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Full Length Research Paper

Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of *Lagenaria Breviflora* Roberts

Adebowale Bernard Saba[1]*, Olayinka Ayotunde Oridupa[1], Matthew Olugbenga Oyeyemi [2] and Oluwaseun Dapo Osanyigbe[1]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.
[2]Department of Veterinary Surgery and Reproduction, University of Ibadan, Nigeria.

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This study was aimed at determining the effects of the ethanolic extract of the whole fruit of *Lagenaria breviflora* Robert on male fertility by evaluating some andrological parameters of the Wistar rat such as morphology of spermatozoa, sperm count, motility, liveability and volume of the semen. Histopathology of the testis was carried out. 20 adult male Wistar rats were randomly divided into 5 groups. The rats in group A (Control) were administered with 0.9% physiological saline, while rats in groups B, C, D, and E were administered with the extract of 1000, 2000, 4000 and 8000 mg per Kg body weight, respectively, once daily for 14 days. The extract caused morphological alterations of sperm cells in this study, predominantly of secondary abnormality. These include bent mid-piece, curved mid-piece, bent tail, curved tail, normal tail without head, normal head without tail, looped tail, and coiled tail, which are indications of interference with maturation stage of spermatogenesis in the seminiferous tubules. The deleterious effect of the extract on the seminiferous tubules is corroborated by histopathology, which revealed degeneration of epithelium of the seminiferous tubules. The degree of sperm cell motility was significantly lowered in animals administered with the extract compared to animals in the control group. However, the lifeability of the spermatozoa was not affected by the extract but it altered the structure of sperm cells produced. Consequently, the sperm count was lowered ($P<0.05$) in the animals administered with the fruit extract in comparison with animals in the control group, as a result of decline in the production of normal, viable sperm cells in the test animals. There was no significant ($P>0.05$) difference in the mean values of semen volume of rats in the control and test groups. It was concluded that extract of *L. breviflora* exerts toxic effect on the seminiferous tubular epithelium with concomitant reduction in the reproductive potential of the male rats. Herbal preparation of *L. breviflora* Robert should therefore be used cautiously in both man and animal. The potential of the plant as an anti-fertility drug in man can be carefully explored.

Key words: *Lagenaria breviflora*, testis, morphology, spermatozoa, fertility, rat.

INTRODUCTION

Lagenaria breviflora Robert is a widely used medicinal plant in West Africa. It belongs to the family Cucurbitaceae with five other species in its genus. *L. breviflora* and *Lagenaria siceraria* are two species in this genus that are known to have medicinal effects (United States Department of Agriculture, 2001; Morimoto et al., 2005). Certain medicinal effects of *L. breviflora* Robert have been demonstrated or confirmed in the laboratory and these include broad-spectrum antibacterial (Tomori et al., 2007), miracicidal and cercaricidal (Ajayi et al., 2002), abortificient (Elujoba and Hymete, 1986), and anti-implantation activities (Elujoba et al., 1985).

Currently, effort is being made to evaluate the toxic

*Corresponding author. E-mail: ab.saba@mail.ui.edu.ng. Tel.: +234 805 413 8127.

effect of *L. breviflora* so as to weigh its untoward effects against its reported therapeutic efficacy. This is quite necessary before further step towards chemical characterization and formulation is attempted. The effect of the fruit extract on the female reproductive system was documented by Elujoba et al. (1985), and Elujoba and Hymete (1986), some of which were thought could be employed for beneficial effects. However, there is no available literature on the effect of the fruit extract on the male reproductive system.

This study is aimed at evaluating the effect of the fruit extract of *L. breviflora* Robert on male fertility by evaluating some andrological parameters of the Wistar rat such as sperm volume, motility, count, liveability and morphology which are some of the indices that determine the ability of a male to produce viable spermatozoa (Garner and Hafez, 1993, Oyeyemi et al., 2000).

MATERIALS AND METHODS

Extraction of the fruit

Fresh fruits of *L. breviflora* Robert were obtained from local vendors in Ibadan and identified at the Department of Botany and Microbiology, University of Ibadan, Nigeria. The fruits were washed, cut in small pieces and weighed out into small portions. These portions were tied in cloth sieves and placed in plastic containers. Sufficient ethanol that covered each portion was poured into the plastic container. The fruit cuttings were allowed to soak for about 3 days and the ethanol was drained off and replaced with fresh ethanol. The ethanol with the fruit extract were stored in plastic containers and kept in the refrigerator at 4^{o}C. The extract obtained was clarified by filtration through celite on water pump and was then concentrated *in vacuo* using a rotation evaporator at low temperatures. The remaining moisture was finally removed by placing small volumes in porcelain dishes in the oven set at low temperatures at 4^{o}C. The extract came as semi-solid greenish brown paste. A stock solution was afterward prepared by dissolving 100 g of the extract in 50 ml of distilled water.

Experimental protocol

20 male adult Wistar rats bred and maintained at the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan were used in this study. Water and feed were provided *ad libitum.* The rats were randomly divided into 5 groups; 1 control and 4 treatment groups. The rats in group A (Control) were administered with 0.9% physiological saline, while the treatment groups B, C, D, and E were administered with graded doses of the fruit extract of 1000, 2000, 4000 and 8000 mg/kg body weight, respectively. The animals were dosed orally once daily for 14 days using rat canula.

Sample collection

The rats were anaesthetized using ether and afterward sacrificed by cervical dislocation. Orchidectomy was performed by open castration method. A midline or pre-scrotal incision was made and the testicles were milked out of the incision site. The testicles were exposed by incising the tunica vaginalis. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter cllected from the cauda epididymis. The methods of collection were similar to that described by Akusu et al. (1985) and Oyeyemi and Ubiogoro (2005). The samples were analyzed immediately after collection.

Sperm volume

The volume and the color were determined by reading out the volume in a calibrated measuring cylinder, while the color was determined by visual assessment.

Sperm count and motility assay

Sperm motility was assessed by the method described by Zemjanis (1977). The spermatozoa were counted by hemocytometer using the improved Neubauer (Deep 1/10 mm, LABART,Germany) chamber as described by Pant and Srivastava (2003). A total of 400 spermatozoa from each rat were examined for morphological changes.

Morphological abnormalities and percentage viability assay

These were determined from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains (0.2 g of Eosin and 0.6 g of Fast green dissolved in distilled water and ethanol in ratio 2:1). Live/dead ratio was determined using 1% Eosin and 5% Nigrosin in 3% sodium citrate dehydrate solution according to the method described by Wells and Awa **(**1970).

Statistical Analysis

Student t-test was used to analyze the data (Steel and Torrie, 1996). The difference of the means were considered significant at $p<0.05$.

RESULT

Sperm morphology

Rudimentary tail

The population of the sperm cells with rudimentary tail was non-significantly ($P>0.05$) lower than the values obtained for the rats in the test groups (Table 1).

Bent mid-piece

The population of spermatozoa with bent mid-piece sperm abnormality occurred significantly ($P<0.05$) more in the rats in each of the treatment groups than in the control rats (Table 1).

Curved mid-piece

Occurrence of curved mid-piece sperm abnormality was

Table 1. Percentage occurrence of different sperm abnormalities observed in the rats of the control and test groups.

Mean Sperm Abnormality (%)	Group A Control	Group B 1000 mg/kg	Group C 2000 mg/kg	Group D 4000 mg/kg	Group E 8000 mg/kg
Tailless head	3.00±0.41^{ab}	5.00±0.41^{a}	4.75±0.63	5.00±0.41^{b}	4.50±0.65
Headless tail	4.25±0.48	4.50±0.65	5.00±0.41	4.50±0.65	4.75±0.25
Bent tail	5.00±0.41^{abcd}	9.25±0.48^{ae}	8.25±0.25^{b}	8.00±0.41^{ce}	9.00±0.41^{d}
Curve tail	6.25±0.25^{abcd}	9.25±0.65^{ae}	8.25±0.48^{ae}	9.00±0.41^{cf}	9.75±0.48^{d}
Bent mid-piece	5.25±0.63^{abcd}	9.50±0.65^{a}	9.00±0.58^{b}	9.50±0.50^{c}	9.75±0.48^{d}
Coiled tail	0.50±0.29	0.50±0.29	0.75±0.48	0.75±0.25	0.75±0.25
Looped tail	0.00±0.00^{ab}	1.75±0.48^{a}	1.00±0.41	1.25±0.63	1.25±0.25^{b}
Rudimentary tail	0.25±0.25	1.25±0.25	1.00±0.41	1.00±0.41	1.25±0.25
Curved mid-piece	6.50±0.29^{abcd}	10.00±0.41^{a}	9.75±0.48^{b}	8.75±0.48^{c}	9.50±0.25^{d}
Total mean sperm abnormality (%)	31.00±3.01	51.00±4.27	47.75±4.13	47.75±4.15	50.50±4.27

Means with the same superscript on the same row are significant at the level of $p<0.05$.

significantly ($P<0.05$) higher in rats in the treatment groups compared with rats in the control group (Table 1).

Bent tail

The rats in each of the treatment groups were observed to have significantly ($P<0.05$) more spermatozoa with bent tail abnormality when compared with those of the control rats. Rats in group B (9.25±0.48%) also had significantly ($P<0.05$) more spermatozoa with this abnormality than those of group D (8.0±0.41%) (Table 1).

Curved tail

Fewer spermatozoa of rats in the control group ($P<0.05$) had curved tail sperm abnormality than those of the rats in each of the treatment groups (Table 1).

Normal head without tail/tailless head

The rats in the control group had lesser number of spermatozoa with normal head without tail abnormality compared with rats in each of the treatment groups. The percentage of this abnormality in the rats in group B (5.0±0.41%) or D (5.0±0.41%) was higher than that of the control rats (3.0±0.41%). The differences of the means were significant ($P<0.05$) for both groups (Table 1).

Normal tail without head/headless tail

The spermatozoa of rats in each of the treatment groups were observed to slightly have more of the normal tail without head/ headless tail abnormality than those of the control rats. The differences of the means were however not significant ($P>0.05$) between the control and any of the test groups (Table 1).

Coiled tail

The occurrence of coiled tail sperm abnormality was slightly higher in rats in the treatment groups C (0.75±0.48%), D (0.75±0.25%) and E (0.75±0.25%) when compared with that of rats in the control group (0.50±0.29%). Rats in group B (0.50±0.29%) had same population of spermatozoa with coiled tail abnormality as those of the control group (Table 1).

Looped tail

Looped tail was not observed on the spermatozoa in the rats in the control group and very few of the spermatozoa exhibited this abnormality in the rats in treatment groups B (1.75±0.48%), C (1.00±0.41%), D (1.25±0.63%) and E (1.25±0.25%) as well. The differences of the mean were not significant between the control and any of the test groups ($P>0.05$) (Table 1).

Sperm motility

The degree of motility of spermatozoa of rats in the control group was significantly ($P<0.05$) higher than what was observed in rats in each of the treatment groups (Table 2).

Percentage liveability/live : dead ratio

The mean value of percentage liveability of sperm cells was not significant ($P>0.05$) for rats in each of the treat-

Table 2. The mean values of sperm motiltiy, liveability and count, and seminal volume of rats in control and test groups.

Sperm parameter	Group A Control	Group B 1000 mg/kg	Group C 2000 mg/kg	Group D 4000 mg/kg	Group E 8000mg/kg
Motility (%)	80.00±4.08^{abc}	40.00±4.08^{a}	30.0±4.08^{b}	47.50±10.31^{c}	50.00±4.08
Percentage liveability	88.75±2.39	85.00±2.04	81.25±4.27	87.50±3.23	92.50±1.44
Volume (cm^3)	5.15±2.89^{-2}	5.15±2.89^{-2}	5.15±2.89^{-2}	5.13±2.50^{-2}	5.15±2.89^{-2}
Count (%)	83.25±2.32^{abcd}	59.75±4.55^{ae}	60.00±5.15^{b}	53.00±2.12^{c}	52.75±3.75^{de}

Means with the same superscript on the same row are significant at the level $p<0.05$.

Table 3. Histopathology of testis of rats in the control and test groups.

Histopathology	Group A Control	Group B 1000 mg/kg	Group C 2000 mg/kg	Group D 4000 mg/kg	Group E 8000 mg/kg
Changes in meminiferous interstitium and epithelium	0	1+	2+	2+	2+

0 = No visible lesion; 1+ = mild degeneration; and 2+ = moderate degeneration.

ment groups compared with that of rats in the control group (Table 2).

Sperm volume

The mean sperm volume was almost constant for the rats in the control group and each of the test groups (Table 2).

Sperm count

The mean value of sperm count of rats in group B (59.75±4.55%), C (60.00±5.15%), D (53.00±2.12%), and E (52.75±3.75%) were significantly ($P<0.05\%$) lower compared with that of the control rats (83.25±2.32%) (Table 2).

Histopathology of testicles of rats

Group A: There was no visible lesion observed
Group B: Section revealed slight or mild degeneration of seminiferous epithelium
Group C: There was moderate degeneration of seminiferous epithelium
Group D: There was mild degeneration of seminiferous epithelium
Group E: There was marked degeneration of the seminiferous tubular epithelium (Table 3).

DISCUSSION

The sperm cell count, motility, live/dead sperm cell ratio, morphology, and the seminal volume were used in this study to evaluate the effect of prolonged administration of *L. breviflora* Robert on male reproductive system using the Wistar rat as animal model. These andrological parameters are usually evaluated to determine the fertility of a male subject (Garner and Hafez, 1993). When critical percentages (i.e. ≥10%) of sperm cell abnormalities are present in the semen, the male subject is usually considered infertile (Zemjanis, 1977).

Alteration of sperm cell morphology caused by *L. breviflora* Robert in this study can be grouped into primary or secondary abnormalities according to the classification by Noarkes et al. (2004). Sperm cell abnormalities observed in the rats in treatment groups in this study were: 1 primary and 8 secondary sperm cell abnormalities. Rudimentary tail abnormality was the only primary sperm abnormality, while bent mid-piece, curved mid-piece, bent tail, curved tail, normal tail without head, normal head without tail, looped tail, and coiled tail were the secondary abnormalities observed. The occurrence of rudimentary tail sperm abnormality observed was significantly ($P<0.05$) higher in rats in treatment group relative to the control rats. Primary sperm abnormality is observed due to aberrations in the process of spermatogenesis (Hafez, 1987; Moss et al., 1979). Sperm cells with rudimentary tails are usually immotile, and are unable to fertilize mature ovum.

Bent mid-piece sperm cell abnormality had the highest occurrence, followed by curved mid-piece, bent tail and curved tail of all the secondary sperm abnormalities observed. These were about two-fold higher than that observed in control rats. The other secondary sperm abnormalities observed were normal tail without head, normal head without tail, looped tail, and coiled tail.

Although rats in the treatment groups had higher percentages of these abnormalities, this second category of secondary sperm abnormalities was within range of that observed in rats of the control group.

Secondary abnormalities arise as a result of a fundamental problem with the process of maturation where abnormal sperm cells are matured from damaged seminiferous tubules (Thomas and Thomas, 2001). Prolonged administration of the fruit extract of *L. breviflora* Robert can be inferred to have deleterious effects on the seminiferous tubules which is reflected in this study by the high percentage of secondary sperm cell abnormalities observed. This observation is supported by histopathological findings of the seminiferous tubules whereby rats administered with *L. breviflora* Robert exhibit varying degrees of lesions of the seminiferous tubules.

The sperm motility of rats in the treatment groups were significantly ($P<0.05$) lower, but the live/dead sperm cells ratio were within the same range as those of the control rats, which shows that the extract did not affect the liveability of spermatozoa but it caused deformation of the cells and rendered them less motile or immotile.

These observations are contrary to what was reported for *Curcuma longa* from the same family Cucurbitaceae and *Garcia kola*. Curcumin (from the rhizome; *Curcuma longa* L.) and Kolaviron (a biflavonoid from the seeds of *Garcia kola*) were discovered to prevent peroxidative changes in the sperm and testicular membrane, thus enhancing sperm motility and decreasing spermatozoa abnormalities (Farombi et al*.,* 2007; Ishihara et al., 2000). *Tribulus terrestris* with Protodioscin as its active component is another plant proven to improve spermatogenesis, sperm motility and morphology (Adimoelja et al., 1995).

In this study, the sperm count was observed to have reduced significantly ($P<0.05$) which is an indication that the fruit extract of *L. breviflora* Robert reduced or inhibited spermatogenesis. This is similar to what was observed in some medicinal plants with detrimental effects on male fertility such as *Carica* papaya and *Quassia amara*. *C. papaya* was reported by Chinoy and Padman (1996) to have ant fertility effect by reduction of testicular mass, sperm count and sperm motility when the benzene extract of the seeds was administered to male albino rats.

The chloroform extract of the bark of *Q. amara* has been shown to decrease sperm count, motility and viability in albino rats (Parveen et al., 2003), while on the contrary, aqueous extracts of root, leaf, or whole plant of *Withania somnifera* Dunai is known to increase sperm count (Abdel-Magnied et al., 2001). Phthalate esters, which were suspected to have originated from medications given to some patients, have been implicated in the decline of human sperm count (Hauser et al*.,* 2004).

Conclusion

Findings from this study show that administration of the fruit extract of *L. breviflora* Robert caused increased sperm cell abnormalities with more of secondary than primary sperm cell abnormalities. The sperm cell abnormalities tended towards critically high percentage occurrence. The toxic effect of *L. breviflora* Robert on the sperm cells was as a result of degeneration of seminiferous tubules. This indicates that the prolonged administration of this fruit extract will induce infertility in the male. Herbal application of *L. breviflora* Robert should therefore be taken with caution in both man and animal, especially in male animals used in breeding programs. The potential of the plant as an ant-fertility drug in man could be carefully explored.

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