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Methanol Extract of *Azanza garckeana* Fruit Pulps Protects against Formalin-Induced Reproductive Toxicity in Adult Albino Male Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The aqueous extract of *Azanza garckeana* was recently reported of exhibiting ameliorative and pro-fertility properties however the protective effects on formalin testicular toxicity have not been studied.
Objective: This study investigated the protective effect of methanol extract of *Azanza garckeana* on formalin-induced testicular toxicity.
Methods: Forty male albino mice were randomly divided into 8 groups of 5. Animals in the first group (1) served as control and administered normal saline (1 ml/kg) by the oral route daily for 40 days. In similar manner, animal in groups 2 received formalin (10 mg/kg) by the IP route, while animals in groups 3; 4 and 5 concurrently received formalin (10 mg/kg IP) and extract at doses of 125; 250 and 500 mg/kg respectively by the oral route. Mice in groups 6; 7 and 8 received the extract at doses of 125; 250 and 500 mg/kg respectively. Phytochemical analysis was conducted for each constituent using specific methods. Gonadotropin and sperm analysis were carried out using standard methods.
Result: Phytochemical screening revealed the presence of various constituents, but notably flavonoids. Induced-toxicity with formalin and concurrent treatment with extract at doses of 250 and

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500 mg/kg from day 20 to 40 caused significant body weight increase compared to baseline ($p < 0.05$). Similarly, treatment with the extract alone at all doses caused significant increase in body weight from day 20 to 40 ($p < 0.05$). Treatment with the extract at 250 and 500 mg/kg, caused a significant increase in weight of testes and epididymis compared to control and untreated group ($p < 0.05$).The extract induced significant increase in gonadotropin levels of animals compared to control and the untreated group ($p < 0.05$).The extract at 125 mg/kg demonstrated the highest fecundity potential, but there was no any consistent relationship between GSI and fecundity.
Conclusion: This investigation was able to establish the protective and pro-fertility potentials of methanol extract of *Azanza garckeana*.

Keywords: Azanza garckeana; gonadotropin; reproductive toxicity; spermatogenesis; gonadosomatic index; semen.

1. INTRODUCTION

Reproductive toxicology has been of major concern to the general public. It is an issue that is concerned with the interference of both physical and chemical agents on fertility. Reproductive toxicology is usually expressed in both genders through decreased production of reproductive parameters such as semen and the gonadotrophins [1]. Chemical injuries arising from inhalation and other modes of transmission particularly from work places or by way of inadvertent ingestion do directly inflict damages especially in the male folks. The reproductive system is basically a very delicate system and it constitutes a variety of targets for many toxicants. Thus, normal gonadal processes such as production of gonadotrophins and spermatogenesis are usually afflicted by such chemicals. Similarly, reproductive structures that constitute the anatomy of the reproductive system such as the testes or epididymis are most at risk to the harmful effects of such toxicants.

One example of such toxic chemicals is formaldehyde (formalin) which is a chemical that is widely used as solvent or reagent for various purposes such as in resins, hospitals, textile, construction, laboratories and chemical industries for its preservative, sterilizing and stabilizing functions. Formalin is said to have an annual commercial value that runs into billions of dollars, which suggests its economic importance and applications [2]. Though it is produced *de novo* by all living organisms, including humans, exposure to exogenous formalin which is found almost everywhere poses great danger to public health [3, 4].

Formalin has long been associated with male reproductive toxicity, even though studies in humans have remained debatable. However, some limited studies have indicated the toxic effects of formalin on sperm viability parameters [5]. Consequently, formalin has been classified as a xenobiotic carcinogen as well as an environmental pollutant. Indeed, several pre-clinical studies have reported the toxiferous effects of formalin on fertility in experimental animals [6-9].

Male infertility has been a growing concern around the world. Indeed, in some countries, male infertility accounts for between40-50 % of infertility cases [10, 11]. Curiously, there has been a global decrease in semen quality over the last 6-7 decades [12-14].

The aqueous extract of *Azanza garckeana* was recently reported of exhibiting ameliorative and pro-fertility properties [15]. The aim of the present study was to investigate the protective effects of the methanol extract of *Azanza garckeana* on some reproductive parameters in formalin induced-toxicity in male mice.

2. MATERIALS AND METHODS

2.1 Animals

Forty (40) adult male albino mice with mean weight of 26.30± 6.10g were purchased from the Animal Experimental Unit of the University of Jos. They were approved and certified for the experiment by the Committee on use of experimental animals' protocol of the Department of Pharmacology and Toxicology, University of Jos under the certificate number F17.00379 issued to the authors (FE) and signed on 1st August, 2019. The mice were then handled under ethical conditions for the use and care of laboratory animals [16]. The animals were fed with standard solid nutritional pellets and water *ad libitum* until the commencement of the experiment. The experimental room was illuminated with natural light.

2.2 Preparation of Extract

Azanza garckeana pulps were purchased from a reputable dealer in a local market in Tula town of Kaltungo Local Government Area, Gombe state of North East Nigeria in the month of November September 2018. The pulps were re-authenticated by a taxonomist, Mr. Christopher John of the Federal College of Forestry, Jos, Plateau state and a herbarium voucher specimen (number FHJ/260) was prepared.

The pulps were removed, washed, and carefully crushed in small pieces to enhance drying. They were then dried under shade in the laboratory. Thereafter, they were grounded to a finer powder and extracted according to the method described by Adegboye, 2008 [17].

600 g of the powdered pulp was extracted continuously with distilled water in a Soxhlet extractor for 25 hours at 55 ^{0}C. The extract was evaporated to dryness in a vacuum evaporator at 50 ^{0}C until a constant yield of 348.2 g (representing 58.03 %) following repeated weighing was obtained. The extract was reconstituted in normal saline for the purpose of the experiment.

The same solvent extraction procedure was used with another quantity of 340 g of the powdered pulps using methanol (80 %) for 96 hours at 40 ^{0}C.Thereafter, the extract was evaporated to dryness by vacuum evaporator at 50 ^{0}C until a constant yield of 194.5 g (representing 57.2 %) was obtained following repeated weighing. The extract was dissolved with dimethyl sulfoxide (DMSO) solvent and reconstituted with distilled water for the purpose of the experiment.

2.3 Phytochemical Analyses of the Fractions

The phytochemical analyses were conducted using various and respective methods for each constituent which include alkaloids, saponin, flavonoids, and carbohydrates [18], tannins [19], anthraquinones [20], steroids [21], and terpenes [22].

2.4 Treatment of Animals

The mice were randomly divided into eight groups of five mice each. Mice in the first group (1) were considered as the control group and were administered normal saline (1 ml/kg) by the oral route every morning daily for 40 days. In similar manner, animals in groups 2 received formalin alone by the IP route (10 mg/kg) while groups 3; 4 and 5 received in addition to formalin, the extract at doses of 125; 250 and 500 mg/kg respectively by the oral route. Mice in groups 6; 7 and 8 were administered the extract alone at doses of 125; 250 and 500 mg/kg by the oral route for same duration of 40 days. The weights of each mice in the respective groups were determined periodically at 10-day intervals until the end of the 40 days treatment.

2.5 Determination of Serum Gonadotropin Levels and Testicular Weights

The microwell enzyme-linked immunoassay (ELISA) method that is based on competitive binding of the gonadotropins on immobilized specific antibody as described by Braide *et al* [23] and also that by Gan and Patel [24] was used. This method allows the detection of very small quantities of antigens such as protein peptides and hormones in a fluid sample. It utilizes enzyme-labeled antigens and antibodies to detect the biological molecules. The antigen is allowed to bind a specific antibody which is itself subsequently detected by a secondary enzyme-coupled antibody. A chromogenic substrate for the enzyme yields an antigen that allows for quantitative measurement based on such colorimetric readings.

Following the daily administration of the drug and extract, and 24 hours after the last dose, the animals were anesthetized using chloroformand blood samples obtained through cardiac puncture. The blood samples from each mouse were collected in a non-heparinized tube and allowed to stand for 3 hours in iced water. Thereafter, it was centrifuged at 3000 rpm for 10 minutes to separate the serum from clots and the serum was collected and stored at -20 ^{0}C for two days. Bioassay using the micro well ELISA method was carried out for the gonadotropins that include luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone.

2.6 Sperm Concentration/Motility Assay

The method of Bavister and Andrews [25] was used with little modifications. Following the last dose of the daily administrations of the extract and normal saline, the mice were allowed to rest for 24 hours. Thereafter, the distal caudal

epididymis was dissected and placed in an equilibrated 2 ml medium in a petri dish. A needle was used to release the sperm cells from the caudal epididymis into the medium and the sperm cells were allowed to swim out into the medium for about 4 minutes. Sperm concentrations were then determined by physical count under the electronic microscope at a magnification of x400. Thereafter, the solution was added to the culture for sperm motility assay. The rapid motility was determined after 4 hours as the cut-off time.

2.7 Determination of Testicular Profile and Epididymal Weights

The right and left testes for each mouse were carefully removed and their lengths and weight measured separately for each group. In similar manner, the epididymis were isolated and weighed for each animal.

2.8 Analysis of Data

Data were collected and expressed as Mean ± SEM. The data were analyzed statistically using student's t-test, two-way ANOVA and correlation method at a significance level of p = 0.05.

3. RESULTS

3.1 Phytochemical Composition

The result for the phytochemical screening of the methanol extract of *Azanza garckeana* is presented on table 1. The result revealed the presence of flavonoids in high quantity, followed with alkaloids and cardiac glycosides. Steroids and tannins were present in much lower quantity while saponins, terpenes and anthraquinones were absent.

3.2 Effect of *Azanza garckeana* on Body Weight of Mice

The results of the effect of daily administration of *Azanza garckeana* on body weight of mice induced with formalin toxicity are presented on Table 2 and Fig. 5. The results indicate that the body weight of mice that were treated with formalin alone was significantly lower compared to control group ($p < 0.05$), but not so when compared with the baseline values ($p > 0.05$). Treatment with the extract alone and concurrent administration of formalin with the extract at the doses of 250 and 500 mg/kg from day 20 to 40 induced significant body weight increased compared to baseline value ($p>0.05$). Generally, while treatment with formalin alone did not cause any significant change in body weight ($p < 0.05$), the extract exhibited significant and positive change in weight gain ($p < 0.05$) which was both dependent on dose and duration of treatment when compared to the baseline (Table 2) or the group that received formalin alone (Fig. 5).

3.3 Effect of *Azanza garckeana* on Testicular and Epididymis Weight

The results as shown on table 3 indicated that administration of the methanol extract of*Azanza garckeana* alone or with formalinat all doses produced a significant increase in testicular and epididymis weight compared to group 2 ($p < 0.05$).

3.4 Effect of *Azanza garckeana* on Gonadosomatic Index (GSI)

Results of the gonadosomatic index following formalin-induced toxicity and concurrent treatment with the extract are presented on table 4. The methanol extract of *Azanza garckeana* at the doses of 250 and 500 mg/kg produced significant increase (($p < 0.05$) in GSI in animals induced with formalin toxicity compared to those left untreated (group 2). Similarly, the extract when administered alone produced a dose-dependent and significant increase ($p < 0.05$) in GSI of animals at all doses used for this experiment compared to that of the untreated animals (group 2).

3.5 Effect of *Azanza garckeana* on Gonadotropins

The results of the effect of the extract on gonadotropins are presented on table 4. The results indicated that the extract alone at doses of 125 mg/kg induced significant increase in LH compared to those of control (group 1) and untreated formalin-induced toxicity (group 2) ($p < 0.05$). In a similar manner, the extract alone at doses of 500 mg/kg caused significant increase in FSH compared with the control and the group that received formalin alone ($p < 0.05$). However, the extract alone at the doses of used caused did not cause any significant increase in testosterone levels compared with the group that received formalin alone ($p > 0.05$). Generally, the extract caused significant increase in both LH and FSH in animals induced with formalin toxicity

compared to the untreated group (group 2), but caused non-significant decrease ($p > 0.05$) in testosterone levels when compared with control (group 1) or the untreated group (group 2). There was significant correlation between testicular weight and testosterone level ($r = 0.97$. $p < 0.05$).

3.6 Effect of *Azanza garckeana* on Sperm Concentration and Progressive Motility

3.6.1 Effect on sperm concentration

The effects of the aqueous extract of *Azanza garckeana* on sperm concentrations are shown on Fig. 1. The extract alone at doses of 250 and 500 mg/kg caused significant increase on sperm concentrations compared to both the control and formalin alone-treated groups ($p < 0.05$). The extract at the dose of 125 mg/kg did not demonstrate any significant ameliorative effect in sperm concentration on formalin-induced toxicity ($p > 0.05$). However, there was an observed ameliorative effect of the extract at the doses of 250 and 500 mg/kg of the extract by way of significant increase in sperm concentrations on formalin-induced toxicity ($p < 0.05$).

3.6.2 Effect on rapid progressive motility

The effects of the aqueous extract of *Azanza garckeana* on some sperm rapid progressive motility are shown on Fig. 2. The extract alone at doses of 250 and 500 mg/kg caused significant increase on sperm cell motility compared to both the control and formalin alone-treated groups ($p < 0.05$). The extract at the dose of 125 mg/kg did not demonstrate any significant ameliorative effect on motility on formalin-induced toxicity ($p > 0.05$). However, there was an observed ameliorative effect of the extract at the doses of 250 and 500 mg/kg of the extract by way of significant increase in motility on formalin-induced toxicity ($p < 0.05$).

Table 1. Phytochemical Composition and Yield ofMethanol Extracts of*Azanza garckeana* Fruit Pulps

Constituents	Composition
Alkaloids	++
Tannins	+
Flavonoids	+++
Steroids	+
Saponins	-
Carbohydrates	++
Cardiac glycosides	++
Terpenes	-
Anthraquinones	-
Yield (%)	57.20

Key: + Low
++ Moderate
+++ High
-- None

Table 2. Effect of Daily Administration of Methanol Extract of*Azanza garckeana* Fruit Pulpson Body Weight during formalin-induced toxicity in Mice

Group	Baseline	Day 10	Day 20	Day 30	Day 40
1	28.03 ± 1.60	28.47 ± 1.28	30.56 ± 1.95	35.93 ± 3.09	36.15 ± 3.05[a,b]
2	26.10 ± 1.66	29.62 ± 2.14	30.40 ± 3.09	26.77 ± 1.74	26.43 ± 1.80
3	25.90 ± 1.81	27.38 ± 2.50	31.30 ± 1.42	27.53 ± 1.99	27.87 ± 1.92
4	30. 35 ± 1.97	28.04 ± 1.41	29.01 ± 2.23	31.60 ± 2.57	31.23 ± 2.19
5	28.48 ± 1.41	28.11 ± 2.36	32.33 ± 2.06	32.86 ± 1.93	33.64 ± 1.32[a,b]
6	27.98 ± 1.21	30.58 ± 1.55	33.82 ± 1.33	34.43 ± 1.33	34.82 ± 1.70[a,b]
7	29.36 ± 1.90	30.87 ± 1.68	34.78 ± 1.94	34.92 ± 1.91	35.84 ± 1.74[a,b]
8	26.61 ± 2.34	32.12 ± 1.58	32.87 ± 2.12	34.31 ± 3.08	34.16 ± 2.73[a,b]

N = 5, values are Mean ± SEM
[a] = Treatment duration has significant effect on weight compared to baseline value (p<0.05).
[b] = Changes in treatment in any group also has significant effect compared to group2 (p<0.05).
1 = Control, Normal saline, 1 mg/kg
2 = Formalin, 10 mg/kg
3 = Formalin, 10 mg/kg & Extract, 125 mg/kg
4 = Formalin, 10 mg/kg & Extract, 250 mg/kg
5 = Formalin, 10 mg/kg & Extract, 500 mg/kg
6 = Extract, 125 mg/kg
7 = Extract, 250 mg/kg
8 = Extract, 500 mg/kg

Table 3. Testicular and epididymis weights of mice treated with methanol seed pulp extract of*Azanza garckeana* during formalin-induced toxicity

Group	Weight of testes (g)	Length of testes (mm)	Epididymis Weight (g)
1.	0.39 ± 0.06*	4.72 ± 0.24	0.28 ± 0.02
2.	0.24 ± 0.08	4.53 ± 0.09	0.18 ± 0.05
3.	0.19 ± 0.08	4.70 ± 0.26	0.39 ± 0.18
4.	0.32 ± 0.01	4.68± 0.21	0.20 ± 0.04
5.	0.33 ± 0.06*	4.64 ± 0.67	0.63 ± 0.13*
6.	0.34 ± 0.08*	4.75± 0.71	0.24 ± 0.01
7.	0.35 ± 0.02*	4.83 ± 0.24*	0.24 ± 0.03
8.	0.34 ± 0.12*	5.73 ± 0.58*	0.69 ± 0.03*

*N = 5, values are mean ± SEM, * = p < 0.05 compared to group 2.*
1 = Control, Normal saline, 1 mg/kg
2 = Formalin, 10 mg/kg
3 = Formalin, 10 mg/kg & Extract, 125 mg/kg
4 = Formalin, 10 mg/kg & Extract, 250 mg/kg
5 = Formalin, 10 mg/kg & Extract, 500 mg/kg
6 = Extract, 125 mg/kg
7 = Extract, 250 mg/kg
8 = Extract, 500 mg/kga

Table 4. Effect of Methanol Extract of *Azanza garckeana* on Gonadotropins of mice induced with formalin toxicity

Group	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/dl)
1	7.60 ± 3.69	8.37 ± 0.98	8.83 ± 0.32
2	6.70 ± 3.08	4.13 ± 0.85	8.03 ± 1.84
3	17.27 ± 0.74*	7.33 ± 1.52	5.93 ± 1.80
4	11.13 ± 1.32	5.60 ± 0.42	6.10 ± 1.51
5	14.18 ± 3.92*	13.63 ± 2.72*	6.23 ± 1.06
6	18.80 ± 2.12*	5.43 ± 1.30	6.70 ± 2.02
7	14.17 ± 1.12*	7.97 ± 1.66*	6.27 ± 1.28
8	10.44 ± 4.72*	28.96 ± 4.32*	8.04 ± 1.54*

** = p<0.05 compared with group 2, N=5 ,Values are expressed as Mean ± SEM.*
1 = Control, Normal saline, 1 mg/kg
2 = Formalin, 10 mg/kg
3 = Formalin, 10 mg/kg & Extract, 125 mg/kg
4 = Formalin, 10 mg/kg & Extract, 250 mg/kg
5 = Formalin, 10 mg/kg & Extract, 500 mg/kg
6 = Extract, 125 mg/kg
7 = Extract, 250 mg/kg
8 = Extract, 500 mg/kg

3.7 Effect on Fecundity Potentials

The results for the fecundity profiles are shown on Fig. 3. Animals in the control group exhibited the highest fecundity potentials followed by the group that received the extract alone at 250 mg/kg. The group with the least reproductive profile was that which received formalin alone.
The fecundity potential is simply explained in this context as testosterone production per unit of GSI which is expressed as:

$$\text{Fecundity Potential} = \frac{\text{Amount of Testosterone (ng/dL)}}{\text{GSI (\%)}}$$

3.8 Comparison of Gonadosomatic Index with Fecundity Potential

The results for the comparative values of GSI with those of the Fecundity Potential are shown on Fig. 5. The results showed that there was no any consistent or direct relationship between the GSI and fecundity potential.

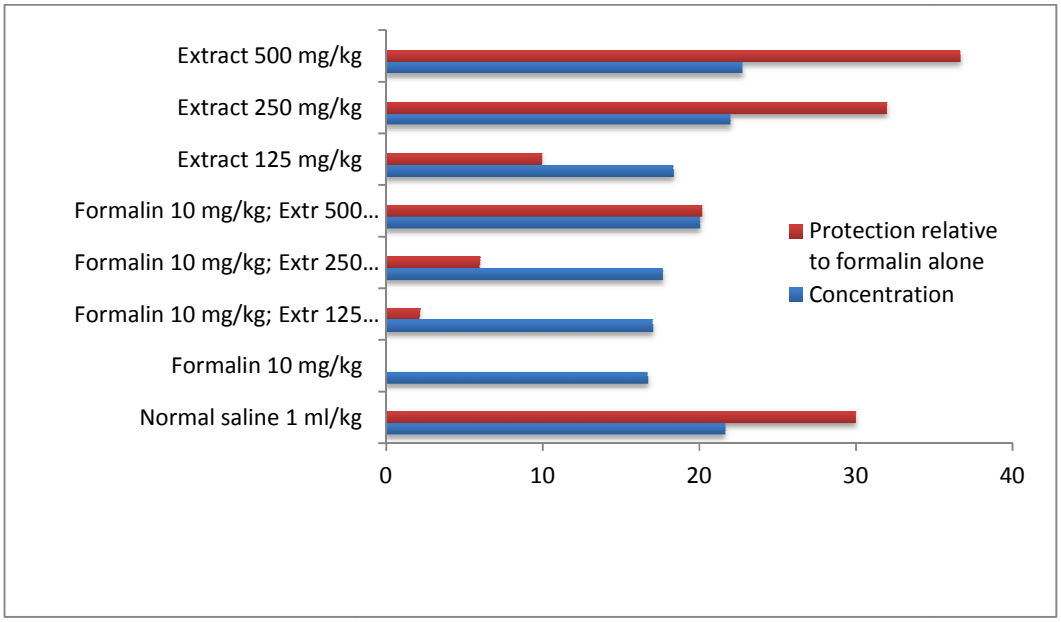

Fig. 1. Protective potential (%) of *Azanza garckeana* extract against formalin-induced toxicity on sperm concentrations (x10^6/mL) in Adult Albino Mice

N = 5

Value are expressed as Mean ± SEM

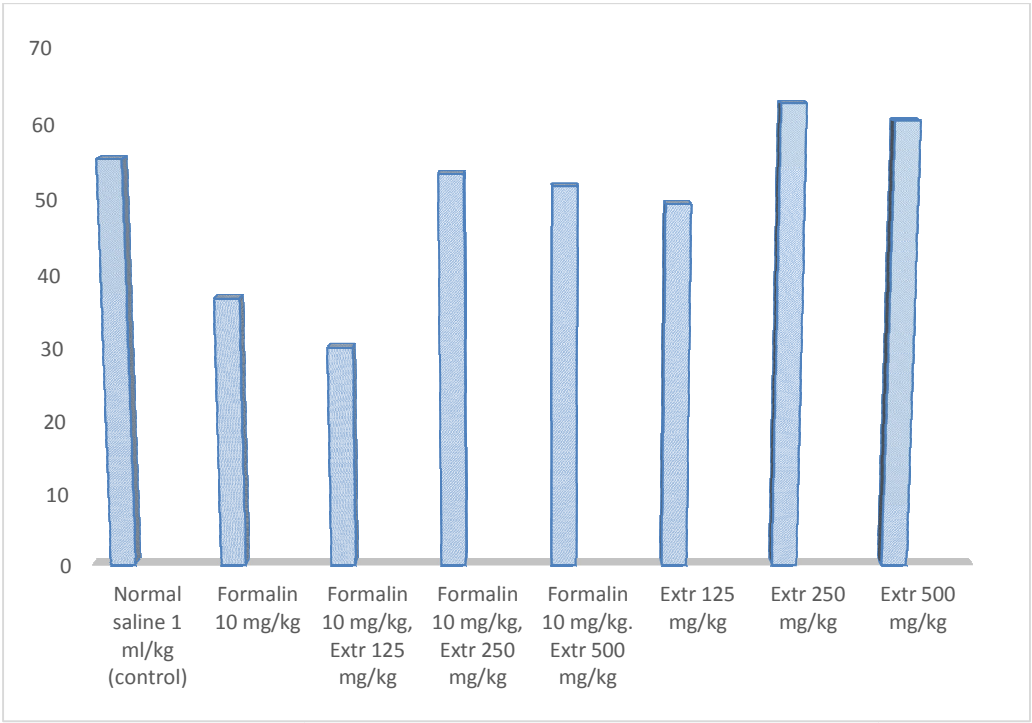

Fig. 2. Rapid Progressive Motility of Sperm Cells of Adult Albino Mice treated with Methanol Extract of *Azanza garckeana* Seed Pulps in formalin-induced toxicity

N = 5

Value are expressed as Mean ± SEM

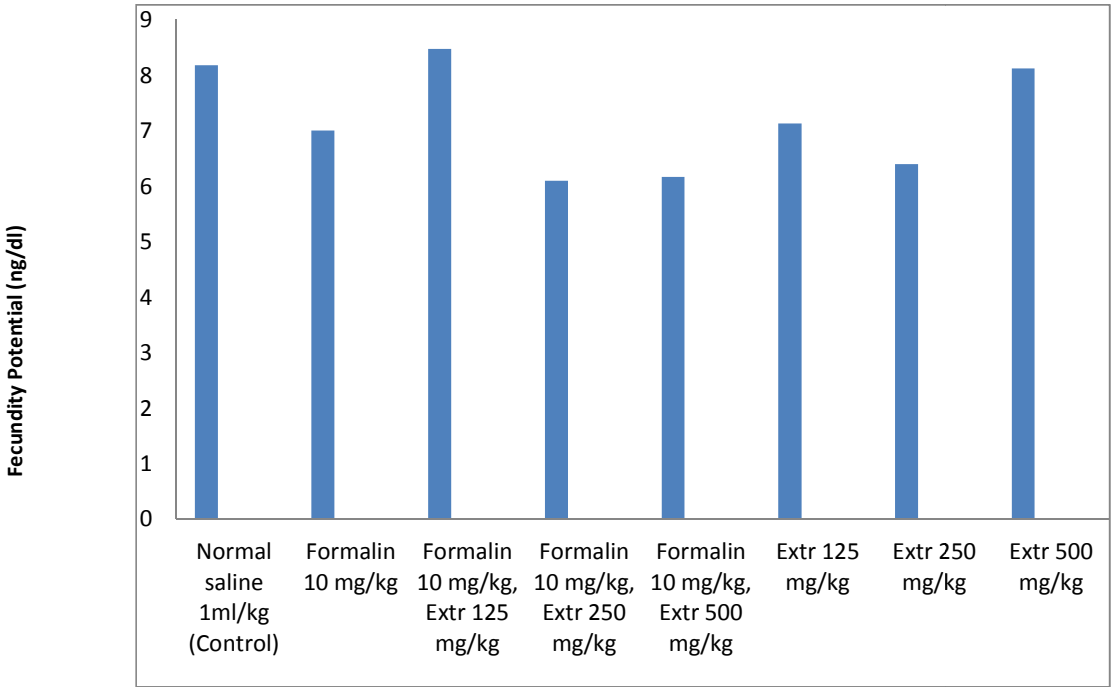

Fig. 3. Fecundity Potentials (ng/dl) of Adult Albino male mice Treated with Methanol Extract of*Azanza garckeana*Seed Pulps in formalin-induced toxicity

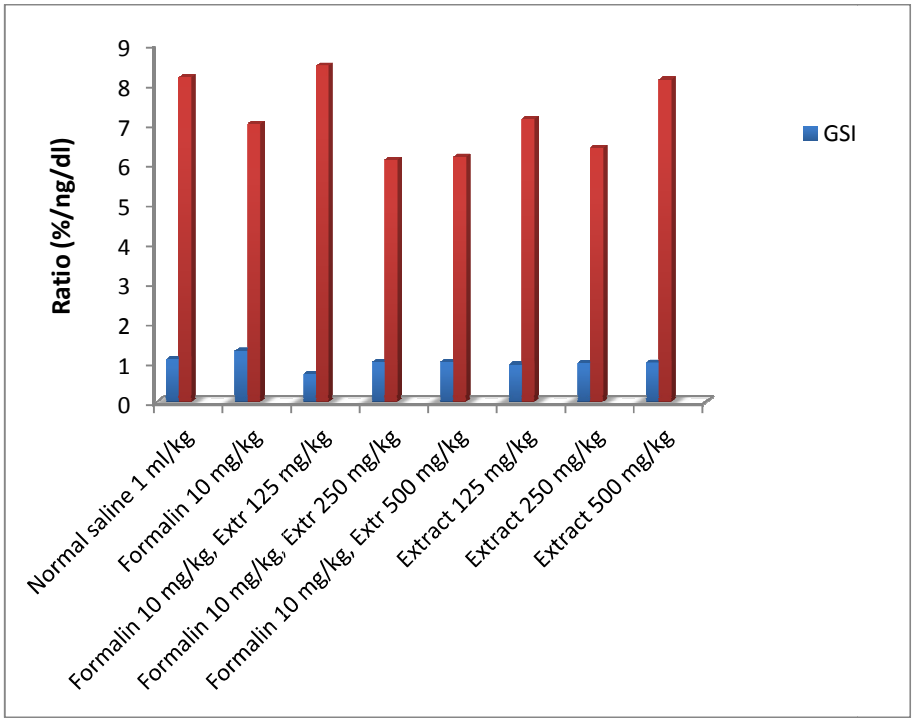

Fig. 4. Comparative Gonadosomatic index (GSI) With Fecundity of Adult Albino male mice treated with Methanol Extract of *Azanza garckeana* Seed Pulps during formalin-induced toxicity

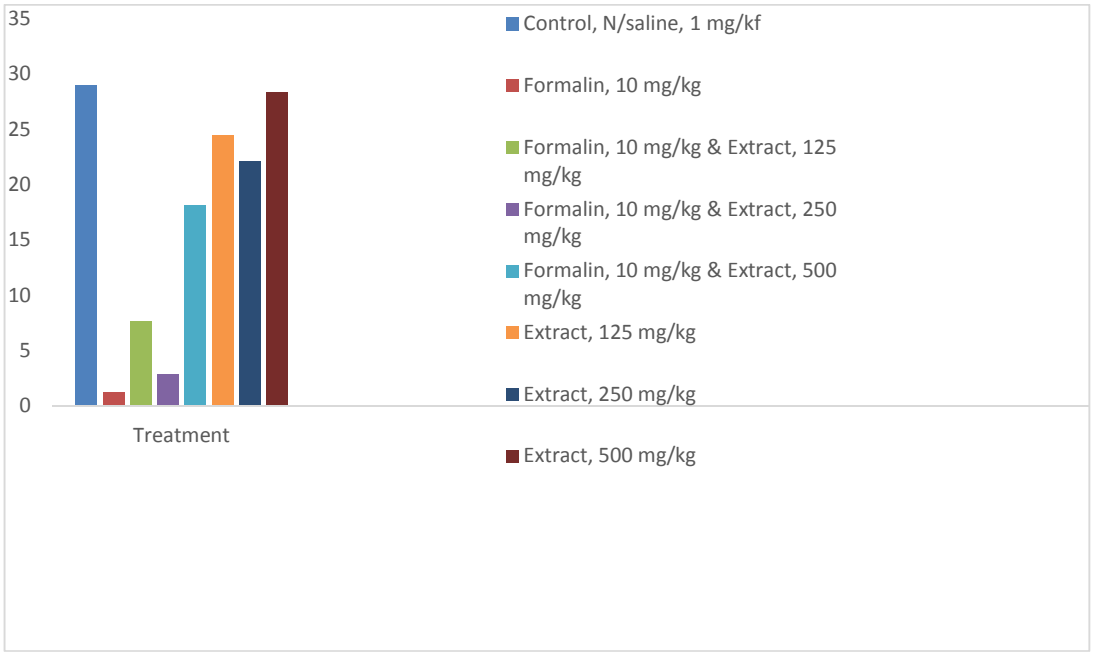

Fig. 5. Protective potential (%) of *Azanza garckeana* against formalin-induced toxicity on body weights of adult albino male mice. Values are calculated relative to those of their respective baseline values

4. DISCUSSION

The use of medicinal plants to treat infertility, particularly male infertility has been in existence for a long time. Phytochemicals are known to possess antioxidant properties that confer protective and beneficial effects on some reproductive parameters arising from insults such as oxidative stress, inflammation, obesity or chemical toxins [26-29].

Based on the results from this study, the methanol extract of Azanza garckeana offered protective mechanism against formalin-induced toxicity on male reproductive profiles. The result revealed the presence of the phytochemical flavonoids in high quantity, followed with alkaloids and cardiac glycosides. This may contribute to the protective effects of *Azanza garckeana* because phytochemicals are most often used for the treatment of male infertility, and this has increased particularly in recent years [11, 30,31]. The results also indicate that the body weight of mice that were treated with formalin alone was significantly lower compared to control group ($p < 0.05$), but not so when compared with the baseline values ($p > 0.05$). Treatment with the extract alone and concurrent administration of formalin with the extract at the doses of 250 and 500 mg/kg from day 20 to 40 induced significant body weight increased compared to baseline value ($p > 0.05$). Generally, while treatment with formalin alone did not cause any significant change in body weight ($p < 0.05$), the extract exhibited significant and positive change in weight gain ($p < 0.05$) which was both dependent on dose and duration of treatment when compared to the baseline or the group that received formalin alone. Several studies have shown that both obesity and overweight may affect male reproductive function [11, 32, 33]. In our study, it was shown that though Azanza garckeana reversed the weight loss caused by formalin induced-toxicity, it did not cause significant increase in weight gain compared to control.

The methanol extract of *Azanza garckeana* at all doses produced a significant increase in testicular and epididymis weight compared to group 2 ($p < 0.05$).Testicular sizes are positively and highly correlated with male fertility such that poor fertility is usually related with small testes [34]. Similarly, sperm post-maturation and motility are related to effective epididymal secretions such that a dysfunctional epididymis may result in the production of immature spermatozoa [35, 36]. The result of this investigation therefore suggests that *Azanza garckeana* may increase male reproductive

functions. Furthermore, the extract at the doses of 250 and 500 mg/kg produced significant increase (($p < 0.05$) in GSI of animals induced with formalin toxicity compared to those left untreated (group 2). Similarly, the extract when administered alone produced a dose-dependent and significant increase ($p < 0.05$) in GSI of animals at all doses used for this experiment compared to that of the untreated animals (group 2). In spite of the promotion of the GSI as an index of reproductive function, its usefulness remains controversial particularly when applied to humans. This is because of its variation among animal species. In view of this, the study assessed the reproductive function in relation to what was termed *fecundity potential* using the expression as shown below in order to compare the GSI with testosterone production. Curiously, it was observed that the animals in group that exhibited the lowest GSI correspondingly showed the highest fecundity potential (Fig. 4). However, there was no consistent relationship between GSI and fecundity potential.

The results also indicated that the extract alone at doses of 125 mg/kg induced significant increase in LH compared to those of control (group 1) and untreated formalin-induced toxicity (group 2) ($p < 0.05$). In a similar manner, the extract alone at doses of 500 mg/kg caused significant increase in FSH compared with the control and the group that received formalin alone ($p < 0.05$). However, the extract alone at the doses of used caused did not cause any significant increase in testosterone levels compared with the group that received formalin alone ($p > 0.05$). Generally, the extract caused significant increase in both LH and FSH in animals induced with formalin toxicity compared to the untreated group (group 2), but caused non-significant decrease ($p > 0.05$) in testosterone levels when compared with control (group 1) or the untreated group (group 2). There was significant correlation between testicular weight and testosterone level ($r = 0.97$. $p < 0.05$). Interestingly, there was a significant correlation between testicular weight and testosterone production ($r = 0.97$, $p < 0.05$), suggesting a strong relationship in variability (r^2 = 94.09 %)between testicular weight and testosterone production. Therefore, the extract of Azanza garckeana was able to protect against the formalin-induced toxicity on the production of gonadotrophins.The effect of the extract on sperm concentration showed that the extract alone at doses of 250 and 500 mg/kg caused significant increase on sperm concentrations compared to both the control and formalin alone-treated groups ($p < 0.05$). The extract at the dose of 125 mg/kg did not demonstrate any significant ameliorative effect in sperm concentration on formalin-induced toxicity ($p > 0.05$). However, there was a dose-dependent protective effect of the extract by way of significant increase in sperm concentrations on formalin-induced toxicity ($p < 0.05$). This observation suggests that Azanza garckeana may improve reproductive potential in chemical-induced toxicity. In similar manner, the extract caused significant increase on sperm cell rapid progressive motility compared to both the control and formalin alone-treated groups ($p < 0.05$). However, at the dose of 125 mg/kg, the extract did not demonstrate any significant protective effect on motility of sperm cells from animals induced with formalin toxicity ($p >0.05$). However, there was improvement at the doses of 250 and 500 mg/kg where the rapid progressive motility was significant from animals induced with formalin toxicity ($p < 0.05$).

5. CONCLUSION

In conclusion, this investigation revealed the reproductive potential and protective effect of methanol extract of*Azanza garckeana* against formalin-induced toxicity on some reproductive parameters of male rats. This could justify its traditional use by its local consumers as a remedy for some infertility cases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental protocols were subjected to the scrutiny and approval of Institutional Ethics Committee

COMPETING INTERESTS

There was no any conflict of interest declared by the Authors.

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