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Effects of ethanol extract of Garcinia kola Seed on the mammary gland: mode of action on lactation and gestational weight.

Olu SI[1], Olotu EJ[1], Oghenemavwe EL[1]
[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria

Corresponding Author: Olu SI
Email: swinogirl@yahoo.co.uk ; +2348056403347

ABSTRACT
This study investigated the effect of Garcinia kola seed on the mammary gland of adult female Wistar rats. Twenty five adult female Wistar rats with average weight of 170g were used for this study. They were divided into five groups (A-E) of five animals each; all animals were given rat feed and water ad libitum. Group A served as the control and received distilled water, groups B, C and D received ethanol extract of Garcinia kola seed at 30mg/kg, 100mg/kg and 1000mg/kg respectively, group E served as the standard control and was given 2.5mg of bromocriptine orally for a period of 20days. The animals were weighted weekly and their gestational weights were recorded. On the 20th day, Animals were sacrificed by cervical dislocation and the mammary glands harvested for histological assessment. The results showed no significant ($p>0.05$) increase in gestational weight of the treated groups. The histological sections of the mammary gland showed a massive decline in the size and number of lactiferous ducts in group B, C, D and E when compared to group A. The results therefore infer that ethanol extract of Garcinia kola seed reduces the lactation efficiency of the mammary glands in adult female wistar rats by reducing the sizes of the lactiferous ducts.

Keywords: *Ethanol, Garcinia kola, mammary gland,lactation, gestational weight*

INTRODUCTION

Breastfeeding provides numerous benefits to newborns and mothers. Breast milk provides abundant nourishment to a growing child[1], offering optimal nutrition, improved cognitive performance and neurological development[2] and enhanced immunity[3]. It reduces the high incidence of sudden infant death syndrome (SIDS), allergic diseases and development of type 1 and type 2 diabetes mellitus[4] relative to the use of infant formula. Because of the importance of breast milk and breast feeding, there are major concerns about lactating women who are under medications since Post partum activities such as lactation are affected by substances consumed by women; medicine circulating in the mother's bloodstream can potentially be transferred into the breast milk, exposing the infant to medicines that could be harmful[4]. The medicine might not only affect the baby but can also reduce the quality and quantity of milk produced by the breast which may deter the success and aim of breastfeeding[5]. Typical examples of such drugs include bromocriptine[6], pseudoephedrine[7]etc. Sometimes these medicines are not conventional drugs but are natural substances that reduce the supply of breast milk. They include peppermint, sage, garlic, parsley, yarrow, black walnuts[8].

Garcinia kola seed popularly known as bitter kola is a nut valued in Nigeria because of its medicinal and socioeconomic value. The name bitter kola is derived from its bitter taste. In Nigeria, *Garcinia kola* seed is known by different names in the different ethnic group; the Hausas call it Miji-goro, the Yorubas call it Orogbo while the Igbos call it Akiilu. It is popularly known for its medicinal function where it is used by African medicine men because of its purgative, anti-parasitic, and antimicrobial property[9]. Also the seeds are used for curing bronchitis, throat infection, colic, head or chest colds, cough and liver disorders[9]. Hence it is called an anti-poison. However there is paucity of scientific information on the effect of *Garcinia kola* seed on lactation. Hence this study is therefore aimed at determining the effect of ethanolic extract of *Garcinia kola* seed on the histology of the mammary gland of adult female wistar rats.

MATERIALS AND METHODS

Plant Material

Garcinia kola seeds were obtained from its natural habitat in Degema, Rivers State. They were identified and authenticated at the Herbarium of Plant Science and Biotechnology Department of the University of Port Harcourt and a voucher specimen number UPH/ C/076 was recorded for ease of identification.

Preparation of Extract

G*arcinia kola* seeds were washed, peeled and cut into small pieces, air dried and ground into powder form using a manual grinding machine. The extraction was done using a Soxhlet apparatus with ethanol as the solvent according to the method of Abioye (2003 *et al*)[10]. In brief; 1.5 kg of the powder

was put into the thimble of the Soxhlet apparatus containing 1L of ethanol. At the end of the procedure, the extract was dried at 40ºC. The yield obtained was stored in sterile bottles and kept in a refrigerator at 4ºC until use. Three different sub-lethal doses (30mg/kg, 100mg/kg, and 100mg/kg were used since the LD50 was 5000mg/kg body weight of rat.

Experimental Animals

Twenty-five female Wistar rats weighing between 150-170g were used for the experiment. The animals were obtained from the animal house of Department of Pharmacology, University of Port Harcourt. They were housed in wire mesh cages under standard condition of temperature and illumination with 12 hours light and 12 hours dark cycle and left to acclimatize for a period of two weeks in Madonna university animal house before the experiment commenced. The rats were given commercial rat chow ((Eastern Premier Feed Mills Limited, Calabar) and water *ad libitum.*

The mating process and confirmation of pregnancy

The oestrous cycles of the female Wistar rats used for the study 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 ratio (2 females to 1 male)[11]. For mating to be ascertained, vaginal smears were done in the mornings to show clumps of spermatozoa and the sperm-positive day was regarded as day zero of pregnancy[12].

Experimental Design

The rats which were confirmed pregnant were divided into five (A-E) groups (n = 5).Group A served as the control and received distilled 1ml of distilled water. Groups B-D received 30mg/kg, 100mg/kg and 1000mg/kg extract of Garcinia kola respectively, while group E was treated with a standard drug (bromocryptine) at 2.5mg. Administration was done per oral for 20 days. At the end of the treatment regime, the animals were sacrificed by cervical dislocation.

Histological Studies

A ventral laparotomy was done; the mammary glands were dissected and fixed in bouin's fluid for 72 hours. Tissues were processed for microscopic examination using a standard protocol and 5µm thick paraffin sections were were made. Slides were stained with routine hematoxylin and eosin stains photomicrographs were made at a magnification of X500 using leica microscope. **Statistical analysis:** Data were expressed as Mean±SEM, one way analyse of variance was used to analyze the experimental data. Turkey HSD was used to compare the group means obtained with the control. Differences were considered significant at $p<0.05$.

RESULTS

Effect on the Maternal Weight during gestation

Table 1 revealed the effect of ethanol extract of *Garcinia kola* seeds on mean weight of the female rats during gestation for each trimester. The result showed that there was a decrease in the mean weights of rats in group C compared to Group A, while there was limited weight gain in groups D and E compared to group A. However, none of these was statistically significant.

Effect on Mammary Gland Histological result: The group A section showed prominent lactiferous duct (LD), skin covering with smooth muscles (SM) and loose connective tissue (LC). The groups B and C sections show reduced sizes and number of lactiferous ducts while that of group D were all shrunken. The group E section shows smooth muscles(SM) and loose connective tissues (LC) with few lactiferous ducts.

TABLE 1: Effect of ethanol extract of the seeds of *Garcinia kola* on mean weight of the female rats during gestation for each trimester

Group (n=5)	Initial weight(g)	1st trimester Weight (g)	2nd trimester Weight(g)	3rd trimester Weight (g)
A(CONTROL)	176.8±14.0	182.2±14.1	189.4±14.7	209.0 ±14.6
B(GK30mg/kg)	186.6±29.1	187.6±31.6	197.4±33.7	219.4±38.7
C(GK100mg/kg)	268.0±10.4	283.6±12.4	251.6±15.1	264.4±17.3

D(GK1000mg/kg)	184.2±14.9	183.8±15.3	191.0±11.9	195.8±12.0
E(BROMOCRIPTINE2.5mg)	165.0±12.7	165.0±12.9	166.6±13.0	170.6±15

Values are expressed as mean ± SEM;* = p<0.05 considered significant relative to control

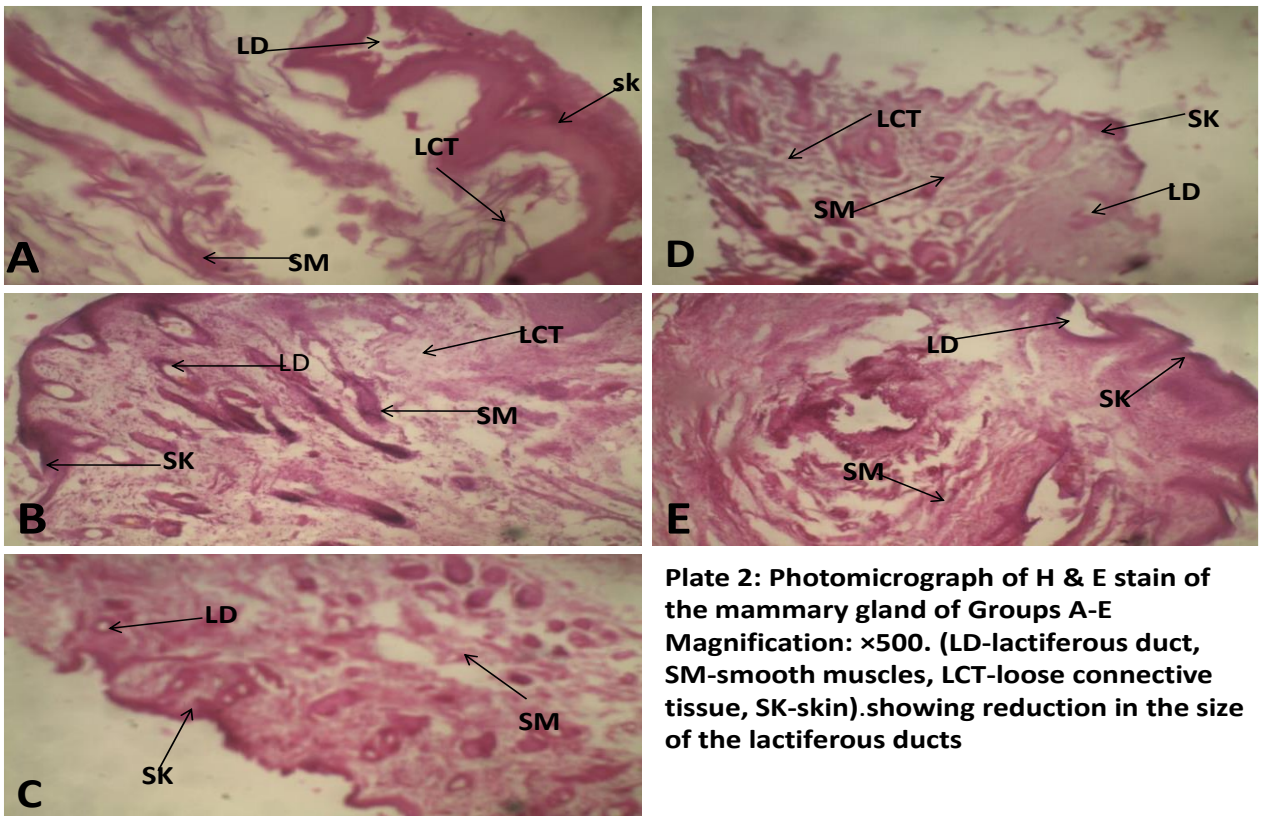

Plate 2: Photomicrograph of H & E stain of the mammary gland of Groups A-E Magnification: ×500. (LD-lactiferous duct, SM-smooth muscles, LCT-loose connective tissue, SK-skin).showing reduction in the size of the lactiferous ducts

DISCUSSION:

Studies have revealed that maternal body weight tend to increase as gestational age increases[14]. However, the weight gained or lost during gestation determines the outcome of the pregnancy[15]. Gestational diabetes and resultant birth defects have been shown to be outcomes of surplus weight in pregnancy; On the other hand insufficient maternal weight during the last trimester of pregnancy could bring about IUGR[14,16]. A steady weight increase was observed across all the trimesters of gestation in the standard and low dose groups. It was observed that those who received higher amounts of the extract and the bromocriptine recorded very small increase in weight across the trimesters and the differences in weight between these groups and the standard group was not significant. This may be due to the high dose effect of *Garcinia kola* on their weight. [17] in their study revealed that *Garcinia kola* seed helps in weight loss. [18] had also shown that *Garcinia kola* seed is able to suppress hunger and increase thirst hence increasing weight loss. Flavonoids from the plant have been reported to decrease lipid levels both in normal and hypercholesterolemic rats as reductions in triglycerides, phospholipids, and free fatty acids are well documented [19]. The suggested mechanism of action for flavonoids in weight and lipid profile reduction action may involve inhibiting lipogenesis, increasing lipid oxidation, and reducing food intake[20]. Food intake reduction may be because of the leptin like action as reported by[21].These suggested actions of flavanoids which are also present in *Garcinia kola* may also have accounted for the insignificant weight gain following administration of the low dose of the extract as well as the observed little or no increase in maternal weight higher dose of *Garcinia kola* seed extract.

There is variance in the histology of the lobules and ducts of the mature female breast which occurs as a result of fluctuations in hormonal levels, arising at different phases of physiological activities such as menstrual cycle, pregnancy, lactation and menopause[22]. During lactation, the hormone, prolactin stimulates proliferation of lactiferous duct making them prominent as observed in the control group. There was a decline in the lactiferous ducts across the treated groups with the high dose group having a higher decline in lactiferous ducts. The decline in the size of lactiferous duct was observed to be dose dependent. Lactiferous ducts also appeared not to be prominent in the bromocriptine group. This result agrees with the previous study by[6] that bromocriptine reduces the quantity of milk produced during lactation and could also be used for the treatment of hyperprolactinaemia. Hence, it can be inferred from this result that the seed of *Garcinia kola* could reduce the quantity of breast milk by decreasing the size and number of the lactiferous ducts. [23]had reported lactation suppression with synthetic ergot alkaloids such as Bromocriptine used in the current study. This has been reported by[24] also, they showed significant serum prolactin depression following administration of bromocriptine. This effect could be traced to the presence of alkaloids in the extract as revealed by their phytochemical analysis.

Conclusion

This study shows that *Garcinia kola* seed is able to interfere with lactation by reducing the lactiferous ducts of the mammary glands.

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