



PATHOLOGICAL, BIOCHEMICAL AND HORMONAL CHANGES ASSOCIATED WITH PROLONGED ADMINISTRATION OF METHANOL EXTRACT OF *GARCINIA KOLA* SEED IN ADULT MALE WISTAR RATS.

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SUMMARY

Garcinia kola (GK) seed is believed to possess aphrodisiac properties hence its traditional use to manage erectile dysfunctions in man. However, there has been conflicting reports of its long-term effects on the male reproductive organs. In this study, the effect of varying doses of methanol extract of GK on testicular function and structure in Wistar rats was evaluated. Forty adult male rats (350-360g) were randomly divided into 4 groups. Groups 1, 2 and 3 received oral doses of 600mg/kg, 400mg/kg and 100mg/kg of the extract daily, respectively for 70 days while group 4 (control group) received normal saline (0.9% NaCl). Blood samples were collected fortnightly from each rat for serum chemistry and testosterone analysis. At termination, the testes, epididymides and livers were harvested, weighed and fixed in 10% neutral buffered formalin and processed for histopathology. The results were presented as mean \pm standard error of mean (SEM) and analyzed using one way analysis of variance (ANOVA) at $P < 0.05$. Dose-related pathological changes were observed in the testes and epididymides. Testicular weight decreased significantly ($p < 0.05$) in 400mg/kg and 600mg/kg groups compared to the control group. There was testicular necrosis associated with distortion of the seminiferous tubular wall and reduced seminiferous tubular diameter. Seminiferous tubules had reduced germinal epithelial thickness along with an absence of viable spermatids and

spermatozoa. Serum testosterone levels decreased progressively ($p < 0.01$) in groups 1 and 2 while those of groups 3 and 4 remain unchanged. The results established that ingestion of methanol extracts of GK seed at the dose of 400mg/kg and 600mg/kg produced significant gonadal pathology in adult male Wistar rats despite the absence of remarkable hepatic biochemical and structural changes.

Keywords: *Garcinia kola*; Methanol extract; Histopathology; Testicular degeneration and Necrosis; Testosterone; Liver enzymes.

INTRODUCTION

Garcinia kola is a flowering plant species in the *Guttifera* family that grows to a height of about 12 meters and abounds in subtropical and tropical lowland forests; a detailed description and distribution of this plant have been documented (Iwu *et al.*, 1999). The seed is widely consumed as masticatories because it contains a wide variety of biologically active substances including antioxidants (Iwu *et al.*, 1998). It is commonly called “bitter kola” or “male kola” due to its bitter taste, and for its acclaimed aphrodisiac activity, respectively (Uko *et al.*, 2001). The potential benefits of GK seed as a therapeutic agent have been widely reported in literature, these include hepato-protective (Akintonwa and Essien, 1990; Braide 1991; Farombi *et al.*, 2000; Nwankwo *et al.*, 2000; Farombi *et al.*, 2005; Dare *et al.*, 2012), antithrombotic (Olajide, 1999) and antimicrobial (Iwu *et al.*, 1999; Nwankwo *et al.*, 2000; Ezeifeke *et al.*, 2004; Adegboye *et al.*, 2008) activities. The seed is also acclaimed in folklore to possess aphrodisiac properties in terms of enhancement of sexual activity in males and thus consequently been

extensively used in the treatment of erectile dysfunctions in man (Ralebona *et al.*, 2012). Several authors including Adesanya *et al.* (2007), Ralebona *et al.* (2012), Atsukwei *et al.* (2015) and Sewani-Rusike *et al.* (2016) reported that GK seed enhanced sexual activity in normal male rats; however, Yakubu and Quadri (2012) found no convincing evidence of the sexual-activity enhancing potential of GK seed in their studies. In recent time, there has been increase in consumption and indiscriminate use of GK seed as an antidote to a number of disease conditions ostensibly due to numerous reports of the potential beneficial therapeutic effects and safety attributed to this seed. However, several studies have reported adverse effects on various body organs (Braide and Gill, 1990; Atawodi *et al.*, 1995; Nottidge *et al.*, 2008), especially the male reproductive organs (Akinloye *et al.*, 1999; Akpantah *et al.*, 2003; Chilaka *et al.*, 2009; Abua *et al.*, 2013; Mesembe *et al.*, 2013, Obi and Nwoba, 2013; Ovuakporaye and Odokuma, 2018). In previous studies, varying degrees of testicular and epididymal lesions were seen in rabbits (Akinloye *et al.*, 1999; Obi and Nwoba, 2013), and a significant reduction in rat sperm concentration (Akpantah *et al.*, 2003) following a prolonged administration of aqueous extracts of GK seed, suggesting possible anti-fertility consequence (Braide *et al.*, 2003). According to Braide *et al.* (2003), alkaloids such as bioflavonoids, contained in the methanol extract of GK seed, maybe the compounds responsible for precipitating organ damage. The divergent and conflicting views especially regarding the safety, or likelihood of GK seed to induce detrimental outcomes on the male reproductive system has continued to generate controversy and concern. There is a need for

more studies to clarify or establish the level of safety in especially prolonged use of the plant product as an antiviral or aphrodisiac agent in man or animals. Therefore, this study is an attempt to further expound the mechanisms by which GK seed exerts its effect on the male reproductive organs and weigh the potential benefits against the adverse effects.

MATERIALS AND METHODS

Experimental animals

Forty adult male Albino rats (350-360g) obtained from the animal house of the Federal University of Agriculture, Abeokuta, Nigeria and were randomly allocated to 4 treatment groups of 10 rats each. The rats were allowed to acclimatize for 2 weeks before the commencement of the experiment and were maintained under standard laboratory conditions. Approval was obtained from the Institutional animal care and use research committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria (Reference NO: FUNAAB/ACUREC/21/0021). They were housed in standard plastic cages containing 5 rats each and kept in a room with ambient temperature not exceeding 30°C. The rats were fed a commercial rat chow (Vital feed®) *ad libitum* and free access to drinking water for the period of 70 days. Animals were adequately restricted from crossing to other cages.

Preparation of *G.kola* seed extract

Fresh seeds of *G.Kola* were purchased from a local market in Abeokuta, Ogun State Nigeria and were authenticated at the herbarium of the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta where a voucher specimen

already exists. Methanol extract was prepared using the method described by Ralebona *et al.* (2012) with modifications. The seeds were peeled, cut into small pieces, air dried for two weeks, and then crushed into a powder using an electric grinder. A 300g of the paste was dissolved in 500ml of methanol at room temperature. The mixture was continuously agitated for until an even suspension was obtained using a magnetic stirrer for 3days. The suspension was sieved and the solution was concentrated using a rotary evaporator (Buchi RE111) to remove the methanol. The concentrated filtrate of the extract was stored at -70°C until used for analysis. The extract was reconstituted in Tween-80 (10 % w/v) for animal oral administration.

Phytochemical screening

A small quantity of the extract was subjected to the phytochemical screening to check for presence alkaloids and flavonoids as described by Adegboye *et al.* (2008) with slight modification. For alkaloids, 0.5g of the extract was dissolved in 5ml of 1% HCl on water bath at 55°C bath. The filtrate was then treated with a few drops of Dragendorff's reagent. Turbidity was indicative of presence of alkaloids. For flavonoids, 0.2g of the extract was dissolved in 2ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. A red colouration was indicative of presence of flavonoids.

Collection of blood

After every two weeks, blood was collected from each rat from the medial canthus of the

eyes into plain sample bottles; serum was obtained by centrifugation (3000 ×g for 10 min) and stored at -20°C until testosterone analysis.

Serum Biochemistry

The serum levels of total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercial reagent kits (ELITech Group, Puteaux, France) and Flexor E automated clinical chemistry analyser (Vital Scientific NV, Dieren, The Netherlands) according to the recommendations of the manufacturers.

Histopathology

Formalin-fixed tissues were embedded in paraffin wax, processed by routine histological method as previously described by Bancroft and Gamble (2014). Testes, epididymides and liver were sectioned at 4-5µm and stained with haematoxylin and eosin (H&E). Sections were examined carefully with the light microscope (Olympus, CX21FS1) at X10 and X40 objective lens to evaluate the testes, epididymides and liver.

Histomorphometry

The slides were examined under the microscope and the measurement of seminiferous tubular diameter and germinal epithelium thickness were taken. For these parameters, seven measurements were made per section using a calibrated eye-piece micrometer (Graticules Ltd. Tonbridge Kent, England). The mean value of the measurement of the parameter in each section was recorded for each animal.

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). Analysis of variance (ANOVA) test was used to evaluate difference between groups. The GraphPad prism 7.3 software package (GraphPad software Inc., San Diego, CA) was employed for this analysis. Differences were considered significant at $p < 0.05$ and $p < 0.01$.

RESULTS

Body and organ weights

No significant differences ($p > 0.05$) in the body weight or absolute liver weights were observed for the treatment groups and control group. In contrast, the testes of groups 1 and group 2 weighed $1.47 \pm 0.32g$ and $1.61 \pm 0.28g$ (Mean ± SEM), respectively. These figures were significantly ($p < 0.05$) different when compared to control testes which weighed 2.91 ± 0.10 (Table 1).

Table 1: Absolute and relative body, testes and liver masses of Wistar rats administered *G. kola* methanol extract at termination of the experiment.

Treatment groups	Body mass (g)	Testes mass (g)	Liver mass (g)	Testes mass/body mass x10 ² (g)	Liver mass/body mass x10 ² (g)
Group 1 (600mg/kg)	360.6 ± 11.5	1.47 ± 0.32*	12.98 ± 1.12	0.41 ± 0.02*	3.60 ± 0.20
Group 2 (400mg/kg)	355.8 ± 17.6	1.61 ± 0.28*	12.85 ± 1.16	0.45 ± 0.03*	3.61 ± 0.12
Group 3 (100mg/kg)	356.4 ± 13.6	2.95 ± 0.24	12.88 ± 1.56	0.83 ± 0.15	3.61 ± 0.32
Group 4 (0mg/kg)	354.9 ± 17.5	2.91 ± 0.10	12.84 ± 1.23	0.82 ± 0.13	3.62 ± 0.51

Results are expressed as mean ± SEM

*Figures are significantly different when compared to the control group (p < 0.05)

Gross appearance and histological characteristics of the testis, epididymis and liver

Grossly, there was severe atrophy of the testes of the rats that received 600mg/kg and 400mg/kg. Histologically, no significant difference was observed in group 3 that received 100mg/kg of the extract. Severities of these changes were related to the dosage of extract administered. The germinal epithelial thickness also followed the same pattern. The testes of groups 1 and 2 that received 600mg/kg and 400mg/kg had small seminiferous tubules many of which were abnormal morphologically with distorted wall and reduced numbers of germ cells (Plate 2) when compared to those of the control group (Plate 1).

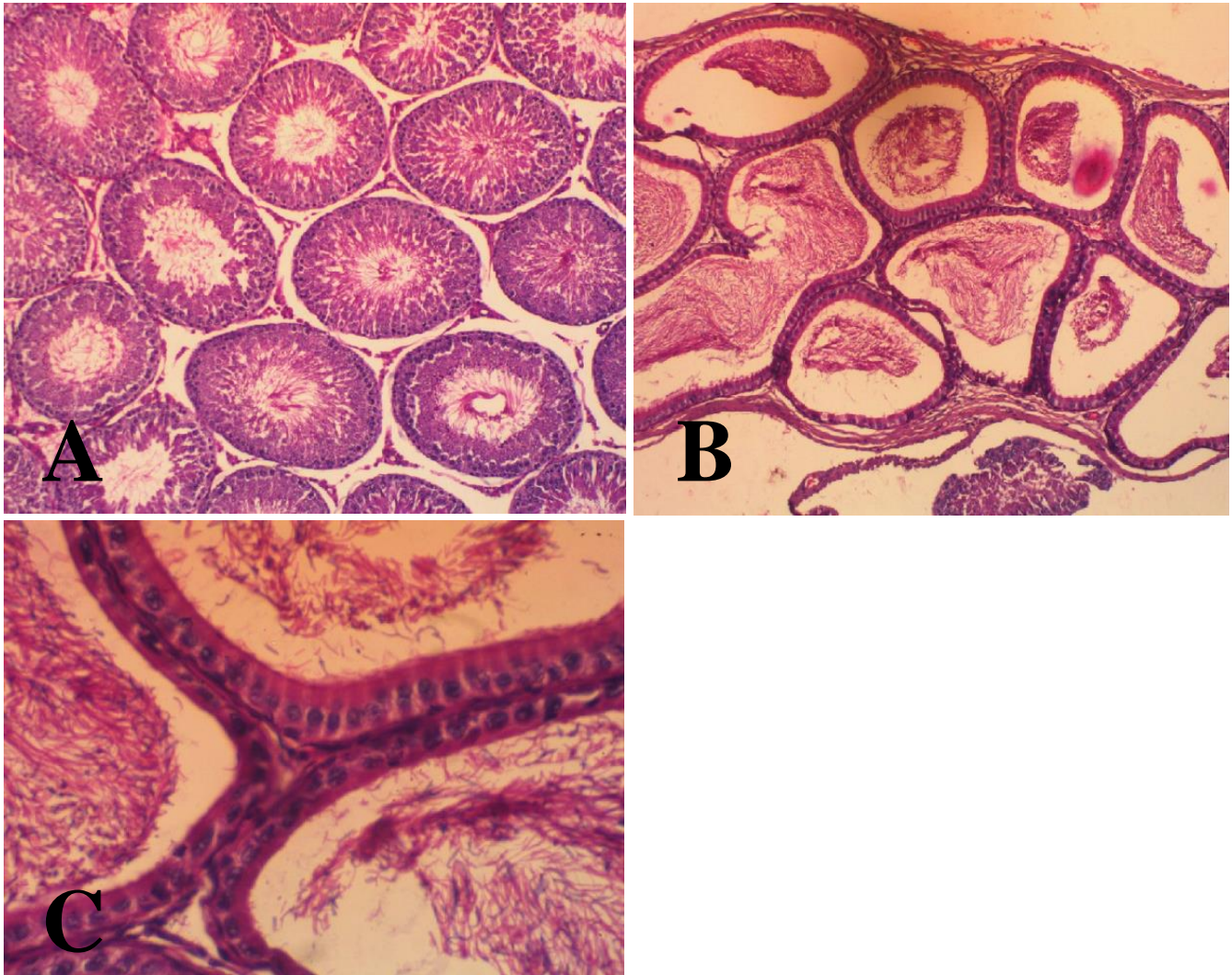


Plate 1: Photomicrograph of the testes, epididymis of control group showing normal histological appearance of seminiferous tubules (A) and epididymis (B) (X400, H&E stain. Plate 1C is a higher magnification of epididymis (X1000, H&E stain).

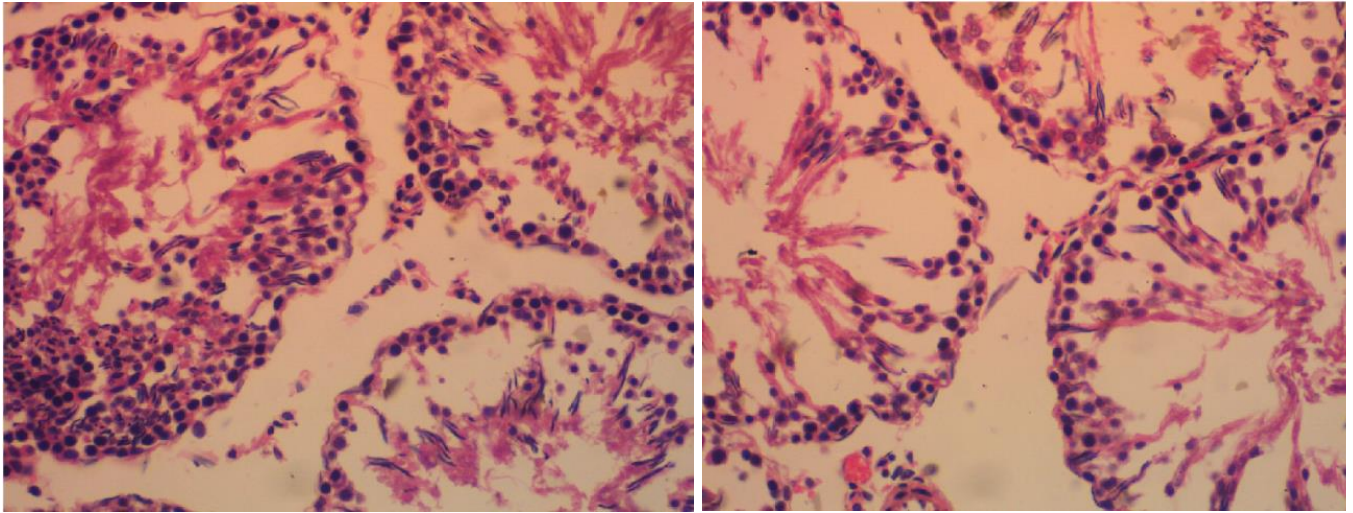


Plate 2: Photomicrograph of testis showing moderate diffuse testicular degeneration in group 2 rats that received 400mg/kg of the extract (X1000, H&E stain).

These pathological changes led to absence of mature spermatozoans in the epididymis in groups 1 and 2 (Plate 3).

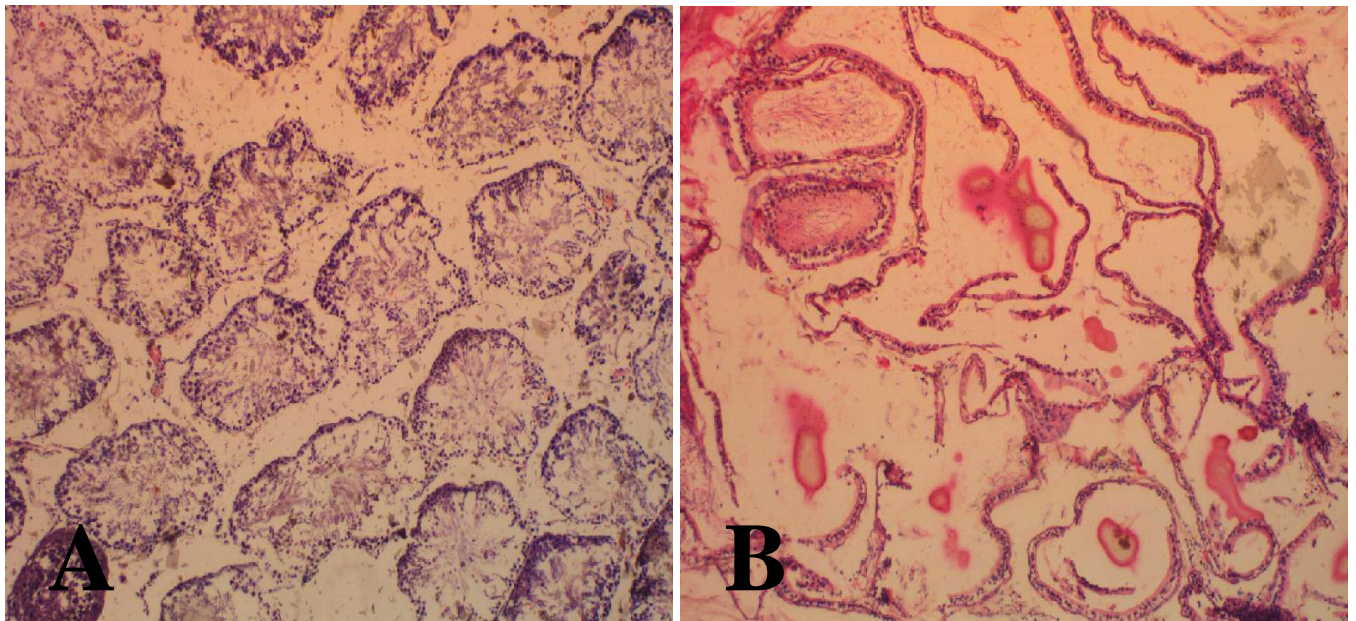


Plate 3: Photomicrograph of testes of group 1 rats showing markedly distorted basement membrane. The seminiferous tubules are reduced in diameter and germinal epithelia are severely degenerated (A). Marked dilation of epididymides with absence of viable sperm in the lumina (B) (X400, H&E stain).

No significant gross lesions in the liver from experimental and control groups. At histology, no structural changes in the liver from the treated and control groups (Plate 4).

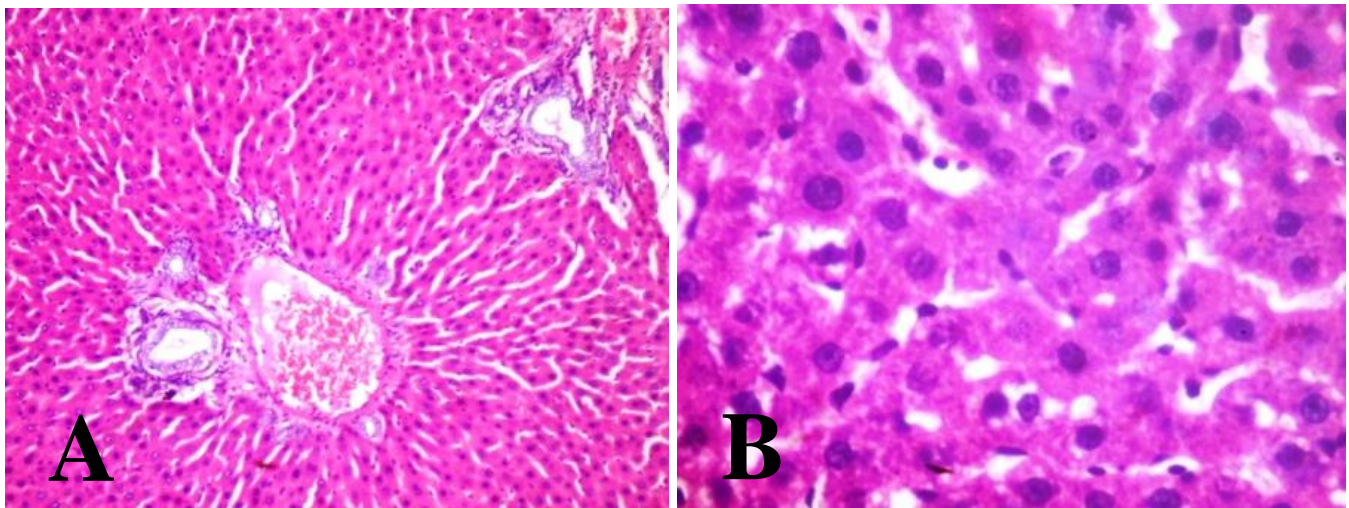


Plate 4: Representative photomicrograph of liver sections from rats that were administered bitter kola methanol extract and control rats showing normal histological appearance. Plate 4A is showing mild vascular congestion (arrow) (X100 & X400, H&E stain).

Histomorphometry showed that the seminiferous tubular diameters were significant difference ($p < 0.01$) in groups 1 and 2 when compared to control (Table 2).

Table 2: Testicular histological characteristics of Wistar rats administered bitter kola methanol extract.

Treatment groups	Seminiferous tubular diameter (μm)	Germinal epithelial thickness (μm)
Group 1 (600mg/kg)	142.43 \pm 5.87*	6.4 \pm 0.3*
Group 2 (400mg/kg)	147.61 \pm 4.26*	7.2 \pm 0.5*
Group 3 (100mg/kg)	170.42 \pm 8.03	12.2 \pm 1.3
Group 4 (0mg/kg)	168.41 \pm 4.71	11.1 \pm 1.5

Results are expressed as mean \pm SEM

*Figures are significantly different when compared to the control group ($p < 0.01$).

Biochemical Profile and Testosterone concentration

There was absence of significant change in the biochemical profile (total protein, albumin, ALP, AST and ALT) in both experimental and control groups (Table 3).

Table 3: Biochemical parameters of Wistar rats administered bitter kola methanol extract.

Treatment groups	Total Protein (g/dl)	Albumin (g/dl)	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)
Group 1 (600mg/kg)	5.2 ± 1.3	3.3 ± 0.4	20.8 ± 4.3	24.5 ± 3.5	55.2 ± 5.7
Group 2 (400mg/kg)	5.1 ± 0.8	3.5 ± 0.2	21.2 ± 3.3	25.4 ± 3.1	54.8 ± 7.1
Group 3 (100mg/kg)	5.4 ± 1.4	3.2 ± 0.4	21.5 ± 5.3	24.7 ± 3.6	54.6 ± 4.9
Group 4 (control)	5.6 ± 1.2	3.4 ± 0.3	21.3 ± 3.5	25.5 ± 5.5	55.2 ± 4.8

Values are presented as mean ± SE

However, there was a significant decrease ($p < 0.001$) in testosterone levels in *G. kola* group that received the highest dose (600mg/kg) of the extract compared to control. Serum testosterone concentrations in groups that received 600mg/kg and 400mg/kg were 2.36 ± 0.05 ng/ml and 4.07 ± 0.06 ng/ml, respectively while 5.13 ± 1.40 ng/ml was obtained for control group (Figure 1).

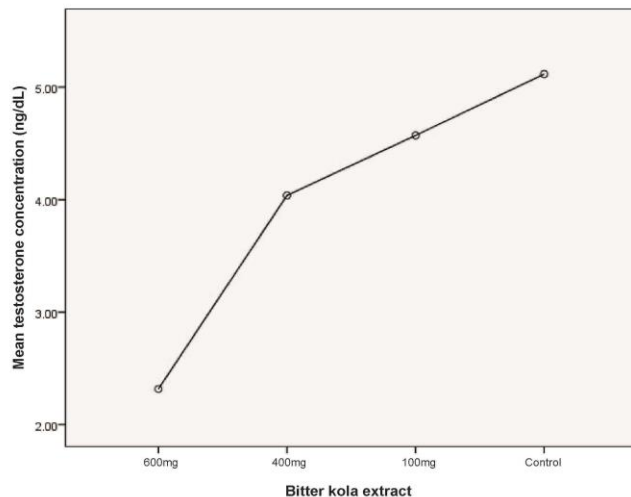


Figure 1: Testosterone concentrations in different groups of rats administered methanol extract of *G. kola*.

DISCUSSION

Garcinia kola is a popular medicinal plant that has made significant contributions to human health and well-being (Iwu *et al.* 1999). The seed of the plant is widely consumed by many people for various reasons and is believed to possess aphrodisiac activities and capable of enhancing sexual drive and consequently improving fertility in man (Ralebona *et al.*, 2012; Sewani-Rusike *et al.*, 2016). However, there have been conflicting reports on this claim. The present study reports the effect of methanol extract of *G. kola* seed on adult male rats treated for 70 days. The results of this study showed that despite the reported potential benefits of *Garcinia kola* seed, prolonged consumption could be injurious to reproductive organs. The present study is consistent with earlier findings on the effect of prolonged and high dose administration of the aqueous extract of *G. kola* on rabbit testes (Akinloye *et al.* 1999). Although *G. kola* is traditionally used as an aphrodisiac, the observed changes on histological sections of the testes and epididymides and the negative effect on

the testosterone concentration in this study suggests a possible anti-fertility activity. In the present study, there were no significant differences ($p>0.05$) in mean body weight and absolute liver weights in both treatment and control groups. The result is in variance with the report of Braide (1990) and Uko *et al.* (2001) who reported decreased body mass gain in rats fed diet containing *G. kola* powder. This was suggested to be associated with reduced feed consumption and or inhibition of gastrointestinal motility (Braide, 1990). Although, weight loss without decreased feed intake had been previously documented (Wight *et al.* 1987), both inappetence and decreased feed efficiency ratios appeared to have affected weight gain negatively (Uko *et al.*, 2001). As a result of these findings, Uko *et al.* (2001) suggested that the consumption of the GKS should be restricted to adults, as the young may suffer growth retardation. In the present study, severe atrophy of the testes occurred grossly in the rat that received 600mg/kg and 400mg/kg, and histological examination of the affected testes revealed severe testicular and epididymal pathology with absence of mature spermatozoans in the epididymides. The severities of these changes were related to the dosage of extract ingested. Microscopically, in the testes of group 1 and 2 rats, almost 100% of the seminiferous tubules were hypoplastic with disorganized and degenerated germinal epithelial cells and marked fibrosis with no viable spermatids and spermatozoa. However, these changes were less severe in group 2 compared to group 1. In this study, there was a marked reduction in the serum testosterone concentration of rats administered the methanol extract of *G. kola* especially in the groups that received 600mg/kg and 400mg/kg body weight.

This might have been as a result of the direct action of *G. kola* on the testicular tissue (Aprioku *et al.*, 2018). The result on testosterone was similar to many previous 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 that *G. kola* seed extract possesses potent aphrodisiac activity in male albino rats hence the resultant increase in sperm count and testosterone levels. Furthermore, the present study showed that the lowest dose of the extract (100 mg/ kg) did not alter either testicular function or structure of the testes and epididymides while higher doses of 400 and 600 mg/ kg extract caused significant pathological changes. Therefore, variations in the dosage administered and duration of treatment could influence the nature of the damage caused. Drugs that enhance sexual function have been reported to act via an increase in circulating level of testosterone, which is the male sex hormone responsible, among others, for enhancing sexual drive via central and peripheral effects (Mills *et al.* 1996). Indeed, testosterone supplementation has been shown to improve libido, orgasm and ejaculation (Fabbri, 2001). In this study, GK seed treatment at high dosage resulted in decreased testosterone levels, the mechanisms for decreased serum testosterone levels may be via central influences to decrease gonadotropins or locally via decrease in the number of Leydig cells or their sensitivity to luteinizing hormone (LH) (Braide *et al.* 2003). This study revealed that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were not altered ($P>0.05$) and histological examination of liver of both experimental and control rats showed no significant lesion. This observation in the liver is in variant with earlier report on the effects of prolonged ingestion of *G. kola* where degenerative

changes were observed in the liver, kidney and small intestine of the experimental dogs (Nottidge *et al.* 2008). It is therefore, plausible to suggest that inhibition of hepatic metabolism in rats treated with methanol extract of *G. kola* seeds did not occur as earlier reported (Braide and Gill, 1990). The present study in sexually mature adult male rats further expounded earlier studies by demonstrating the development of gonadal atrophy with severe abnormalities of both testes and epididymides in the absence of hepatic biochemical and histological changes. Further evaluation of the mechanisms responsible for the deficit in testosterone secretion is warranted. This may entail a more elaborate and direct evaluations of the anatomical and biochemical changes occurring in the hypothalamic-pituitary circuit using immunochemical or more advanced techniques.

CONCLUSION

The report presented in this paper demonstrated that sub-chronic ingestion of *G. kola* can produce gonadal atrophy and necrosis in the testes and epididymides associated with a significant reduction in serum testosterone concentrations in the absence of important biochemical and structural changes in the liver. However, this depends significantly on the amounts ingested. The results of the study also demonstrated that low concentrations of GKS extract at dose of 100mg/kg body weight neither produced testicular and epididymal structural alterations, nor induce adverse effects on the testosterone secretion.

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