Antifertility Potentials of Aqueous Seed Extract of *Xylopia aethiopica* in Adult Male Wistar Rats

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Abstract
Background: There has been increased ingestion of concoctions and products from plants origin to boost male sexual functions. *Xylopia aethiopica is one of such plant with wide uses and acclaimed sexual importance*. The aim of this study is to determine the effects of aqueous extract of *Xylopia aethiopica* on the male sexual function and histology of the testes of adult Wistar rats. **Materials and Methods:** Twenty (20) adult male Wistar rats weighing between 180g and 200g were used for this study. They were divided into four groups. Group A served as the control group, while treatment groups (B, C and D) were administered 250mg/kg, 500mg/kg and 1000mg/kg body weight of the extract respectively for a period of 56 days, after which the testes were excised, weighed and fixed in Bouin's fluid for histological analysis. Semen was aspirated and analyzed for sperm motility, morphology and total count. **Results:** Results obtained showed that there was significant increase ($P<0.05$) in body and testicular weight in the treated groups when compared with the control group. A significant reduction was observed in sperm characteristics ($P<0.05$) in groups treated with 500mg/kg and 1000mg/kg of the extract compared to the control group. Histologically, the testes showed decreased tubular density and tubular atrophy in the groups administered with the extract. **Conclusion:** High doses of *Xylopia aethiopica* may have antifertility potentials in the male rats.

Keywords: *Xylopia aethiopica,* Testes, Antifertility, Semen, Fluid

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Introduction
Medicinal plants constitute valuable resources in low income countries and are often popular in rural communities. It was reported by WHO that 80% of world's developing populations depend on traditional herbs for treatment (1). A good number of medicinal plants have shown beneficial effects as contraceptives, antioxidants, anti-inflammatory, anticancer and anti-microbial agents (2,3,).

Xylopia aethiopica is a common Africa plant, predominant in the low land rainforest and savanna zones especially in Nigeria, Ghana, Cameron, Ethiopia, and Senegal. It is widely distributed in the humid forest zones, particularly along rivers (4). *Xylopia aethiopica* is commonly used in the treatment of cough (fruits and roots), bronchitis, dysentery and sores (leaves and bark, also, the fruit is employed in the treatment of malaria while the powdered root is used in local dressing of wounds (3).

Result from previous investigations by researchers in terms of the bioactive components of *Xylopia aethiopica* revealed the presence of alkaloid, cardiac glycosides, saponins, tannins, flavonoids, polyphenols. The stem bark contained high amount of

saponins (8.33%), alkaloid (5.67%) and flavonoid (5.24%), as reported by (Aguoru *et al.,* (5). These bioactive components are known to be bactericidal, pesticidal and fungicidal in nature and also a veritable source of drugs (6).

The testis is the male gonad in animals. It consists of two oval shaped organs located in the scrotum (7). It measures about 4cm in length, 2.5cm in breadth, 3 cm in anteroposterior diameter and weighs 10–15g (8). While the average testicular volume is between 15ml and 25 ml (9). The testis is vital in spermatogenetic and androgenetic processes and these important functions of the testis are controlled by gonadotropic hormones produced by the anterior pituitary gland (10). This gland produces luteinizing hormone which facilitate testosterone release. The presence of both testosterone and follicle-stimulating hormone (FSH) is essential for spermatogenesis (11).

Materials and methods

Plant Material

Negro pepper (*Xylopia aethiopica)* was purchased from the popular new market in Aba, Abia State, Nigeria. It was identified and authenticated by the Department of Plant Biology and Biotechnology (PBB), University of Benin, Benin City, Nigeria. *Xylopia aethiopica* was air dried at a room temperature for two weeks, and was then pulverized into powdered form using grinding machine (specimen grinding machine 55-CO202 Bench model version). 500g of the powdered *Xylopia aethiopica* was soaked in 1.5L of distilled H_2O for 24hrs with constant stirring. It was filtered and the filtrate poured into crucibles in water bath at 45^0C to expel the H_2O content. The eventual paste extract was then concentrated and preserved in a refrigerator until when needed-

Experimental Animals

Twenty (20) adult male Wistar rats weighing between 180-200g were used for this study. The animals were purchased and maintained in the Animal House Unit of the Department of Anatomy, University of Benin. They were kept in clean cages and allowed to acclimatize for two weeks under standard laboratory conditions (temperature 24-28°C and 12-hour light-dark cycle) before commencement of the experiment. They were allowed access to standard rat chow and water *ad libitum* throughout the experimental period.

Experimental Design

The rats were randomly assigned into a control group (A) and three treatment groups (B, C and D) of five (5) rats each. Rats in group A served as control; rats in group B were administered 250mg/kg *X. aethiopica*; rats in group C were administered 500mg/kg body weight of *X. aethiopica* and rats in group D were administered 1000mg/kg body weight of X. aethiopica. Extract was administered orally for fifty-six (56) days to animals in the various groups.

Sperm Analysis

Sperm cells were collected from the epididymis during the sacrificing of the rats by ligating the extremities of the vas deferens to a length of about 36nm. To a sterile petri dish, 10 µl of normal saline already adjusted to 37+2°C was added to the semen in the vas deferens in a Petri dish viewed under the microscope for the evaluation of semen parameters: sperm motility, sperm viability, sperm count and sperm morphology (12).

Sperm Count and Motility

The sperm count and motility were determined as described by Saalu et al. (13). Two drops of semen were placed on a slide, two drops of warm 2.9% sodium citrate were added and covered with cover slip and examined with microscope using × 40 objectives for motility. Sperm count was done using the improved Neubauer's counting chamber (Haemocytometer). The sperm concentration was then calculated and recorded in million and expressed as (X) $\times 10^6$/ml, where X is the number of sperm in a 16-celled square.

Sperm Viability
Viability test was carried out to evaluate living and dead spermatozoa. One volume of semen (a drop) was milted into two volumes of eosin solution (1% diluted water). After 30 seconds three volumes of nigrosine solution (10% nigrosine) was added and the sample homogenized. A thin smear was then made immediately and air dried. The Stained slide was examined under the oil immersion objective lens (x100). Live spermatozoa were unstained (white) and the dead ones were red (14).

Sperm Morphology.
The sperm cell morphology was assessed by staining the slide with the Improved Eosin and Leishman stain (15). A drop of the sperm cells was dispensed on a grease free clean slides and a smear was made. The slide was left to air dry. The slide was flooded with the Improved Eosin and Leishman stain for 15 mins. The stain was rinsed and the back was blotted dry with cotton wool and left to air dry. The slide was placed in a microscope with the magnification lens at x100. The slide was viewed with at least 30 magnification fields, the normal and abnormal sperm cells were spotted and scored in percentage. Normal sperm shows a normal sperm characteristic of head, middle piece and a tail. Abnormal sperm cells were characterized by large heads; headless, tailless, bulgy mid-piece curved tail and joined head.

Statistical Analysis
The results obtained were statistically analyzed using descriptive and inferential statistics and reported as Mean ± Standard Error of Means (S.E.M) in bar charts. The statistical analysis was performed using Graph pad Prism version 20. The significance of the difference in the mean of all parameter was determined using one-way analysis of variance (ANOVA; 95% confidence interval). Correlation between variables was evaluated using Pearson's correlation coefficient with level of significant difference of (<0.0001), significant difference of (<0.007) and significant difference of (<0.05)

Histological Analysis
The testes from the control and experimental groups were dissected out and fixed in Bouin's fluid. The tissues were processed using the routine methods for histological examination. Paraffin sections were stained with hematoxylin and eosin and qualitative microscopic examination was made.

Results
Figure 1 shows the comparison of initial and final body weights of animals in control group (A) and treatment groups (B, C and D). Analysis of data showed that There was statistically significant increase ($P<0.05$) in body weight in all the groups. On the other hand, figure 2 shows the testicular weight change across the treatment groups (B, C and D) when compared with control group. Analysis of data showed that there was no significant difference ($P>0.05$) in testicular weight in 250mg/kg, 500mg/kg and 1000mg/kg groups when compared to the control group. Figure 3 shows the progressive motility of sperm in treatment groups (B, C and D) when compared with control. Analysis of data showed that there was significant difference ($P<0.05$) in progressive motility of sperm in groups treated with 500mg/kg and 1000mg/kg of extract when compared to the control group, unlike group treated with 250mg/kg. Similarly, figure 4 shows the percentage of immotile sperm in treatment groups (B, C and D) when compared with control. Analysis of data showed that there was a significant increase ($P<0.05$) in immotile sperm levels in groups treated with 500mg/kg body weight and 1000mg/kg body weight of aqueous extract of *Xylopia aethiopica* when compared to the control group, unlike group treated with 250mg/kg body weight.
Figure 5 shows the percentage sperm viability in treatment groups (B, C and D) when compared with control. Analysis of

data showed that there was a significant decrease ($P<0.05$) in percentage viability in the group treated with 500mg/kg body weight and 1000mg/kg body weight of aqueous extract of *Xylopia aethiopica* when compared to the control group, unlike group 250mg/kg body weight. Similar result was obtained in figure 6 which showed the normal sperm morphology in treatment groups (B, C and D) when compared with control. Analysis of data showed that there was a significant decrease ($P<0.05$) in sperm normal morphology in the groups treated with 500mg/kg body weight and 1000mg/kg body weight of aqueous extract of *Xylopia aethiopica* when compared to the control group, unlike group 250mg/kg body weight. Also, figure 7 shows the abnormal morphology of sperm in treatment groups (B, C and D) when compared with control. Analysis of data showed that there was significant increase ($P<0.05$) in sperm abnormal morphology levels in groups treated with 500mg/kg b.wt, and 1000mg/kg b.wt of aqueous extract of *Xylopia aethiopica* when compared to the control group. Figure 8 shows the sperm count of rats in treatment groups (B, C and D) when compared with control. Analysis of data shows that there was a significant decrease ($P<0.05$) in total sperm count in 500mg/kg and 1000mg/kg group when compared to the control, unlike group treated with 250mg/kg.

Histology

Photomicrographs of histological sections of the testes of rats in Control (Plate 1a and 1b) show the following features; normal histological features with normal spermatogenic series in the wall of the seminiferous tubules, mature spermatozoa in the lumen of the seminiferous tubules and Leydig cells in the interstitial space. However, photomicrograph of histological sections of the testes of rats in group B (Plate 2a and b) shows reduced amount of spermatozoa in the lumen of seminiferous tubule. Also, Photomicrographs of histological sections of the testes of rats in group C (Plate 3a and b) show the following features; increased tubular atrophy, Leydig cell hyperplasia, reduced amount of spermatozoa in the lumen of the seminiferous tubules. In similar manner, photomicrographs of histological sections of the testes of rats in group D (Plate 4a and b) show the following features; tubular atrophy, Leydig cell hyperplasia and marked depletion of luminal content.

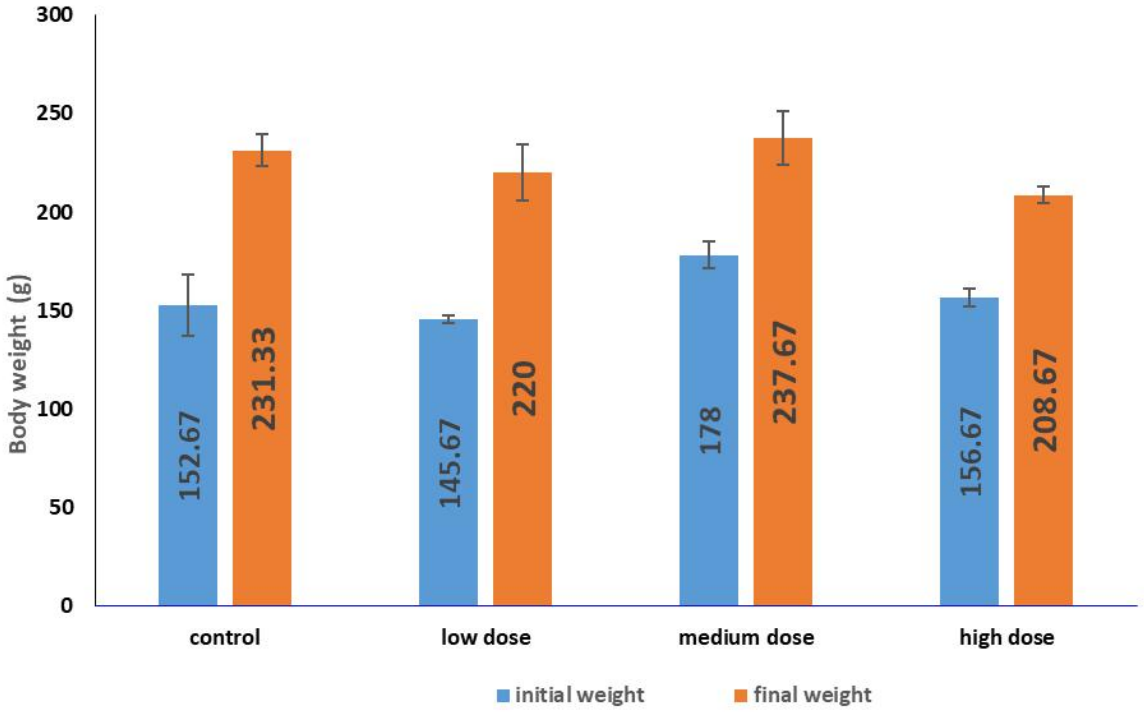


Figure 1: **Effects of *Xylopia aethiopica* on the initial and final body weight (g)**
Key: Low dose= 250mg/kg body weight. Medium dose= 500mg/kg body weight
High dose= 1000mg/kg body weight.

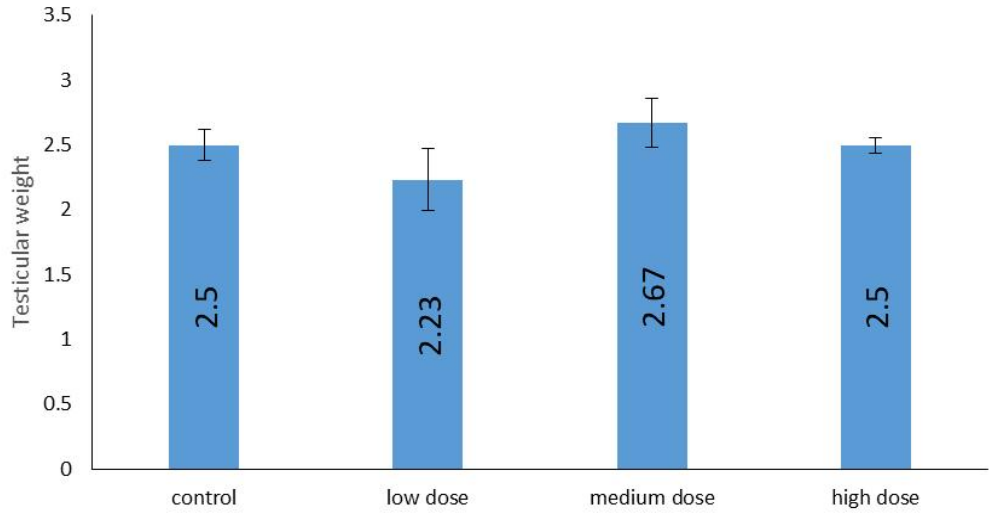


Figure 2: **Effects of *Xylopia aethiopica* on testicular weight (g)**

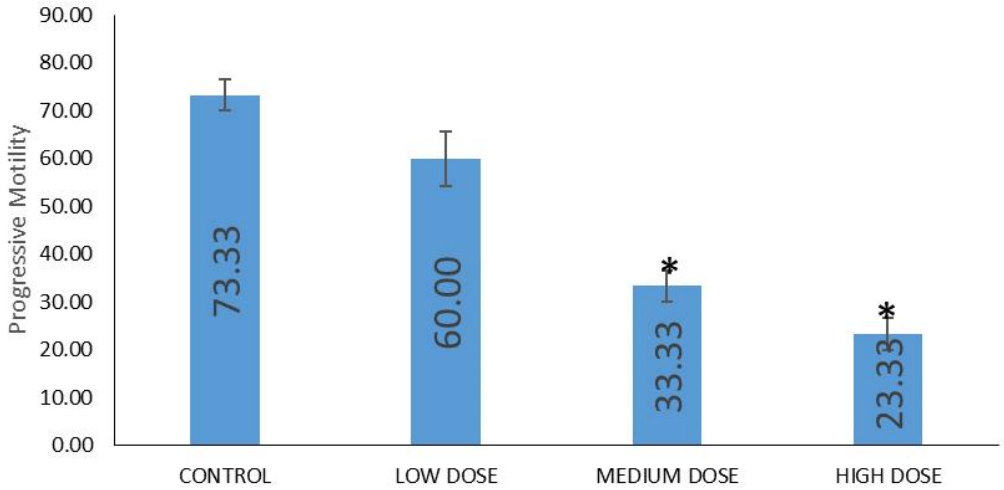


Figure 3: **Effects of *Xylopia aethiopica* on progressive motility (%)**

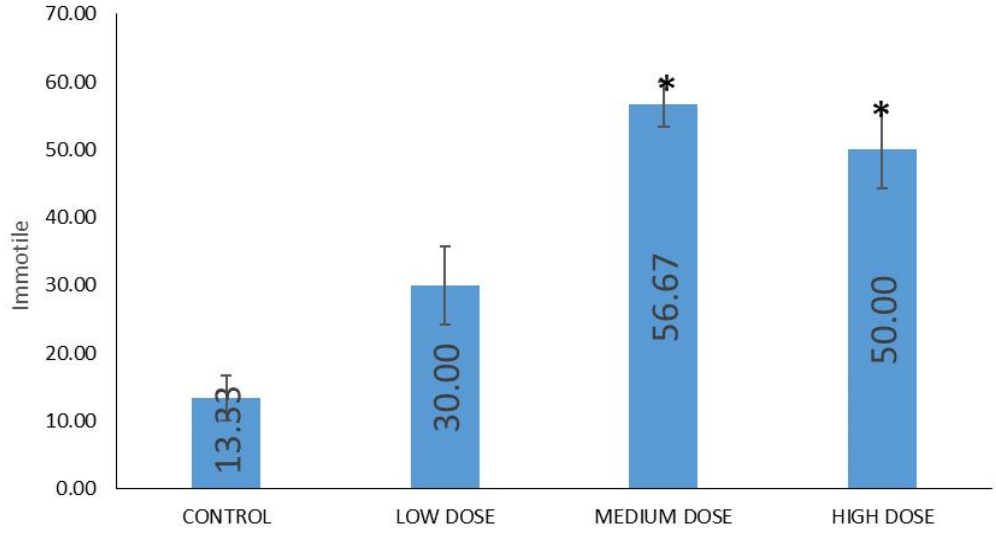


Figure 4: **Effects of *Xylopia aethiopica* on immotile sperm (%)**

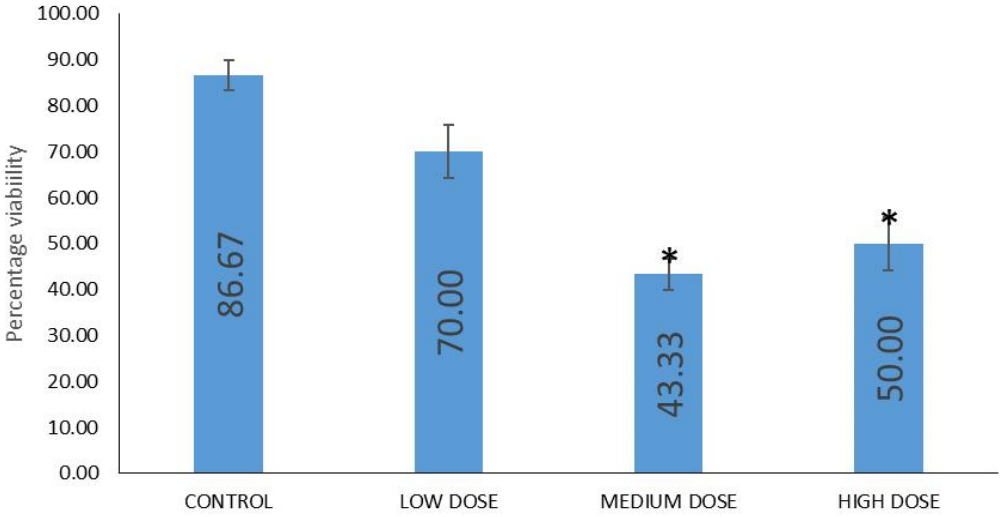


Figure 5: Effects of *Xylopia aethiopica* on percentage viability (%)

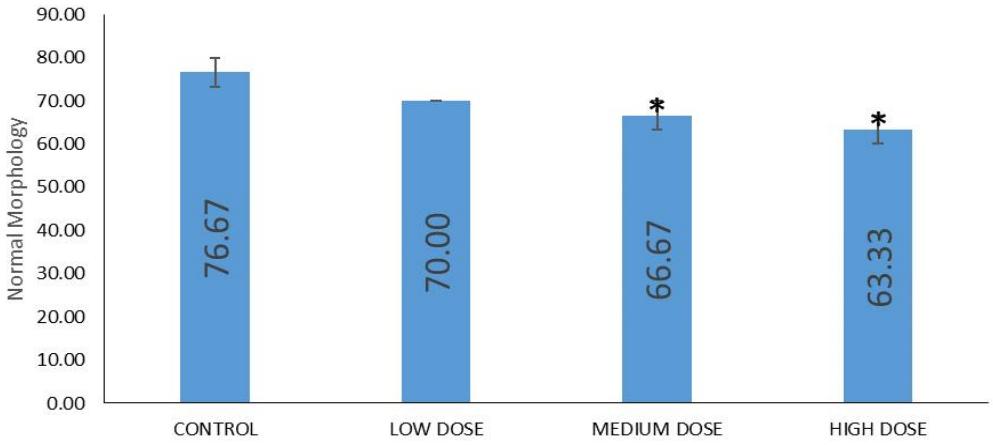


Figure 6: Effects of *Xylopia aethiopica* on normal morphology (%)

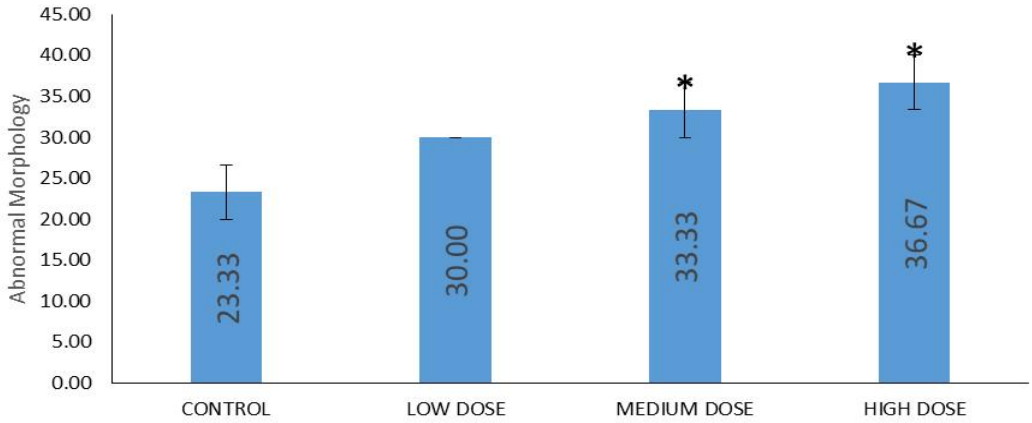


FIGURE 7: Effects of *Xylopia aethiopica* on abnormal morphology (%)

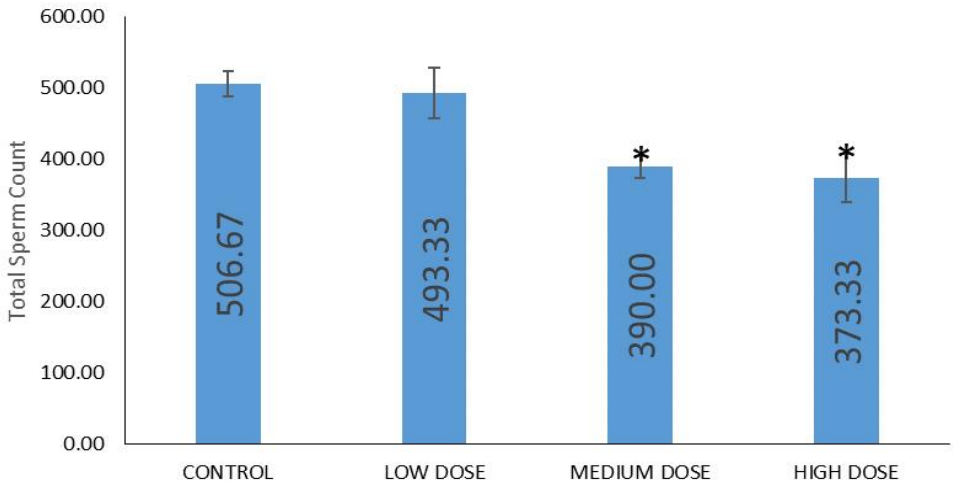


Figure 8: Effects of *Xylopia aethiopica* on total sperm count (X10^6 cells/mm^3)

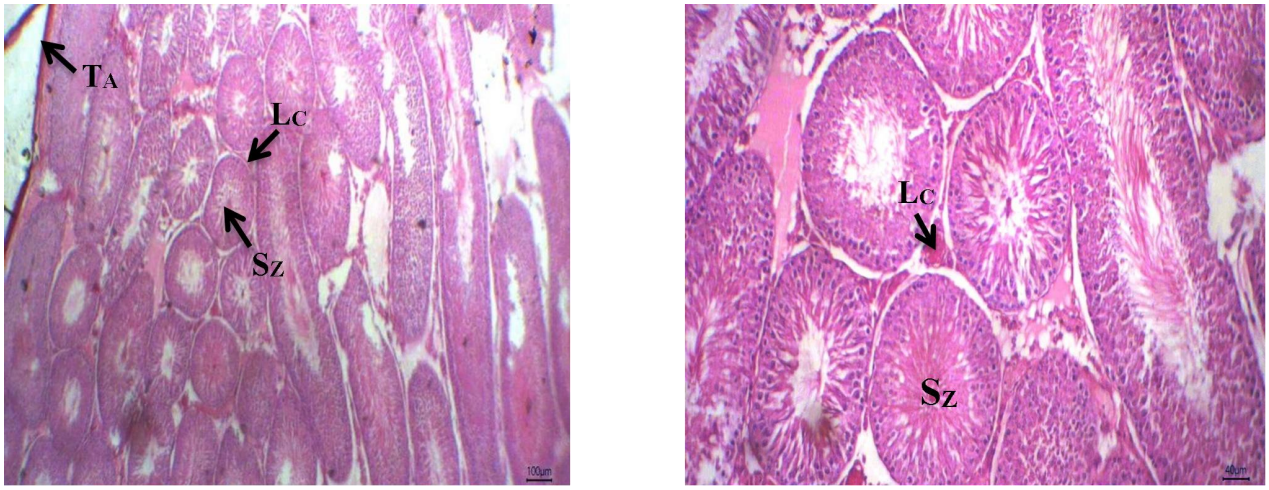


Plate 1 (a & b): Photomicrographs of the testis of the animals in control group showing: normal histological features with normal spermatogenic series in the seminiferous tubules, mature spermatozoa (Sz) in the lumen of the seminiferous tubules and Leydig cells (Lc) in the interstitial space and the tunica albuginae (T_A). H&E ×40 and ×100 respectively.

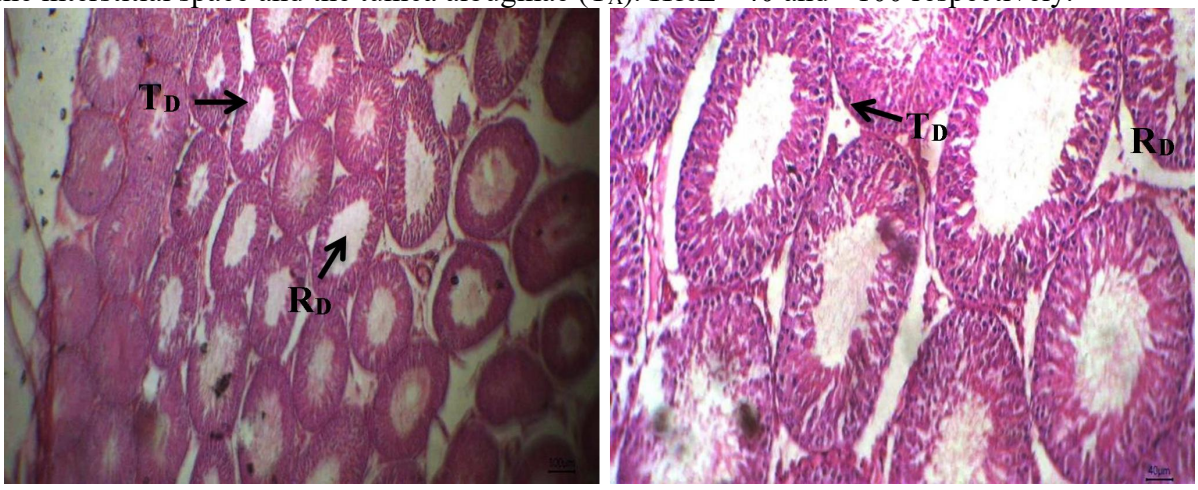
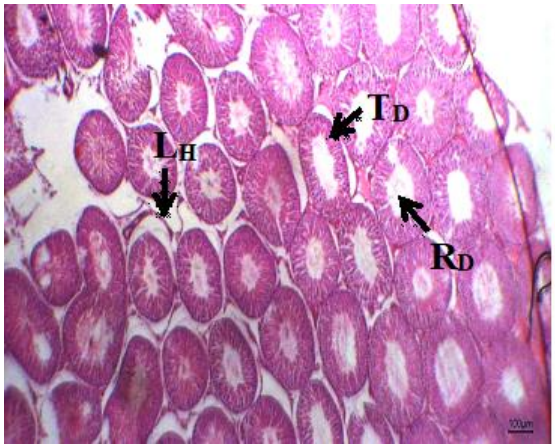


Plate 2 (a & b): Photomicrographs of the testis of the animals in group B treated with 250mg/kg body wt of *Xylopia aethiopica* showing; reduced amount of spermatozoa in the lumen of seminiferous tubules (R_D) and decreased tubular density (T_D) of sperm cells.. H&E ×40 and ×100 respectively.



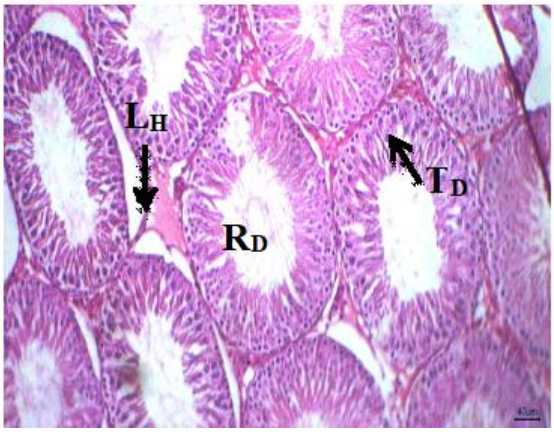
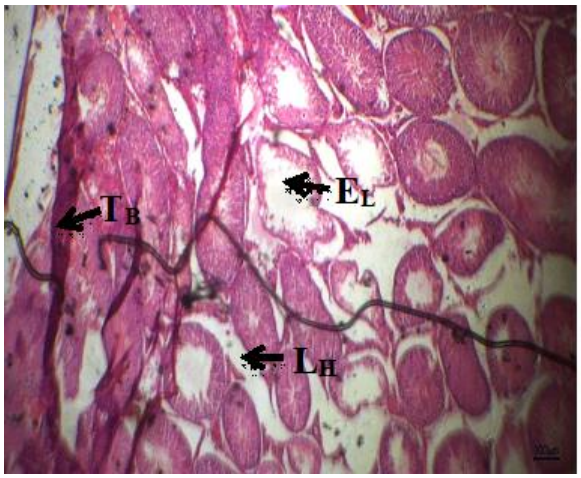


Plate 3 (a & b): Photomicrographs of the testis of the animals in group C treated with 500mg/kg body wt of *Xylopia aethiopica* showing: decreased tubular density (T_D) of sperm cells, Leydig cell hyperplasia (L_H)) reduced amount of spermatozoa in the lumen of the seminiferous tubules (R_D). H&E ×40 and ×100 respectively.



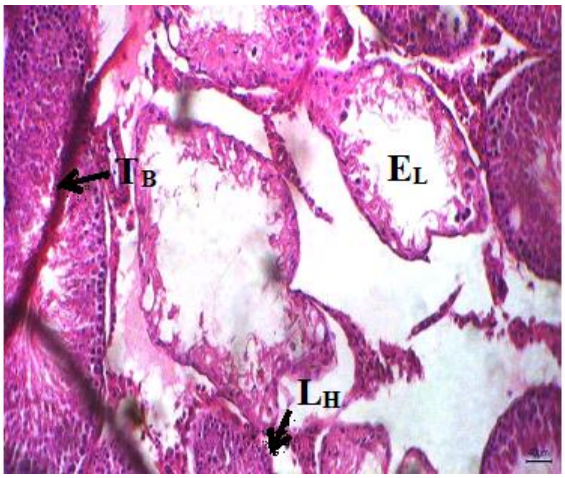


Plate 4 (a & b): Photomicrographs of the testis of the animals in group D treated with 1000mg/kg body wt of *Xylopia aethiopica* showing: tubular atrophy (T_B), Leydig cell hyperplasia (L_H) and some empty lumen (E_L). H&E ×40 and ×100 respectively.

Discussion

The findings of this study indicate that *Xylopia aethiopica* causes increase in the animal body weight as well as weight of the reproductive organs such as the testis and there was no significant difference in body weight of the animals in all treatment groups when compared to the control group. The findings in this study corroborate the previous work done by Woode *et al* (16). This study also indicates that *Xylopia aethiopica* significantly reduced the total sperm count and sperm motility in the groups that received medium dose and high dose of the extract when compared to the control group. This finding is in agreement with the previous study done by Nwangwa *et al*., (17) and in contrast to the previous study done by Woode *et al* (16) where it was shown to increase sperm count. There was significantly increased number of non-progressive sperm cells in the group administered medium and high dose extract of *Xylopia aethiopica* when compared with the control group. The increase in the number of immotile sperm cells and decrease in the number of progressively motile sperm cells in the groups could be as a result of direct toxic effects of the extract on the sperm cells at high doses. In terms of morphology, there was a significant decrease in normal morphology and increase in abnormal morphology of sperm cells in the groups administered with 500mg/kg and 1000mg/kg bodyweight of *Xylopia aethiopica* extract when compared to the control group. This collaborates the work of Uyovwiesevwa et al (18) where it was stated that prolonged administration of this extract could adversely affect male reproductive parameters. However, the group that received 250mg/kg body weight and the control rats had normal counts, motility, and morphology. This is in line with the work of Ansa et al (19), were they posited that consumption of low dose extract of *Xylopia aethiopica* could be beneficial to rabbit bucks. In fact, in the experimental groups that received *Xylopia aethiopica* aqueous extract, the sperm parameters showed evidence of dose dependent toxicity. In addition, high dose intake of *Xylopia aethiopica* reduces spermatogenesis with consequent decrease in quantity and quality of sperm cells as well as depleted sperm cells in lumen of seminiferous tubules. This finding is similar to earlier findings by Onyebuagu et al (20).

Conclusively, the effects of aqueous extract of *Xylopia aethiopica* on the testis were dose dependent and could result in deleterious effect at high dose. It shows that excessive and uncontrolled dietary intake of *Xylopia aethiopica* may be harmful to spermatogenesis in the testes, thus impairing testicular functions and possibly compromised male fertility.

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