Effect of Aqueous *Garcinia Kola* Seed Extract on The Histopathology of The Reproductive Organs of Wistar Rats

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Abstract

Background: Despite the wide acceptance and consumption of herbal decoction from medicinal plants, there is a need to scientifically assess their possible adverse effects on organs of the body. This study aimed at determining the effect of Garcinia kola extract (GKSE) on the reproductive organs of Wistar rats.
Materials and Methods: Forty male and female rats weighing 200 – 250g were randomly divided into four (4) groups with groups B, C and D subdivided into three based on the dose. The control group (A) was fed on standard diet and physiological saline orally. Groups B to D were fed on standard diet and administered 200, 400, and 800mg/kg body weight/day of GKSE orally for 3 months. Blood samples were collected for reproductive hormone assays and the gonads harvested for histopathological analysis.
Results: The female rats experienced significant decrease in the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and ER ($p<0.0001$) following the administration of varied concentrations of GKSE whereas testosterone (TST) significantly increased in male rats treated with 800mg/kg body weight/day of GKSE for 3 months ($p=0.0001$). Sections of the ovaries of female rats administered 400mg/kg of GKSE for 3 months revealed atypical hyperplasia whereas those treated with 800mg/kg of GKSE showed luteinized stromal cells with atypia. Sections of testicular tissue of male rats treated with 400 and 800mg/kg of GKSE for 3 months showed hypo-spermatogenesis.
Conclusion: Further studies to elucidate the pathological effects of prolonged consumption of Garcinia kola in the reproductive organs are advocated.

Keywords: Histopathological effect, *Garcinia kola,* testes, ovaries, Wistar rats.

Introduction

The oldest form of health care known to mankind is the use of plants for medicinal purposes (1). Many of these plants contain substances that can be used for therapeutic purposes (2, 3). In developing countries, the World Health Organization reported that about 80% of the population relies on traditional medicine for their health care (4, 5). Furthermore, it has also been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins possess medicinal properties (6, 7).

Garcinia kola plant is commonly found in the subtropical and tropical forests of some countries of West and Central Africa such as Benin, Ghana, Ivory Coast, Nigeria, Sierra Leone, Liberia, Cameroon and other countries in Central Africa, Asia and Europe. It is a dicotyledonous plant and belongs to the *Clusiaceae* or Guttiferae family (8, 9).

The phytochemical constituents of *G. kola* include dimeric flavonoid, biflavonoid, xanthone and benzophenones (9). The biological activities of flavonoids as potent water-soluble super antioxidants and free radical scavengers could be beneficial in alleviating some nutritional risk factors such as atherosclerosis which is a disease that often leads to hypertension and subsequently often develop into cardiovascular complications. It starts from ineffective metabolic management of cholesterol and triglyceride constituents of the blood (10).
Previous studies affirmed the positive role of *Garcinia kola* seed in ameliorating renal damage (11) and also reducing induced kidney dysfunction in rats (12). The widespread benefits of *G. kola* for its medicinal properties and the dearth of information on its potential toxicity justify the need for this study in order to investigate its potential histopathological effects on the reproductive organs of the Wistar rats. Thus, this study was conducted to determine the histopathological effects of aqueous *G. kola* seed extract on the reproductive organs of Wistar rats.

Materials and methods

Plant Collection and Identification

The dried seeds of *Garcinia kola* were procured from the New Benin market, Benin City, Edo State and sent to the Department of Pharmacognosy, University of Benin, Benin City, Edo State for identification.

Plant Extract

Two hundred and fifty grams of the dried seeds of *Garcina kola* were sliced into bits, blended and soaked in 500 ml of distilled water in a corked flask for 48 hr at room temperature with intermittent shaking. The mixture was sieved into another clean conical flask and filtrate was centrifuged at 3500 rpm for 10 min and sieved again through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure in a Rotary Vacuum Evaporator until a semisolid substance was obtained (7).

Animal

A total of 40 male and female Wistar rats weighing between 220 - 250g were used in this study. The rats were randomly divided into groups and kept under standard conditions of temperature ($23^0C \pm 2^0C$) and humidity. The animals were allowed to acclimatize for 2 weeks in the Animal house of the Department of Anatomy, University of Benin, Benin City and maintained on unrestricted supply of food and water. In handling the rats, the National Institutional Guidelines for the Protection of Animals were followed in the experiment. The protocol for this study was approved by the Ministry of Agriculture and Natural Resources, Benin City, Edo State with reference number V.1041/27.

Experimental Design

Group A (Control) rats that were fed and administered distilled water for the period of 3 months. Group B were treated with 200 mg/kg body weight/day of aqueous GSKE for 3 months. Group C were administered 400 mg/kg body weight/day of aqueous GKSE for 3 months. Group D were administered 800 mg/kg body weight/day of aqueous GKSE for 3 months. A set of 4 rats from each group were sacrificed at the end of each month. The administration of aqueous GKSE was done orally using gavage daily for 3 months following which blood samples were collected for reproductive hormone assays. The animals were anaesthsized with chloroform fume and subsequently sacrificed. The testes and ovaries were harvested and fixed in 10% formal saline for histopathological analysis.

Collection of specimens

The blood specimen was collected by cardiac puncture under anesthesia into plain tubes for reproductive hormone assays, allowed to clot and retract at room temperature and serum was separated into micro tubes. The testes were harvested and immediately fixed in freshly prepared Bouin's fluid whereas the ovaries were preserved in 10% formal saline.

Hormone assay follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and ER

All reagents, test sera, serum references and controls were brought to room temperature preceding the assays. The following parameters were assayed namely follicle stimulating hormone, luteinizing hormone, estrogen receptor, progesterone receptor, prolactin and testosterone by using a previously described method of Vitt *et al.* (13). Briefly, the micro plate wells for each serum reference, control and the test specimen to be assayed in duplicate were formatted. Approximately 50µl of the appropriate serum reference, control and specimen were pipetted into the assigned wells while 100 µl of follicle stimulating hormone, luteinizing hormone, estrogen receptor, progesterone receptor, prolactin and testosterone-enzyme reagent solution was added to each well respectively. The micro plate was swirled gently for 30 sec to mix and incubated at room temperature for 60 min. The content of the micro plate was aspirated and discarded while the plate was blot dried with absorbent paper. About 350 µl of wash buffer was added, aspirated, discarded and the plate blot dried. The contents of the micro plate were discarded by aspiration and the plate blotted dry. This process was repeated thrice. One hundred microliter of working substrate solution was added to all wells, incubated at room temperature for 15 min. About 50 µl of stop solution was added to each well and mixed for 20 sec. The absorbance of each well was read at 450 nm (using a reference wavelength of 620-630nm to minimize well imperfection) in a micro plate reader and the result read in 30 min on the addition of the stop solution.

Histopathological analysis

Following the harvest and grossing of the testes and ovaries, the specimens were further fixed in 10% formal saline for 24 hr. Tissues were processed in an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). Tissues were dehydrated in varying grades of alcohol, cleared in toluene and impregnated in molten paraffin wax for specified periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Trimmed tissue blocks were sectioned at 3 µ and dried on a hotplate for 15 min. Dried sections were taken to water and stained in Cole's haematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene and mounted in Canada balsam (14). Sections were examined microscopically using x10 and x40 objective lenses.

Data Analysis

Results were expressed as the mean ± SEM of ten determinations. All analyses were done using SPSS 24.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). The differences was considered statistically significant at $p<0.0001$.

Results

There was a significant decrease in the serum levels of FSH, LH, PRL and ER of the female rats in groups treated with different concentrations (200, 400 and 800mg/kg body weight/day) of aqueous GKSE for 3 months when compared with the control ($P < 0.0001$). However, the effect of the aqueous GKSE did not significantly affect the serum levels of PR and TST ($P>0.0001$) (Table 1). The administration of 200, 400 and 800mg/kg body weight/day of aqueous GKSE for 3 months produced a reduction in the values of FSH, LH, PRL, PR, and ER in the male rats but not statistically significant ($P > 0.0001$). However, there was a significant increase in the serum level of TST in male rats treated with 800mg/kg body weight/day of aqueous extract of *Garcinia kola* ($P < 0.0001$) and significant decrease in the serum level of PRL ($P < 0.05$) (Table 2).

In female rats treated with 200mg/kg body weight/day of aqueous GKSE for 3 months, sections of the ovaries revealed normal cellular appearance with haemorrhagic areas. However, sections of the ovaries of rats administered 400mg/kg body weight of

aqueous GKSE for 3 months showed highly proliferating large atypical cells disposed in nests, cords and diffuse sheets. Many mitotic figures, tumour giant cells and bizarre large cells were also observed suggestive of decidualized cells with atypical hyperplasia. Similarly, sections of ovaries of Wistar rats treated with 800mg/kg body weight revealed areas of enhanced atypical cells that are punctuated by clusters and dispersed large cells with high nuclear grade. The stromal cells are characterized by high nuclear cytoplasmic ratio, inflammatory cells, prominent nucleoli, mitotic figures and multinucleation and suggestive of luteinized stromal cells with atypical (Figure 1).

Section of the testicular tissue from the control group without treatment with aqueous GKSE showed many normal appearing seminiferous tubules populated by cells of spermatogenic series with prominent spermatocytes and many terminal forms which is suggestive of hyper-spermatogenesis. Similarly, sections of the testes revealed normal architecture with well-preserved seminiferous tubules in the male rats treated with 200mg/kg body weight/day of aqueous GKSE for 3 months. However, section of the testicular tissue after treatment for 3 months with 400mg/kg body weight of GKSE revealed normal seminiferous tubules but with scanty terminal forms as well as few spermatocytes and is suggestive of hypo-spermatogenesis. Section of the

testicular tissue administered 800mg/kg body weight GKSE for 3 months revealed

absence of terminal forms spermatocytes which is suggestive of hypo-spermatogenesis (Figure 2).

Table 1: Impact of *Garcinia kola* seed extract on hormonal profile of female wistar rats in 3 months of administration.

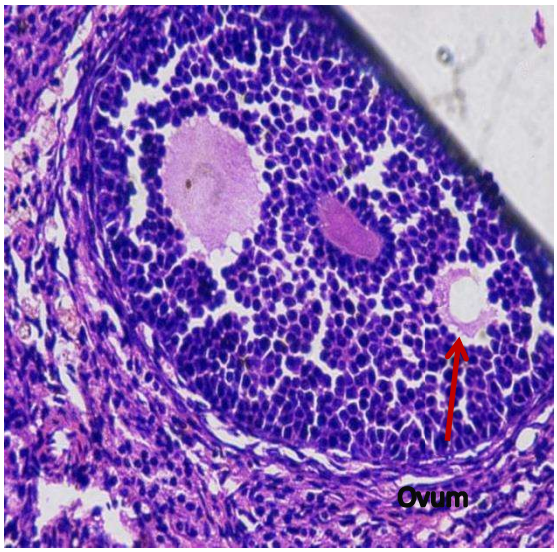
Groups	FSH (mIU/ml)	LH (mIU/ml)	PRL (ng/ml)	PR (nM/L)	TST (nM/L)	ER (pM/L)
A Control	**3.0**	**2.0**	**1.8**	**4.2**	**0.5**	**68.5**
B (200mg/kg)	0.6±0.1	0.8±0.2	1.5±0.0	4.3±0.2	0.5±0.1	21.5±0.4
C (400mg/kg)	0.6±0.1	0.7±0.0	1.3±0.1	4.5±0.4	0.6±0.0	17.6±0.7
D (800mg/kg)	0.5±0.2	0.5±0.2	1.2±0.1	4.6±0.1	0.7±0.0	12.5±0.8
P-values	**0.0001**	**0.0001**	**0.0001**	**0.15**	**0.10**	**0.08**

P-values were expressed as Mean ± SEM for four animals (n=4) in each group after treatment with the extract of Garcinia kola for three months.

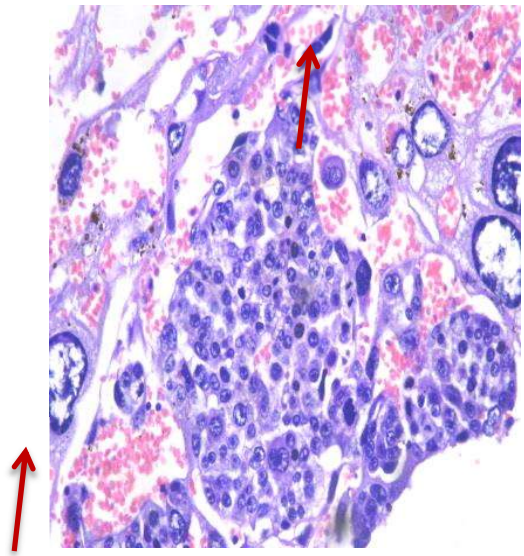
Table 2: Impact of *Garcinia kola* seed extracts on hormonal profile of male rats in 3 months of administration.

Groups	FSH (mIU/ml)	LH (mIU/ml)	PRL (ng/ml)	PR (nM/L)	TST (nM/L)	ER (pM/L)
A Control	**1.0**	**0.7**	**1.2**	**0.4**	**2.5**	**4.0**
B (200mg/kg)	0.7±0.1	0.6±0.1	0.8±0.1	0.6±0.1	3.0±0.2	3.7±0.1
C (400mg/kg)	0.7±0.1	0.6±0.1	0.7±0.1	0.4±0.1	3.7±0.3	3.5±0.2
D (800mg/kg)	0.6±0.1	0.5±0.1	0.6±0.3	0.4±0.1	4.2±0.2	3.4±0.2
P-values	**0.30**	**0.22**	**0.04**	**0.80**	**0.0001**	**0.29**

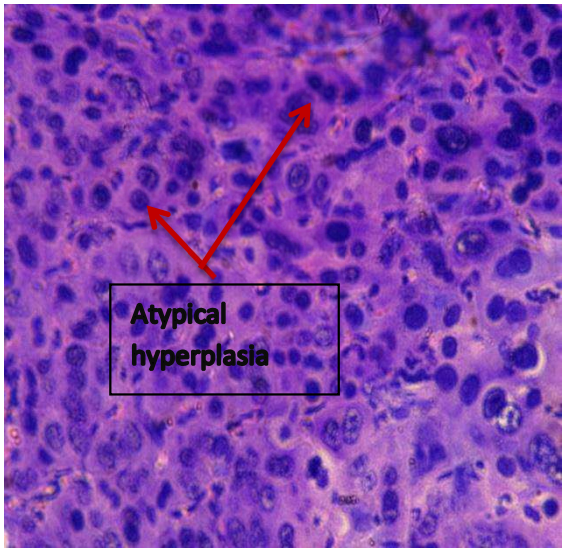
P-values were expressed as Mean ± SEM for four animals (n=4) in each group after treatment with the extract of Garcinia kola for 3 months.



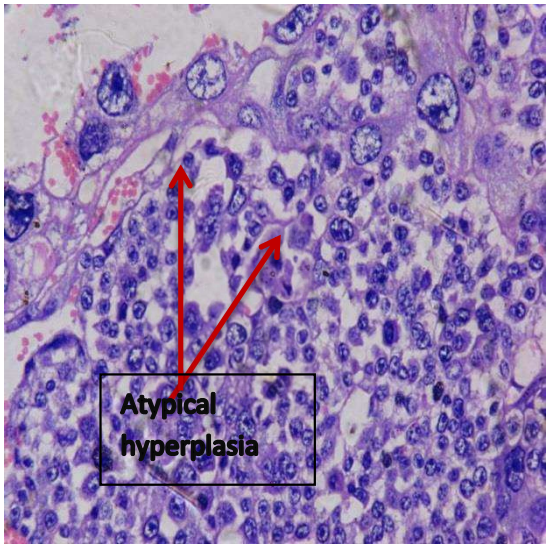
Group A: Section of ovary appeared normal (control) Without GKSE. H&E. Magx400.



Group B: Sections of ovary of Wistar rats administered 200mg/kg bw showed mild haemorrhage. H&E. Mgax400

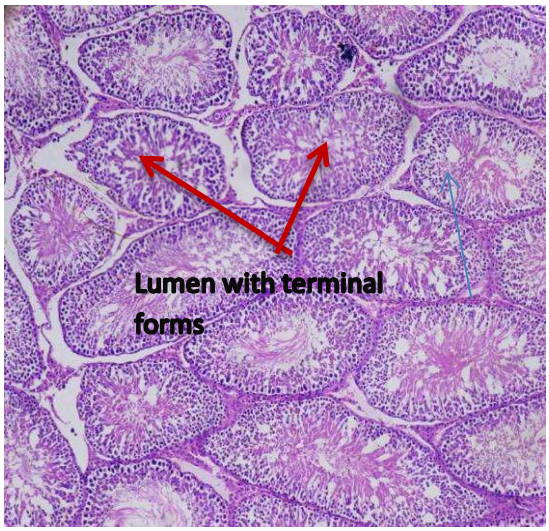


Group C: Section of ovary of rats administered 400mg/kg body weight of GKSE showed atypical cells. H&E. Magx400

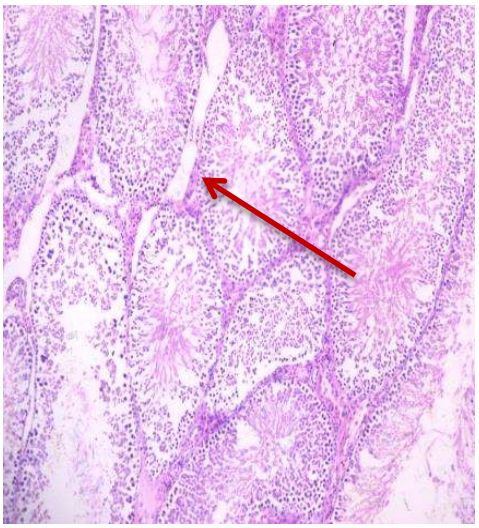


Group D: Section of ovary of rats administered 800mg/kg bw of GKSE showed atypical cells with haemorrhage. H&E. Magx400cells.

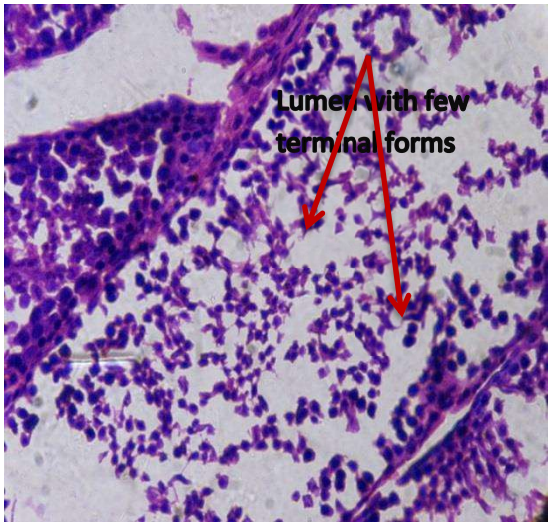
Figure 1: Sections of the ovaries following treatment of Groups B, C and D with 200mg/kg, 400mg/kg and 800mg/kg body weight/day of the aqueous GKSE respectively for 3 months.



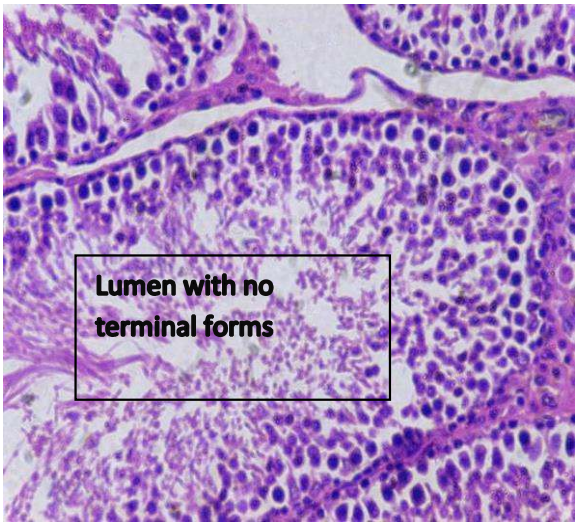
Group A: Section of testis showed normal features 200mg/kg (Control) H&E. Mag x400.



Group B: Section of testis treated with GKSE showed well-preserved seminiferous tubules H&E. Mag x400.



Group C:Section of testis treated with 400mg/kg b.w. GSKE revealed mild testicular atrophy H&E. Mag x400



Group D: Section of testis treated with 800mg/kg b.w. GSKE showed mild testicular atrophy. H&E. Mag x400

Figure 2: Sections of the testes following treatment of Groups B, C and D with 200mg/kg, 400mg/kg and 800mg/kg body weight/day of the aqueous GKSE respectively for three (3) months.

Discussion

Numerous scientific research studies have been conducted for several years on natural remedies for a wide variety of ailments. However, there have been a lot of improvements in the field of herbal medicine leading to wide acceptance of these herbal decoctions both in developing and developed countries due to their natural origin and less side effects (15). Several studies have shown the vital role of medicinal plants including *Garcinia kola* seed in improving the functions of the body organs and systems (16).

In this study, administered aqueous GKSE at 200, 400 and 800mg/kg body weight significantly reduced the serum levels of FSH, LH, PRL and ER in female rats that

were exposed for 3 months. Similar observation was made by Braide *et al.* (17) where significant reduction in the levels of FSH, LH and PRL in rats. However, we did not observe a significant increase in the values of ER and PR as observed by Braide *et al.* (17). Flavonoid which has been reported as one of the active ingredients in *Garcina kola* seed extract is believed to block cyclo-oxygenase enzymes otherwise known as COX-2 from initiating follicular rupture resulting into multiple female reproductive failures (18, 19, 20, 21). This may be the reason for this finding. The FSH in humans is known to be responsible for the stimulation of the ovarian follicle to release estrogen whereas the LH is thought to stimulate corpus luteum to secrete progesterone (17, 21). These processes may have influenced the values of ER and PR reported in our study and consequently led to our finding.

Endometrial hyperplasia is a disordered proliferation of endometrial glands which results from the unopposed estrogenic stimulation of the endometrial tissue with a relative deficiency of the counterbalancing effects of progesterone (22). It is believed that endometrial hyperplasia arises when estrogen, unopposed by progesterone which stimulates endometrial cell growth by binding to estrogen receptors in the nuclei of endometrial cells (23). Consequently, the exposure of these estrogen-induced proliferating cells to progesterone help in prompting the shedding of endometrial tissue both by reducing the number of estrogen receptors and by increasing the rate of conversion of estradiol to estrone through an increase in the activity of estradiol dehydrogenase (24; 25). In this study, the ovarian sections revealed atypical hyperplasia in female rats that were administered 400 and 800mg/kg body weight (respectively) in 3 months. The significant reduction in the level of PR occasioned by the direct impact of the GKSE on the FSH may have resulted in the development of atypical hyperplasia in the ovarian sections. This may explain the observation in this study.

Hypothalamus synthesizes and releases gonadotrophin releasing hormone (GnRH) into the hypothalamo-hypophyseal portal system. This hormone stimulates the release of pituitary gonadotrophins that move across the testis to regulate testosterone synthesis and spermatogenesis, respectively (26). The interstitial cells of Leydig produce testosterone when the testis is stimulated by LH from the pituitary gland of which the quantity of testosterone secreted varies in proportion to the quantity of LH available (27). In this study, there was a significant increase in the serum TST of male rats administered with the varied doses of GKSE which peaked in the 800mg/kg bodyweight ($P<0.0001$). This observation is in tandem with the report of Sewani *et al.* (28) that reported elevated serum testosterone in male rats with increased dose of GKSE. However, the report of Eyong and Braide (29) is inconsistent with our observation as a significant reduction in the level of TST was reported in male rats that were administered GKSE. In addition, the GKSE was reported to have led to a decrease in the levels of FSH and LH but not statistically significant in this study. This means that prolonged exposure of GKSE enhances a rise in the serum value of testosterone in male rats. The testicular section of the male rats revealed normal cellular architecture in male rats treated with 200mg/kg body weight/day of aqueous GKSE for 3 months. However, rats treated with 400 and 800mg/kg body weight/day of aqueous GKSE for 3 months showed hypo-spermatogenesis. This finding is inconsistent with the report of Sewani *et al.* (28) where sections of testicular tissue did not indicate obvious pathological changes on treatment of rats with GKSE. It is suggested that the consumption of *Garcina kola* at higher dose for a prolong period of time may act as a testicular toxicant.

Conclusion

The serum levels of FSH, LH, PRL and ER of the female rats significantly reduced on

treatment with different concentrations (200, 400 and 800mg/kg body weight/day) of aqueous GKSE for 3 months. The serum level of TST in male rats treated with 800mg/kg body weight/day of aqueous extract of *Garcinia kola* significantly increased in the 3 months group. Sections of the ovaries of female rats administered 400mg/kg body weight of aqueous GKSE for 3 months revealed highly proliferating large atypical cells disposed in nests, cords and diffuse sheets and many mitotic figures suggestive of atypical hyperplasia. Ovarian sections of Wistar rats treated with 800mg/kg body weight of aqueous GKSE revealed areas of enhanced atypical cells with high nuclear grade, stromal cells with high nuclear cytoplasmic ratio, mitotic figures and multinucleation that is suggestive of luteinized stromal cells with atypical. Sections of testicular tissue of male Wistar treated with 400 and 800mg/kg body weight/day of aqueous GKSE for 3 months showed hypo-spermatogenesis. Further studies to elucidate the pathological effects of prolonged consumption of *Garcinia kola* in the reproductive organs are advocated.

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