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# Evaluation of antispermatogenic effect of *Garcinia kola* seed extract in Albino rats

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ABSTRACT

**Objective:** To determine the effects of aqueous extract of *Garcinia kola(G. kola)* seed on serum sex hormones and epididymal functions of Wistar albino rats. **Methods:** Fifteen male Wistar albino rats were randomly assigned into three groups of five rats per group. The animals in the treatment groups were administered orally aqueous extract of *Garcinia kola* seed at the doses of 200 and 400 mg/kg body weight once daily for 30 days. Rats in the control group received normal saline (0.9%). The body weights of the rats were determined at the beginning and end of the experiment. Sperm characteristics of each rat were determined. Hormonal assay for FSH, LH and testosterone were done using Enzyme-Linked Immunosorbent Assay (ELISA). **Results:** There were significant ($P<0.05$) decreases in relative organ weights of extract-treated rats compared to the control. *G. kola* seed extract decreased sperm motility, sperm concentration and sperm viability as well as affected normal morphology of sperm cells in dose-dependent manner. Furthermore, the extract reduced the serum concentrations of sex hormones of the treated rats relative to the control. **Conclusion:** The findings suggest that aqueous extract of *G. kola* seed may have antispermatogenic property.

## 1. Introduction

*Garcinia kola* (bitter kola or male kola) *(G. kola)*of the family, Guttiferae is a medium-sized tree growing up to 12 m tall and 1.5 m wide and usually distributed in the forest zone of Cameroon, Ghana, Nigeria, Sierra Leone and other West African countries. In Nigeria, it is known by various local names such as "Namijin Goro" (Hausa), "Orogbo" (Yoruba) and "Aku Ilu" (Igbo). The plant has characteristic astringent, bitter and resinous taste and is mainly grown around homesteads and its seeds are chewed for medicinal or ceremonial purposes. Every part of the plant (bark, leaves and root) is adjudged to be of medicinal importance. It is commonly used to treat cases of asthma, cough, poisoning or vomiting [1], for its supposed aphrodisiac activity, nervous alertness and induction of insomnia [2] and improved bowel movement [3].

Experimental studies have confirmed anti-bacterial [4, 5], anti-hepatoxic [6], antioxidant [7], anti-inflammatory [8] and hypoglycemic [9] properties of *G. kola* seed. However, recent scientific study raises doubt about its aphrodisiac property [10]. Furthermore, there have been reports of adverse effects of *G. kola* seed extract on testis functions [11, 12] raising concerns as to whether the consumption of bitter kola has any effect on spermatogenesis. This study was designed to evaluate the effects of *G. kola* water extract on sperm characteristics of Wistar albino rats.

## 2. Materials and methods

### 2.1. Plant collection and preparation of the extract

The seeds of *G. kola* used in this study were purchased from the local market in Makurdi, Nigeria and confirmed in the College of Forestry Herbarium, University of Agriculture, Makurdi, Nigeria where a voucher specimen already exists. The outer coats of the seeds were peeled off, shade dried under room temperature and pounded into a

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powder. The dried powder (100 g) was macerated in 500 mL of distilled water for 48 h at room temperature with frequent agitation or stirring and filtered through No. 1 filter paper (Whatman's) in a funnel. The filtered extract was concentrated over water bath (45 ℃), then stored at 4 ℃ in air-tight containers until required for animal treatments.

### 2.2. Animals

This study was approved by the Departmental Ethics Committee of the University of Agriculture, Makurdi. Male Wistar rats weighing between 140 and 150 g and obtained from the Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria were used in this study. The rats were maintained on a 12 h light and 12 h dark cycle, and provided with pellet feed (Grand Cereals and oils Mills Ltd, Jos, Nigeria) and water *ad libitum*. The rats (5/group) were orally administered 0.9 % saline solution (control), 200 and 400 mg/kg body weight doses of *G. kola* seed extract for 30 days respectively. The final weights of the animals were recorded a day after the last dose administration. Blood was collected by cardiac puncture (under ether anaesthesia) in plain tubes for serum separation and stored at −20 ℃ until used for hormone assay. The rats were then autopsied.

### 2.3. Body and reproductive organ weights determination

Body weights of the rats at the end of the experimental period were recorded. At autopsy, reproductive organs (testes, epididymis, seminal vesicles and ventral prostate) were carefully removed, cleared of adhering tissues and weighed.

### 2.4. Cauda epididymal sperm motility, concentration, viability and morphology determination

Cauda epididymis was separated and minced using a pair of small scissors in a beaker containing 1 mL warm physiological saline to allow motile sperm to swim up from the epididymis. Five microlitres (5 μL) of epididymal fluid was delivered onto a glass slide covered with a (22×22) mm cover slip and motile sperm were counted under the light microscope at a magnification of ×40. The microscope stage and slides were prewarmed prior to assessing motility. The procedure was repeated and the two readings averaged. Sperm motility was expressed as a percent of motile sperm of the total sperm counted.

Sperm count was determined using the improved Neubauer haemocytometer as previously described [13]. For sperm counts, five counts per sample were made and averaged. Sperm was expressed as sperm/mL of suspending solution.

Sperm viability (percentage of live spermatozoa) was determined using the eosin/nigrosin stain. The stained (dead) and the unstained (live) sperm cells were counted using ×40 objectives of the microscope. Sperm morphology was determined by examining air-dried slides under oil immersion after staining with Wells and Awa stain [14]. The abnormal sperm cells were counted and the percentage calculated.

### 2.5. Estimation of serum FSH, LH and testosterone

Blood obtained through cardiac puncture was centrifuged at 300 rpm for 10 min; serum was collected and stored at −20°C until assayed. Serum FSH, LH and testosterone concentrations were assayed using the Enzyme-Linked Immunosorbent Assay (ELISA). The AccuBind® ELISA kits used were from Monobind Inc., Lake Forest, California USA; the manufacturer's instruction were followed.

### 2.6. Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and $P<0.05$ was regarded as significant. All statistical analyses were carried out using Graph Pad Prism Version for windows (Graph Pad Software, San Diego, California USA).

## 3. Results

### 3.1. Body weight and relative weights of organs

There were no significant differences ($P>0.05$) in body weights of the treated rats when compared with the control group (Table 1). However, treatment with *G. kola* seed extract showed dose-dependent reductions in the weights of testis, epididymis, ventral prostate and seminal vesicle of the animals as shown in Table 1.

### 3.2. Results of sperm motility, concentration, viability and morphology

Administration of *G.kola* seed extract for 30 days significantly decreased ($P<0.05$) sperm motility, concentration, viability and morphology of spermatozoa in extract treated rats when compared with the control (Table 2). The decreases were dose-dependent.

**Table 1**
Effect of *G. kola* extract on body weights and reproductive organ weights of Wistar rats ($n$=5).

| Treatment group | Change in body weight (g) | Weight of testes (g/100 g) | Weight of epididymis (g/100 g) | Weight of ventral prostate (g/100 g) | Weight of seminal vesicles (g/100 g) |
|---|---|---|---|---|---|
| Control | 82.40 ± 1.20 | 0.57±0.04 | 0.24±0.01 | 0.26±0.02 | 0.39±0.01 |
| 200 mg/kg | 72.60 ± 2.40 | 0.47±0.02 | 0.16±0.02 | 0.07±0.01 | 0.25±0.01 |
| 400 mg/kg | 65.30 ± 2.40 | 0.36±0.03 | 0.10±0.04 | 0.05±0.01 | 0.11±0.02 |

Data are expressed as mean ± SEM; $P < 0.05$.

**Table 2**
Effect of *G. kola* extract on sperm characteristics and sex hormones of Wistar rats ($n$=5).

| Parameters | Control | 200 mg/kg | 400 mg/kg | $P$-value |
|---|---|---|---|---|
| Sperm motility (%) | 77.40 ± 0.80 | 63.50 ± 1.20 | 48.60 ± 1.20 | < 0.05 |
| Sperm concentration(x $10^6$) | 58.00 ± 1.40 | 42.75 ± 1.80 | 36.40 ± 1.70 | < 0.05 |
| Viability (%) | 72.00 ± 1.80 | 56.00 ± 1.80 | 51.20 ± 1.60 | < 0.05 |
| Normal morphology (%) | 68.80 ± 0.80 | 46.50 ± 2.30 | 39.40 ± 1.80 | < 0.05 |
| FSH (mIU) | 11.20 ± 0.80 | 6.75 ± 0.40 | 5.49 ± 0.40 | < 0.05 |
| LH (mIU) | 5.80 ± 0.30 | 3.00 ± 0.40 | 3.40 ± 0.30 | < 0.05 |
| Testosterone (ng/mL) | 4.40 ± 0.20 | 2.50 ± 0.20 | 2.40 ± 0.20 | < 0.05 |

Data are expressed as mean ± SEM.

### *3.3. Hormone assay*

Administration of *G. kola* seed extract for 30 days significantly decreased ($P$<0.05) serum FSH, LH and testosterone in a dose-related manner when compared with the control (Table 2).

## 4. Discussion

The results of the present study showed that there were no significant alterations in body weights of rats that were treated with G. kola seed extract, suggesting that the extract had no systemic toxic effects in rats. This finding agrees with previous study [15] which showed the non-toxic effect of G. kola seed. The observed decrease in body weights of the treated rats could be attributed to reduced feed consumption [2].

In our study, we observed marked reductions in concentration, motility, viability and morphology of spermatozoa in treated rats. The reductions in sperm concentration and motility as observed in the present study agreed with an earlier study [11], but at variance with the findings of other investigators [16] who reported an increase in the sperm concentration in Wistar rats after treatment with ethanolic extract of *G. cambogia* for 8 weeks. It is well established that motile spermatozoa in sufficient concentration and free from abnormalities are highly correlated with fertility [17] because sluggishly motile or immotile spermatozoa are not likely to penetrate the cervical mucus and fertilize the ova [18, 19]. The reductions in epididymal parameters suggest an impairment of the hypothalamo- pituitary-gonadal axis [20]. The reduced weights of testis, epididymis, ventral prostate and seminal vesicles lend credence to the observed epididymal functions. It is probable that the reduced cauda epididymal sperm concentration and motility may be due to a decline in the levels of gonadotophins which in turn suppressed testosterone secretion.The significant decreases in FSH, LH and testosterone levels of Wistar rats as observed in the present investigation are in agreement with the findings in a similar study on *G. kola* [21], but contrary to the results obtained in another study [22]. A decline in testosterone secretion is reported to be responsible for impaired spermatogenesis as characterized by reduced sperm concentration, motility, viability and morphology. Some studies [21] confirmed that the marked reduction in the serum testosterone concentration of rats administered the methanolic extract of *G. kola* seed might due to direct action on the testicular tissue. Although *G. kola* is traditionally used as an aphrodisiac, the adverse effects observed in the present study as well as that earlier reported [12] may suggest a possible antispermatogenic property.

The oral administration of aqueous extract of *G. kola* seed for 30 days produced dose-related reductions in serum sex hormones and cauda epididymal sperm parameters of Wistar rats. However, further work is required to determine whether these antispermatogenic effects are reversible.

## Conflict of interest statement

We declare that we have no conflict of interest.

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