

# Micromorphometric and Stereological Effects of Ethanolic Extracts of *Garcinia cambogia* seeds on the Testes and Epididymides of Adult Wistar Rats

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## Abstract

The objective of this study was to evaluate testicular and epididymal alterations resulting from the administration of ethanolic extract of *Garcinia cambogia* by morphometric methods. Fifteen (15) rats weighing between 120-135g were used for the study. These were divided into control and experimental groups of 5 rats each. They were given ethanolic extract at doses of 0.00mg/kg B.W (control), 100mg/kg B.W (Group B), 200mg/kg B.W (Group C) respectively by gastric lavage for 6 weeks. Five slides chosen at random from the testicular slides of control and experimental were evaluated and analyzed. There was a reduction in the germinative cell thickness of the seminiferous tubules in the treated group compare with control. There was a significant increase in the sperm counts but reduction in motility in the treated groups in a dose dependent manner compare with control ( $P < 0.05$ ). The volume density ratio of lumen was increased in the treated groups which receive the higher dose of extract compared with the control.

## INTRODUCTION

"*Garcinia cambogia* (G.kola; bitter kola) is a popular plant in southern Nigeria and extensively used as food and herbal medicine". It is found in the tropical rain forest of West-Africa<sub>1</sub>. "The height of the plant is approximately 14m and it produces reddish, yellowish or orange colour fruits containing 2 to 4 seeds. The seeds when chewed have a bitter astringent taste. Several works done on G. Kola have confirmed its hypolipidemic<sub>2,3,4</sub> antifungal<sub>5</sub>, anti-histaminic<sub>6</sub>, spermatogenic<sub>7</sub>; aphrodisiac and anti-microbial<sub>8,9</sub>, erythropoietic<sub>4</sub>, antiviral<sub>8,10</sub>; anti-bacteria against *Neisseria gonorrhoeae*<sub>11</sub> and anti-ulcerogenic effects<sub>12</sub>. Chewing sticks produced from G. Kola tree is very effective and reliable for cleaning teeth when used without toothpaste<sub>13,14</sub>. The seeds as well as chewing sticks are important products often seen in West African markets<sub>3</sub>. The chewing stick is used to cure halitosis among the Yorubas of Nigeria.

The presence of biflavonoids and xanthone in G. kola seeds have been confirmed<sub>15</sub>. These compounds are potent antioxidants<sub>3,7,16</sub>. Administration of G. kola seed extracts

caused an increase in testosterone production in Sprague-Dawley rats<sub>17,18</sub> due to the anti-oxidant properties of its constituents.

The use of quantitative methods of light and electron microscope levels has increased remarkably over the last few years. These methods collectively called "morphometry" or "stereology" allow the number, volume, surface area and lineal extent of structures in three dimensions to be assessed from two dimensional images of light and electron microscopy<sub>19,20</sub>.

The aims of this study were to investigate the effects of methanolic extract of G. Kola seeds on testes and epididymides using morphometric and stereologic methods.

## MATERIALS AND METHODS

### ANIMALS

Fifteen (15) adult male wistar rats weighing between 120-135g obtained from the animal house of the Igbinedion University, Okada, Edo State were used for this experiment. The rats were kept in the animal control room, acclimatized for two weeks before the experiment commenced. The rats

were fed standard diet (Rat's pellet, Bendel feeds and flour mills Limited, Edo state, Nigeria. Water was given ad libitum and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25-27°C under day/night 12-12 hour photoperiodicity. The rats were randomised into three groups of 5 rats each: A (Control, received 0.00mg/kg B.W of extract), B (received 100mg/kg B.W of extract), and C (received 200mg/kg B.W of extract).

## PLANT MATERIALS

The plant material, *Garcinia cambogia* seed, was obtained from a local market in Ile-Ife, Osun State and authenticated by the botany Department, Igbinedion University. The outer coats were removed and the seed sun-dried. The dried seeds were grounded into fine powder and the crude ethanolic extraction done using 70% ethanol. The solution was filtered after 24 hours while the filtrate was concentrated to a semi-solid form using the rotary evaporator, weighed and the solutions were prepared as 100mg/ml and 200mg/ml respectively.

## EXPERIMENTAL DESIGN

The administration of the extract was totally done by gavage using metallic oropharyngeal canula and calibrated hypodermic syringe. The administration of *G.kola* extract was done once in a day, 6 days of the week and for the period of 6 weeks. The control group received no extract while group B and C received 100mg/kg B.W and 200mg/kg B.W. of the extracts respectively. The animals were sacrificed under chloroform anaesthesia a day after the administration of extracts stopped.

The testes and the epididymides were dissected free, weighed in a torsion balance and fixed in 10% buffered formalin. Routine histological slides preparations were done using Erlich's haematoxylin and eosin method as described by Oluyemi et al<sub>7</sub>. The stained sections were subjected to micro-morphometric analysis according to Huttunen et al<sub>21</sub>

The sperm count was analyzed using Neubauer's counting chamber.

## STATISTICAL ANALYSIS

The values are recorded as mean  $\pm$  standard deviation. The statistical significance of difference in the mean and standard deviation ( $p < 0.05$ ) was analyzed by two-way ANOVA comparison of each of the test groups and the control.

## RESULTS

Morphometric analysis of *Garcinia kola* treated Testis: The diameter of seminiferous tubules showed an increment in the treated group as compared with the control (Table 1). This observation was pronounced in group C which received 200mg/kg B.W of the extract (Table 1). The germinative cell thickness and germinative cell thickness/ radius, showed a gradual reduction in the treated group as compared with the control. This observation is dose dependent.

Semen Analysis: The sperm concentration presents a dose dependent significant increment ( $P < 0.05$ ) as shown in Table 2 in contrast to dose dependent significant decrement observed in sperm motility.

Volume Density of Seminiferous Tubules: The volume density of seminiferous tubules was observed to present a non significant increment in the epithelium and lumen of the treated group as shown in Table 3. a dose dependent reduction was observed in the treated group (Table 3)

Volume Density of Epididymis: Table 4 presents the volume density of the epididymis. It shows a significantly higher volume of epithelium in a dose dependent manner when compared with the control. The luminal volume of Group B is not significantly different from that of the control while the volume of the Group C is significantly lower than that of the Group A and Group B. The connective tissue volume shows a dose dependent increase in both treatment groups compared to the control.

### Figure 1

Table 1: Morphometric analysis of *Garcinia kola* treated Testis

Dose	Diameter Of seminiferous tubules ( $\mu$ m)	Germinative cell thick-ness ( $\mu$ m)	Germinative cell thickness / radius
Group A (0.00 mg/kg B.W)	168 $\pm$ 19	45 $\pm$ 3.5	0.54 $\pm$ 0.19
Group B (100mg/kg B.W)	173 $\pm$ 5	41 $\pm$ 5.5	0.47 $\pm$ 0.11
Group C (200mg/kg B.W)	181 $\pm$ 24*	39 $\pm$ 13.1	0.41 $\pm$ 0.72

\* Significantly different when compared with control ( $P < 0.05$ ); n = 5

### Figure 2

Table 2: Semen Analysis

	Group A (0.00 mg/kg B.W)	Group B 100mg/kg B.W	Group C 200mg/kg B.W
SPERM CONCENTRATION	59.8 $\pm$ 2.14	70.0 $\pm$ 2.98*	81.5 $\pm$ 3.6*
MOTILITY (%)	77 $\pm$ 2.54	70 $\pm$ 2.74*	65 $\pm$ 2.41* *

\* Significantly different when compared with Group B ( $P < 0.05$ ); \* Significantly different when compared with Group A (Control) ( $P < 0.05$ ); n = 5

**Figure 3**

Table 3: Volume Density of Seminiferous Tubules (ST) (mm/mm)

	Epithelium	Lumen	Connective tissue
Group A (0.00 mg/kg B.W)	0.52 ± 0.03	0.14 ± 0.03	0.21 ± 0.03
Group B (100mg/kg B.W)	0.54 ± 0.08	0.16 ± 0.08	0.36 ± 0.10
Group C (200mg/kg B.W)	0.56 ± 0.07	0.19 ± 0.06	0.53 ± 0.07**

\* Significantly different when compared with Group B ( $P < 0.05$ );  $n = 5$

**Figure 4**

Table 4: Volume Density of Epididymis (mm/mm)

	Epithelium	Lumen	Connective tissue
Group A (0.00 mg/kg B.W)	0.19 ± 0.08	0.33 ± 0.04	0.29 ± 0.10
Group B (100mg/kg B.W)	0.50 ± 0.06*	0.30 ± 0.10	0.13 ± 0.07*
Group C (200mg/kg B.W)	0.56 ± 0.07**	0.19 ± 0.06*	0.56 ± 0.09**

\* Significantly different when compared with Group B ( $P < 0.05$ ); \* Significantly different when compared with Group A (Control) ( $P < 0.05$ );  $n = 5$

## DISCUSSION

The morphometric analysis of the seminiferous tubules (Tables 1) of the present work justifies the increment in sperm counts as found in the epididymal lumen (Table 2). The increase in the diameter of the seminiferous tubules and the decrease in the germinative cell thickness could be as a result of rapid spermiogenesis resulting in the depopulation of the peripherally placed spermatids and increase in luminal sperm concentration. Spermatids develop into mature spermatozoa through the process of spermiogenesis<sup>22</sup>. Rapid spermiogenesis is facilitated by antioxidants and increase in the peripheral testosterone level. These two factors are properties of *G.kola*'s biflavonoids and xanthenes<sup>7,23</sup>. The decrease in the motility of the spermatozoa could be due to the rapidity of development. The spermatozoa may need a moderate but progressive development for them to have excellent motility. This may also be due to the presence of some toxic component like benzophenone in the ethanolic extracts of *G. kola*. Cotterih et al<sup>24</sup>, has reported the antioxidant potency of Kolaviron- an antioxidant extract of *G.kola*. Other antioxidant like carotenoid has been found to protect spermatogenesis in animals exposed to toxicant<sup>25</sup>. Anti-oxidants such as carotenoids are well known as highly efficient scavengers of singlet oxygen and other excited species (oxidants). Apigenin-based flavonoids represent 60% of the total flavonoids present in the diethyl-ether fraction of *G. kola*<sup>26</sup>. Other studies in man, have shown that lycopene helps men with idiopathic infertility, with an improvement in male fertility especially sperm characteristics<sup>25</sup>.

However, the diameter of the ST is largest in the group

receiving the 200mg/kg B.W of ethanolic extracts of *G. kola*. The diameter of the group receiving 200mg/kg B.W of ethanolic extracts of *G. kola* was significantly higher than that of 100mg/kg B.W extracts and the control. The volume density of the epithelium and lumen of the seminiferous tubules (Table 3) as assessed by the Weibel counting grids (point counting method) increase non-significantly as compared with the control ( $P > 0.05$ ). The volume density of C.T of the seminiferous tubules shows a dose dependent increase when compared with the control (Table 3). In relation to a report by Oluyemi et al<sup>7</sup>, there is a need for the development of more connective tissues to support the contractile mechanism involved in the migration of immotile spermatozoa, formed in the seminiferous tubule, into the epididymis where they acquire motility. This mechanical contraction is brought about by the fibroblasts in the connective tissue walls of the seminiferous tubules. Young and Heath<sup>27</sup> affirmed that the contractile activity of fibroblasts increases in response to the testosterone level in the testis. This androgen (testosterone) is produced in the testis by the interstitial cells of Leydig in response to follicle stimulating hormone (FSH) produced by the anterior pituitary<sup>27</sup>. Akpantah et al<sup>23</sup> found out that *G. Kola* extract increases the peripheral testosterone levels in wistar rats treated with 100mg/Kg B.W of the extract. Increased spermatogenic activity in seminiferous tubules associated with the administration of *G. kola* extract is hence due to the ability of the antioxidant compounds in the latter to increase peripheral testosterone levels<sup>7,23</sup>.

The significant decrease in the volume density of the lumen of the epididymis (Table 4) is most likely due to the significant increase in the epithelium (pseudostratified) of the epididymis with the corresponding increase in the volume density of the connective tissue of the epididymal wall. This is a very strong indication that *G.Kola* extract influences the epididymis in a positive direction. The ciliated epithelium is also important in the movement of the spermatozoa from the proximal to the distal portion of the tube prior to ejaculation which under the influence of sympathetic innervations. "The proximal portion of the muscular wall of epididymis exhibits slows rhythmic contractility which gently moves spermatozoa towards the ductus deferens. Distally the smooth muscle is richly innervated by the sympathetic nervous system which produces intense contractions of the lower part of the epididymis during ejaculation<sup>27</sup>.

In conclusion, this work further confirmed the spermatogenic and tissue enhancing effects of *G. Kola* extract in male Wistar rats.

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