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*Full Length Research Paper*

# Anti-progestational, anti-ovulatory and anti-implantation potentials of methanolic extract of *Garcinia kola* seed in female rats

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**Abstract**

***Garcinia kola* seed, used in traditional medicine is known to have effects on reproduction. The study was designed to ascertain the phytochemical constitiuents and toxicity level of *Garcinia kola* seed, the progestational, anti-progestational and post-coital effects of methanolic extract of *Garcinia kola* seed in rats. Phytochemical screening and acute toxicity study were done using standard methods, progestational and antiprogestational activities were evaluated by traumatization method while laparectomy was used to determine the number of implantation sites in the uteri. The extract was found to contain tannins, saponins, flavonoids, alkaloids and cardiac glycosides and the mean lethal dose was calculated to be 1000 mg/kg. The extract exhibited anti-progestational effect in all rats treated with it. The extract also inhibited ovulation in all animals treated with 200 mg/kg and 300 mg/kg of the extract however; there was positive deciduoma formation in 67% of rats pretreated with 200mg/kg extract in the presence of progesterone. The extract significantly impaired implantation, caused resorption of implantation sites and also delayed delivery for 4-5 days. This study reveals that *Garcinia kola* seed possesses anti-ovulatory, anti-progestational and anti-implantation effects thus, corroborating the rationale behind the traditional use of *Garcinia kola* seed as contraceptive.***

**Keywords:** *Garcinia kola,* phytochemical constitiuents, toxicity, reproduction, anti-progestational, post-coital.

## INTRODUCTION

In the last decades, there is increasing pharmacological evaluation of medicinal plants that could be of benefits as contraceptive and fertility control agents as many plants are known to have promising contraceptive properties (Farnsworth *et al*., 1980). *Garcinia kola* plant Heckel (Guttiferae) known in commerce as “bitter kola” is a highly valued ingredient in African traditional medicine. In traditional medicine, *Garcinia kola* is used in treatment of ailments such as common cold, cough, hoarseness of voice, dysentery, diarrhoea and colic pain (Dalziel, 1956). Preliminary investigations of the action of alkaloid and biflavonoid fractions of the *G kola* seed indicated marked, dose-dependent spasmolytic and antispasmogenic effects on uterine and gastro intestinal smooth muscle (Braide, 1989). Other studies using methanolic extracts showed that the phytochemical principles exhibited antihepatotoxic biochemical effects (Akintonwa and Essien, 1990; Braide 1991), hypoglycaemic anti-diabetic activity (Iwu *et al*., 1990) and antipyretic, anti-inflammatory 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 medical practitioners in Nigeria. In another study, the alkaloid fraction of *Garcinia kola* seed altered serum levels of gonadal hormones and histology of both male and female reproductive organs in rats (Braide *et al.,* 2003). There are increased adverse effects resulting from the use of hormonal contraceptives; the present study was therefore conducted to determine whether *Garcinia kola* seed has progestational, anti-progestational and/or post-coital effects on female rats.

## MATERIALS AND METHODS

### Preparation of Plant Extract

Fresh *Garcinia kola* seeds were harvested from a *Garcina kola* tree in Ukpom village, in Ini Local Government Area of Akwa Ibom State, Nigeria. The plant was identified by Dr. (Mrs.) Margaret Bassey, a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. A herbarium specimen voucher number Essien UUH2038 (Ikono) assigned to it and deposited in the Department of Botany and Ecological Studies. 1 kg weight of the *Garcinia kola* seeds was used for the extract preparation. The outer coat of the seeds was removed and the seeds were dried in an electric oven, thermostatistically controlled at 40$^{o}$C ± 10$^{o}$C for 12 hours. The dry seeds were pulverized to fine powder with a mortar and pestle. The pulverized powder was exhaustively de-fatted with n-Hexane for 12 hours. The de-fatted fraction was further extracted by cold maceration in methanol for 72 hours. The filtrate was concentrated and evaporated to dryness and stored in a refrigerator at -4$^{0}$C until used. 100g of the extract was used to obtain four different fractions: Chloroform, Ethyl acetate, n-butanol and Aqueous fractions. All fractions were also stored in the refrigerator until used.

### Animal Stock

Adult albino rats and mice of both sexes were obtained from the animal house of the Department of Pharmacology and Toxicology of the University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted Feed (Guinea Feed) and water *ad libitum* under standard laboratory conditions (Temperature 25± 5oC, Relative humidity 50-60%, and a 12/12h light/dark cycle).

### Phytochemical screening

The extract was screened for the following constituents – Reducing sugars, flavonoids, saponins, tannis, anthraquinones, phlobatannins, alkaloids, terpenes and cardiac glycosides using the standard methods of Trease and Evans (1989) and Sofowora (1993).

### Acute Toxicity Testing

The method of Miller and Tainter, 1944 was used to determine the Median Lethal Dose ($LD_{50}$). Thirty-six healthy albino mice weighing (18-25g) were divided into six groups of six mice per group. Different doses (100-2000mg/kg) of the extract were administered, intraperitoneally (i.p). Signs of physical toxicity were observed and values obtained were used to plot log probit versus concentration graph.

### Determination of Progestational and Anti-Progestational Effects of Extract

Progestational and antiprogestational activities were evaluated in mature female rats by traumatization method of Ohta (1982), with slight modification, taking decidual response as an end point. Rats at estrus were overiectomized and the day designated D-1.This was followed by injection of 17-β-estradiol subcutaneous injection at a dose of 0.1µg/day for 3 consecutive days. Thereafter, the rats received a single subcutaneous injection of progesterone daily for 7 consecutive days (4-10). On the 6th day, all rats received a single subcutaneous dose of 0.1µg/day of 17-β- estradiol in 0.05ml in corn oil between(1800 and 1900) hours. Sixteen hours after the 17-β-estradiol injection, the animals were laparectomized under light ether anaesthesia and decidual stimulus applied to the right horn of the uterus by traumatizing it with needle. For evaluating progestational activity of the extract different doses (100 – 300 mg/kg, i.p) were administered for 7 days instead of standard progesterone and for anti-progestational activity, 200 mg/kg of the extract was administered concurrently with progesterone for the same length of time (shukla *et al*., 1988).

### Determination of Postcoital Effects of Extract on Female Rats

In this experiment, adult female rats in the proestrus phase (confirmed by vaginal smear analysis) were left overnight with males of proven fertility in the ratio 3:1(F/M). The presence of clumps of spermatozoa in the vaginal smear the next morning confirmed that mating had occurred and was considered D-1 of pregnancy. The extract was administered on days 1- 4 at three doses (100 - 300 mg/kg, i.p). Control group received Tween 80 (5ml/kg, i.p). Laparectomy of all rats was done under light ether anaesthesia on D-10 of pregnancy and the number of implantation sites in the horns of the uteri was recorded. The wounds were sutured and all animals allowed to go to term and number of pups recorded (Bhargava 1988).

### Determination of Effect of Extract on Ovulation

To establish the effect of the extract on ovulation, twenty-four female cycling rats were randomized and divided into four groups of six rats per group. Group I received Tween 80 (5ml/kg, i.p) and served as the control.

Groups II–IV received the extract in different doses (100 - 300mg/kg,i.p). All injections were administered at late proestrus. At the end of estrus, the animals were laparectomized and the ovaries were examined with a hand lens to observe if there was any interference with ovulation (Telleria *et al.,* 1997).

**Table 1.** Result of phytochemical screening

| | TEST | OBSERVATION | INFERENCE |
|---|---|---|---|
| | **Alkaloid Test** | | |
| (a) | Dragendorff's reagent | Brick red precipitate Formed | ++ |
| (b) | Mayer's reagent | Yellow Precipitate formed | ++ |
| (c) | Wagner's reagent | Brownish Precipitate formed | ++ |
| | **Saponin Test** | | |
| (a) | Frothing test | Formed frothing, that lasted for a while | +++ |
| (b) | Fehling's test | Brown Precipitate formed | +++ |
| (c) | Haemolysis test | Haemolysis in tubes with extract | +++ |
| | **Tannins** | | |
| (a) | Ferric Chloride test | Turned blue black | +++ |
| (b) | Bromine test | Decolourized bromine water | +++ |
| | **Anthraquinones** | | |
| (a) | Borntrager's test | No violet colour observed in the ammonia phase | _ |
| (b) | Combined Anthraquinones test | No violet colour observed in ammonia phase | _ |
| (a) | **Cardiac Glycoside** | | |
| (b) | Salkowski test | Steroidal ring present | +++ |
| (c) | Keller Killiani test | Brown ring formed at interface | +++ |
| | Lieberman's test | Colour change from violet to blue to green | +++ |
| | **Flavoniod Test** | Crimson colour precipitate | +++ |
| | **Terpenes** | No pink colour in the interface | — |
| **Key:** | **+ Trace**<br>**++ Positive**<br>**+++ Strongly positive**<br>**— Absent** | | |

## RESULTS

### Phytochemical Screening

The phytochemical screening of methanolic extract of *Garcinia kola* seed as shown in Table1, revealed the presence of the following secondary metabolites: tannins, saponins, flavonoids, alkaloids and cardiac glycosides. However phlobatanins and anthraquinones were absent.

### Acute Toxicity Testing

The mean lethal dose ($LD_{50}$) was calculated to be 1000 ± 66.40mg/kg. The physical signs of toxicity included excitation, paw-licking, decreased motor activity. Others were increased respiratory rate, convulsion and death.

### Progestational and anti-progestational effects of extract

The progestational and anti-progestational effects of extract are as shown in Table 2. The extract exhibited anti-progestational effect in all rats treated with different doses of the test drug (100 – 300mg/kg) respectively. In the control group pretreated with Tween 80 and progesterone, all animals showed progestational effect with formation of positive deciduoma. However, there was positive deciduoma formation in 67% of rats pretreated with extract (200mg/kg), in the presence of standard hormone (progesterone).

### Post-coital effect of extract on pregnant rats

As shown in Table 3 the extract significantly impaired implantation in rats. This inhibition was dose-dependent. It caused resorption of implantation sites and delayed delivery for 4-5 days was also observed among the groups that littered

### Effect of Extract on Ovulation

The effect of extract on ovulation is as shown in Table 4. It was observed that there was ovulation in all the animals in the control group. On the contrary, there was no ovulation in 30% in animals pretreated with 100mg/kg, while there was no ovulation in all animals pretreated with 200mg/kg-300mg/kg of extract, indicating 100% inhibition.

**Table 2.** Progestational and Anti-progestational effect of extract

| Dose (mg/kg) | Progesterone (mg/kg) | Initial Weight (g) | Final Weight (g) | Quantal ratio showing positive residual responses |
|---|---|---|---|---|
| Control Tween 80 (5ml/kg) | 3mg | 126.30 ± 4.30 | 128.70 ± 2.90 | 6/6 |
| l00 | No Progesterone | 129.00 ± 4.50 | 131.80 ± 3.60 | 0/6 |
| 200 | No Progesterone | 112. 20 ± 5.10 | 115.50 ± 5.50 | 0/6 |
| 300 | No Progesterone | 115.50 ± 4.80 | 117.00 ± 3.70 | 0/6 |
| | 3mg +200 extract. | 127. 20 ± 2.40 | 129.30 ± 1.60 | 4/6 |

Values represent mean ± SEM, n = 6

**Table 3.** Post-coital effect of extract of extract on female pregnant rats

| DOSE of extract (mg/kg) | NO. OF RATS PREGNANT ON D-10 (%) | NO. OF IMPLANTATION SITES | NO. OF MATED RATS THAT LITTERED (%) | NO. OF PUPS LITTERED |
|---|---|---|---|---|
| Control Tween 80 (5ml/kg) | 100 | 11.33 ± 0.22 | 100 | 11.33 ± 0.22 |
| 100 | 83.33 | 6.00 ± 0.18 | 66.67 | 2.83 ± 0.47 |
| 200 | 83.33 | 4.50 ± 0.04 | 50 | 1.17 ± 0.25 |
| 300 | 50 | 2.00 ± 0.00 | 16.67 | 0.33 ± 0.54 |

n=6

**Table 4.** Effect of Extract on Ovulation

| DOSE (mg/kg | ANIMAL WEIGHT | DAY-0 | DAY-1 | INFERENCE |
|---|---|---|---|---|
| Control TWEEN 80 (5ml/kg) | 119g | At late proestrous | ROS | Evidence of ovulation |
| | 101g | At late proestrous' | ROS | Evidence of ovulation |
| | 121g | At late proestrous | ROS | Evidence of ovulation |
| | 116g | At late proestrous | ROS | Evidence of ovulation |
| | 122g | At late proestrous | ROS | Evidence of ovulation |
| | 105g | At late proestrous | ROS | Evidence of ovulation |
| 100 | 116g | At late proestrous | RNOS | Evidence of ovulation |
| | 141g | At late proestrous | ROS | Evidence of ovulation |
| | 121g | At late proestrous | RNOS | Evidence of ovulation |
| | 124g | At late proestrous | NRNOS | No ovulation |
| | 135g | At late proestrous | RNOS | Evidence of ovulation |
| | 132g | At late proestrous | NRNOS | No ovulation |
| 200 | 114g | At late proestrous | NRNOS | No ovulation |
| | 108g | At late proestrous | NRNOS | No ovulation |
| | 118g | At late proestrous | NRNOS | No ovulation |
| | 117g | At late proestrous | NRNOS | No ovulation |
| | 126g | At late proestrous | NRNOS | No ovulation |
| | 104g | At late proestrous | NRNOS | No ovulation |
| 300 | 102g | At late proestrous | NRNOS | No ovulation |
| | 126g | At late proestrous | NRNOS | No ovulation |
| | 100g | At late proestrous | NRNOS | No ovulation |
| | 125g | At late proestrous | NRNOS | No ovulation |
| | 131g | At late proestrous | NRNOS | No ovulation |
| | 118g | At late proestrous | NRNOS | No ovulation |

NRNOS - No rupture of ovary, no ovum seen ROS - Rupture of ovary, ovum seen RNOS - Rupture of ovary, no ovum seen D-0 - Day of administration of extract D-l - Subsequent day after.

## DISCUSSION

The median lethal dose ($LD_{50}$) value of 1000±66.67 mg/kg, indicated that the extract was practically non-toxic, hence a high safety margin and tolerability, this correspond to a report by Homburger, (1989).

From the phytochemical screening, the extract contains flavonoids. Studies have shown that flavonoids, such as apigenin, which forms the major component of *G.* kola seed (Iwu and Igboko, 1982) has anti-inflammatory property (Braide, 1993, Madubunyi, 1995). Ovulation is a type of inflammatory reaction and anti-inflammatory drugs were employed to block ovulation (Gaytan *et al*., 2002) which might explain the observed reduction in the number of eggs released in this research. Also analysis of the extract's phytochemistry revealed the presence of polyphenols such as saponins. This secondary metabolite also inhibits estrous cycle and reduces fertility (Tamura *et al*., 1997).

For many decades, *G.kola* seeds have been used as contraceptive by women of some parts of Itam clan in Itu Local Government area of Akwa-Ibom State of Nigeria (Essien, E. Itam clan, Nigeria. personal communication). The present study was instigated by the folkloric reports that *G. kola* seed is used locally as an aphrodisiac by men and as female contraceptive to control fertility (Ajibola and Satake, 1992).

In the anti-implantation model, the extract caused resorption of implantation sites in pregnant rats. Fetuses confirmed on Day-10 through laparectomy failed to develop/litter at term. There was no deciduoma formation in the uterus by traumatization, indicating the anti-progestational property of the extract; this was in consonance with a similar work carried out by Braide *et al*., (2003) on female rats to determine the effects of the *G.kola* seed on the female reproductive system. It was observed in their work that the seed caused a decrease in serum concentration of the gonadotropins (FSH and LH) and prolactin, while coincidentally causing marked increase in serum level of estradiol and progesterone in female rats.

One of the main steps in the elucidation of the mechanism of implantation is to determine how the blastocyst stimulates the uterus to undergo a decidual reaction. This reaction is particularly marked in rodents and without it ovum implantation does not occur (Finn and Keen, 1963). In this work, the finding that the extract could not cause deciduoma also resulted in an unfavorable endometrium environment, since degeneration at the implantation site has been demonstrated in animals (Kinol and Dorfman, 1965). Bratde *et al*., (2003) reported that the seed also caused marked proliferation of the uterine endothelial cells and dilation of the lumen which was in consonance with the finding in this research.

Phytoestrogens, which are present in *Garcinia kola* seed, are plants' derived xenoestrogens, which structurally and functionally mimic circulating estrogen in mammalian reproductive system. They are also known to induce estrogenic and anti-estrogenic effects in the pituitary-gonad axis and peripheral reproductive organs (Yildiz, 2005). Typical estrogenic compounds possess ability to increase the wet weight, induce cornification and vaginal opening in immature rats. It is known that administration of estrogen has uterotrophic effects in several animal species, including rats and mice (Edgren and Calhoun, 1957; Jacobs and Lewis, 2002). Such effects are associated with growth and proliferation of endometrial cell number, vaginal opening and cornification (Ljungkvist, 1971). Estrogens are also known to increase uterine contractility, which may lead to premature expulsion of eggs (Jonathan *et al.,* 1995).

Contrary to the control group in this work; there was no ovulation in 30% in animals pretreated with 100mg/kg, while there was no ovulation in all animals pretreated with 200mg/kg-300mg/kg of extract indicating a100% inhibition of ovulation. Similarly in another study, Akpanta *et al.* (2005) reported that ethanolic extract of *G. kola* seed blocked ovulation in female rats.

From the above observations, the contraceptive activity of the extract might be connected with; the estrogenicity of the extract, anti-progestational effect, anti-ovulatory effect and anti-implantation effects. These observed effects of the extract are predicated on its phytochemical constituents as the antifertility activities of many plants are known or suspected to be due to their secondary metabolites (such as phytoestrogens or other compounds of known structure and biological activities (Gupta and Rakhi, 2006).

## CONCLUSION

The findings in this study reveal that *Garcinia kola* seed possesses anti-progestational, anti-implantation and anti-ovulatory effects on female rats. Therefore, this work corroborates the rationale behind the traditional use of *Garcinia kola* seed as contraceptive for women.

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