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Effect of *Garcinia kola* Seed Extract on Female Reproductive Functions in Rats

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Abstract: The effects of *G. kola* seed extract on oestrous cycle, ovulation, implantation and pregnancy were studied in adult female rats with the aim of its possible use as female contraceptive. The three experimental groups were fed with *G. kola* seed extract (200 mg kg⁻¹ body weight) (GKSE) and the control received distilled water once daily. Group 1 was fed with GKSE for three weeks and the estrous cycle monitored and number of ova released recorded. Group 2 consisted of pregnant rats and received GKSE from day one of pregnancy. Equal numbers of the rats were sacrificed on days six, eight and 19th of pregnancy. The total number of implants, resorption and viable foetuses were weighed, examined and recorded. Group 3 animals received GKSE daily for three weeks; half of them were sacrificed after three weeks and the others were allowed three weeks to recover from the effect of the extract. Histology of the ovary, uterus and fallopian tubes were done. The results showed that the oestrous cycle was altered with a significant reduction in the occurrence of estrous and metestrous phase. Ovulation was partially blocked (70% blockage). No significant difference between the number and weight of the implantation sites in both pregnant control rats and the GKSE rats. No significant difference in both the foetal number and weight on day 19 of pregnancy. GKSE made the uterine endometrial gland inactive, stopped maturation of the follicles, caused acute inflammation of the tubes but all the organs showed full recovery after administration of GKSE was stopped. GKSE may be use as a contraceptive with a possible advantage of reversibility.

Key words: *Garcinia kola*, *Heckel guttiferae*, estrous cycle, ovulation, implantation and pregnancy

INTRODUCTION

Traditionally, in Africa the seed is used for the treatment of bronchitis, throat infections, antipurgative and antiparasitic (Madubunyi, 1995; Okunji and Iwu, 1991; Adefile-Ositelu *et al.*, 2004). It is known to possess anti-atherogenic effects (Adaramoye *et al.*, 2005b) antilipoperoxative effect (Emerole *et al.*, 2005), aphrodisiac effect (Uko *et al.*, 2001) and gastroprotective effects (Olaleye and Farombi, 2006). The biflavonoids complex from *Garcinia kola* has also been shown to have anti-diabetics, antihepatotoxic and hypolipidemia effect (Tita *et al.*, 2001; Adaramoye and Adeyemi, 2006a, b). Kolaviron (a natural antioxidant) was isolated as a defatted ethanol extract from *Garcinia kola* seed and it contains a mixture of three compounds Garcinia bioflavonoid GB-1, GB-2 and *G. kola* flavanone. (Farombi *et al.*, 2002; Adaramoye *et al.*, 2005a). Other constituent of *Garcinia kola* seed include, 1-3, 8-11 benzophenones (Olatunde *et al.*, 2004). Traditionally *G. kola* seed is believed to destroy sperm cell and make

male infertile and this has been proven scientifically. The *G. kola* seed extract has been found to raise peripheral testosterone levels in plasma and reduce sperm count without damage to the germinal epithelium; thus the proposed used as a male contraceptives (Akpantah *et al.*, 2003). Ovulation an important aspect of fertility is an inflammatory process (Epsey, 1994; Akpantah *et al.*, 2003). According to Braide (1993) the presence of flavonoids in *G. kola* seed confirms its anti-inflammatory property. Thus, this study was carried out to discover the effect of *G. kola* seed on female reproduction and its possible use as female contraceptive.

MATERIALS AND METHODS

Plant material: The *Garcinia kola* seed was purchased from the local market in Lagos, Nigeria in 2006 and authenticated by The Botany Department, University of Lagos.

The outer coats were removed and the seed was cut into pieces and air-dried. The dried seeds were grounded

into fine powder and extraction was done using 70% alcohol in a soxhlet extract. The yield is then concentrated into a solid form by evaporation using a rotary evaporator at 40°C. The resulting residue was further air-dried. Two gram of the extract was dissolved in 100 mL of distilled water to give 20 mg mL⁻¹ and stored under -4°C until required for use.

Animal grouping: Female pubertal adult rat and 4 pubertal male were collected from the animal house of the College of Medicine, University of Lagos. They were acclimatized for 2 weeks in the rat room of Physiology department under standard condition of temperature and illumination. The rats were fed with the rat pellets and had access to drinking water *ad libitum*. The average weights of the animals used were 145-170 g. The female rats that have undergone two successive four days cycle were divided into three experimental groups.

Estrous and ovulation study: Five animals received 200 mg kg⁻¹ b.wt. of GKSE once daily for three weeks. Another 5 animals receive equal volume of distilled water daily for three weeks and serve as the control. The pattern of the estrous cycle was studied in all animals by taking the vaginal smear. At the end of three weeks, each animal was sacrificed on the morning of estrous by cervical dislocation. The fimbriated part of the oviduct was dissected out, suspended in normal saline and placed on a microscopic slide to count the number of ova in the oviduct.

Implantation and pregnancy study: Thirty animals in this group were mated on the afternoon of proestrus and the male remain in the cage until the morning of estrus. The presence of spermatozoa (determined by microscopic examination of the vaginal smear) the next morning indicated conception and day one of pregnancy (Oderinde *et al.*, 2002). The pregnant rats were divided into two groups of 18 rats each. Rats in group 1 receive 200 mg kg⁻¹ b.wt. of GKSE orally from day four of pregnancy while rats in group 2 were given equal volume of distilled water starting from day four of pregnancy serving as the control. Localized changes in endometrial vascular permeability, indicative of implantation were assessed on days six and eight of pregnancy. Six rats each from each group were randomly selected and given 0.3 mL of 0.5% Evans blue dye via the tail vein. They were killed 15 min later and the uteri were separated from fat and connective tissues and opened to ascertain the implantation sites as described by Bolarinwa and Olaleye (1997). Where present, the dye sites were carefully dissected out with a scalpel blade and weighed to the nearest 0.01 mg on a mettler balance. The corned out sites

were counted and the number of uterine dye sites per rat recorded. The remaining 12 rats were sacrificed on day 19 of pregnancy, foetuses were removed from the pregnant rats by ventral laboratory and fetal weight, number and the resorption sites were determined.

Histological study: Twenty four rats were divided into two groups. The 12 rats in group 1 received 200 mg kg⁻¹ b.wt. of GKSE for three weeks. The remaining 12 rats serve as control and received equal volume of distilled water. At the end of 3 weeks, six rats from group three were sacrificed by cervical dislocation. The remaining 12 rats from both groups were allowed another 3 weeks for recovery before they were sacrificed. The fallopian tube, ovary and uterus were removed from each animal, weighed and processed for histological study. The weights of all the animals were monitored and recorded through-out the 3 weeks of study.

Histological processing: The organs were cut in slabs of about 0.5 cm thick transversely and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. After 6 h it was transferred to 90% alcohol and left overnight. From 90% to 3 changes of absolute alcohol for 1 h each, then into chloroform for about 10 h and later transferred into fresh chloroform for about 30 min. The tissues were placed in two changes of molten paraffin wax for 20 min each in an oven at 57°C. They were placed vertically in molten paraffin wax inside a metal mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using rotary microtome at 5 microns. Sections were floated on a water bath to spread out and later picked into albumenised slides and dried on a hot plate at 52°C. Slides were put in staining rack and placed in staining wells containing xylene to dewax; absolute alcohol (2 changes); 70% alcohol and then to water for 5 min after which they were stained with Haematoxylin (Hx) for 3 min. Excess Hx was washed off with water and differentiated with 1% acid alcohol. Sections were rinsed in running tap water and left for 5 min for blueing. Sections were stained with 1% eosin and washed off with water. They were dehydrated with 70 and 90% absolute alcohol and cleared in xylene to remove all traces of water. Mountant (a drop) was placed on the surface of slide and covered with a 22 by 22 mm cover slip.

Statistical analysis: Values were expressed as Mean±SEM. The statistical analysis was carried out using student's t-test. The level of significance for all experiment was p≤0.05. Simple percentages were used to present the alteration in the different phases of estrous cycle.

RESULTS

All the dams survived and no death was recorded through-out the study. GKSE had no significant effect on the weight of the animals studied (Table 1).

Estrous and ovulation studies: The estrous pattern was also significantly altered following administration of GKSE. The occurrence of estrous reduced drastically from 20% in control to 7.5% in GKSE. Metestrus also reduces from 44.8% in control to 31.3% in GKSE and Proestrus and diestrus increases from 20.9 and 14.3% in control to 26.5 and 34.7% in GKSE (Table 2).

The number of ova in the oviduct was significantly reduced ($p < 0.05$) in the GKSE treated rats when compared with the control (10.80 ± 2.6 for the control and 3.2 ± 0.8 for GKSE). Thus GKSE produces 70.4% blockage in ovulation.

Implantation and pregnancy studies: There is no significant difference between the number of the implantation site and the weight of the implantation sites in both pregnant control rats and the GKSE treated rats (Table 3).

The observations on foetal condition on day 19 of pregnancy are shown in Table 4. Observation of the

uterus of the GKSE treated and control rats showed no significant difference in both the foetal number and weight.

Histological studies: The histological section through the fallopian tube of the GKSE treated rats showed acute inflammation of the tubes which was absent in the control groups of rats (Fig. 1a, b). The various follicular stages are visible in the sections through the ovary of the control and recovery rats (Fig. 2a, c) with no developing follicles but corporal albican seen in the *G. kola* treated rats (Fig. 2b). The uterus of the *G. kola* treated rats showed an

Table 1: Effect of normal saline (0.9%) and *Garcinia kola* seed extract (200 mg kg⁻¹ b.wt.) on weekly body weight

	1st week weight (g)	2nd week weight(g)	3rd week weight (g)	*Growthrate per week
Control	150±3.53	159±3.15	170.4±1.20	6.80
GKSE	147±1.87	158±2.09	168.0±1.80	7.00
	NS	NS	NS	NS

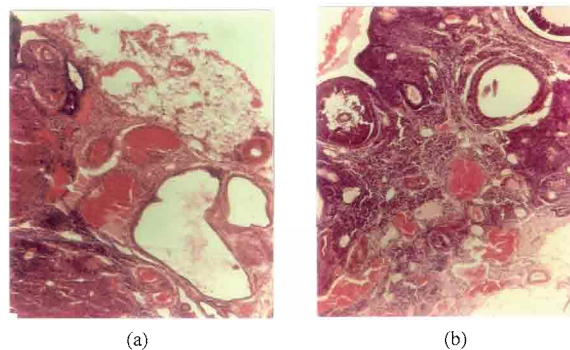
NS: Means not significant ($p > 0.05$), *Growth rate per week = Weight by week 3-Weight by week 1

Table 2: Effect of administration of 200 mg kg⁻¹ *Garcinia kola* seed extract on the phases of estrous cycle

Phases of the cycle	Control (%)	GKSE (%)
Proestrus	20.9	26.5
Estrus	20.0	7.5
Metestrus	44.8	31.3
Diestrus	14.3	34.7

Table 3: Effect of normal saline (0.9%) and *Garcinia kola* seed extract (200 mg kg⁻¹ b.wt.) on implantation in rat (days 6 and 8 of pregnancy)

	Day 6		Day 8			
	Mean No. of dye site±SEM	Mean weight (mg) of dye site±SEM	Mean No. of free blastocyst±SEM	Mean No. of dye site±SEM	Mean weight (mg) of dye site±SEM	Mean No. of free blastocyst±SEM
Control (0.9% normal saline)	7.8±0.32	4.67±0.38	NF	8.0±0.22	14.82±0.02	NF
GKSE treated (200 mg kg ⁻¹ b.wt.)	7.4±0.58	4.42±0.18	0.2±0.08	7.8±0.31	14.78±0.03	NF



Mag. X100 (H and E)

Fig. 1: Photomicrographs of the ovary from the GKSE treated (a) and recovery (b) rats
 a: Sections showing histology of ovary with no developing follicles, old degenerate luteum
 b: Sections showing histology of the ovary at various follicular stages, Graafian follicles and numerous Corpus luteum and congestion of the stroma

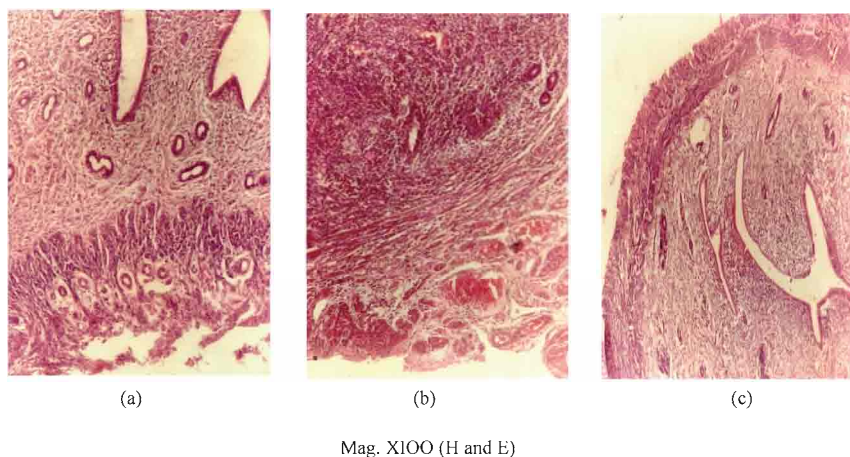


Fig. 2: Photomicrographs of the fallopian tube from the control (a), *G. kola* treated (b) and recovery (c) rats
 a: Section showing normal histology of the fallopian tube except for edema and congestion in the control rats
 b: Sections showing acute inflammation of the fallopian tube in GKSE treated rats
 c: Section showing histology of fallopian tube in the recovery rats with unremarkable feature except for oedema and congestion

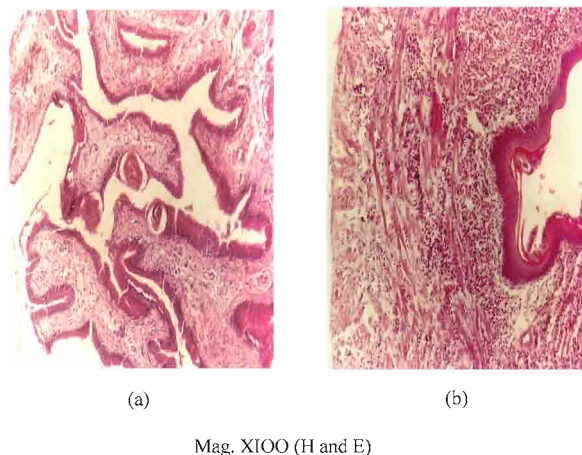


Fig. 3: Photomicrographs of the uterus from the *G. kola* treated (a) and recovery (b) rats
 a: Sections through the uterus of the GKSE treated rats showing inactive endometrial glands
 b: Sections showing histology of the uterus, endometrium at secretory phase in the recovery rats

Table 4: Effect of normal saline (0.9%) and *Garcinia kola* seed extract (200 mg kg⁻¹ b.wt.) on pregnancy at day 19 of gestation

Treatment	Mean fetal weight (g)	Resorption	No. of viable fetus
Control	4.41±0.10	2.2±0.51	6.3±0.84
<i>G. kola</i> treated	4.48±0.08	2.8±0.58	5.8±0.75

inactive endometrial gland (Fig. 3b). The uterus showed full recovery after administration of GKSE was stopped (Fig. 1b, 2c, 3b).

DISCUSSION

GKSE has no significant effect on the weight of growth rate of the rats similar result was reported by

Akpantah *et al.* (2003). But this is contrary to the report of Braide and Grill (1990) who reported retarded growth in a group of rats fed with 10% w/w dry powder seed of *Garcinia kola* for six weeks. The difference in observed weight may be due to the mode of administration (in their study, *G. kola* seed powder was mixed with the rat's feed) or the presence of tannin which as explained by them, causes malabsorption and thus nutritional deficit. But in this study the process of extraction would have reduce the effectiveness of tannins in the extract used.

Garcinia kola seed have been reported to have anti-inflammatory (Madubunyi, 1995). In the past, anti-inflammatory drugs have been employed in blocking

ovulation (Gaytan *et al.*, 2002), thus this explains the observed reduction in number of egg released and the occurrence of the estrous phase in the *G. kola* treated rats.

Phytochemical analysis has shown that *Garcinia kola* contains flavonoids and some cardiac glycosides especially cardenolides (Cotterih *et al.*, 1978). Studies have shown that flavonoids such as apigenin are a major component of *Garcinia kola* seed (Iwu and Igboko, 1982). Flavonoids will inhibit cyclo-oxygenase enzymes (Liang *et al.*, 1999). It has been revealed that all traditional non-steroid anti-inflammatory drugs produce most of their effects by blocking COX-2 (Stand, 2000). Mice with COX-2 deficiency suffer defective reproductive functions as COX-2 plays an important role in follicular rupture (Lim *et al.*, 1997). Thus GKSE may be blocking ovulation as reported in this study by suppressing the activity or formation of COX-2 thereby preventing follicular rupture. Estrous phase is the period of vagina cornification and the drastic reduction its occurrence following GKSE treatment may be due to the presence of apigenin an effective inhibitor of the enzyme aromatase. Aromatase is important in estrogen synthesis i.e., in the conversion of androgen to estrogens (Jeong *et al.*, 1999).

CONCLUSION

GKSE significantly reduces the occurrence of estrous while producing 70% blockage of ovulation without damage to the reproductive tissues (ovary, uterus and fallopian tubes). Thus it may be use as a contraceptive having the advantage of reversibility.

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