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EFFECT OF ETHANOLIC EXTRACT OF GARCINIA KOLA SEEDS ON FOETAL DEVELOPMENT

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ABSTRACT
The incidence of children born with birth defects is 20% higher in Sub Saharan Africa than in developed countries and care must be taken to check the risk factors correlated to birth defects. *Garcinia kola* is used anecdotically as an anti-emetic during early pregnancy by many Nigerian women. In this study *Garcinia Kola* was evaluated for its potential to produce teratogenicity in rats following oral administration. Twenty five female Wistar rats with weight ranging from 170-250g were divided into 5 groups (n=5). They were mated with males in a 2:1 (female: male) ratio and mating was ascertained the following morning by the presence of spermatozoa in vaginal smear and that day was designated Day 0 of pregnancy. Group A(control) received feed and distilled water *ad libitum*, groups B, C and D were administered orally with 30, 100 and 1000mg/kg b. w of *Garcinia kola* Seed Extract respectively while group E received 200mg/kg b. w of Valproic Acid throughout gestation. On day 20, the animals were sacrificed and the following parameters were examined and recorded; uterine horns, resorption sites, live and dead foetuses, foetal weight, crown-rump length(C-RL), crown-heel length(C-HL) and abdominal circumference (ABD. Cir)The results showed resorption sites in the uterine horns of some animals in Groups C, D, and E. Foetal weight values in groups C(1.58±0.67), D(0.39±0.26) and E(1.40±0.59) were significantly lower ($p<0.05$) than the values in group A(3.97±0.20). The mean values of C-RL, C-HL and ABD. Cir. in goups B, C, and E were lower than A but not significant ($p<0.05$). However, C-RL, C-HL and ABD Cir. values in group D (0.79±0.49, 0.83±0. 51,0.92±0.58) were significantly lower ($p<0.05$)than group A(3.07±0.77,3.28±0.82,3.48±0.79) respectively. The findings suggest that *Garcinia Kola* may induce a dose dependent teratogenic effect in the rat model.

KEYWORDS: Garcinia kola, Teratogenic, foetal weight, crown-rump length, Wistar rats.

INTRODUCTION
Teratogens are substances that cause birth defects. Throughout human history, there has been evidence that exposure to toxic chemicals cause birth defects, however, this did not generate a lot of public concern until the 1961 Thalidomide episode which became an eye opener. Exposure to drugs during the period of pregnancy is very common among women in the society and many of these drugs may pose some risks to the developing embryo or foetus. Studies have shown that approximately 3-5% of live births are born with birth defects each year in the USA.[1] The incidence of children born with birth defects has been shown to be 20% higher in Africa than in developed countries.[2] Other researchers have also studied the incidence of birth defect in the Nigerian population and shown that in the South-South region there is an observed 0.4% incidence of birth defect,[3] in the South-East region , it is shown to be about 0.42%,[4] in the South-West it is about 1.58%, [5] and about 5.8% in the North-East region.[6] With the growing statistical reports on the incidence of birth defects, care must be taken to check the risk factors correlated to birth defects.

Garcinia kola Heckel (Clusiaceae), commonly called Bitter kola is a largely cultivated tree indigenous to sub-Saharan Africa and has been referred to as a "wonder plant"because almost every part of it has been found to be medicinal.[7] It is used traditionally in the treatment of cough, as a purgative, anti-parasitic, anti-microbial,[8,9] The seed has also been reported in the treatment of diarrhea,[10] bronchitis and throat infections,[11,12] It also possesses hypoglycemic properties,[13,14] anti-hepatotoxic properties,[15] and antioxidant properties.[16,17] Researchers have confirmed the contraceptive, anti-ovulatory, anti-inflamatory and anti-implantation properties of *Garcinia kola* (GK).[18, 19, 20] Studies have also shown that the alkaloid fraction of GK could alter serum level of gonadal hormones and histology of both male and female reproductive organs in rats[21] and may also produce duration dependent teratogenicity in rats.[19]

Since GK is used traditionally as a contraceptive,it is important to know what effect it will have on the foetus in an event that it fails as a contraceptive. This study was therefore carried out to evaluate its potential to produce teratogenicity in rats following oral administration.

MATERIALS AND METHODS

Test Material

Garcinia kola seeds were collected from their natural habitat in Degema town, Degema Local Government of Rivers State, Nigeria. The plant sample was identified by Dr. Chimezie, a Taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. A herbarium specimen voucher number UPH/C/076 was assigned to it and deposited in the Department of Plant Science and Biotechnology. The seeds were peeled, cut into small pieces and air-dried. The Dried seeds were pulverized and the powdered samples were stored in tightly closed reagent bottles for subsequent extraction and bioassay.

Extraction Method

The extraction of *Garcinia kola* seed was carried out using ethanol, methanol, acetone and distilled water as extracting solvents. The cold maceration extraction method of Cowan [22] used. 50g of the powdered sample was weighed and dissolved in 1000ml of the extracting solvent inside a 2litre conical flask and covered with parafilm.[23] The flasks were shaken vigorously at 30 minutes interval and left to stand for 24 hours at room temperature. The resultant mixture was then filtered with a filter paper (Whatman's No. 4 filter paper) and cotton wool to remove particles of plant sample. The clear solution obtained was distilled at 65^{o}C under low pressure on a steam bath. The semi-solid concentrations of the extracts were then collected in sterile pre-weighed screw capped bottles and labeled accordingly.[24] The extracts were stored at 4^{o}C until when needed.

Determination of phytochemical constituents

The extract was screened for the following constituents-, alkaloids, flavonoids, saponins, phenols and steroids using standard methods described by Onwuka.[24]

Animals

Twenty five (25) female Wistar rats weighing between 170-250g were used. The animals were allowed to acclimatize for two weeks prior to use. All rats were identified by unique markings on the ears as tags. Rats were housed in wire-mesh cages, with saw dust laid in as beddings which are changed every day to maintain a clean environment and reduce odour. The animals were housed in a cross-ventilated room under standard conditions of temperature and illumination (temperature 25±2.0°c, 12hr light /12hr dark cycle) and were fed with standard rat feed (Eastern Premier Feed Mills Limited, Calabar) and water *ad libitum* throughout the study.

Experimental Design

The oestrous cycles of Adult virgin female Wistar rats, approximately 12 weeks of age and 175—220 g were determined by daily vaginal smear analysis and the animals were selected after two consecutive 4-5day oestrous were confirmed in each of them. Each animal was smeared daily until at proestrous and then mated with males in a 2:1 (female/male) ratio. For mating to be ascertained, the animals were smeared the following morning and the presence of clumps of spermatozoa in the vaginal smears confirmed mating and the sperm-positive day was considered to be day zero of pregnancy. The animals were then divided into 5 groups (n=5) and treated as follows: Group A(control) received feed and distilled water *ad libitum*, groups B, C and D were administered orally with30, 100 and 1000mg/kg body weight of *Garcinia kola* Seed Extract respectively while group E received 200mg/kg body weight of Valproic Acid throughout gestation. On day 20, the animals were sacrificed. In order to sacrifice the animals, they were anaesthetized by chloroform inhalation method with the aid of a desiccator. Laparotomy was carried out and the foetuses removed, blotted dry and the following parameters were examined and recorded; uterine horns, resorption sites, live and dead foetuses, foetal weight, crown-rump length(C-RL), crown-heel length(C-HL) and abdominal circumference (ABD. Cir).

Statistics

The results were expressed as mean ± SEM and test of statistical significance was carried out using one-way analysis of various (ANOVA). The post hoc testing was performed for inter-group comparison using Turkey HSD multiple test. The statistical packaged used was the statistical package for social sciences (SPSS), (IBM®, version 20, Armonk, New York, USA).

RESULTS

Phytochemical Screening

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

Acute Toxicity Testing

This was done according to Lorke, 1983. 30 female rats weighing between 150-200g were used. The test was carried out by single oral administration of *Garcinia kola* seed extract(GKSE) in Distilled water at doses of 10, 100, 1000, 3000, 5000 and 6000 mg/kg to different groups of rats (5 rats per group). Mortality and general behaviour was observed continuously for one, three and intermittently for the next six hours and again at 24 and 48 hours. The parameters observed were gross behavioural changes, grooming, alertness sedation, and loss of righting reflex, tremors and convulsions. The LD50 of *Garcinia kola* seed extract was found to be 6741.40mg/kg and doses up to 1000mg/kg body weight

were found to be safe. All doses used in this study were carefully chosen to exclude the lethal dose.

Table 1: Result of Phytochemical Screening.

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

Maternal observations: All animals survived until the end of the experiment. Clinical signs of maternal toxicity, including excessive salivation, urine staining in the perineal region, porphyrin deposits about the eyes, vaginal bleeding, and tremors, were not observed during the dosing period among animals in the treatment groups. No effects were noted on the general appearance or demeanor of animals in the control or treatment groups. No appreciable changes in feed consumption were noted among any of the dose groups (data not presented).

Fetotoxicity: Values of foetal growth parameters taken are presented in Table 2. No effects on the pregnancy rate, or sex ratio were observed among any of the treatment groups.

The results showed resorption sites in the uterine horns of some animals in Groups C, D, and E. Foetal weight values in groups C, D and E were significantly lower ($p<0.05$) than the values in group A. The mean values of C-RL, C-HL and ABD. Cir. in groups B, C, and E were lower than but not significant ($p<0.05$).However, C-RL, C-HL and ABD. Cir. values in group D were significantly lower ($p<0.05$) than group A respectively.

Table 2: Effect of *Garcinia kola* Seed Extract (GKSE) on foetal growth parameters.

S/n	Groups	Foetal Weight(g)	CRL(cm)	CHL(cm)	ABD.CIR.(cm)	Resorption
1	Group A (Control)	3.97±0.02	3.066±0.77	3.28±0.82	3.48±0.79	NIL
2	Group B (GK30mg/kg)	2.01±0.59	3.54±0.05	3.05±0.71	3.07±0.69	NIL
3	Group C (GK100mg/kg)	1.60±0.68*	2.73±0.81	2.35±0.96	2.38±0.87	2/5
4	Group D (GK1000mg/kg)	0.39±0.26*	0.99±0.52*	0.83±0.52*	0.92±0.51*	3/5
5	Group E (VA200mg/kg)	1.39±0.59*	1.73±0.77	1.84±0.82	1.93±0.75	3/5

(*) Significantly different from controls ($p<0.05$), the values represent means± SEM, Numerator indicates Total resorption

DISCUSSION

G. kola seeds extract has been reported to cause resorption of implantation sites in pregnant rat.[20] These studies revealed that GKSE possess an antiprogestational property which prevents deciduoma formation in the

uterus thereby hindering foetal development. This is in consonance with the present study, confirmed pregnancies failed to develop when laparectomy was done, instead resorption sites were found. In another study carried out by[19] on effect of *G kola* seeds on pregnant female rats, it was observed that the extract may cause a dose dependent adverse effect on foetal development in rats. This is similar to the present study as seen in the low mean values of the foetal parameters with the lowest values on the highest dose of the extract.

Studies have shown that the exposure of a developing embryo or fetus to alkaloids from plants, plant products, or plant extracts has the potential to cause developmental defects in humans and animals.[27,28,21] carried out a study 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 alkaloid fraction of *G kola* seeds 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 phytochemical analysis carried out on the extract revealed the presence of alkaloids in appreciable amounts, although the mechanism of action was not part of this study, it could be that the alkaloid fraction of the *G kola* seed was responsible for the differences observed in foetal development with increasing doses as compared to the control group.

G kola has been reported by several authors to improve glucose tolerance to a degree similar to the oral hypoglycaemic drug, glibenclimide and dianil. [29, 30] However its management in gestational diabetes still remains a controversial issue since even a minor degree of hypoglycemia can adversely affect the reproductive outcome.[31] Significant morphological changes were observed in the developing fetus in the female Wistar rats treated with GK seed extract during gestation. Induced prenatal growth deficiencies observed in the litters include low mean birth weights, low mean crown rump length, low mean crown heel length and abdominal circumferences of fetuses compared to their control counterpart. This indicates intrauterine growth retardation (IUGR). The IUGR in this study was not due to the reduction of gestational length or preterm delivery, and was probably not due to intrinsic fetal factors such as chromosomal abnormalities or other malformations. However, it may be attributed to impaired glucose supply to the fetuses.

CONCLUSION

In summary, the present study shows that oral administration of Garcinia kola seed extract to pregnant rats causes intrauterine growth retardation, this effect is dependent on the dose administered for it to be toxic to the conceptus. This finding obliges to introduce caution in the use of Garcinia kola during pregnancy.

REFERENCES

1. Centers for Disease Control and National Birth Defects Prevention Network (NBDPN). Preventing Birth Defects. Available at http://www.nbdpn.org/current/2008pdf/PrevBDBroch.pdf (Accessed 15/1/2008.
2. Wolkam A. The Burden of Birth Defects and Genetic Disease in Sub-Saharan Africa, 2017.
3. Ekanem TB, Okon DE, Akpantah AO, Mesembe OE, Eluwa MA, Ekong MB. Prevalence of congenital malformations in Cross River and Akwa Ibom states of Nigeria from 1980–2003. *Congenit Anom (Kyoto)*, 2008; 48(4): 167–170.
4. Onyearugha CN, Onyire BN. Congenital malformations as seen in a secondary healthcare institution in Southeast Nigeria. *J Med Investig Pract*, 2014; 9: 59–62.
5. Iroha EO, Egri-Okwaji MTC, Odum CU, Anorlu ROI, Oye-Adeniran B, Banjo AAF. Prenatal outcome of obvious congenital malformation as seen at the Lagos University Teaching Hospital, Nigeria. *Niger J Paediatr*, 2001; 28(3): 73–77.
6. Mukhtar-Yola M, Ibrahim M, Belonwu R. Prevalence and perinatal outcome of obvious congenital malformations among inborn babies of Aminu Kano University Teaching Hospital, Kano. *Niger J Paediatr*, 2005; 32(2): 47–51.
7. Hutchinson J., Dalziel J.M. Flora of West Tropical Africa. 2nd ed. H.M.S.O; London, 1956; 11: 295.
8. Madubunyi II. Antimicrobial Activities of the Constituents of Garcinia Kola Seeds. Int. J. Pharmacognosy, 1995; 33(3): 232-237.
9. Oloyede OI, Afolabi IS. Antioxidant potential of Garcinia kola (leaf). Acad. Res. Int., 2012; 2(2): 49-54.
10. Braide VP. Antihep atotoxic Biochemical effects of kolaviron, a Biflavonoid of *Garcinia kola* seeds. *Phytotherapy Res*; 1991; 5: 35 - 37.
11. Orie NN, Ekon EU. The Bronchodilator effects of *Garcinia kola*. *East. Afr. Med. J*, 1993; 70: 143 - 145.
12. Adesina SK, Gbile ZO, Odukoya OA., Akinwusi DD, Illoh HC, Jayeola A A. Survey of indigenous useful plants of West Africa with special emphasis on medicinal plants and issues associated with their management. The United Nations University Programme on natural resources in Africa; 2nd edn, 1995; 84-85.
13. Iwu MM, Igboko OA, Elekwa OK, Tempesta MS, "Prevention of thioacetamide-induced hepatotoxicity by biflavanones of *Garcinia kola*," *Phytotherapy Research*, 1990; 4(4): 157–159.
14. Odeigah, PG, Taiwo IA, Akomolafe EO, Durojaiye OO. Hypoglycemic action of medicinal plants with tolbutamide in the albino rats. *Diabetes Intern*; 1999; 9: 71 - 73.
15. Akintowa, A. and Essien, A. Protective effects of *Garcinia kola* seed extracts against paracetamol induced hepatotoxicity in rats. *J. Ethnopharmacol*, 1990; 29: 207-11.

16. Olatunde, F. E., Akanni, O. O., and Emerole, G. O. Antioxidant and scavenging activity of flavonoid extract (kolaviron) of *Garcinia kola* seeds. *Pharmaceut. Biol*, 2002; 40: 107-116.
17. Terashima K, Takaya Y, Niwa M. Powerful antioxidative agents based on garcinoic acid from *garcinia kola*, *Bioorg Med Chem*, 2002; 10(5): 1619–25.
18. Braide VB. Anti-inflammatory effect of kolaviron, a biflavonoid extract of *Garcinia kola*. Fitoterapia; 1993; 433-36.
19. Akpantah AO, Oremosu AA, Noronha CC, Ekanem TB, Okolawon AO Effects of *garcinia kola* seed extract on ovulation, oestrous cycle and foetal development in cyclic female sprague - dawley rats *Niger. J. Physiol. Sci. 2005*.20 (1-2): 58-62.
20. Essien GE, Effiong GS, Anti-progestational, anti-ovulatory and anti-implantation potentials of methanolic extract of Garcinia kola seed in female rats. Int. Res. J. Pharm and Pharmacol, 2014; 4(2): 22-27.
21. Braide VB, Agube CA, Essien G E and Udoh FV. Effect of *Garcinia kola* seed alkaloid extract on levels of gonadal hormones and pituitary gonadotrophins in rat serum. *Niger. J. Physiol. Sci.ences*, 2003; 18: 59-64.
22. Cowan M.M. Antimicrobial Activity. Clinical Microbiology Reviews, 1999; 12(4) 564 – 582.
23. Duke JA, Wain KK Medicinal plants of the world. Computer enteries, 1981; 3.
24. Ogunjobi AA, Nnadozie N.Comparative effect of antimicrobial activites of *Ocimumgratissium*and *Vernoniaamygdalina. Bulletin of Science Association of Nigeria*, 2004; 25: 165-170.
25. Ogunjobi AA, Fagade OE, David OOAntimutagenic and potential Anticarcinogenic activities of Aloe-vera gel and aqueous Garlic extract in the Bacterial reverse mutation test (Ames assay) *Afr. J. Biomed. Res*, 2007; 10: 275 – 278.
26. Onwuka GI. Food Analysis and Instrumentation: Theory and Practice, Naphtali prints, Lagos, 2005; 136-139.
27. Lorke DA. New approach to practical acute toxicity testing. Arch Toxicol, 1983; 54: 275-287.
28. Yakubu MT and Musa IF Effects of Post-coital Administration of Alkaloids from *Senna alata* (Linn. Roxb) Leaves on some Fetal and Maternal Outcomes of Pregnant Rats J Reprod Infertil, 2012; 13(4): 211–21
29. Benedict TG, Stephen TL, Kevin DW and Kip EP, Plant alkaloids that cause developmental defects through the disruption of cholinergic neurotransmission, Birth Defects Research Part C: Embryo Today: Reviews, 2013; 99-4.
30. Adaramoye OA, Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats, Afr Health Sci, 2012; 12(4): 498–506.
31. Ofor CC,Oguwike F N, Onubueze D.P.M, Olisa M.C. Effects of Bitter Cola (Garcinia Kola) On Haemostatic and Biochemical Indiced Male Diabetic Albino Wistar Rats. Journal of Dental and Medical Sciences, 2013; 11(3): 53-57.
32. Ratnasooriya WD, Jayakody JRAC, Premakumara GAS.Adverse pregnancy outcome in rats following exposure to a Salacia reticulata (Celastraceae) root extract. Braz J Med Biol Res, 2003; 36(7): 931–5.