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

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

Abstract

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

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

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

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

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

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INTRODUCTION

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

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

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

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

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

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

Methods

Plant Authentication

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

Plant Preparation

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

Ethical Approval

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

Experimental Animals

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

and Use of Laboratory Animals.

Treatment Groups

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

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

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

Specimen Collection

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

Haematological assays

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

Serological tests

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

Statistical Analysis

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

RESULTS

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

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

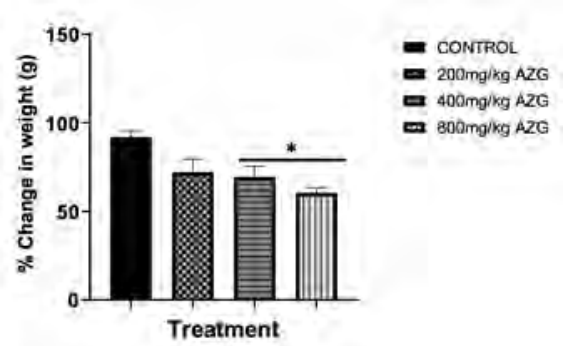


Figure 1: Effect of AZG-HE on animal weight in rats after 60 days oral treatment

*p <0.05 in comparison to untreated group

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

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

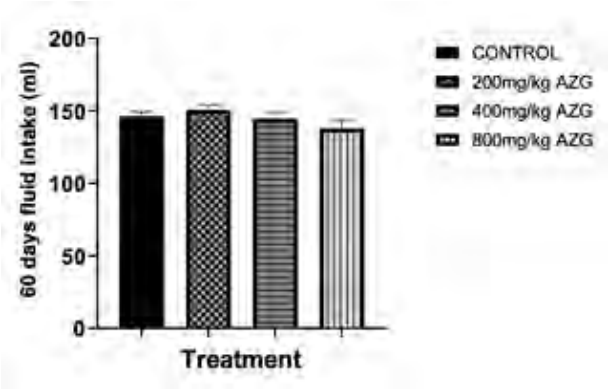


Figure 3: Effect of AZG-HE on fluid intake in rats after 60 days oral treatment

p 0.05 in comparison to untreated group

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

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

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

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

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

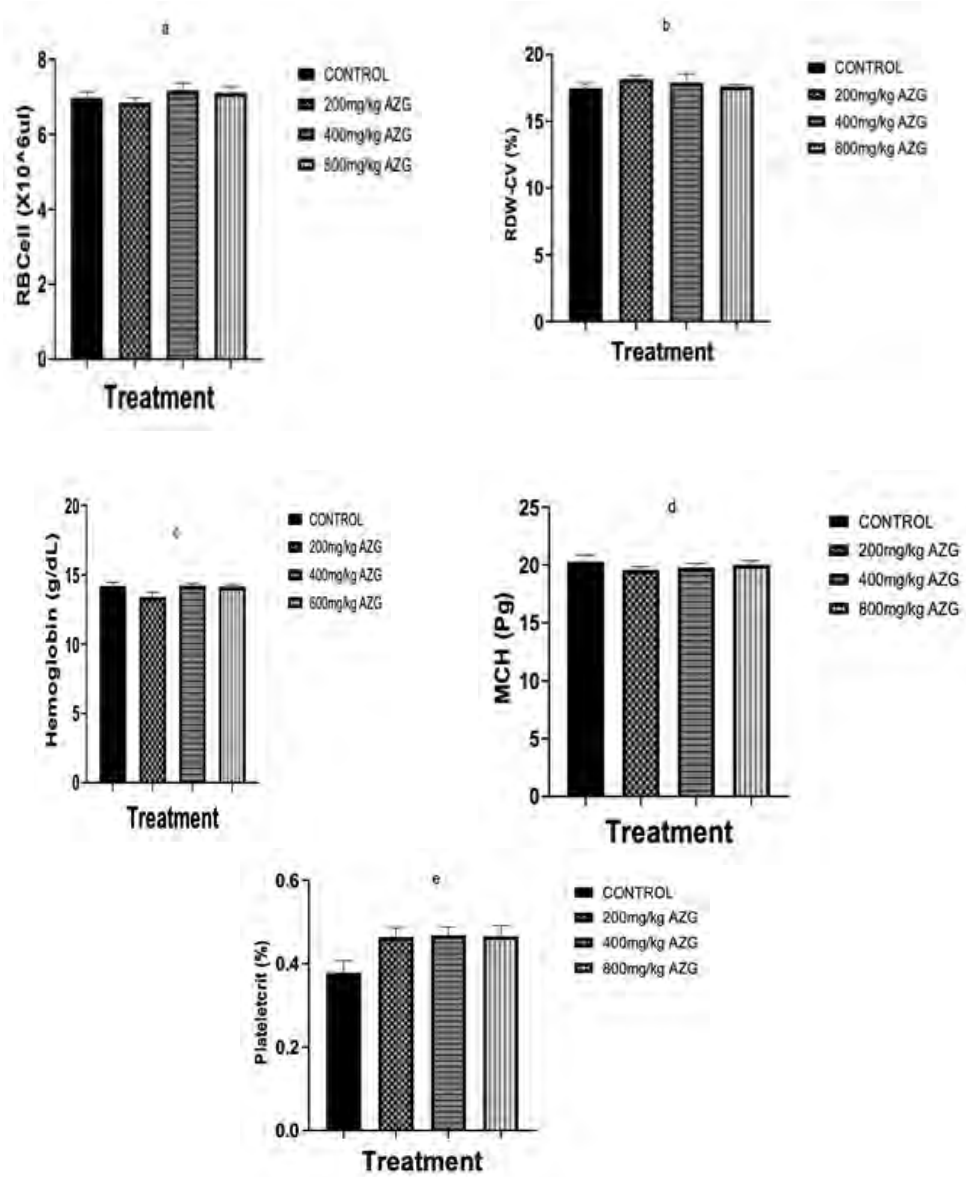


Figure 4: Effect AZG-HE on (a) RBC, (b) RDW-CV, (c) HB, (d) MCH and (e) PCT in rats after 60 days oral treatment
P >0.05 in comparison to untreated group

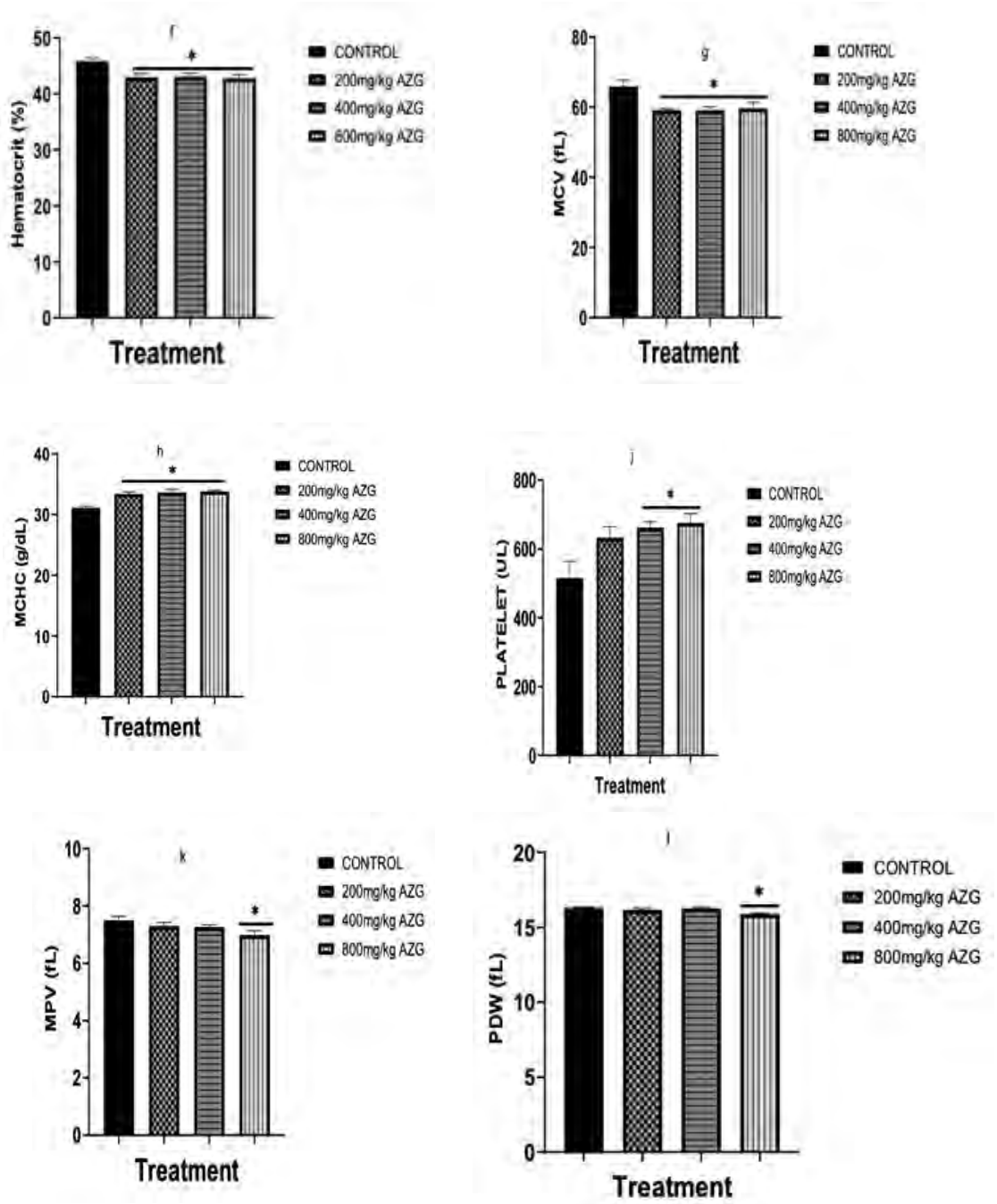


Figure 4: Effect of AZG-HE on (f) hematocrit, (g) MCV (h) MCHC, (i) RDW-SD, (j) PLT, (k) MPV and (l) PDW in rats after 60 days oral administration
$p < 0.05$ in comparison to untreated group

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

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

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

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

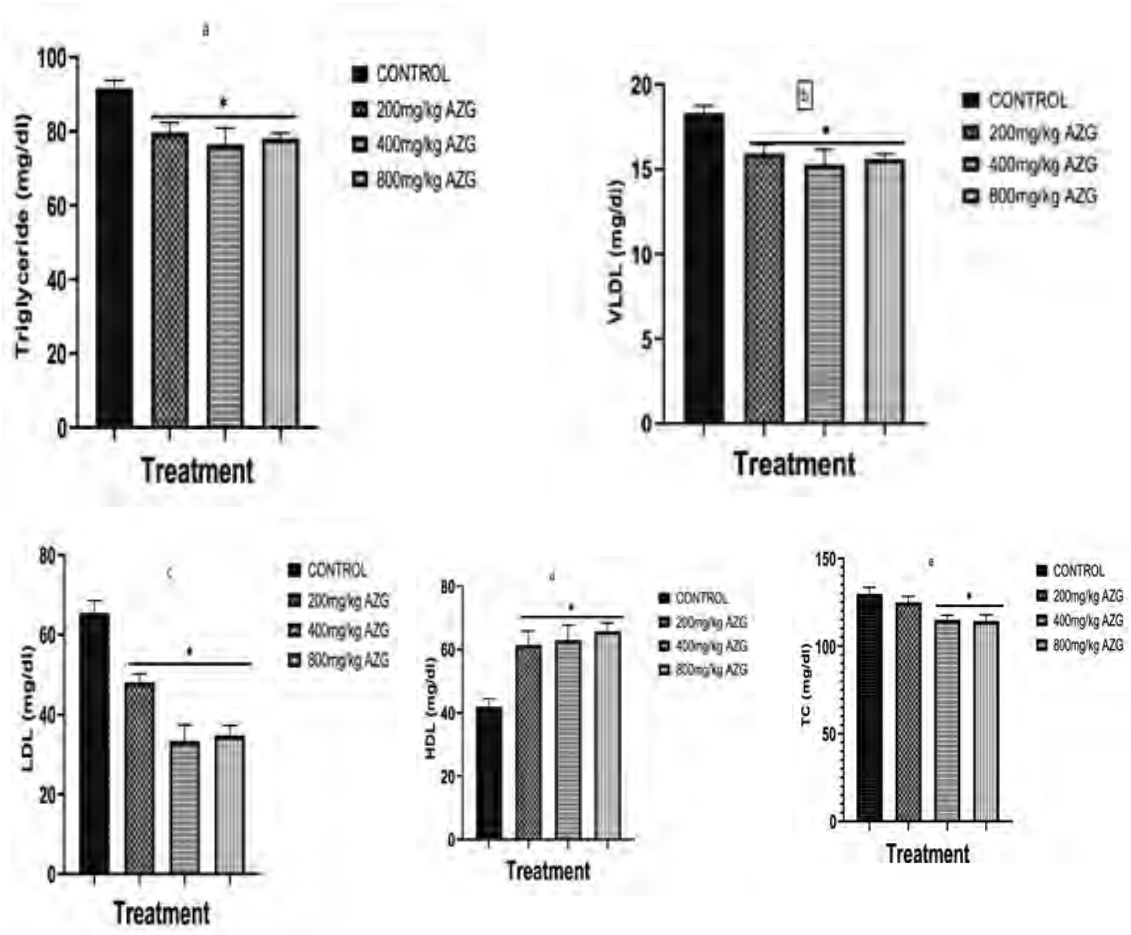


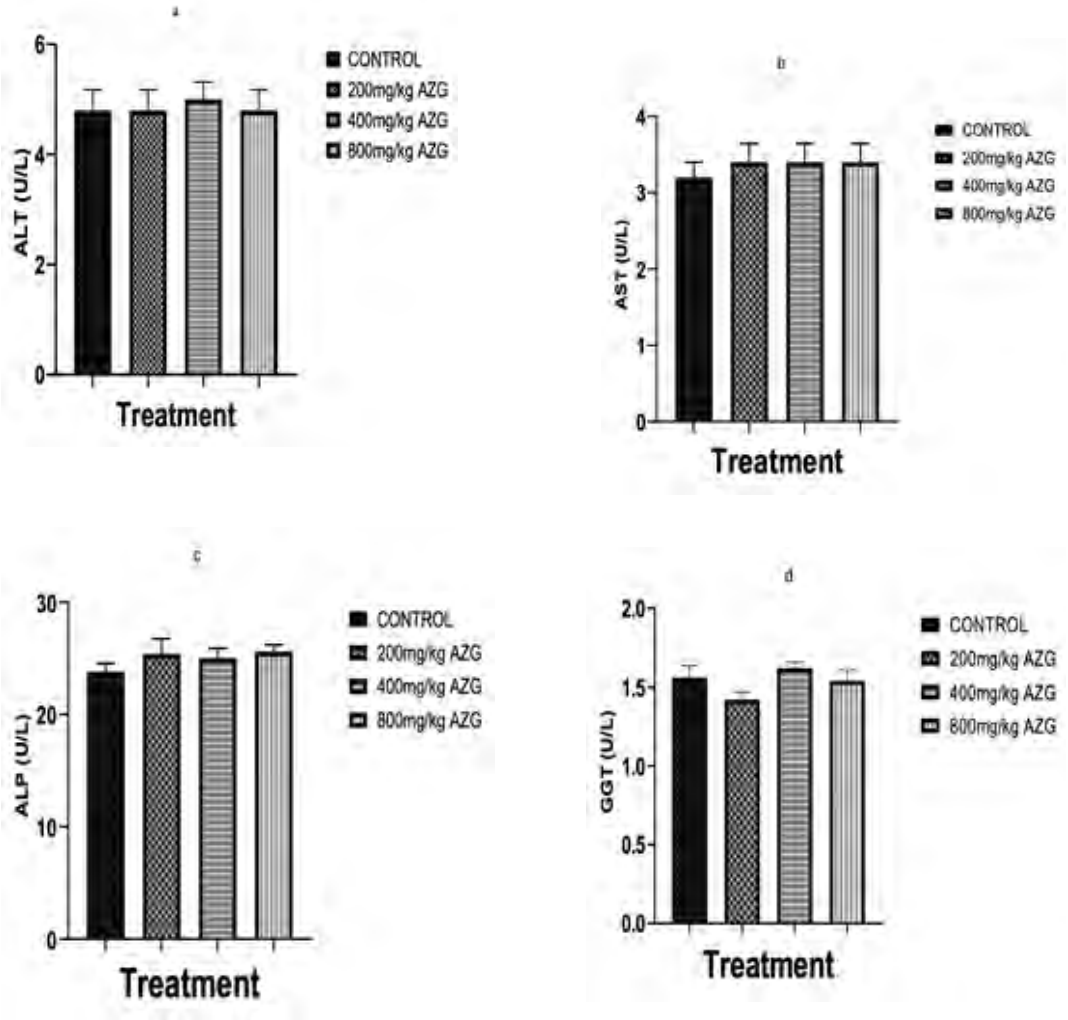
Figure 5: Effect of AZG-HE on (a) TG, (b) TC, (c) HDL, (d) LDL, and (e) VLDL level in rats after 60 days oral administration

* $p<0.05$ in comparison to untreated group

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

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

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



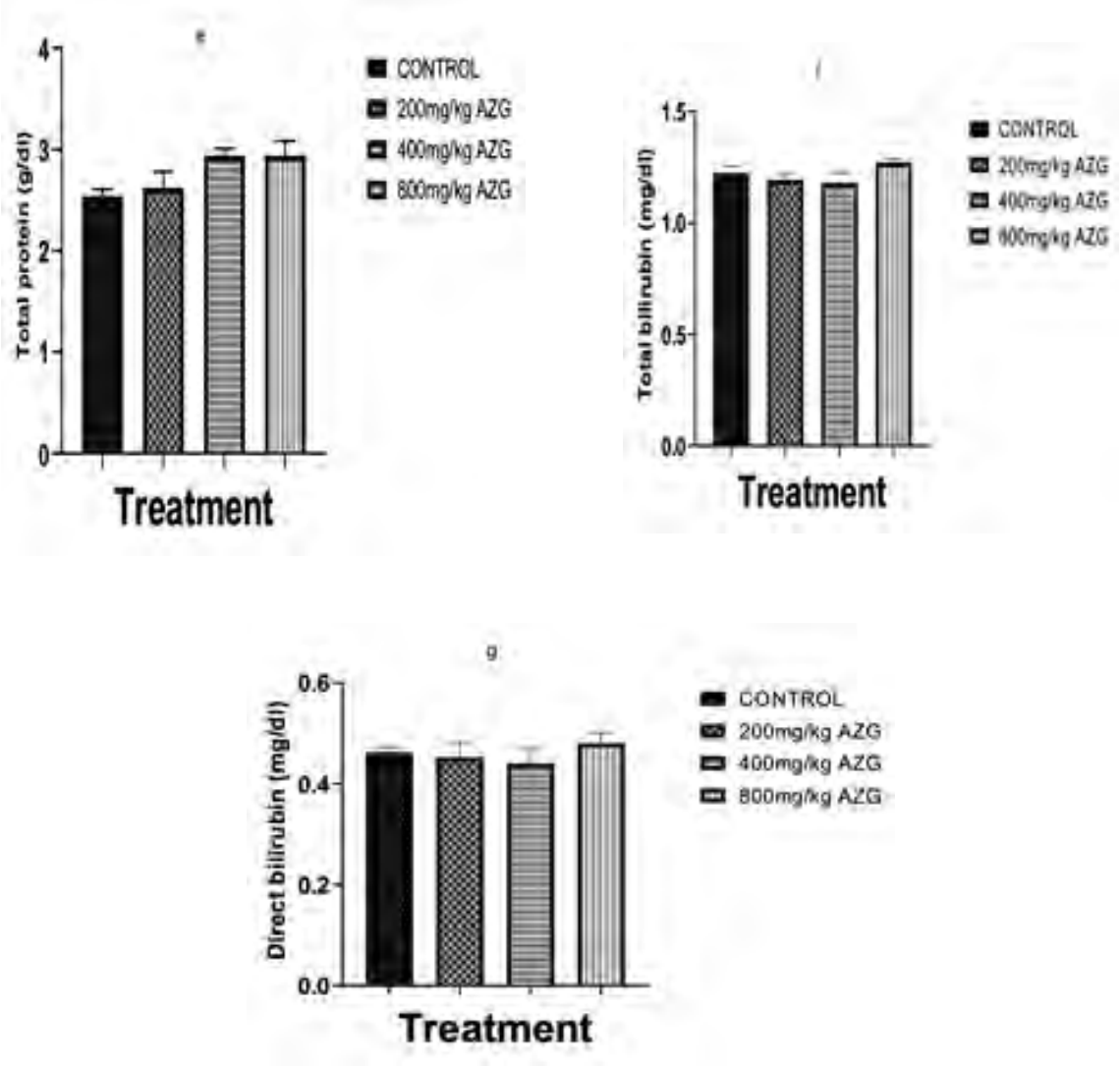


Figure 6: Effect of AZG-HE on (a) ALT level, (b) AST level, (c) ALP level, (d) GGT level, (e) T. protein level, (f) T.bil level, (g) D.bil level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

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

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

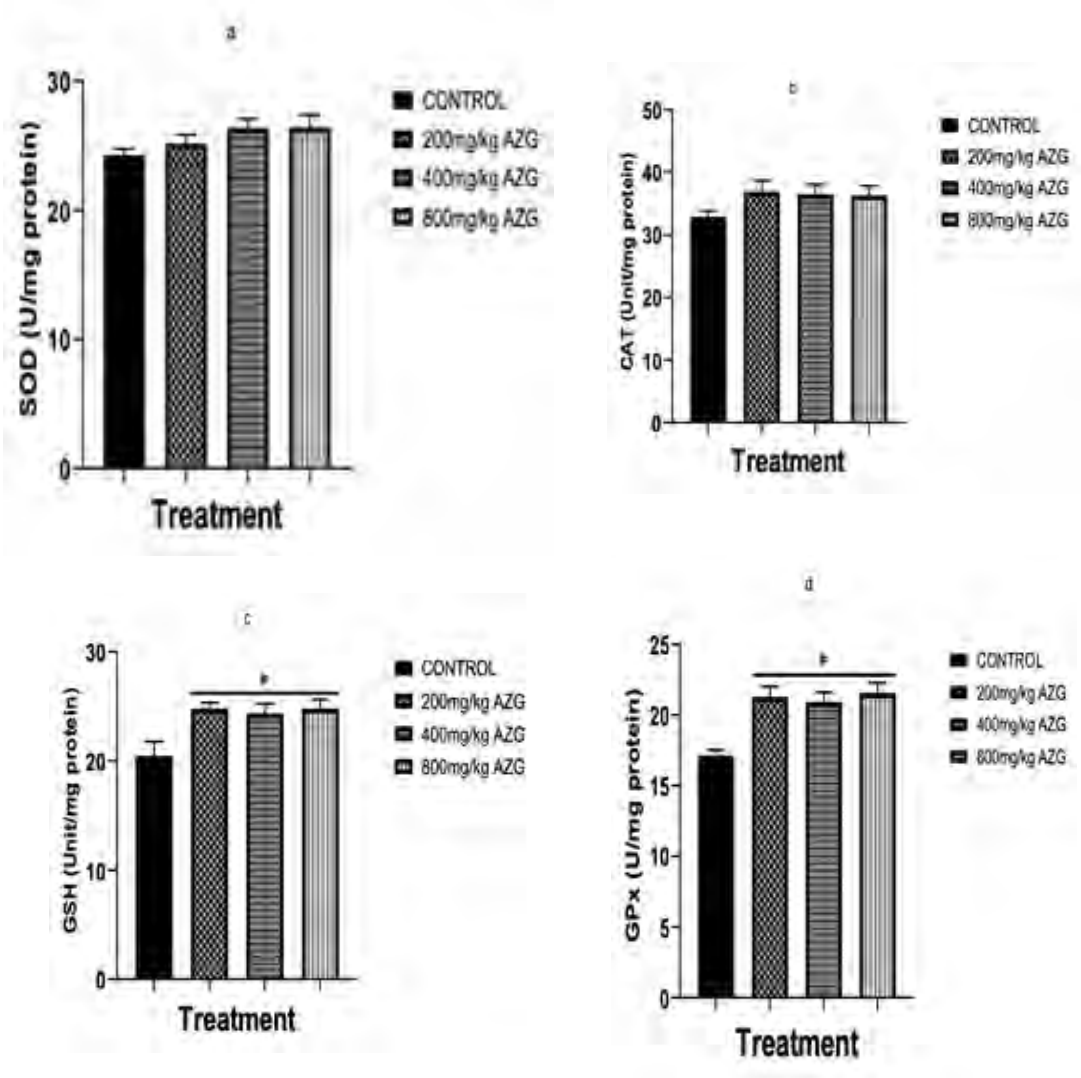


Figure 7: Effect of AZG-HE on (a) SOD level (b) CAT level (c) GSH level, and (d) GPx level in rats after 60 days oral administration
* $p < 0.05$ in comparison to untreated group

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

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

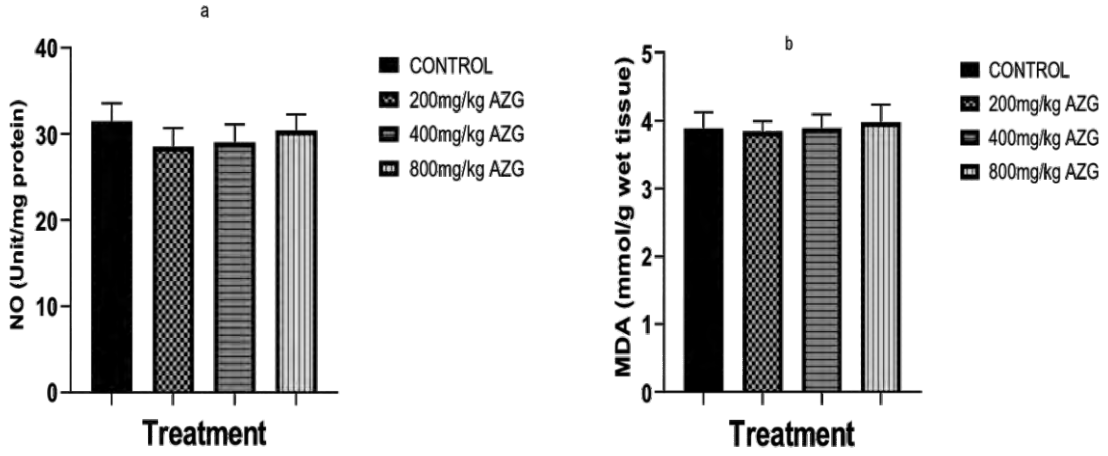


Figure 8: Effect of AZG-HE on (a) liver NO level and (b) liver MDA in rats after 60 days oral administration
p >0.05 compared to untreated group

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

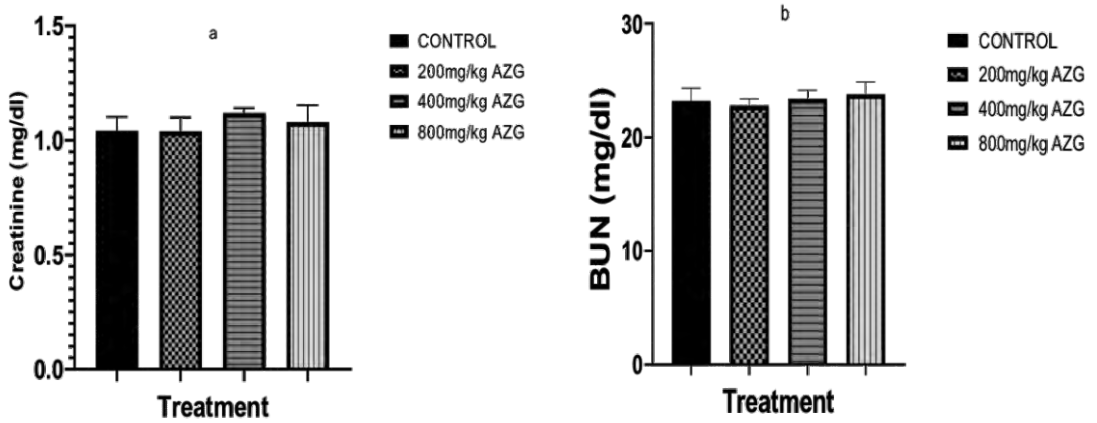


Figure 9: Effect of AZG-HE on (a) serum creatinine level and (b) BUN level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

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

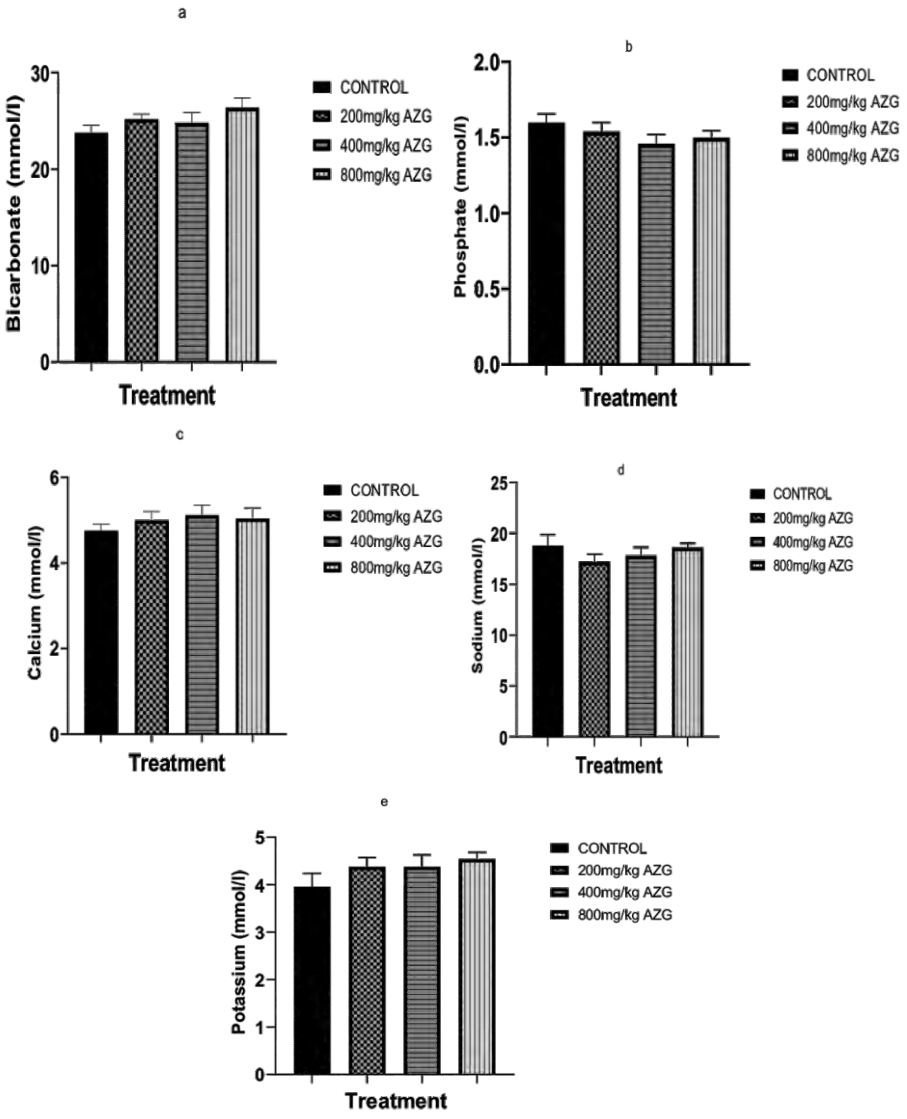


Figure 10*:* Effect of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum sodium level, and (e) serum potassium level in rats after 60 days oral administration
p>0.05 in comparison to untreated group

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

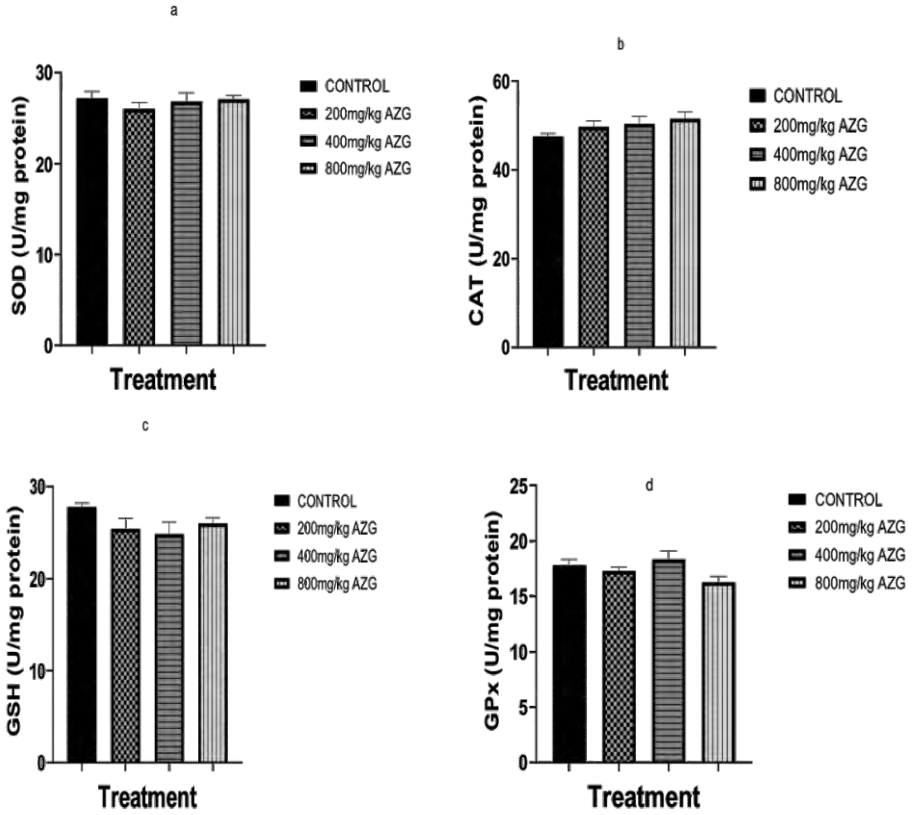


Figure 11: Effect of AZG-HE on (a) kidney SOD level, (b) on kidney CAT level, (c) kidney GSH level, and (d) kidney GPx level in rats after 60 days oral administration
p>0.05 in comparison to untreated group

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

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

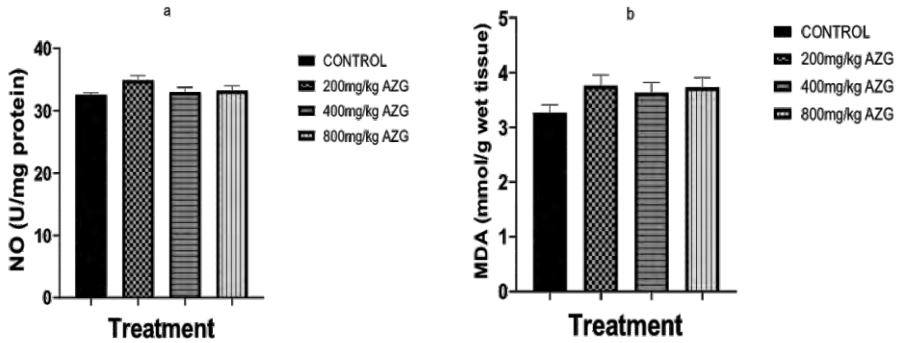


Figure 12: Effect of AZG-HE on kidney (a) nitric oxide level and (b) Malondialdehyde level in rats after 60 days oral administration

p>0.05 in comparison to untreated group

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

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

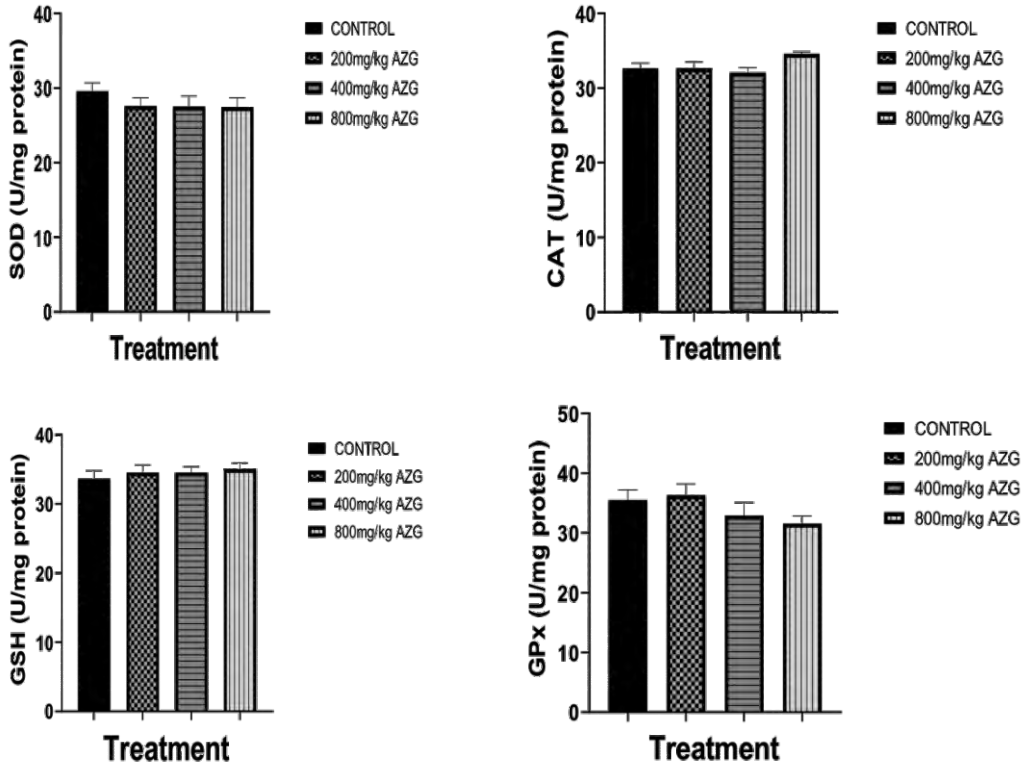


Figure 13: Effect of AZG-HE on (a) heart SOD level, (b) heart CAT level, (c) heart GSH level, and (d) heart GPx level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

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

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

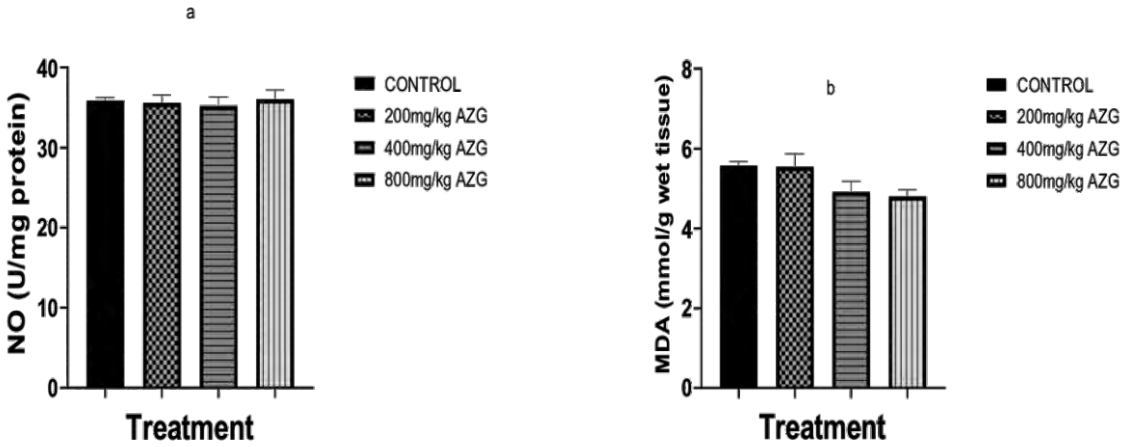


Figure 14*:* Effect of AZG-HE on heart homogenate (a) NO level, and (b) MDA level in rats after 60 days oral administration
p >0.05 in comparison to untreated group

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

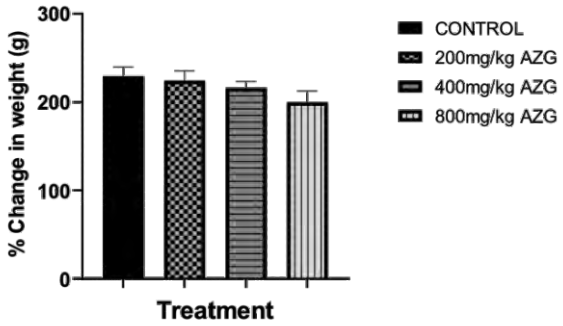


Figure 15: Effect of AZG-HE on body in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

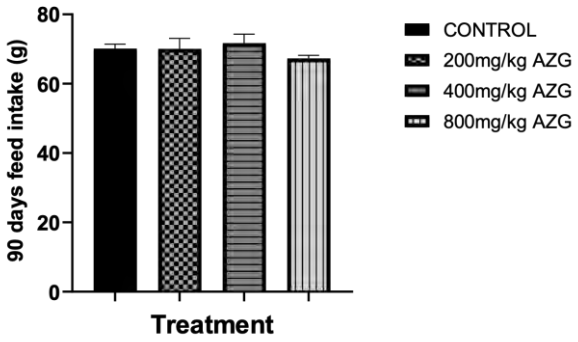


Figure 16: Effect of AZG-HE on feed intake in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

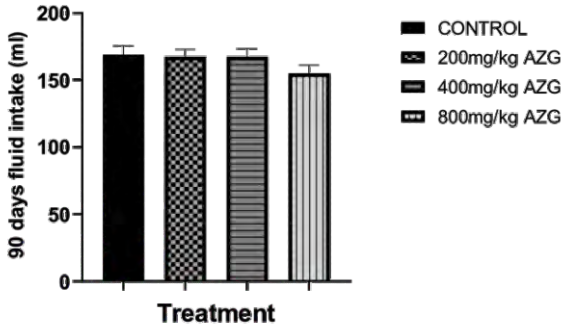


Figure 17: Effect of AZG-HE on fluid intake in Wistar rats after 90 days
p >0.05 in comparison to untreated group

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

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

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

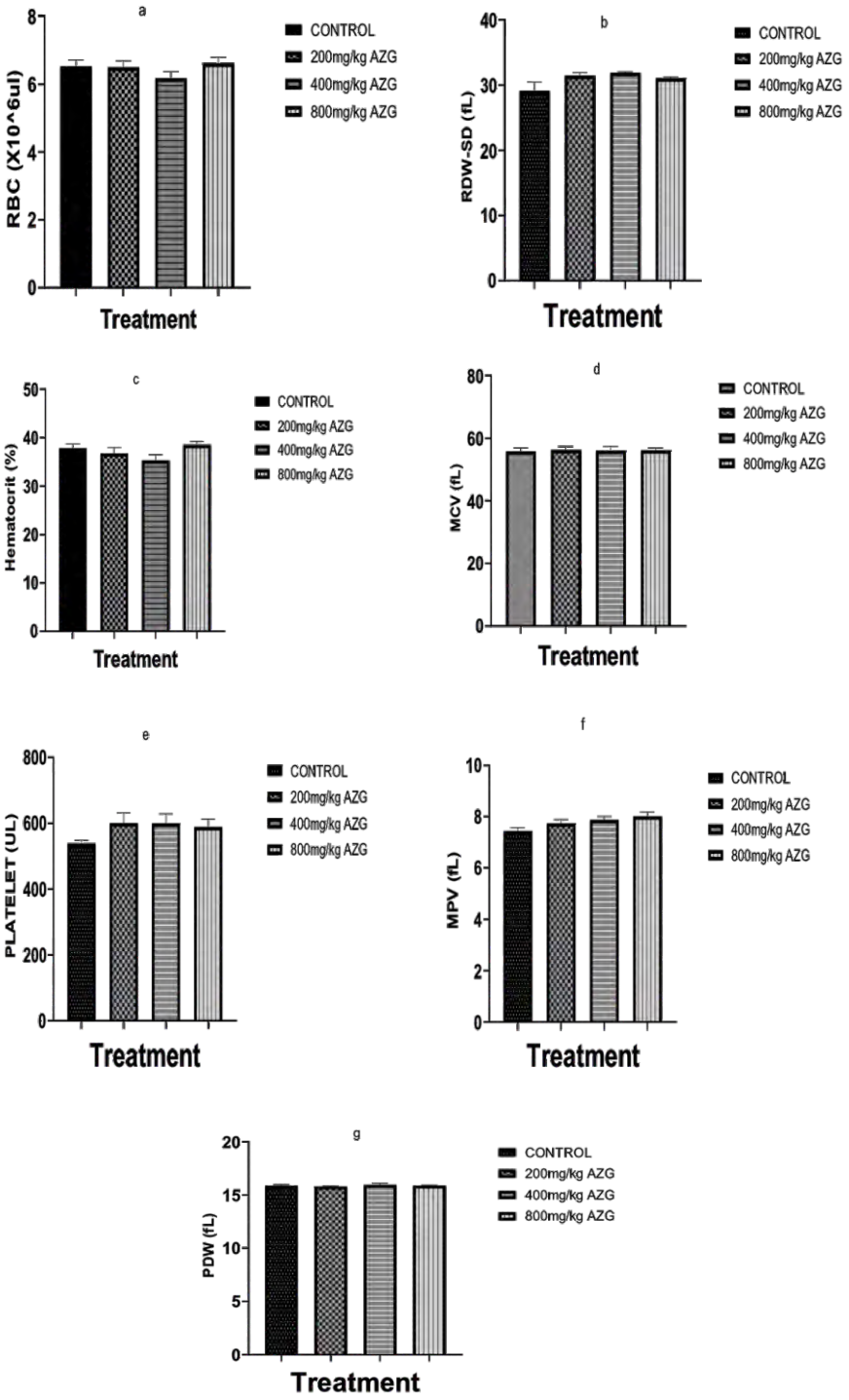


Figure 18*:* Effect of AZG-HE on (a) RBC count, (b) RDW-SD, (c) hematocrit, (d) MCV, (e) PLT count, (f) MPV, (g) PDW in rats after 90 days oral administration
p >0.05 in comparison to untreated group

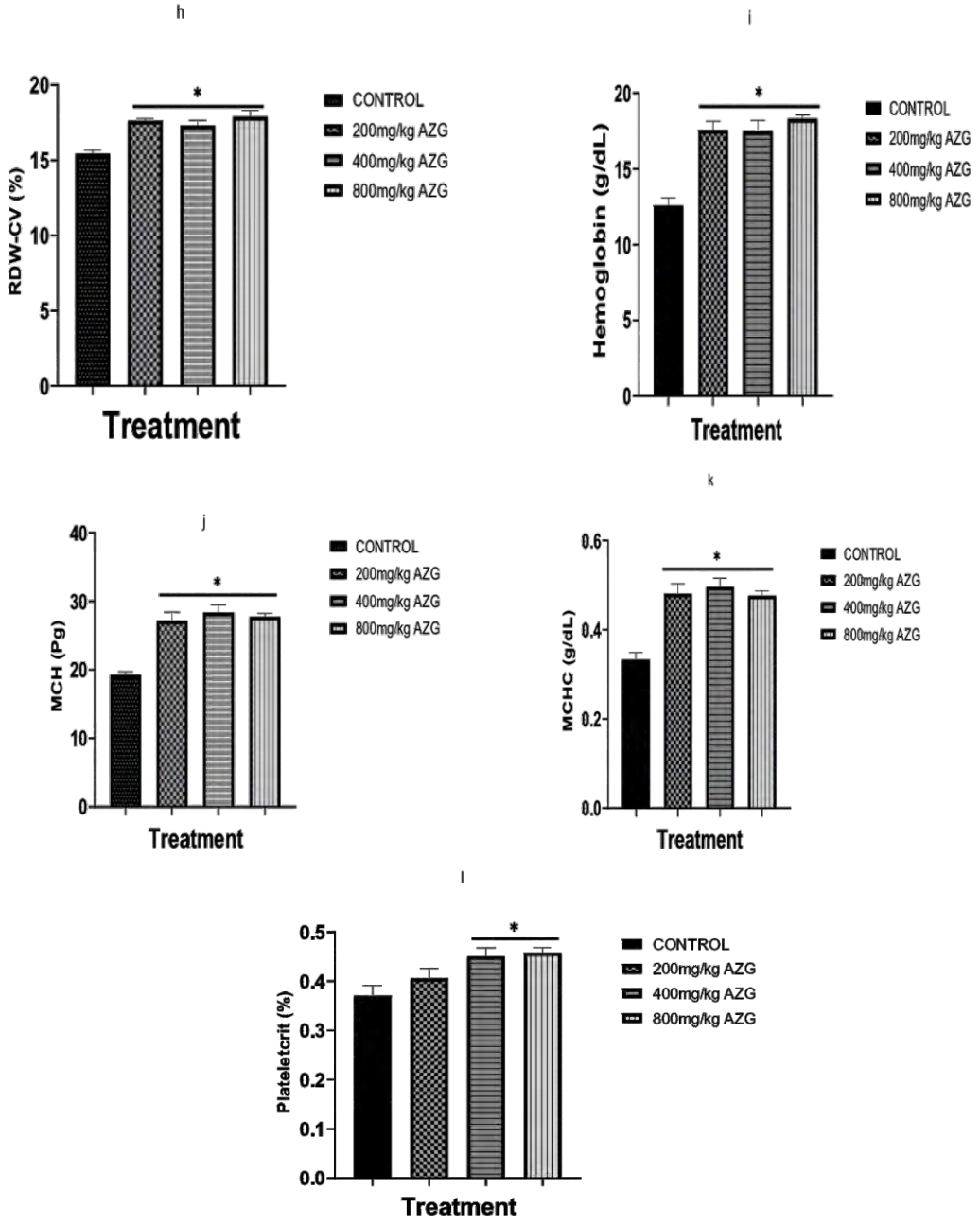


Figure 18*:* Effect of AZG-HE on (h) RDW-CV, (i) HB level, (j) MCH levels, (k) MCHC and (l) PCT in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

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

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

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

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

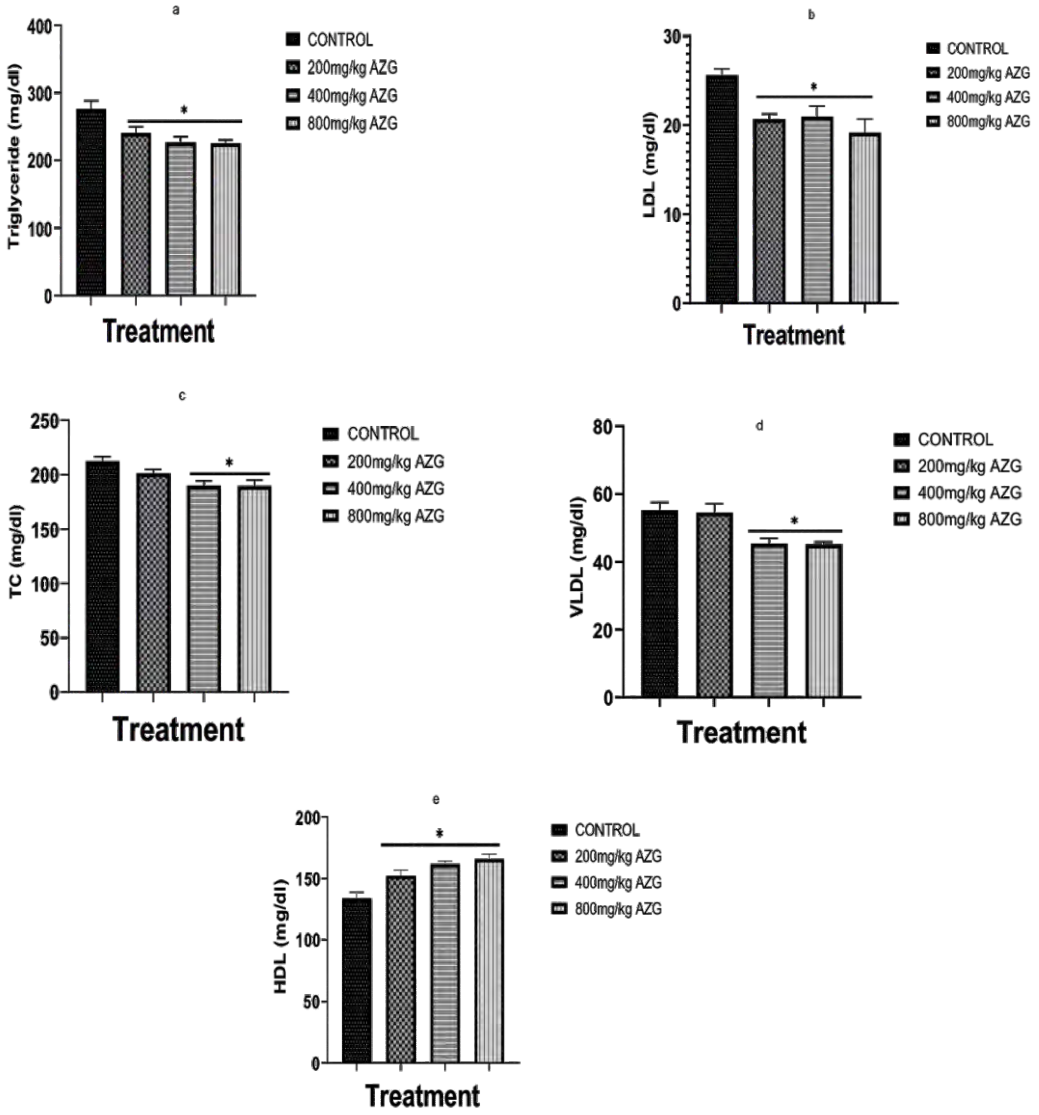


Figure 19*:* Effect of AZG-HE on (a) TG level, (b) TC level, (c) HDL level, (d) LDL level, (e) VLDL in rats after 90 days oral administration
* $p<0.05$ in comparison to untreated group

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

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

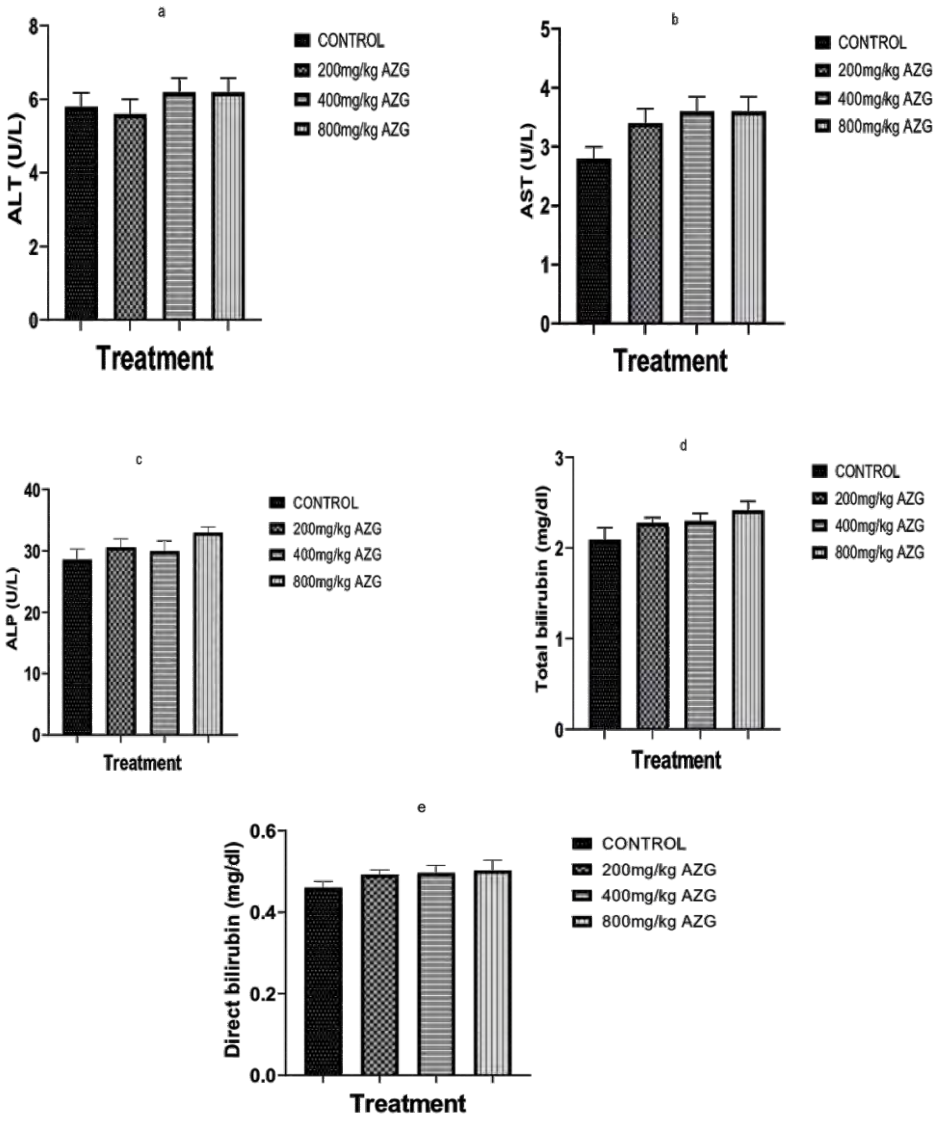


Figure 20*:* Effect of AZG-HE on (a) ALT, (b) AST, (c) ALP, (d) T.bil, and (e) D.bil levels in rats after 90 days oral
p >0.05 in comparison to untreated group

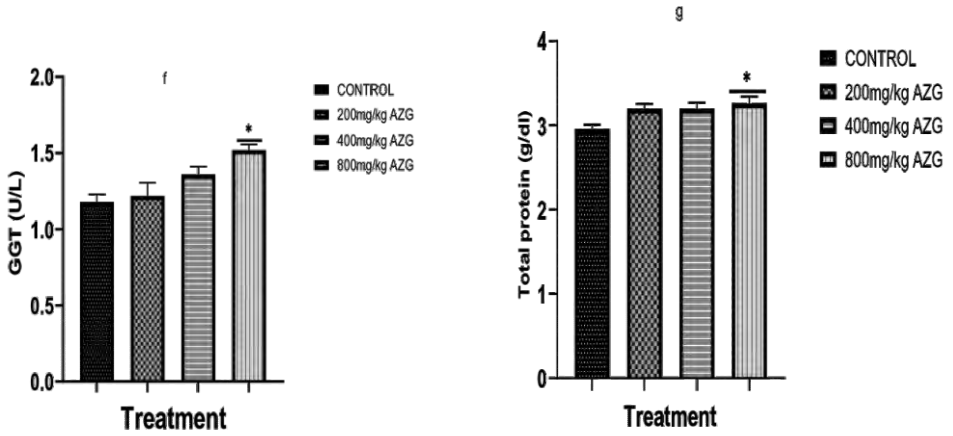


Figure 20*:* Effect of AZG-HE on (f) gamma-glutamyl transferase level and (g) total protein level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

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

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

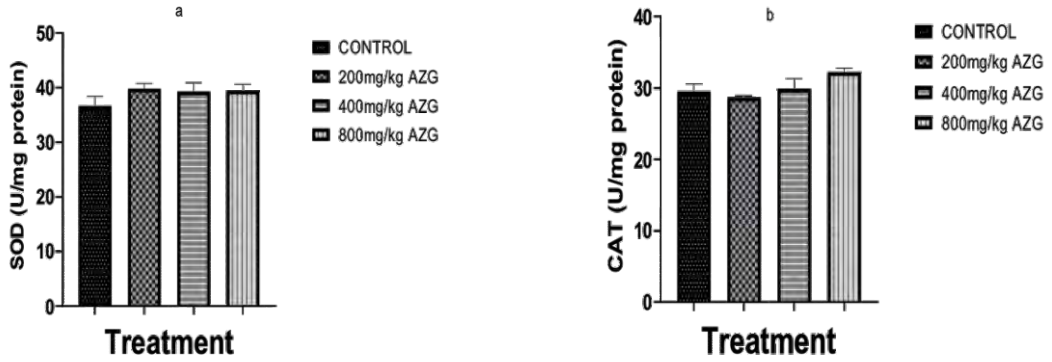


Figure 21*:* Effect of AZG-HE on (a) liver SOD level, (b) liver CAT level in rats after 90 days oral administration
p>0.05 in comparison to untreated group

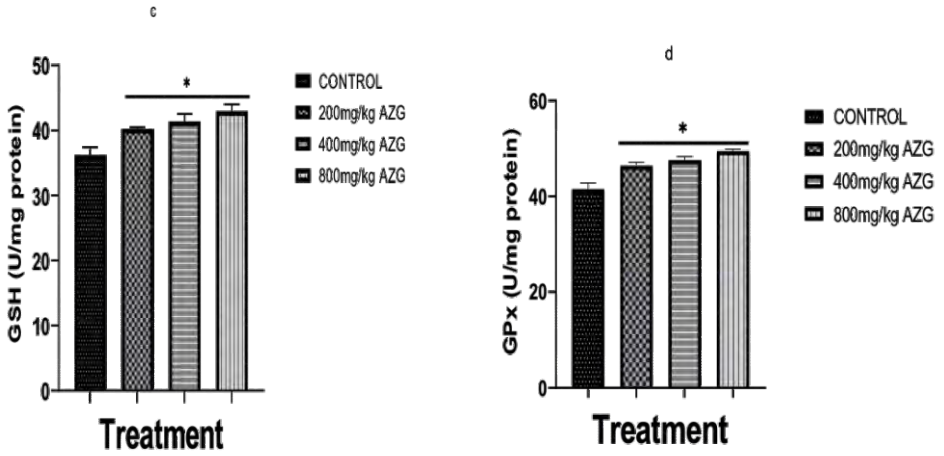


Figure 21*:* Effect of AZG-HE on (c) liver glutathione level, (d) liver glutathione peroxidase level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

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

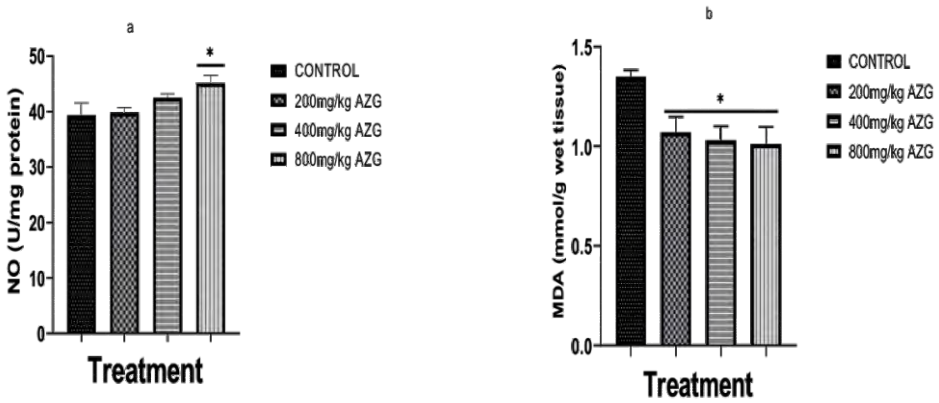


Figure 22*:* Effect of AZG-HE on (a) liver NO level, (b) liver MDA level in rats after 90 days oral administration
* p <0.05 in comparison untreated group

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

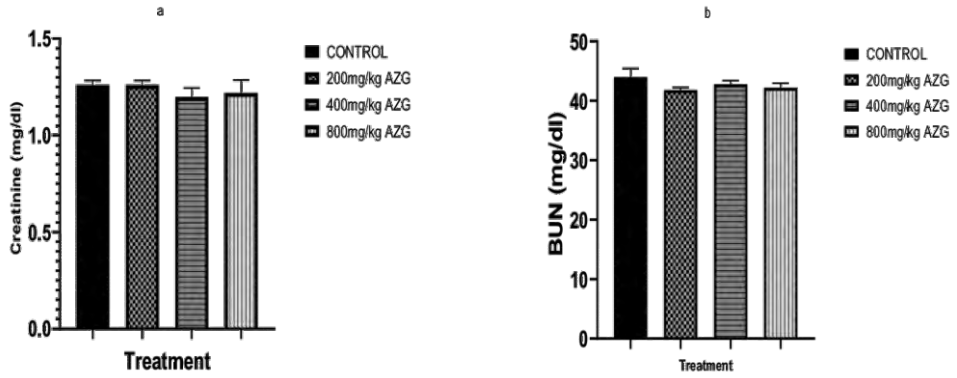
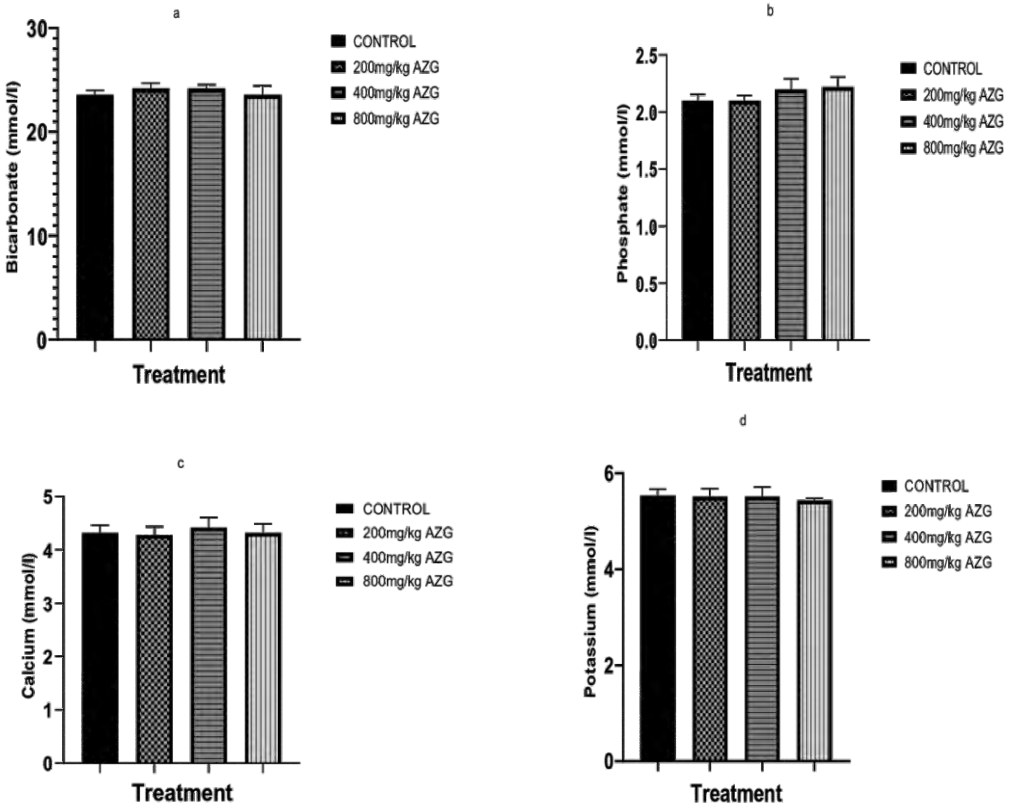


Figure 22*:* Effect of AZG-HE on (a) serum creatinine, (b) BUN level in rats after 90 days oral administration
p >0.05 compared to untreated group

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



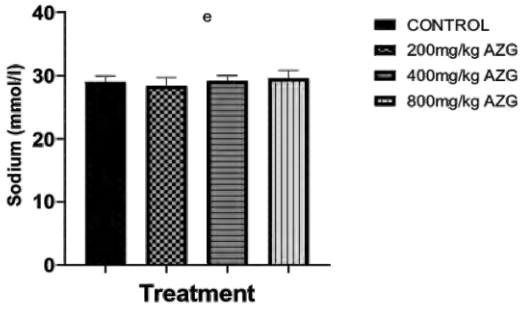


Figure 23*:* Effect of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum potassium level, (e) serum sodium level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

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

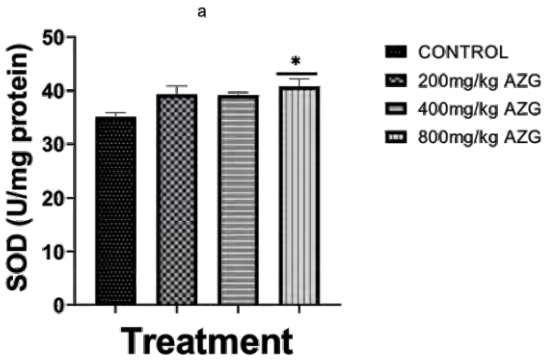


Figure 24a*:* Effect of AZG-HE on kidney SOD level in rats after 90 days oral administration
* p <0.05 in comparison to untreated group

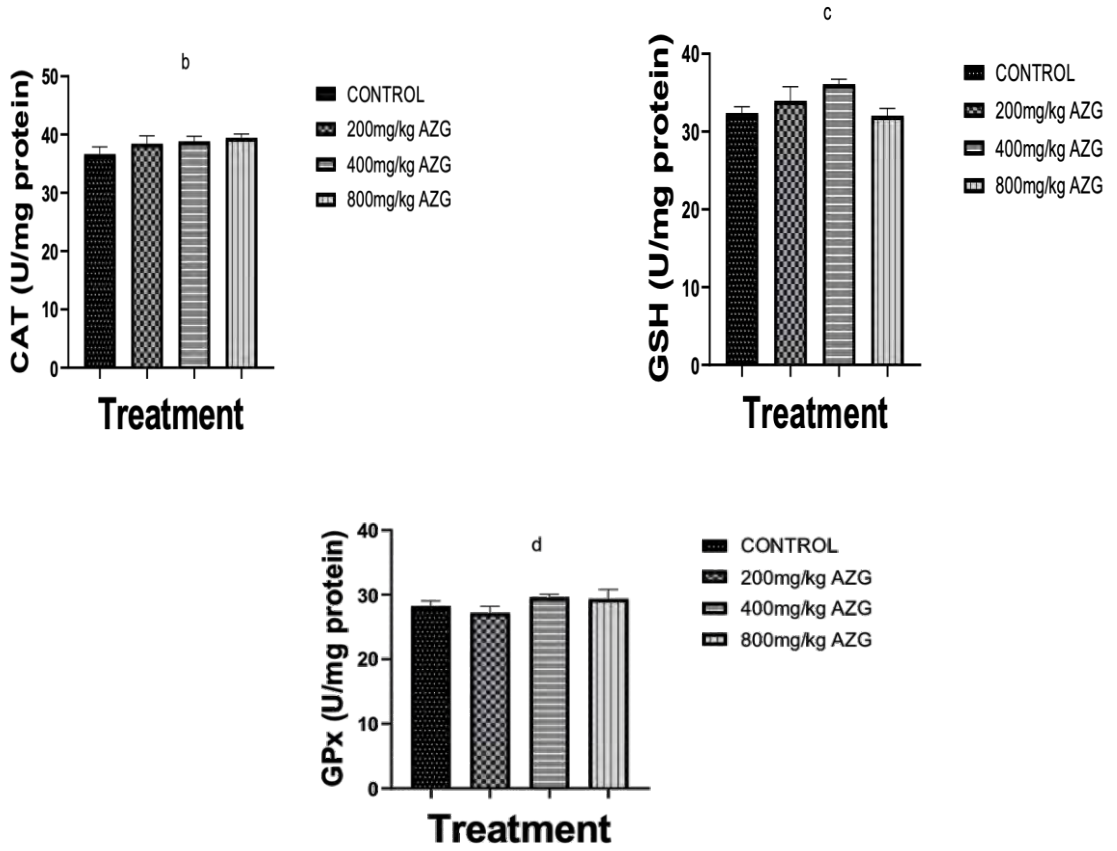


Figure 24*:* Effect of AZG-HE on (b) kidney catalase level, (c) kidney reduced glutathione level, (d) kidney glutathione peroxidase level in rats after 90 days oral administration
p >0.05 compared to untreated group

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

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

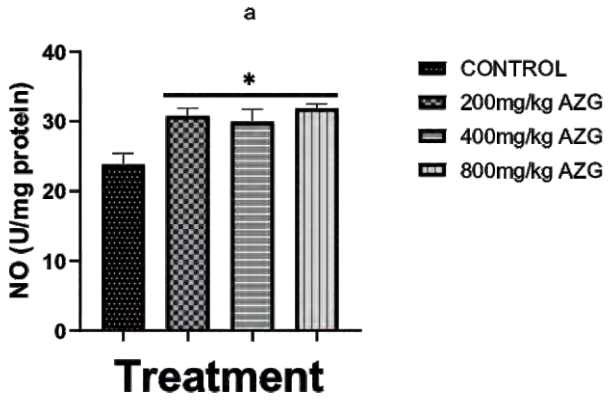


Figure 25a*:* Effect of AZG-HE on kidney NO level in rats after 90 days oral administration
*p <0.05 in comparison to untreated group

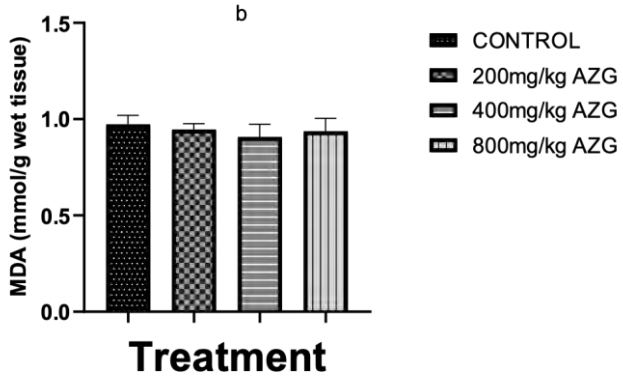


Figure 25b*:* Effect of AZG-HE on kidney MDA level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

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

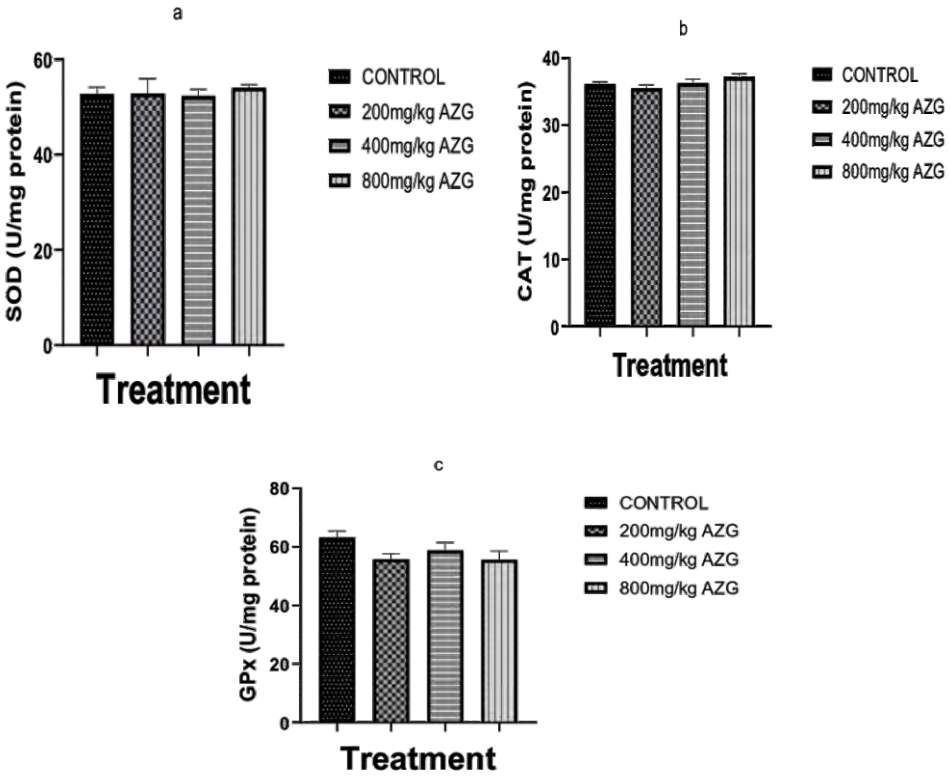


Figure 26*:* Effect of AZG-HE on (a) heart SOD level, (b) heart CAT level, (c) heart GPx level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

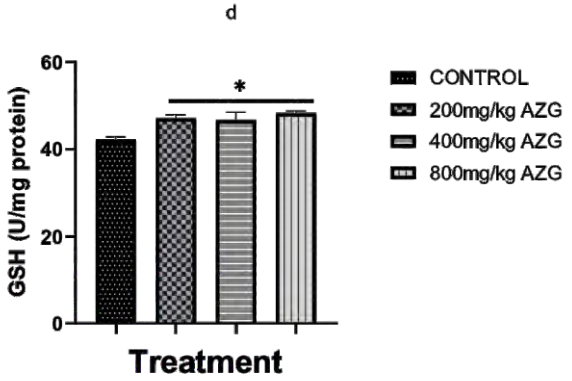


Figure 26d*:* Effect of AZG-HE on heart GSH level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

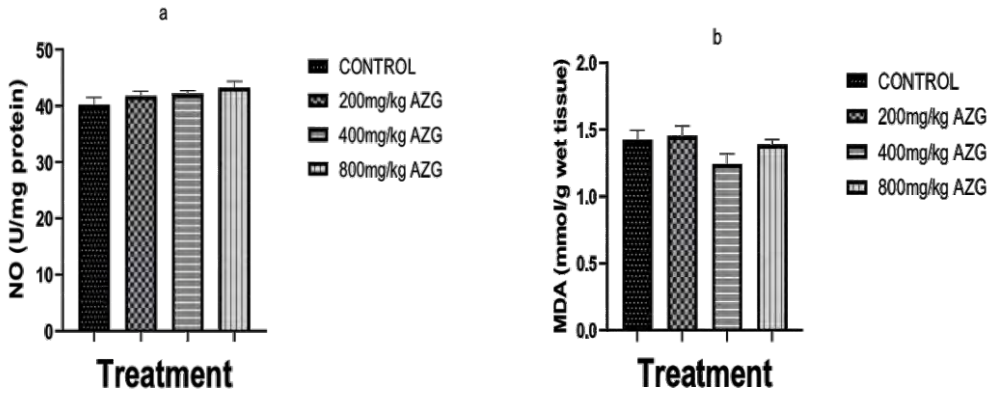


Figure 27*:* Effect of AZG-HE on (a) heart NO level and (b) heart MDA level in rats after 90 days oral administration
p >0.05 in comparison to untreated group

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

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

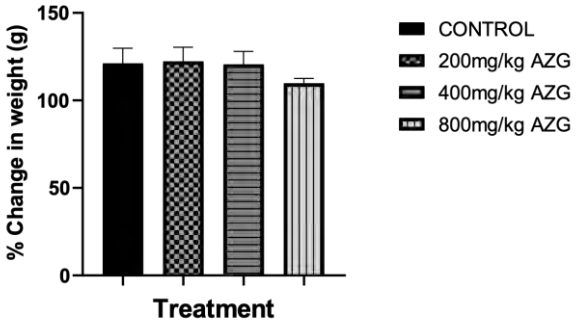


Figure 28: Effect of 14 days of toxicity reversibility of AZG-HE on body weight changes in rats
p >0.05 in comparison to untreated group

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

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

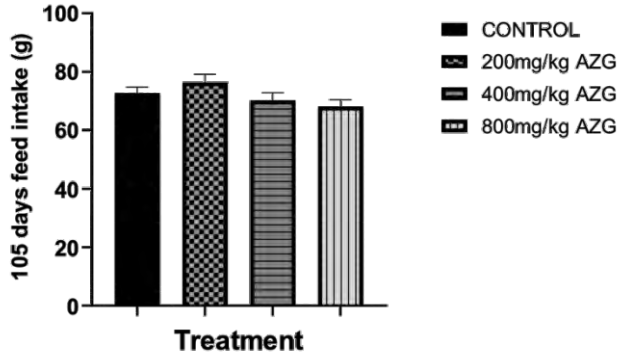


Figure 29: **Effect of 14 days of toxicity reversibility of AZG-HE on feed intake in rats**
p >0.05 in comparison to untreated group

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

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

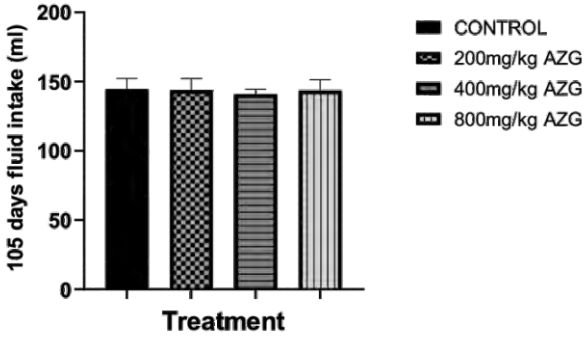


Figure 30: Effect of 14 days of toxicity reversibility of AZG-HE on fluid intake in rats
p >0.05 in comparison to untreated group

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

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

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

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

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

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

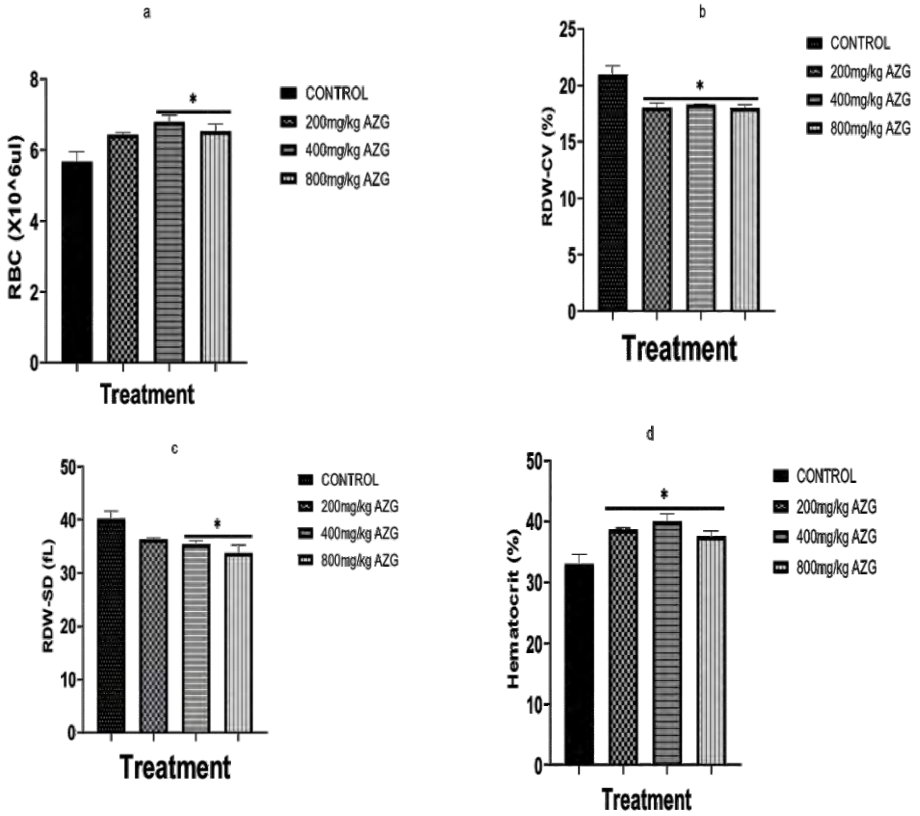
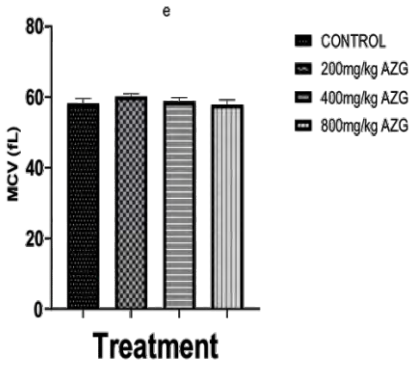
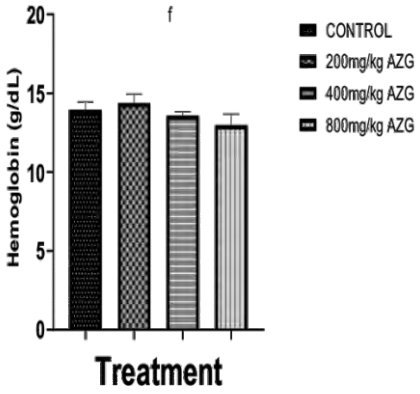
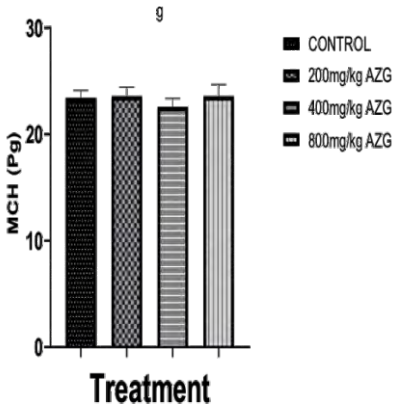
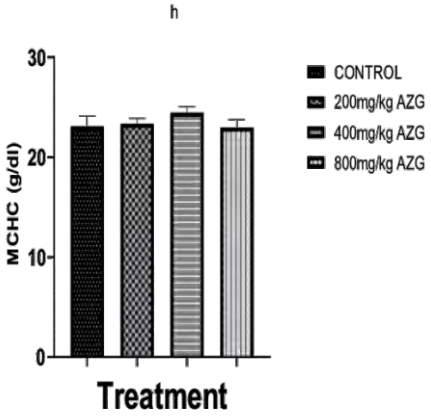


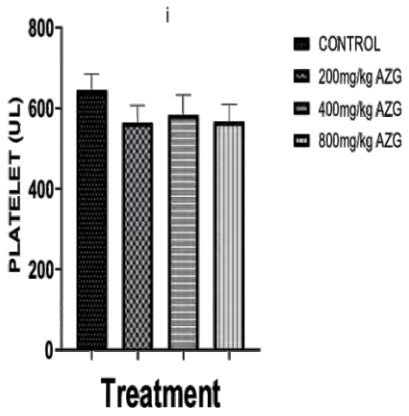
Figure 31*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) RBC count, (b) RDW-CV, (c) RDW-SD, (d) hematocrit in rats
* $p<0.05$ in comparison to untreated group

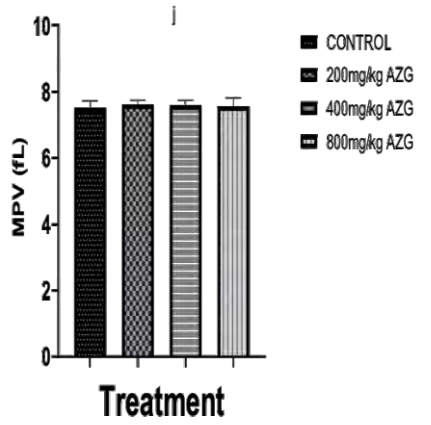

e
MCV (fL)
80
60
40
20
0
Treatment
CONTROL
200mg/kg AZG
400mg/kg AZG
800mg/kg AZG


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


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


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


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


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

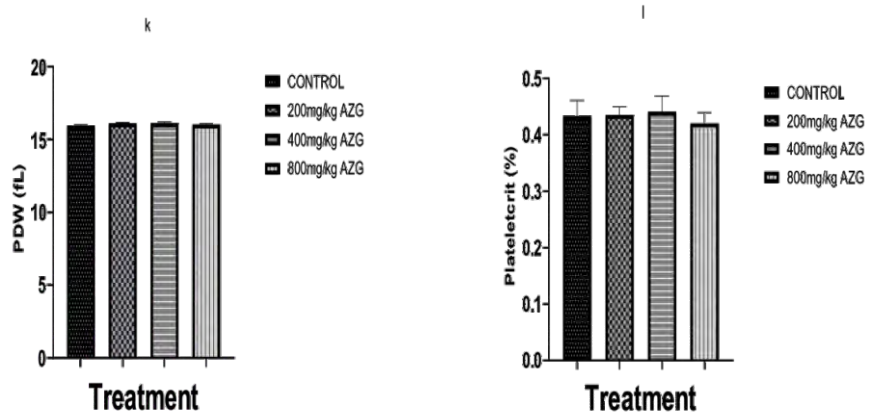


Figure 31*:* Effect of 14 days of toxicity reversibility of AZG-HE on (e) MCV, (f) HB level, (g) MCH, (h) MCHC, (i) PLT count, (j) MPV, (k) PDW, and (l) PCT in rats
p>0.05 in comparison to untreated group

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

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

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

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

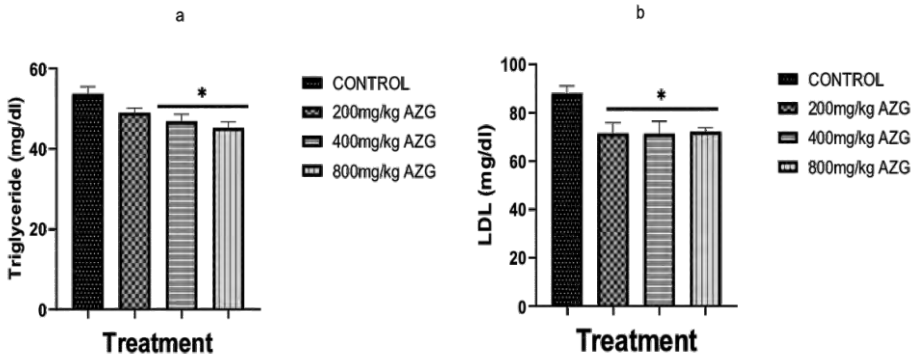


Figure 32: Effect of 14 days of toxicity reversibility of AZG-HE on (a) TG levels and (b) LDL in rats
*p <0.05 in comparison to untreated group

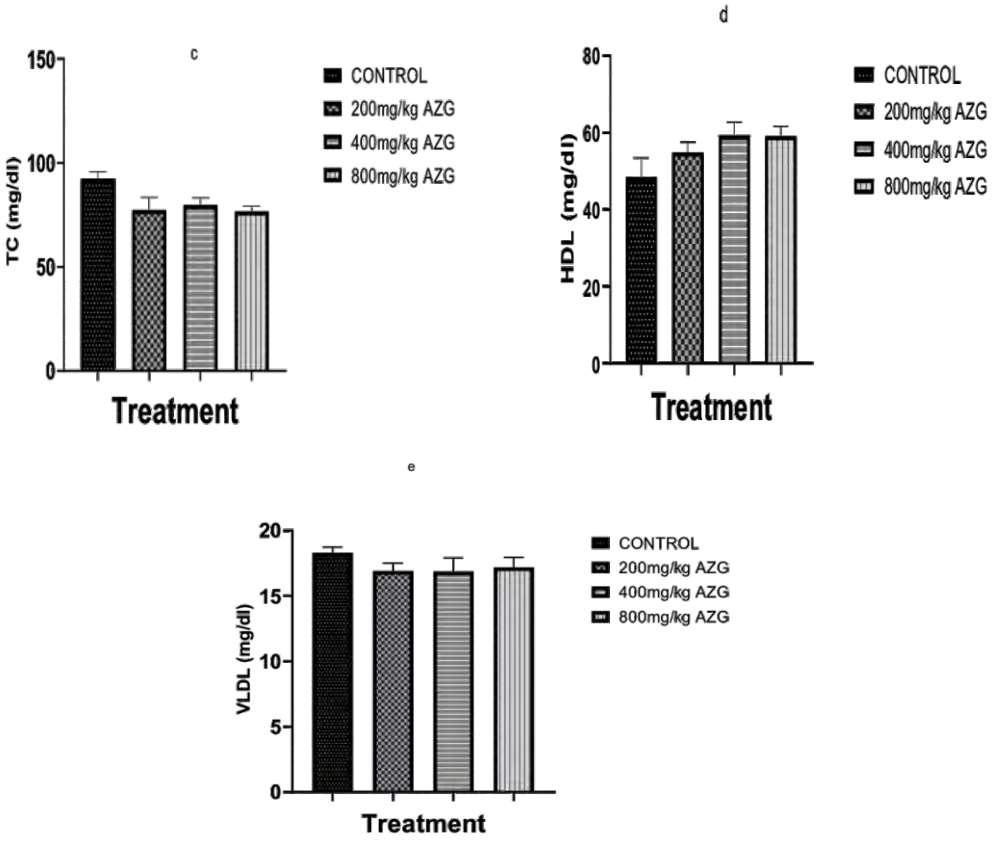
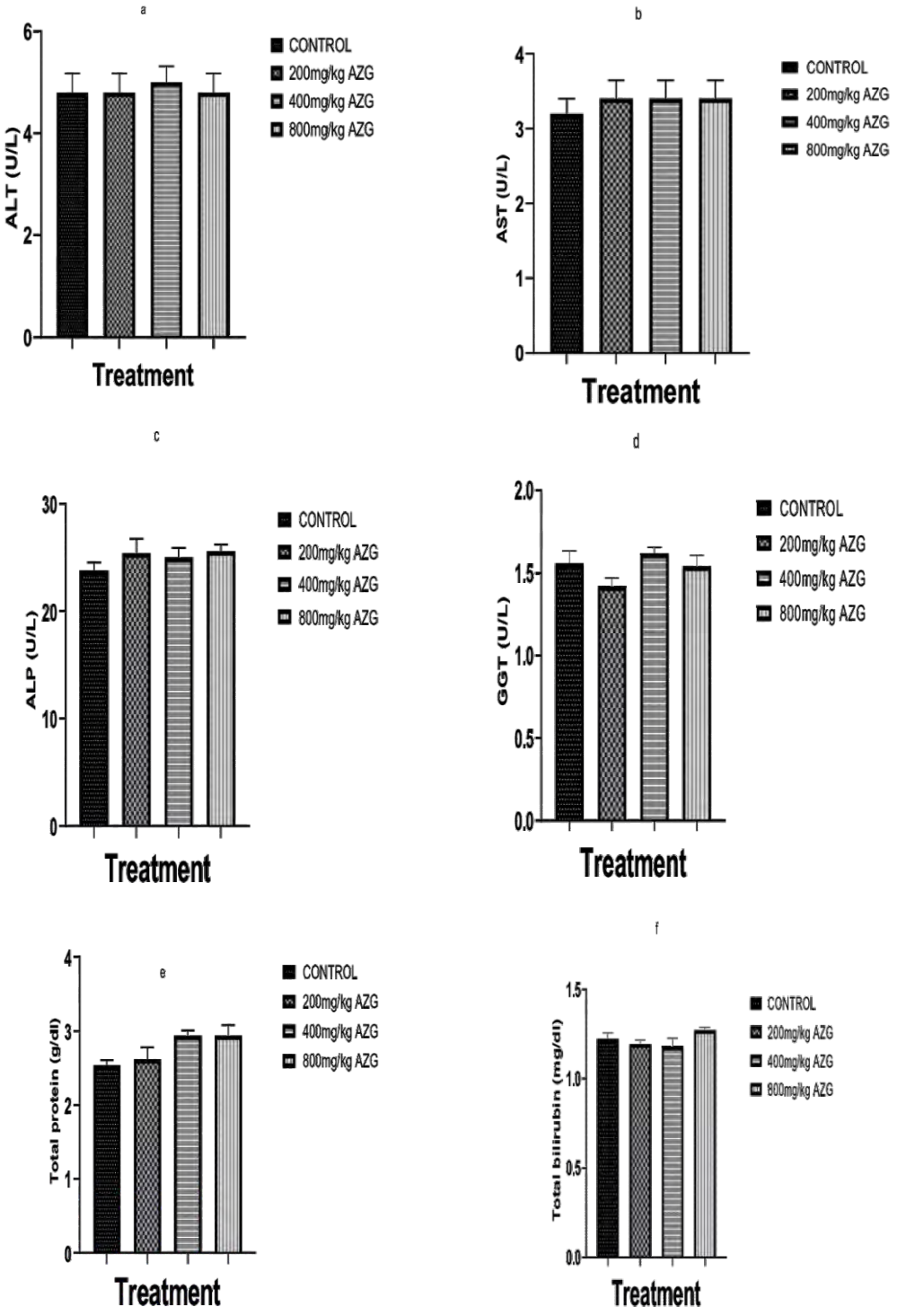


Figure 32*:* Effect of 14 days of toxicity reversibility of AZG-HE on (c) TC levels, (d) HDL levels, and (e) VLDL level in rats
p >0.05 in comparison to untreated group

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

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


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

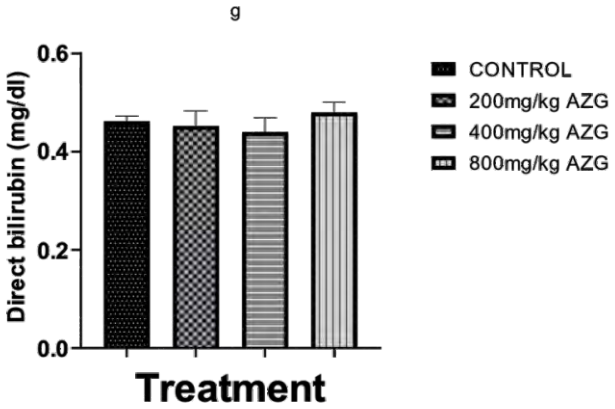
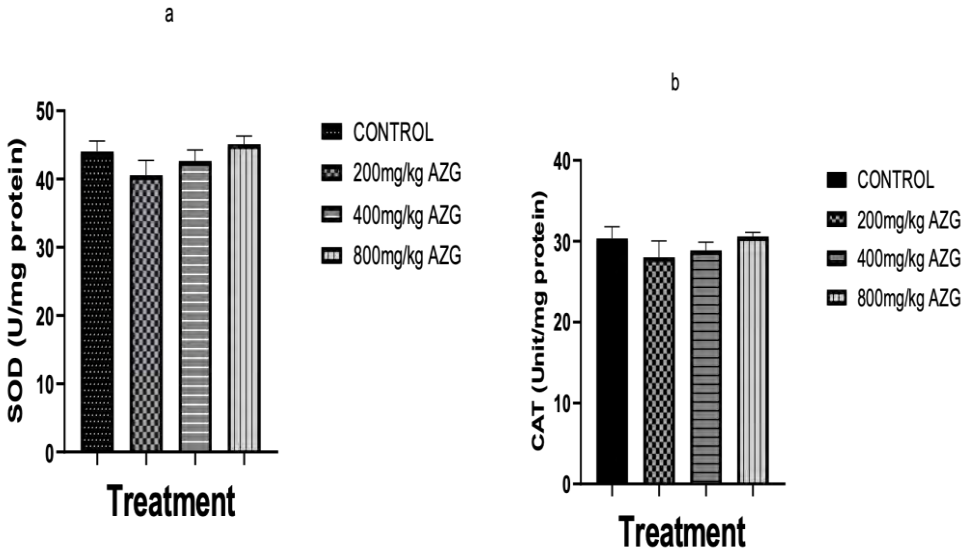


Figure 33: Effect of 14 days of toxicity reversibility of AZG-HE on (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) T.protein, (f) T.bil, and (g) D.bil levels in rats
p >0.05 in comparison to untreated group

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



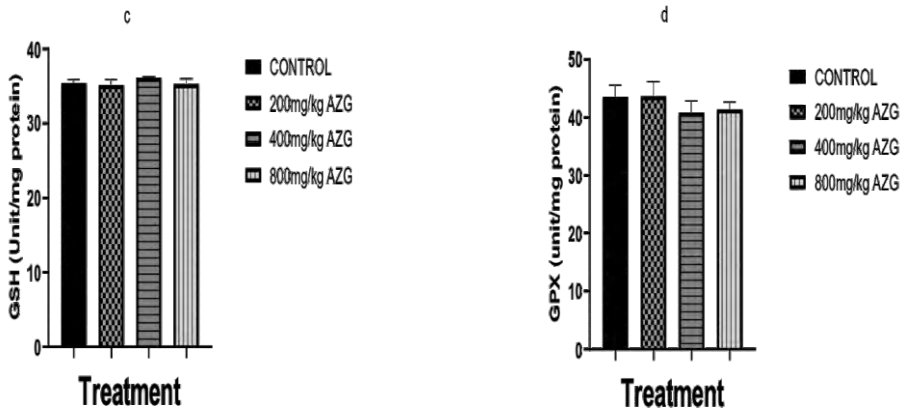


Figure 34*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) liver SOD level, (b) liver catalase level, (c) liver GHS level, (d) liver GPx level in rats
p >0.05 in comparison to untreated group

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

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

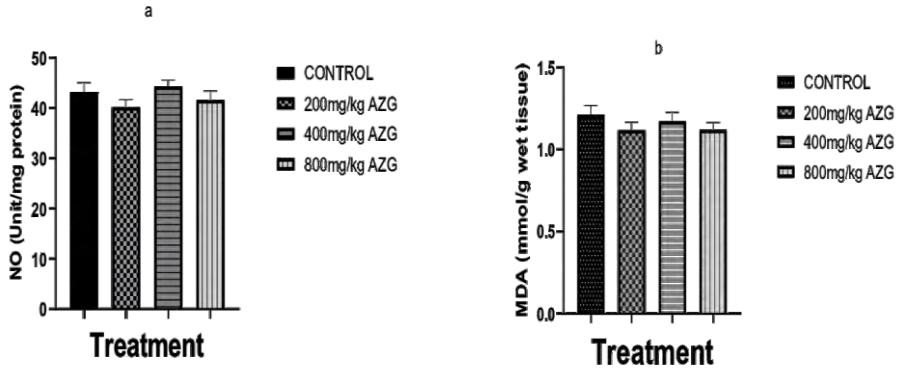


Figure 35*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) liver NO level and (b) liver MDA in rats
p >0.05 in comparison to untreated group

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

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

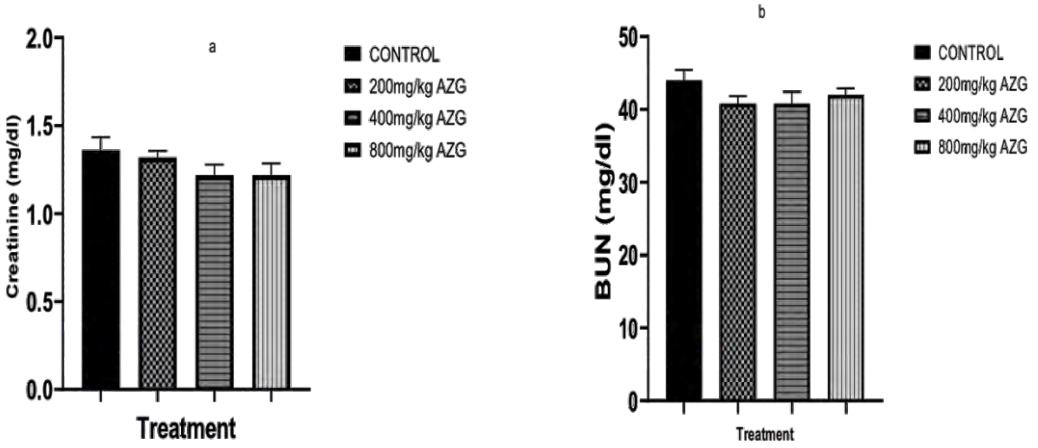
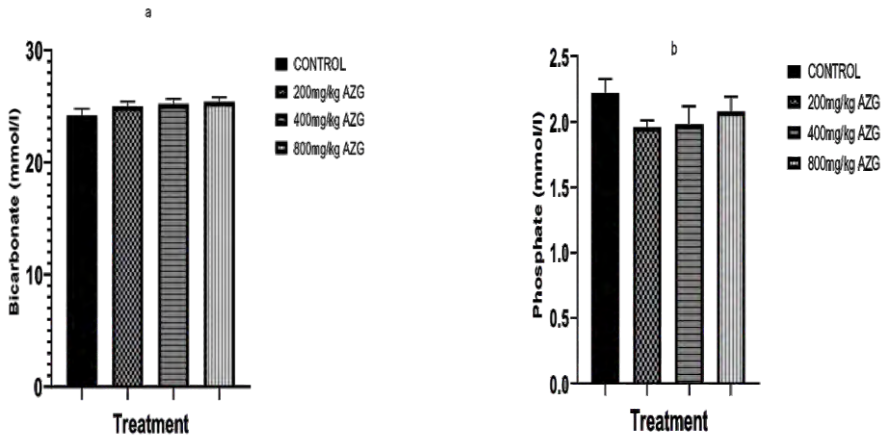


Figure 36: Effect of 14 days of toxicity reversibility of AZG-HE on (a) serum creatinine level and (b) BUN level in rats
p >0.05 in compared to untreated group

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

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



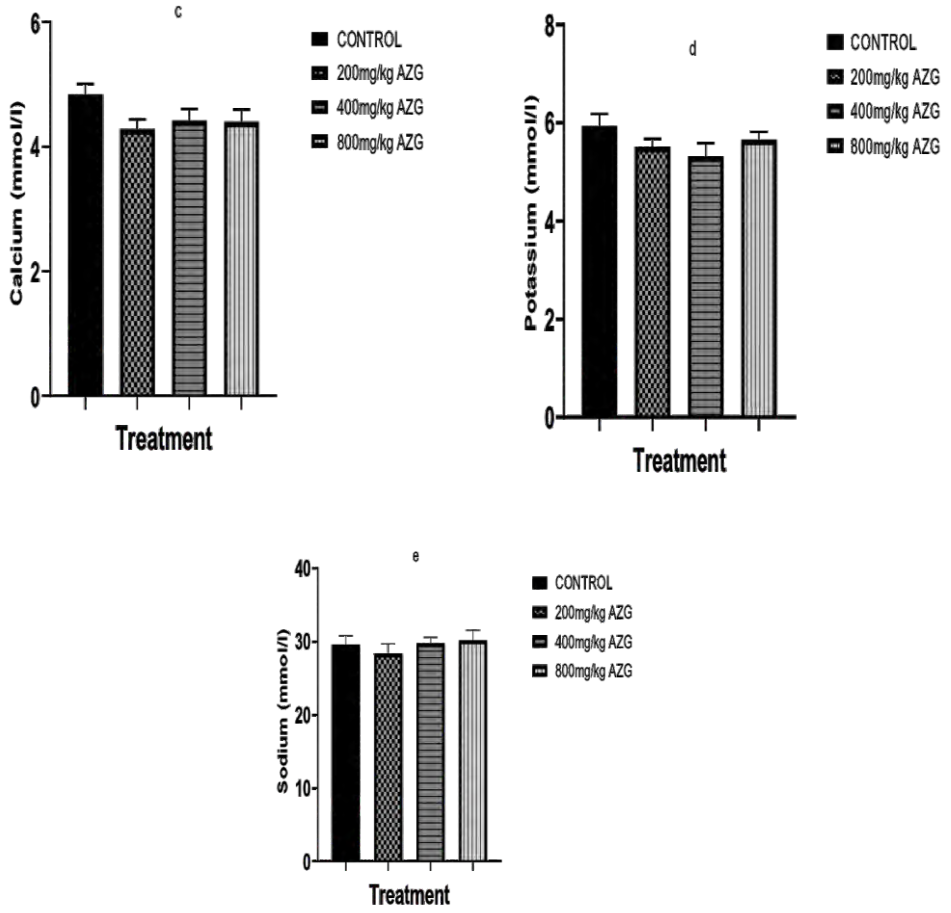


Figure 37: Effect of 14 days of toxicity reversibility of AZG-HE on (a) serum bicarbonate level, (b) serum Phosphate level, (c) serum calcium level, (d) serum potassium level, and (e) serum sodium level in Wistar rats after 105 days
p >0.05 in comparison to untreated group

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

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

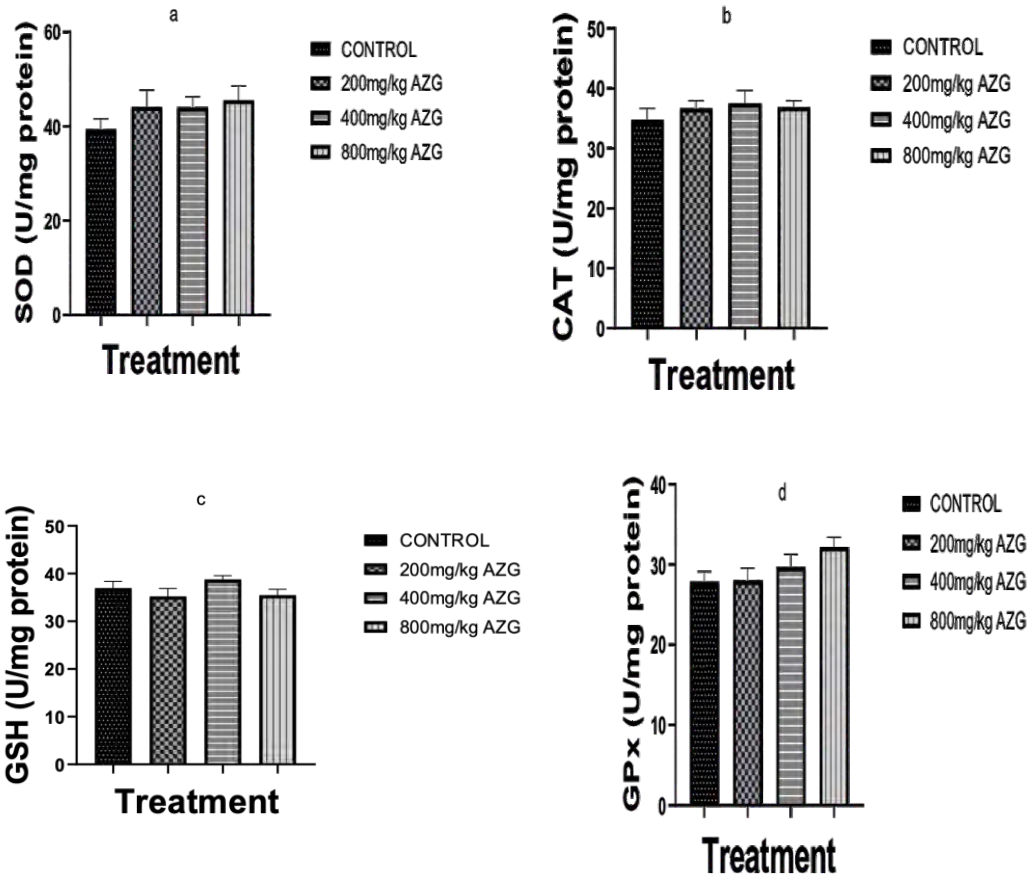


Figure 38: Effect of 14 days of toxicity reversibility of AZG-HE on (a) kidney SOD level, (b) kidney CAT level, (c) GSH level, and (d) kidney GPx level in rats
p >0.05 in comparison to untreated group

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

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

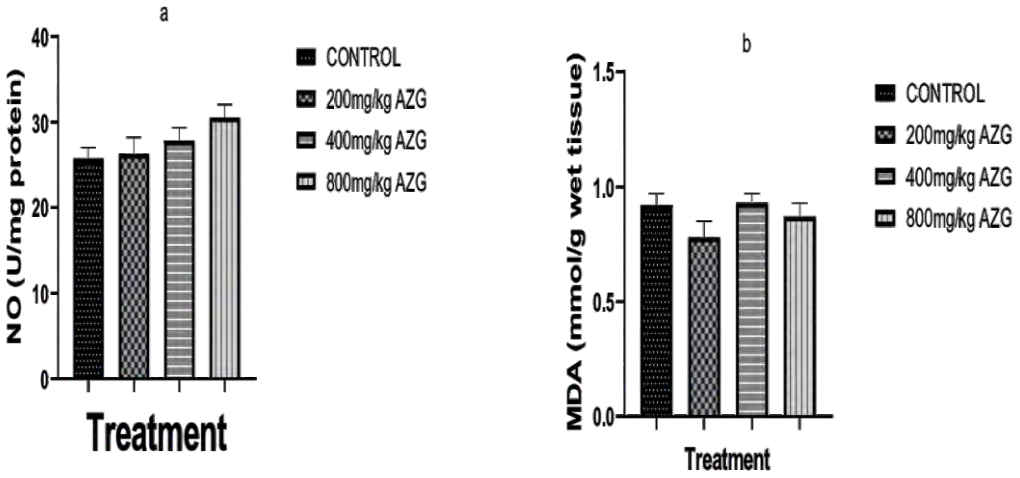
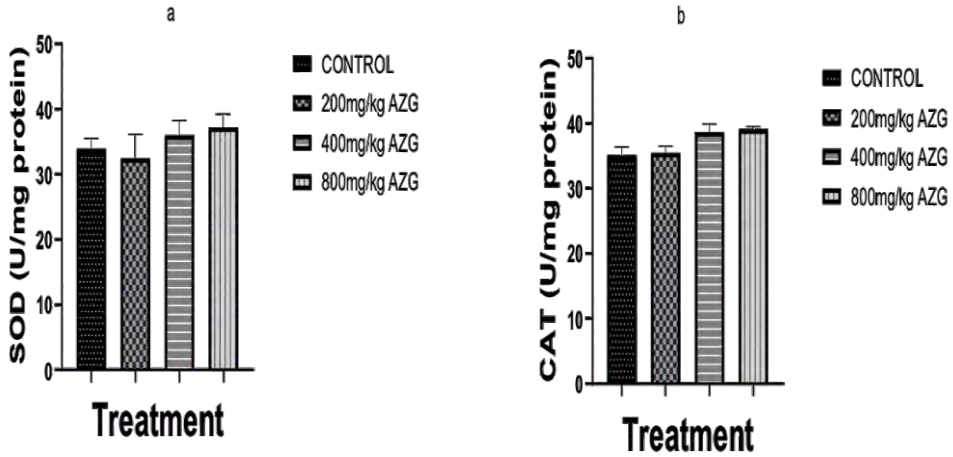


Figure 39*:* Effect of 14 days of toxicity reversibility of AZG-HE on (a) kidney NO level and (b) kidney MDA level in rats
p >0.05 in comparison to untreated group

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

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



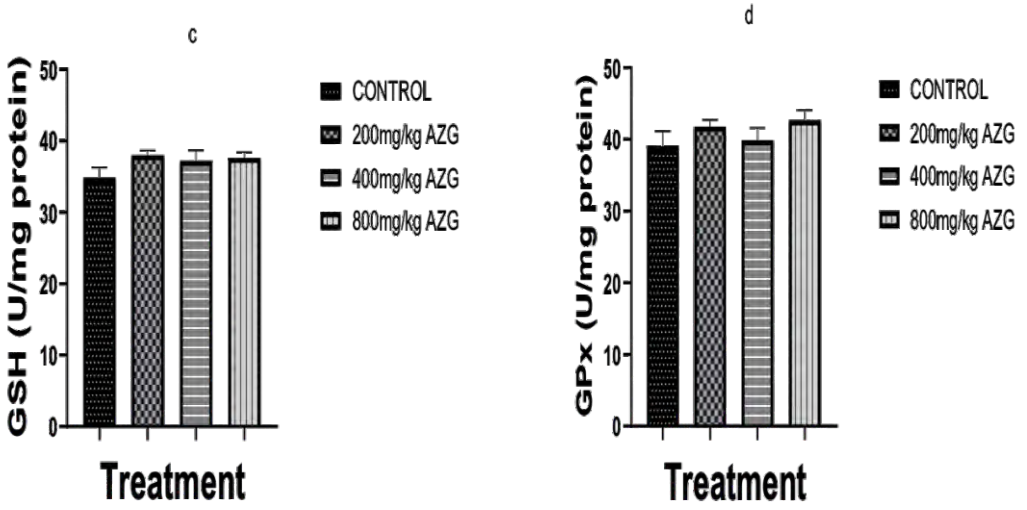


Figure 40: Effect of 14 days of toxicity reversibility of AZG-HE (a) heart SOD level, (b) heart CAT level, (c) heart GSH level, and (d) heart GPx level in rats

p >0.05 in comparison to untreated group

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

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

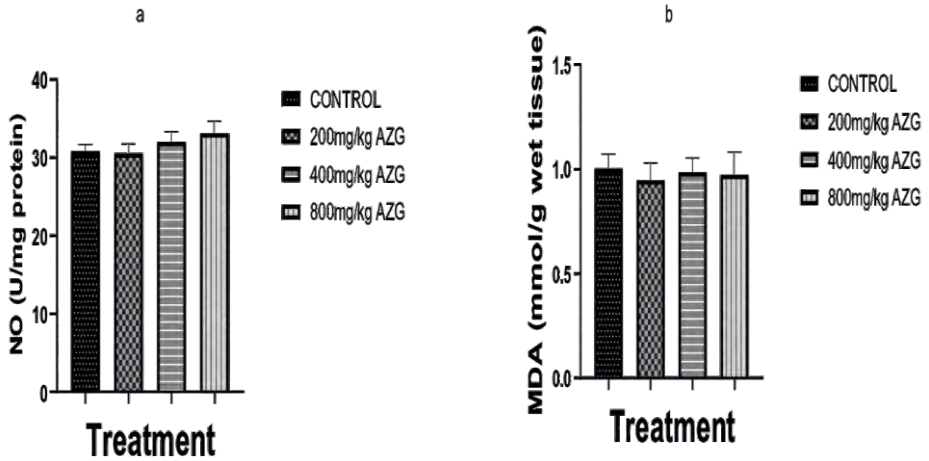


Figure 41: Effect of AZG-HE on (a) heart N0 level and (b) heart MDA in Wistar rats 15 days post administration

p >0.05 in comparison to untreated group

DISCUSSION

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

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

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

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

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

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

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

Conclusion

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

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