione and glutathione peroxidase) in liver, kidney, and heart, with no evidence of organ damage. After 30 days, changes included improved lipid profile (decreased total/LDL-cholesterol, increased HDL), mild hematological modulations (e.g., decreased red cell distribution width), reduced food intake (400–800 mg/kg) without weight loss, and sustained catalase elevation in heart (800 mg/kg). No hepatotoxicity or nephrotoxicity was observed.

Conclusion: AZG exhibited no significant toxicity at doses up to 800 mg/kg/day for 30 days. The no-observed-adverse-effect level (NOAEL) under the present experimental conditions is 800mg/kg/day. These findings support its relative safety, with observed changes suggesting no adverse effects but potential hypolipidemic, antioxidant, and hematological modulations.

Keywords: *Azanza garckeana*, Acute toxicity, Sub-acute toxicity, Oxidative stress, Hematology,

1. Introduction

Azanza garckeana (F. Hoffm.) Exell & Hillc. is a deciduous tree belonging to the Malvaceae family, found in tropical and subtropical regions of Africa [1,2], across Nigeria, Zambia, Zimbabwe, and South Africa [3]. Where, it is commonly known as snot apple, African chewing gum, or gorontula [4], and serves as an important food source during times of scarcity, with both the fruits and seeds being consumed [5]. In traditional medicine, various parts of *Azanza garckeana* (fruits, leaves, bark, and roots) have been utilized to treat numerous ailments; including respiratory diseases (like

cough and chest pain)[6], reproductive health concerns (such as infertility and menstrual irregularities), liver disorders, sexually transmitted infections, and diabetes [7]. Additionally, *Azanza garckeana* has also been reported to display antimicrobial, antifungal, and anti-inflammatory activities by inhibiting mediators like COX-2 and NF-κB [8,9]. Recent studies have identified several active compounds in *Azanza garckeana*, including flavonoids, alkaloids, tannins, and specific metabolites such as phenol, 24-bis (1, 1-dimethylethyl) [10], vitamins, and sugars (glucose, fructose, maltose), [11] and pectins [12]. These various compounds contribute to the plant pharmacological properties, as phenols, flavonoids, and tannins exhibit strong antioxidant effects by scavenging DPPH and FRAP [13, 14] while, alkaloids and saponins present in *Azanza garckeana* enhance analgesic, anti-arthritis, and wound-healing effects [15,16], whereas carotenoids and ascorbic acid promote nutritional benefits and exhibit antiproliferative (anticancer) properties [17]. These diverse bioactivities highlight *Azanza garckeana* potential as a source of natural medicine.

Despite the promising pharmacological profile and increasing use of *Azanza garckeana* as a complementary therapy for enhancing sexual performance and libido, its safety profile remains under-researched. Initial toxicity assessments of methanol extracts from the fruits, roots, bark, and aqueous leaf extract of *Azanza garckeana* in rodent models suggest low acute and sub-acute toxicity [18], with no significant adverse effects at doses up to 5000 mg/kg body weight in acute toxicity evaluations using Lorke's method. However, in-depth studies on hydroethanolic extracts commonly used for capturing a wide range of both polar and semi-polar phytoconstituents [19] are limited. Acute toxicity studies are crucial for determining the safety margins of herbal extracts, providing important insights into potential adverse effects from exposure [20]. This is especially relevant for plants like *Azanza garckeana*, which may transition from traditional applications to formal pharmaceutical use, requiring compliance with regulatory guidelines such as those set by the Organization for Economic Co-operation and Development (OECD). The current study aimed to assess the acute toxicity of hydroethanolic extracts of *Azanza garckeana* fruits in Wistar rats. In particular, we examined hematological parameters, biochemical markers for kidney and liver function, and the antioxidant status of the heart, kidney, and liver to clarify any possible toxicological effects and contribute to the scientific validation of its safe use.

2. Method

2.1 Plant Material

Fresh fruits of *Azanza garckeana* were purchased from Gombe State, Nigeria and subjected to botanical identification at the University Herbarium of the Department of Botany Science, Delta State University, Abraka, Delta State, Nigeria. The university referencing and voucher specimen number for the plant DELSUH279 was obtained and documented.

2.2 Plant Identification and Extraction

The fresh fruits of *Azanza garckeana* were completely air-dried at room temperature (28-33 °C) until constant weight was achieved. The dried fruits were cracked open and pulverized using milling machine. About 1.0 kg of the pulverized fruit was completely macerated in hydroethanolic solvent "80% ethanol (v/v) prepared with distilled water" at room temperature for 72 hours with intermittent shaking to assist and ensure complete extraction. Thereafter, the solution was filtered through cotton wool and then 110 mm Whatmann filter paper, and the resultant filtrate concentrated *in vacuo* using rotary evaporator (B'U'CHI Rotavapor® Model R-215, Switzerland) with Vacuum Module V-801 EasyVac®, Switzerland) set at a revolution of 70 rpm and a temperature at 37 °C. The resulting concentrate was then dried over water bath preset at 40 °C, and the resultant residue weighed, stored in air- and water-proof container and kept in a refrigerator at 4 °C. Fresh solutions were prepared from this stock whenever required. The percentage yield of the extract was calculated using the formulae below:

$$\begin{aligned} \text{\% Yield of extract} &= \frac{\text{Weight of crude extract obtained (g)}}{\text{Weight of starting pulverized dry fruit extracted (g)}} \times 100 \\ &= (83.9/1000) \times 100 \\ &= 8.39\% \\ \text{The percentage yield of the extract was } &8.39\% \end{aligned}$$

2.3 Experimental Animals and treatment groupings

Fifty-two (52) male Wistar rats (150–200 g) were used in this study: twelve (12) for acute toxicity (Lorke's method) and forty (40) for the sub-acute toxicity study. Treatment for sub-acute toxicity study was carried out in different sub-groups as outlined below:

- Group IA, and IB: Distilled water *p.o.* for 15 and 30 days respectively.
- Group IIA, and IIB: Dose 200 mg/kg/day *p.o.* *Azanza garckeana* for 15 and 30 days respectively.
- Group IIIA, and IIIB: Dose 400 mg/kg/day *p.o.* *Azanza garckeana* for 15 and 30 days respectively.
- Group IVA and IVB: Dose 800 mg/kg/day *p.o.* *Azanza garckeana* for 15 and 30 days respectively.
- The study animals were allowed to acclimatize for two (2) weeks.

2.4 Sample Collection

Twenty-four hours (24 h) post administration of *Azanza garckeana* extract; overnight-fasted rats were humanely euthanized by cervical dislocation. Blood was collected through cardiac puncture via a 21-G needle fixed to a 5 mL syringe. The blood was transferred into EDTA-coated tubes and plain tubes (BD Vacutainer®, BD-Plymouth, UK). Blood in the EDTA tubes was gently mixed by inversion to ensure proper anticoagulation. Hematological assays such as red blood cell count (RBC), red blood cell distribution width-coefficient of variation (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, plateletcrit, platelet distribution width (PDW), and mean platelet volume (MPV), were determined using an automated hematology analyzer (Sysmex KX-21N). Serum was separated from the clotted blood in the plain tubes by centrifugation at 5000 rpm and used for biochemical analyses, including lipid profile, kidney function tests, and liver function tests using standard bioassay procedures and commercial kits. The heart, kidneys, and liver were dissected, harvested, weighed, and prepared for biochemical analysis.

2.5 Statistical Analysis

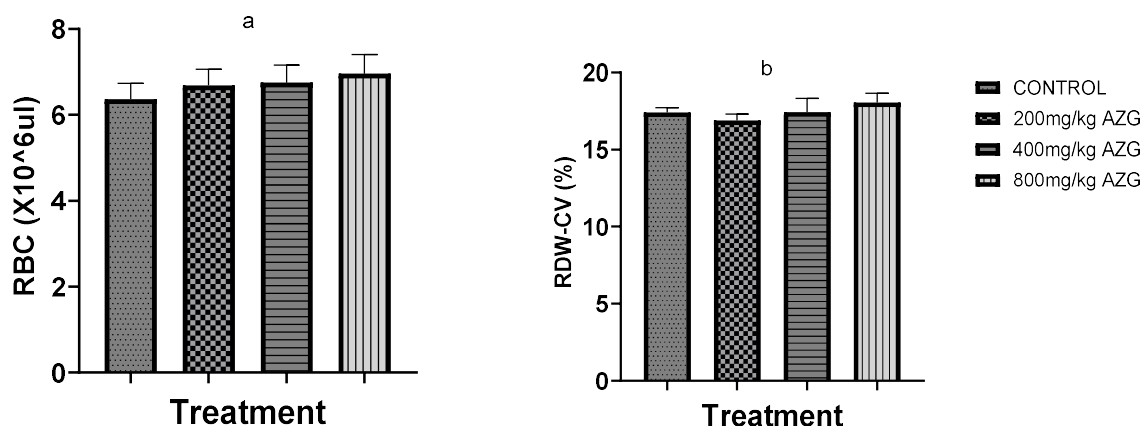
Data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered significant. Analyses were performed using GraphPad Prism software version 10.4.2.

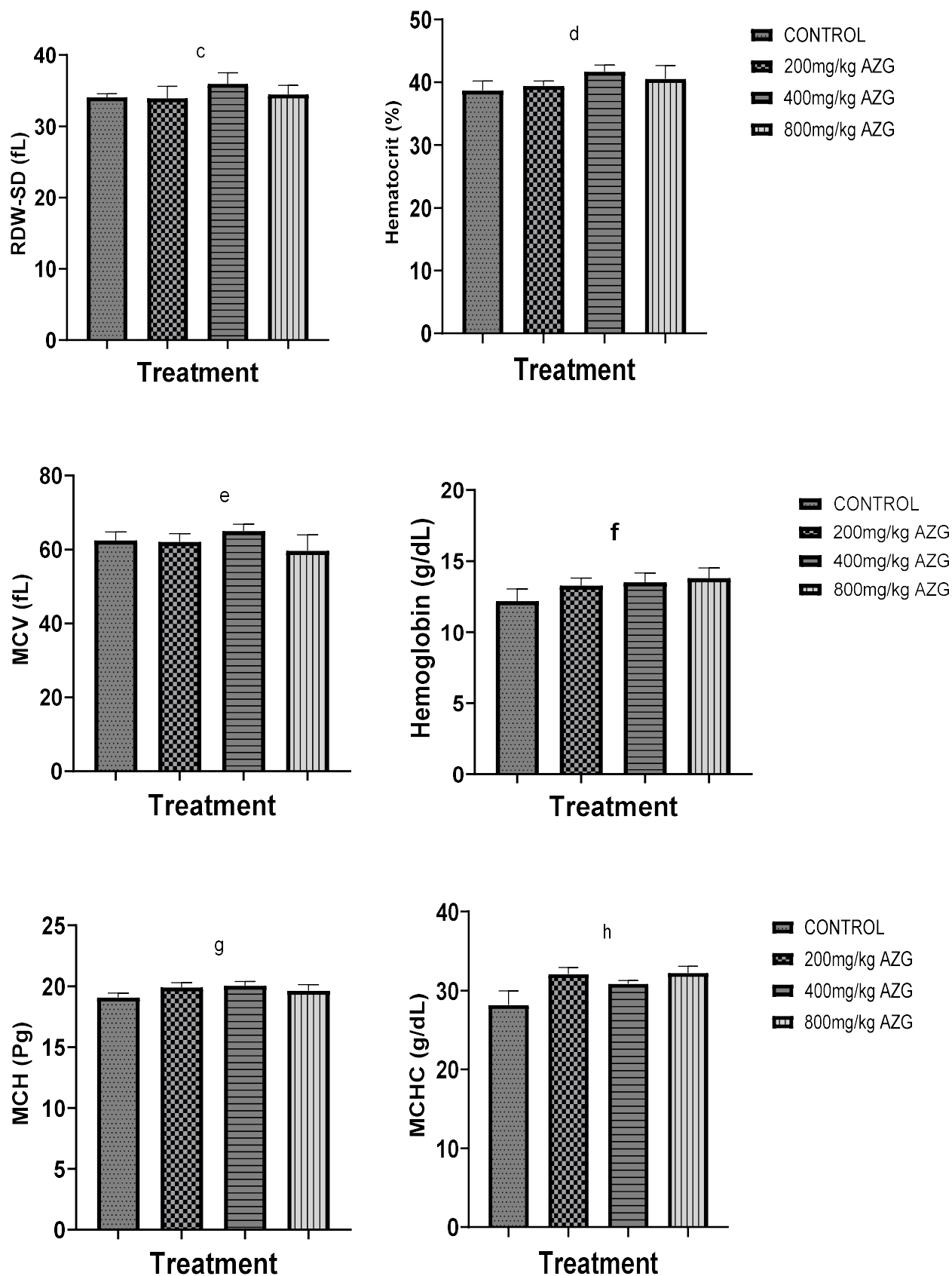
3. Results

No mortality or overt signs of toxicity were observed in the acute toxicity study, the oral LD₅₀ was estimated to be greater than 5000 mg/kg, indicating low acute toxicity. In sub-acute studies ($n = 5/\text{group}$), hydroethanolic fruit extract of *Azanza garckeana* administration for 15 or 30 days showed no significant adverse effects on most parameters, with some modulations suggesting absence of toxicity.

3.1 The effect of *Azanza garckeana* hydroethanolic extract on hematological indices (15 Days)

No significant differences in most hematological parameters (RBC, RDW-CV, RDW-SD, HCT, MCV, Hb, MCH, MCHC, platelet count, and plateletcrit) across groups ($p > 0.05$; Fig. 1a-j). However, PDW decreased at all doses ($p < 0.05$; Fig. 1K), and MPV decreased significantly at 800mg/kg ($p < 0.05$; Fig. 1L). These changes suggest modulation without pathological alterations.





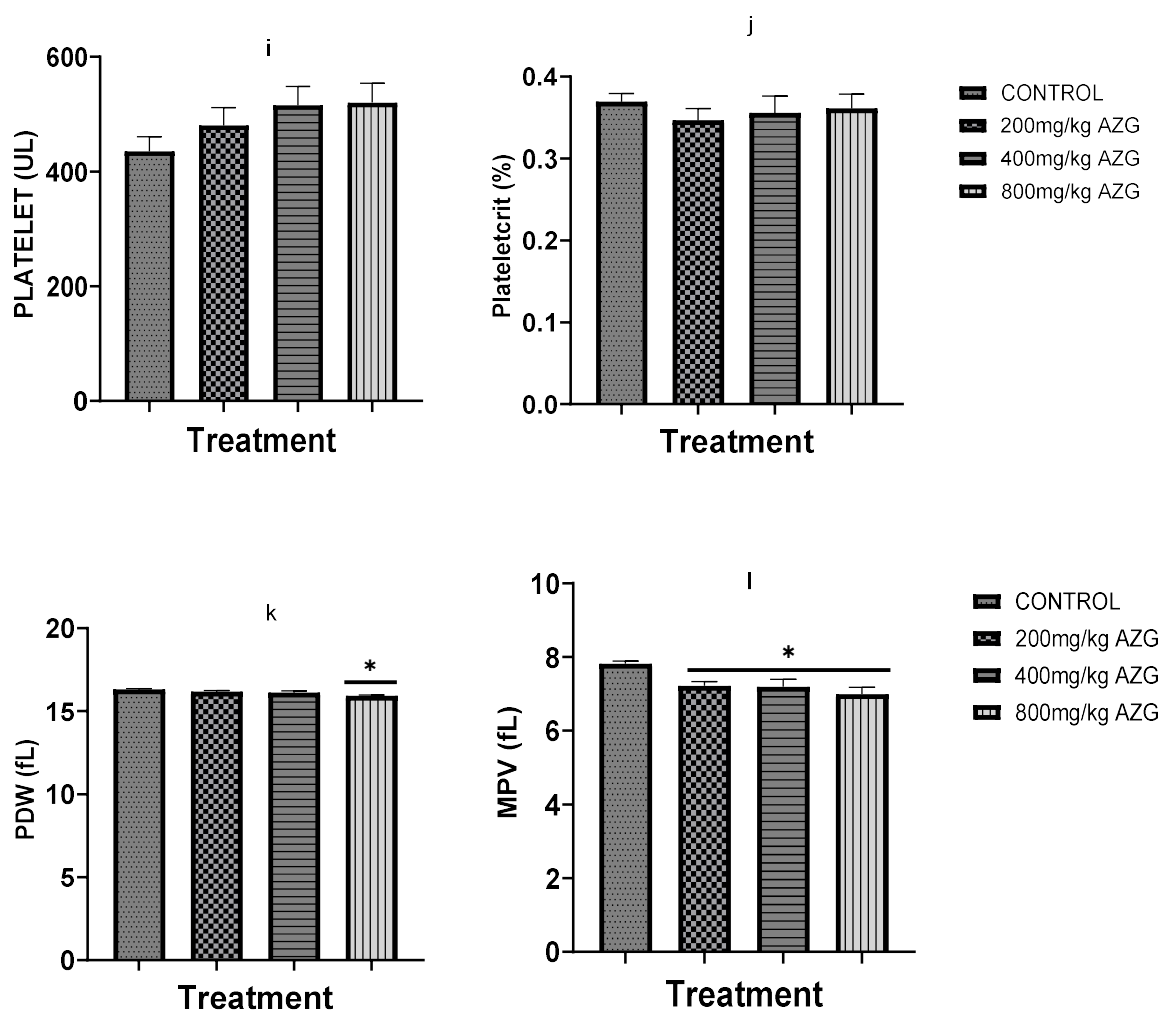
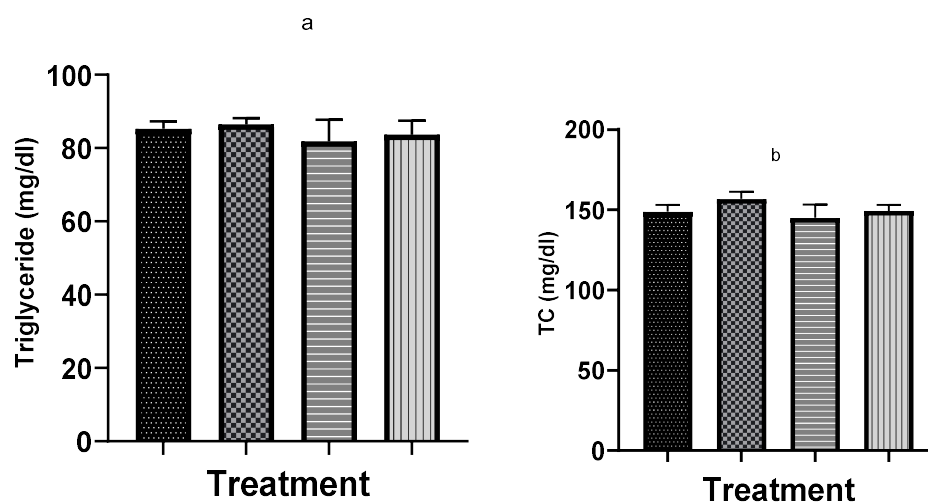


Figure 1: Effects of AZG (15 days) on hematological parameters in male Wistar rats. (a) RBC, (b) RDW-CV, (c) RDW-SD, (d) HCT, (e) MCV, (f) Hb, (g) MCH, (h) MCHC, (i) platelet count, (j) plateletcrit, (k) PDW, (l) MPV.

Values are mean $\pm$ SEM (n = 5). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.2 The effect of *Azanza garckeana* hydroethanolic extract on Lipid Profile (15 Days)

No significant changes in triglycerides, total cholesterol, LDL, or VLDL ($p > 0.05$; Fig. 2a-d). HDL increased at 800 mg/kg ($p < 0.05$; Fig. 2e).



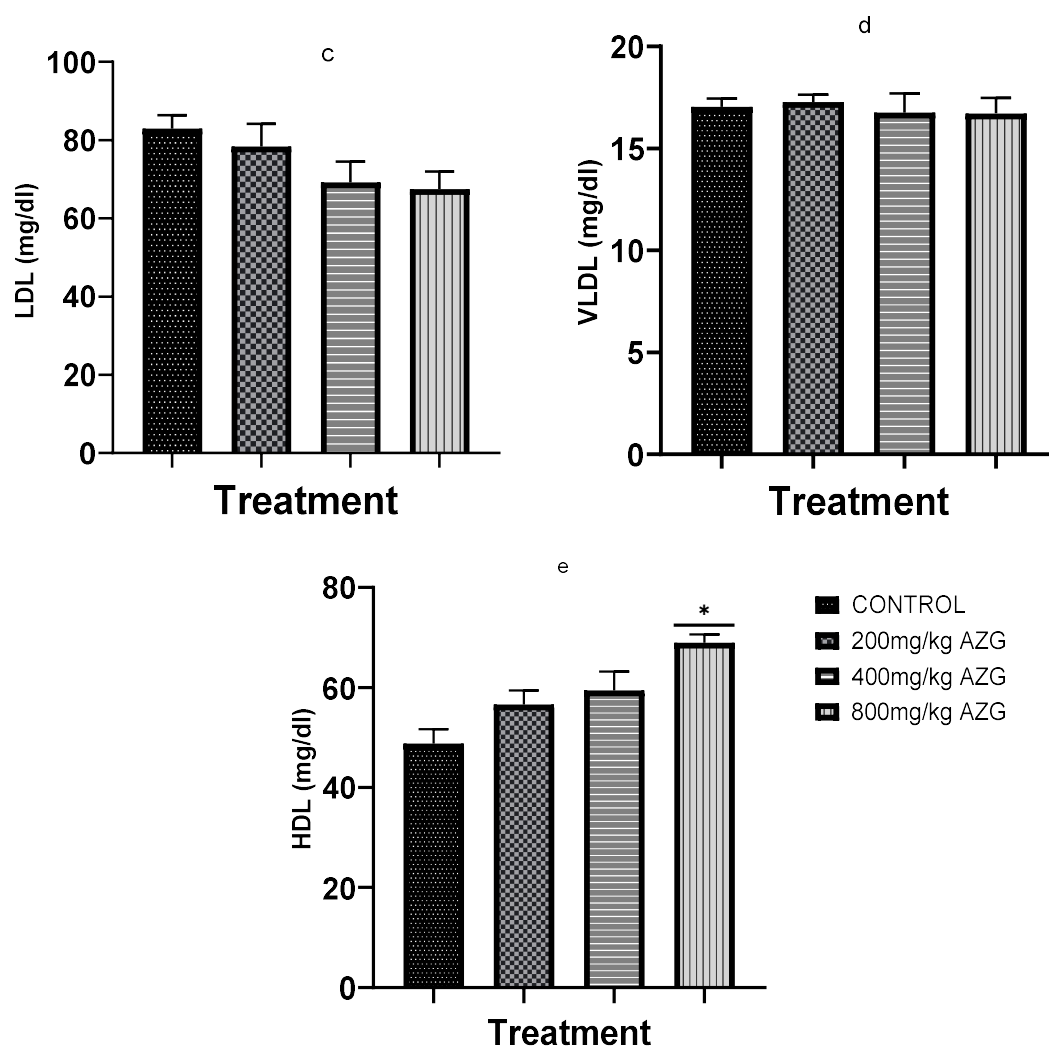
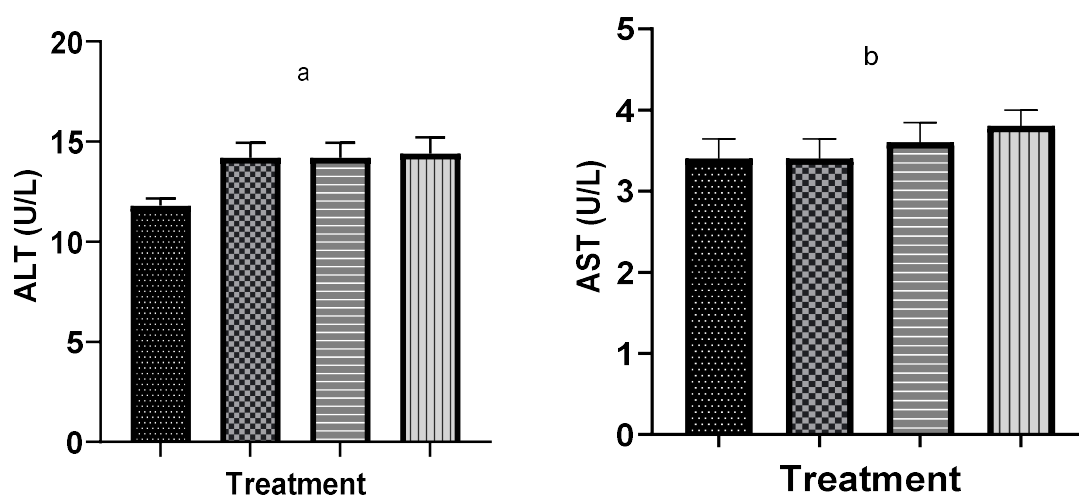


Figure 2: Effects of AZG (15 days) on lipid profile. (a) Triglycerides, (b) total cholesterol, (c) LDL, (d) VLDL, (e) HDL. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where $*p < 0.05$ vs. control.

3.3 The effect of *Azanza garckeana* hydroethanolic extract on Liver Function (15 Days)

No significant changes in ALT, AST, ALP, GGT, total protein, total bilirubin, or direct bilirubin ($p > 0.05$; Fig. 3a-g).



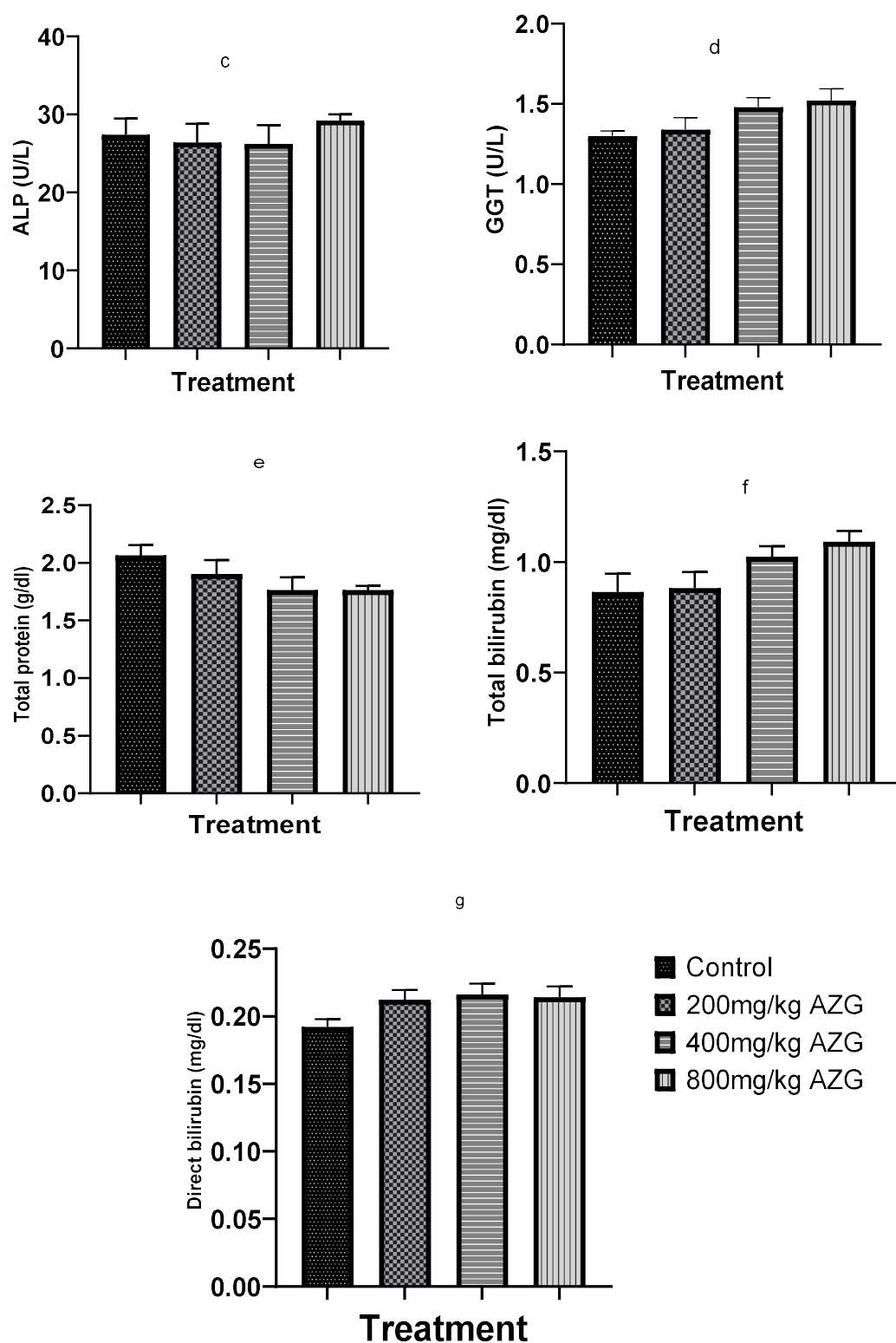


Figure 3: Effects of AZG (15 days) on liver function parameters. (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) total protein, (f) total bilirubin, (g) direct bilirubin.

Values are mean $\pm$ SEM (n = 5). No significant differences (p > 0.05).

3.4 The effect of *Azanza garckeana* hydroethanolic extract on Liver Oxidative Stress (15 Days)

No changes in SOD, CAT, GPx, NO, MDA (p > 0.05; Fig. 4a-e). GSH increased at 800 mg/kg (p < 0.05; Fig. 4f).

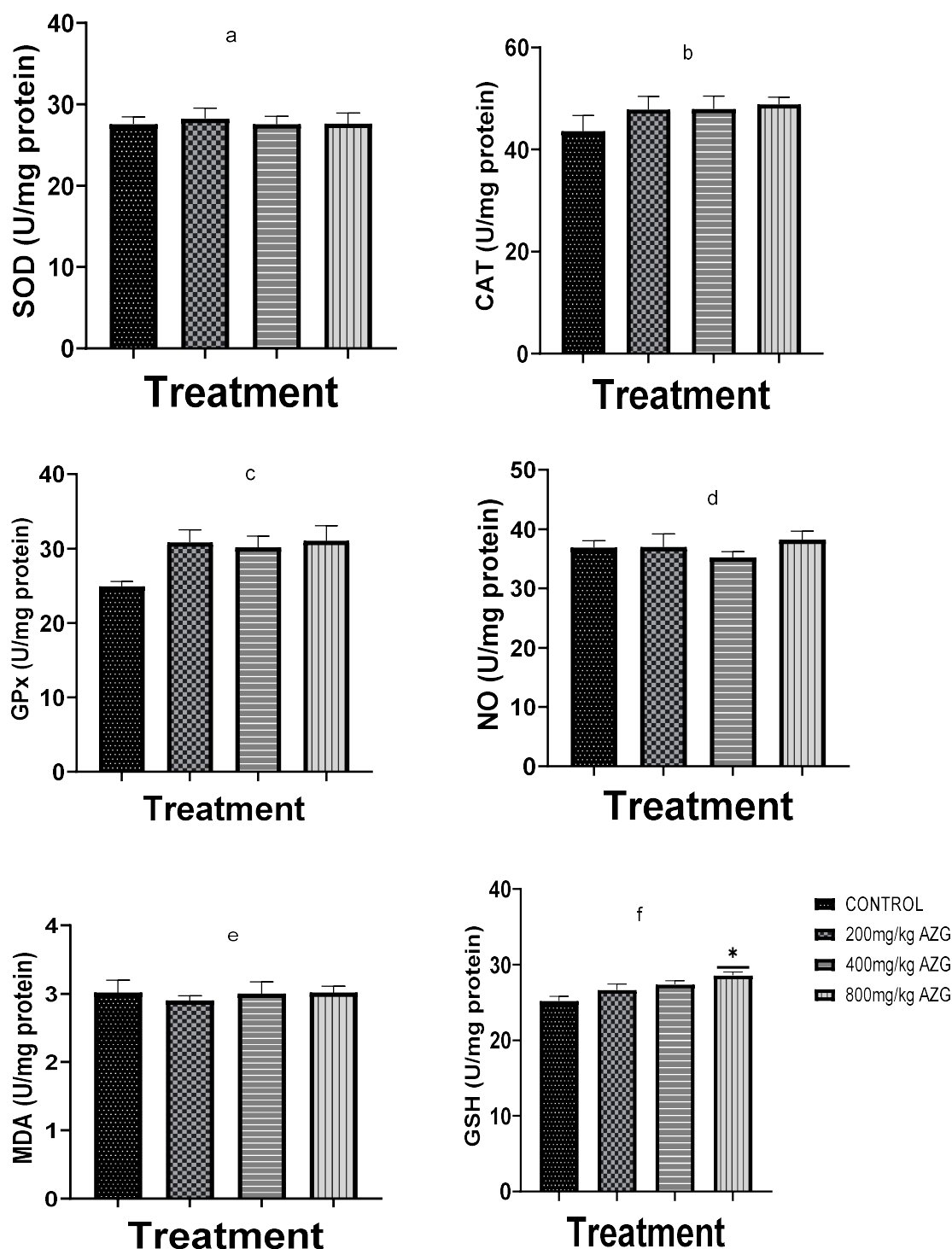
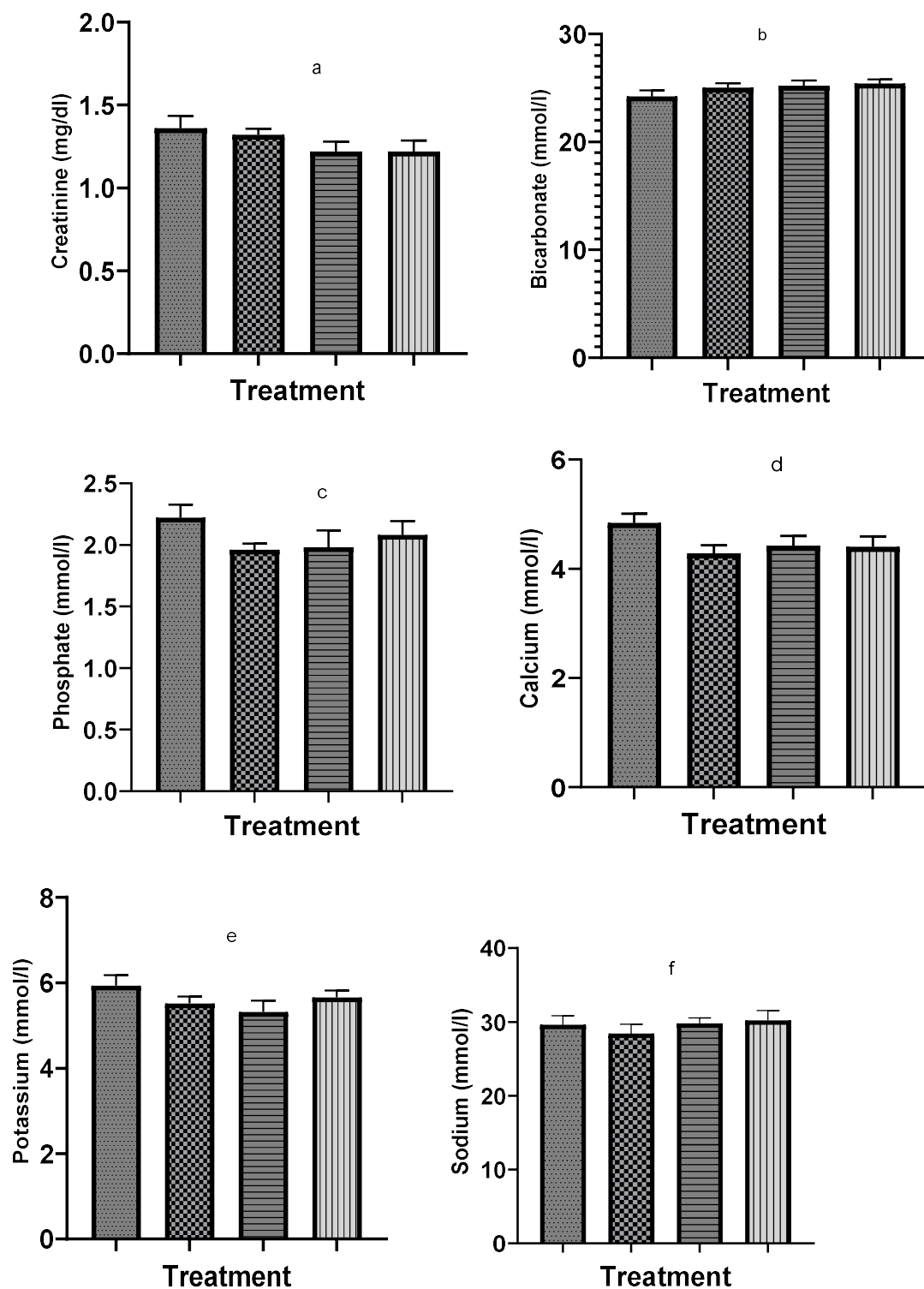


Figure 4: Effects of AZG (15 days) on liver oxidative stress markers. (a) SOD, (b) CAT, (c) GPx, (d) NO, (e) MDA, (f) GSH. Values are mean $\pm$ SEM (n = 5). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.5 The effect of *Azanza garckeana* hydroethanolic extract on Kidney Function (15 Days)

No changes in creatinine, bicarbonate, phosphate, calcium, potassium, sodium ($p > 0.05$; Fig. 5a-f). BUN decreased at all doses ($p < 0.05$; Fig. 5g).



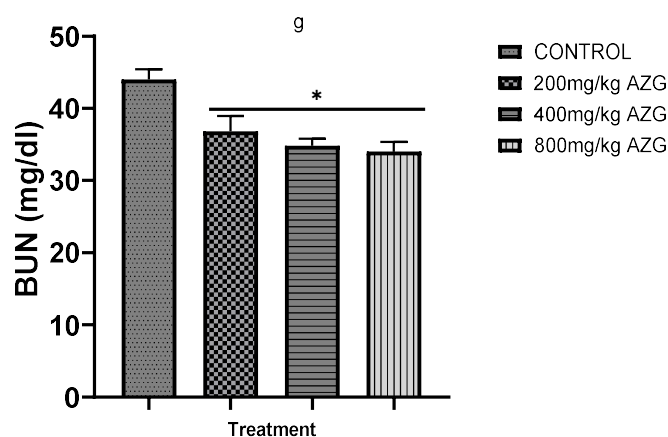
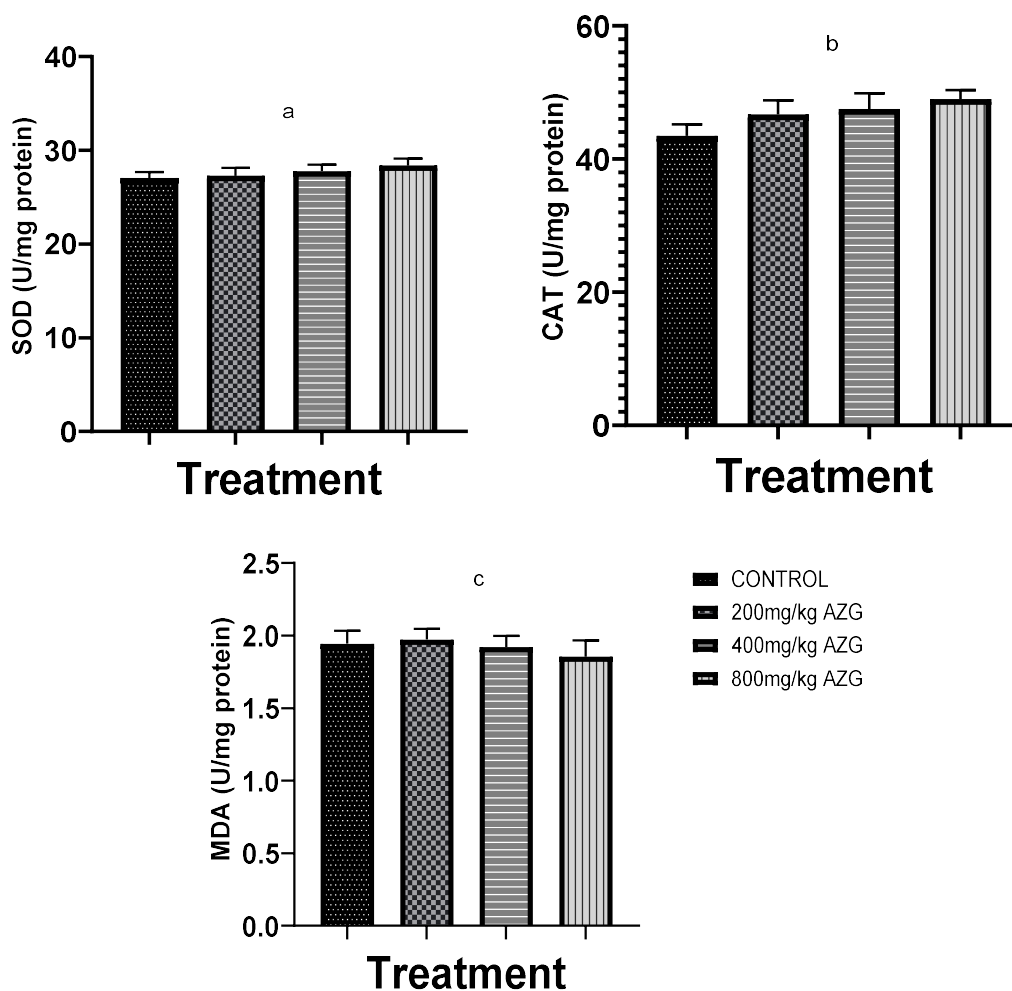


Figure 5: Effects of AZG (15 days) on kidney function parameters. (a) Creatinine, (b) bicarbonate, (c) phosphate, (d) calcium, (e) potassium, (f) sodium, (g) BUN.

Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where $*p < 0.05$ vs. control.

3.6 The effect of *Azanza garckeana* hydroethanolic extract on Kidney Oxidative Stress (15 Days)

No changes in SOD, CAT, MDA ($p > 0.05$; Fig. 6a-c). GSH increased at 400 and 800 mg/kg, GPx at all doses, NO at 800 mg/kg ($p < 0.05$; Fig. 6d-f).



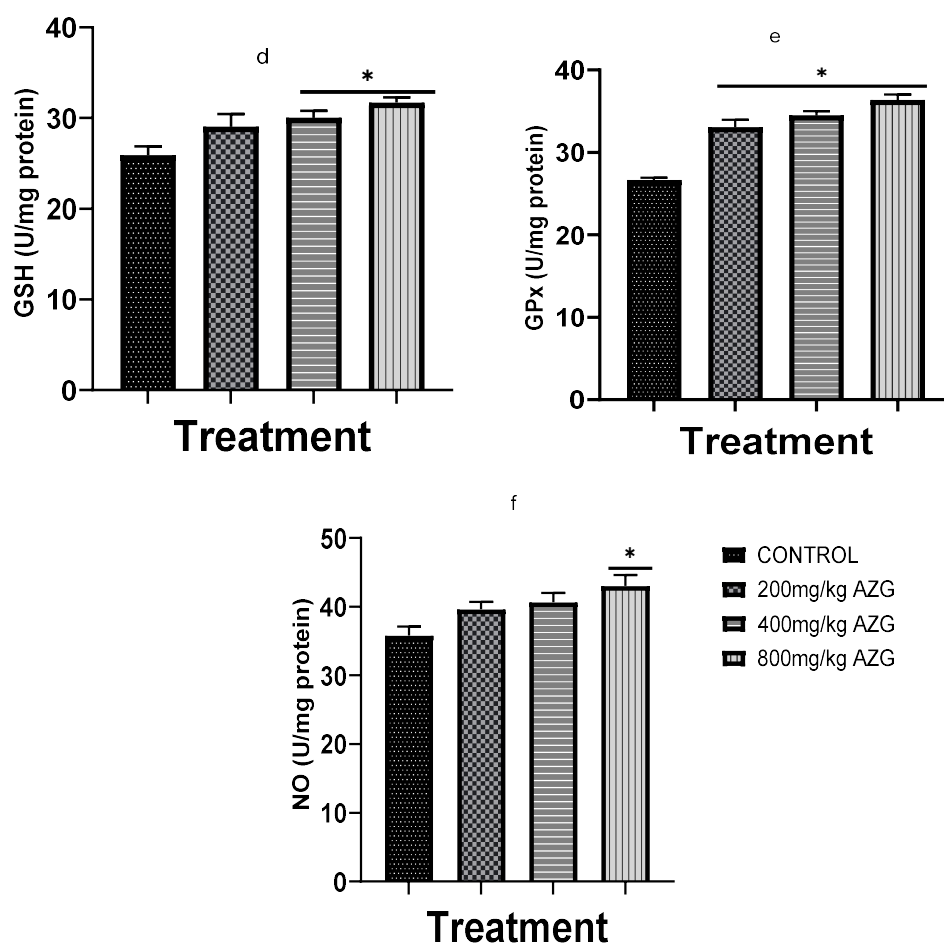
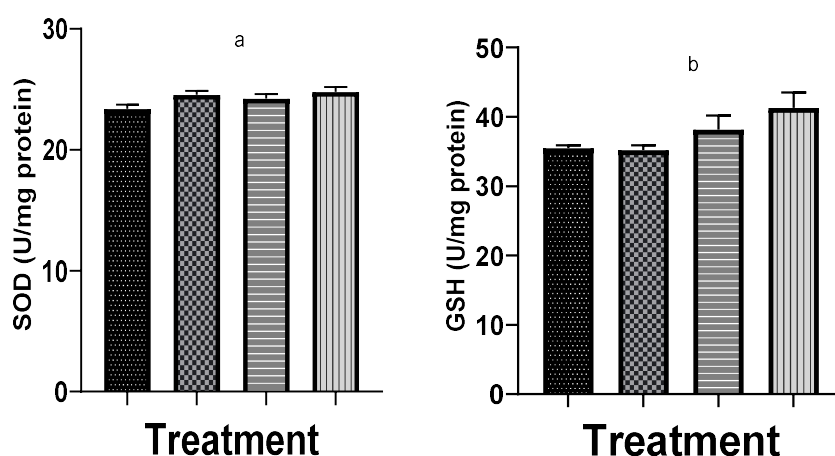


Figure 6: Effects of AZG (15 days) on kidney oxidative stress markers. (a) SOD, (b) CAT, (c) MDA, (d) GSH, (e) GPx, (f) NO. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.7 The effect of *Azanza garckeana* hydroethanolic extract on Heart Oxidative Stress (15 Days)

No changes in SOD, GSH, GPx, MDA ($p > 0.05$; Fig. 7a-d). CAT increased at 400 and 800 mg/kg, NO at all doses ($p < 0.05$; Fig. 7e-f).



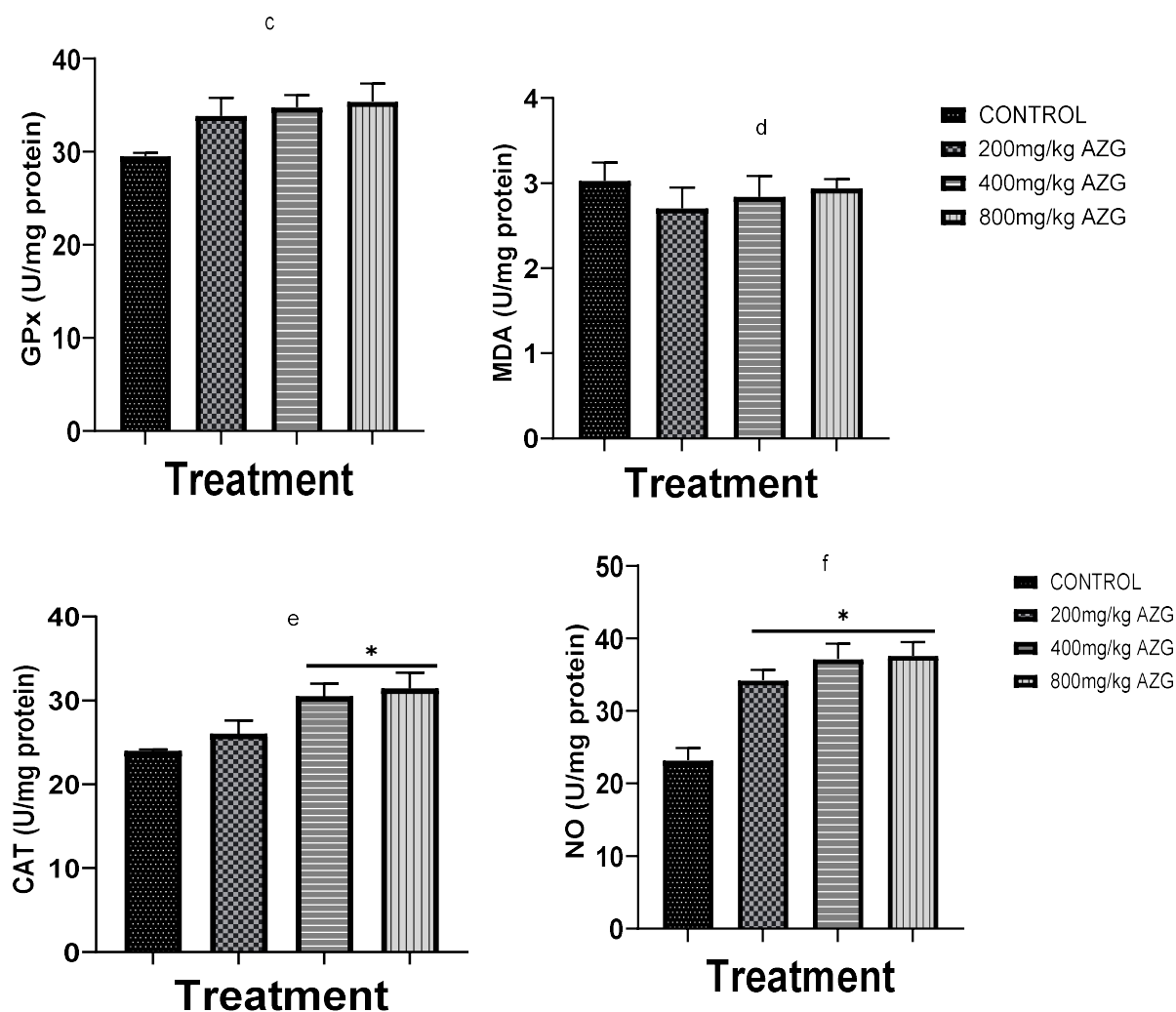
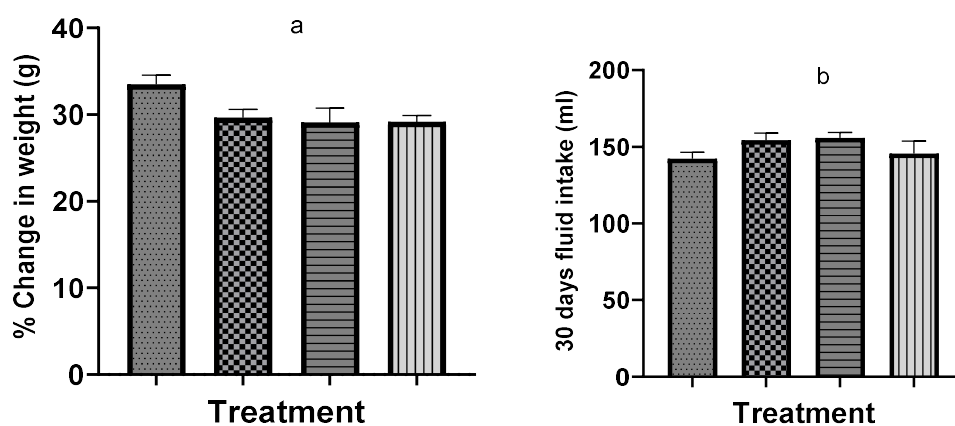


Figure 7: Effects of AZG (15 days) on heart oxidative stress markers. (a) SOD, (b) GSH, (c) GPx, (d) MDA, (e) CAT, (f) NO. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.8 The effect of *Azanza garckeana* hydroethanolic extract on Weight, Food, and Fluid Intake (30 Days)

No changes in weight or fluid intake ($p > 0.05$; Fig. 8a-b). Food intake decreased at 400 and 800 mg/kg ($p < 0.05$; Fig. 8c).



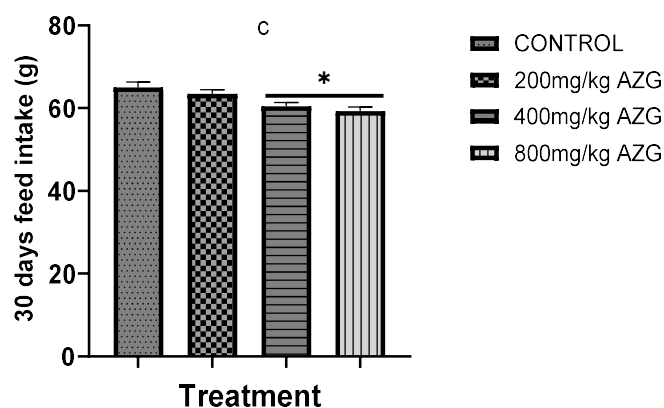
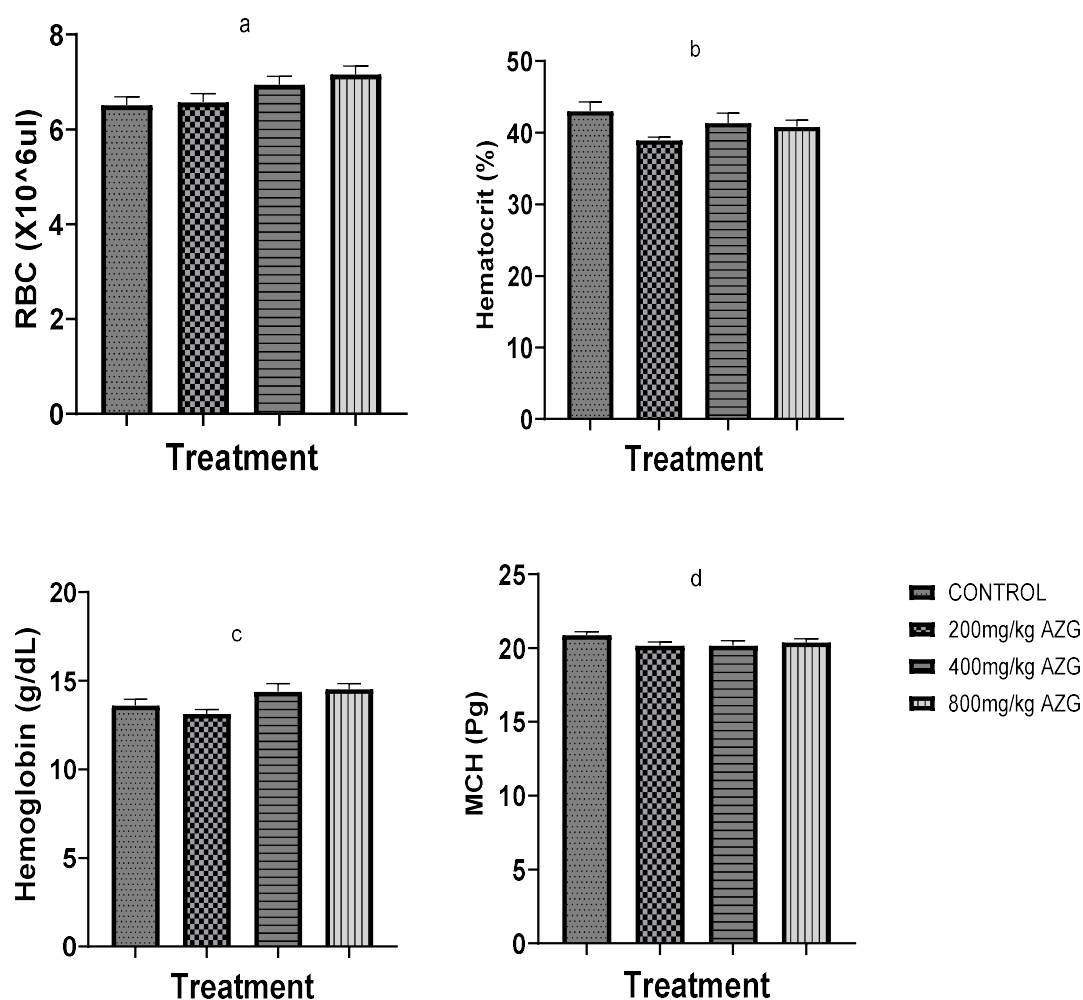
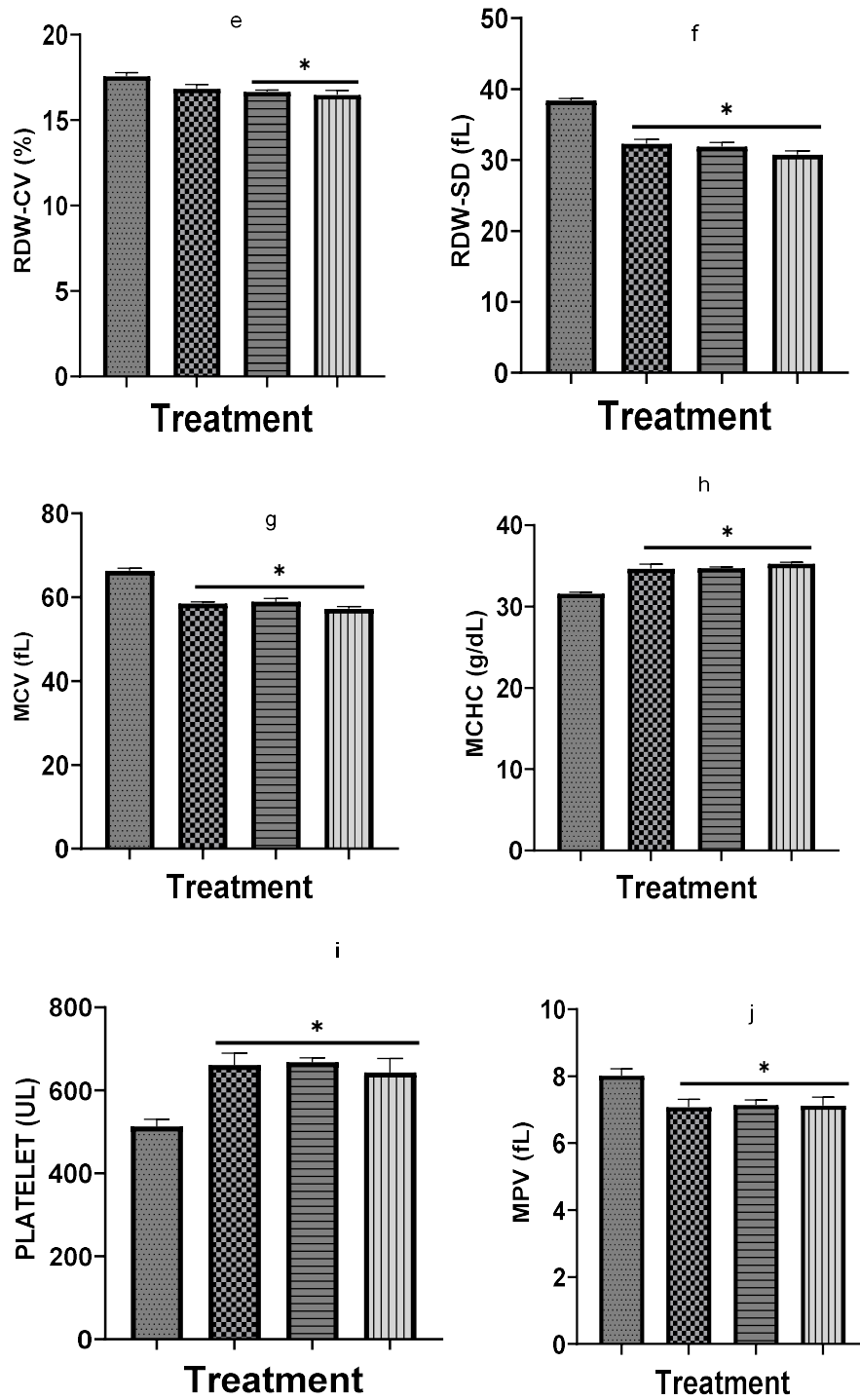


Figure 8: Effects of AZG (30 days) on (a) weight changes, (b) fluid intake, (c) food intake. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.9 The effect of *Azanza garckeana* hydroethanolic extract on Hematological Parameters (30 Days)

No significant changes in RBC, HCT, Hb, MCH ($p > 0.05$; Fig. 9a-d). Significant modulations in other parameters (e.g., decreased RDW-CV/SD, increased MCHC, platelet count/platelet crit; decreased MPV/PDW) at various doses ($p < 0.05$; Fig. 9e-l), suggesting no pathological effects.





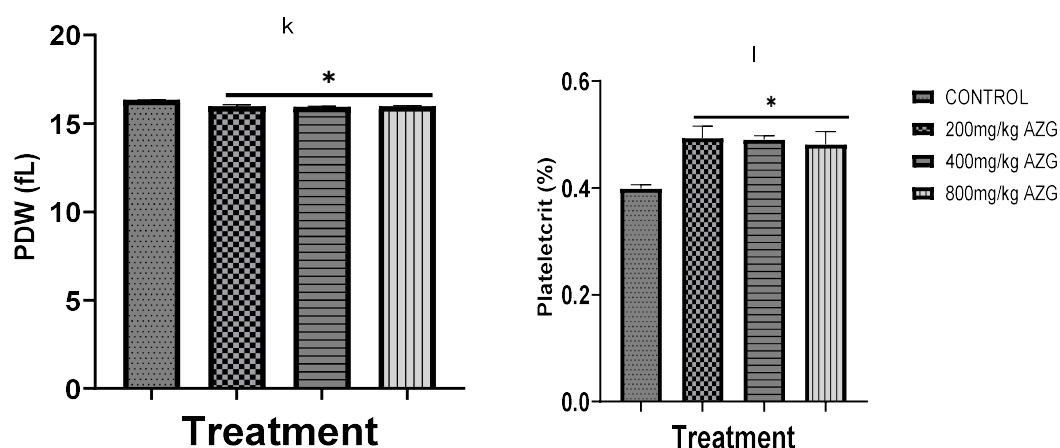
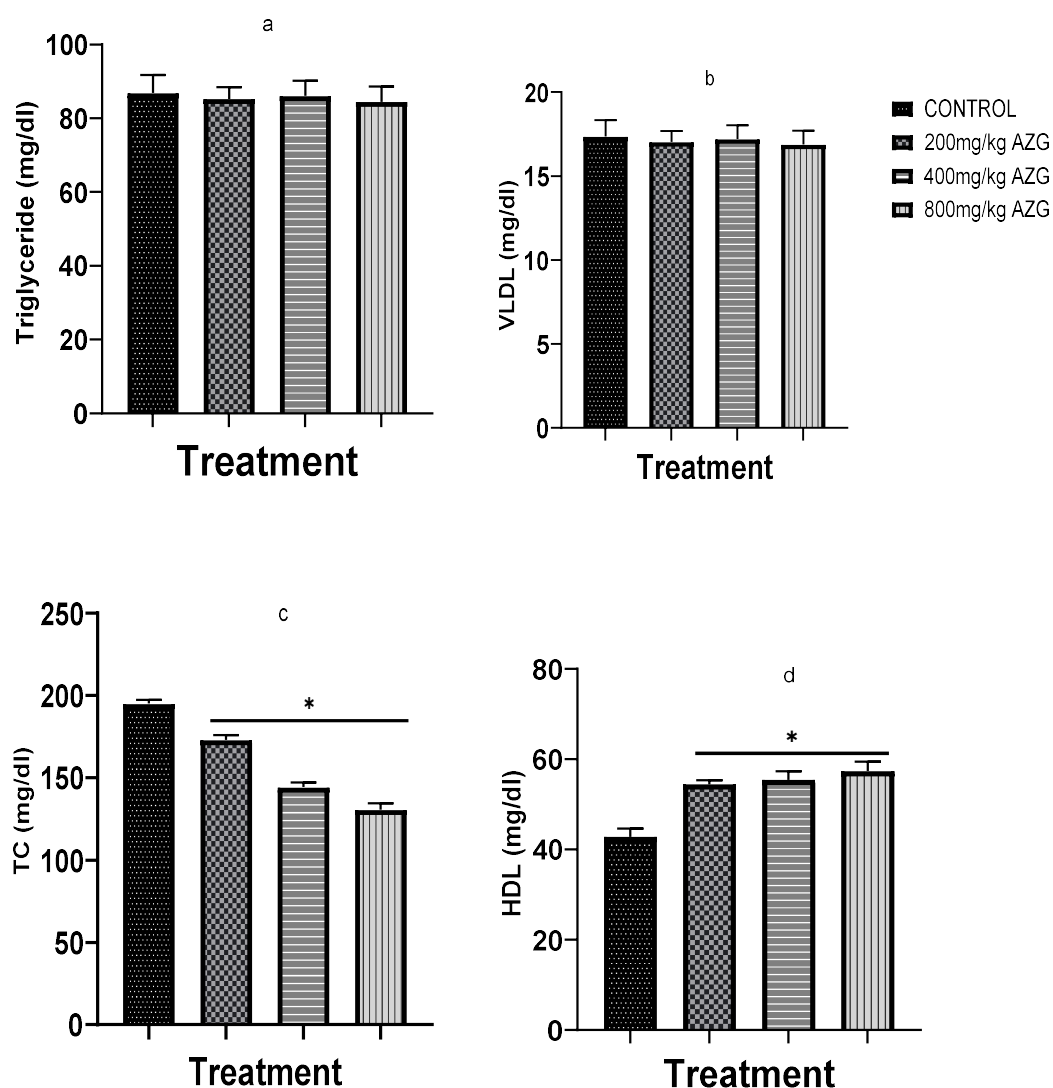


Figure 9: Effects of AZG (30 days) on hematological parameters. (a) RBC, (b) HCT, (c) Hb, (d) MCH, (e-l) other indices. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.10 The effect of Azanza garckeana hydroethanolic extract on Lipid Profile (30 Days)

No changes in triglycerides or VLDL ($p > 0.05$; Fig. 10a-b). Total cholesterol and LDL decreased; HDL increased at various doses ($p < 0.05$; Fig. 10c-e).



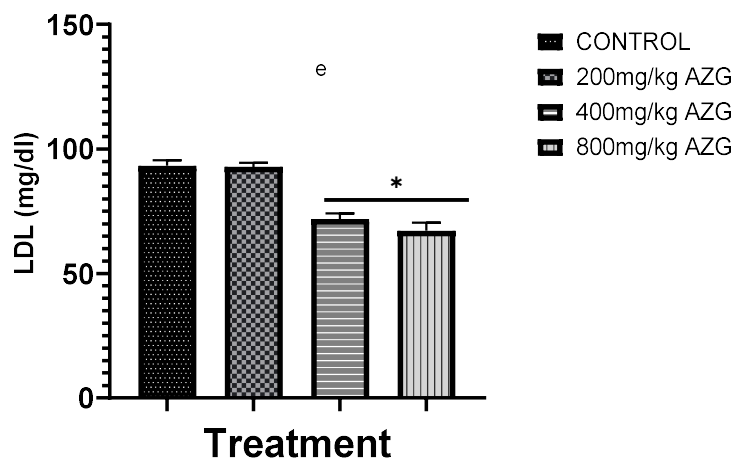
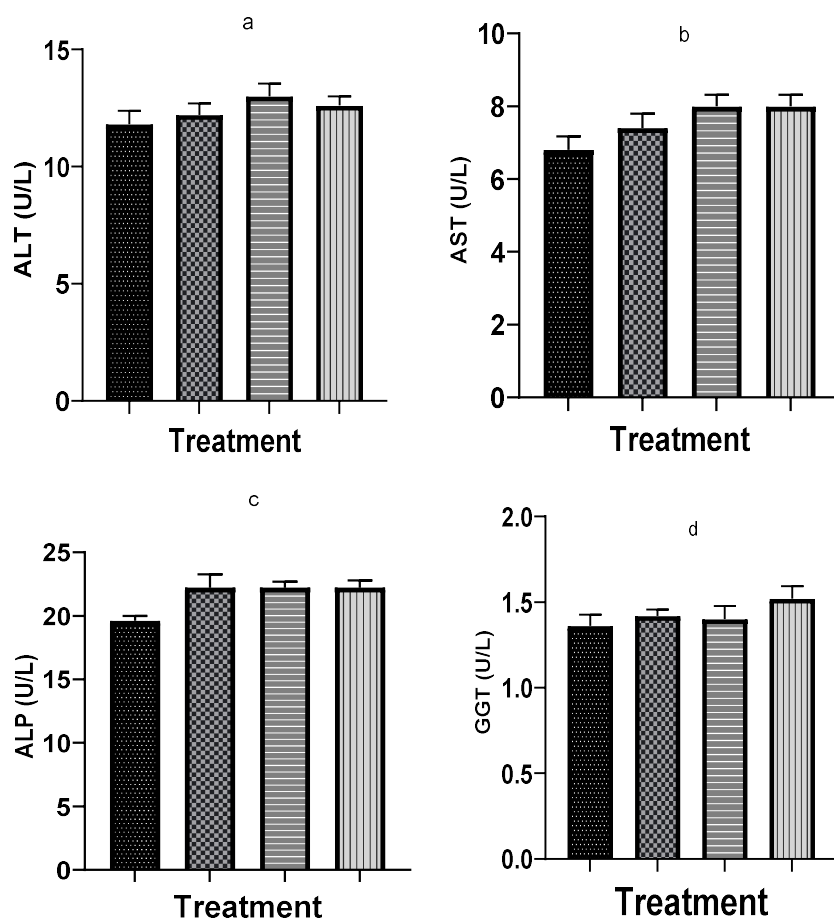


Figure 10: Effects of AZG (30 days) on lipid profile. (a) Triglycerides, (b) VLDL, (c) total cholesterol, (d) HDL, (e) LDL. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

3.11 The effect of *Azanza garckeana* hydroethanolic extract on Liver Function (30 Days)

No significant changes in ALT, AST, ALP, GGT, total protein, total/direct bilirubin ($p > 0.05$; Fig. 11a-g).



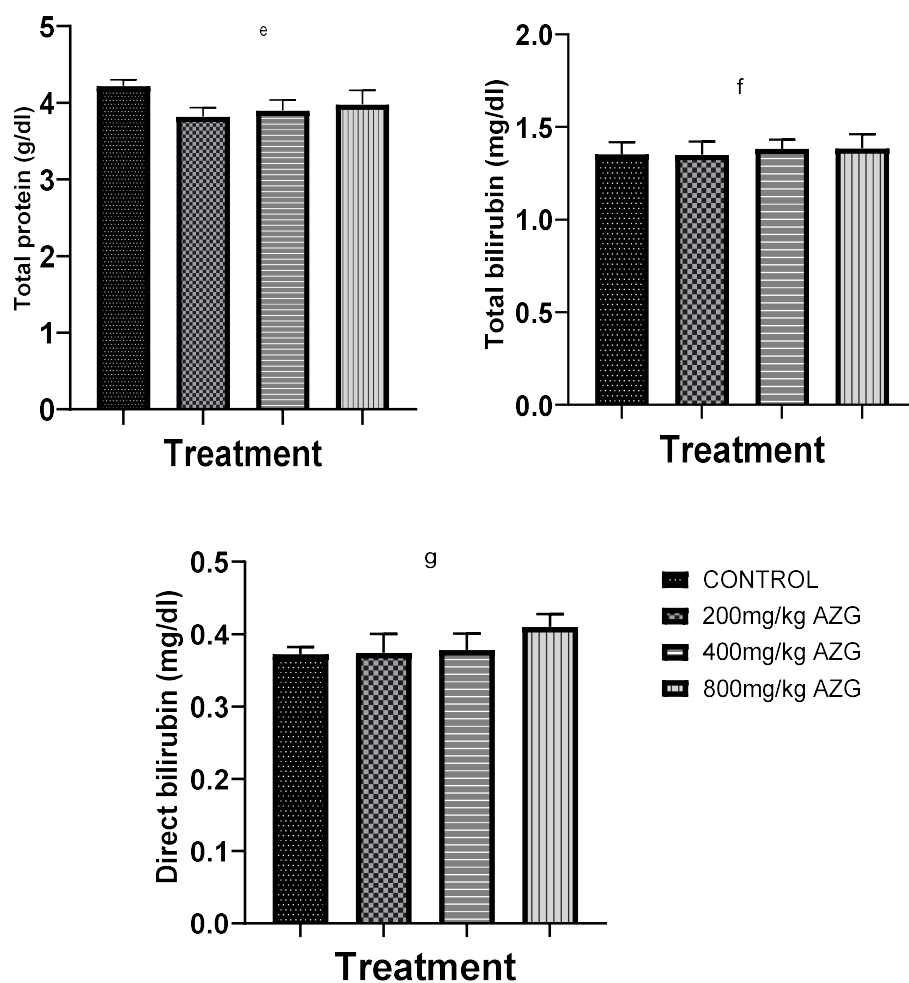
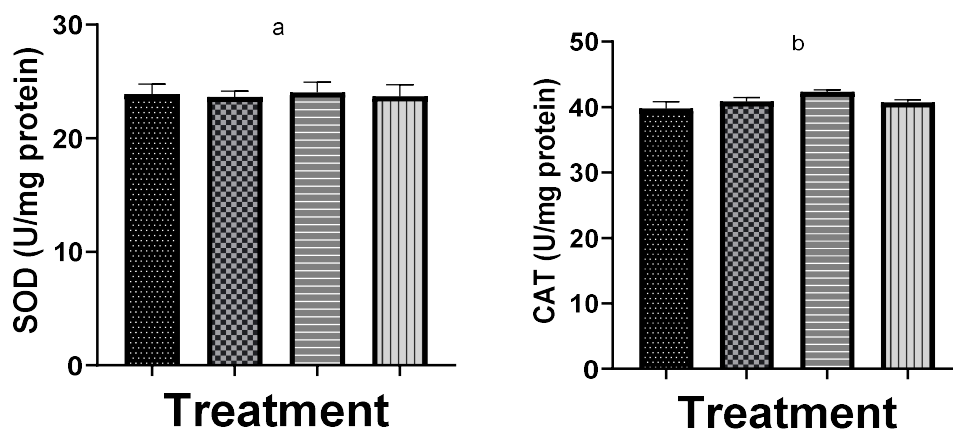


Figure 11: Effects of AZG (30 days) on liver function parameters. (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) total protein, (f) total bilirubin, (g) direct bilirubin.

Values are mean $\pm$ SEM (n = 5). No significant differences ($p > 0.05$).

3.12 The effect of *Azanza garckeana* hydroethanolic extract on Liver Oxidative Stress (30 Days)

No significant changes in SOD, CAT, GSH, GPx, NO, MDA ($p > 0.05$; Fig. 12a-f).



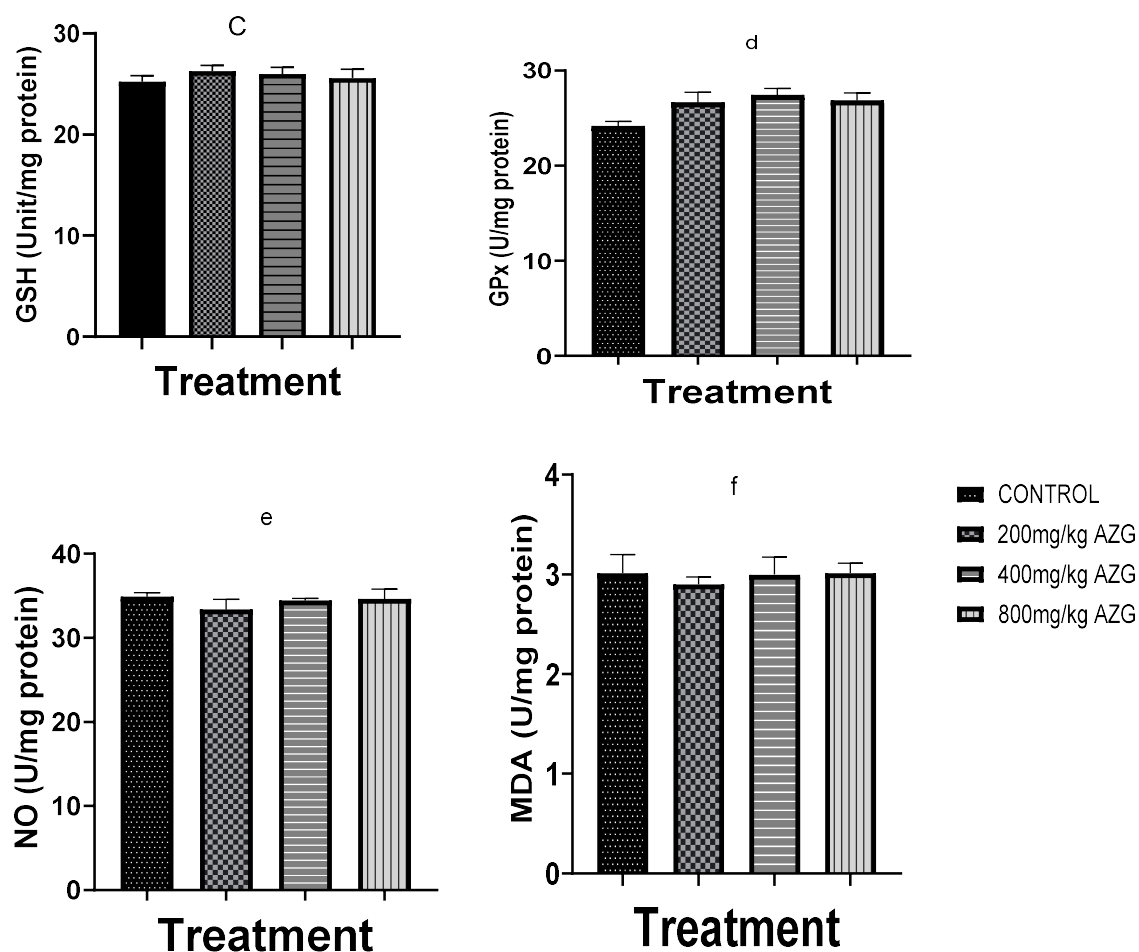
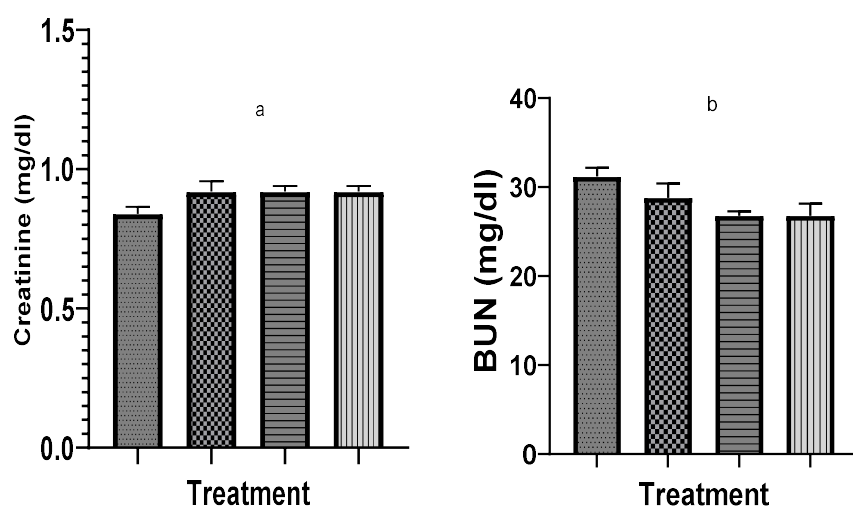


Figure 12: Effects of AZG (30 days) on liver oxidative stress markers. (a) SOD, (b) CAT, (c) GSH, (d) GPx, (e) NO, (f) MDA. Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$).

3.13 The effect of *Azanza garckeana* hydroethanolic extract on Kidney Function (30 Days)

No significant changes in creatinine, BUN, bicarbonate, phosphate, calcium, potassium, sodium ($p > 0.05$; Fig. 13a-g).



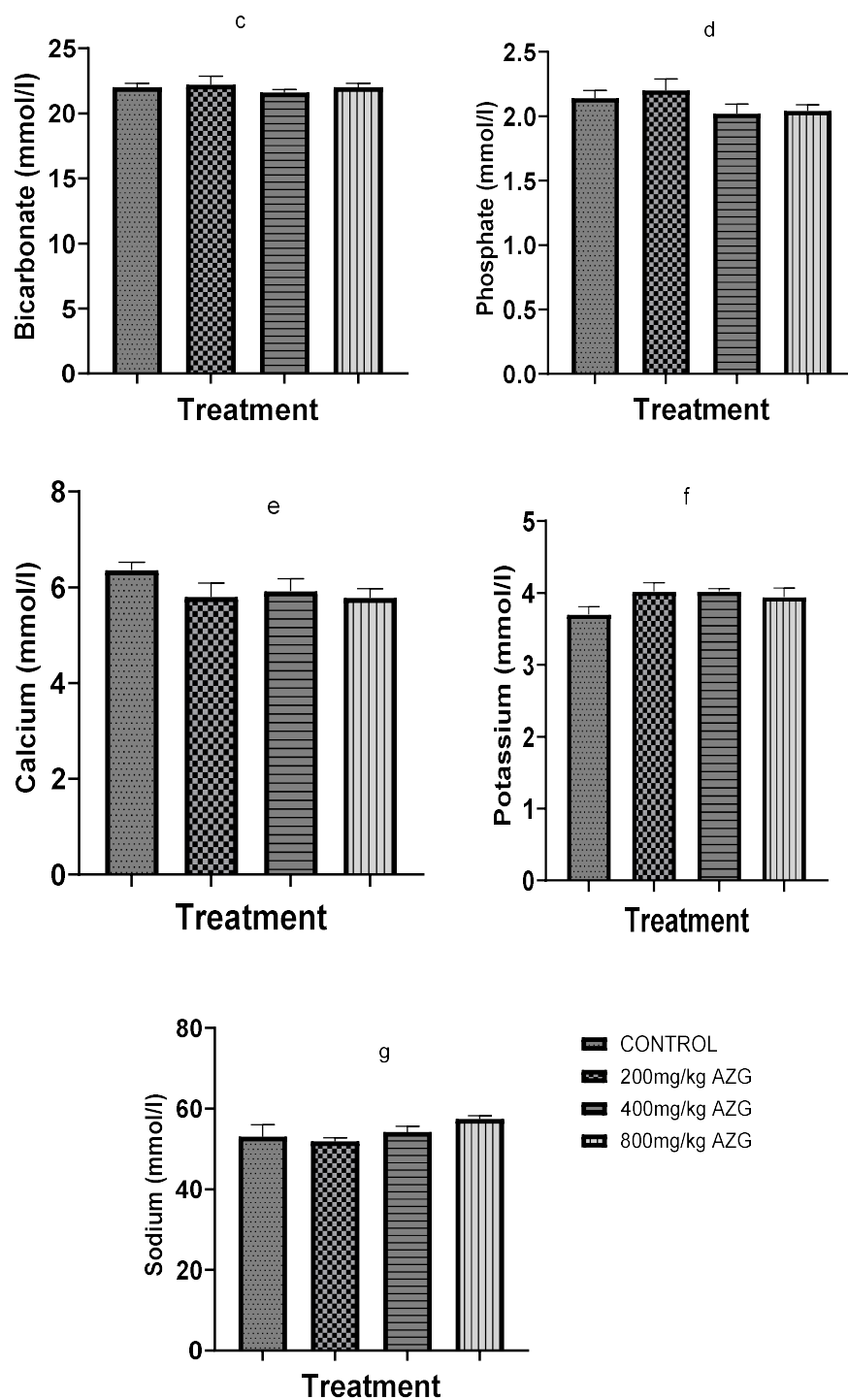


Figure 13: Effects of AZG (30 days) on kidney function parameters. (a) Creatinine, (b) BUN, (c) bicarbonate, (d) phosphate, (e) calcium, (f) potassium, (g) sodium.

Values are mean $\pm$ SEM ($n = 5$). No significant differences ($p > 0.05$).

3.14 The effect of *Azanza garckeana* hydroethanolic extract on Kidney Oxidative Stress (30 Days)

No significant changes in SOD, CAT, GSH, GPx, NO, MDA ($p > 0.05$; Fig. 14a-f).

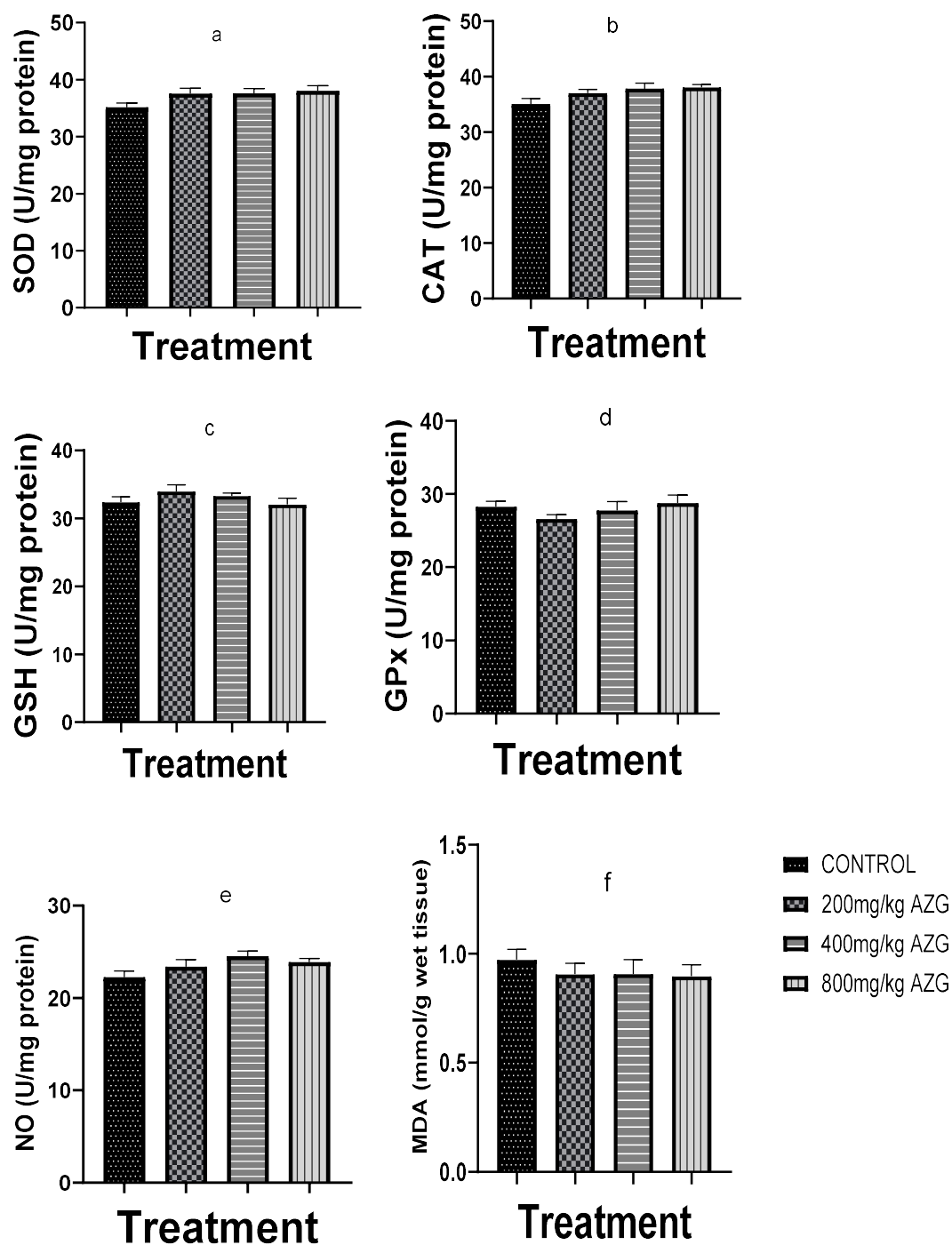


Figure 14: Effects of AZG (30 days) on kidney oxidative stress markers. (a) SOD, (b) CAT, (c) GSH, (d) GPx, (e) NO, (f) MDA. Values are mean $\pm$ SEM (n = 5). No significant differences ($p > 0.05$).

3.14 The effect of Azanza garckeana hydroethanolic extract on Heart Oxidative Stress (30 Days)

No changes in SOD, GSH, GPx, NO, MDA ($p > 0.05$; Fig. 15a-e). CAT increased at 800 mg/kg ($p < 0.05$; Fig. 15f).

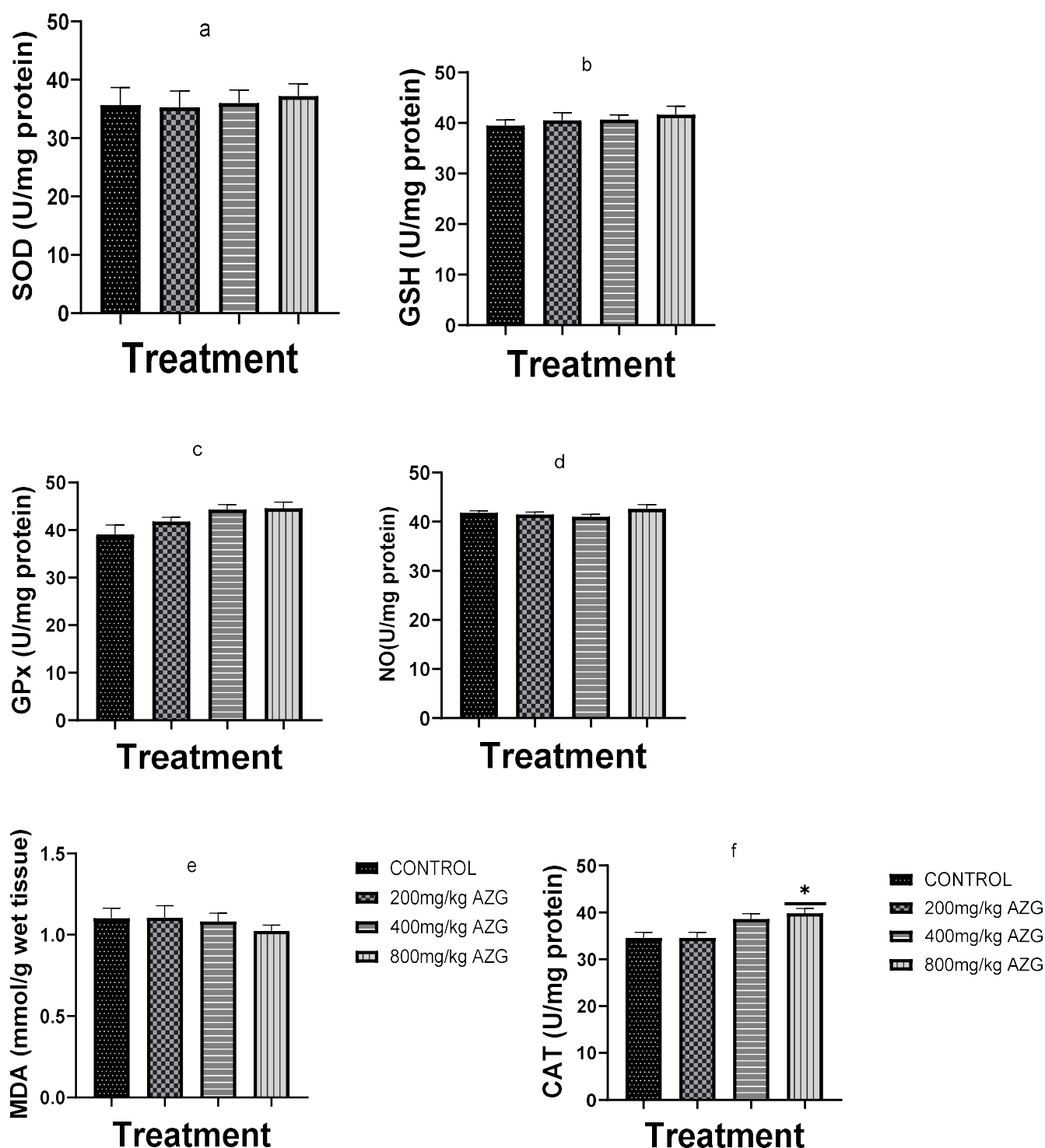


Figure 15: Effects of AZG (30 days) on heart oxidative stress markers. (a) SOD, (b) GSH, (c) GPx, (d) NO, (e) MDA, (f) CAT. Values are mean $\pm$ SEM (n = 5). No significant differences ($p > 0.05$) except where * $p < 0.05$ vs. control.

4. Discussion

The use of complementary and alternative medicine (CAM), particularly herbal preparations, is widespread globally and should not be underestimated, as many have contributed significantly to reducing the incidence of various diseases [21,22]. However, serious adverse reactions and organ toxicities associated with certain herbal remedies have been increasingly reported [23]. Comprehensive toxicological studies are therefore essential to identify the potential hazards of commonly used medicinal plants [24]. Such evidence will enable regulatory authorities and policymakers to provide accurate, evidence-based guidance to the public, facilitate the standardization of herbal products, and support the development of safe and effective herbal-derived drugs. Accordingly,

the present study evaluated acute and sub-acute toxicity profile of the AZG for 15 and 30 days in male Wistar rats at doses of 200, 400, and 800 mg/kg. The oral LD₅₀ of AZG was estimated to be greater than 5000 mg/kg in Wistar rats. Consequently, *Azanza garckeana* can be considered relatively safe with respect to acute oral toxicity as plant extract or compound with LD₅₀ greater than 2000mg/kg suggesting a wide margin of safety [25].

After 15 days of administration, AZG produced minimal changes in hematological indices across all dose ranges (200, 400, and 800 mg/kg/day). Most erythrocyte parameters (RBC, RDW-CV, and RDW-SD), red cell indices (hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), and platelet parameters remained comparable to those of the vehicle-treated control, indicating no adverse effects on erythropoiesis or thrombopoiesis. These findings suggest that AZG does not adversely alter erythrocyte morphology or size distribution. Since anisocytosis is primarily determined by an increase in red blood cell distribution width (RDW), specifically both RDW-CV (coefficient of variation) and RDW-SD (standard deviation), which both reflect variation in erythrocyte volume [26]. Megaloblastic anemias (e.g., due to vitamin B₁₂ or folate deficiency, which typically present with macrocytes, elevated MCV, and increased RDW) and iron deficiency anemia (characterized by microcytic hypochromic erythrocytes, low RBC count, reduced hemoglobin, elevated RDW, and marked anisocytosis) were not seen in this present study. This observation agrees with a study on nutritionally induced iron deficiency in Wistar albino rats by Ahmed *et al.* (2016), which confirmed that oral administration of *Azanza garckeana* aqueous fruit extract at a dose of 2 g/kg body weight over three weeks caused no significant changes in key hematological indices, including hemoglobin levels, MCV, or mean corpuscular hemoglobin (MCH), when compared to untreated iron-deficient rat controls [27].

In contrast, after 30 days, more pronounced but still non-adverse changes were observed, particularly in erythrocyte distribution indices (decreased RDW-CV and RDW-SD) and an increase in mean corpuscular hemoglobin concentration (MCHC) across all doses. These findings indicate a mild modulation in erythrocyte homogeneity and hemoglobin packaging efficiency, possibly attributable to the phytochemicals and antioxidant content of *Azanza garckeana* [28, 29]. Platelet count and plateletcrit increased, while MPV and PDW decreased, changes that collectively suggest modulation of platelet production and uniformity without evidence of thrombocytosis or bleeding diathesis. Such hematological modulations have been documented with several African medicinal plants possessing antioxidant properties and may indicate a potential hematinic effect rather than toxicity [30, 31].

Changes in lipid profile were evident with prolonged administration. At 15 days, only a modest increase in HDL-cholesterol was observed at the highest dose (800 mg/kg), with no changes in triglycerides, total cholesterol, LDL, or VLDL. By 30 days, however, all doses significantly reduced total cholesterol, increased HDL-cholesterol, and the higher doses (400 and 800 mg/kg) reduced LDL-cholesterol. This progressive, time-dependent effect underscores a lack of acute adverse changes. The observed modulations align with reports on various solvents of *Azanza garckeana* in disease models, including attenuation of diabetes-induced dyslipidemia in rats (reduced total cholesterol, triglycerides, and LDL with elevated HDL) [32], and dietary fruit meal supplementation in rabbits (reductions in total cholesterol, triglycerides, and VLDL) [33]. The effect may be hypothesized to involve inhibition of HMG-CoA reductase, enhanced LDL-receptor expression, or bile acid sequestration, mechanisms previously described for related Malvaceae species [34, 35]. These findings suggest no toxicity in lipid metabolism.

No evidence of hepatotoxicity was observed at either time point. Liver function enzymes (ALT, AST, ALP, GGT) and bilirubin fractions remained unchanged after both 15 and 30 days, indicating preserved hepatocellular integrity and biliary function. Total protein levels were also unaffected, ruling out impaired synthetic capacity. Regarding hepatic oxidative stress markers, 15-day administration increased reduced glutathione (GSH) at 800 mg/kg without altering enzymatic antioxidants (SOD, CAT, GPx) or lipid peroxidation (MDA). After 30 days, however, all hepatic antioxidant parameters returned to control levels, suggesting an early adaptive enhancement of non-enzymatic defense that normalized with continued exposure [36]. The absence of increased MDA at any dose or time point demonstrates lack of pro-oxidant or peroxidative damage to the liver [37].

Kidney function parameters showed remarkable safety. Serum creatinine, electrolytes, and bicarbonate remained stable throughout. A significant reduction in blood urea nitrogen (BUN) was noted after 15 days (all doses) that disappeared by 30 days, possibly reflecting transient modulation

in protein catabolism or glomerular filtration rather than impairment [38]. Renal antioxidant status was enhanced rather than compromised: increased GSH (400 and 800 mg/kg), GPx (all doses), and nitric oxide (800 mg/kg) after 15 days, with complete normalization by 30 days. These transient elevations in antioxidant defenses, in the absence of elevated MDA, indicate no deleterious effect on renal tissue [39]. These observations align with findings by Itodo *et al.*, who noted protective effects of *Azanza garckeana* in models of cytotoxicity [40].

Similar to liver and kidney, heart antioxidant profile showed enhancement without evidence of oxidative injury. Increased catalase (400 and 800 mg/kg) and nitric oxide (all doses) after 15 days, with sustained catalase elevation at 800 mg/kg after 30 days, suggest improved free radical scavenging capacity in cardiac tissue. Nitric oxide elevation may also reflect preserved or improved vascular function rather than any adverse effect [41]. The observed reduction in food intake at 400 and 800 mg/kg after 30 days, without concomitant body weight loss, may reflect satiety promoting effects possibly mediated by saponins or fibers present in the extract, as reported for several plants [42]. The absence of weight loss despite reduced caloric intake further supports lack of systemic toxicity.

5. Conclusion

The sub-acute toxicity study of AZG for up to 30 days at doses as high as 800 mg/kg produced no frank toxicity in male Wistar rats. The extract demonstrated a favorable safety profile, with observed changes indicating absence of adverse effects on hematological, biochemical, and oxidative stress indices. These findings support the relative safety of AZG and suggest potential hypolipidemic, antioxidant, and hematological modulatory benefits supporting its continued traditional use. The no-observed-adverse-effect level (NOAEL) under the present experimental conditions was 800 mg/kg/day. Potential modulations in lipid parameters, antioxidant defenses, and hematological indices warrant further investigation.

Declarations

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Conflict of interest: The authors declare that they have no conflicts of interest.

Funding: This study was self-funded

Contribution of authors: AAO, OMO and EAT designed the experimental protocol, participated in the execution of the experiments, and were involved in writing and editing the manuscript. ACO analyzed the data, assisted in conducting the experiments throughout the study, and also participated in editing the manuscript. All authors read and approved the final version of the manuscript.

Ethical approval: All experimental procedures were approved by the Research and Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka (Approval No: RBC/FBMC/DELSU/25/971).

Availability of data and materials: Upon request, the corresponding author will provide the data sets created and/or analyzed during the current study.

Use of artificial intelligence (AI): The authors used (Grammarly AI) solely to improve language, grammar, and readability of the text. No AI tool was used for any other aspect of this manuscript. All authors have reviewed and take full responsibility for the final content.

Use of research reporting tools: No specific research reporting guidelines were applied

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Review Article

The Role of Pharmacy and Pharmacology in Hospital Management and Healthcare Administration: A Mini Review

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Abstract:

Background: Pharmacy and pharmacology are integral to modern healthcare systems, influencing clinical outcomes, patient safety, medicines management, and institutional efficiency. Their roles extend beyond traditional functions, contributing significantly to hospital administration, policy development, and rational pharmacotherapy. Despite this evolving scope, the strategic importance of these disciplines in healthcare management remains underemphasized, particularly in developing health systems.

Methods: This mini-review is based on secondary data obtained from peer-reviewed literature accessed through databases including PubMed, ScienceDirect, Google Scholar, ResearchGate, and HINARI. Relevant studies addressing pharmacy practice, pharmacology, healthcare administration, and interprofessional collaboration were critically evaluated.

Key Findings: Evidence indicates that pharmacy practice has transitioned from a product-centered model to a patient-oriented clinical role involving medicines management, pharmacovigilance, patient counseling, and medication therapy management. Pharmacology contributes through drug development, rational therapeutics, safety evaluation, and mechanistic insights that inform clinical decision-making. Both disciplines play essential roles in optimizing healthcare delivery, resource utilization, and patient safety frameworks.

Conclusion: The participation of pharmacy and pharmacology plays a critical role in strengthening healthcare systems through improved medicines management, patient safety, and rational pharmacotherapy.

Keywords: Pharmacy, Pharmacology, Healthcare Administration, Hospital Management, Patient Safety

Introduction

Hospital management and administration are pivotal components of healthcare that helps to coordinate an integration of medical professionalism, essential healthcare supplies and definitive attention [1]. This improves the quality of life of patients by effective cooperation by various department of the medical care system [2]. The placement and utilization of resources at its size in an

organization, including hospitals, it is necessary to consider resource sustainability as a strategic approach to improving performance as one of these resources is a drug store such as a pharmacy that requires technological support in managing it [3]. Though pharmacies responsibilities is high due to the demand for drugs support, how to provide a number of these drugs to the right patient with proper precision of beneficence [4]. This could be jeopardized due to high healthcare demand especially in public medical facilities. The appropriateness of efficiency of pharmacy and other medical professional become effective with involvement of a precise knowledge of pharmacology [5].

Pharmacy service is an essential part of a healthcare system as the profession is well recognized, placed and practiced to its full potentials in developed countries. It has improved from a product focused practice to a patient-oriented care [6]. They have a primary duty of providing drug information, medicines management, preparation and dispensing of medicines, counseling of patients, and formulating pharmaceutical care plan for patients. Pharmaceutical care plan is an individualized service provided to improve the quality of patient's health. Collaborative relationship between the pharmacist and the physician to improve the health status of patients is visible through shared pharmacological knowledge. Pharmacologist involvement in clinical drug discovery and development in emerging disease management helps to synergize administrative roles of pharmacist in traditional roles of pill counting, packaging, and dispensing [7]. The condition is different in developing countries as the method of pharmaceutical care is derailed due to less access to patients' records, time constraints, lack of standard reimbursement, poor communication among healthcare professionals, insufficient number of qualified pharmacists, trained pharmacologists and absence of policies adherence and involvement [8].

There is a need to enhance collaborative relationship between pharmacist and pharmacologist to solve medication problems and health related problems as it will foster standardized quality of medication for precise treatment [9]. Treatment progression is recognized with an effective documentation system. This study helps to define the role of pharmacology and pharmacy in building effective healthcare delivery system through fascinating patient oriented clinical care. This could be actualized by healthcare practice to ensure maximally that there is a clinical setting work force synergism among health workers by pharmacological knowledge and standards [10].

Materials and Methods

This mini-review was conducted using secondary data derived from peer-reviewed literature. Over 100 articles were screened over a six-week period, of which 60 relevant studies were selected based on scope, relevance, and availability of full texts. Literature searches were performed using databases including PubMed, Google Scholar, ScienceDirect, ResearchGate, and Elsevier. Search terms included: *Pharmacy, Pharmacology, Healthcare Administration, Hospital Management, and Pharmaceutical Care*. Studies outside the thematic focus or lacking accessible full texts were excluded.

Hospital Management and Administration

The healthcare sector plays a crucial role in prioritizing wellbeing seen in service delivery by healthcare providers through defining of pathological condition and medical-surgical interventions [11]. Medical specialization through professional handling is capacitated through financial subsidization to efficiently deliver treatment cost that is handled by patients. Management of healthcare sector has an expanded horizon from the traditional staffing capacity through thorough medical supply chain and ensuring optimal utilization of the pharmaceuticals [12]. Technicalities of pharmacist and pharmacologist from the primary to tertiary levels of health institutions engage public satisfaction via modern trends of facilitating communication in telemedicine and social media especially in developing countries [13]. To actualize these administrative management strategies it involves careful rendering of partnership to ensure availabilities of supplies within the core ethics of pharmaceutical responsibilities. This will aid attending to healthcare priorities and emerging disease surveillance with complicated pathophysiology from the point of emergencies to medical satisfactory discharge [14].

Effectively, healthcare calls for more careful attention as it ensures that important micro to macro part of patient oriented care and wellness are attended to while also focusing on harnessing staff strength to meet up with better outcome. The availabilities of pharmaceutical supplies for daily medical attention of patients both in and out, as first emergency contact involves the use of these

pharmaceutical inductions by doctors and nurses with a knowledgeable pharmacological approach [15]. Optimizing efficiency in healthcare delivery by healthcare providers in the best financial status of healthcare seekers helps to maintain stable financial records and continuity of health institutions in meeting with dwindling cost of purchase of pharmaceutical [16]. Medical care is wellness outcome through quality attention strategies and emergence enhancing the availability of specialized skills professional with maximum remuneration of their services to facilitate smooth running of the system [17].

Evolving Role of Pharmacy and Pharmacology

The concept of pharmaceutical care has been advanced from the drug centeredness to meeting a goal of patient centered oriented care which bridges the communication gap of effective healthcare [18]. It also engages identifying drug related therapy problems and necessitating effective outcome of clinician taking responsibility through effective patient's medical records, comorbid conditions history and synergism for medication therapy management. More effective approach to enhance the administration of pharmaceutical care has enabled a sequential feedback mechanism system emancipated by preformation of definitive therapeutics [19]. Proper diagnosis and how to effectively adhere to prescribed medication outline for maximum recovery which is being built up especially in developing nations where there seems to be a gap. The advancement of knowledge disposal through the aid of artificial intelligence broadens assessment of wide range of emerging disease conditions [20]. The invention of new drug discovery and development through computational pharmacology has quickened physicians to extend confidence and uniqueness of medicines, brand overviews and superiority especially in hospital environment [21]. Enabling pharmacologists to scientifically propose best pharmacotherapy measures to enhance proper treatment outcome and minimizing possible adverse drug reactions [22]. With the ever increasing healthcare needs, pharmacist effective communication of medicines usage due to shortage of staffing capacity especially in public healthcare facilities with high teeming numbers of patients visiting medical facilities will enhance treatment [23]. Hence, the need to actively engage pharmacist to collaborate with other healthcare providers towards providing appropriate medication to the right patient with most deserving compliance with mutual respect, ethics and professionalism [24].

Pharmacology helps in proper medication usage through effective selection, choice of use, effective delivery system, maximizing safety and efficacy on the patient health [25]. This is ensured by ascertaining standardized drug procurement, storage, dispensing, and administration forming the basis for ethical clinical decision making by clinicians for pharmacotherapy patient care. The different roles of pharmacologist and pharmacist involves rational drug usage through well-defined clinical decisions and avoiding drug misuse and abuse with setting the boundaries for safety, efficacy and optimizing outcomes [26]. The absorption, distribution, metabolism, excretion, receptor binding and receptor-like drug action which form the basis for pharmacokinetics and pharmacodynamics helps minimize harmful drug reaction and unnecessary poly-pharmacy [27]. Pharmacologist helps ensure proper procurement plan for quality medicines usage through justifiable inventory and monitoring appropriate drug dispensing and medication error management by awakening patients [28].

The use of medicines has been broadened by the introduction of integrative and complementary medicines combining co-administration of traditional remedies with orthodox medicines [29]. Most synthetic drugs do have specific potency, efficacy and receptor binding affinity determination unlike most remedies that are used especially in developing countries though has been practice over hundreds of years before the advancement of synthetic drug formulation. Setback of traditional medicines has been tagged to poor research findings about plant identification, preparation, compounding pattern, administration and monitoring usage [30]. Showing potency but its specific receptor drug binding like action has low quantification with less promotion but highly used in developing countries [31]. Natural medicines are often perceived to be safer with broader range of remedy coverage have poor preparation based on pharmaceutical and industrial approved standards [33]. However, synthetic drug usage also have more adverse drug reaction reports especially in advanced countries when compared to natural medicines though pharmacologist has played more scientific rebranding role in employing food and drug administration prerequisites [32].

Comparison of Hospital and Community Pharmacy Roles

Different pharmacy dispositions play a helpful path in healthcare depending whether it is a hospital or community based pharmacy. The community pharmacy known as chemist shop traditionally has dominated over the years due to their usefulness to societal wellness and health values

[33]. Its prominence in effective administration of medication advice, health information, proper patient assessment, counseling, observation of drug interaction, supervision of stocks and procurement has brought about pharmacotherapy and health promotion [34]. Collaboration with a trained pharmacologist enhances patient safety vaccination advice, guidance to medication and duration of treatment, awareness of emerging diseases, precision medicine and advocacy, over the counter medicine management and subscription to alternative remedies [35]. Pharmacologist in hospital settings enables medicine dispensing with optimal collaboration with other healthcare providers in clinical trials and aiding personalized medicine for individuals [36]. They engage in medical procedures of suitable procurement, stocking and adequate shelving for half-life sustainability, educational medical counseling, evaluating benefits that outweighs risk [37]. Though proper medication history scrutiny and medication reviews by pharmacist in ward rounds to ensure proper medication profiling and safety suffers limitations in developing countries [38].

Pharmacy and Pharmacology in Management

Healthcare involves effective leadership qualities and equity with an optimal goal for the betterment of the patient's wholeness through resolving devastating health challenges by efficient teamwork [39]. Enriching patient's satisfaction by mitigating possible challenges following code of ethical conduct and jurisdiction, technological drug screening is carried out based on pharmacological investigations [40]. The role of a pharmacist and pharmacologist could be embedded in directions of management which comprises of clinical, administrative and executive. Clinical system helps to maximize inter professional synergy in meeting patient's needs, administrative focuses on orderly plans in motivating staffing via remunerations rewards, while executive establishes goals that could transcend overtime in promoting good successive structures [41]. The impacts of health services management are diversified yet achievable with tremendous outcomes through good influence of good governance and organized political interference [42]. Maximizing the peculiarities of individual countries in disease tackling techniques and Pharmaceutical care should be enhance through adequate checks of medication profiling [43]. There is need to incorporate the private health sector into vital health ministry organograms to enable universal health coverage and solving other environmental factors in essential health demand [44].

The system of healthcare management ensures welfare are optimize with appropriate planning, controlling, remuneration, staffing, critical thinking by various managerial focus achieved by community engagements, initiatives and programs [45]. Determinants of healthcare involving social-cultural, personal and environmental contribute to direct and indirect impacts of leadership skills [46]. Effective health communication is realized in cordial prescriber-patient interaction as patient's priority and health needs are attained through critical diagnosis [47]. Consistent areas being consider by medical expertise on diagnosis, physical examination, treatment of disease based on scientific training and consideration of patients personal belief system has impact on health [48]. Mental, social, work life, education, marital and physical cognizance of health seekers impacts critical health decision making [49]. Incorporating empathy into care is adjudged by conscience while training pharmacist and pharmacologist in shaping the future of pharmaceuticals [50].

Scope of Pharmacy and Pharmacology in Pharmaceutical Process

Quality of medicines based on pharmaceutical standard procedures ensures products undergoes various testing and assays before getting to the patient leading to potency, efficacy and shelf life [51]. Pharmaceutical products are set on stage by various process of quality assurance through various documented procedures to bring quality to patient's health while avoiding fake products by regulatory agencies [52]. It entails risk characterization, assessment, deviation management, supplies, preventive and corrective measures to ensure standards, improve patient medical delivery and administration [53]. Quality of product visibility is due to its effects in management of disorders without reducing life expectancy [54]. Producing quality products involves planning for specifications with structured outlines, assurance of beating production acceptable standards, control measures for safety and efficacy of medicines for patients use and improvements to set excellence through best practices [57].

Challenges and Perspectives of Healthcare System

The Nigeria healthcare system is pluralistic in management strategy based on public, private and joint partnership healthcare system [58]. Private sector funding depends on substantive ownership capital base systems [59]. Public sector which the larger population percent is able to afford base on cost of pharmaceutical products and expenses of healthcare is under staff with medical personnel's, poorly financed and inadequate technological equipment for various specialties [60]. Control, ad-

ministration and management of health sector in Nigeria is regulated by the federal ministry of health through policies, formulations and established acts of institutions in federal teaching hospitals, research institutes and medical centers. While States highlights secondary healthcare services in establishment of general hospitals but also tertiary teaching hospitals while promoting primary health centers in line with local government collaboration in the campaign for fight against killer diseases such as malaria, HIV/AIDS, tuberculosis and engaging in effective vaccination programs [61]. Local government authority engages rural in effective healthcare support, attention and services through antenatal care and immunization against childhood killer diseases. Health insurance services are also run by the state government but with less patronage from the population due to low remuneration and lack of confidence in the public healthcare institutions [62].

Policy and Legal Frameworks

Individual's good health brings better productivity base on physical reflection, psychological stability, financial viability, emotional balance relief from painful events and good behavior sanity for a better life. Medical and pharmaceutical industries are modernized by effective legislature, policies, constructive governance and defined professional responsibilities through stakeholder's engagement, pharmaceutical ethics of informed consent, beneficence, non-maleficence and equity frontiers disease management and control[63]. Legal framework helps in establishment of healthcare and medical institutions to launch universal health strategies through proper provisional funding [64]. Formulating goal oriented policies, provision of pharmaceutical supplies, management of health information, networking of health institutions, risk assessments leads to standardized healthcare system [65]. Health workers and patient's integrity are guided by policies in recognition of critical health procedures, research, reproductive health matters and mental rehabilitation proficiency [66].

Health policies are also political strategies in pharmaceutical care seen in adhering to lifestyle modification therapy through avoidance of tobacco usage to reduce respiratory diseases, proper dietary in reducing diabetes [67]. Poor infrastructural development, low level of income, poor educational status are part of measures to be properly checkmated to ensure a healthy society [68]. Policies formulations depend on duration of organizational targets which influences the society by effective governance implementations and developed behavior irrespective of socio-political grounds [68]. Discretion of information sharing with other healthcare providers, benefit for the community, source of financing, health protection, registration of healthcare facilities, training and practice conduct, safety and efficacy of medicines upholds patients right in addressing communicable and non-communicable diseases [69].

Conclusion

Pharmacy and pharmacology play indispensable roles in healthcare administration and hospital management. Their integration enhances patient safety, rational pharmacotherapy, and institutional efficiency. Strengthening collaborative frameworks and policy recognition of these disciplines is essential for advancing healthcare systems.

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