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EFFECT OF GARCINIA KOLA SEED ALKALOID EXTRACT ON LEVELS OF GONADAL HORMONES AND PITUTARY GONADOTROPHINS IN RAT SERUM

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Summary: The effects of three tolerated oral doses (350 mg/kg, 1500 mg/kg, 2000 mg/kg) of methanolic alkaloid extract of Garcinia kola seed (GKA) on serum levels of estradiol, progesterone, prolactin, FSH and LH were observed in female rats (125-170 g). The control animals received 2ml oral doses of methanolic saline (0.9% NaCl) daily and the treatment period of dosing for all animals lasted 3, 7 or 30 days, at the end of which they were exsanguinated to collect serum for hormonal assays. In another study, the effects of daily oral doses of GKA (350 mg/kg or 2000 mg/kg for days; 300 mg/kg, 1300 mg/kg or 2000 mg/kg for days; 1500 mg/kg for 14 days) on serum levels of testosterone, LH and FSH were observed in male rats (150 – 175). The experiments showed that serum LH, FSH and prolactin levels were lower, while estradiol and progesterone levels were higher, than control values in females. There was marked reduction in serum testosterone and a concomitant elevation of serum FSH and LH in males. The findings suggest a possible antifertility consequence of treatment with GKA.

***Key Words**: Garcinia kola seeds; alkaloids; gonadotrophins; sex hormones.*

Introduction

Garcinia Kola Heckel (Guttiferae) is a large fruit tree that abounds in the rain forest belt of Southern Nigeria. The seed ("bitter kola") is used by traditional Nigerian herbal doctors to treat ailments such as diarrhea, hepatitis, asthma, dysmenorrhea or menstrual cramps (Dalziel, 1937). Preliminary investigations of the action of alkaloid and bioflavonoid fractions of the G. kola seed indicated marked, dose – dependent, reversible spasmolytic and antispasmogenic effects on uterine and gastrointestinal smooth muscle (Braide, 1989).

Chronic ingestion of G. kola seed was observed to induce histopathological changes in liver parenchymal cells, renal tubular epithelium and duodenal villous epithelium (Braide, 1990; Braide and Grill, 1990). These changes were surmised as being attributable to the biflavonoids contained in G. kola seed. Other studies using methanolic extracts, or isolated alkaloid and biflavonoid fractions of the seed, showed that these phytochemical principles stimulated an increase in gastric acid secretion (Oluwole and Obatomi, 1991); exhibited antihepatotoxic biochemical effects (Iwu, 1985; Akitonwa and Essien, 1990;Braide, 1991 a, b; Adegoke et al., 1998; Adaramoye and Akinloye, 2000; Farombi, 2000; Farombi et al., 2000), hypoglycaemic anti-diabetic activity (Iwu et al., 1990) and antipyretic, antiinflammatory effects (Braide, 1993). It has also been observed that ingestion of G. kola seed caused mild bronchodilatation in man (Orie and Ekon, 1993) thus justifying its use in therapy of asthmatic patients by traditional herbal medicine practitioners in Nigeria.

Plant extracts have been found to induce testicular atrophy with consequent deterioration of reproductive or sexual function, as has been documented in the case of gossypol (Udo and Patil, 1992). Also, an earlier study observed that animals fed with subterranean clover, rich in isoflavones, suffered serious breeding impairment (Cheng et al., 1995).

Since G. kola seed is an important ingredient in material medica of traditional herbal medical practice, it was considered relevant to investigate its effect on male and female reproductive systems of experimental animals, in order to have some inkling as to possible effects in humans. The objective was to determine if the alkaloid extract of G. kola seed (GKA) directly affected the gonads and other parts of the reproductive system in male and female rats, or if the effects were secondary to alterations in central gonadotrophin regulation.

Materials and Methods

Preparation of Plant Extracts.

Fresh seeds of Garcinia kola, purchased in season from the local markets in Calabar, were peeled to remove the testa, washed and air-dried for 8h, then subsequently dried in an electric oven (Astell Hearson, England) thermostatically

controlled at 40°C, for 12h. The dry seeds were ground to a fine powder with the aid of a mortar and pestle. Herbarium specimens were deposited in the ethnopharmacology unit of the Department of Pharmacology of the University of Calabar.

Batches of the G. kola seed powder (100g wt.) were separately wraped in a thimble and placed a Soxhlet extractor (M & G Scientific Co., England) fitted to a 1,000 ml round-bottom flask containing 500ml of either petroleum ether (40 – 60°C) or methanol as extracting solvents.

The seed powder was extracted first with petroleum ether for 12h to remove fat and other organic constituents soluble only in ether. The ether extract in the flask was decanted and then replaced with 500 ml methanol. The ether-extracted powder residue was then resubjected to Soxhlet extraction in methanol for 72h. The methanol extract was evaporated to dryness at 45°C in vacuo, using a rotary evaporator and the powder so obtained was subsequently processed by Soxhlet extraction in petroleum ether at 50°C for 8h, to further remove and discard unwanted substances soluble in petroleum ether. The power residue containing substances not soluble, and therefore not extractable, in petroleum ether contained bioflavonoid and alkaloids of the G. kola seed (Brain and Turner, 1975) and was then partitioned in equal volumes of chloroform and water for 24h in a separating funnel. The water soluble phase, which contains alkaloid constituents, was evaporated in vacuo, to powder form, in a rotary evaporator. The alkaloid powder was stored in a refrigerator at 4°C until used for experiments reported in this study.

Animals.

The animals used in the study were young adult, virgin male (150 – 175g) and female (125 – 170g) albino Wistar rats of a strain obtained from the National Veterinary Research Institute at Vom, near Jos, in Plateau State, Nigeria. The animals were allowed one week of acclimatization to conditions of the animal housing facility (26 – 28°C; 60 – 80% relative humidity; 14 light: 10h dark cycle). The animals were housed individually in wire mesh cages and received food (Agrofeed Mills, Ikot Omin, Calabar, Nigeria with composition: protein, 18%: fats, 3.5%; fibre, 3.8%; calcium, 1%; phosphorus, O.68%; metabolizable energy, 2905 kcal/kg) and tap water ad libitum.

Preparation of Extract for Administration.

The powder extract of G. kola seed (GKA) which contained only alkaloid constituents (water – soluble but chloroform – insoluble), was made into a stock solution of 1 g/ml concentration in 0.9% NaCl, prior to oral administration to the experimental animals.

Collection and Handling of Blood Serum:

The animals were anesthetised in a chloroform chamber at the end the treatment period, and blood was obtained through cardiac puncture. Blood samples from each animal were put in well-labelled nonheparinized sample tubes which were then allowed to stand for 3h in iced water and later centrifuged at 7,000g for 10 minutes. The serum was then collected and stored at -15°C for two days before hormonal assay.

Hormonal Assay

Serum samples were assayed for the following hormones: follicle stimulating hormone (FSH); luteinizing hormone (LH): estrogen (estradiol); progesterone; prolactin; and testosterone. The method used involved the microwell enzyme-linked immunoassay (ELISA) using analytical grade reagents (Syntron Bioresearch Inc., USA).

Statistical Analysis

All data for control and experimental animals were subjected to statistical evaluation, using the student's t-test for significant differences, between control and experimental groups, at values of $p < 0.05$.

Results

Effect of G. kola seed diet on serum gonadotrophins and testosterone in male rats.

Male rats fed for 6 weeks on diets containing various levels of G. kola seed powder (GKP) were studied at the end of the feeding period. The diets contained GKP at levels of 10% w/w (18g/kg/day), 30% w/w (54 g/kg/day) and 60% w/w(108 g/kg/day). The GKP diet caused an increase in serum concentrations of pituitary gonadotrophins (FSH and LH) and a concomitant decrease in serum levels of testosterone in a dose – related manner (Table 1). The most significant changes were observed in rats fed on the 30% w/w and 60% w/w GKP diets.

Effects of oral doses of G. kola alkaloid extract (GKA) on serum gonadotrophins and testosterone in male rats.

Male rats receiving daily oral doses (1500 mg/kg/day) of GKA for 14 days showed statistically significant increases in FSH and LH concentrations in serum and decreased serum testosterone levels (Table 2).

Effect of oral doses of G. kola alkaloid extract (GKA) on serum levels of gonadotrophins, prolactin and ovarian hormones in female rats.

Female rats receiving daily various oral doses of GKA (350, 1500, 2000 mg/kg/day) for various periods of treatment (3, 7, 30 days) exhibited increased levels of serum FSH, LH and prolactin and concomitant decreased levels of serum estradiol and progesterone. These changes were statistically significant and dose – related. The data in respect of treatment lasting for 30 days are shown in Table. 3.

Table 1. Effects of G. kola seed powder (GKP) diet on follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels in serum of male rats after 6 weeks feeding.

Diet	Serum concentration (ng/ml)		
	FSH	LH	Testosterone
Control, O% GKP (5)	0.7 ± 0.001	4.2 ± 0.01	400 ± 2
10% GKP, w/w or 18 g/kg/day(5)	1.5 ± 0.01 (*)	4.5 ± 0.05	0.11 ± 0.01(*)
30% GKP, w/w or 54 g/kg/day(5)	4.0 ± 0.1 (*)	5.1 ± 0.1(*)	0.05 ± 0.001(*)
60% GKP, w/w 0r 108 g/kg/day(5)	5.5± 0.05(*)	5.1 ± 0.1(*)	0.04 ± 0.005(*)

The values are means ± SEM; and the number in parenthesis represents the number of animals in each treatment group. (*) significantly different from control values ($P < 0.05$, Student's t- test).

Table: 2 Effect of oral GKA (1500 mg/kg/day) on serum concentrations of follicle stimulating hormone (FSH), liteinizing hormone (LH) and testosterone in male rats, after treatment for 14 days.

Treatment	Hormones	Conc. in Serum (ng/ml)
Control(5)	FSH	0.80 ± 0.01
GKA (5)	FSH	2.33±0.012 (*)
Control (5)	LH	4.26 ± 0.04
GKA (5)	LH	4.92 ± 0.10(*)
Control (5)	Testosterone	13.06 ± 0.30
GKA (5)	Testosterone	2. 13 ± 0 .06(*)

Values are expressed as means ± SEM. Number in parentheses represents number of animals per treatment group. (*) Significantly different from controls ($p < 0.05$, student's t –test).

Table: 3 Effect of oral GKA (1500 mg/kg/day) on serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol (EST) and progesterone (PRG) in female rats, after treatment for 30 days.

Treatment	Hormones	Conc. in Serum
Control (5)	FSH	14. 72 ± 0.52 mlu/ml
GKA (5)	FSH	5.60 ± 0. 12 mlu/ml (*)
Control (5)	LH	2. 16 ± 0. 35 mlu/ml
GKA (5)	LH	1.50 ± 0. 12 mlu/ml(*)
Control (5)	PRL	12.82 ± 0.30 ng/ml
GKA (5)	PRL	10. 10 ± 0.38 ng/ml (*)
Control (5)	PRG	10. 8 ± 0.50 ng/ml
GKA (5)	PRG	14. 0 ± 0. 65 ng/ml(*)
Control (5)	EST	19.0 ± 0.1 pg/ml
GKA (5)	EST	38.0 ± 0.2 pg/ml (*)

Values are expressed as means ± SEM. Number in parentheses represents number of animals per treatment group.
(*) Significantly different from controls ($p < 0.05$, Student's t –test).

Discussion

The study herein presented was instigated by an earlier finding that *Garcinia kola* seed diets, fed for durations lasting 6 weeks or longer, caused testicular atrophy, reduced relative testis weight, induced spermatogenesis arrest, and resulted in degeneration of spermatozoa. These effects were replicated by oral doses of crude alkaloid extracts of the *G. kola* seed (Udoh, 1998). The present study is an attempt to explain the observations by Udoh (1998) on the basis of changes in the profiles of serum gonadal and gonadotrophin hormones.

In this study it was observed that GKA caused a decrease in serum concentration of gonadotrophins (FSH and LH) and prolactin, while coincidentally causing marked increase in serum level of oestradiol and progesterone in female rats. On the other hand, in male rats, GKA decreased the serum concentration of testosterone while increasing significantly the levels of FSH and LH.

The above observation may be interpreted variously, depending on the sex of animals involved. In the male rat, following long-term ingestion of *G. kola* seed, or oral administration of the alkaloid extract (GKA) there is marked spermatogenesis arrest (Udoh, 1998). Possible explanations for such observation include a direct action of GKA on the testis, thereby causing inhibition of gonadotrophic action in that organ. Other possibilities include preventing the release of pituitary gonadotrophins and/or elevation of blood levels of testosterone (via inhibition of hepatic metabolism) thereby inducing negative feedback effect on gonadotrophin release. It is not plausible that prevention of gonadotrophin release is the likely mechanism in operation; because GKA actually was observed to enhance serum levels of FSH and LH in male rats. Furthermore, elevation of blood testosterone levels is not a good explanation because GKA actually decreased serum levels of testosterone in male rats. The most plausible explanation of the observations on male rats in this study is the possibility of GKA inhibition of gonadotrophic action on the testis. This is in consonance with an earlier study indicating that phenolic compounds are antispermatogenic (Udoh and Patil, 1992). Any direct damage to the test is likely to impair gonadal response to FSH and LH; such as diminished testosterone production due to lack of gonadal response to LH. Observations in this study indicated that serum levels of testosterone were remarkably low, despite the marked elevation of LH levels in blood.

The study of GKA treatment in female rats gave somewhat different results from those in male rats: decrease, instead of increase, in gonadotrophin levels and concomitant escalation, instead of diminution, of gonadal (ovarian) hormone (estradiol and progesterone)levels in serum. The serum level of prolactin was also decreased. The most plausible explanation for these observations is that GKA may have a direct adverse effect on the female gonads, thus blocking their response to gonadotrophin (FSH and LH). Follicle-stimulating hormone (FSH) in female

mammals stimulates maturation of the ovarian follicle and the release of oestrogen. Luteinizing hormone (LH) stimulates the release of progesterone from the ovarian corpus luteum. The diminished levels of gonadotrophins in serum should have resulted in decreased blood concentrations of the gonadal (ovarian) hormones. This was not the case in this study. Rather, GKA caused elevation of serum levels of estradiol and progesterone in female rats. This could be due to a possibility of GKA causing impairment of hepatic catabolism of these ovarian hormones, and thus enhancing their accumulation and concentrations in serum. Earlier studies had indicated that *Garcinia kola* seed constituents could inhibit hepatic metabolism of drugs (Braide, 1991 a, b; Farombi *et al.*, 2000). The high levels of ovarian hormones presumably would cause, via negative feed-back mechanism on the hypothalamo-pituitary axis, decreased serum levels of FSH, LH and prolactin. This would be in line with observations that crude alkaloid extracts of the alligator ***pepper (Macroterms bellicosus)*** decreased prolactin levels in blood (Ebong *et al.*, 1998). Furthermore an earlier study had demonstrated that the alkaloid drug bromocriptine reduced prolactin levels in blood via a central mechanism (Sandorama, 1986). It is unlikely that the GKA effects observed in this study have any direct central component; since *Garcinia kola* alkaloid has no effect on the CNS (Dalciel, 1956).

It is concluded that prolonged high-level exposure to *Garcinia kola* seed alkaloid induces profound changes in serum concentrations of gonadotrophins and gonadal hormones in rats; and that the direction of alteration (increase or decrease) depends significantly on the sex of animals under observation. These effects on the levels of gonodal hormones are most likely due to a primary peripheral action of GKA on the male and female reproductive systems, rather than being secondary to alterations by GKA in central gonadotrophin regulation.

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