

Original Research Paper

The Influence of *Garcinia kola* Seed on Sexual Behavior and Testis Physiology in Wistar Rats

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Abstract: *Garcinia kola* seed is believed by some to enhance male sexual performance but existing scientific data show divergent results. In this study, the sexual and reproductive influence of ethanol (70%) seed extract of *G. kola* was evaluated in Wistar rats. Adult male rats were gavaged with 0, 50, 100, 200 or 300 mg kg⁻¹ day⁻¹ of extract for 30 days and mated with female rats after sexual behavior characteristics were carefully evaluated. Serum concentrations of testosterone, LH and FSH, as well as epididymal sperm indices were analyzed after mating, while pregnancy rate and litter size of female rats were recorded. Extract (50 mg kg⁻¹) treatment produced no effect on sexual activities, but higher doses caused reduction relative to control. Additionally, except at 50 mg kg⁻¹, extract treatment caused reduction in sperm count (p<0.0001), sperm viability (p = 0.0011), testosterone and FSH (p<0.0001). LH was unaltered, while abnormal sperm morphology was elevated (p<0.05). Furthermore, pregnancy rate and litter size in extract (100-300 mg kg⁻¹) treated rats were lower when compared to control. The results suggest that low dose of *G. kola* seed may not affect sexual activity, whereas high doses may affect fertility by negatively altering sexual behavior, testosterone level and sperm indices.

Keywords: Bitter Kola, *Garcinia kola*, Infertility, Sperm Parameters, Gonadal Function

Introduction

Medicinal plants are increasingly relied upon for maintenance of personal health and wellbeing. It has been estimated by the World Health Organization (WHO) that a large global population rely on herbal medicines for some aspect of their health care needs, partly because they are cheap and easily accessible compared to orthodox medicines and also because of the belief that they are effective in the treatment of a wide range of ailments (Barata *et al.*, 2016). *Garcinia kola* (Family, Guttiferae) is one of the most widely consumed medicinal plants in Africa. It is popularly known as ‘‘Bitter Kola’’ in Nigeria because of the bitter taste of the seed, but is also identified by other local names in different regions of the country. The plant has been referred to as a ‘‘Wonder Plant’’ because every part of it is found to be of medicinal importance (Okoye *et al.*, 2014). The numerous ethno-medicinal uses of the plant

include, treatment of abdominal discomfort, headache, cough, throat infections, vomiting and impotence (Adesina *et al.*, 1995; Ofor *et al.*, 2004; Okoye *et al.*, 2014). It has equally been used locally for the treatment liver diseases, diarrhea, microbial infections and as a general antidote (Ibiblio, 1983; Iwu, 1989; Ofor *et al.*, 2004; Adegboye *et al.*, 2008). Most of the folkloric uses of *G. kola* have been authenticated, but its beneficial role in the treatment of male infertility is yet to be substantiated with scientific proof.

Infertility is a global problem, with a global prevalence rate of 10.5% (Mascarenhas *et al.*, 2012) and second most prevalent health care problem in sub-Saharan Africa (Chinnoch, 1996). In Nigeria, prevalence of infertility was alarmingly reported to be 40-50% (Emokpae *et al.*, 2005; Okonofua *et al.*, 2005). *G. kola* seed is consumed by some to increase sexual desire or performance (Iwu, 1989; Ngueyem *et al.*, 2009) but unfortunately, existing data show conflicting reports on

this in experimental animal studies (Ralebona *et al.*, 2012; Yakubu and Quadri AL, 2012; Sewani-Rusike *et al.*, 2016). Additionally, some studies have indicated that *G. kola* negatively affects spermatogenic indices (Chilaka *et al.*, 2009; Abua *et al.*, 2013; Mesembe *et al.*, 2013), while others concluded that it enhances male reproductive indices (Adesanya *et al.*, 2007; Atsukwei *et al.*, 2015; Sewani-Rusike *et al.*, 2016). More so, despite the wide consumption of *G. kola*, infertility rate is relatively high in regions where it is highly distributed with the implication that the plant may contribute to infertility.

The present study aims to evaluate the influence of *G. kola* on male reproductive function by investigating the effect of 30 days oral treatment with ethanol extract of *G. kola* seed on sexual behavior, sperm parameters, serum reproductive hormone concentrations and fertility potential in Wistar rats.

Materials and Methods

Animals

Thirty male (200-250 g body weight) and thirty female Wistar rats (200-220 g body weight) were obtained from the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria. All animals were acclimated for a period of 14 days before commencement of the study. They were fed with rat pellets and allowed access to tap water *ad libitum*. They were housed and maintained under standard laboratory conditions at ambient temperature (26±5°C) and humidity with a 12 h photoperiod. The animals were handled humanly in compliance with the guidelines for care and use of laboratory animals in biomedical research (CCAC, 2009). All experimental procedures were approved by the Research Ethics Committee of the University of Port Harcourt, Nigeria.

Methods

Extraction of Plant Material

Fresh *Garcinia kola* seed was purchased from a local fruit market in Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated by a botanist, Mr. M. Suleiman of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria and assigned a voucher number, DPH048 which was deposited at the herbarium of the Faculty. Following identification, the seeds were allowed to air dry for one week and later dried in an oven at 40°C until a constant weight was obtained. The dried seeds were then ground to powder and macerated in 70% ethanol (1:5) for 72 h with regular agitation. The mixture was filtered using Whatman number 51 filter paper and the resulting filtrate was evaporated using a rotary evaporator to obtain a pasty extract. This was evaporated

further in a steam bath (40°C) to obtain a brownish-black dry extract and stored in a refrigerator until used for experiments. The percentage yield was calculated, 19.4% w/w (1000 g powder yielded 194 g extract).

Phytochemical Screening of *G. kola* Seed Extract

Phytochemical screening was conducted on the extract to determine the presence of various chemical constituents like alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardiac glycosides and carbohydrate using standard procedures (Sofowora, 1993; Trease and Evans, 2009).

Experimental Design

Sixty rats (thirty males and thirty females) were used in the study. The male rats were randomized into five groups (n = 6 rats per group) and administered vehicle (DMSO plus water, 1:2); or extract (50, 100, 200 or 300 mg kg⁻¹) once daily by oral gavage (2 ml kg⁻¹) for 30 days. The doses chosen were based on previous works (Braide *et al.*, 2003; Sewani-Rusike *et al.*, 2016) and well below the oral LD₅₀ value of the extract (5000 mg kg⁻¹) reported (Atsukwei *et al.*, 2015). At the end of extract treatment, they were paired (1:1) and sexual behavior characteristics in all groups were closely monitored and recorded as described by Yakubu *et al.* (2012). After sexual behavior testing, animals were allowed further to mate freely with female rats for an hour twice daily. Mating was confirmed by the observation of vaginal plug in female rats. Thereafter, male rats were sacrificed by cervical dislocation under deep diethyl ether anesthesia. Blood was collected via cardiac puncture with sterile syringes and needles, serum was separated and testosterone, LH and FSH levels were assayed by Enzyme Linked Immunoassay (ELISA) method using commercially available kits. Testis was removed and epididymal sperm was analyzed. Also, the female rats were closely monitored after mating. Pregnant rats, confirmed by the presence of vaginal plug, were separated and monitored until littering. The number of pregnancies and number of pups delivered by animals in each group were recorded.

Sexual Behavior Test

Female rats were given single dose of estradiol benzoate (25 µg/kg, sc in 0.1 mL sesame oil) 48 h prior to pairing and progesterone (500 µg, sc in 0.1 mL propylene glycol) 5 h before commencement of the evaluation to bring the animals to estrus phase in order to make them receptive (Kutzler, 2007; Lopez *et al.*, 2007). Sexual behavior characteristics monitoring was conducted in a dark room 2 h after the last dose of extract treatment. To familiarize the animals to the behavior testing environment, animals were brought to the laboratory and exposed to dim light around 8 pm

daily for 6 days before the monitoring. Female rats were introduced into cages containing male rats one at a time and observation for sexual behavior was started immediately after the introduction of female rats and recorded as they occurred over 20 min. The sexual behavior parameters measured include: precoital behavior characteristics (qualitative observation of chasing, nosing, sniffing and genital grooming); mount frequency (MF, number of mounts without intromission); intromission frequency (IF, number of intromission or penetration); and ejaculation frequency (EF, number of ejaculations). The qualitative observations of chasing, nosing, sniffing and genital grooming were scored as absent, mild, moderate or high depending on the frequency of occurrence of the events.

Sperm Analysis

Sperm was expressed from the epididymis into a clean glass slide and emulsified with equal volume of 1% NaHCO₃ buffered Tyrodes Lactate solution on a clean dry glass slide. The slide was viewed under a light microscope to analyze sperm motility, count, viability and morphology using standard methods (Ochei and Kolhatker, 2002; Baker, 2007). Briefly, sperm motility was determined by counting both motile and non-motile spermatozoa in 10 randomly selected fields using 40x magnification. The percentage of motile sperm was calculated from the mean percentage motility for all the fields counted. Sperm count was determined using the new improved Neubauer counting chamber. The chamber was prepared and charged with diluted seminal fluid (1:20) and allowed to stand in a moist chamber for 15 min. This was then viewed under the microscope and complete morphologically mature sperm cells were counted using 40x magnification. For sperm morphology, sperm smears were stained on microscopic slides with two drops of Walls and Ewas after air-drying. The slides were examined under brightfield optics at 100x magnification with oil immersion. Two hundred spermatozoa were counted and the percentage of abnormal forms (morphology) was calculated.

Statistical Analysis

All the values (data) obtained from the study were analyzed by one way ANOVA followed by Dunnett's

posttest using GraphPad Prism Version 5 software (GraphPad Software Inc., San Diego, CA, USA). Student's *t*-test was also carried out where applicable. The values are expressed as Mean ± Standard Deviation of Mean and *p* values <0.05 were considered significant.

Results

Phytochemical Analysis

Garcinia kola seed extract contained flavonoids, alkaloids, tannins, steroids, saponins and carbohydrates, but did not show presence of cardiac glycosides and anthraquinones (Table 1).

Effect of Extract on Sexual Behavior

Precoital behavior characteristics (chasing, nosing, sniffing and genital grooming) were observed and recorded qualitatively. Sniffing and nosing occurred highly, whereas chasing and genital grooming were observed to be moderate among control rats (Table 2). All four precoital behavior indices were highly exhibited by rats that received the lowest dose (50 mg kg⁻¹) of extract. Conversely, precoital activities were absent in rats that were administered the highest dose (300 mg kg⁻¹), except chasing and genital grooming that occurred mildly. In addition, sniffing, chasing and genital grooming occurred moderately, while nosing was mild in 100 mg kg⁻¹ extract treated rats. Further, chasing was moderate, sniffing and genital grooming were mild, but there was no nosing observed in 200 mg kg⁻¹ extract treated rats (Table 2).

Table 1: Phytochemical constituents of ethanol seed extract of *Garcinia kola*

Phytochemical constituent	Observation
Alkaloids	++
Saponins	++
Tannins	++
Flavonoids	+++
Steroids	+
Anthraquinones	-
Cardiac glycosides	-
Terpenoids	++
Carbohydrates	++

- Absent; + Mildly present; ++ Moderately present; +++ Abundantly present

Table 2: Effect of 30 days treatment with ethanol seed extract of *Garcinia kola* on precoital behavior in male Wistar rats

Dose (mg/kg)	Precoital behavior parameters			
	Sniffing	Nosing	Chasing	Genital Grooming
Control	+++	+++	++	++
50	+++	+++	+++	+++
100	++	+	++	++
200	+	-	++	+
300	-	-	+	+

- Absent; + Mild; ++ Moderate; +++ High

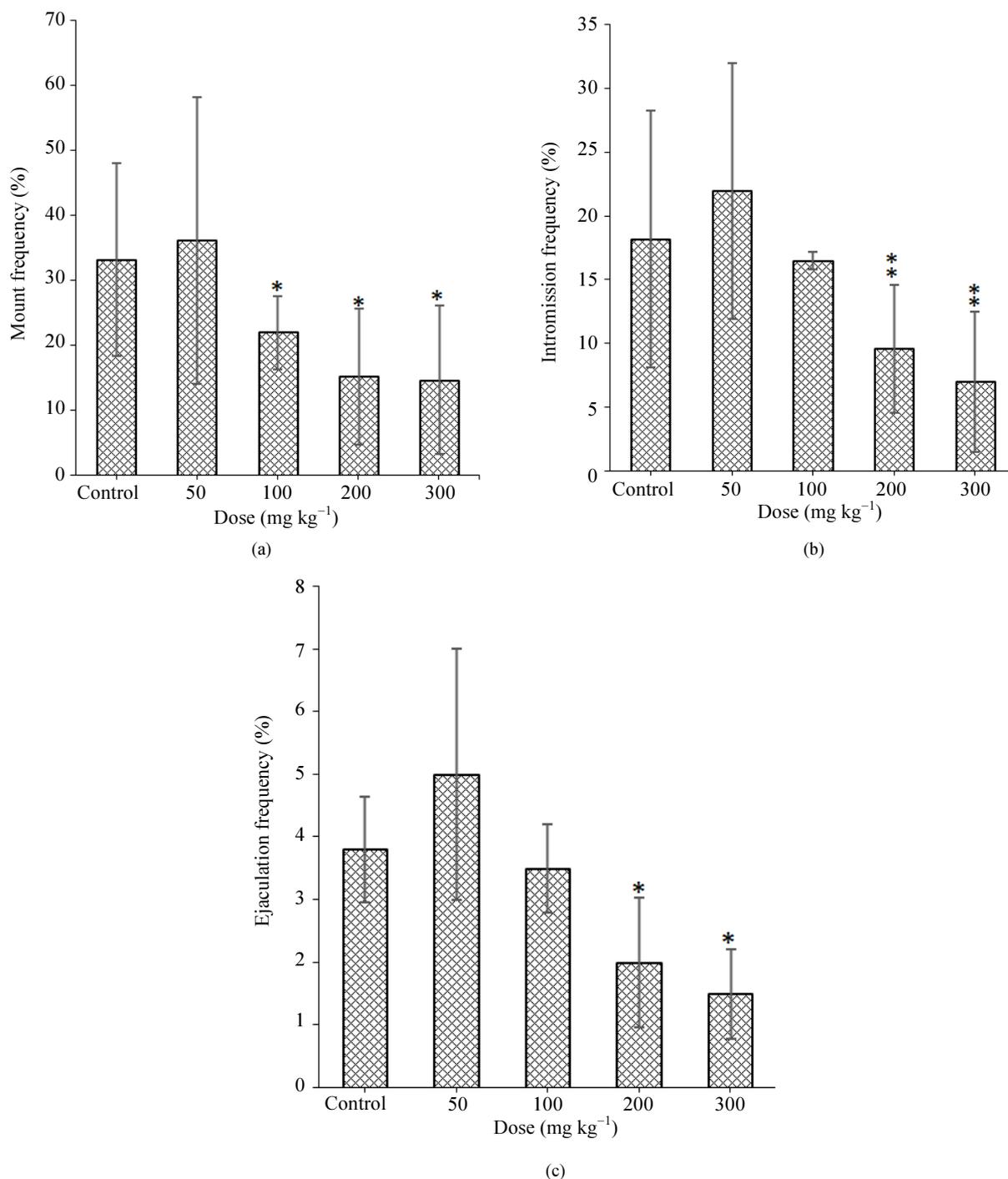


Fig. 1: Effects of ethanol *G kola* seed extract on sexual behaviors: (a) Mount frequency (b) Intromission frequency and (c) Ejaculation frequency in Wistar rats; Values expressed as mean \pm SD, n = 6 animals per group; *p<0.05, **p<0.01

Mount Frequency (MF) was reduced ($p < 0.05$) in rats that received 100-300 mg kg⁻¹ extract compared to control; but MF in 50 mg kg⁻¹ extract treated rats was normal (Fig. 1a). Similarly, intromission frequency (number of intromissions) in all extract administered rats was reduced, but only values in 200 and 300 mg kg⁻¹ treated rats were

significant ($p < 0.0023$) when compared to control (Fig. 1b). Additionally, ejaculation frequency (number of ejaculations) in extract administered rats was reduced ($p = 0.0344$). The values were also significant ($p = 0.0346$) in 200 and 300 mg kg⁻¹ treated rats, but not in 50 and 100 mg kg⁻¹ treated rats when compared with control (Fig. 1c).

Effect of Extract on Sperm Parameters

There was no significant difference ($p = 0.4015$) in sperm motility between extract administered rats and control (Fig. 2a). Comparison of the different test groups with control did not also show any significant difference (Fig. 2a). Sperm count, viability and morphology in rats that received 50 mg kg^{-1} extract were not altered (Fig.

2b-2d), but sperm count and viability in $100\text{-}300 \text{ mg kg}^{-1}$ extract treated rats were decreased ($p < 0.0001$, $p = 0.0011$) in a dose-dependent fashion when compared with control (Fig. 2b and 2c). Percentages of sperms with structural abnormalities (abnormal sperm morphology) in $100\text{-}300 \text{ mg kg}^{-1}$ extract treated rats were elevated ($p < 0.001$) compared to control (Fig. 2d).

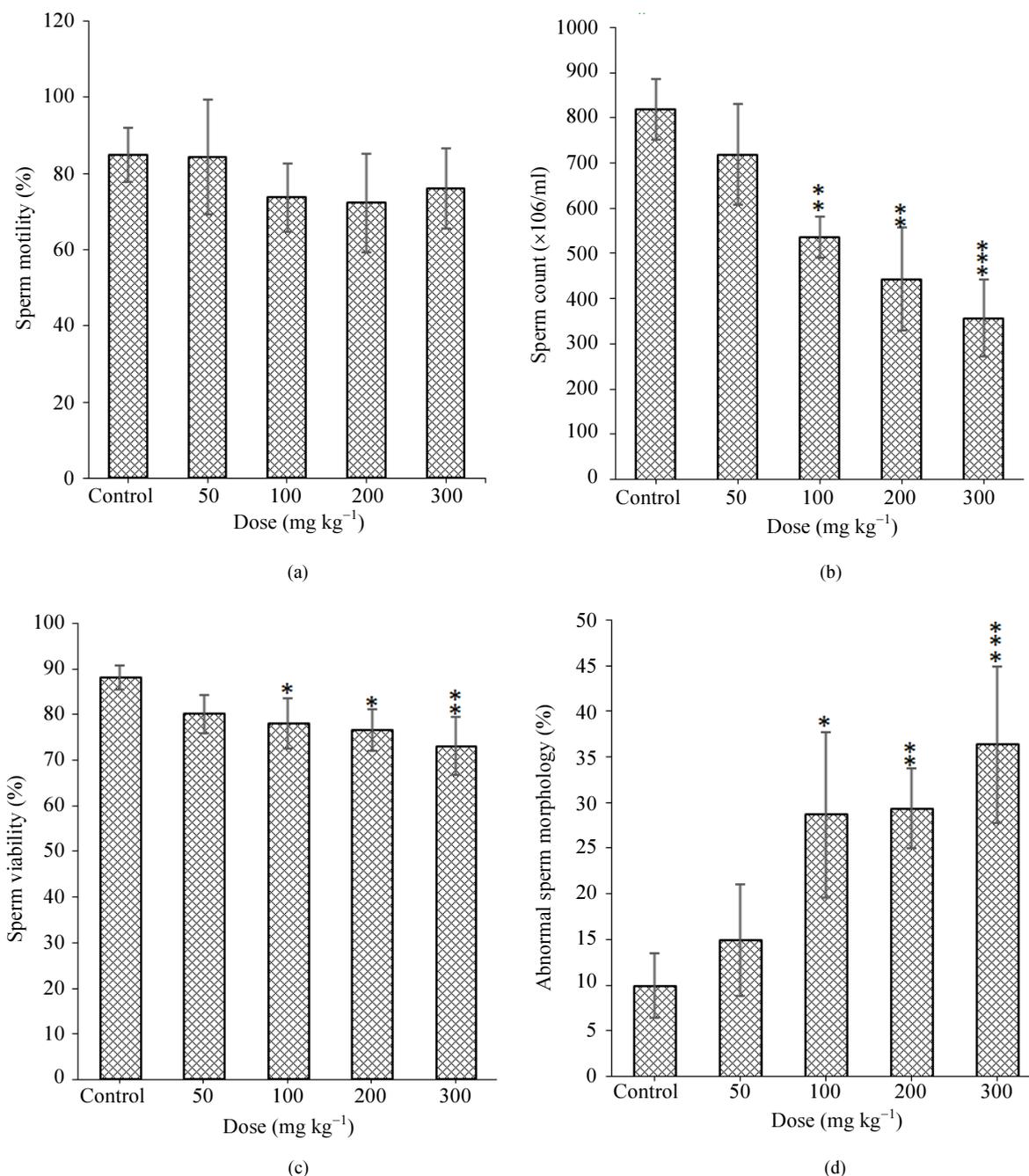


Fig. 2: Effects of ethanol seed extract of *G kola* on sperm parameters: (a) Sperm motility (b) Sperm count (c) Sperm viability and (d) Abnormal sperm morphology (percentage of morphologically abnormal sperm cells) in Wistar rats; Values expressed as mean \pm SD, $n = 6$ animals per group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

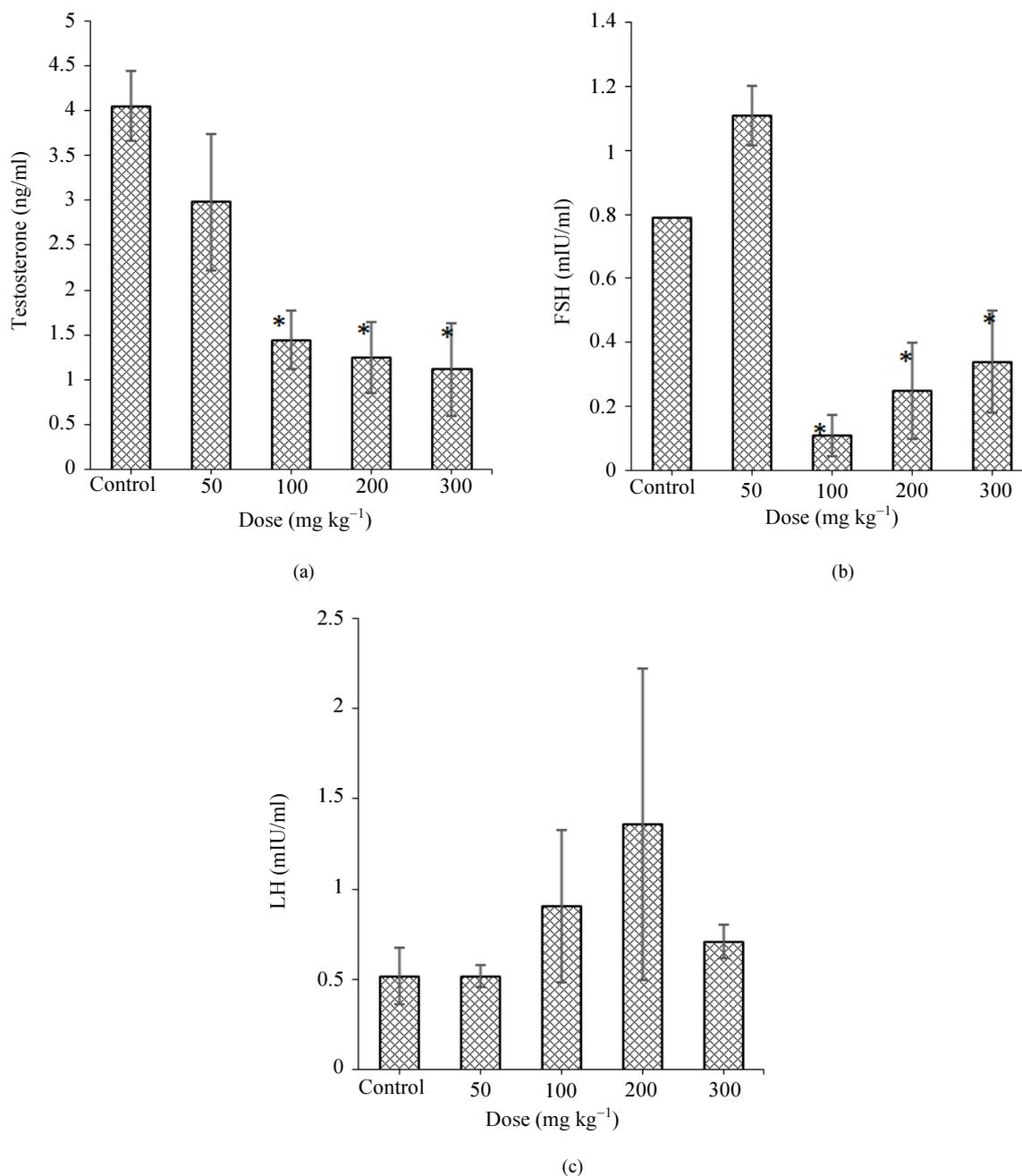


Fig. 3: Effects of ethanol seed extract of *G kola* on serum levels of (a) Testosterone (b) Follicle stimulating hormone (FSH) and (c) Luteinizing hormone (LH) in Wistar rats; Values expressed as mean \pm SD, n = 6 animals per group; *p<0.0001

Table 3: Pregnancy rate and litter size of female rats mated with vehicle (control) and *Garcinia kola* seed extract treated male Wistar rats

Dose (mg/kg)	Number of pregnancies that occurred	Percentage of pregnancies that occurred	Litter size (Mean \pm SD)
Control	6 (6)	100	8.67 \pm 1.80
50	6 (6)	100	8.33 \pm 2.44
100	2 (6)	33.33	2.50 \pm 0.71* [‡]
200	0 (6)	0	0
300	0 (6)	0	0

n = 6 per group; * Significant at p = 0.0072, compared to control (*t*-test); [‡]Significant at p = 0.0165, compared to 50 mg kg⁻¹ extract (*t*-test)

Effect of Extract on Reproductive Hormones

Serum concentrations of testosterone, FSH and LH were not affected by the lowest dose of the extract compared to control (Fig. 3a-3c). But in rats that were administered higher doses of the extract (100-300 mg kg⁻¹), serum testosterone level was decreased ($p < 0.0001$), FSH level was decreased ($p < 0.0001$); whereas LH was not altered (Fig. 3a-3c).

Effect of Extract on Fertility

Pregnancy occurred in all female rats (100%) that were mated with control or 50 mg kg⁻¹ extract treated male rats (Table 3). Pregnancy was observed in only two rats (33.33%) in 100 mg kg⁻¹ extract administered rats, whereas no pregnancy (0%) occurred in 200 or 300 mg kg⁻¹ extract administered rats (Table 3). In addition, average number of pups (litter size) that was delivered by pregnant animals in control (8.67±1.16) and 50 mg kg⁻¹ administered rats (8.33±1.53) were comparable and not significantly different from each other, but their values were both higher ($p = 0.0072$) compared to the litter size (2.50±0.50) in 100 mg kg⁻¹ extract treated rats (Table 3).

Discussion

Garcinia kola is a popular medicinal plant that is consumed by many for diverse purposes. The seed of the plant is claimed locally in some areas to enhance fertility and sexual activities (Iwu, 1989). However, there is no established scientific evidence of this claim as results from previous studies are simply contrasting. The present study reports the effect of *G. kola* ethanol seed extract treatment on sexual behavior, sperm characteristics, reproductive hormones and fertilizing capacity in male rats.

Precoital behaviors with mounting, intromission and ejaculation activities are useful factors that are used to assess male sexual dysfunction in animal models (Cimanga *et al.*, 2016). From our results, the lowest dose of the extract (50 mg kg⁻¹) produced high precoital behaviors of chasing, nosing, sniffing and genital grooming in the animals comparable to the levels that were exhibited by control rats. Similarly, the extract at this dose did not alter animals' Mount Frequency (MF), Intromission Frequency (IF) or Ejaculation Frequency (EF) of the rats during the observation period of 20 min. Surprisingly, the higher doses of the extract (100-300 mg kg⁻¹) produced reduction of these sexual activities in the rats. This was characterized by reduced expression or absence of precoital behaviors, as well as low MF, IF and EF which demonstrate low sexual desire. Previously, Ralebona *et al.* (2012) had reported that ethanol extract (200-400 mg kg⁻¹) given for 28 days enhanced sexual activity and increased testosterone in Wistar rats. Sewani-Rusike *et al.* (2016) demonstrated that ethanol extract at 100-400 mg kg⁻¹ when

administered for 28-50 days possesses aphrodisiac activity in Wistar rats, but effect was more at lower doses. On the other hand, Yakubu and Quadri (2012) showed that *G. kola* (25-100 mg kg⁻¹), administered over a period of 200 h does not enhance sexual activity. From the result obtained in the present study, low dose levels of *G. kola* seed may have no effect, whereas high dose levels may slow sexual desire in rats.

The results equally indicated that daily extract treatment for 30 days reduced sperm count and viability. Our study equally showed that *G. kola* treatment increased percentage of abnormally shaped sperm cells (abnormal sperm morphology). This finding is in agreement with some earlier studies (Akpantah *et al.*, 2003; Chilaka *et al.*, 2009; Abua *et al.*, 2013; Mesembe *et al.*, 2013), but differ from a few others who reported increase in sperm count after 6 weeks of extract treatment in Wistar rats (Adesanya *et al.*, 2007; Sewani-Rusike *et al.*, 2016). The extract had been reported to cause sperm motility reduction (Adesanya *et al.*, 2007; Chilaka *et al.*, 2009; Mesembe *et al.*, 2013), but there was no change in motility in the present study which is consistent with the result of Sewani-Rusike *et al.* (2016). The negative spermatogenic effects observed in this study suggest that the extract is likely to alter spermatogenesis (sperm production), which occurs in the seminiferous tubules of the testis. Testosterone, the androgen hormone, plays an essential role in spermatogenesis and its reduction as observed in this study could contribute to the reduced sperm count obtained. Further, the non-alteration in serum LH concentration that was seen indicates that feedback control response of anterior pituitary to the testosterone reduction signal in the testis was absent (Neill and Herbison, 2006). This suggests that the extract might affect the testis, partly via interference of the endocrine regulatory function. This observation is in agreement with an earlier study which demonstrated that *G. kola* treatment caused gross cellular depletion and desquamation of cells of the testis, hypothalamus and pituitary with resultant alteration in the histology of the hypothalamic-pituitary-gonadal axis (Obi and Nwoha, 2014). In addition, our result on testosterone was similar with many earlier findings (Braide *et al.*, 2003; Chilaka *et al.*, 2009; Abua *et al.*, 2013), but was not consistent with the studies of Ralebona *et al.* (2012) and Sewani-Rusike *et al.* (2016) who reported the opposite. Although, variations in extraction vehicle and duration of treatment in some of the studies cited above could influence the nature of the results obtained, the wide range of contrasting results suggest that studies on the subject remains in-exhaustive at the moment.

Finally, our results showed that the lowest dose of the extract (50 mg kg⁻¹) did not alter fertility, but higher doses reduced fertility capacity of male rats. Animals that received 200 and 300 mg kg⁻¹ extract

failed to cause pregnancy, whereas at 100 mg kg⁻¹, extract reduced pregnancy rate as well as number of pups delivered. This suggests that high concentrations of the extract possesses antifertility potential, which correlates positively with the adverse spermatogenic effects produced by the extract. Phytochemicals that were found in the plant extract includes, flavonoids, alkaloids, saponins, tannins, terpenoids and steroids. The composition of these compounds may be partly responsible for the results that were obtained.

Conclusion

The results of the study demonstrate that low concentrations of *G. kola* seed extract neither alter sexual behavior nor exhibit adverse effect on the testis. But high concentrations of the extract may reduce sexual behavior, alter sperm characteristics, and reduce testosterone and fertility potential in male rats following prolonged administration.

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Authors' Contributions

Aprioku conceived and designed the study. Okeke, Emakpor and Nwachukwu managed the literature searches. All authors contributed in conduction of the experiments and collection of the data. Aprioku, Okubuike and Igbo handled the analysis of the data. Aprioku wrote the first draft of the manuscript and all authors approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest with respect to the research, authorship and/or publication of this article.

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