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HISTOMORPHOMETRIC STUDIES OF GARCINIA KOLA, MUSA PARADISIACA AND KHAYA IVORENSIS VS SURE MALE CAPSULE FORMULA DRUG (TRIUMVIRATE COMBINATION OF G. KOLA, M. PARADISIACA AND K. IVORENSIS) ON TESTICULAR FUNCTION IN ADULT MALE WISTAR RATS

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ABSTRACT

This study evaluated the histomorphometric effects of *Garcinia kola*, *Musa paradisiaca*, *Khaya ivorensis*, and the Sure Male Capsule Formula—a commercial sexual stimulant comprising a combination of these three botanicals—on testicular function in adult male Wistar rats. Thirty rats were divided into five groups (n=6): Group A (control), Group B (500 mg/kg *M. paradisiaca*), Group C (200 mg/kg *G. kola*), Group D (200 mg/kg *K. ivorensis*), and Group E (400 mg/kg Sure Male Capsule Formula). Treatments were administered orally for 28 days. Post-treatment assessments included weight changes, semen parameters, histological and histopathological evaluations of testicular tissue using Johnsen's scoring system. Results revealed a significant increase in body weight in groups D and E ($P<0.05$). All treatment groups demonstrated varying degrees of testicular degeneration and reduced semen quality, including significant reductions in sperm count, motility, morphology, and viability. Histologically, all extracts showed degenerative changes in the seminiferous tubules, with the Sure Male Capsule Formula group showing pronounced architectural distortion and degeneration of interstitial and Leydig cells. However, Johnsen's scores were significantly lower in groups B and C, indicating severe spermatogenic disruption, while scores in groups D and E were comparable to the control, suggesting relatively preserved spermatogenesis. While individual plant extracts exhibited anti-spermatogenic and spermatotoxic effects, the Sure Male Capsule Formula showed some spermatogenic activity based on Johnsen's score alone. Nonetheless, its adverse histological profile cautions against its efficacy as a safe sexual stimulant. These findings underscore the need for further toxicological evaluation before clinical application.

Keywords: Sure, Male Capsule Formula, testicular function, *Garcinia kola*, *Musa paradisiaca*, *Khaya ivorensis*, spermatogenesis, Wistar rats.

INTRODUCTION

Methanolic extract of *M. paradisiaca* flower showed significant reduction in blood glucose levels in streptozotocin induced diabetic rats and reduced oxidative stress, the superoxide dismutase, lipids, peroxide and catalase in nephropathy and in testicular groups. Therefore, the extract has reno-protective and testicular-protective effect [1]. Mallick *et al.* [2], showed that hexane fraction of root of *M. paradisiaca* has the potential to correct diabetes induced testicular germ cell apoptosis. Ethanolic extract of Garcinia kola on the histology of the testes of male adult Wistar indicated that it had some adverse effects on the testis of male Wistar rats which was dose dependent [3]. G. kola has however been reported to cause deterioration of the reproductive system by Braide *et al.* [4]. Nottidge *et al.* [5] also reported prolonged administration of bitter kola extract to have destructive effects on the testes of dogs.

Akingboye *et al.* [6] reported that the consumption of the ethanolic extract of Garcinia kola at high doses may cause histopathological lesions in the testicular cells. Udoh [7] reported that the long-term ingestion of Garcinia kola seed would result in the arrest of spermatogenesis and sperm degeneration. Reports have shown that components of Garcinia kola seeds caused a toxic effect on the sertoli cells, have a negative impact on the leydig cells thereby interfering with testosterone production leading to reduction in spermatogenesis [8].

Khaya ivorensis (African mahogany or Lagos mahogany) is used as a bitter tonic, remedy for fever, vermifuge and for treating venereal diseases [9]. It is used as an antimalaria, as well as having anthelmintic effects [10]. Extracts of the stembark of *K. ivorensis* are used as anticonvulsants and treatment of hemorrhoids, heat rash, boils and arthritis [11].

Male Capsule Formula is a drug used for stimulating male reproductive hormone in man. It Contains 30 capsules, average weight of one capsule 0.33g. Constituents include K*haya ivorensis, Garcinia kola and Musa paradisiaca. Note:* The drug is still on trial basis to ascertain its potency on sexual function by examining the various constituents.

Therefore, the aim was to evaluate the histomorphometric studies of *garcinia kola, musa paradisiaca, khaya ivorensis versus* Male Capsule Formula drug (triumvirate combination of *garcinia kola, musa paradisiaca, khaya ivorensis*) on testicular function in adult male Wistar rats.

METHODOLOGY

Ethical Review

These investigations were carried out in conformity with the rules and guidelines of the

Animal Ethics Committee of the University of Ilorin, Ilorin. Kwara State, Nigeria.

Animal Grouping

Thirty (30) adults male Wistar rats, average weight of 200g were obtained from a commercial Animal Holding Facility in Ogbomoso, Oyo state, Nigeria. The rats were maintained in the Animal House of the Basic Medical Science, College of Health Sciences, University of Ilorin. Kwara State. The rats were fed with food and water ad libitum. They were acclimatized for one week before commencement of the experiment and weekly weighing of the animals was done using an Electronic Weighing Balance in the Department of Anatomy, College of Health Sciences, University of Ilorin Kwara State. The rats were grouped into four, with each group consisting of four rats each. The grouping was as follows:

Group A – Control group; received 1ml of distilled water, Group B- *Musa paradisiaca* 500 mg/kg body weight daily, Group C- Garcinia Kola 200mg/kg body weight daily, group D- *Khaya Ivorensis* 200mg/kg body weight daily and group E- Sure Male Capsule Formula 400mg/kg body weight daily.

Preparation and Administration of the Extract

Musa paradisiaca

Green plantain fruits were obtained from Ipata Market in Ilorin, Nigeria. The fruits were cut longitudinally into chips of about 5 mm thickness and air-dried for 4 days after which they were grinded and made into flour [12]. The *musa paradisiaca* flour was dissolved in 2 ml of double distilled water, for easy administration while a dosage of 500 mg/kg/day was given to the group using an oral cannula [12]. The duration of administration was 28 days.

Garcinia Kola

Garcinia Kola seeds were bought from the Ipata Market in Ilorin, Nigeria. The outer coats were removed and the seeds cut into pieces and air-dried. The dried seeds were ground to fine powder. The dried powdered 21g *Garcinia kola* was dissolved in 421ml of distilled water and shaken vigorously; it was allowed to stand on the bench shaking at intervals. The solution was then refrigerated for twenty-four (24) hours and then sieved using laboratory sieve. This was then allowed to stand for at least one (1) hour to allow the heavier particles to settle down after which, it was decanted and filtered using Whatman filter paper while the filtrate was collected into a bottle [9]. Extract was administered orally using an oral cannula. Dosage of 200mg/kg body weight daily was administered for 28 days, and the animals were sacrificed 24 hours after [9, 13].

Khaya Ivorensis

K. Ivorensis stem bark was purchased from Ipata Market, Ilorin, Nigeria. The *Khaya Ivorensis* extract was prepared at the Chemistry Laboratory, Department of Chemistry, University of Ilorin.

Sure, Male Capsule Formula

The Sure Male Capsule used was purchased from a dealer (fig 1). Sure Male Capsule formula contains 66 grams which was dissolved in 1 Liter of water to obtain a concentration of 0.06g/L. Rats in this group received 400mg/kg body weight of Male capsule formula daily.

The solution was prepared by using the formula.

$$\text{Volume of X (ml)} = \frac{\text{Weight of rat (kg) x dosage (mg/ml)}}{\text{Concentration of X (mg/ml)}}$$

Assuming X represented male capsule formula

Sacrifice of Animals

Twenty-four hours after the 28th day of administration the rats were sacrificed by cervical dislocation. Thereafter, the testis was identified and excised while the caudal epididymis was immediately removed for semen analysis. The testis removed were fixed in 10% formalin to prevent putrefaction and autolysis for histological analysis.

Semen Analysis

The epididymis was placed in normal and used for evaluation of sperm quality (i.e. sperm count, sperm motility and sperm morphology). The concentration of spermatozoa was determined using the improved Neubauer Chamber Haemocytometer (Deep 1/10 mm, LABART, Germany)

Histological Analysis

The testes of all the rats were fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5μm thick paraffin section of each testicular tissue was stained with hematoxylin and eosin (H &E), followed by examination under a Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0MP Amscope Camera (Amscope Inc, USA.) at ×100 magnification.

Histopathological Evaluation of the Testis

In addition to qualitative descriptions, the testicular histology was analyzed quantitatively using modified

Johnsen’s Score [14]. The Johnsen’s Score grades seminiferous tubules on a scale of 1-10 so that the best histological appearance with evidence of full spermatogenesis is rated 10 while the worst histological appearance with absent seminiferous tubule is given a score of One.

The score allocated to each seminiferous tubule is based on the descriptions summarized below. The modified Johnsen’s scores were applied to a minimum of 25 randomly chosen seminiferous tubules from each animal. The mean score for each animal was obtained by multiplying the numbers of tubules awarded a score with the score and then, the total number of tubules graded was divided by the sum of all the multiplications. The group mean was obtained by dividing the sum of means by number of animals per group.

Modified Johnsen's Scores as described by Holstein *et al*. [14].

10	*Represents intact spermatogenesis with many mature spermatids and zones of spermiation.*
9	*Describes modestly reduced spermatogenesis: reduced number of mature spermatids, a few zones of spermiation.*
8	*Implies a distinct reduction in spermatogenesis: few mature spermatids, no spermiation.*
7	*Is awarded when there is considerably reduced spermatogenesis: no mature spermatids, only immature spermatids, no spermiation.*
6	*Means severely reduced spermatogenesis: only few immature spermatids, reduced height of germinal epithelium.*
5	*Indicates arrest of spermatogenesis at the stage of primary spermatocytes: many spermatocytes border the lumen of the seminiferous tubules.*
4	*Implies arrest of spermatogenesis at the stage of primary spermatocytes: a few primary spermatocytes are present.*
3	*Depicts arrest at the stage of spermatogonia: A-type spermatogonia multiply but do not develop to maturing cells of spermatogenesis*
2	*Means there are no germ cells but only Sertoli cells are present.*
1	*Awarded when neither germ cells nor Sertoli cells are found. The seminiferous tubule is also replaced by connective tissue ground substance.*

Statistical Analysis

Data from both groups were statistically analyzed using Student's t-test followed by subsequent analysis by GraphPad Prism software (version 5.02) with statistical significance set at P<0.05* and P<0.001**. The mean, the standard error of mean and standard deviation of the data were calculated.

RESULTS

Physical Observations

The treated groups exhibited some behavioral changes such as aggressiveness in group B, agitation, restlessness, and disorientation in group C while group E exhibited calmness and tranquility compared to the control group. Reduced food intake was observed in all the treatment groups (B, C, D and E) compared to the control group.

Weight Changes

Significant increase (P<0.05) in weight changes was observed in groups D and E when compared to the control (table 1).

Semen Analysis

Significant decrease (P<0.05) in sperm count was observed in groups B, C and E when compared to the control group. Significant decrease (P<0.05) in

Sperm Motility was observed in groups D and E compared to the control group (Fig. 1). Sperm morphology experienced a significant decrease (P<0.05) in groups C and D compared to the control group (Fig. 2). Significant decrease (P<0.05) in Life and Death ratio was observed in groups D and E compared with the control group (Table 2).

Table 1: Showing the weight changes of the treatment groups compared to the control group.

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)
A	175.0 ± 1.3	185.0 ± 3.0	10.0 ± 0.72
B	165.0 ± 3.22	180.0 ± 3.56	15.0 ± 0.34
C	170.0 ± 3.22	180.0 ± 3.23	10.0 ± 0.55
D	175.0 ± 3.54	195.0 ± 3.23	20.0 ± 0.42*
E	160.0 ± 3.34	185.0 ± 3.78	25.0 ± 0.34*

* = P <0.05 = Significant difference.

Table 2: Semen analyses of rats in the treatment groups compared to the control group.

Groups	Sperm Count (%)	Sperm motility (%)	Sperm Morphology (%)	L/D ratio (%)
A	70.70±0.94	86.66±2.75	86.87±2.14	86.96±2.64
B	55.76±3.63*	76.3±1.36	79.40±1.40	76.40±3.64
C	58.4±1.22*	83.9±2.84	76.53±2.63*	72.79±2.33
D	61.9±3.63	75.14±1.43*	69.14±1.34*	71.14±1.44*
E	60.4±3.51*	65.44±2.25*	81.40±2.31	69.78±2.21*

*= (P<0.05) - significant difference.

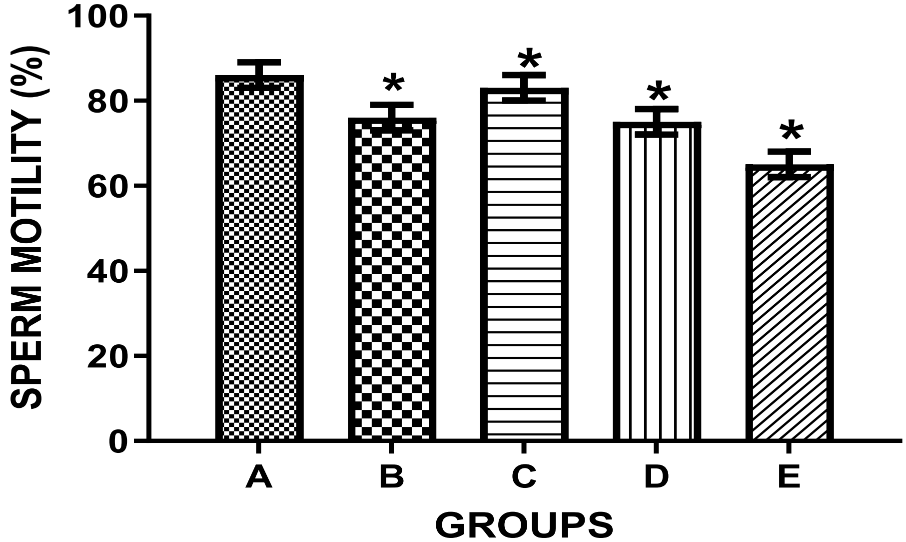

Fig 1: Sperm motility of rats in the treatment groups compared with the control group. *(P<0.05)-significant difference.

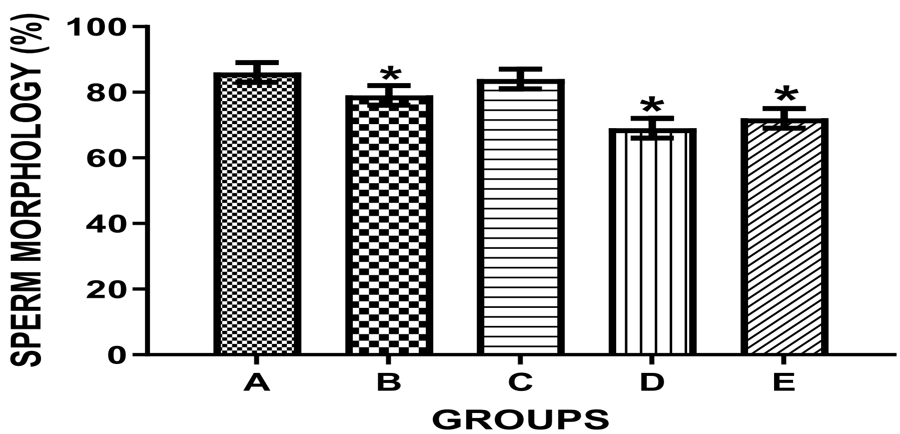

Fig 2: Sperm morphology of rats in the treatment groups compared with the control group. *(P<0.05)-significant difference.

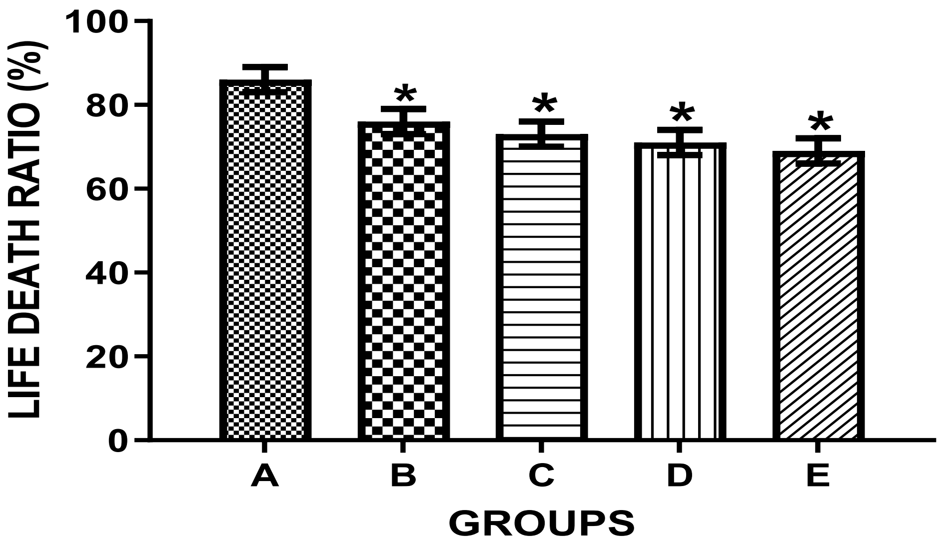

Fig 3: showing the Life Death Ratio of rats in the treatment groups compared with the control group.
*(P<0.05)-significant difference.

Histological Observation

The control group (A) showed well-defined seminiferous tubules, interstitial cells (IC) and a well-spaced lumen (L). The *Musa paradisca* (B) group showed slight degeneration of seminiferous tubules and epithelium, degenerated and widened lumen with variable cells around the tubules. The *Garcina kola* (C) group showed a defective seminiferous epithelium, degenerated basement membrane, detachment of sertoli cells and reduced numbers of spermatogenic cells. The *Khaya ivorensis* (D) group showed a severe degenerated seminiferous epithelia highlighting severe alterations in their architecture.

The Male Capsule Formula (E) group showed poor seminiferous tubule profile, distorted arrangement of the spermatogenic cells, increased interstitial space, detachment of sertoli cells, severe degeneration of the interstitial cells and Leydig cells (Fig. 4).

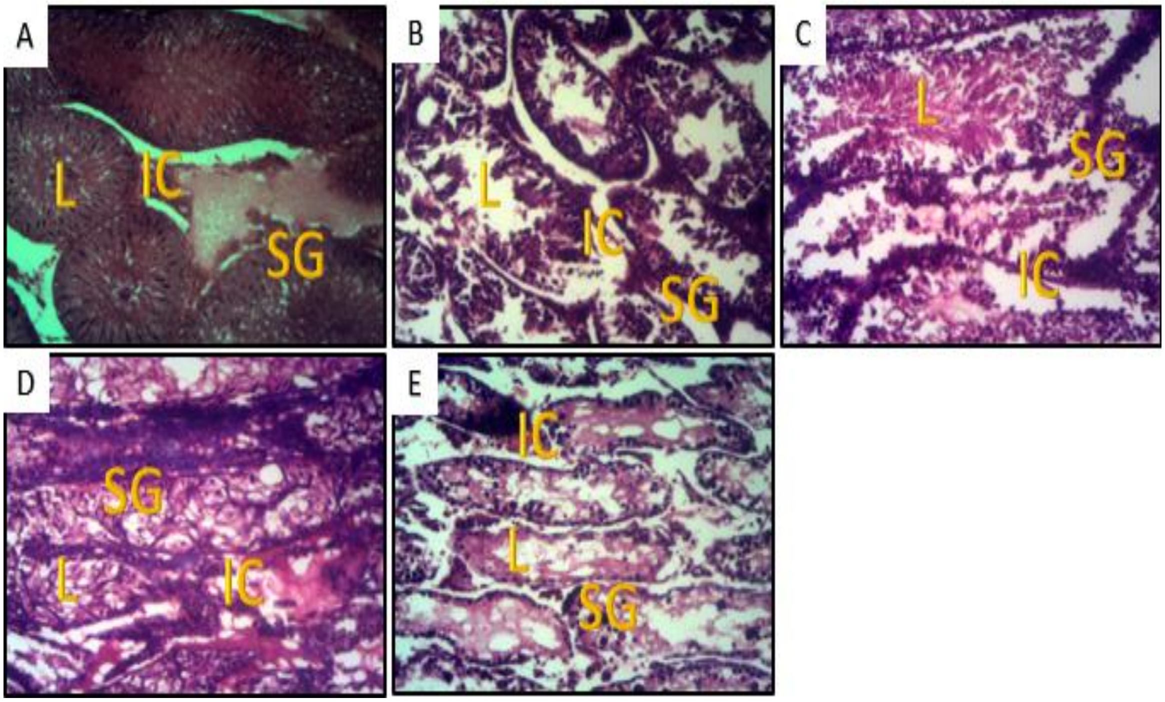

Fig. 4: (A-E) Photomicrograph representative of the testis of adult male Wistar rats treated with normal saline, control (A). Musa paradisca (B), Garcina kola (C), Khaya ivorensis (D), and Male Capsule Formula (E). L-lumem, IC- Interstitial Cells, SG-spermatogonia. (H and E x100).

Histopathological Observation

Histopathological observations using Johnsen's Score showed that were significant reduction (***P<0.001) in groups B and C when compared to the control group while no significant difference was observed in groups D and E which showed normal spermatogenesis in some seminiferous tubules (Table 3). This depicted a slight reduction in the number of germ cells present in all treatment groups (B, C, D and E).

Table 3: Histomorphometric observation across treatment and control groups using Johnsen's Score

A	B	C	D	E
8.4	5.5	6.4	8.7	8.8
8	4.6	6.1	8.8	8.9
7.56	5.3	5	8.7	8.8

DISCUSSION

Significant increase (P<0.05) in weight differences was observed in groups D and E when compared to the control (table 1). This negates studies by Akingboye *et al.,* [9] who observed significant decrease (P<0.001) in the final body weights and body weight differences after investigating the effects of ethanolic extract of *Garcinia kola* on the testes of adult male Wistar rats. Results from groups B and C were in consonance with Abu e*t al.* [15], Ralebona *et al*. [16] and Sumita *et al.* [17] who observed no significant difference in body weight. However, dose dependent reduction was observed in the weight of the testis, epididymis, prostate and seminal vesicle after investigating the effects of ethanolic extract of *Garcinia kola* and *Musa paradisiaca* on serum sex hormones and testicular function respectively.

The significant decrease in semen parameters observed in all treatment groups were in consonance with studies by Akpantah *et al*., [15], Adesanya *et al.* [18] and Abu *et al.,* [19] who observed marked reduction in sperm concentration, motility, viability and morphology after administration of ethanolic extract of G. *kola* on the testis to investigate its antispermatogenic effects (table 2) (Fig 1) (Fig 2). Significant decrease in Life and Death ratio was observed in groups D and E compared with the control group was consistent with the findings of Iwuji and Herbert [20] who demonstrated significant decrease in abnormal sperms in all the treated groups (Fig 3) [20]. They further stated that a decrease in the abnormalities of the head and tail in all the treated groups when compared with the controls was noted. It contradicted Ralebona *et al.* [16] who reported that G. kola treatment enhances testosterone secretion and increases testicular weights which accounts for the enhanced sexual activity, improve libido and intensifying orgasm and ejaculation and it's likely due to the presence of antioxidants such as kolaviron in the G kola extract. Therefore, these reductions in sperm parameters may suggest an impairment of the hypothalamo-pituitary-gonadal axis [21]. It is also probable that the reduction in sperm concentration and motility may be due to a reduction in gonadotropin levels which in turn suppressed testosterone secretion thus suggesting an antispermatogenic property.

The degenerated seminiferous tubules observed in all treatment groups agreed with Braide *et al*. [4] who reported that G. kola caused a severe deterioration of the reproductive system. Nottidge *et al.* [5] also reported prolonged administration of bitter kola extract to have destructive effects on the testes of dogs. Udoh [7] reported that the long-term ingestion of *Garcinia kola* seed would result in the arrest of spermatogenesis and sperm degeneration. Reports have also shown that components of *Garcinia kola* seeds caused a toxic effect on the sertoli cells, having a negative impact on the leydig cells thereby interfering with testosterone production leading to reduction in spermatogenesis [7, 8].

According to Johnsen's Score reported in this study, groups B and C had a significantly low score compared to the control. This is an indicator that administration of extracts in both groups was not very healthy for male reproductive health. This was corroborated by the poorly arranged histoarchitecture of the testicular profile in this group. This result agrees with Udoh [7] who reported that the prolonged use of *Musa paradisica* could lead to disruption of the spermatogenic process. While Johnsen's Score in groups D and E was at par with the control thereby suggesting both extracts might be good for reproduction, but this negates reports from the histological observation in both groups.

The investigation was conducted in adherence to the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the study protocol was approved by the University Ethical Review Committee (UERC). Consent to participate is not applicable.

Competing interests

The authors declare no competing interest.

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