International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 05 Issue 03 March 2025

Page No : 187-194

DOI: https://doi.org/10.47191/ijpbms/v5-i3-07, Impact Factor: 8.163

Impact of Azanza Garckeana Seed Extract on Body Weight and Basic Reproductive Profiles in Lead-Intoxicated Male Wistar Rats

Dagbota Dan-Jumbo[1], Elizabeth Eepho Krukru[2], Fortune Somiari Amah-Tariah[3], Buduka Justice Otto[4]

[1,2,3,4] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nigeria.

ABSTRACT

Azanza garckeana (Gorun Tula) is a commonly consumed edible plant, in parts of Nigeria, with claims by many that it possesses several medicinal potencies like aphrodisiac effects and more. Thus, the present study investigated the effects of the Azanza garckeana hydromethanolic seed extract (AgSE) on percentage body weight changes, reproductive hormonal levels and testicular histology in male Wistar rats. Thirty (30) adult male Wistar rats were procured and randomly selected into 6 groups of 5 rats each. Group 1 served as negative control and had only 1ml distilled water, group 2 served as positive control and was treated with 25mg/kg lead chloride only, group 3 was administered Clomide (50mg/kg) as standard drug, group 4 had 250mg/kg AgSE and $PbCL_2$ 25mg/kg, group 5 had 500mg/kg AgSE and $PbCl_2$ 25mg/kg and group 6 750mg/kg AgSE and PbCl2 25mg/kg for 28 days. At the end of treatments, blood and testicular tissue samples were harvested from the rats after proper sedation with chloroform. Numerical data obtained from the study were subjected to statistical analyses using analyses of variance and Post Hoc test tools of the SPSS Version 29.0.10. AgSE and clomide treated rats showed significantly ($p<0.05$) increased percentage body weight changes when compared to the positive control group. Groups 4 (treated with 250mg/kg AgSE) and 6 (treated with 750mg/kg AgSE) showed significant increase in reproductive hormonal levels (testosterone) when compared to the Groups 1, 2 and 3. The outcome on the testes histological analysis revealed that group 4 (treated with 250mg/kg AgSE) had only mild sloughing off of germinal cells, intact sertoli and leydig cells when compared to that of Group 2 (positive control) that showed obvious degenerative changes in germ cells with Leydig cells lesions. Thus, the AgSE may possess ameliorative potentials on $PbCl_2$ altered percentage body weight, reproductive hormones and testicular tissues.

KEYWORDS: Azanza garckeana; percentage body weight changes; reproductive hormonal levels; testicular histology; ameliorative potentials; $PbCl_2$ intoxications

ARTICLE DETAILS

Published On:
21 March 2025

Available on:
https://ijpbms.com/

I. INTRODUCTION

Infertility is a reproductive disorder that indicates failure to attain a viable pregnancy after a year or more of regular exposed sexual intercourse. It could be of primary (when a pregnancy has never been achieved by a person) or secondary (failure to conceive or carry a baby to term after previously giving birth) origins (WHO, 2024; Sharma et al., 2024). Infertility is one common global factor affecting approximately ≤8% of couples ((Okonofua et al., 2021). The condition is more common in Sub-Saharan Africa, with Nigeria accounting for ≤30% of couples affected (Chimbatata & Malimba, 2016).

Infertility is a major reason for seeking Reproductive Physicians consultations amongst couples (Sami & Ali, 2006; Zheng et al., 2019). The cause of the condition in Nigeria has been identified to be primarily connected to post infectious causes; sexually transmitted infections, post abortal and puerperal sepsis (Mohammed-Durosinlorun et al., 2019). Irrespective of the cause of the condition, two main modes of treatment are popular in Nigeria: herbal and orthodox medications (Okonofua et al.,

1997; Ombelet et al., 2008; Panti & Sununu, 2014). A good analysis of male fertility profile evaluation includes checks on reproductive hormonal levels (i.e. endocrine disorder), total and progressive motility, the number of normally shaped sperm cells, and the level of sperm count (Arab et al., 2019; Pizzol et al., 2021; Agboola et al., 2021).

Further, the rate of herbal medicine use amongst fertility clinic attendees is said to be high (79.9%) in a Nigerian study (Maduka & Okubor, 2024). It was therefore suggested that further enquiry be made about the use of herbal medicines in order to understand the potential advantages and provide council against the possible health risks associated with its use (Maduka & Okubor, 2024).

Amongst the numerous medicinal plants with significant potentials of reproductive system stimulations, Azanza garckeana is outstanding (Itodo et al., 2022; Nurudeen et al., 2023). The plant is locally known as "Goron Tula" or "Kola of Tula and commonly called "African chewing gum or snot apple''. it is a tree plant belonging to the family of Malvaceae and commonly found in tropical Africa in wooded grasslands, open woodland and thickets (Bioltif et al., 2020; Olayiwola et al., 2021). The plant is often found on or near termite mounds in old fields (Malombe et al., 2004).

Specifically, the fruit extract of Azanza garckeana (A. garckeana) has been reported to exert positive effects on male sexual act and improved their fecundity; these characteristics were attributed to the plant's high content of flavonoids in the submission (Nonso et al., 2007). Similarly, Agarwal & Sekhon, (2010) emphasized that antioxidants could possibly preserve the equilibrium between reactive oxygen species (ROS) production and clearance, thereby improving sperm quality. In fact, it was also found that men who consume more antioxidants in their diet do have a lower incidence of sperm aneuploidy than those who consume less (Agarwal & Sekhon, 2010).

Consequently, considering the much-acclaimed benefits of the A. garckeana plant (Bioltif et al., 2020; Adenowo et al., 2022; Momodu et al., 2022), the present study set out to investigate the aqueous seed extract of the plant on basic reproductive profiles (including reproductive hormonal and testicular histology changes) in lead-intoxicated male Wistar rats.

II. MATERIALS AND METHODS

This study was a laboratory-based experiment study using male Wistar rat models. The study was carried out in the Animal studies Unit of the Department of Human Physiology, University of Port Harcourt. Prior to the commencement of the research, ethical approval was sought and obtained from the Central University Ethical Committee.

A. Acquisition and Preparation of Azanza garckeana Seed Extract

Azanza garckeana seed was obtained from Tula community in Kaltungo local government area of Gombe state. The seeds were properly washed, and air-dried under shade for fourteen days. After the seeds got dried, they were then pulverized with the help of an industrial motorized grinder. The powdered sample of the plant was extracted using the cold maceration method. The powder was then dissolved in 1.5 liters of water in a 2.5L conical flask for seventy-two (72) hours. The ratio of ethanol to water solvents was 70:30. Thereafter, the extract was filtered using Whatman paper 1, and the solvent was evaporated at 45°c using a rotary evaporator. The obtained crude extract was stored at 4°c until used.

B. Animal Handling

A total of 30 adult male Wistar rats weighing 120g-150g were procured from the Animal house of the Department of Human Physiology, University of Port Harcourt and allowed access to feed and water ad libitum. They were housed in wire-gauzed plastic cages, with saw dust beddings and maintained at room temperature under the 12hour light/dark cycle. The animals were acclimatized for 14 days prior to commencement of experimentations. The study animals were handled according to the guidelines of the National Institute of Health, Use and Care of Experimental Animals (NRC, 2010).

C. Treatment Protocol

The total of 30 adult male Wistar rats was randomly selected into six different groups of five rats each. The specified extract doses were prepared based on the average body weights of the respective groups. The administration was par oral with the aid of oral gavage. The distribution of the treatment groups are as follows:

Group 1: Negative control that received normal rat chow and water.

Group 2: Positive control that received 1ml 25mg/kg $PbCl_2$(Lead chloride).

Group 3: received the standard drug (clomiphene citrate) in 50mg/kg and 1ml 25mg/kg $PbCl_2$.

Group 4: received 1ml 250mg/kg.bw of Azanza garckeana hydromethanolic seed extract (AgSE) +
25mg/kg $PbCl_2$.

Group 5: received 1ml 500mg/kg.bw of AgSE + 25mg/kg $PbCl_2$.

Group 6: received 1ml 750mg/kg.bw of AgSE + 25mg/kg $PbCl_2$.

D. Harvesting of Samples from the Study Models

At the end of twenty eight (28) consecutive days of the respective treatments, blood samples were harvested from the study animals under light anesthesia via cardiac puncture and put into well labelled plain sample bottles. Thereafter, the blood was allowed to clot at room temperature and the

samples were centrifuged at 3000t/min for 10 minutes. The serum was separated and stored at about -85°c prior to further analysis.

E. Hormonal Assay

Subsequently, the obtained serum from the study models' samples were transferred to separate vials and tested for FSH, LH and Testosterone using the Enzyme-linked Immunoabsorbent Assay (ELISA) method according to the manufacturer's instructions.

F. Histological Analysis

Testicular tissues were carefully harvested from the study models by dissection and were observed for any possible obvious pathological changes before being fixed in a 10% buffered formalin solution. The tissues were then dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections were cut using a rotary microtome at 5mm thickness and then stained using hematoxylin and eosin (H & E).

III. RESULTS

The results of the present study are presented in table, figure and photomicrographs with appropriate interpretations.

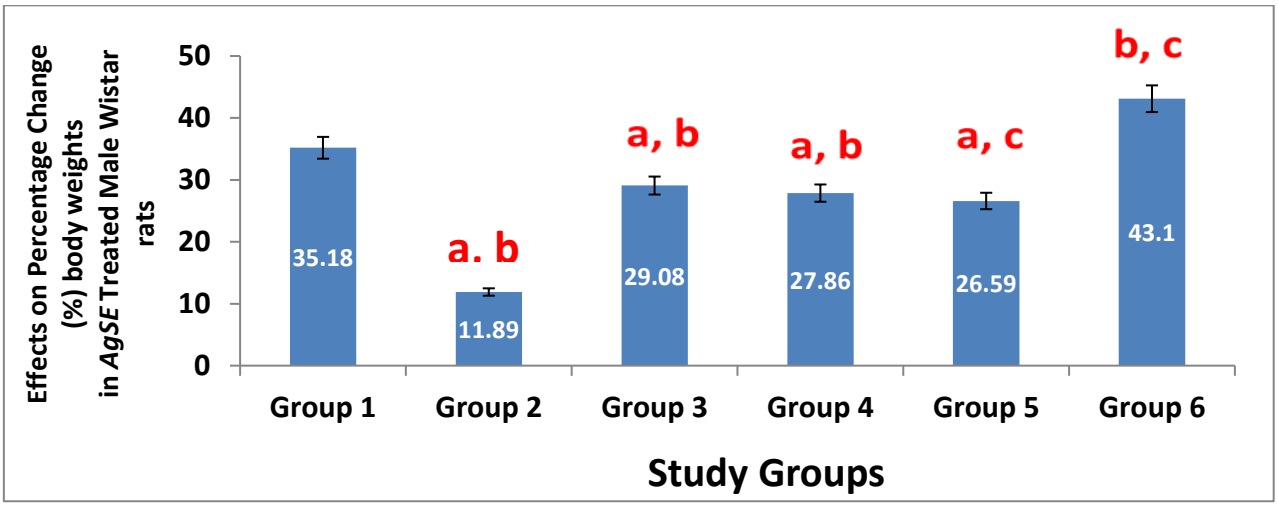

Figure 1: Effects on Percentage Change (%) body weights in AgSE Treated Male Wistar rats

Note: All values are expressed as mean ± SEM. [a]= p<0.05 compared to Control. [b]= p<0.05 compared to $PbCl_2$ Only. [c]= p<0.05 compared to Clomid + $PbCl_2$.

KEY:

GROUP 1: Negative Control (untreated)
GROUP 2: Positive Control (25mg/kg $PbCl_2$ only treated)
GROUP 3: Clomide (50mg/kg) + $PbCl_2$
GROUP 4: AgSE 250mg/kg + $PbCl_2$
GROUP 5: AgSE 500mg/kg + $PbCl_2$
GROUP 6: AgSE 750mg/kg + $PbCl_2$

Table I: Effects of on male reproductive hormones in AgSE treated male Wistar rats

TREATMENTS	FSH	LH	TET
GROUP 1: Negative Control (untreated)	1.54±0.42	1.68±0.44	1.96±0.41
GROUP 2: Positive Control (25mg/kg $PbCl_2$ only treated)	1.97±0.33	2.28±0.40	1.50±0.24
GROUP 3: Clomide (50mg/kg) + $PbCl_2$	0.73±0.08[ab]	0.90±0.08[b]	0.87±0.11
GROUP 4: AgSE 250mg/kg + $PbCl_2$	1.03±0.14[b]	1.17±0.11[b]	3.20±0.64[bc]
GROUP 5: AgSE 500mg/kg + $PbCl_2$	2.15±0.30[c]	1.73±0.23	1.70±028
GROUP 6: AgSE 750mg/kg + $PbCl_2$	1.89±0.17[c]	1.75±0.34	2.71±0.61[c]

Note: All values are expressed as mean ± SEM. [a]= p<0.05 compared to Control. [b]= p<0.05 compared to $PbCl_2$ Only. [c]= p<0.05 compared to Clomid + $PbCl_2$.

The data on Figure 1 shows the effects on percentage change in body weights of AgSE treated male wistar rats. Aside from group 6 (AgSE 750mg/kg + $PbCl_2$ treated) with significantly ($p<0.05$) raised level of percentage change in body weight, all other groups had theirs' significantly ($p<0.05$) reduced when respectively compared to that of the control. It was found that both the clomide treated (group 3) and the AgSE 750mg/kg + $PbCl_2$ treated (group 4) had significantly ($p<0.05$) elevated percentage change in body when compared to that of group 2 (positive control (25mg/kg PbCl2 only treated). And then, groups 5 and 6 (the 500mg/kg and 750mg/kg AgSE treated groups) significant reduced and increased respectively when compred to that of group 3 (clomide treated).

Table I shows data on male reproductive hormones changes in AgSE treated male Wistar rats. It is noteworthy to state that the administration of lead chloride only to group 2 rats significantly increased their FSH and LH concentrations but decreased testosterone level when compared to group 1 rats ($p<0.05$). Considering the outcome on follicle stimulatory hormone (FSH) level, only group 3 (clomide only treated) had significantly ($p<0.05$) reduced level when compared to that of the control value; both groups 3 and 4 (treated with AgSE 250mg/kg + PbCl2) had significantly ($p<0.05$) reduced levels when compared to that of group 2. And then, it was seen that groups 5 and 6 had markedly raised FSH level when compared to that of group 3.

In view of the changes on luteinizing hormone (LH) level, only groups 3 and 4 indicated significantly ($p<0.05$) dropped values when compared to that of group 2. On the testosterone (TET) level changes, group 4 showed significantly ($p<0.05$) raised level when respectively compared to those of groups 2 and 3. And again, group 6 indicated marked raised level of TET when compared to that of group 3.

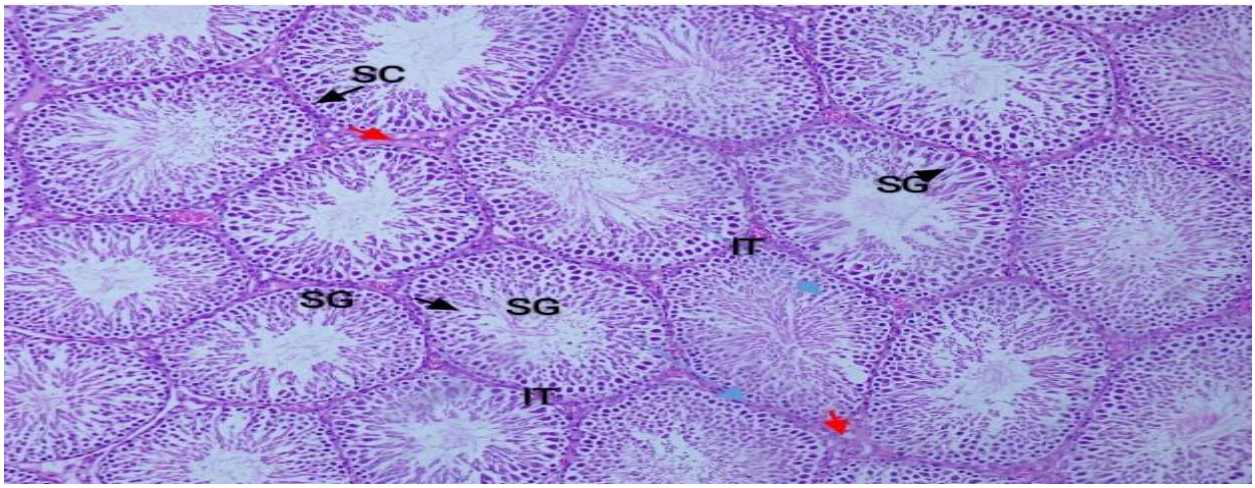

Plate 1: Photomicrograph section of testes of Wistar rat from control group given distilled water and normal rat chow. Section showed seminiferous tubules with sertoli cells (SC), spermatogonia, primary spermatocytes (SPC) and spermatids. The interstitium (IT) showed Leydig cells. Germ cell showed varied maturation around the tubule. H&E X100.

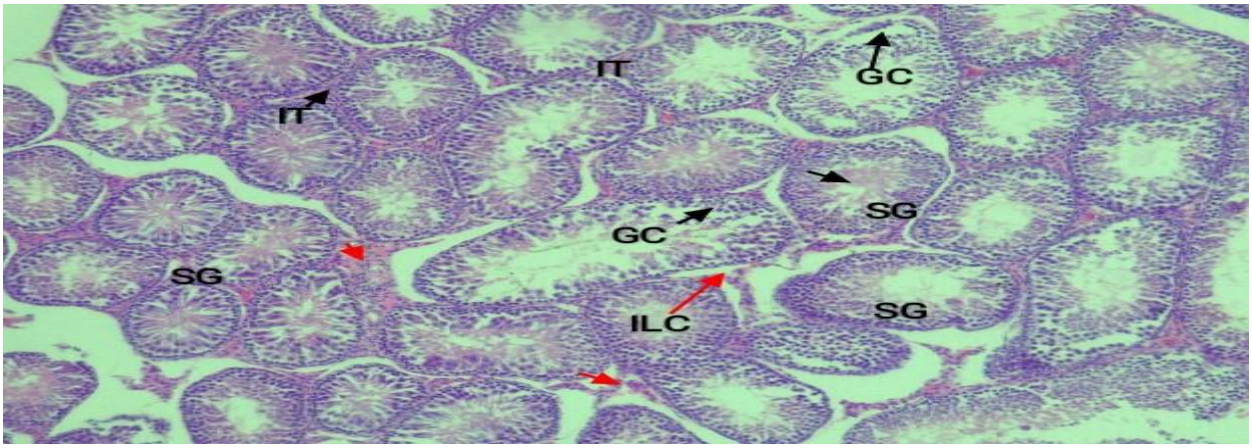

Plate 2. Photomicrograph section of seminiferous tubule of Testes of Wistar rats treated with lead Chloride (PbCl2) 25mg/kg alone. Section showed artefactual sloughing of germ cell (GCS) elements (black arrows) into tubule lumen showing hypolastic and degenerative changes in germ cells (LS). IT (Intersitium), Leydig cells lesions (ILC, red arrows)). H&E X100

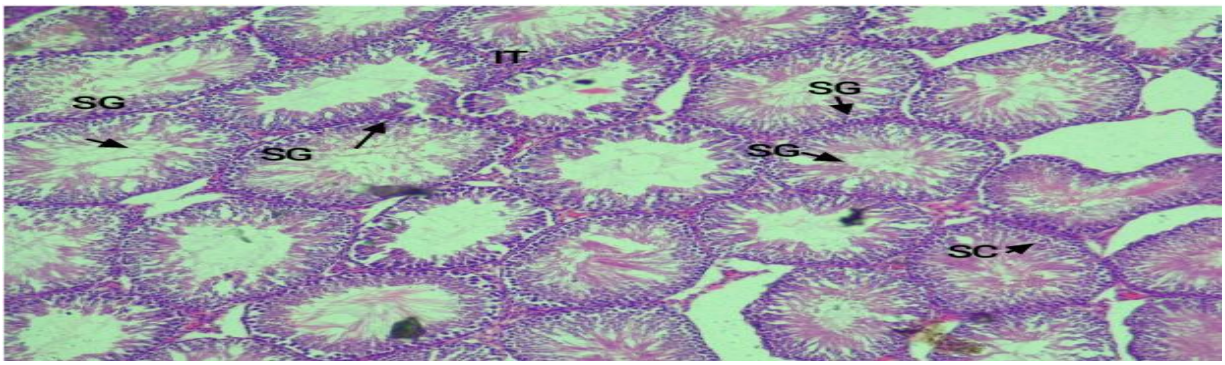

Plate 3. Photomicrograph section of seminiferous tubule of Testes of Wistar rats treated with Clomiphene citrate (50mg/kgbw) + Lead Chloride (PbCl2) 25mg/kg. Section displayed some recovered germ cells (Sperm cell, (SC), Spermatogonia (SG) with less sloughing of sertoli cells and interstitium (IT). (H&E X100).

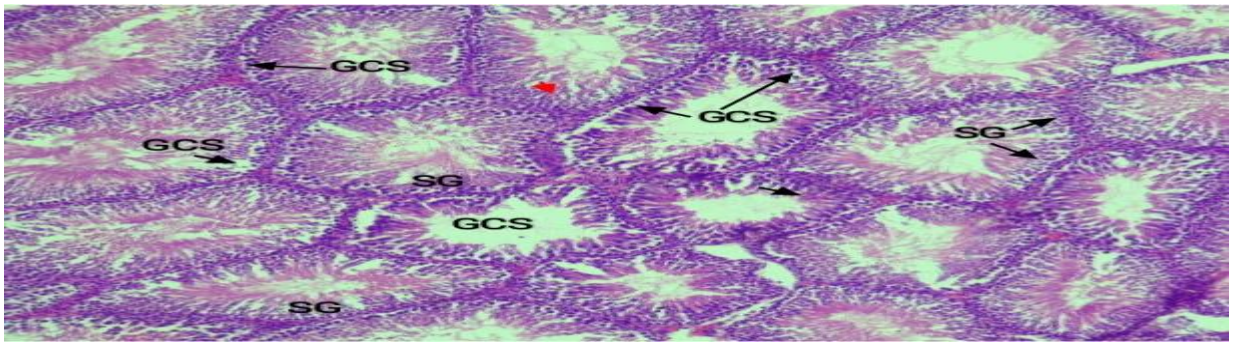

Plate 4. Photomicrograph section of testis tissue of rats taken from group four (AgSE 250/kg + $PbCl_2$ 25mg). Section showed seminiferous tubules mild germinal cell (GCS) sloughing off into the lumen. The interstitium (IT) are intact with Leydig cells (LC). Spermatogonia (SG), IT (interstitium). H&E X100.

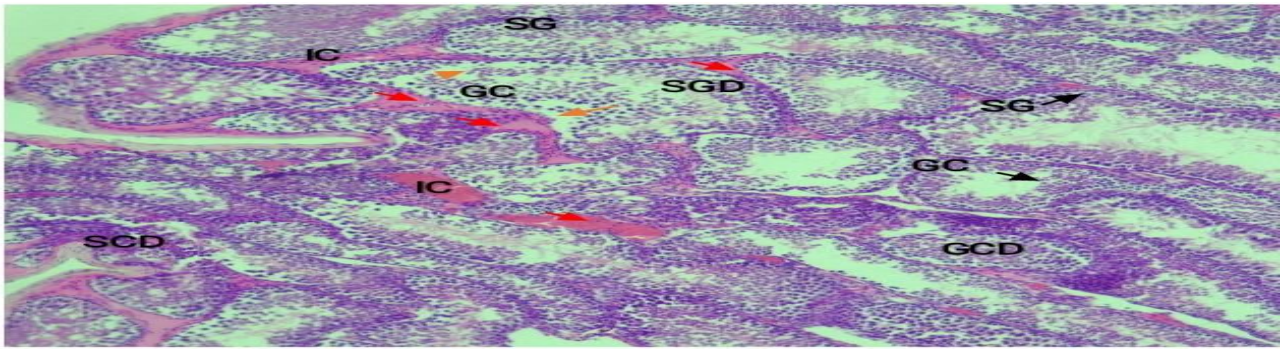

Plate 5. Photomicrograph of testicular tissue taken from Wistar from group five (500mg/kg AgSE+ PbCl2 25mg). Section showed testicular cells and tissue necrosis, degeneration and distortions of sertoli cells (SC), Germ cells degeneration (SCD), Spermatogonia (SG) and interstitial cells degeneration (ICD) at the interstitium (IT). H&E X400.

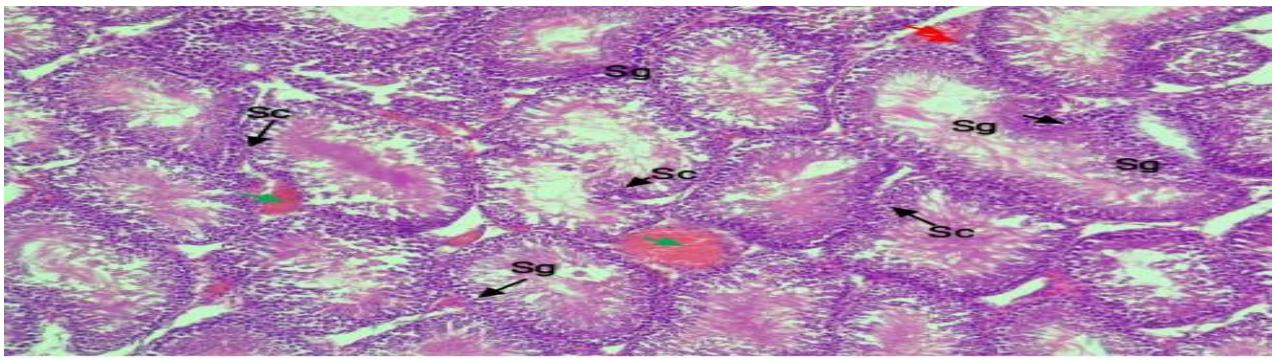

Plate 6. Photomicrograph of testicular tissue taken from Wistar from group six (750mg/kg AgSE + PbCl2 25mg). Section showed testicular cells and tissue necrosis, degeneration and distortions of sertoli cells (Sc), Germ cells degeneration (Scd), Spermatogonia (Sg) and interstitial cells necrosis (ICD-green arrows) at the interstitium (red arrows -IT). H&E X400.

IV. DISCUSSIONS

In general, nutraceuticals are foods or food components that play an important role in regulating and preserving normal physiological function that keeps humans healthy; on the other hand, the great shift in the lifestyle of most global populations has brought about health trends that calls for the identification and proper application of nutraceuticals (Nonso et al., 2025). Accordingly, as an edible fruit, Azanza garkeana, is taken for many nutraceutic possesses (Adenowo et al., 2022). Hence, the present study investigated the seed extract of the plant on body weight and basic reproductive profiles changes and major findings are discussed in the following paragraphs.

As expected, lead chloride presumably reduced body weights of the study models in the present study; this is consistent with earlier submission of Yu et al., (2021), that stated the potency of lead causing gut dysbacteriosis and disrupting gut physiological homeostasis, thereby leading to oxidative stress, immune-inflammatory responses, and increased intestinal barrier permeability and thus, the difficulty in nutrient absorption. Interestingly, in both the clomide and the AgSE treated models, the depressive impact of lead chloride on the body weight was possibly reversed and improved on. This was most with the highest dose of AgSE treated models. It is therefore suggestive to state that A. garckeana plant may be rich in phytochemicals that perhaps worked synergistically to bring about the positive effect on body weight. Such active compounds would need further investigations to properly profile them and better understand the functions.

The results obtained from this study showed that the administration of lead chloride to group 2 rats (PbCl2 25mg/kg) did not affect follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels but statistically decreased testosterone levels with respect to the untreated rats. This indicates that the pathophysiology of the toxicant was more on the testicular tissues (Kumar, 2018; Yu et al., 2021). But the AgSE treated models had their depressed TET levels markedly ameliorated. This finding is consistent with earlier reports that show how antioxidant-rich foods, such as vitamins C and E, may assist with protecting the reproductive cells and increase fertility (Torres-Arce et al., 2021; Walke et al., 2023). This attribute of the plant extract can be connected to earlier identified active compounds (such as saponins, alkaloids and phenol) of the plant (Ajayi et al., 2013). These compounds possess a variety of biological properties, including antioxidant, anti-tumor, hypocholesterolemic, hypoglycemic, and anti-inflammatory properties (Sharma et al., 2023). From the foregoing outcome of the present study, it can be said that the plant may be a good candidate for testosterone enhancement potentials. Thus, more investigations may be required to profile such active compounds.

The results obtained from this study as seen in Plate 2 from the group treated with PbCl2 25mg/kg shows artefactual sloughing of germ cell elements into tubule lumen showing hypolastic and degenerative changes in germ cells, Leydig cells lesion when compared to group 1(negative control) which showed seminiferous tubules with sertoli cells, spermatogonia, primary spermatocytes and spermatids. The interstitium showed Leydig cells, germ cell showed varied maturation around the tubule. The Clomide and 250mg/kg AgSE treated models had improved testicular cells and less tissue necrosis and degeneration but these were rather adverse in the models treated with increasing doses of the extract.

The above histological finding of the current study is consistent with some earlier submissions that noted how medicinal plants with rich active beneficial components could protect against degeneration and infection of distorted tissues; stimulate angiogenesis and promote differentiation of keratinocytes as well as re-epithelialization (Dzobo et al., 2018; Radmehr et al., 2020; Sharma et al., 2021). It is thus suggestive to state that beyond the beneficial effects of the AgSE on body weight and reproductive hormonal changes in the study models, the plant extract, at moderate doses may be able to improve intoxicated or disordered testicular tissues in such models.

V. CONCLUSION

In conclusion, the present study has shown that sub-chronic treatments with Azanza garckeana hyromethanolic seed extract (AgSE) in male Wistar rat models were able to reverse and improve dysfunctional effects of lead chloride intoxication on the percentage body weight, reproductive hormones and testicular tissues in the models. While the higher doses of the AgSE (500mg/kg and 750mg/kg) exerted even better outcomes on the percentage body weight and reproductive hormonal parameters, only the low dose (250mg/kg AgSE) was helpful on the histological changes. Consequently, more investigations on identifying and profiling the actual bioactive compounds of the AgSE would be helpful in advancing the potential therapeutic attributes of the plant. Further, caution should be taken in the use of increasing doses of the AgSE for the sake of a safe testicular tissue conditions.

REFERENCES

I. Okonofua F, Balogun JA, Odunsi K, Chilaka VN, editors. Contemporary obstetrics and gynecology for developing countries. Springer; 2021 Aug 5.
II. Chimbatata NB, Malimba C. Infertility in sub-Saharan Africa: a Woman's issue for how long? A qualitative review of literature. Open J Soc Sci. 2016;4:96–102.
III. Mohammed-Durosinlorun A, Adze J, Bature S, Abubakar A, Mohammed C, Taingson M, Airede L.

Use and pattern of previous care received by infertile Nigerian women. Fertility Research and Practice. 2019 Dec;5:1-8.

IV. Sharma R, Bakshi H, Patel P, Patel B, Gajjar S, Dave R, Bapat N, Mehta R, Mehta L, Chaudhary P. Burden of Infertility, Its Risk Factors, Perceptions and Challenges Faced by Women of Peri-urban Community from Ahmedabad City: Mixed Method Study. Indian Journal of Community Medicine. 2024 Sep 1;49(5):687-94.

V. WHO (World Health Organization), Infertility. [Retrieved online on Tuesday 25 February, 2025 from: https://www.who.int/news-room/fact-sheets/detail/infertility]

VI. Sami N, Ali TS. Health seeking behavior of couples with secondary infertility. Journal of College of Physicians and Surgeons Pakistan. 2006;16(4):261.

VII. Zheng D, Zhou Z, Li R, Wu H, Xu S, Kang Y, Cao Y, Chen X, Zhu Y, Xu S, Chen ZJ. Consultation and treatment behaviour of infertile couples in China: a population-based study. Reproductive biomedicine online. 2019 Jun 1;38(6):917-25.

VIII. Maduka NR, Okubor OP. Prevalence and Predictors for use of Herbal medicine in the treatment of Infertility in a secondary Health facility in South-South Nigeria. Nigerian Medical Journal. 2024 Jul 1;65(3):255-65.

IX. Okonofua FE, Harris D, Odebiyi A, Kane T, Snow RC. The social meaning of infertility in Southwest Nigeria. Health transition review. 1997 Oct 1:205-20.

X. Ombelet W, Cooke I, Dyer S, Serour G, Devroey P. Infertility and the provision of infertility medical services in developing countries. Human reproduction update. 2008 Nov 1;14(6):605-21.

XI. Panti AA, Sununu YT. The profile of infertility in a teaching Hospital in North West Nigeria. Sahel Medical Journal. 2014 Jan 1;17(1):7-11.

XII. Itodo JI, Rekwot PI, Aluwong T, Allam L, Jolayemi KO, Kyari S, Abah KO, Ibrahim S, Dogara MU, Yusuf AT, Musa MM. Azanza garckeana ameliorates Bisphenol A-induced reproductive toxicities in rabbit bucks. Theriogenology. 2022 Oct 15;192:150-65.

XIII. Nurudeen QO, Asinmi MR, Falana MB, Dikwa MA. Aphrodisiac Potentials of Aqueous Extract of Azanza garckeana Fruit Pulp in Fluoxetine-induced Sexually-impaired Female Rats. Journal of Science and Technology. 2023 Dec 13;15(2):11-22.

XIV. Bioltif YE, Edward NB, Tyeng TD. A chemical overview of Azanza garckeana. Biology, medicine, & natural product chemistry. 2020 Nov 17;9(2):91-5.

XV. Olayiwola VA, Abiodun FO, Ojedokun RO, Kolawole IO, Ihediuche CI. Early Growth Response of Azanza garckeana (Exell & Hillc) as Influenced by Organic and Inorganic Fertilizers. Nigeria Agricultural Journal. 2021 Aug 31;52(2):284-8.

XVI. Malombe I, Mutangah JG. Assessment of conservation value of natural habitat relics in Thika district. Final Report. National Museums of Kenya, Nairobi. 2004;21.

XVII. Nonso EE, Ekenechukwu U, Bethel DC, Frances OI, Serah NW, Chukwudi AF, Uche NC, Ekenedirichukwu AK. Impact Of Ethanolic Extract of Azanza garckeana (Goron tula Fruit) On Semen Quality and Hormonal Profile Of Adult Wistar Rats.

XVIII. Agarwal A, Sekhon LH. The role of antioxidant therapy in the treatment of male infertility. Human fertility. 2010 Dec 1;13(4):217-25.

XIX. Adenowo AF, Salisu FT, Akinsanya MA, Kazeem MI. Nutraceutical Potentials of Azanza garckeana (Snot Apple): A Review. Current Nutraceuticals. 2022 May;3(2):e210322202460.

XX. Bioltif YE, Edward NB, Tyeng TD. A chemical overview of Azanza garckeana. Biology, medicine, & natural product chemistry. 2020 Nov 17;9(2):91-5.

XXI. Momodu IB, Okungbowa ES, Agoreyo BO, Maliki MM. Gas chromatography–mass spectrometry identification of bioactive compounds in methanol and aqueous seed extracts of Azanza garckeana fruits. Nigerian Journal of Biotechnology. 2022 Jun 6;38(1):25-38.

XXII. Arab A, Hadi A, Moosavian SP, Askari G, Nasirian M. The association between serum vitamin D, fertility and semen quality: A systematic review and meta-analysis. International Journal of Surgery. 2019 Nov 1;71:101-9.

XXIII. Pizzol D, Foresta C, Garolla A, Demurtas J, Trott M, Bertoldo A, Smith L. Pollutants and sperm quality: a systematic review and meta-analysis. Environmental Science and Pollution Research. 2021 Jan;28:4095-103.

XXIV. Agboola B, Fisher S, Vithala S, Maheshwari A. Association between lifestyle factors and semen parameters: An overview of systematic reviews. Fertility science and research. 2021 Jul 1;8(2):111-21.

XXV. NRC [National Research Council, Division on Earth, Life Studies, Institute for Laboratory Animal Research, Committee for the Update of the Guide for the Care], 2010 Use of Laboratory Animals. Guide for the care and use of laboratory animals.

XXVI. Nonso EE, Ekenechukwu U, Bethel DC, Frances OI, Serah NW, Chukwudi AF, Uche NC, Ekenedirichukwu AK. Impact of ethanolic extract of

Azanza garckeana (gorontula fruit) on semen quality and hormonal profile of adult Wistar rats. 2025. International Journal of Recent Research in Life Sciences. 12 (1):1-4.

XXVII. Adenowo AF, Salisu FT, Akinsanya MA, Kazeem MI. Nutraceutical Potentials of Azanza garckeana (Snot Apple): A Review. Current Nutraceuticals. 2022 May;3(2):e210322202460.

XXVIII. Yu L, Yu Y, Xiao Y, Tian F, Narbad A, Zhai Q, Chen W. Lead-induced gut injuries and the dietary protective strategies: A review. Journal of Functional Foods. 2021 Aug 1;83:104528.

XXIX. Kumar S. Occupational and environmental exposure to lead and reproductive health impairment: an overview. Indian journal of occupational and environmental medicine. 2018 Sep 1;22(3):128-37.

XXX. Yu L, Yu Y, Xiao Y, Tian F, Narbad A, Zhai Q, Chen W. Lead-induced gut injuries and the dietary protective strategies: A review. Journal of Functional Foods. 2021 Aug 1;83:104528.

XXXI. Walke G, Gaurkar SS, Prasad R, Lohakare T, Wanjari M. The impact of oxidative stress on male reproductive function: exploring the role of antioxidant supplementation. Cureus. 2023 Jul 27;15(7).

XXXII. Torres-Arce E, Vizmanos B, Babio N, Marquez-Sandoval F, Salas-Huetos A. Dietary antioxidants in the treatment of male infertility: counteracting oxidative stress. Biology. 2021 Mar 20;10(3):241.

XXXIII. Ajayi IA, Ojelere OO. Phytochemical screening, proximate analysis and antimicrobial activity of aqueous extract of Megaphrynium macrostachyum seeds. Int. J. Eng. Res. Technol. 2013;2:2123-31.

XXXIV. Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde SV, Kumar V. Saponins: A concise review on food related aspects, applications and health implications. Food Chemistry Advances. 2023 Oct 1;2:100191.

XXXV. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Future Journal of Pharmaceutical Sciences. 2021 Dec;7(1):1-3.

XXXVI. Dzobo K, Thomford NE, Senthebane DA, Shipanga H, Rowe A, Dandara C, Pillay M, Motaung KS. Advances in regenerative medicine and tissue engineering: innovation and transformation of medicine. Stem cells international. 2018;2018(1):2495848.

XXXVII. Radmehr M, Shadmehr A, Haghbeen M, Abiri S, Taheri L, Rahmanian E, Nezhad AK, Ghaedi M, Rahmanian Z, Foroughian M, Sadeghi SE. Network meta-analysis of some herbal medicines for osteoarthritis pain control: a methodology for the efficacy assurance of new pharmaceutical products. EurAsian Journal of BioSciences. 2020 Aug 1;14(2).