



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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**Citation:** Akinsola A.O.; Ojezele M.O.; Eduviere A.T.; Akpovwre, C.O. Hematological and Biochemical Evaluation of Acute and Sub-acute Oral Toxicity of *Azanza garckeana* Hydroethanolic Fruit Extract in Male Wistar Rats. *Emerg. Front. Transl. Biomed. Health Sci.* **2025**, *1*(2): 72-96.

**Academic Editor:** B.C Nwoguzee

Received: 18<sup>th</sup> November, 2025.

Revised: 27<sup>th</sup> November, 2025.

Accepted: 10<sup>th</sup> November, 2025.

Published: 19<sup>th</sup> December, 2025.



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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 LD<sub>50</sub> 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, 2,4-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:

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

### 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

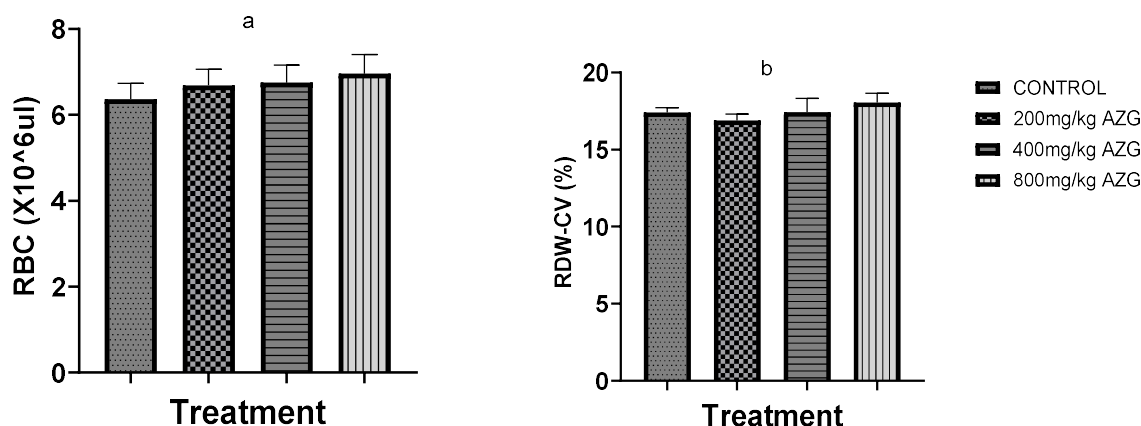
Data were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with  $p < 0.05$  considered significant. Analyses were performed using GraphPad Prism software version 10.4.2.

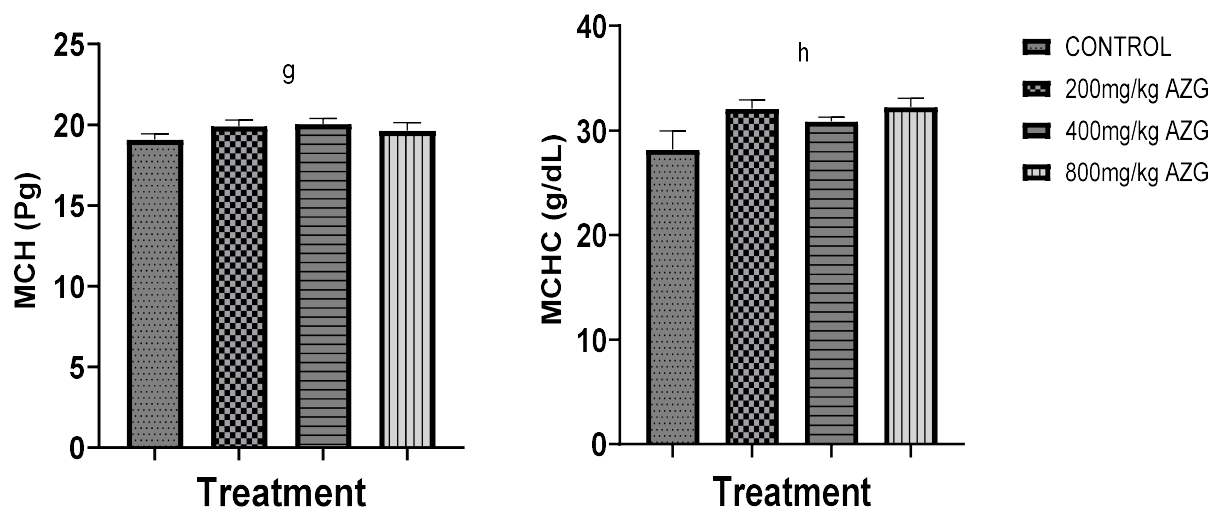
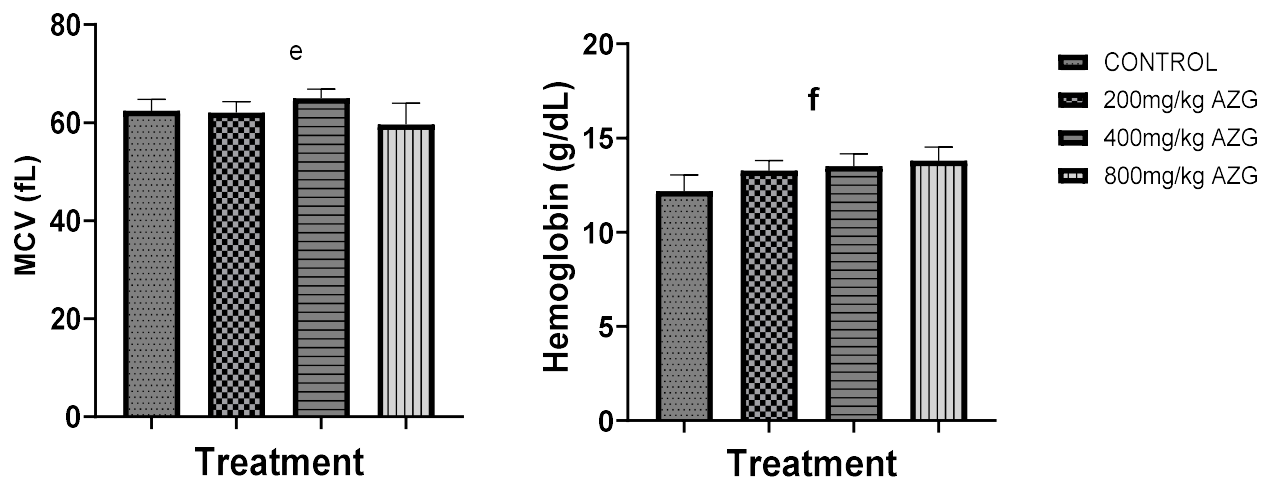
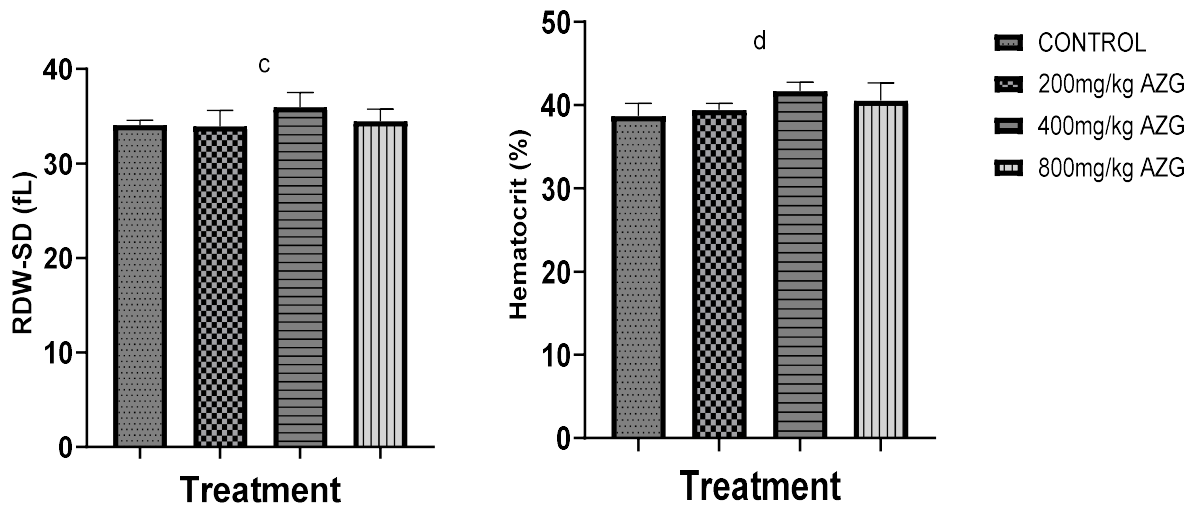
### 3. Results

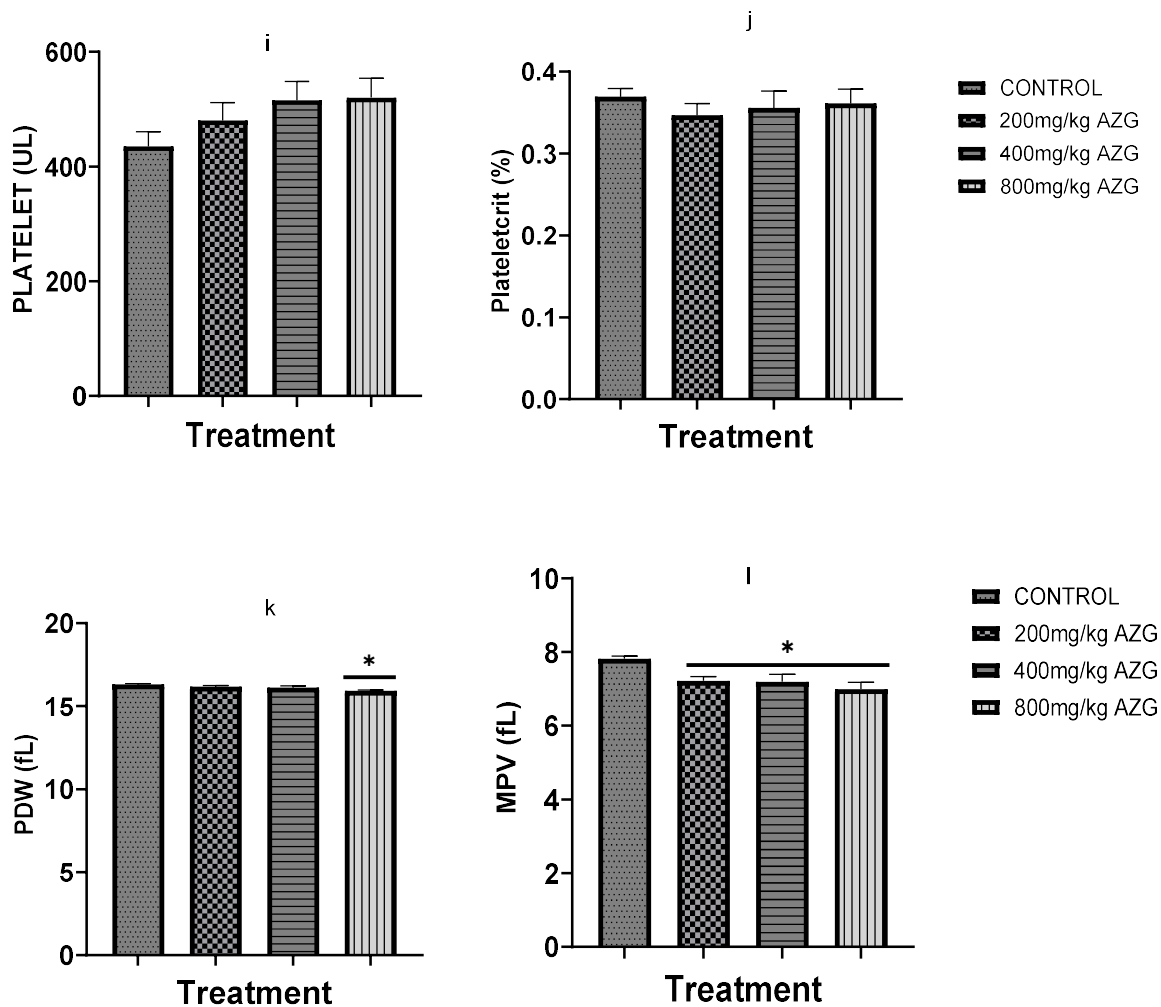
No mortality or overt signs of toxicity were observed in the acute toxicity study, the oral LD<sub>50</sub> was estimated to be greater than 5000 mg/kg, indicating low acute toxicity. In sub-acute studies ( $n = 5/\text{group}$ ), hydroethanolic fruit extract of *Azanza garckeana* administration for 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.



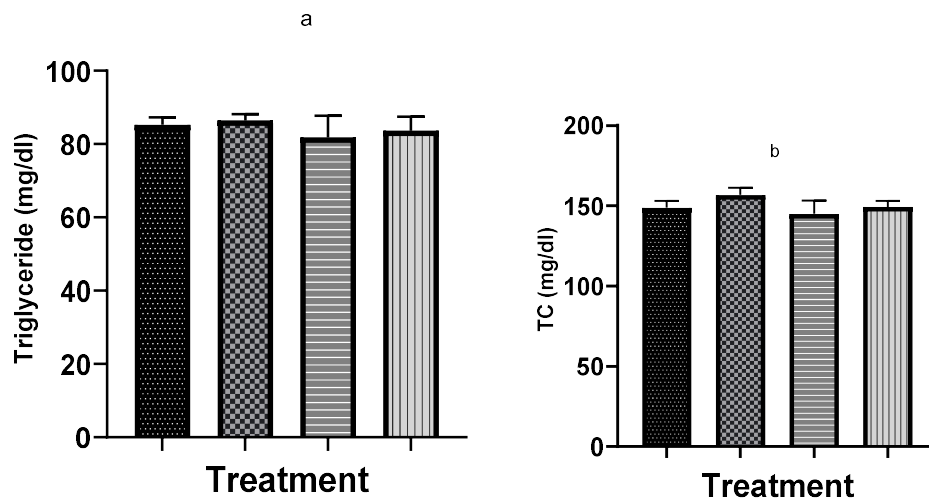




**Figure 1:** Effects of AZG (15 days) on hematological parameters in male Wistar rats. (a) RBC, (b) RDW-CV, (c) RDW-SD, (d) HCT, (e) MCV, (f) Hb, (g) MCH, (h) MCHC, (i) platelet count, (j) plateletcrit, (k) PDW, (l) MPV. Values are mean  $\pm$  SEM (n = 5). No significant differences ( $p > 0.05$ ) 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).



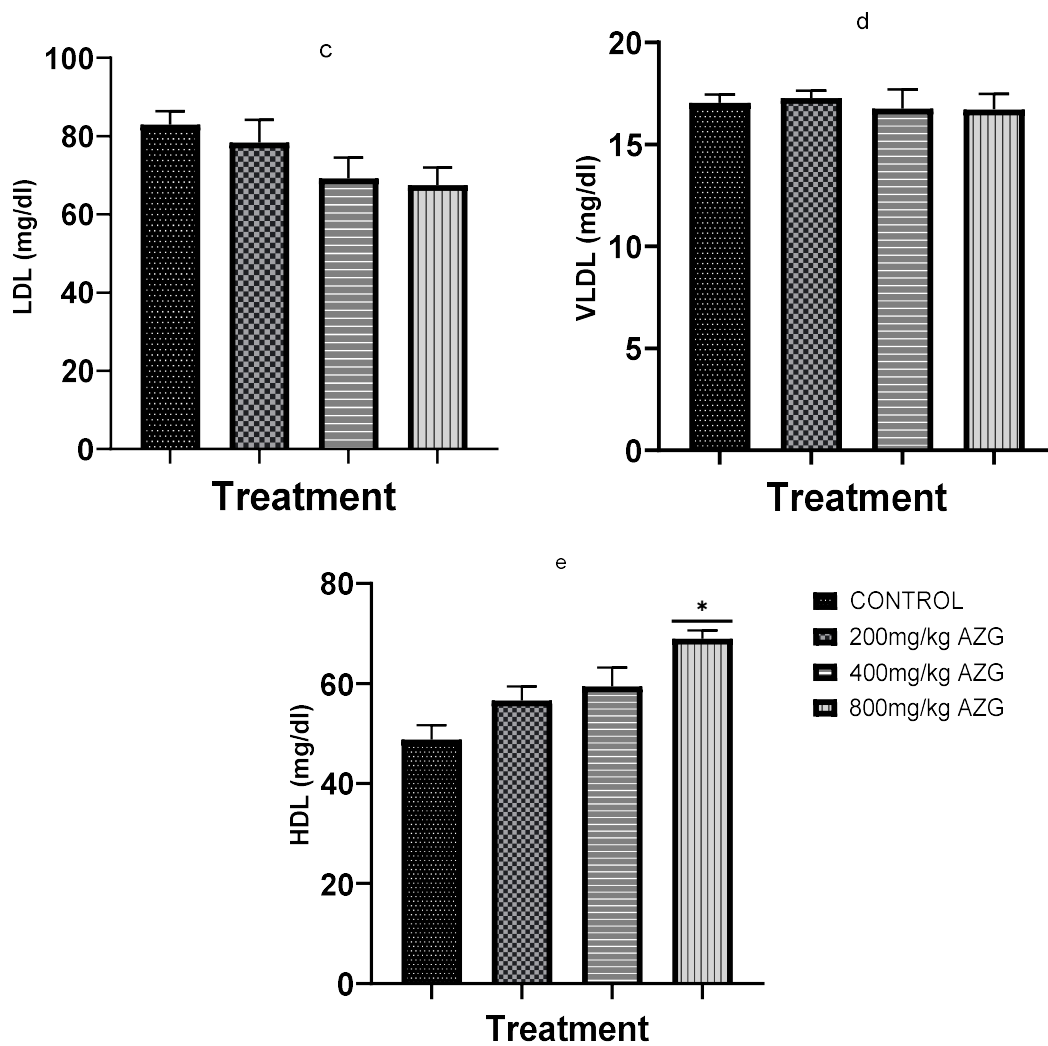
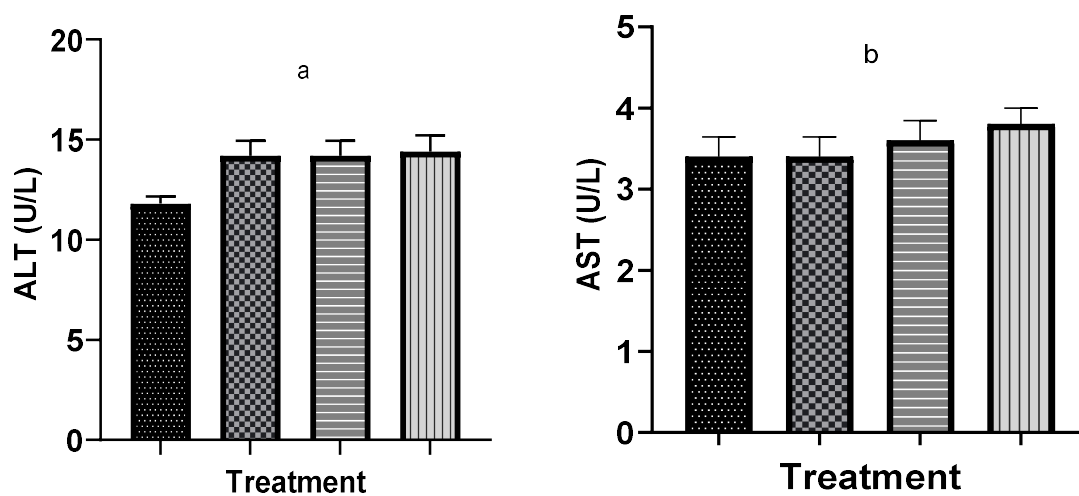


Figure 2: Effects of AZG (15 days) on lipid profile. (a) Triglycerides, (b) total cholesterol, (c) LDL, (d) VLDL, (e) HDL. Values are mean ± SEM (n = 5). No significant differences ( $p > 0.05$ ) except where  $*p < 0.05$  vs. control.

### 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).



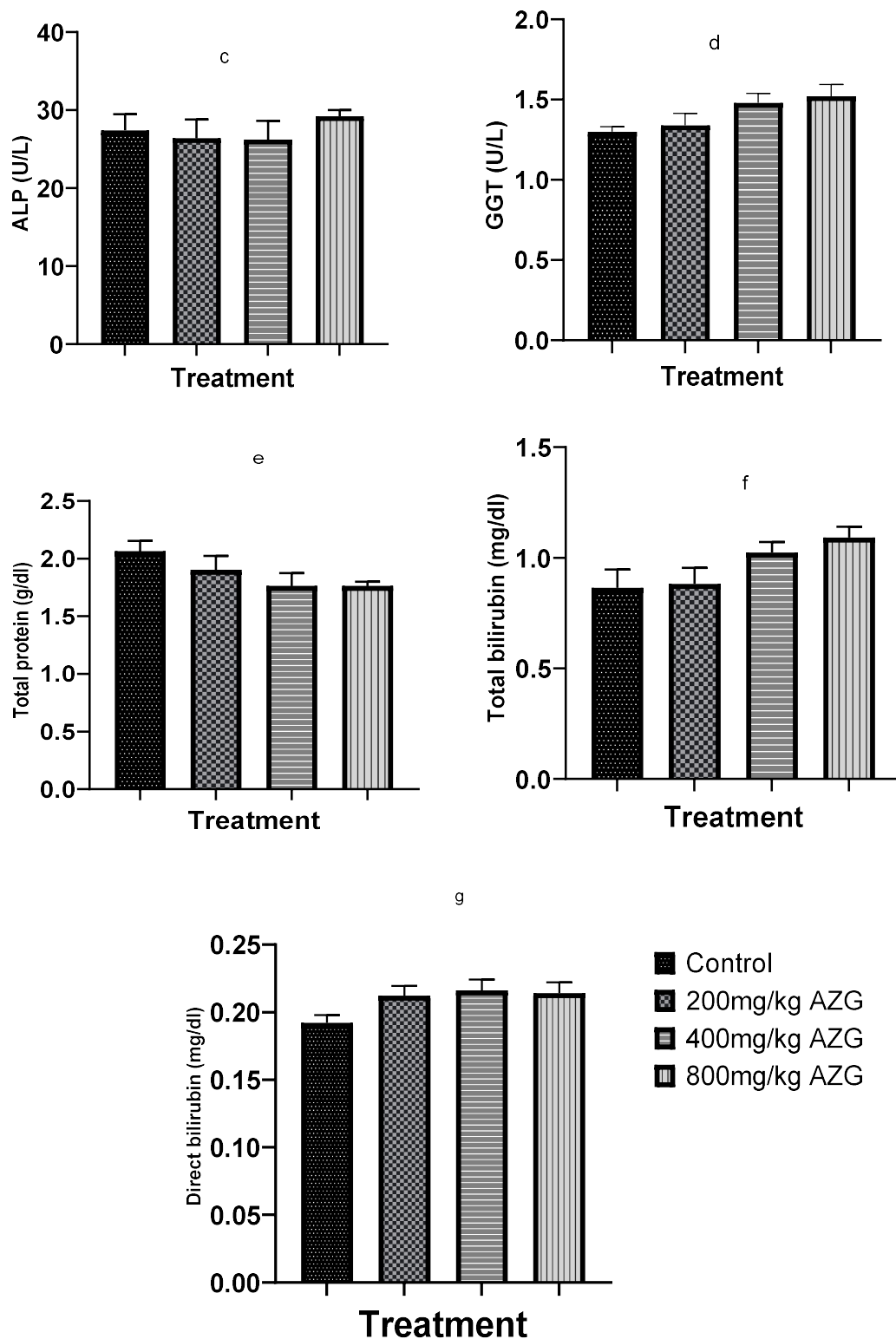


Figure 3: Effects of AZG (15 days) on liver function parameters. (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) total protein, (f) total bilirubin, (g) direct bilirubin.

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).

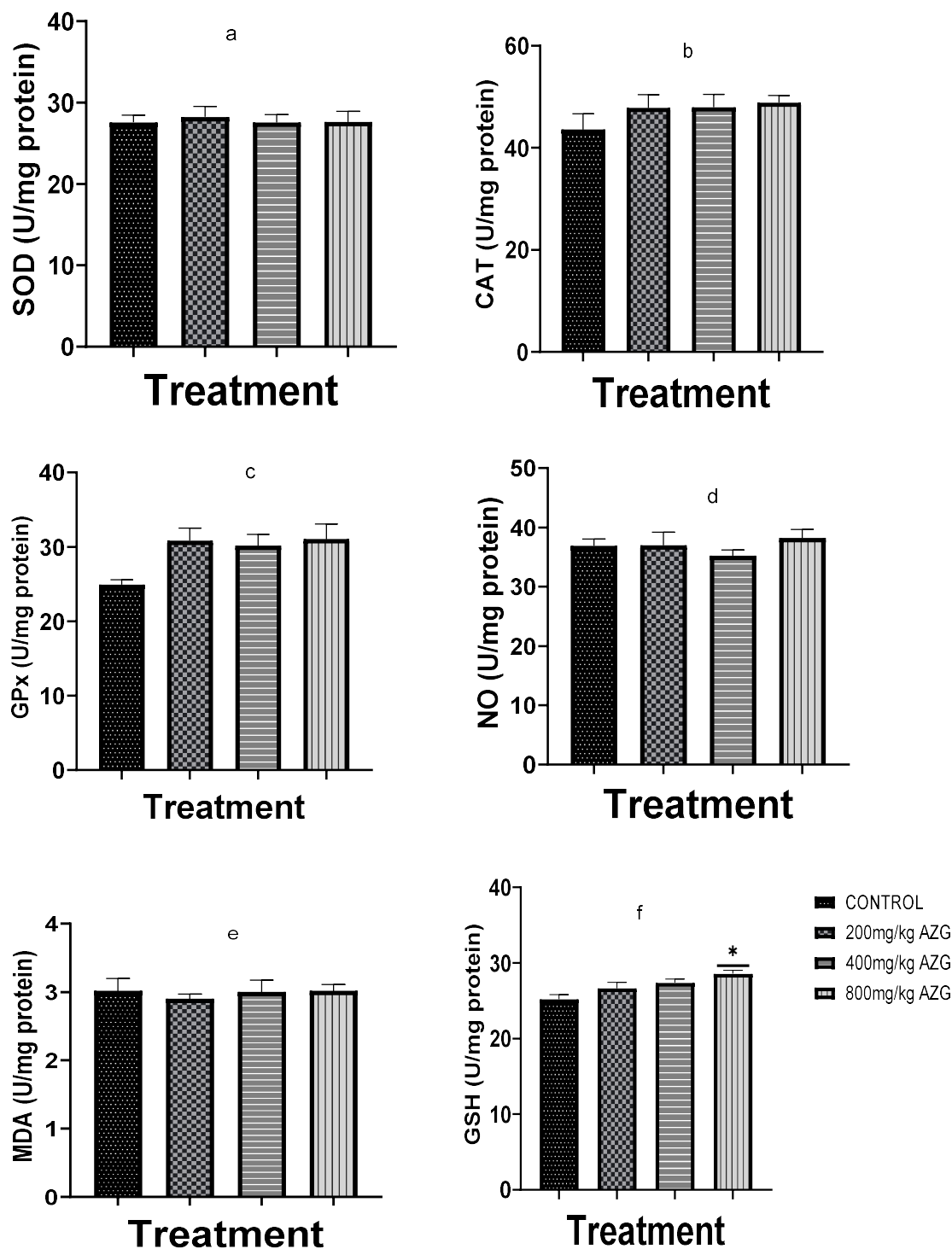
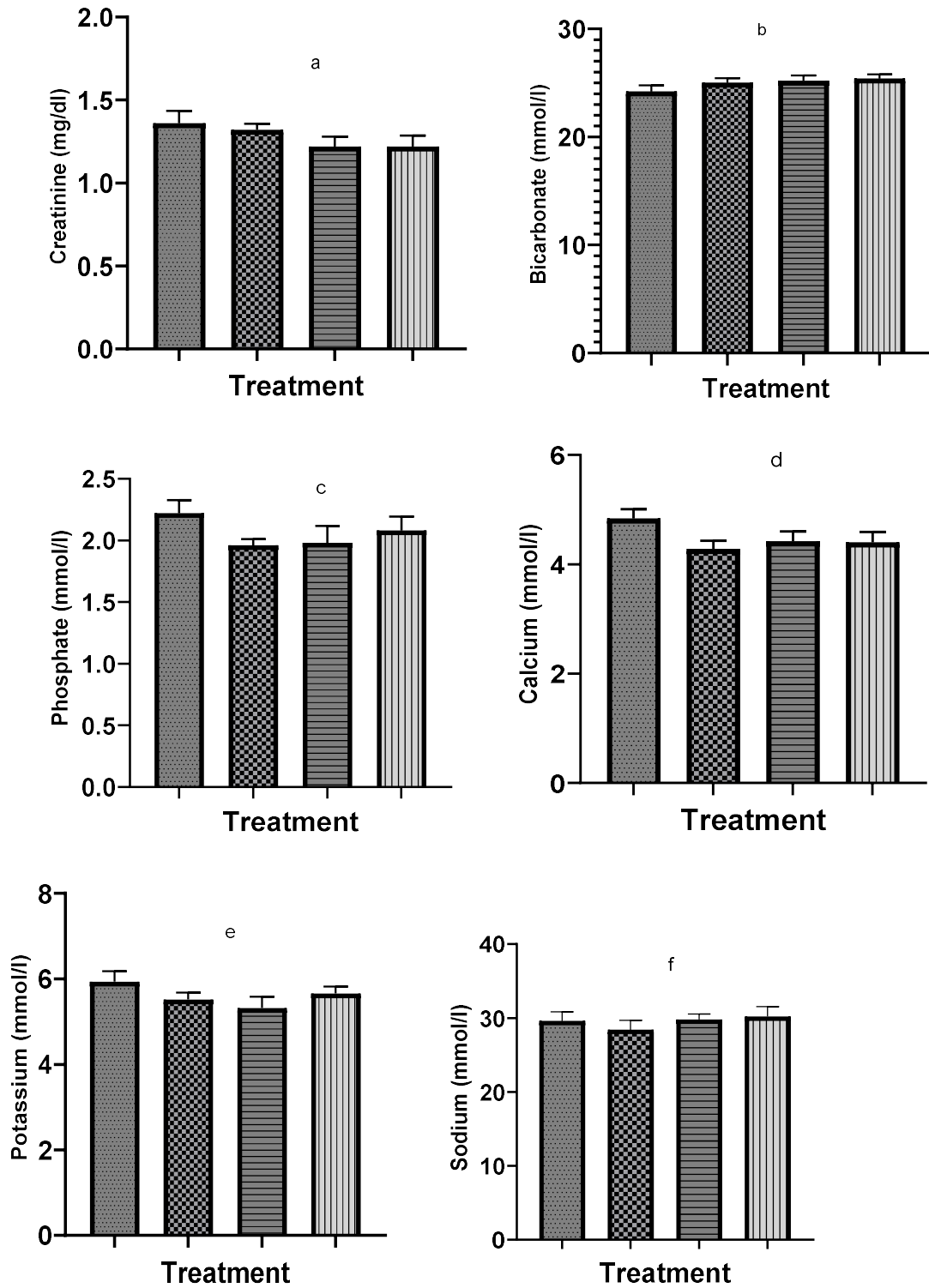


Figure 4: Effects of AZG (15 days) on liver oxidative stress markers. (a) SOD, (b) CAT, (c) GPx, (d) NO, (e) MDA, (f) GSH. Values are mean  $\pm$  SEM ( $n = 5$ ). No significant differences ( $p > 0.05$ ) except where \* $p < 0.05$  vs. control.

### 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).



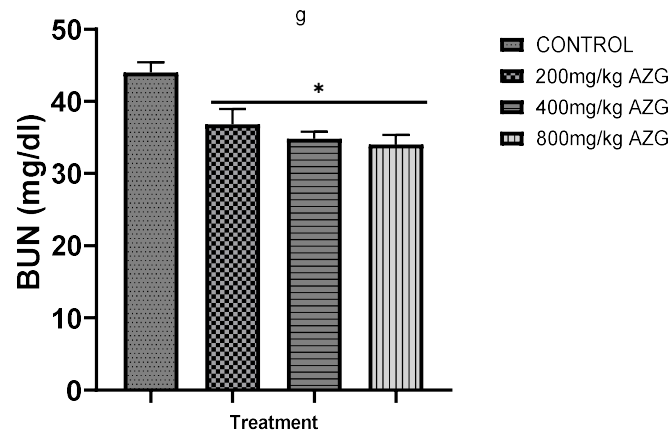
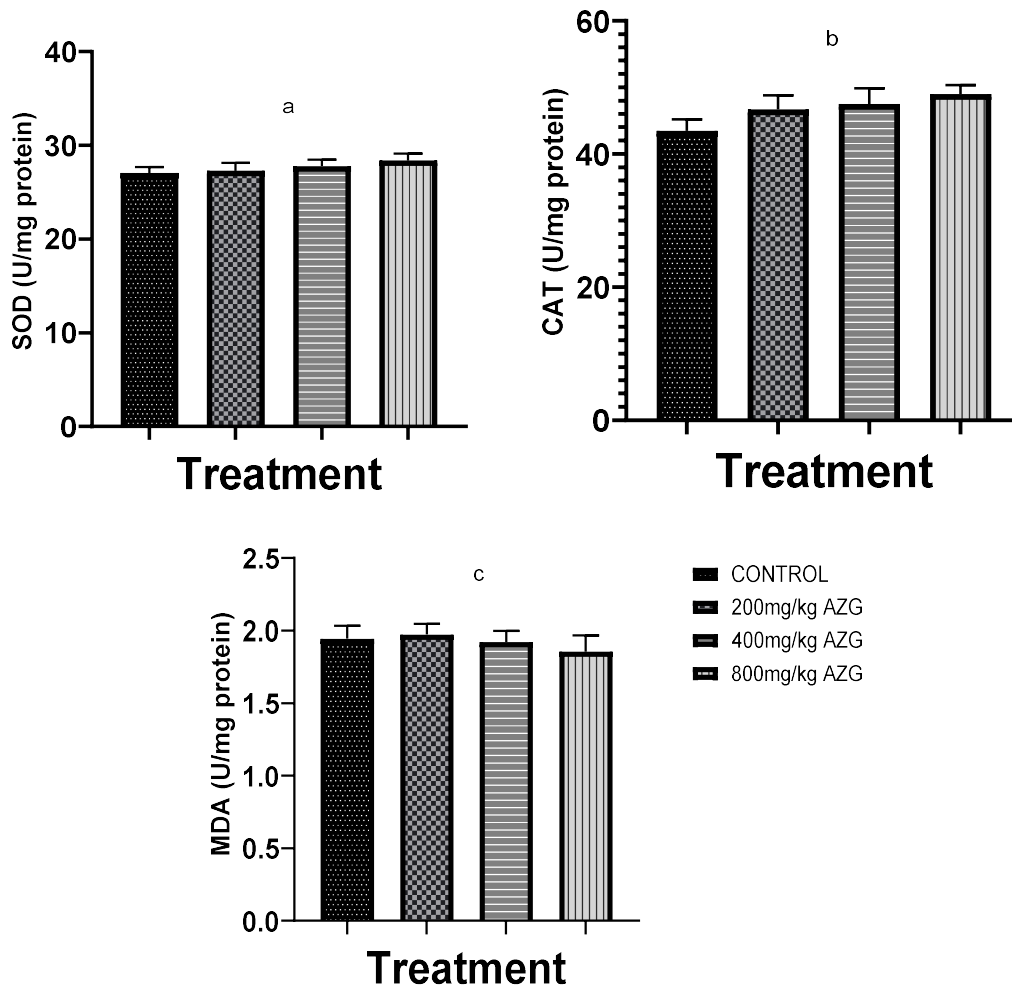


Figure 5: Effects of AZG (15 days) on kidney function parameters. (a) Creatinine, (b) bicarbonate, (c) phosphate, (d) calcium, (e) potassium, (f) sodium, (g) BUN.

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).



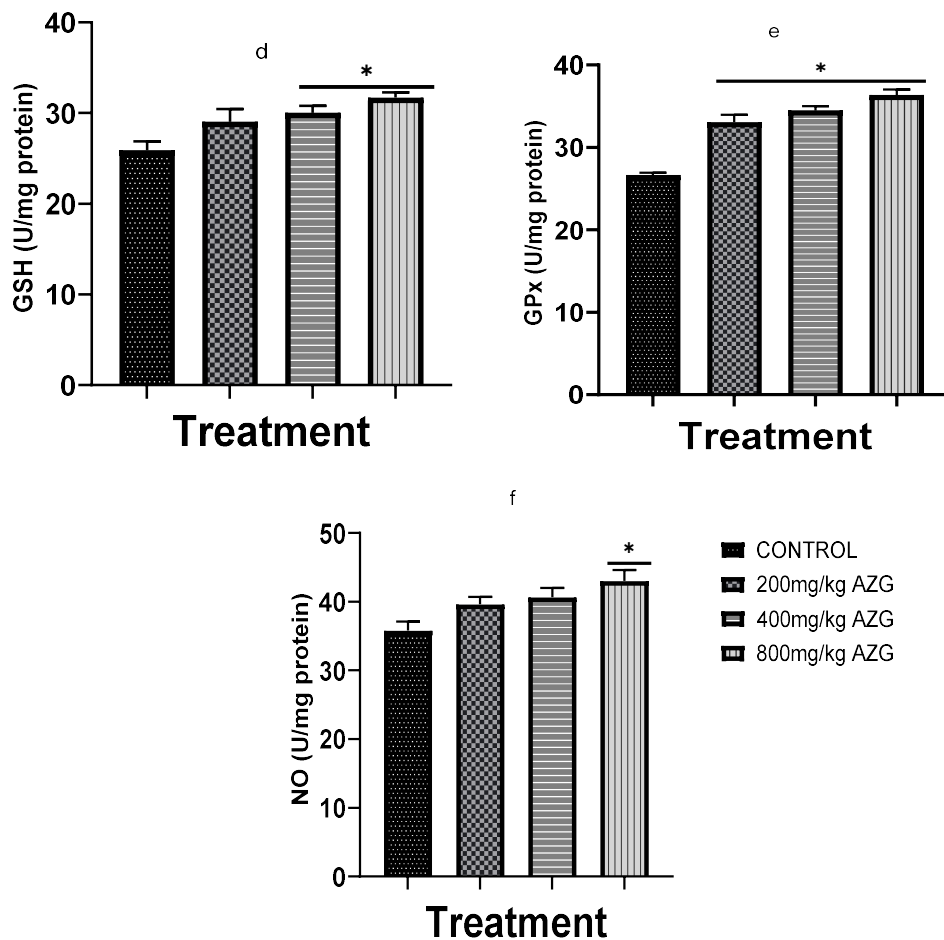
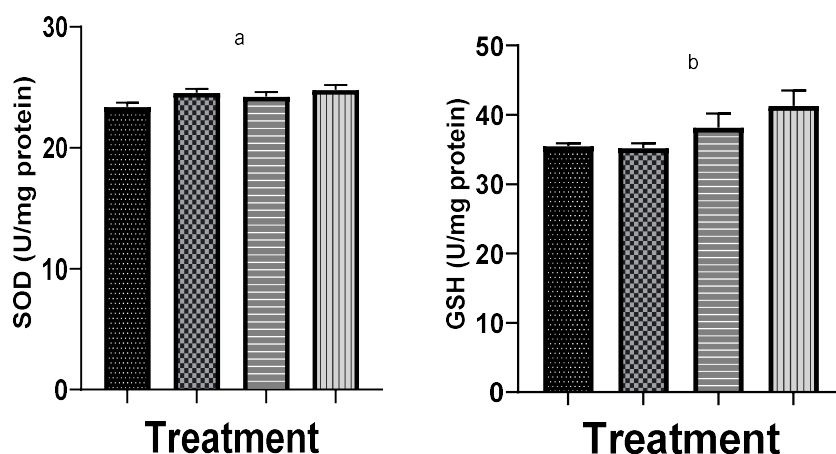


Figure 6: Effects of AZG (15 days) on kidney oxidative stress markers. (a) SOD, (b) CAT, (c) MDA, (d) GSH, (e) GPx, (f) NO. Values are mean ± SEM (n = 5). No significant differences (p > 0.05) except where \*p < 0.05 vs. control.

**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).



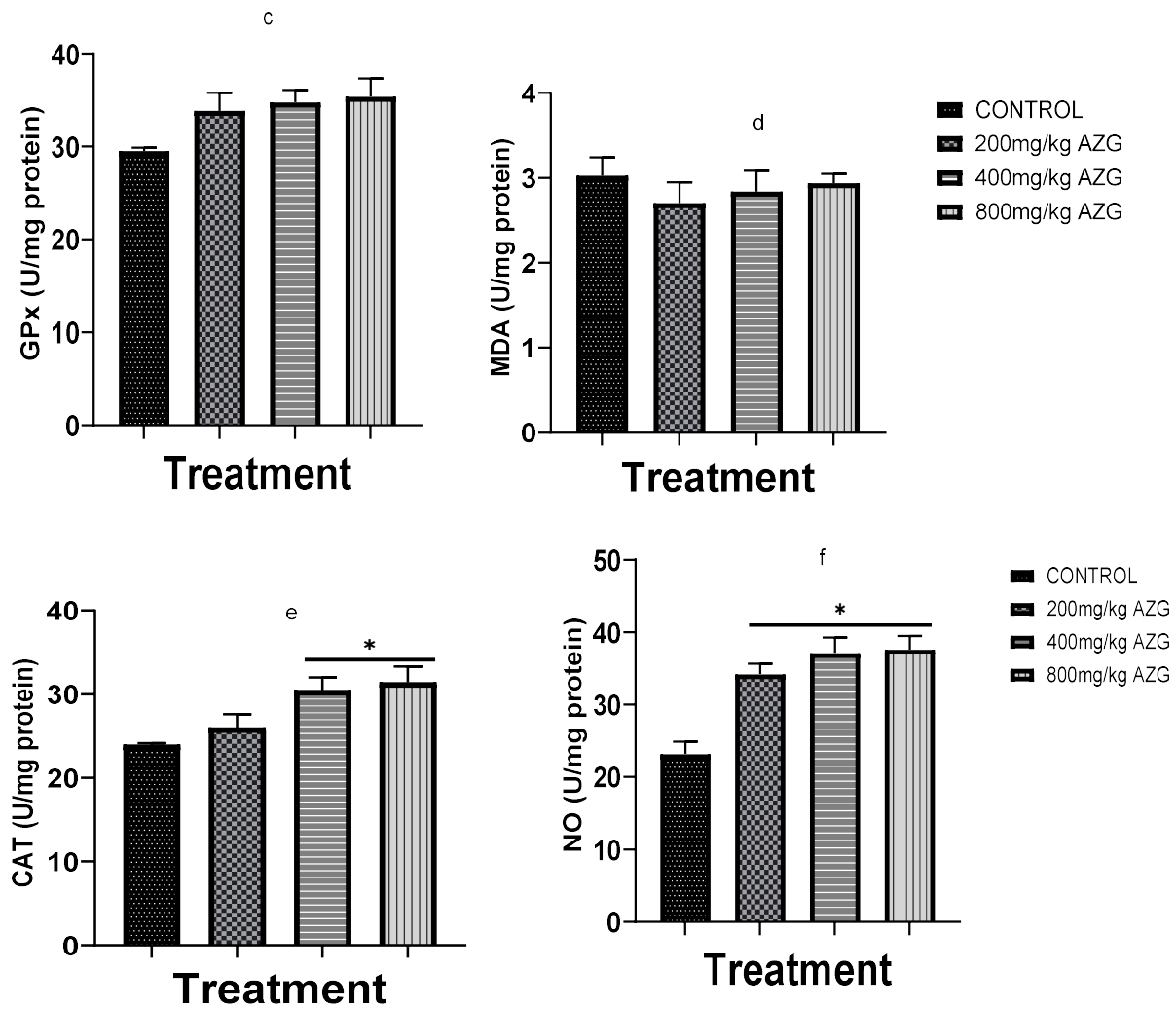
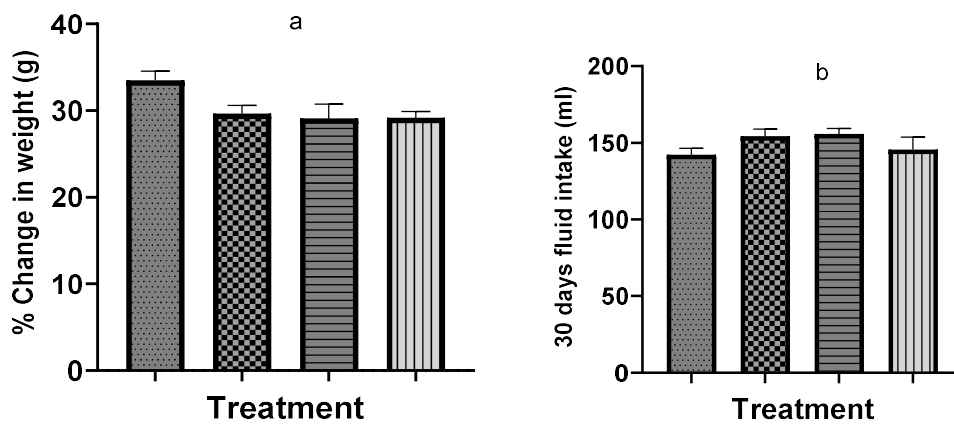


Figure 7: Effects of AZG (15 days) on heart oxidative stress markers. (a) SOD, (b) GSH, (c) GPx, (d) MDA, (e) CAT, (f) NO. Values are mean ± SEM (n = 5). No significant differences (p > 0.05) except where \*p < 0.05 vs. control.

### 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).



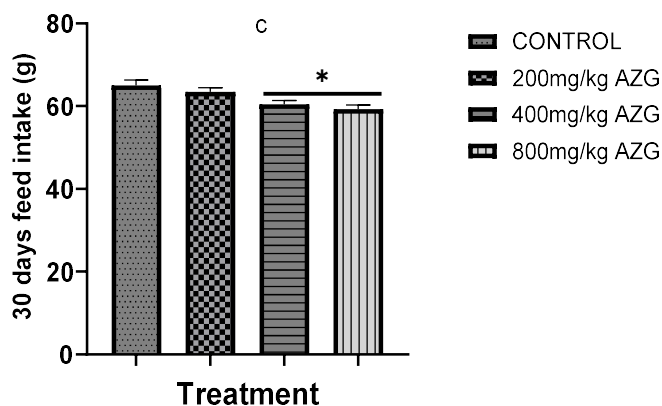
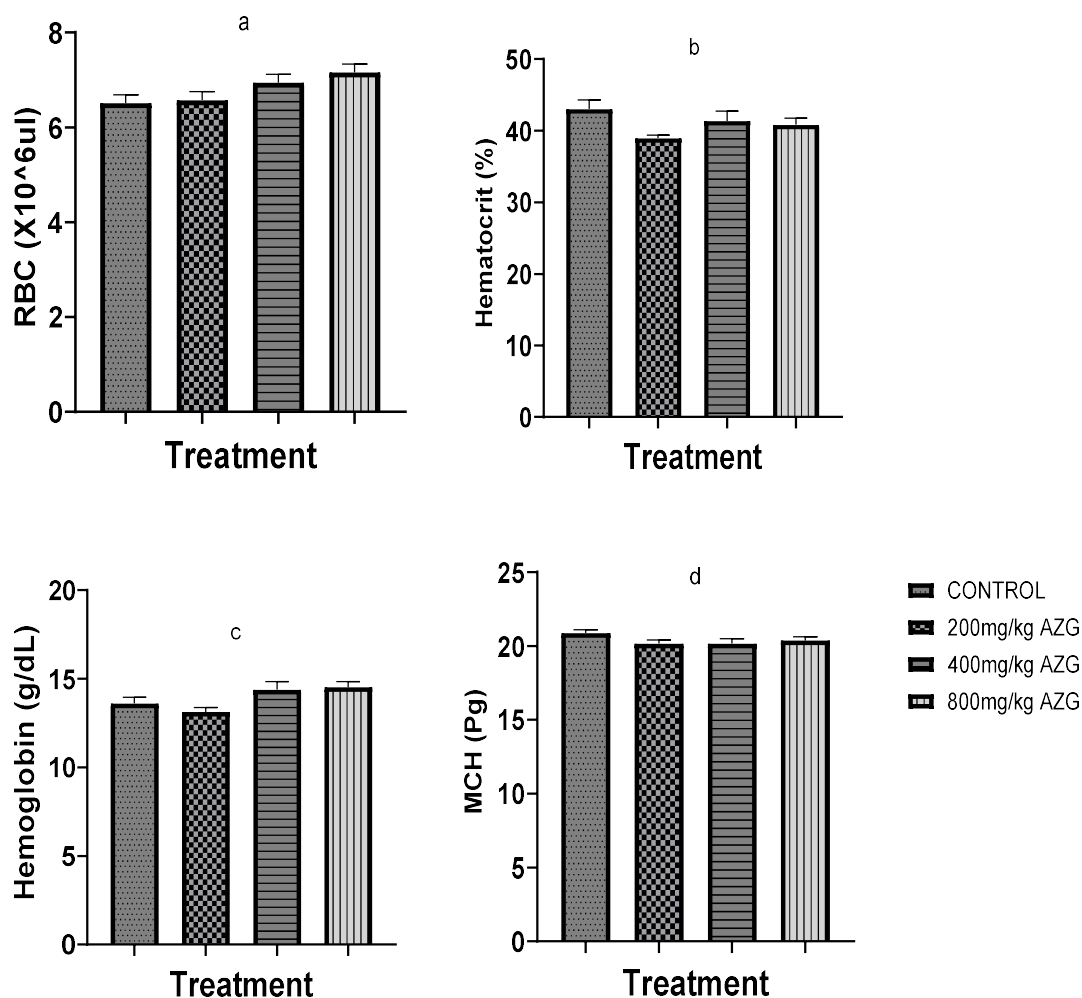
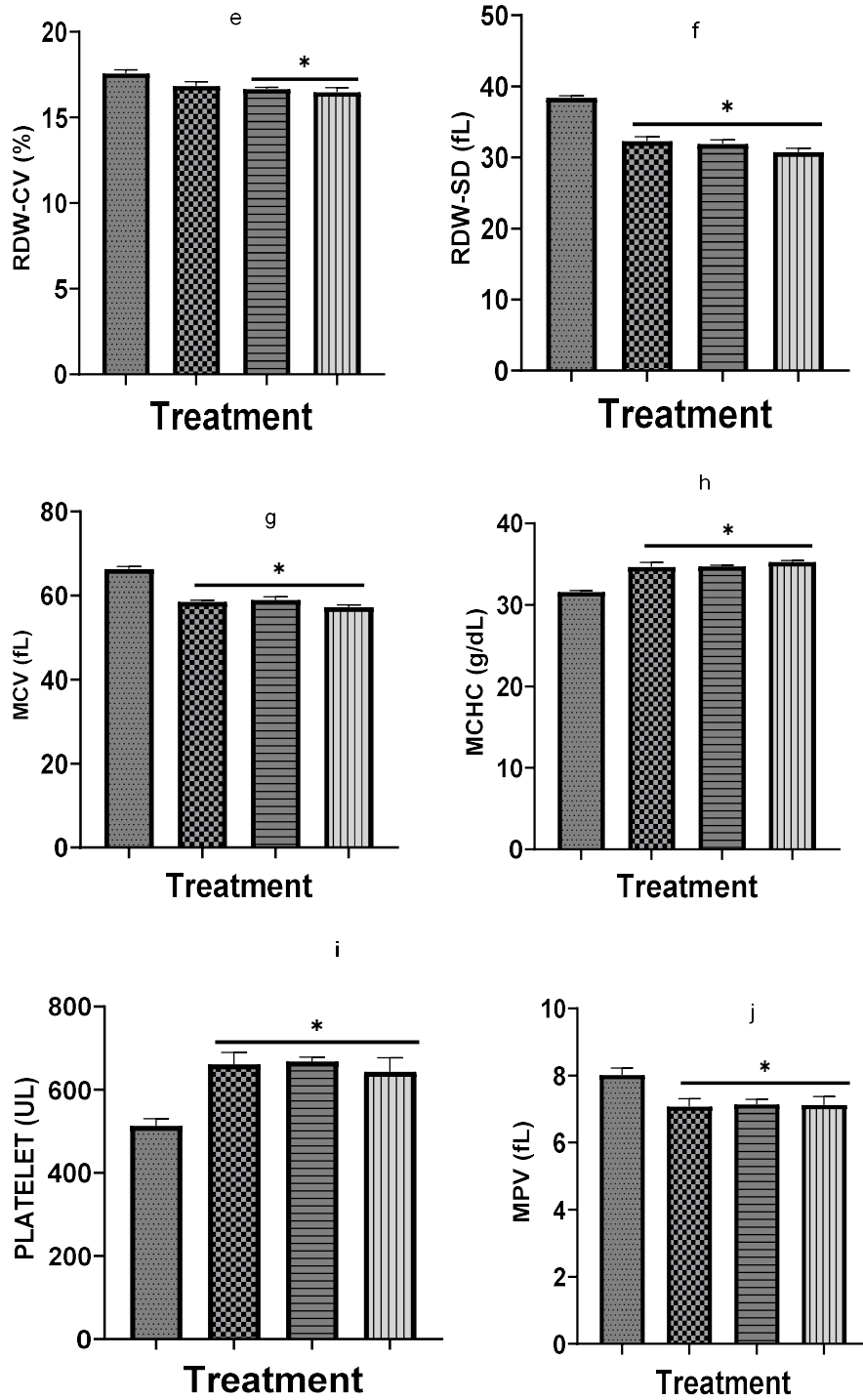


Figure 8: Effects of AZG (30 days) on (a) weight changes, (b) fluid intake, (c) food intake. Values are mean ± SEM (n = 5). No significant differences ( $p > 0.05$ ) except where  $*p < 0.05$  vs. control.

**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.





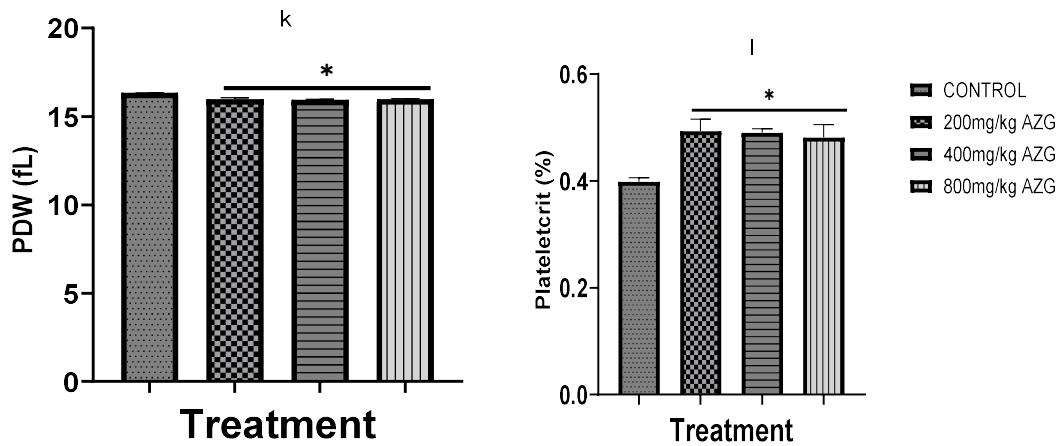
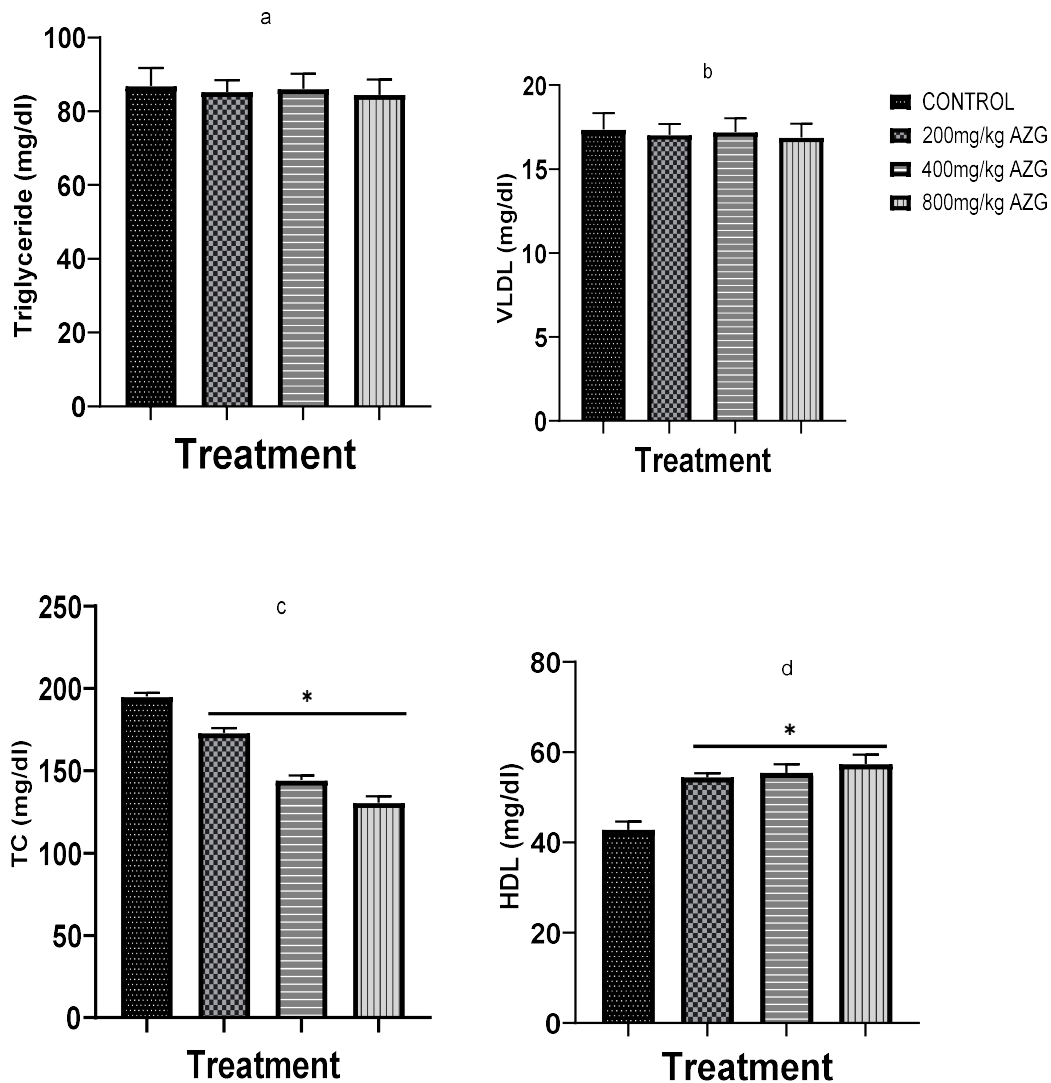


Figure 9: Effects of AZG (30 days) on hematological parameters. (a) RBC, (b) HCT, (c) Hb, (d) MCH, (e-l) other indices. Values are mean  $\pm$  SEM (n = 5). No significant differences ( $p > 0.05$ ) except where \* $p < 0.05$  vs. control.

### 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).



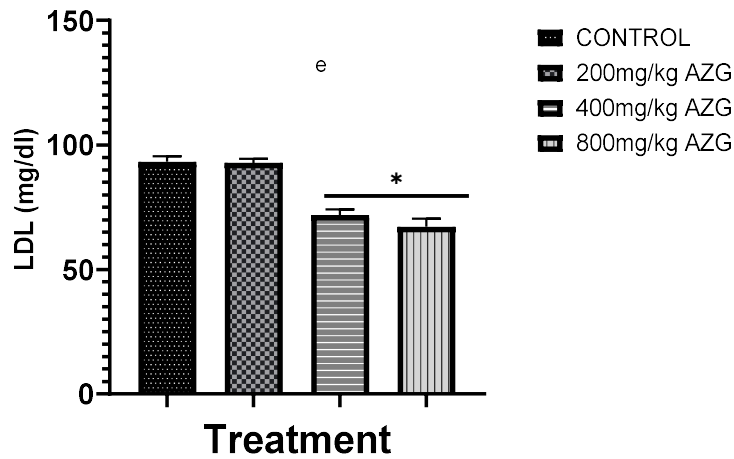
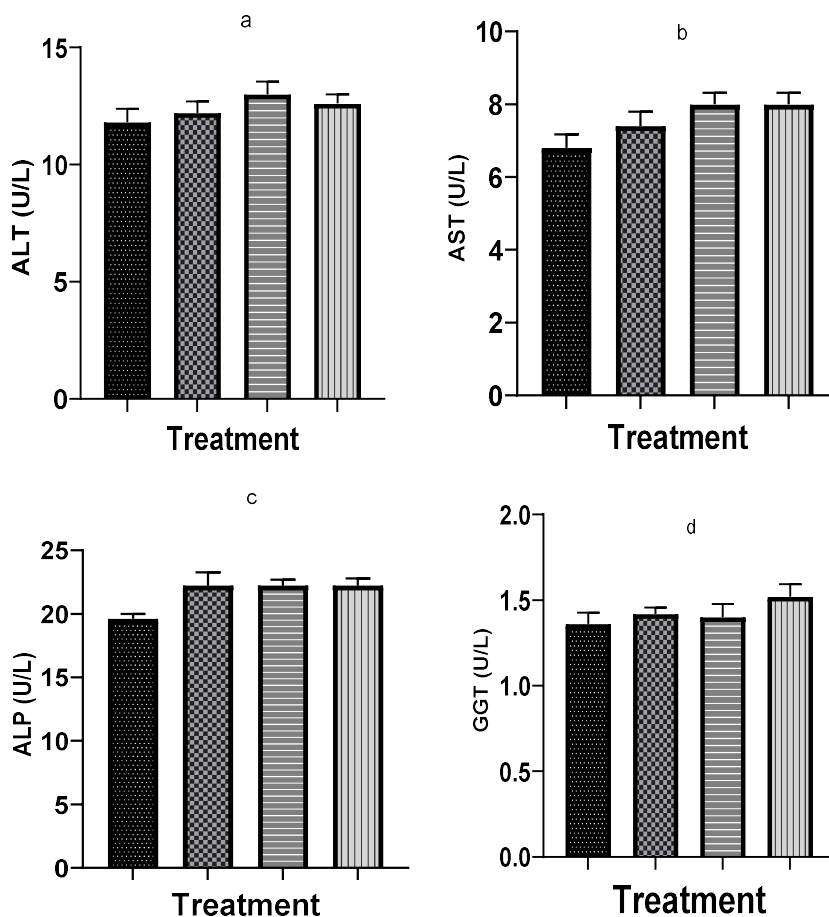


Figure 10: Effects of AZG (30 days) on lipid profile. (a) Triglycerides, (b) VLDL, (c) total cholesterol, (d) HDL, (e) LDL. Values are mean ± SEM (n = 5). No significant differences ( $p > 0.05$ ) except where  $*p < 0.05$  vs. control.

**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).



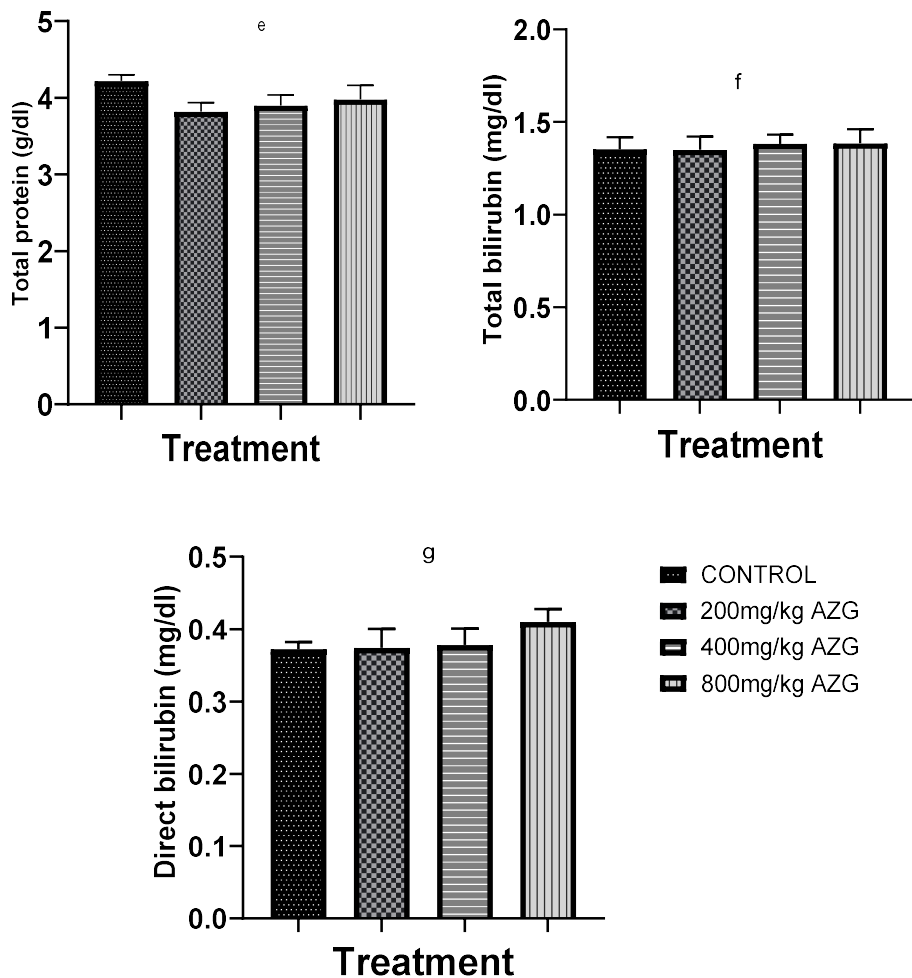
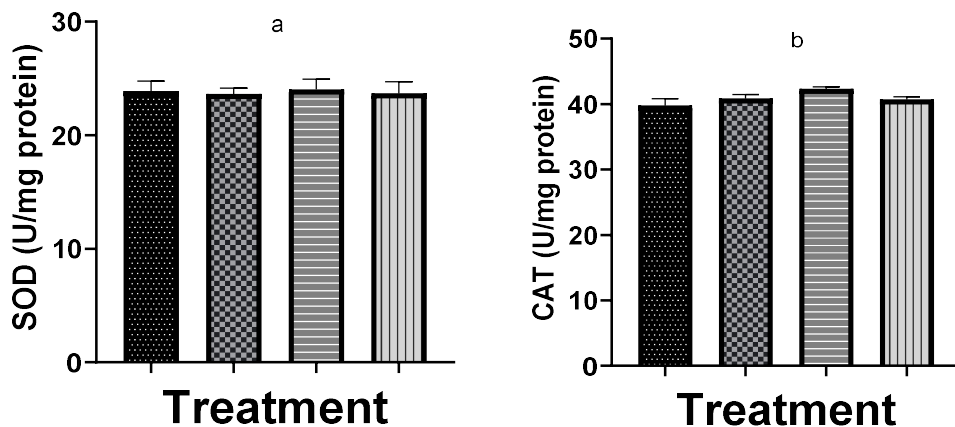


Figure 11: Effects of AZG (30 days) on liver function parameters. (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) total protein, (f) total bilirubin, (g) direct bilirubin. Values are mean ± SEM (n = 5). No significant differences (p > 0.05).

**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).



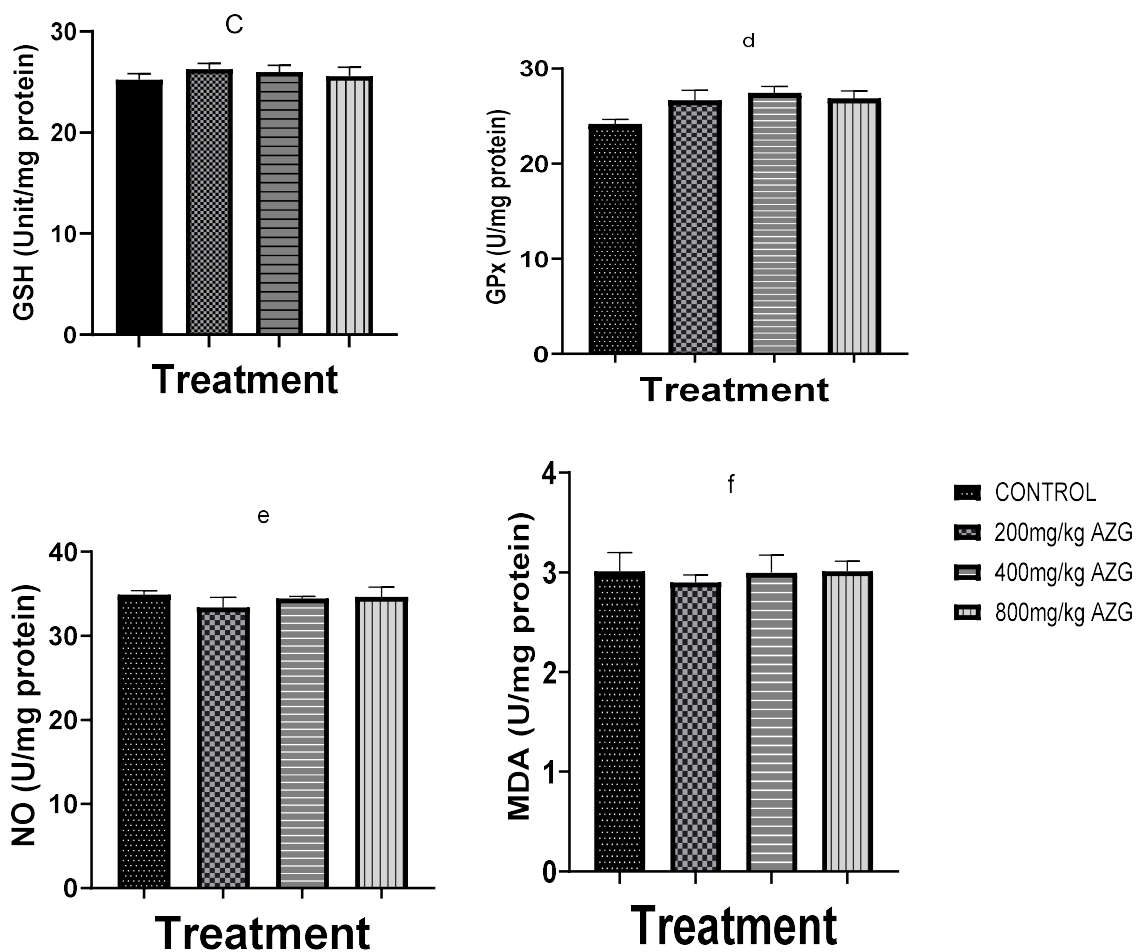
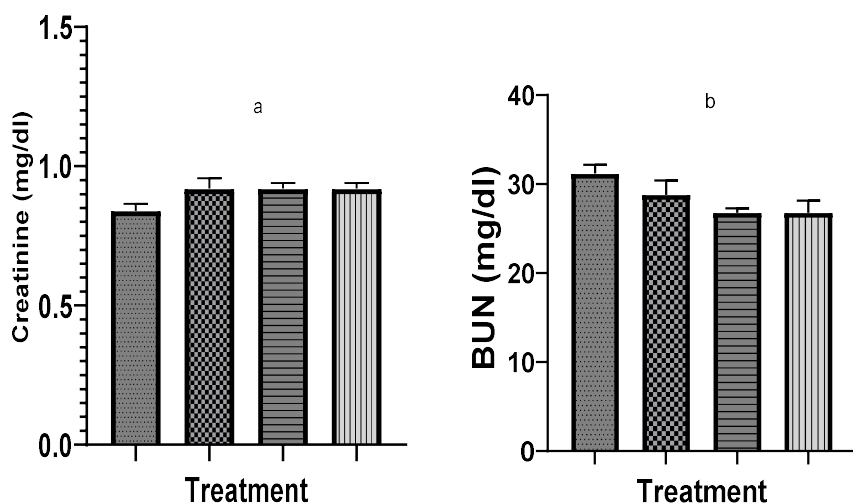


Figure 12: Effects of AZG (30 days) on liver oxidative stress markers. (a) SOD, (b) CAT, (c) GSH, (d) GPx, (e) NO, (f) MDA. Values are mean ± SEM (n = 5). No significant differences (p > 0.05).

**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).



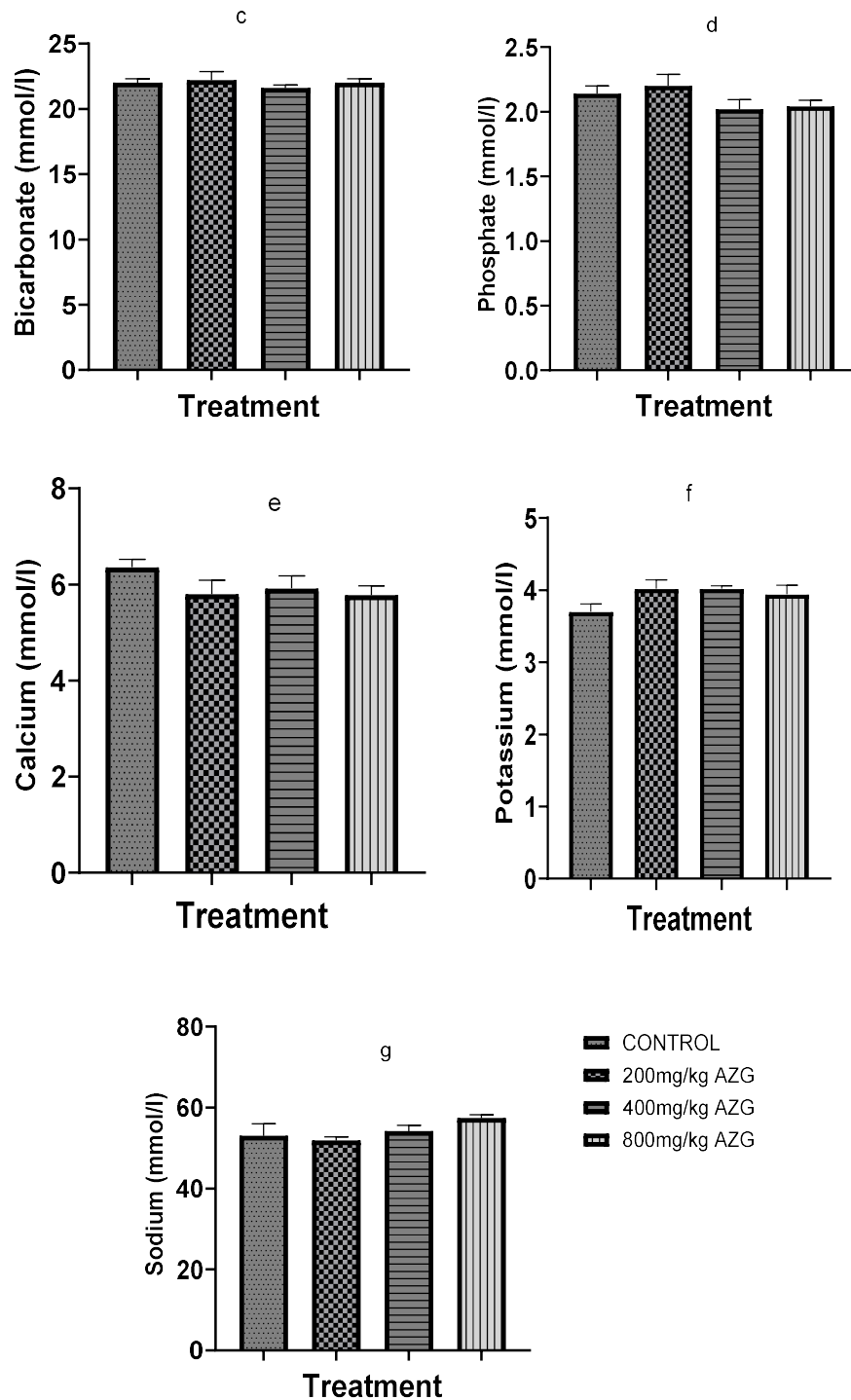


Figure 13: Effects of AZG (30 days) on kidney function parameters. (a) Creatinine, (b) BUN, (c) bicarbonate, (d) phosphate, (e) calcium, (f) potassium, (g) sodium.

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).

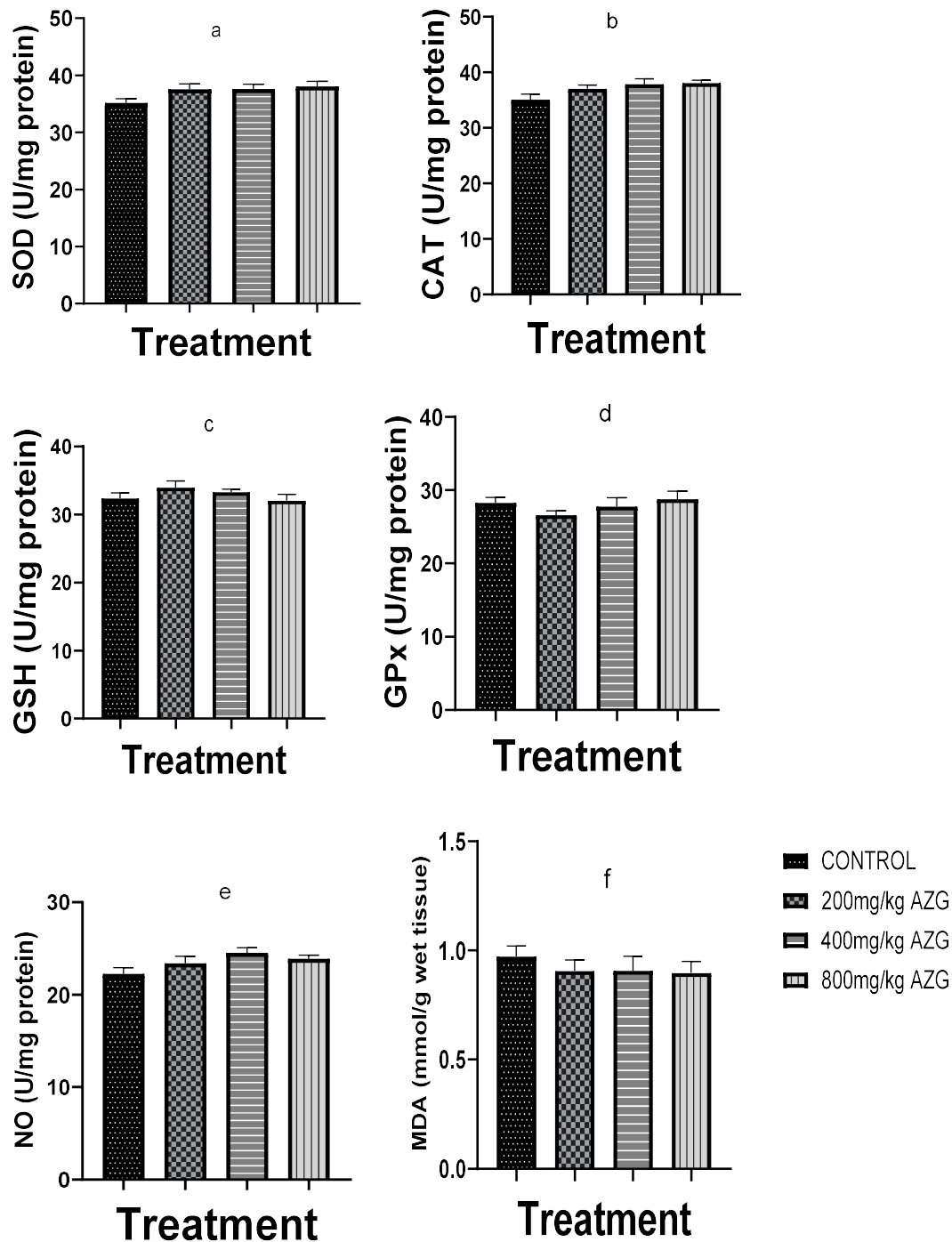


Figure 14: Effects of AZG (30 days) on kidney oxidative stress markers. (a) SOD, (b) CAT, (c) GSH, (d) GPx, (e) NO, (f) MDA. Values are mean  $\pm$  SEM ( $n = 5$ ). No significant differences ( $p > 0.05$ ).

### 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).

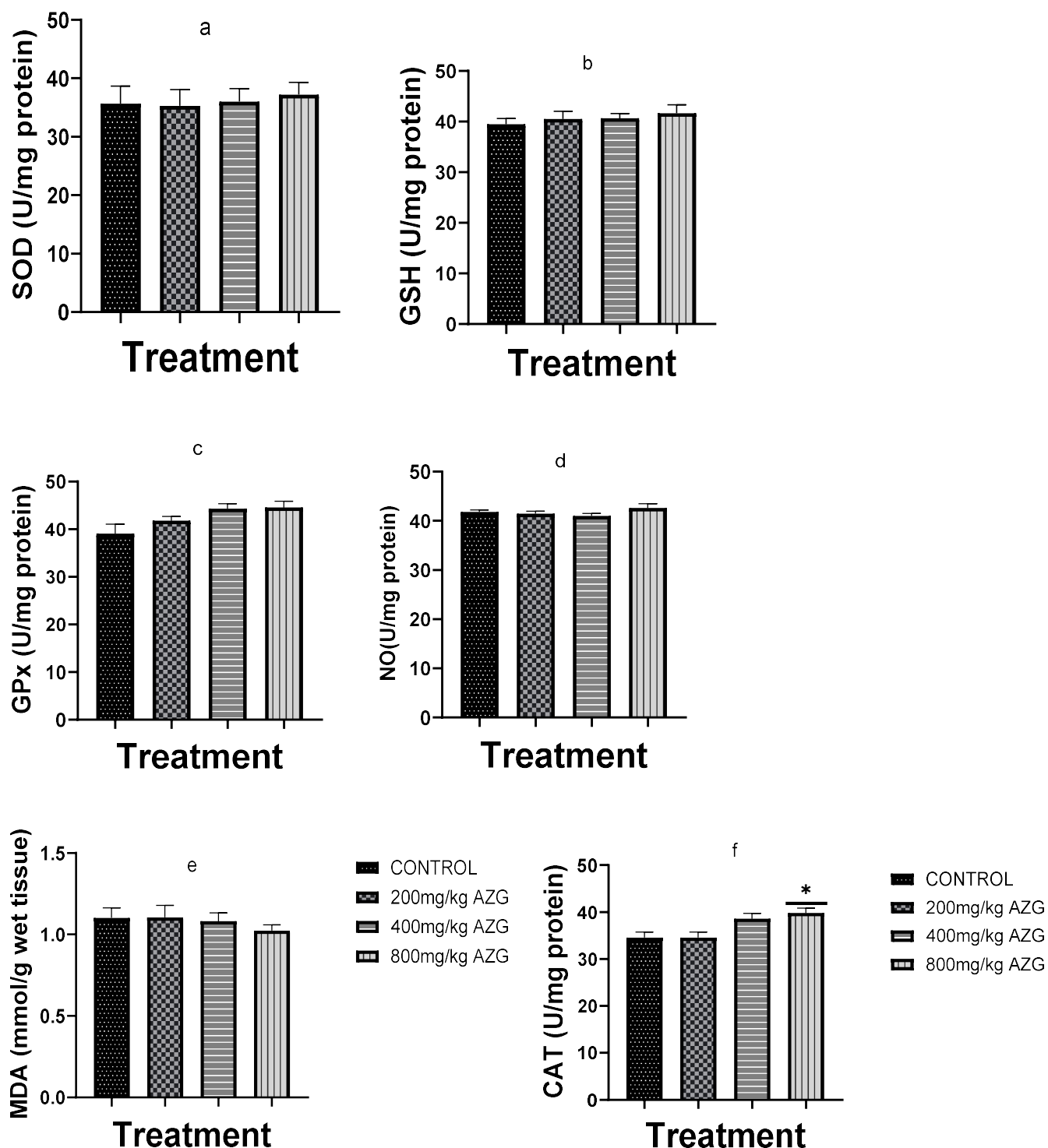


Figure 15: Effects of AZG (30 days) on heart oxidative stress markers. (a) SOD, (b) GSH, (c) GPx, (d) NO, (e) MDA, (f) CAT. Values are mean  $\pm$  SEM ( $n = 5$ ). No significant differences ( $p > 0.05$ ) except where \* $p < 0.05$  vs. control.

#### 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 LD<sub>50</sub> 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 LD<sub>50</sub> 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 B<sub>12</sub> or folate deficiency, which typically present with macrocytosis, 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

**Acknowledgements:** Authors wish to express their sincere gratitude to the technical staff of the Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria. We also extend our appreciation to Vicklin Laboratory and Emma-Maria Laboratory, Abraka, Delta State, for their invaluable technical support and assistance throughout this study.

**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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