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EFFECTS OF CRUDE ALKALOID EXTRACT OF *GARCINIA KOLA* SEED ON SERUM GONADOTROPHINS AND TESTOSTERONE LEVELS IN MALE RATS

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ABSTRACT

The effects of daily oral administration of alkaloid extract of *Garcinia kola* seed (GKA) at 1500mg/kg on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (TST) were studied in male rats (100-150g) for six to seven weeks. The pair-fed control rats received oral doses of methanolic saline daily for six to seven weeks. The animals were injected i.m. with Menotrophin (1.07IU/kg) or Testosterone (2.6mg/kg) during the seventh week of receiving GKA or following six weeks of treatment with GKA. The control rats were injected with normal saline or olive oil vehicles during the seventh week. At the end of the seventh week, all animals were exsanguinated to collect serum for hormone assays. Results showed that levels of serum gonadotrophins (LH and FSH) were significantly elevated, while serum TST levels were markedly reduced, compared to the pair-fed controls.

RUNNING TITLE: *G. kola* alkaloid effects on endocrine organs.

KEYWORDS: *Garcinia kola; alkaloids, gonadotrophins testosterone*

INTRODUCTION

Garcinia kola (family: Guttiferae) is a plant that grows wild in the southern areas of Nigeria. The seeds, called *efiat* (Efik) or *bitter kola* (common English) are served to guests, as an alternative to the regular kolanuts (*Cola nitida:* family Sterculaceae); and are also used by some Nigeria herbal doctors to treat various ailments, including diarrhea and menstrual cramps (Dalziel, 1937). Preliminary investigation of the action of alkaloid fractions of the *Garcinia kola* seeds indicated marked dose dependent reversible spasmolytic and antispasmogenic effects on smooth muscle activity (Braide, 1989). Orie and Ekon (1993) showed that ingestion of the *G. kola* seeds caused bronchodilation, justifying its use for antiasthma therapy in traditional medicine. The chronic ingestion of *G. kola* seeds could result in male reproductive organs (Udoh, 1998; Agube, 2001; Essien, 2002; Braide et al; 2003). In the studies cited above, it was observed that *G. kola* seeds caused damage to the female reproductive organs as indicated by histological findings which revealed extensive damage to the germinal epithelium of the ovary. *G. kola* seed alkaloids were found to cause significant reduction in serum levels of gonadotrophins (FSH and LH) and prolactin but marked elevation of oestradiol and progesterone levels in serum (Essien, 2002; Braide *et al.,* 2003). An earlier study (Agube, 2001) had shown that *G. kola* may influence reproductive activities through its action on gonadotrophins and testosterone by reducing the serum level of testosterone and markedly elevating the levels of FSH and LH in male rats. The present study was designed to further elucidate the antifertility effect of crude alkaloid extract of *G. kola* seeds in an experimental model.

MATERIALS AND METHODS

Preparation of Plant Extracts

This was achieved by the methods used, as described by Braide *et al*. (2003), starting with

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finely ground powder of *Garcinia kola* seeds.

Animals

Male albino Wistar rats (100-150g) purchased from the animal house in the Department of Pharmacology, University of Calabar, Nigeria, were housed in groups of six, one rat per cage, and maintained for two weeks prior to the experiments, to allow for uniform husbandry conditions of light (14 hours light and 10 hours darkness) and ambient temperature of $28^oC\pm 1^oC$ and they were fed with rat mash (Pfizer Feeds) and tap water *ad libitum.*

Preparations of Extract for Administration

The dried alkaloid extract was dispersed in a solution of 0.9% NaCl to obtain a concentration of 1.0g/ml.

Drug Treatment of Animals

Eight paired groups (A-H) were tested as follows:

Group A received daily oral doses (1500mg/kg) of the alkaloid extract of *Garcinia kola* seed (GKA) for six weeks only but fed on the basal diet for seven weeks. The controls (*Group* A_o) while also being fed on basal diet for seven weeks, received orally the same volume of vehicle (methanolic saline) for six weeks.

Group B received GKA orally (1500mg/kg) for six weeks only. Then, during the seventh week, received daily i.m. injections of Menotrophin at 1.07IU/kg. The controls (Group B_o) also received GKA orally (1500mg/kg) for six weeks only; then, during the seventh week, received daily i.m. injections of the same volume (0.9% NaCl solution). All rats in groups B and B_o were fed on basal diet for seven weeks.

Group C received daily GKA orally (1500mg/kg) for six weeks only, followed by two i.m. injections of testosterone (2.6mg/kg) during the seventh week. The controls (Group C_o) also received daily GKA orally (1500mg/kg) for six weeks only; then during the seventh week, received two i.m. injections of the same volume of vehicle (olive oil). All rats in groups C and C_o were fed on basal diet for seven weeks.

Group D received daily GKA orally (1500mg/kg) for seven weeks, while the control (Group D_o) received daily the same volume of vehicle (methanolic saline) for seven weeks.

Group E received daily GKA orally (1500mg/kg) for seven weeks but received, during the seventh week, daily i.m. injections of Menotrophin (1.01 IU/kg). The control (Group E_o) also received daily, GKA orally (1500mg/kg) for seven weeks and, during the seventh week, daily i.m. injections of the same volume of vehicle (0.9% NaCl solution).

Group F received daily oral doses of GKA (1500mg/kg) for seven weeks and, during the seventh week, two i.m. injections of testosterone (2.6mg/kg). The control (Group F_o) had similar treatment but received only the same volume of vehicle (olive oil) instead of testosterone.

Group G received methanolic saline orally, daily, for seven weeks and also received Menotrophin (1.07IU/kg) i.m. daily during the seventh week. The controls (Group G_o) while receiving 2ml methanolic saline orally, daily, for seven weeks, also received during the seventh week daily i.m. doses of same volume of vehicle (0.9% NaCl solution).

Collection of blood serum

At the end of the seven weeks experiment period, the rats were anaesthetized in the chloroform chamber, to facilitate blood sampling via cardiac puncture. Blood samples, collected in nonheparin tubes, were allowed to stand for 3h, then centrifuged at 7,000g for 10 minutes. The serum so obtained was stored at 4^oC for two days before hormonal assay.

Hormonal assay

Serum samples were analyzed by the microwell enzyme linked immunoassay method.

Statistical analysis

The data were computed and analyzed using group comparison and Student's t-test; and differences were considered significant at $P<0.05$.

RESULTS

Effects of daily oral dose (1,500mg/kg) of alkaloid extract of *Garcinia kola* seed (GKA) for seven weeks on serum LH, FSH and TST in male albino rats. Male rats receiving daily oral dose (1,500mg/kg) of GKA for seven weeks showed marked increase in serum LH and FSH concentrations but decreased serum TST level compared with untreated controls (Table 1).

Effects of Menotrophin treatment (1.07IU/kg) on serum levels of LH, FSH and TST in male rats receiving GKA (1,500mg/kg/day) for seven weeks.

Male rats receiving oral doses of methanolic saline showed markedly higher serum levels of LH, FSH and TST following treatment with Menotrophin, compared to the controls that did not receive Menotrophin (Table 2). The serum levels of LH and TST were markedly higher and that of FSH were markedly lower in animals treated with both Menotrophin and GKA compared with animals that did not receive Menotrophin (Table 3).

Effects of Testosterone on serum levels of LH, FSH, and TST in male rats receiving oral daily doses of GKA (1,500mg/kg) for seven weeks.

In animals treated with only methanolic saline (the control counterparts of GKA - treated rats) Testosterone treatment resulted in markedly higher levels of LH, FSH and TST in serum, compared with animals that did not receive Testosterone (Table 4). In animals treated with GKA the injections with Testosterone caused slight increase in serum LH but marked decrease in serum levels of FSH and TST, compared to the GKA - treated rats that did not receive the Testosterone injections (Table 5).

Effects on One Week's suspension of GKA treatment after six weeks of treatment with GKA (1,500mg/kg/day) on serum LH, FSH and TST levels.

At the end of the seventh week (one week suspension of GKA treatment) after six weeks of treatment with GKA (1,500mg/kg/day orally) the serum levels of LH and FSH were significantly higher while TST was lower, in comparison to data from control rats that received methanolic saline, instead of GKA, for six weeks (Table 6).

Effects of Menotrophin (1.07IU/kg) post-treatment for seven days on serum levels of LH, FSH and TST in male rats following six weeks treatment with GKA (1,500mg/kg/day orally).

The results indicate that post-treatment with Menotrophin, in control animals that received six weeks treatment with methanolic saline (instead of GKA) caused significantly higher levels of LH, FSH and TST in serum (Table 7). However, in animals that received GKA treatment for six weeks, followed by one week post-treatment with Menotrophin, the levels of LH and FSH were significantly lower, and TST level was significantly higher, compared with their pair-fed controls which did not receive Menotrophin (Table 8).

Effects of Testosterone treatment (2.6mg/kg, 2 injections in seven days) following six weeks treatment with GKA (1,500mg/kg/day orally) on serum levels of LH, FSH and TST in male rats.

The results indicate that post-treatment with Testosterone, in control animals that received six weeks treatment with methanolic saline (instead of GKA) caused marked increase in serum levels of LH, FSH and TST, compared with animals that did not receive testosterone (Table 9). On the other hand, in animals that received GKA treatment for six weeks, followed by two injections of Testosterone during the seventh week, the serum levels of LH, FSH and TST were significantly higher than in rats that also received GKA treatment but without Testosterone post-treatment (Table 10).

TABLE 1
Effects of alkaloid extract of *Garcinia kola* seed (GKA) 1,500mg/kg orally for seven weeks on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.40±0.020(6)	0.19±0.030(6)	0.31± 0.025(6)
GKA	0.75*±0.007(6)	1.85*±0.230(6)	0.22*±0.030(6)

* Significantly different from control values ($p<0.05$, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 2
Effects of Menotrophin (1.07IU/kg) on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male albino rats treated with methanolic saline for seven weeks.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.40±0.030(6)	0.20±0.020(6)	0.32±0.010(6)
Menotrophin Only,	1.85*±0.030(6)	1.60*±0.035(6)	0.73*±0.070(6)

* Significant difference from control was calculated using Student's t-test, $P<0.05$. Number in parentheses represents number of animals per treatment group.

TABLE 3
Effects of Menotrophin (1.07IU/kg) on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male albino rats exposed to alkaloid extract of *Garcinia kola* seed (GKA) 1,500mg/kg/day for seven weeks.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
GKA	0.70 ± 0.010(6)	1.38±0.320(6)	0.26±0.030(6)
GKA + Menotrophin	1.50*±0.003(6)	0.70*±0.006(6)	0.46*±0.002(6)

* Significantly different from control value ($P<0.05$ Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 4
Effects of testosterone on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats orally treated with methanolic saline for seven weeks.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.46±0.030(6)	0.21±0.005(6)	0.33±0.020(6)
Testosterone only	7.50*±0.600(6)	5.40*±0.520(6)	0.39*±0.020(6)

* Significant difference from controls was calculated using Student's t-test, P<0.05. Number in parentheses represents number of animals per treatment group.

TABLE 5
Effects of testosterone (2.6mg/kg) on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats treated with alkaloid extract of *Garcinia kola*_ seed (GKA) 1,500mg/kg for seven weeks.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
GKA	1.25 ± 0.12(6)	0.78 ± 0.01(6)	0.20±0.003(6)
GKA + Menotrophin	1.40 ± 0.05(6)	0.60 ± 0.03(6)	0.10±0.001(6)

* Significantly different from control values (p<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 6
Effects of one week 0% GKA treatment on serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), after six weeks treatment with alkaloid extract of *Garcinia kola* seed (GKA) at 1,500mg/kg/day.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.40±0.010(6)	0.19±0.003(6)	0.31±0.025(6)
GKA	0.70*±0.045(6)	0.77*±0.050(6)	0.20*±0.030(6)

* Significantly different from control values (p<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 7
Effects of Menotrophin (1.07IU/kg) post treatment for seven days on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats following six weeks exposure to methanolic saline

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.40±0.001(6)	0.20±0.014(6)	0.32±0.005(6)
Menotrophin only	1.85*±0.250(6)	1.60*±0.380(6)	0.73*±0.020(6)

* Significant difference from controls (P<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 8
Effects of Menotrophin (1.07IU/kg) post treatment for seven days on serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats following six weeks exposure to alkaloid extract of *Garcinia kola*_ seed (GKA) at 1,500mg/kg/day

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
GKA	0.70±0.030(6)	0.77±0.040(6)	0.22±0.010(6)
GKA + Menotrophin	0.30*±0.001(6)	0.26*±0.010(6)	4.90*±0.360(6)

* Significantly different from control values (p<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 9
Effects of testosterone (2.6mg/kg) post treatment for seven days on serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats following six weeks treatment with methanolic saline (0%GKA).

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
Controls	0.50±0.050(6)	0.30±0.030(6)	0.35±0.001(6)
Testosterone only	9.30*±0.700(6)	8.10*±0.650(6)	0.42*±0.030(6)

* Significantly different from control values (P<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

TABLE 10
Effects of testosterone (2.6mg/kg) post treatment for seven days on serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and Testosterone (TST), in male rats following six weeks treatment with Alkaloid extract of *Garcinia kola seed* (GKA) at 1,500mg/kg/day.

	LH mIU/ml	**FSH mIU/ml**	**TST ng/ml**
GKA	1.28 ± 0.200(6)	0.90±0.030(6)	0.18±0.020(6)
GKA + Testosterone	13.90*±0.350(6)	4.20*±0.520(6)	0.73*±0.010(6)

* Significantly different from control values (P<0.05, Student's t-test). Number in parentheses represents number of animals per treatment group.

DISCUSSION
In this study, it was observed that in male rats, following seven weeks treatment with alkaloid extract of *Garcinia kola* seed (GKA) administered orally, daily, there was a significant elevation of serum concentrations of gonadotrophins (LH and FSH) but a reduction in serum levels of testosterone (TST) compared to their pair-fed controls. This is in consonance with the observations earlier published (Braide et al., 2003). Also, treatment with GKA for six weeks, gave almost the same pattern with seven weeks treatment; except in the serum levels of FSH that showed a profound decrease after six weeks vis-à-vis seven weeks of treatment. Possible explanations for these observations include the feed-back effect of testosterone on the hypothalamus. The hypothalamus synthesizes and releases gonadotrophin releasing hormone (GnRH) into the hypothalamo-hypophyseal portal system. GnRH stimulates release of pituitary gonadotrophins which traverse to the testis to regulate testosterone synthesis and spermatogenesis, respectively (Craige and Stitzel, 1994). The administration of GKA led to insensitivity of the testis to gonadotrophin stimulation; hence there was a direct action of GKA on the testis, thereby causing inhibition of gonadotrophin action on the testis.
It is not probable that GKA could have acted on the pituitary, to prevent the release of gonadotrophins. This is because GKA was observed to actually enhance serum levels of LH and FSH in male rats. Treatment of male rats, that had been exposed to GKA for seven weeks, with exogenous Menotrophin during the seven weeks, caused significant increase in serum levels of LH and testosterone, but a decrease in FSH levels. Likewise, the treatment of male rats with exogenous Menotrophin after six weeks exposure of GKA produced profound elevation in serum levels of testosterone compared to seven weeks; and significant decrease in serum concentration of LH and FSH. The above observations could imply that exogenous Menotrophin caused increase in testosterone production through testicular response to Menotrophin; but the effect was more remarkable when Menotrophin was given alone after six weeks of exposure to GKA. This could be as a result of non-interference by GKA with Menotrophin action on the testis compared to when given together during the seventh week.
The decrease in serum LH after one week of treatment with Menotrophin, without GKA, could be as a result of negative feed-back mechanism from the high level of testosterone. Testosterone is produced by the interstitial cells of Leydig when the testis is stimulated by LH from the pituitary gland and the quantity of testosterone secreted varies in proportion to the amount of LH available (Guyton 1979, 1982). Following GKA treatment not much effect of exogenous testosterone was observed, as there was no significant change in LH levels in serum.
It is concluded that prolonged high level of *Garcinia kola* seed alkaloid extracts (GKA) induced profound increase in serum concentration of gonadotrophins with decreased level of testosterone.

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