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Ethanolic Seed Extract of *Garcinia kola* Reduces Epididymal Sperm Count and Some Serum Reproductive Hormone Concentrations in Adult Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEO designed the study, performed the statistical analysis, wrote the protocol. Authors ECO and IOU wrote the first draft of the manuscript and managed the analyses of the study. Author FNU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to elucidate the effect of *Garcinia kola* on serum reproductive hormones and sperm count in adult male albino Wistar rats.
Study Design: Albino rats were randomly assigned into 4 groups containing 7 rats each.
Place and Duration of Study: Department of Human Physiology, Madonna University, Nigeria.

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Methodology: Group 1 served as the control group, while groups 2, 3 and 4 received *Garcinia kola* extract orally at the rate of 100mg/kg, 200mg/kg and 300mg/kg body weight, respectively once daily for 6 weeks (n=7). After 6 weeks of treatment, reproductive hormonal assay was carried out using the rat serum. Epididymal spermatozoa were collected and sperm count was determined using heamocytometer.
Results: The experimental groups had significantly lower weights of testes (P<0.05), as compared with the control group. The weights of epididymis in the experimental groups where significantly (P<0.05) higher when compared to the control group. There was a significant decrease in the serum concentration of testosterone (P<0.05) in the experimental groups when compared to the control group. Semen analysis also showed a significant decrease in the sperm density (P<0.05) in the groups treated with *Garcinia kola* extract when compared to the control group.
Conclusion: Ethanolic seed extract of *Garcinia kola* showed a possible anti-spermatogenic consequence on treatment in male Wistar rats, and may be detrimental to male reproductive health, hence need to regulate its consumption rate.

Keywords: Garcinia kola; epididymis; testosterone; sperm count; testes.

1. INTRODUCTION

Garcinia kola (*G. kola*) is a medium size tree up to 12 m high, grown and cultivated in the moist forests of West Africa, South Africa and South East Asia. *G. kola* seeds contain biflavanoids (kolaviron) capable of having anti-inflammatory [1] and natural antioxidant properties [2,3]. *G. kola* have shown to have anti-fertility consequences [1,4,5], found to reduce testosterone secretion and sperm volume, but increased LH and FSH secretion [1,6] and reduced sperm volume [4,6]. Alterations of serum concentrations of reproductive hormones are implicative of disordered spermatogenesis, which are undoubtedly major determinants of male fertility [7]. Thus, following the increased usage of *G. kola* in African traditional medicine and its consumption, especially amongst the male population in Nigeria, this study aimed to discover the possible effects of *G. kola* on serum reproductive hormones and sperm count, which are major determinants of male fertility.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

Fresh *G. kola* seeds were purchased from a local market in Anambra state, Nigeria and authenticated in the botanical unit of the Department of Biological Sciences, Madonna University Nigeria. The crude extract was prepared as described by Nwafor and Kagbo [8]; the seeds were peeled, sliced and dried in Astell Hearson Oven at 45°C. The dried seeds were then grounded into fine powder using an electric blender.

50 g of the powdered *G. kola* was macerated in 250 mls of ethanol for 72 hrs, filtered using Whatman filter paper into a 500 ml Beaker and the filtrate obtained was then homogenized and concentrated to dryness in a water bath at 45°C. The filtrate was left to evaporate until the extract was made into a solid form. Weighed samples (1 g in 10 ml distilled water) of the extract were then used to prepare the stock solution (100 mg/ml).

2.2 Experimental Animals and Feeding Protocol

Twenty-Eight male Albino Wistar rats were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Enugu Campus, and acclimatized for two weeks before the onset of the experiment. The rats were fed with rat chow from feed store (Growers Vital Feed) and tap water *ad libitum*. The rats were randomly assigned into four groups of seven rats each, housed in wire mesh cages (14 hrs light and 10 hrs dark cycle). Group 1 served as the control group, while groups 2, 3 and 4 received *G. kola* extract orally at the rate of 100 mg/kg, 200 mg/kg and 300 mg/kg body weight, respectively once daily for six weeks.

2.3 Sample Collection

At the end of the 6 weeks experiment, the animals were anesthetized in a chloroform chamber, and blood was obtained via cardiac puncture using a 5ml syringe attached to a needle (21 SWG). Blood samples from each animal were put in a labeled non-heparinized sample tubes, allowed to stand for three hours in iced water and later centrifuged at 5000

revolutions for 10 minutes. Serum was then collected and stored at -15°C for reproductive hormonal assay. After blood collection, the animals were cut open with the aid of a dissection set and some internal organs (Testis and Epididymis) were collected and weighed. The semen from the epididymis was collected for sperm analysis (sperm count).

2.4 Sample Analysis

Serum testosterone and LH concentrations were determined using the enzyme linked immunosorbent assay (ELISA). The epididymis and testes were carefully removed, rinsed in normal saline solution and weighed using an electronic weighing balance. Epididymal spermatozoa were collected and sperm count was done by method of Freud and Carol [9].

2.5 Statistical Analysis

Data was expressed as Mean ± Standard Error of Mean (SEM). Results obtained from this study were analyzed using Statistical Package for Data Analysis (SPSS) version 17.0 for windows. Analysis of Variance (ANOVA) was used to compare means, and values were compared at $P<0.05$. Post Hoc multiple comparisons for difference between groups were established by Tukey's HSD.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of *G. kola* on male reproductive hormones

Result in Table 1 shows significant ($P<0.05$) increase in the serum concentration of LH, between 200 mg/kg *G. kola* treated group (4.41 ± 0.37 µ/ml) and 300 mg/kg *G. kola* treated group (4.51 ± 0.04 µ/ml) when compared to control group (4.21 ± 0.04 µ/ml).

Serum concentrations of testosterone was significantly ($P<0.05$) lower in 200 mg/kg (3) *G. kola* treated group (12.1 ± 0.03 nmol/ml) and 300 mg/kg (4) *G. kola* treated group (10.72 ± 0.39 nmol/ml) than the control group (13.4 ± 0.14 nmol/ml). While there was no significant difference between the control group (13.4 ± 0.14 nmol/ml) and 100 mg/kg (2) *G. kola* treated group (12.9 ± 0.04 nmol/ml).

3.1.2 Effect of *G. kola* on the weight of the testis and epididymis organs

Data in Table 2 shows significant ($P<0.05$) increases in the weights of the epididymis in 200 mg/kg (3) *G.kola* treated group (1.13 ± 0.07 g) and 300 mg/kg (4) *G. kola* treated group (1.46 ± 0.12 g) when compared to the control group (0.64 ± 0.02 g).However, there was no significant difference between the control group (0.64 ± 0.02 g) and 100 mg/kg (2) *G. kola* treated group (0.65 ± 0.11 g).

The weights of the testes decreased significantly ($P<0.05$) in the 100 mg/kg (2) *G. kola* treated group (1.35 ± 0.09 g), 200 mg/kg (3) *G. kola* treated group (1.31 ± 0.05 g) and 300 mg/kg (4) *G. kola* treated group (0.74 ± 0.1g) when compared to the control group (1.53 ± 0.16 g).

3.1.3 Effect of *G. kola* on epididymal sperm count

As presented in Table 3 the sperm count was found to decrease significantly ($P<0.05$) in 100 mg/kg (2) *G. kola* treated group (33.57 ± 0.9 x 10^6/ml), 200 mg/kg (3) *G. kola* treated group (21.44 ± 0.4 x 10^6/ml) and 300 mg/kg (4) *G. kola* treated group (11.14 ± 0.24 x 10^6/ml) as compared to the control group (58.93 ± 0.47 x 10^6/ml).

Table 1. Effects of *G. kola* extract on male reproductive hormones in male Wistar Albino rats

Group	Control	Group 2	Group 3	Group 4
LH (µ/ml)	4.21 ± 0.04	4.33 ± 0.01	4.41 ± 0.37 (*)	4.51 ± 0.04 (*)
Testosterone (nmol/ml)	13.4 ± 0.14	12.9 ± 0.04	12.1 ± 0.03 (*)	10.72 ± 0.39 (*)

Values are expressed in mean ± SEM, (*) statistically significant at P<0.05 compared to control groups

Table 2. Effects of *G. kola* extract on male reproductive organs in male Wistar Albino rats

Group	Control (g)	Group 2 (g)	Group 3 (g)	Group 4
Epididymis	0.64 ± 0.02	0.65 ± 0.11	1.13 ± 0.07 (*)	1.46 ± 0.12 (*)
Testes	1.53 ± 0.16	1.35 ± 0.09(*)	1.31 ± 0.05 (*)	0.74 ± 0.1 (*)

Values are expressed in mean ± SEM, (*) statistically significant at P<0.05 compared to control groups

Table 3. Effects of *G. kola* extract on epididymal sperm count in male Wistar Albino rats

Group	Control (g)	Group 2 (g)	Group 3 (g)	Group 4
Sperm count (x 10^6/ml)	58.93 ± 0.47	33.57 ± 0.9$^{(*)}$	21.44 ± 0.4 $^{(*)}$	11.14 ± 0.24 $^{(*)}$

Values are expressed in mean ± SEM, (*) statistically significant at P<0.05 compared to control groups

3.2 Discussion

In this study, the effect of ethanolic extract of *G. kola* on sperm count, male reproductive hormones and reproductive organs was investigated. *G. kola* has been found to have high concentration of saponins. Saponins decrease plasma concentrations of cholesterol and increase bile acid production [10]. Testosterone is a steroid hormone, therefore decrease in plasma cholesterol will reduce the level at which cholesterol is being synthesized [11]. Direct action of *G. kola* on the testes may have caused inhibition of gonadotropic action on the testes. This was shown by Price et al., [12] who observed an irreversible combination of saponins with membranes in animal cells, thus rendering the membrane non semipermeable. Other possibilities include preventing the release of pituitary gonadotropins and/or elevation of blood levels of testosterone (by inhibition of hepatic metabolism) thereby inducing negative feedback effect on gonadotropin release. This may be the mechanism in which *G. kola* enhances the serum levels of LH in rats. The most plausible explanation of the observations in male rats in this study could be that *G. kola* inhibits gonadotropic action on the testes. This is in collaboration with studies done by Udoh and Patil [13] which showed that phenolic compounds (saponins) are anti-spermatogenic.

The significant increase in the weights of the epididymis of the rats in the treatment groups of *G. kola* is in line with studies done by Oluyemi et al., [6], who reported an increase in the weights of the epididymis in rats treated with *G. kola* at the rates of 100 and 200 mg/kg body weight. Decrease in weights of the testes are in collaboration with studies done by Akinloye et al., [14] who reported a decrease in the weights of testes of male albino Wistar rats fed with *G. kola* extract. This may be due to the reduction of Leydig cells population in the interstitial spaces, slight reduction in the seminiferous luminal spermatozoa concentration and derangement of cells of the spermatogenic series with increase in the interstitial spaces [15].

The sperm density of rats treated with 100 mg/kg body weight of *G. kola* extract showed a marked decrease when compared to the control group. Udoh [15] found out that long term administration of *G. kola* caused marked spermatogenesis arrest. This may be primarily due to the decreased production of testosterone by testes [6]. The decreased sperm count observed in this study may also be an implication of the reduced testosterone and LH concentration, which are major regulators of spermatogenesis [16].

4. CONCLUSION

It can be concluded from this study that ethanolic seed extract of *G. kola* resulted in reduced serum reproductive hormone concentrations, a dose dependent decrease in sperm count in male Wistar rats, and may be detrimental to male reproductive health. Hence, its consumption rate and usage in African traditional medicine needs to properly regulated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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