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Research Article

Effects of Methanol Extract of *Azanza garckeana* (Goron Tula) Fruit on Bisphenol A Induced Reproductive Toxicity in Adult Male Wistar Rats

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Abstract

Introduction: Infertility is increasingly linked to genetic, environmental, metabolic, and lifestyle factors. *Azanza garckeana* (AG), also known as "goron tula", is traditionally used for its aphrodisiac and energy-enhancing properties, though scientific evidence of its protective role against reproductive toxicity is limited.

Aims: This study investigated the protective and restorative effects of methanol extract of *Azanza garckeana* fruit against bisphenol A (BA)-induced reproductive toxicity in adult male Wistar rats.

Study Design: Thirty-six rats (weighing 200 - 250g) were randomly assigned into six groups (n = 6). Groups B and C were administered ethanol extract of *Azanza garckeana* at doses of 125 mg/kg and 250 mg/kg, respectively, Group D received 10 mg/kg Bisphenol A, Group E was co-administered with 10 mg/kg Bisphenol A and 125 mg/kg *Azanza garckeana*, while group F was co-administered with 10 mg/kg Bisphenol A and 250 mg/kg *Azanza garckeana*.

Place and Duration of Study: Department of anatomy, Ladoke Akintola University of technology, Ogbomoso, Oyo state Nigeria. Between January 2024 and June 2024.

Methodology: Treatments were administered orally for 56 days. Hormonal assays (testosterone, LH, FSH) were assessed from serum samples, sperm parameters, oxidative stress markers (SOD, MDA, GSH) were analyzed in testicular homogenate, and histological analyses of testes and epididymis were conducted. Data were analyzed using One way analysis of variance followed by Tukey Post-hoc test and $P < 0.05$ was taken as accepted level of significant difference.

Results: There was significant decrease in bodyweight (0.001), testicular weight (0.002) and also in both reproductive hormones, sperm parameters and antioxidant [LH ($P = 0.0337$), TH ($P = 0.0001$), FSH ($P = 0.0019$), rapid-progressive sperm motility ($P = 0.0493$), count ($P = 0.0001$), GSH ($P = 0.0001$), SOD (0.0001) and increase in MDA (0.0011) of group D while there was significant decrease in MDA level and increase in above parameters in group E and F when compared with control, histo-morphological degeneration of testes and epididymis was seen in group D characterized by distorted seminiferous tubules with irregular outlines, collagen accumulation and fibrotic remodeling within the interstitial spaces, while co-treated groups (E and F) presented normal testicular and epididymis histological architecture.

Conclusion: This study concluded that *Azanza garckeana* possesses protective and therapeutic potential against chemically induced male reproductive toxicity.

Keywords: *Epididymal Architecture; Antioxidant Activity; Hormonal Assays; Azanza garckeana*

Abbreviations

AG: *Azanza garckeana*; AG125g/kg: *Azanza garckeana* given at the dose of 125 g/kg; Ag250g/kg: *Azanza garckeana* given at the dose of 250 g/kg; BA: Bisphenol A; BA+AG125g/kg: Bisphenol A and *Azanza garckeana* given at the dose of 125g/kg; BA+AG250g/kg: Bisphenol A and *Azanza garckeana* given at the dose of 250g/kg; FSH: Follicle Stimulating Hormone; GSH: Glutathione; LH: Lutein Hormone; MDA: Malondialdehyde; SOD: Superoxide Dismutase; TEST: Testosterone

Introduction

Introduction should reflect the background, purpose and significant of the study that is carried out.

Reproductive health has emerged as a cornerstone of global public health priorities, yet impaired fecundity remains a persistent and profound medical and social challenge, affecting an estimated 8% to 12% of the population worldwide [1]. Clinically, male-related factors contribute to approximately 40% to 50% of all infertility cases, with roughly 5% of the male population presenting with suboptimal semen characteristics. The etiological landscape of infertility is highly heterogeneous and varies substantially across geographic and socio-cultural boundaries. In developing nations like Nigeria, this complexity is further compounded by vast disparities in ethnicity, cultural practices, religious beliefs, and uneven access to modern educational and healthcare infrastructures [1].

At the cellular level, critical spermatozoa parameters-specifically count, motility, and viability-are exceptionally susceptible to oxidative injury mediated by free radicals or reactive oxygen species (ROS), which are known to negatively impact male fertility. An overproduction of these volatile radical species inflicts systemic damage across multiple physiological systems, triggering lipid peroxidation of cellular membranes, disrupting structural DNA, and inducing severe oxidative stress that compromises tissue integrity and drives male factor infertility [2].

Concurrently, ubiquitous environmental factors significantly exacerbate these oxidative pathways. Bisphenol-A (BPA) stands as one of the most heavily manufactured industrial chemicals globally, serving as a primary monomer in the synthesis of polycarbonate plastics and epoxy resins [1]. Due to its widespread utility, human exposure to BPA is virtually inevitable, with measurable concentrations detected in everyday consumer goods, canned food linings, micro plastics, and household dust. Emerging toxicological evidence flags BPA as a potent reproductive hazard. Because of its cost-effectiveness, industrial reliance on BPA for food packaging and commercial manufacturing continues to rise globally, expanding the footprint of environmental exposure and heightening its risk as a disruptive reproductive toxicant [3].

To counteract such environmental insults, attention has increasingly shifted toward ethnobotanical interventions. According to the World Health Organization (WHO), nearly 80% of populations in developing economies depend on plant-derived natural products to meet their primary healthcare needs, and over 20% of modern standardized pharmaceuticals are derived directly from phytochemical origins [5]. *Azanza garckeana* (AG), colloquially designated as "goron tula" in Hausa or the "African chewing gum", is an invaluable medicinal and dietary plant indigenous to several ecological zones in Northern Nigeria, including Gombe, Katsina, and Adamawa states [5]. Beyond its versatile domestic value as fodder, fuel, and localized timber, its fruit pulp is widely masticated or processed into syrups and porridges. In traditional medicine, its roots, leaves, and fruits are employed to treat coughs, alleviate internal pains, and notably, serve as a potent aphrodisiac and energy booster to enhance sexual performance [6,7].

Biochemically, *Azanza garckeana* possesses a robust nutritional and phytochemical profile. The fruit pulp is characterized by a slightly acidic pH (5.96) and contains substantial crude protein (12.0%), essential mineral ions-predominantly potassium, phosphorus, and magnesium-and vital micronutrients like ascorbic acid (20.5 mg/100g) [8]. Crucially, the plant is rich in bioactive secondary metabolites, including phenols and flavonoids, which exhibit powerful radical-scavenging capabilities to neutralize ROS, safeguard cellular membranes, and prevent oxidative DNA fragmentation [9].

Given that current therapeutic paradigms seek sustainable, natural agents capable of mitigating xenobiotic-induced gonadal damage, exploring the bioactivity of this plant is highly warranted.

Aim of the Study

The present study was designed to investigate the protective and restorative effects of a methanol extract of *Azanza garckeana* fruit against Bisphenol-A-induced reproductive toxicity in adult male Wistar rat model.

Materials and Methods

COLLECTION AND METHANOL EXTRACTION OF PLANT MATERIAL

AZANZA GARCKEANA FRUITS WAS HARVESTED FROM KALTUNGO LOCAL GOVERNMENT IN GOMBE STATE, NIGERIA THE PLANT WAS IDENTIFIED AND AUTHENTICATED BY PROFESSOR A. J. OGUNKUNLE, A TAXONOMIST FROM THE DEPARTMENT OF PURE AND APPLIED BIOLOGY, LADOKE AKINTOLA UNIVERSITY OF TECHNOLOGY, OGBOMOSO, OYO STATE, NIGERIA. THE FRUITS WERE AIR-DRIED AT ROOM TEMPERATURE AND SUBSEQUENTLY GROUND INTO A POWDERED FORM. COLD MACERATION EXTRACTION WAS PERFORMED USING SOXHLET EXTRACTION METHOD WITH METHANOL AS THE SOLVENT OF EXTRACTION, AFTER WHICH THE FILTRATE IS CONCENTRATED TO DRYNESS TO OBTAIN METHANOL EXTRACT OF *AZANZA GARCKEANA* FRUIT [10].

FIVE HUNDRED GRAMS (500G) OF DRIED AND POWDERED FRUIT PULP OF *AZANZA GARCKEANA* WERE EXHAUSTIVELY MACERATED IN 100% METHANOL FOR 72 HOURS. THE MIXTURE WAS THEN FILTERED USING A BÜCHNER FUNNEL AND WHATMAN NO.1 FILTER PAPER. THE RESULTING FILTRATE WAS CONCENTRATED UNDER REDUCED PRESSURE AT 40°C AND STORED AT ROOM TEMPERATURE, BETWEEN 40 - 60°C. THE EXTRACT WAS FURTHER EVAPORATED INTO THE EXTRACTION COLUMN ALONG WITH THE SAMPLE, AFTER WHICH IT WAS ALLOWED TO SIPHON AND CONCENTRATE.

ANIMALS MATERIAL

THIRTY-SIX (36) HEALTHY ADULT MALE WISTAR RATS WEIGHING 200 - 250G WERE OBTAINED FROM CALVARY BREEDS ANIMAL HOUSE IN OGBOMOSO. THE ADULT WISTAR RATS WERE FED WITH RAT CHOP, GIVEN DISTILLED WATER AD LIBITUM AND ALSO KEPT AND MAINTAINED IN THE LABORATORY FOR THREE WEEKS TO ACCLIMATIZE PRIOR TO THE STUDY. THE WEIGHT OF EXPERIMENTAL RATS WERE CONTINUALLY TAKEN THROUGHOUT THE EXPERIMENTAL PERIOD. ETHICAL APPROVAL FOR THE RESEARCH WAS OBTAINED FROM THE ETHICAL RESEARCH COMMITTEE OF THE FACULTY OF BASIC MEDICAL SCIENCES, LADOKE AKINTOLA UNIVERSITY OF TECHNOLOGY (APPROVAL NUMBER: ERCFBMSLAUTECH: 060/08/2024). ALL EXPERIMENTAL PROCEDURES WERE CONDUCTED IN COMPLIANCE WITH THE GUIDELINES ESTABLISHED BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC). THE WISTAR RATS WERE TREATED IN ACCORDANCE WITH THE; GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS COMPLIED BY THE NATIONAL ACADEMY OF SCIENCE AND PUBLISHED BY THE NATIONAL INSTITUTE OF HEALTH (1992).

EXPERIMENTAL DESIGN

A TOTAL OF THIRTY-SIX (36) ADULT MALE WEIGHING BETWEEN 200-250G, WERE RANDOMLY ASSIGNED TO SIX GROUPS, WITH SIX ANIMALS PER GROUP (N = 6). GROUP A SERVED AS THE CONTROL GROUP AND WAS FED WITH STANDARD FEED AND WATER AD LIBITUM. GROUPS B AND C WERE ADMINISTERED ETHANOL EXTRACT OF *AZANZA GARCKEANA* AT DOSES OF 125 MG/KG AND 250 MG/KG, RESPECTIVELY. GROUP D RECEIVED BISPHENOL A AT 10 MG/KG. GROUP E WAS CO-ADMINISTERED BISPHENOL A AT 10 MG/KG AND OF *AZANZA GARCKEANA* AT 125 MG/KG, WHILE GROUP F RECEIVED CO-ADMINISTRATION OF BISPHENOL A AT 10 MG/KG AND OF *AZANZA GARCKEANA* AT 250 MG/KG. THE ADMINISTRATION OF *AZANZA GARCKEANA* AND BISPHENOL A WERE DONE SIMULTANEOUSLY ORALLY WITH THE AID OF ORAL CANNULA FOR 56 DAYS.

AT THE END OF THE EXPERIMENTAL PERIOD 57TH DAY, BLOOD SAMPLES WERE TAKEN BY CARDIAC PUNCTURE FOR THE ESTIMATION OF LEVELS OF FSH, LH AND TESTOSTERONE. SEMEN PROXIMATE WERE COLLECTED THROUGH THE CAUDAL EPIDIDYMAL SAMPLES

TO DETERMINE SPERM COUNT, MOTILITY AND MORPHOLOGY [11]. TESTES SAMPLES WERE ALSO HOMOGENIZED TO DETERMINE THE ASSESSMENTS OF THE LEVELS OF BIOCHEMICAL STRESS MARKERS GLUTATHIONE (GSH), SUPEROXIDE DISMUTASE (SOD) [12], AND MALONDIALDEHYDE (MDA) [13]. OTHER TESTICULAR AND EPIDIDYMAL SAMPLES WERE CAREFULLY HARVESTED, AND FIXED IN BOUIN'S FLUID FOR ROUTINE HISTOLOGICAL EXAMINATION USING HAEMATOXYLIN AND EOSIN (H&E) AND MASSON'S TRICHROME STAINS.

HISTOLOGICAL PROCEDURE

EACH ANIMAL'S TESTES AND EPIDIDYMIS WERE METICULOUSLY REMOVED, WEIGHED, AND FIXED IN BOUIN'S FLUID BY COMPLETE IMMERSION FOR A WHOLE DAY. THEY WERE THEN CUT INTO SLICES THAT WERE BETWEEN 3 AND 5 MM THICK AND PROCESSED USING THE PARAFFIN WAX EMBEDDING METHOD. AT ROOM TEMPERATURE, THE TISSUE WAS DEHYDRATED USING INCREASING ALCOHOL GRADES. USING A MULTI-BLOCK PLASTIC EMBEDDING MOLD, DEHYDRATED TISSUES WERE CLEANED AT ROOM TEMPERATURE IN TWO CHANGES OF MELTED PARAFFIN WAX. AFTER TRIMMING AND MOUNTING THE PARAFFIN BLOCK TISSUE ON A WOODEN BLOCK FOR SECTIONING ON A ROTARY MICROTOME (BRIGHT B5143, HUNTINGTON, ENGLAND), THE SECTION WAS PLACED IN A WATER BATH AT 40 DEGREES CELSIUS TO ENABLE THE FOLDED SECTIONS TO SPREAD. THESE SECTIONS WERE MOUNTED ON NEW CLEAN GLASS SLIDES WHICH ARE LATER DRIED ON A SLIDE DRIER TO ENHANCE ADHERENCE OF SECTION TO SLIDE

STATISTICAL ANALYSIS

A ONE-WAY ANOVA (ANALYSIS OF VARIANCE) OR A MIXED MODEL WAS EMPLOYED AND DIFFERENCES BETWEEN GROUPS WERE ASSESSED USING TURKEY POST HOC TEST. EXCEL SOFTWARE WAS USED FOR GRAPH CREATION. THE RESULTS OF THE STATISTICAL ANALYSIS WERE DEPICTED USING BAR CHARTS WITH ERROR BARS INDICATING THE MEAN AND STANDARD ERROR OF MEAN (M ± SEM). A SIGNIFICANCE THRESHOLD WAS ESTABLISHED AT $P < 0.05$.

Results

Effect of methanol extract of *Azanza garckeana* on body weight in bisphenol-A-treated rats

The result of the body weight showed that there was significant decrease in groups D ($P = 0.0001$) and significant increase in C, E and F when compared with the control. Also, there was significant increase observed in the body weights of groups E ($P = 0.0001$) and F ($P = 0.0001$) when compared with the Bisphenol A only treatment group (D).

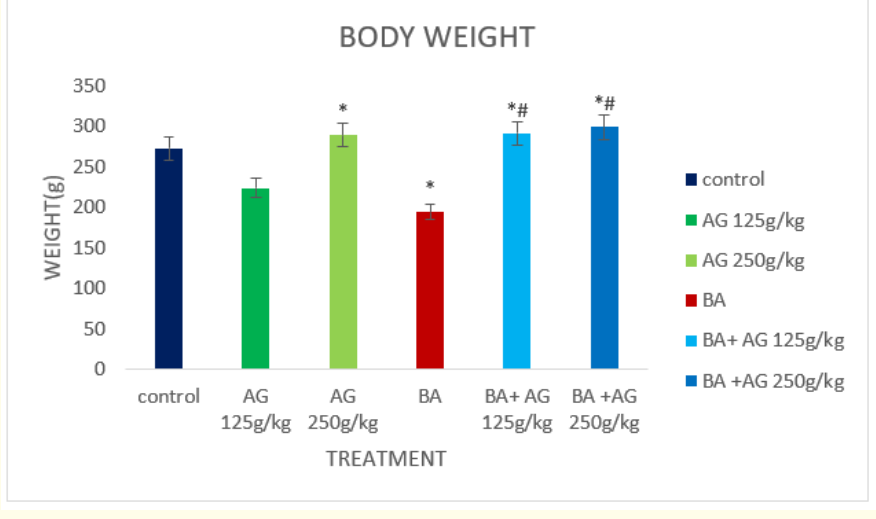

Figure 1: *Effect of Azanza garckeana on body weight in bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, *p < 0.05 vs. control, #p < 0.05 significant difference from Bisphenol-A, number of rats per treatment group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* on the mean testicular weight

The mean testicular weight showed that there was significant decrease observed in group D (P = 0.0320) when compared with the control. Also, there was significant increase observed in groups C, B, E and F when compared with group D.

Groups	Right testes	Left testes	Mean testicular weight
Control	0.97 ± 0.97	1.04 ± 1.93	1 ± 0.01
AG 125 g/kg	1.1 ± 1.65*#	1.14 ± 3.52*#	1.12 ± 0.02*#
AG 250 g/kg	1.27 ± 7.32*#	1.15 ± 1.83*#	1.21 ± 0.04*#
BA	0.85 ± 1.83*	0.87 ± 0.56*	0.86 ± 0.01*#
BA+AG 125 g/kg	1.1 ± 2.01*#	1.08 ± 2.79#	1.09 ± 0.02*#
BA + AG 250 g/kg	1.1 ± 1.83*#	1.09 ± 1.32#	1.09 ± 0.01*#

Table 1: *Presented in Mean ± S.E.M, *p < 0.05 against control, #p < 0.05 from BA, treatment animal per group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* on microscopic sperm count

The result of the percentage sperm count revealed that compared to the control (A) group, the sperm count decreased significantly in D (P = 0.0001). Also, there was significant increase observed in groups C, B, E and F when compared to group D.

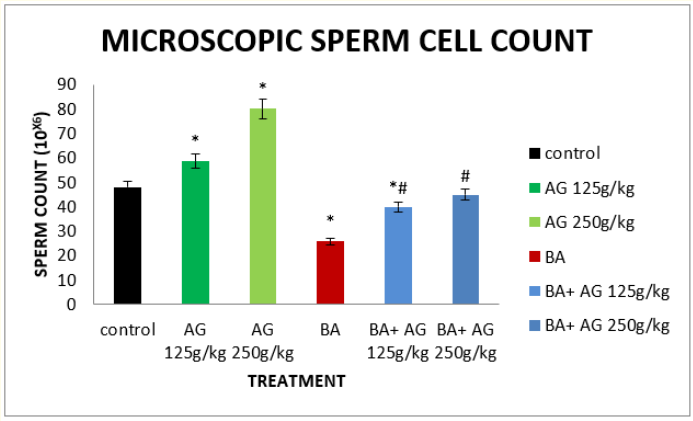

Figure 2: *Effect of Azanza garckeana on microscopic cell count in bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, Significant differences between treatments and control: *p < 0.05; #p < 0.05 significant difference from Bisphenol A. Number of rats per treatment group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* on sperm mobility in bisphenol-A-treated rats

The result of rapid progressive sperm motility percentage revealed that there was significant decrease observed in groups D (P = 0.0157) and significant increase observed in group C (P = 0.0007) when compared to the control. Also, there was significant increase observed in groups C (P = 0.0007), and E (P = 0.0157) and when compared to group D.

Compared to the control (A) group, result of percentage slow progressive sperm motility showed that there was significant increase in groups D (P = 0.0493), and C (P = 0.0493). Also, there was significant decrease observed in groups B (P = 0.0493), E (P = 0.0493) and F (P = 0.0493) when compared with group D.

The non- progressive sperm motility revealed that there was significant increase in group D (P = 0.0457) compared to the control (A) group and other groups.

The dead cell percentage showed that there was significant increase observed in groups D (P = 0.0001) and significant difference in group B, C and F compared to the control (A) group. Also, there was significant decrease observed in groups B and C, E (P = 0.0001) and F when compared with group D.

Groups	Normal spermatozoa	Head defect	Mid piece defect	Tail defect
Control	40 ± 0	40 ± 0	5 ± 0	5 ± 0
AG 125g/kg	40 ± 0	40 ± 0#	5 ± 0	5 ± 0
AG 250g/kg	45 ± 2.24*#	35 ± 2.24*#	5 ± 0	5 ± 0
BA	30 ± 0	55 ± 2.24*	6.67 ± 1.05	6.67 ± 1.05
BA+ AG 125g/kg	35 ± 2.24*#	35 ± 2.24*#	5 ± 0	5 ± 0
BA+ AG 250g/kg	40 ± 0	40 ± 0#	5 ± 0	5 ± 0

Table 2: *Presented in Mean ± S.E.M, *p < 0.05 against control, #p<0.05 from BA, treatment animal per group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* fruit on the levels of Luteinizing hormone (LH), and Testosterone (TH)

Result showed that there was significant decrease in Luteinizing hormone (LH) level of group D (P = 0.0001), E and F while B and C, shows insignificant increase compared to the control (A) group. But when compared to group B there was significant increase observed in groups E (P = 0.0001) and F.

The result of levels of Testosterone (TH) showed that compared to the control (A) group, significant decrease was observed in groups D (P = 0.0019), E and F with significant increase in group B (P = 0.0012), C, E and F when compared with group D.

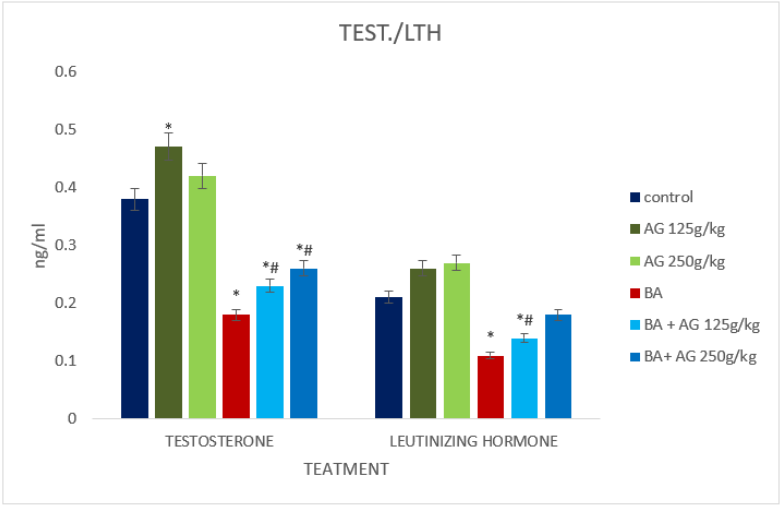

Figure 3: *Effect of Azanza garckeana on testosterone and luteinizing hormone in bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, Significant differences between treatments and control: *p< 0.05; #p < 0.05 significant difference from Bisphenol A. Number of rats per treatment group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* fruit on the levels of follicle-stimulating hormone (FSH)

Result showed that there was significant decrease observed in groups D (P = 0.0337) compared to the control (A) group. Also, there was significant increase observed in groups C (P = 0.0001), E (P = 0.0032) and F (P = 0.0016) when compared to group D.

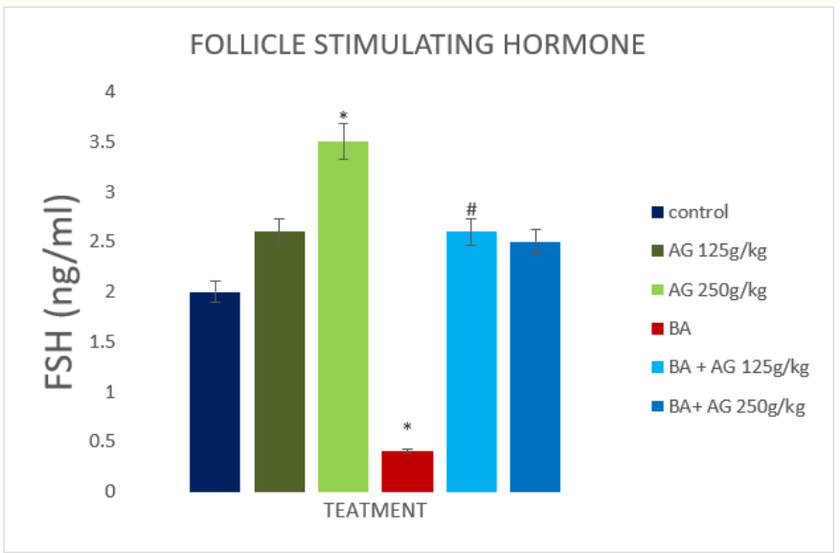

Figure 4: *Effect of Azanza garckeana on follicle stimulating hormone in Bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, Significant differences between treatments and control: *p < 0.05; #p < 0.05 significant difference from Bisphenol A. Number of rats per treatment group = 6.*

Effect of Azanza garckeana on the levels of MDA (Malondialdehyde) and superoxide dismutase (SOD) in bisphenol-A-treated rats

Result revealed that there was significant increase in level of MDA in groups D (P = 0.0001), E and F with no significant increase in B (P = 0.1011) and C (P = 0.8115) when compared to group A control. Also, significant decrease in group B (P = 0.0001), C (P = 0.0001), E (P = 0.0020) and F (P = 0.0002) when compared to group D.

Compared to the control (A) group, there was significant decrease on the levels of superoxide dismutase (SOD) in groups D (P = 0.0001), B (P = 0.0025), C (P = 0.0032), E (P = 0.0001) and F (P = 0.0014). And significant increase in E (P = 0.0018) and F (P = 0.0037) when compared to D.

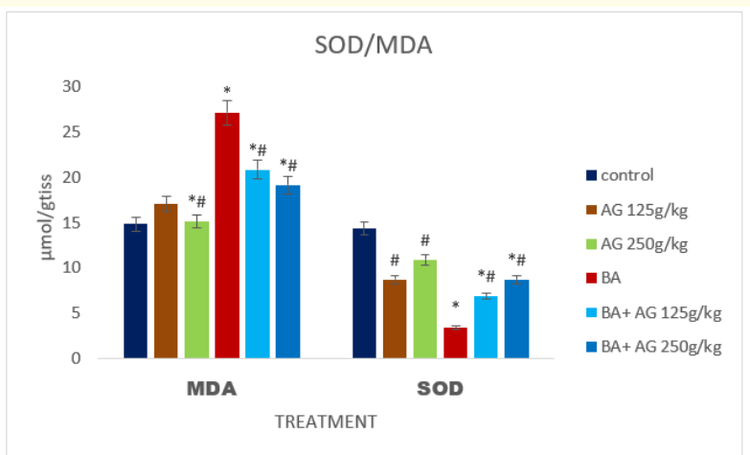

Figure 5: *Effect of Azanza garckeana on the levels of MDA and SOD in bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, Significant differences between treatments and control: *p < 0.05; #p < 0.05 significant difference from bisphenol A. Number of rats per treatment group = 6.*

Effect of bisphenol A and methanol extract of *Azanza garckeana* fruit on the levels of glutathione (GSH)

Result of the level of glutathione (GSH) showed that there was significant decrease in groups D (P = 0.0011) when compared with the control. Also, there was significant increase observed in groups B (P = 0.0001), C (P = 0.0001), E (P = 0.0001) and F (P = 0.0001) when compared with group D.

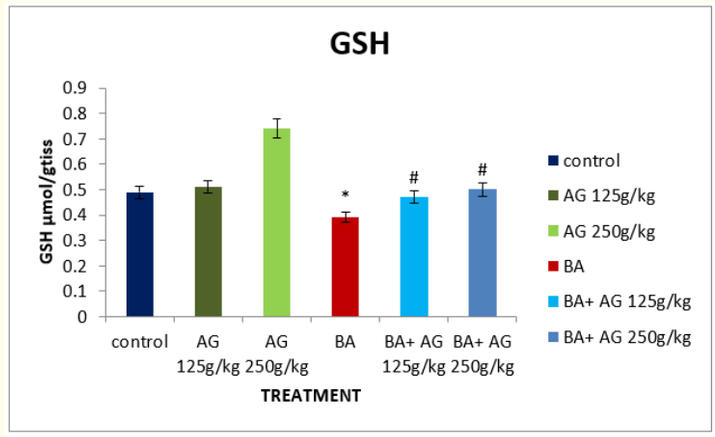

Figure 6: *Effect of Azanza garckeana on the levels of GSH in bisphenol-A-treated rats. Each bar represents Mean ± S.E.M, Significant differences between treatments and control: *p < 0.05; #p < 0.05 significant difference from Bisphenol A. Number of rats per treatment group = 6.*

Histological observation

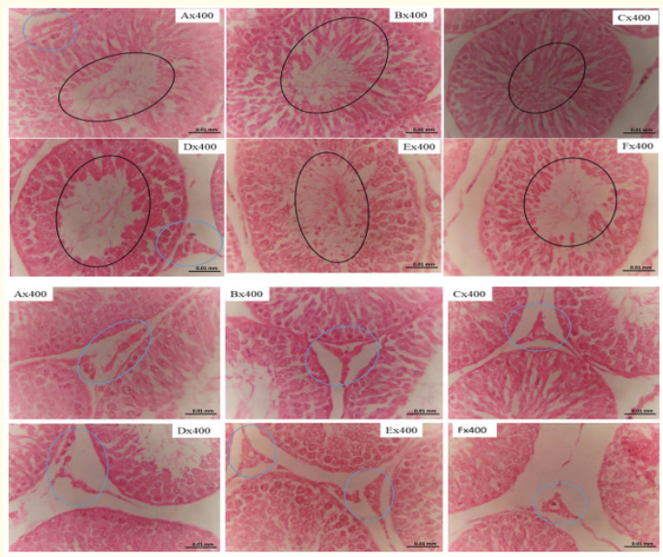

Plate 1: *Photomicrograph of testis-stained section by haematoxylin and eosin (x400).*

The H&E-stained; sections of the testes revealed varying degrees of structural preservation among the experimental groups. In the control and treatment groups (GPA, GPB, GPC, GPE, and GPF), the seminiferous tubules appeared normal with well-preserved architecture. The germinal epithelium showed orderly arrangement of germ cells at various stages of spermatogenesis, ranging from spermatogonia at the basement membrane to mature spermatozoa within the lumina. Sertoli cells were evident among the germinal series, while the interstitial tissue contained numerous normal Leydig cells. The tubular lumina were filled with mature spermatozoa, indicating active spermatogenesis and intact testicular function. In contrast, the GPD group demonstrated distorted seminiferous tubules with irregular outlines and signs of germ cell degeneration. The interstitial spaces were widened, and there was evidence of vascular congestion, although the Leydig cells remained morphologically normal. These findings suggest partial testicular degeneration, possibly resulting from oxidative stress or toxic attack, which might have impaired spermatogenic efficiency.

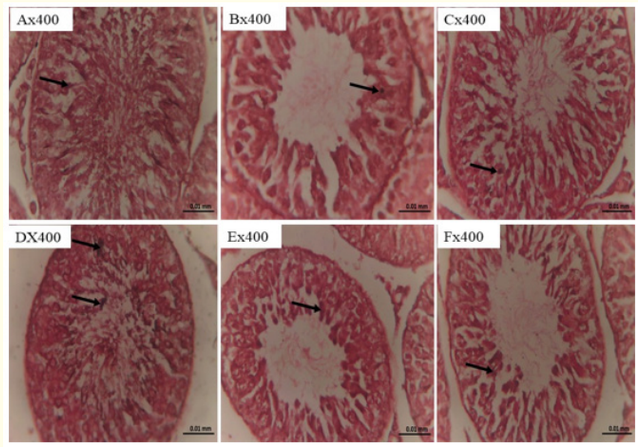

Plate 2: *Photomicrographs of the testis (Masson's Trichrome, Mag: 400 x).*

Arrow shows collagen deposition

Masson's Trichrome staining was used to assess collagen distribution and fibrotic changes within the testicular tissue. Sections from GRP A, GRP B, GRP C, GRP E, and GRP F showed minimal collagen deposition, with fine blue staining limited to the basement membrane and interstitial areas. The seminiferous tubules were well defined with normal connective tissue architecture, suggesting absence of fibrosis. In contrast, sections from GRP D revealed intense blue staining, indicative of collagen accumulation and fibrotic remodeling around seminiferous tubules and within the interstitial spaces. This finding is consistent with degenerative changes observed in the H&E-stained slides and may reflect chronic inflammatory or oxidative injury leading to extracellular matrix remodeling.

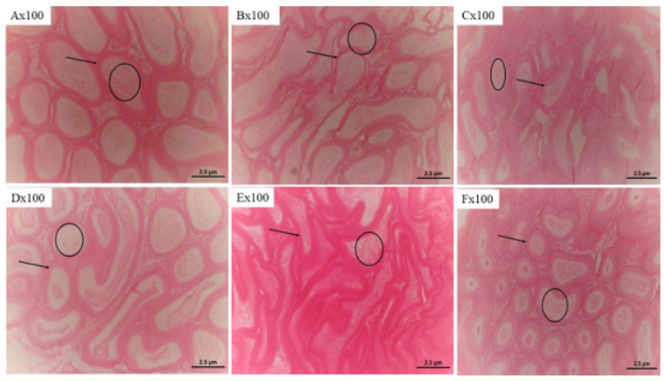

Plate 3: *Photomicrographs of epididymis (H&E, Mag.: x100).*

GRP (A) The epididymis shows preserved histoarchitecture. The tubules (black arrow) are well-outlined with intact epithelium and separated by interstitial space that is free of collection. The tubular lumen (black circle) is filled with normal sperm. GRP (B, C & F) The epididymis shows preserved histoarchitecture. The tubules (black arrow) are well-outlined with intact epithelium and separated by interstitial space that is free of collection. The tubular lumen (black circle) is filled with normal sperm.

GRP (D) The epididymis shows distorted histoarchitecture. The tubules (black arrow) are distorted in shape, though well-outlined with intact epithelium and separated by interstitial space that is free of collection. The tubular lumen (black circle) is filled with scanty sperm. H&E-stained; sections of the epididymis across groups revealed a generally preserved histoarchitecture in all but one group. In the control (GRPA) and treated groups (GRPB, GRPC, GRPE, GRPF), the epididymal ducts were well outlined by a pseudostratified columnar epithelium with stereocilia. The lumina were filled with numerous spermatozoa, while the interstitial spaces were free of cellular infiltration or edema, indicating active sperm storage and maturation. The GRPD group, however, showed distorted epididymal tubules and reduced luminal sperm content, although the epithelium remained largely intact. The distortion and reduction in sperm concentration correspond with the testicular changes observed in the same group, suggesting secondary epididymal dysfunction due to decreased testicular sperm output.

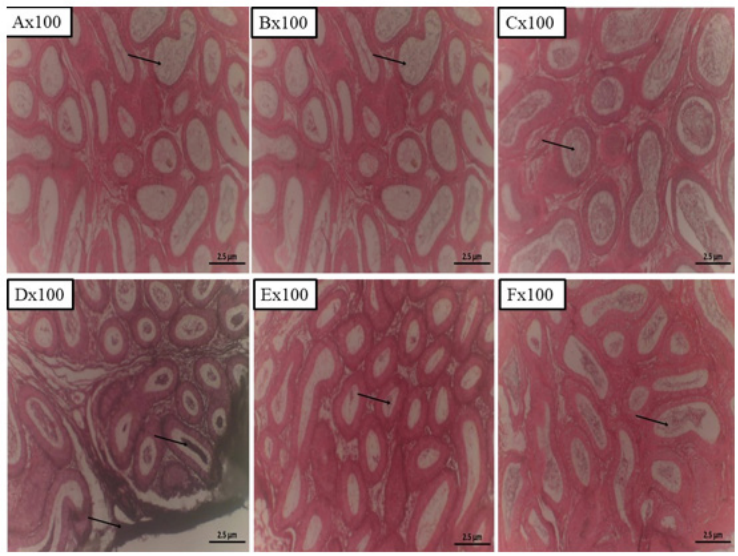

Plate 4: *Photomicrographs of epididymis Masson's Trichrome stain (MT, Mag.: x100).*

Arrow shows collagen deposition

Masson's Trichrome-stained sections of the epididymis showed a pattern similar to that of the testis regarding collagen deposition. In groups Grp A, Grp B, Grp C, Grp E, and Grp F, collagen fibers appeared sparsely distributed within the intertubular stroma, and there was no evidence of fibrosis or thickening of the peritubular connective tissue. The epithelial lining and luminal structure remained well organized, signifying preserved epididymal integrity.

Conversely, the Grp D exhibited noticeable blue-stained collagen deposition around and between the epididymal ducts, denoting fibrotic remodeling. This pattern aligns with the degenerative and congestive features seen in its corresponding H&E; section. The fibrosis observed in Grp D likely reflects chronic inflammatory response following tissue damage, which may impair sperm maturation and motility.

Discussion

The utilization of medicinal plants for the management of infertility, specifically male factor infertility, is a long-standing practice in traditional medicine. This therapeutic potential is largely attributed to phytochemicals, which possess potent antioxidant properties. These bioactive compounds confer protective effects on reproductive parameters by mitigating insults such as oxidative stress, inflammation, and chemical toxicity-factors known to compromise male fecundity.

In the present study, the administration of a methanol extract of *Azanza garckeana* significantly ($p < 0.05$) enhanced body weight gain in treated animals, showing a potential restorative and protective effect against the weight reduction observed in the group exposed to Bisphenol A (BA), aligning with previous findings regarding the plant's ability to attenuate metabolic and systemic impairments [7]. These results indicate that AG contains bioactive constituents capable of neutralizing the adverse physiological effects triggered by endocrine disruptors like BA [14].

Furthermore, treatment with the extract at all dosage levels resulted in a significant increase in testicular and epididymal weights compared to the BA-only group. The extract also exhibited restorative actions in co-treated groups, a finding consistent with prior research documenting the positive influence of AG on experimental testicular mass [6,15]. This observation is clinically significant, as testicular size serves as a positive correlate for male fertility, whereas diminished mass is frequently linked to poor reproductive outcomes.

The investigation further revealed that AG may enhance male reproductive function through the preservation of epididymal health. Effective epididymal secretions are essential for post-testicular sperm maturation and motility; a dysfunctional epididymis often results in the production of immature, non-viable spermatozoa. In this study, BA exposure exerted deleterious effects on sperm parameters, including: Reductions in total sperm count, rapid progressive motility, and the percentage of normal sperm cells, increased prevalence of tail, mid-piece, and head defects and Elevated rates of dead, slow progressive, and non-progressive spermatozoa, Santiago (2021) [16] also reported these effects of BA on sperm parameters. Conversely, AG significantly increased sperm concentration (at 125 and 250 mg/kg), number of sperm cells, and rapid progressive motility (at 250 mg/kg) compared to both control and BA-treated groups ($p < 0.05$). This shows that AG improves reproductive potential even in the presence of chemical-induced toxicity. These effects of AG in this study confirmed what was reported by Oromaiwele and Odaiase, (2025) [17]. AG possess high concentration of bioactive compounds which include flavonoids, tannins, and alkaloids which able enable it to counter the adverse effect of bisphenol A.

The biochemical and hormonal profile further elucidates the extract's mechanism of action. BA exposure significantly increased malondialdehyde (MDA) levels, indicating elevated reactive oxygen species (ROS) generation and lipid peroxidation (LPO). Such oxidative stress disrupts the membranes of the seminiferous tubules and damages DNA, leading to the degeneration of spermatogenic cells. Conversely, animals treated with AG exhibited significantly higher levels of glutathione (GSH) and superoxide dismutase (SOD) activity ($p < 0.05$), alongside reduced MDA concentrations, this is in line with the finding of Bukata., *et al*. (2022) [18]. These findings suggest that the pulp of AG contains substantial quantities of polyphenols, such as flavonoids, which enhance antioxidant defense mechanisms and reduce oxidative stress.

On hormonally investigation, the extract effectively protected against BA-induced toxicity on the hypothalamic-pituitary-gonadal axis. AG treatment groups shows significantly increased levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone compared to the BA-only group. By alleviating oxidative stress-a key contributor to impaired steroidogenesis-the extract likely supports improved Leydig cell performance and endocrine function, thereby enhancing testosterone synthesis and spermatogenesis, this is in agreement with Nanso, (2024) [19].

Histopathological analysis using Hematoxylin and Eosin (H&E) and Masson's Trichrome staining corroborated these findings. H&E staining of BA group demonstrated distorted seminiferous tubules with irregular outlines and signs of germ cell degeneration. The interstitial spaces were widened, and there was evidence of vascular congestion, although the Leydig cells remained morphologically normal. These findings suggest partial testicular degeneration, possibly resulting from oxidative stress or toxic attack, which might have impaired spermatogenic efficiency while Masson's Trichrome staining revealed collagen accumulation and fibrotic remodeling around seminiferous tubules and within the interstitial spaces, This finding is consistent with findings of Alabi., *et al*. (2021) [20]. The degenerative changes observed in the H&E and Masson's Trichrome stained slides reflect chronic inflammatory or oxidative injury leading to extracellular matrix remodeling. In other hand, AG treatment groups maintained normal tissue architecture, preserved germ cell integrity, and minimized fibrotic changes. These results confirm the extract's role in protecting reproductive tissues and maintaining functional spermatogenesis [21].

Conclusion

Azanza garckeana demonstrates promising protective and restorative effects against BA-induced toxicity through its potent antioxidant activity and hormonal regulation. These findings provide scientific validation for its traditional use in promoting male fertility. Future research should focus on isolating the specific active constituents and assessing long-term safety to establish its potential as a natural therapeutic agent.

Further studies are required to identify *Azanza garckeana* active constituents responsible for protective and restoration actions revealed in this study, and also research on it's optimize dosage, and evaluate long-term safety and clinical applicability.

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Conflict of Interest

No conflict of interest from any author.

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Volume 14 Issue 6 June 2026