academicJournals

Vol. 8(42), pp. 1237-1244, 10 November, 2014
DOI: 10.5897/JMPR2014.5556
Article Number: AB0194F48722
ISSN 1996-0875
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Anticonceptive, estrogenic and antiestrogenic potentials of methanol extract of *Garcinia kola* seed in rodents

Grace Emmanuel Essien* and Paul Alozie Nwafor

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.

Received 10 August, 2014; Accepted 6 November, 2014

The anticonceptive, estrogenic and antiestrogenic potential of methanolic extract of *Garcinia kola* seed in rodents was investigated. The anti-conceptive effect of extract showed that the extract dose-dependently protected female mice and rats from conception for two to three gestational periods. Changes observed in the length and weights of pups were not statistically significant relative to control. There were no abnormalities observed in the pups over thirty days. In ovariectomized immature rats treated with extract (100 to 300 mg/kg), there was a significant increase in uterine wet weight. The extract also induced uterotrophic effects, namely, immature vaginal opening and cornification, when comparedwith control. These findings agree with the traditional use of *G. kola* seed in control of fertility. The contraceptive property of the extract may be associated with the direct effects of its chemical constituents.

Key words: *Garcinia kola*, anti-conceptive potential, estrogenic.

INTRODUCTION

In the last two decades, the scientific world has recorded an increased pharmacological evaluation of medicinal plants that could be of benefits as fertility regulatory agents (Farnsworth et al., 1980; Gupta and Rakhi, 2006). The search for these agents became very intense due to some adverse effects of the synthetic drugs. Besides, the high cost and non-affordability of these drugs, especially in developing countries, made it more imperative for an alternative search of drugs that could be accessible and affordable.

A large number of plants which have been screened for contraceptive activity, include among others, Gossypol seeds (Udoh et al., 1992), *Azadirachta indica* (Joshi et al., 1996), *Asparagus pubescens* (Nwafor et al., 1998), *Cassia nigricans* (Nwafor and Okwuasaba, 2001), *Similax krausinia* (Idiong, 2010) and *Carpolobia lutea* (Ettebong et al., 2011). Garcinia kola seed (Guttiferae), also known as bitter kola is one of the medicinal plants used by some indigenes in Nigeria, to control fertility in females. Despite its bitter taste, *G. kola* seed is widely used in African

*Corresponding author. E-mail: graceessien@uniuyo.edu.ng.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Traditional Medical practice. Studies carried out by Iwu and Igboko (1982) showed that the phytochemical principles, in G kola seed stimulated an increase in gastric acid secretion, exhibited anti-hepatotoxic biochemical effects (Iwu, 1985, Akintowa and Essien, 1990). Garcinia kola is also used in the treatment of such conditions as common cold, catarrh, cough, hoarseness of voice (Okunji and Iwu 1991), dysentery, diarrhoea. Other studies using methanolic extracts showed that the phytochemical principles, exhibited antidiabetic effect (Iwu et al, 1990); antipyretic, anti- inflammatory effects (Braide, 1993, Iwu 1993). It has also been shown that ingestion of G. kola seed caused mild bronchodilatation in man (Orie and Ekon, 1993). Udoh (1998) reported that G. kola seed diets fed for durations lasting 6 weeks or longer caused testicular atrophy and degeneration of spermatozoa in male rats. A similar work was carried out by Braide et al. (2003) on female rats to determine the effects of the seed on female reproductive system. It was observed 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. The seed also caused marked proliferation of the uterine endothelial cells and dilation of the lumen. In another study, Akpanta et al. (2005) reported that ethanolic extract of *G. kola* seed blocked ovulation in female rats. The present study, designed to investigate the anticonceptive potential of the plant was instigated by the findings from the works mentioned earlier.

MATERIALS AND METHODS

Plant

Fresh seeds of *G. kola* were purchased from the local markets in Uyo, Akwa Ibom State, Nigeria. The plant was authenticated by Dr (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo and a voucher specimen with number UUH220 was assigned to it and was deposited at the herbarium of Department of Botany and Ecological Studies, University of Uyo.

Extraction procedure

The seeds were peeled to remove testa, washed and air-dried for 8 h, then subsequently dried in an electric oven (Astell Hearson, England) thermostatically controlled at 40°C for 12 h. The dry seeds were pulverized to a fine powder with the aid of a mortar and pestle. The pulverized powder was exhaustively de-fatted and was further extracted by cold maceration in methanol for 72 h. The filtrate was concentrated and evaporated to dryness using the yield calculated and then stored in a refrigerator at -4°C until when needed.

Phytochemical screening

Phytochemical screening of the extract was done to determine the presence of chemical constituents such as flavonoids, simple sugar, alkaloids, tannins, saponins, phlobatannins, cardiac glycosides and anthraquinones and the methods of Odebiyi and Sofowora (1978) and Trease and Evans (1989) were adopted.

Animals

Adults and immature albino rats (weighing 180 to 220 g and 60 to 90 g, respectively) and mice (18 to 25 g) were obtained from the University of Calabar, Calabar, Cross River State, Nigeria. They were not quarantined for 2 weeks and subsequently quarantined for two weeks, and then were maintained strictly under favorable environmental conditions of 12 h light/12 h dark cycle, temperature 22 ± 2.5°C and fed with growers pellets feed (Bendel Feeds and flour Mills Ltd, Edo State) with water *ad libitum*. All animal experiments were conducted in accordance with internationally accepted laboratory animal use and care (Based on Helsinki Convention) and guidelines and rules of Faculty of Pharmacy, University of Uyo, Ethical Committee Report on Animal Experimentation.

Determination of median lethal dose (LD_{50})

The method of Miller and Tainter (1944) was used to determine the median lethal dose of the extract. Thirty-six healthy albino mice weighing 18 to 25 g were divided into six groups of six mice per group. Different doses (100 to 2000 mg/kg) of the extract were administered intraperitoneally (i.p). Physical signs of toxicity were observed for 24 h and recorded. The mortality values obtained were used to plot a graph of log probit versus concentration.

Anticonceptive activity

The anticonceptive activity was determined using the method of Nwafor et al. (1998). Adult albino female rats and mice showing regular estrus cycle through daily vaginal smear analysis and those having at least two successive 4-day estrus cycles were selected. The animals were randomized and separated into six groups consisting of six animals per group. Group I received 5 ml/kg of Tween 80, intraperitoneally in divided doses for 4 days. Groups II, III, and IV received different doses (100 to 300 mg/kg) of the extract intraperitoneally in divided doses for 4 days. Group V received 0.1 µg/rat of 17β-estradiol, while group VI received 17β-estradiol concurrently with 200 mg/kg of extract. On the 5th day, fertile males were introduced in the ratio of one male to three females and allowed to remain until experiment was terminated. The number, weight and length of pups were recorded (Telleria et al., 1997; Nwafor et al., 1998).

Estrogenic and anti-estrogenic potentials

Estrogenic and antiestrogenic activities of the extract were assessed in bilaterally ovariectomized immature albino rats (weighing 70 to 90 g) using the methods of Edgren and Calhoun (1957) and Nwafor et al. (1998). The end point used to determine the estrogenic effects included: uterine wet weight, degree of vaginal cornification and quantal vaginal opening. Exactly one week after bilateral ovariectomy, the rats were randomized and divided into six groups of six animals per group. Group I received 5 ml/kg Tween 80 (s.c) in divided doses for four consecutive days and served as control. Groups II to IV received 100 to 300 mg/kg, respectively by the same route for four consecutive days. Group V received 0.1 µg/rat of 17-β-estradiol dissolved in corn oil by the same route for four consecutive days. Group VI received 200 mg/kg of extract concurrently with 17-β-estradiol for four consecutive days, to evaluate the antiestrogenic activity. The animals were observed for degree of vaginal opening and cornification. All animals were sacrificed 24 h after the last treatment and the uterine wet weight

Table 1. Phytochemical screening of extract.

	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	-
	Cardiac glycoside		
a	Salkowski test	Steroidal ring present	+++
b	Keller Killiani test	Brown ring formed at interface	+++
c	Lieberman's test	Colour change from violet to blue to green	+++
	Flavoniod test	Crimson colour precipitate	+++
	Terpenes	No pink colour in the interface	-

+: Trace; ++: Positive; +++: Strongly positive; -: Absent.

Table 2. Anti-conceptive effect of methanol extract in adult female rats.

Dose (mg/kg)	Mean No. of pups	Protection over n-gestational period	Percentage of animals protected
Control (5 ml/kg Tween 80)	5.60 ± 0.45	(0/6)	0
100	5.17 ± 0.62	2 (3/6)	50
200	4.80 ± 0.00	2 (4/6)	67
300	4.50 ± 0.75	3 (4/6)	67
17-β	4.00 ± 0.52	3 (5/6)	93.33
17- β+ 200	3.85 ± 0.32	3 (5/6)	93.33

Numerator indicates the number of rats protected

for degree of vaginal opening and cornification. All animals were sacrificed 24 h after the last treatment and the uterine wet weight recorded (Rubin et al., 1951).

Statistical analysis

Results were expressed as multiple comparison of mean ± standard error of mean (SEM). Significance was determined using one way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

RESULTS

Acute toxicity test

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

Table 3. Anti-conceptive effective of extract in adult mice.

Dose (mg/kg)	Mean No. of pups	Protection over n-gestational period	Percentage of animals protected
Control (5 ml/kg Tween 80)	4.8 ± 1.06	(0/6)	0.00
100	3.00 ± 1.35	2 (3/6)	33.33
200	2.23 ± 1.24	2 (3/6)	50.00
300	2.00 ± 1.48	3 (4/6)	67.00
17-β	2.15 ± 1.06	3 (3/6)	50.00
17- β + 200	2.10 ± 1.15	3 (3/6)	50.00

Numerator indicates the number of mice protected.

Table 4. Estrogenic and anti-estrogenic effect of extract.

Dose (mg/kg)	Weight of Animals		Uterine wet weight (mg/100 g body weight)	Vaginal opening	Cornification
	Initial (g)	Final (g)			
Control (5 ml/kg Tween 80)	101.30	112.70	0.05 ± 0.01	-	-
100	121.30	123.00	0.07 ± 0.02	+	+
200	102.80	104.80	0.29 ± 0.15^{c}	+	+
300	113.70	116.00	0.31 ± 0.01^{c}	2+	2+
Standard (17-β estradiol)	111.70	115.50	0.42 ± 0.02^{c}	4+	4+
200 + 17-β estradiol	94.00	108.30	0.56 ± 0.01^{b}	4+	4+

Values represent Mean± SEM. Significance relative to control; $^{b}p<0.05$, $^{c}p<0.001$ (n=6).

(Figure 1).

Phytochemical screening

The phytochemical screening of the extract revealed the presence of the following secondary metabolites: tannins, saponins, flavonoids, alkaloids and cardiac glycosides. Phlobatanins and anthraquinones were however absent (Table 1).

Anticonceptive effect of the extract

The extract (100 to 300 mg/kg), protected the rats from conception. The protection lasted for 2 to 3 gestational periods, equivalent to 50 to 67% degrees of protection relative to control (Table 2). Similar effects were observed in mice (33.33 to 67%) (Table 3). Similarly, the effects of the extract on weight and length of pups in both rats and mice, showed no significant discriminatory changes (Figures 2 to 5).

Effect of extract on lengths and weights of pups

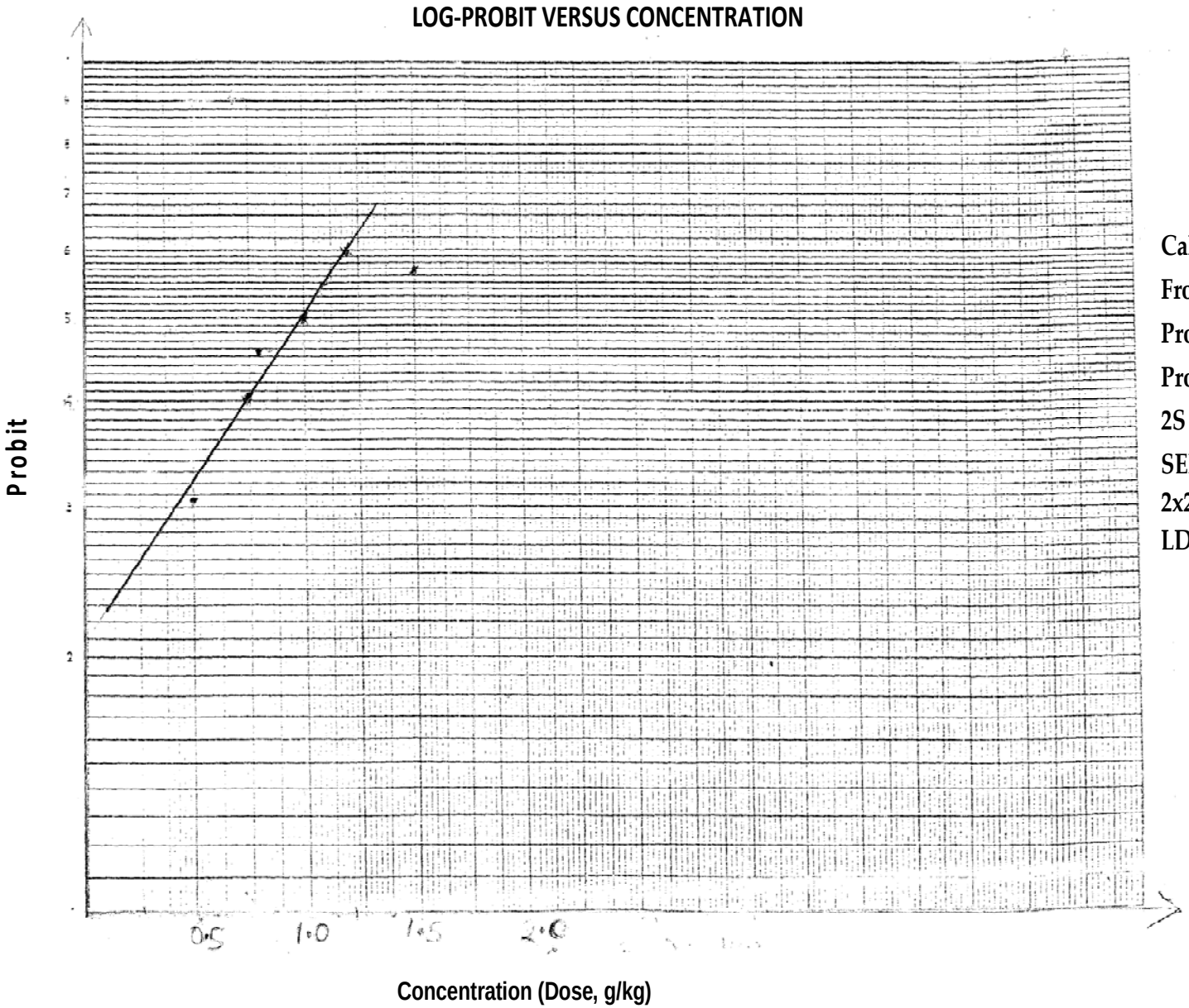
The extract caused changes in lengths and weights of pups, which were statistically not significant relative to control as shown in Figures 2 to 5.

Estrogenic and anti-estrogenic effects of extract

The methanolic extract of *G. kola* showed a dose dependent increase in uterine wet weight. This increase was statistically significant ($p<0.05$ to $p<0.01$). There were also vaginal opening and cornification in a dose-dependent manner. However, the extract showed a weak estrogenic effect relative to the standard (Table 4).

DISCUSSION

These results show that the methanolic extract of *G. kola* seed, possesses anti-conceptive activity in rats and mice. The extract also possesses weak estrogenic potential in rodents. This is predicated upon the fact that animals previously treated with extract and kept with sexually active males were protected over varied gestational periods. There was an increase in uterine weight which was dose dependent. The premature vaginal opening and cornification were observed in young overiectomized rats. These effects are associated with endometrial growth and proliferation (Jacobs et al., 1996). From the phytochemical screening, the extract contains flavonoids, which are known to have anti-inflammatory effects



Calculation of LD_{50}

From probit graph LD_{50} = 1000 mg/kg

Probit 4 = 740

Probit -6 = 1200

2S = Probit 6. – Probit 4 = 1200-740 = 460

SEM = $\sqrt{2}S/2$ = $\sqrt{460}$

2x24 =66.395

LD50 = 1000 ± 66.40 mg/kg

Figure 1. Graph for acute toxicity.

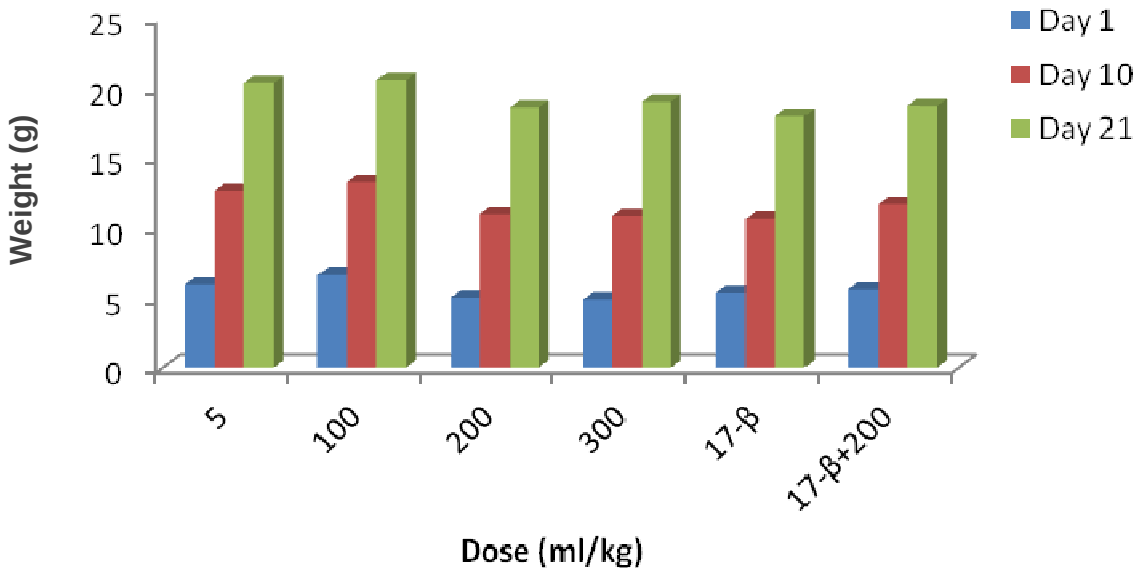


Figure 2. Effect of extract on the weight of rat pups.

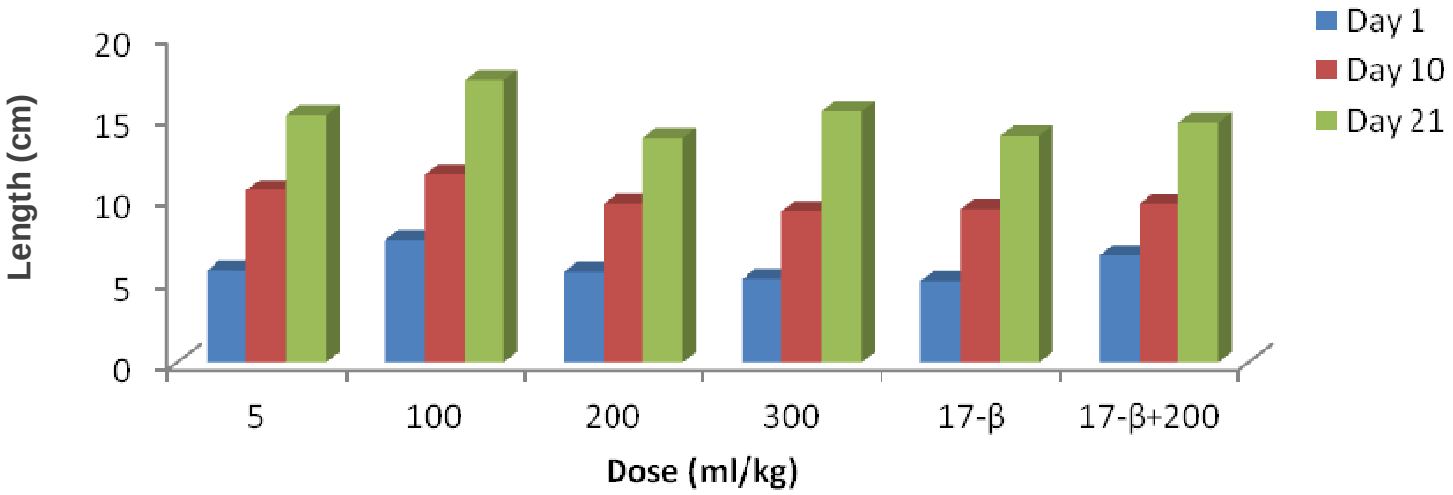


Figure 3. Effect of extract on the length of rat pups.

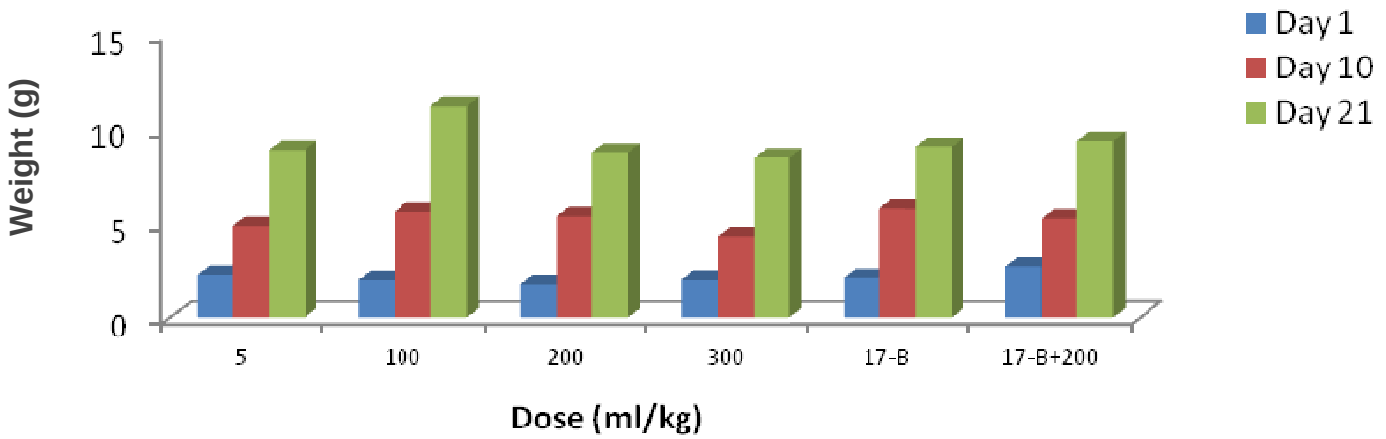


Figure 4. Effect of extract on the weight of mice pups.

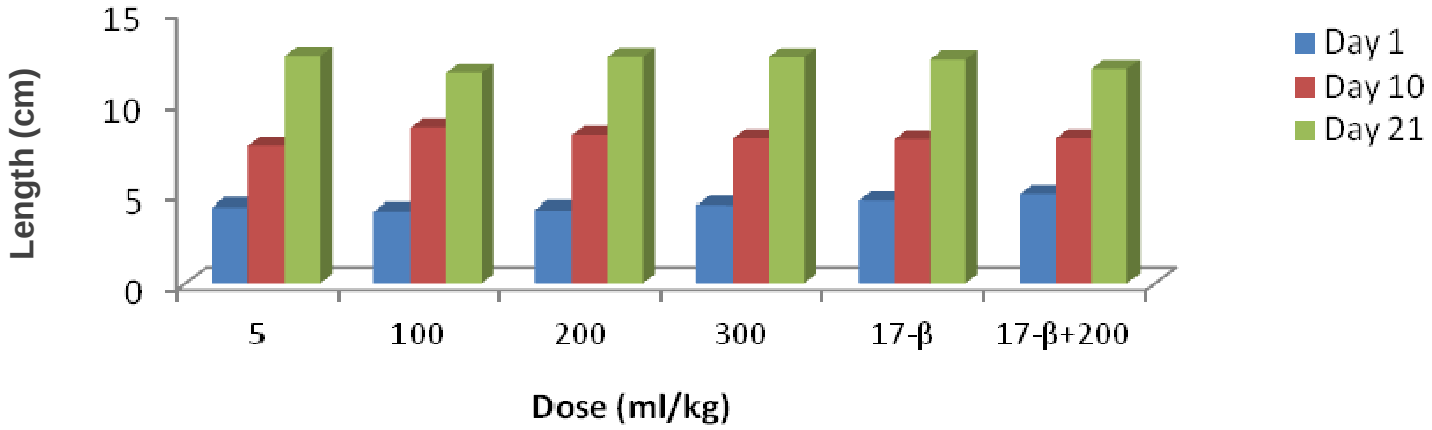


Figure 5. Effect of extract on the length of mice pups.

(Braide, 1993 and Liang et al., 1999). Ovulation is a type of inflammatory reactions which were blocked by anti-inflammatory drugs (Gaytan et al., 2002).

This may explain the anti-conceptive effect observed in the study. The weak estrogenic activity may also be responsible for the anti-conceptive effect of the extract. The study carried out by Braide et al. (2003), showed that the alkaloid fraction of *G. kola* seed, caused changes in gonadal hormones in female rats. It was observed that the seed caused a decrease in serum concentration of the gonadotropins (FSH and LH) and prolactin, while the alkaloid fraction of *G. kola* seed, caused changes in gonadal hormones in

female rats. It was observed 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. In another study, Akpanta et al. (2005) reported that ethanolic extract of *G. kola* seed blocked ovulation in female rats. The findings in the present study, coupled with other works which had been reported earlier, corroborate the rationale behind the traditional use of *G. kola* seed as contraceptive among women in some parts of Nigeria.

Conclusion

The findings in this study reveal that *G. kola* seed possesses anti-conceptive and weak estrogenic properties. Therefore, this work corroborates the rationale behind the traditional use of *G. kola* seed as contraceptive for women.

ACKNOWLEDGEMENT

The authors are grateful to Messrs Bala, Aniefiok Ukpong, Nsikan Malachy and Sifon Akpan for their technical assistance.

Conflict of Interest

Authors have not declared any conflict of interest.

REFERENCES

Akintowa AA, Essien AR (1990). Protective Effects of *Garcinia kola* Seed xtract against Paracetamol-Induced Hepatotoxicity in Rats. J. Ethno Pharmacol. 29(2):207-211.

Akpanta AO, Oremosu AA, Noronha CC, Ekanem TB, Okolawon AO (2005). The Effect of Crude Extract of *Garcinia kola* Seed on Ovulation in Female Rats. Niger. J. Physiol. Sci. 20(1-2):58-62.

Braide VB (1993). Anti-inflammatory Effect of Kolaviron, Bio-flavonoid of *Garcina kola.* Fitoterapia 64:433-436a.

Braide VB, Agube CA, Essien GE, Udoh FV (2003). Effect of *Garcinia kola* seed Alkaloid Extract on Levels of Gonadal Hormones and Pituitary Gonadotropins in Rats Serum. Niger. J. Physiol. Sci. 18(1-2):59-64.

Edgren RA, Calhoun DW (1957). The Biology of Steroidal Contraceptives. In:R.A.Edgren. The Chemical Control of Fertility. New York Marcel Dekker. pp. 537-552.

Ettebong EO, Nwafor PA, Ekpo M, Ajibesin O (2011). Contraceptive, Estrogenic Potentials of Methanolic Root Extract of *Carpolobia lutea* in Rodents. Pak. J. Pharm. Sci. 24(4):445-449.

Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HS (1980). Potential Value of Plants as a Source of New Antifertility Agents. Indian J. Pharm. Sci. (64):535-549.

Gaytan EE, Tinadas C, Morales CB, Ellido C, Sauchez-criado J (2002). Morphological evidence for uncontrolled proteolytic activity during the ovulatory process in indomethacin-treated rats. Reproduction 123(5):639-49.

Gupta RS, Rakhi S (2006). A review on medicinal plants exhibiting antifertility activity in males. Natl. Prod. Radiance 5:389-410.

Idiong OJ (2010). Contraceptive and Non-Estrogenic Effects of Methanolic Extract of *Similax krausiana* Root in Rodents. Master's Thesis.

Iwu MM (1985). Antihepatotoxic Constituents of *Garcinia kola* Seeds. Experintia 41:699-700.

Iwu MM (1993). Handbook of African Medicinal Plants. CRC Press, London. pp. 183-184.

Iwu MM, Igboko AO (1982). Constituents of *Garcinia kola* Seeds. J. Nat. Prod. 45:650-651.

Iwu MM, Igboko OA, Okunji CO, Tempesta MS (1990). Antidiabetic and Aldose Reductase Activities of Biflavonoids of *Garcinia kola.* J. Pharm. Pharmacol. 42:290-292.

Jacob D, Morris JMCL (1996). Estrogenic activity of postcoital anti-fertility compounds. Fertil. Steril. 20:211-222.

Joshi AR, Ahamed RN, Pathan K , Manivannan B (1996). Effect of *Azadirachta indica* Leaves on Testis and its Recovery in Albino Rats. Indian J. Exp. Biol. 34:109-1094.

Liang YC, Huang YT, Tsai OH, Shiau SY, Lin JK (1999). Suppression of Inducible Cyclooxygenase and Non-Inducible Nitric Oxide Synthase by Apigenin and Related Flavonoids in Mouse Macrophages. Carcinogenesis 20:1945-1952.

Miller LC, Tainter ML (1944). Estimation of LD_{50} and Its Error by Means of Logarithmic-Probit Graph Paper. Proc. Soc. Exp. Biol. 57:261.

Nwafor PA, Okwuasaba FK, Onoruvwe OO (1998): Contraceptive and Non-Estrogenic Effects of Methanolic Extract of *Asparagus pubescens* Roots in Experimental Animals. J. Pharmacol. 62:117-1.

Odebiyi OO, Sofowora EA (1978). Phytochemical Screening of Nigerian Medicinal plants. Lloydia 41:234.

Orie NN, Ekon EUA (1993). The Bronchodilator Effect of Garcinia kola. East Africa Med. J. 70(3):143-145.

Okunji CO, Iwu MM (1991). Molluscidal activity of *G. kola* biflavonones. Fitoterapia 67:74-76.

Rubin BL, Dorfman AS, Black L, Dorfman RI (1951). Bioassay of Estrogens Using the Mouse Uterine Response. Endocrinology 49:429-438.

Telleria CM, Mezzardri MR, Deis RP (1997). Fertility Impairment after Mefiprostone Treatment to Rats at Proestrus: Actions on Hypothalamic Ovarian Axis. Contraception 56:267-294.

Trease GE, Evans WC (1978). Pharmacognosy. Bailliere Tindall, London, UK. pp. 229-253,401-404,527-534.

Udoh FV (1998). Effects of Extracts of *Piper guineense* Leaf and *Garcinia kola* Seed on the Histology and Morphology of the Reproductive Organs in Male Rats. Master's thesis.

Udoh P, Patil DR, Deshpande MK (1992). Histopathological and Biochemical Effects of Gossypol acetate on Pituitary-gonadal axis of Male albino rats. Contraception 45:193-509.