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Short Communication

Effects of crude ethanolic extract of *Garcinia cambogia* on the reproductive system of male wistar rats (*Rattus novergicus*)

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15 male rats were grouped and administered crude ethanolic extracts of *Garcinia cambogia* seeds to test the effects on the histology of the testis and sperm counts. Group A served as the control while Groups B and C received 100 and 200 mg/kg body weight of extracts, respectively. The administration was done orally once a day, six days a week for six weeks. The routine histological preparation at the end of administration revealed increase in the interstitial spaces, degeneration of the Ledgid cells and distortion in the arrangement of the cells of spermatogenic series. The sperm counts revealed a significant increase in the experimental groups when compared statistically with the control ($p<0.05$). It was $81.5 \pm 13.62 \times 10^6$/ml and $70 \pm 12.98 \times 10^6$/ml in groups Band C, respectively, as compared to $59.8 \pm 2.14 \times 10^6$/ml in the Control group.

Key words: *Garcinia cambogia*, sperm count, histology, Leydig cells, interstitial spaces.

INTRODUCTION

With the shifting of attention from synthetic drugs to natural plant products, the use of plant extracts for disease treatment is now on the increase. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects. One of such plant is *Garcinia cambogia* (bitter kola), a plant found in the moist forest and grows as a medium size tree, up to 12 meters high in places like Nigeria, United State, South East Asia, India and Central Africa.

The active constituent of *G. kola* is a dimeric flavonoid molecules fused together- biflavonoid. Biflavonoids are potent antioxidants. Other constituents include xanthones and benzophenones (Ebong and Korubo-Owiye, 1996; Encyclopedia, 2002). Several works done on *G. kola* have confirmed its hypolipidermic (Koshy and Vijaya-lakshmi, 2001; Iwu, 1993; BBC News, 1999; Oluyemi et al, 2007), antifungal (Mackeen et al., 2002), anticancerous (Ho et al., 2002; Pan et al., 2001), antihistaminic (Nakatani and Atsumi et al., 2000), antimicrobial (BBC News, 1999: Iwu et al., 1999), erythropoietic (Oluyemi et al, 2007), antiviral (Chen et al., 1996; Iwu et al., 1999) and antiulcerogenic effects (Mahendran et al., 2002). Though its effects on the reproductive system have been investigated by Akpantah et al. (2003) and Akinloye et al. (1999), their findings are however at variance.

The present study aims at furthering tests on the effects of *G. kola* on the reproductive system of male wistar rats and probably resolves the conflicting reports.

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Table 1. Sperm counts of wistar rats treated with crude ethanolic extract of *G. cambogia*.

Parameter	Control A	Group B	Group C
Dose	Saline water	100 mg/kg body weight	200 mg/kg body weight
Sperm density (million/ml)	59.8±0.96	70.0 ± 5.80$^{\Psi}$	81.5 ± 6.08$^{\Psi}$

n= 5 for all group.
Values are recorded as Mean ± SEM.
$^{\Psi}$Values are significantly different from control (P< 0.05).

MATERIALS AND METHODS

Animals

15 Adult male rats weighing between 120 – 135 g were 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 on standard diet (Bendel Feed and Flour Mills Ltd), water was given *ad libitum* and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25 - 27^{o}C under day/night 12-12 h photoperiodicity. The rats were grouped into three groups (5 rats each) A (0 mg/kg), B (100 mg/kg), and C (200 mg/kg).

Plant materials

G. kola 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% alcohol. The solution was filtered after 24 h while the filtrate was concentrated to a semi solid form using the rotary evaporator, weighed and the solutions were prepared as100 and 200 mg/ml.

Experimental design

The administration of the extract was totally by gavage. Proper concentrations were administered by the use of metal 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 groups B and C received 100 and 200 mg/kg body weight of the extracts respectively. The animals were sacrificed after the administration of extracts stopped.

Testis collection and semen analysis

The testes were dissected out via the inguinal region; the caudal epididymis were dissected free and suspended in the semen diluting fluid (sodium bicarbonate-formalin diluting fluid), containing 1% acetic acid, 4% $NaHCo_3$ and 35% Formalin (Monica, 2000). The rest of the testis was fixed in 10% buffered formalin. The sperm count was done using haemocytometer counting chamber of the Neubauer type.

Routine histological preparation

The histology of the testes and epididymis were done by modification of method described by Akpantah et al. (2003). The organs were cut in slabs of about 0.5 cm thick transversely and fixed in 10% buffered formalin for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax for 1 h each in an oven at 65 ^{o}C for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 6 microns. The tissues were fixed into albumenised slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 min. The slides were then stained with haematoxylin and eosin. The slides were mounted in Canada balsam. Photomicrographs were taken using X40 objectives.

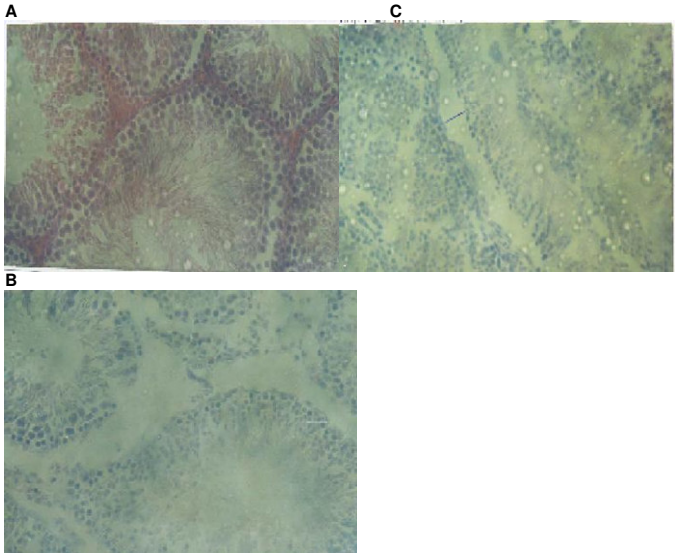
Statistical analysis

All data from the sperm count were compare using a two-way ANOVA at $p<0.05$.

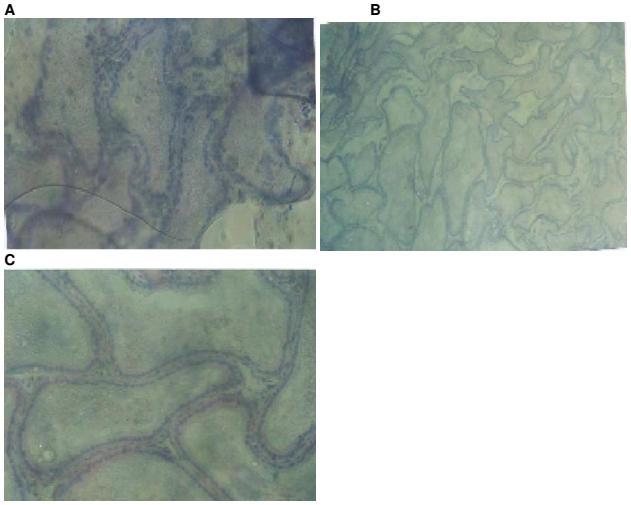
RESULTS AND DISCUSSION

Table 1 shows the effects of the crude ethanolic extract of *G. cambogia* on the sperm count of wistar rats. There were significant increases when compared with the control. This could be as a result of the presence of biflavonoid and xanthone in the plant. These compounds are potent antioxidants which are capable of increasing the production of testosterone, the key hormone involved in the production and maturation of spermatozoa in the seminiferous tubules of the testis (Ganong, 2003; Guyton and Hall, 1998). Akpantah et al. (2003) has found that *G. cambogia* increases the peripheral testosterone level. This result agrees with the work of Akinloye et al. (1999) who recorded an increase in the sperm count after feeding 35 mg/kg of *G. cambogia* to rabbits for 8 weeks.

Figures 1A – C show the effects of graded dosages of *G. cambogia* on the histology of the testes of the control and experimental rats. *G. cambogia* extract causes increase in the interstitial spaces, reduction in the Leidig cells population in the interstitial spaces, slight reduction in the seminiferous luminal spermatozoa concentration, contraction of the seminiferous tubules, and derangement of the cells of spermatogenic of series. These findings agreed with the work of Akinloye et al. (1999) but are at variance with the work of Akpantah et al. (2003) who recorded no histological difference in the testes of wistar rats. The reduction in sizes of the seminiferous tubules might be due to the contractile activities of the fibroblast of the seminiferous tubular wall. This is because testos-



Figures 1A to C. Photomicrograph of the histology of the testis of the animals.



Figures 2A to C. Photomicrograph of the histology of the epididymis of the animals.

terone causes the contraction of the fibroblasts which aid the movement of the non-motile spermatozoa into the epididymal tubule where the final maturation into motile spermatozoa takes place (Peter et al., 1999). This might be the reason for the increase in the interstitial spaces.

Figures 2A - C show the micrographs of the epididymis of the control and experimental rats. They show reduction in the sperm concentration in the tubules of the epididymis of both groups and increase in the interstitial spaces of the group which received 100 mg/kg body weight of extract. These agree with the work of Akpantah et al. (2003). The histological preparations of the testis and epididymis revealed toxic effects which were more pronounced in the group that received 200 mg/kg body weight of extract. This shows a dose dependent toxic effect. This is likely due to the presence of garcinone E in *G. cambogia*. This compound has been found to possess cytotoxic effects against liver cells (Ho et al., 2002).

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