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A study on effects of *Garcinia kola* seeds on the pituitary gland during lactation

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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. On the 20th day, Animals were euthanized by cervical dislocation and the pituitary glands harvested for histological assessment. Blood samples were also collected for hormonal assay. The results of the hormonal assay showed higher values of estrogen, and progesterone compared to the control, while prolactin showed lower values compared to the control. The histological sections of the pituitary gland showed a massive decline in the number of acidophils in groups B, C, D and E when compared to group A and this is reflected in the cell count which showed a significant ($p < 0.05$) decrease in the number of acidophils present in the treated groups. The results therefore show that ethanol extract of *Garcinia kola* seed reduces the lactation efficiency of the pituitary gland in adult female wistar rats by reducing the number of the acidophilic cells of the anterior pituitary gland as well as the lactation hormones.

Keywords: Study, *Garcinia kola*, pituitary gland, lactation

1. Introduction

The pituitary gland in humans is a pea-sized gland. It is also called hypophysis and is an endocrine gland that weighs about 0.5 grams in humans. The pituitary gland is responsible for the secretion of Prolactin (PRL) commonly referred to as luteotropic hormone which is a protein that enables female mammals to produce milk. It is a peptide hormone, encoded by the PRL gene [1]. Milk production in mammals commonly referred to as lactation or breastfeeding provides numerous benefits to newborns and mothers. Breast milk provides abundant nourishment to a growing child [2], offering optimal nutrition, improved cognitive performance and neurological development [3] and enhanced immunity [4]. It reduces the high incidence of sudden infant death syndrome (SIDS), allergic diseases and development of type 1 and type 2 diabetes mellitus [5] relative to the use of infant formula. Studies have shown that synthetic alkaloids like bromocriptine could cause lactation suppression by inhibiting prolactin secretion [6, 7]. Because of the importance of breast milk and breast feeding, there are major concerns about lactating women who are under medications since postpartum activities such as lactation are affected by substances consumed by women; these medicines could affect the quality and quantity of milk produced by the breast which may deter the success and aim of breastfeeding [8]. Typical examples of such drugs include bromocriptine [9], pseudoephedrine 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 [10].

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^[11]. Also the seeds are used for curing bronchitis, throat infection, colic, head or chest colds, cough and liver disorders^[11]. However there is paucity of scientific information on the effect of *Garcinia kola* seed on lactation suppression. Hence this study is therefore aimed at determining the effect of ethanolic extract of *Garcinia kola* seed on the histology of the pituitary gland of adult female wistar rats and the hormones involved in lactation.

2. Materials and Methods

2.1 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.

2.2 Preparation of Extract

Garcinia 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^[12]. 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 40°C. The yield obtained was stored in sterile bottles and kept in a refrigerator at 4°C 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.

2.3 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*.

2.4 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)^[13]. 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^[14].

2.5 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 euthanized by cervical dislocation.

2.6 Histological Studies

The pituitary glands were excised 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 made. Slides were stained with routine hematoxylin and eosin stains photomicrographs were made at a magnification of X500 using leica microscope.

2.7 Cell Count (chromophils) of the Anterior Pituitary

The cellular content of the anterior pituitary gland were analyzed with emphasis on the chromophils (acidophils and basophils) using a software known as Motic Images Plus (MIPlus) 2.0 Set Up as template. The cell count (stereology) was done in order to scientifically ascertain the presence or absence of the chromophils and confirm if the extract either increased or reduced the cells or not. As such this will be an indicator of the extract's ability to either enhance (if it increases) or diminish (if it reduces) lactation. For elimination of bias the lap top screen measuring 25cm (Length) by 15.5cm (Width) was utilized as the photomicrograph screen. The software was then superimposed on three photomicrographs per anterior pituitary tissue slides of 15 animals (figure 3.4). Of the 15 animals the acidophils and basophils were recognized and counted using 3 animals each from the five groups (control and treatment groups). The superimposed software been placed on the photomicrograph was then sectioned into four equal rectangular frames measuring 298.5µm in width, 493.0µm in height, 147160.5 sq.µm in area and 1583.0µm in perimeter. Giving a total screen of 1194.0µm in width, 1972.0µm in height, 588642.0sq.µm in area and 6332.0µm in perimeter. Only cells that fell within the rectangles were counted to avoid error. In all, fifteen (15) photomicrographs of anterior pituitary tissues were analyzed.

2.8 Statistical analysis

Data were expressed as Mean±SEM, one way analyses 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.

3. Results

3.1 Effect of ethanol extracts of the seed of *Garcinia kola* on hormonal profile

Table 1 shows the effects of ethanol extract of the seeds of *Garcinia kola* on the hormonal profile of adult female Wistar rats were presented. The results revealed that the values of Estrogen were significantly higher in group B (242.60±17.41) and D(188.00±1.41) in contrast to Group A (164.20±12.84), while groups C and E were significantly lower compared to group A. The results for progesterone showed that groups C (6.72±1.12) and E (4.76±0.05) were significantly less than group A (10.96±0.87) while group B (12.46±0.50) and D (13.72±0.67) were higher than A but not significant. The results for prolactin showed significant increase in groups C (9.18±0.67) and E (4.90±0.59) compared to group A (1.74±0.20) while groups B (1.24±0.97) and D(1.32±0.12) showed a reduction in the values compared to group A but not statistically significant.

3.2 Effect on the Cell count of the Anterior Pituitary Cells

Table 2 shows the effect of the ethanol extract of the seeds of *Garcinia kola* on Acidophil and Basophil cell count in the five different animal groups using the different treatment agents was presented. The results revealed that all the treated groups were statistically significant, (P < 0.05) from the control in Acidophils while statistical significance was seen only in Groups B and D in Basophils.

3.3 Effect on Pituitary Gland Histology

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 the hormonal profile of adult female wistar rat

Groups (n=5)	E ₂ (pg/ml)	PROG(ng/ml)	PRL(ng/ml)
A(CONTROL)	164.20±12.84	10.96 ±0.87	1.74±0.20
B(GK30mg/kg)	242.60±17.41*	12.46±0.50	1.24±0.97
C(GK100mg/kg)	78.20±1.98*	6.72±1.12*	9.18±0.67*
D(GK1000mg/kg)	188.00±1.41*	13.72±0.67*	1.32±0.12
E(BROMOCRIPTINE2.5mg)	116.40±4.03*	4.76±0.05*	4.90±0.59*

KEY: Values are expressed as mean± SEM; significant @ (p < 0.05)

Legend: E₂-Estrogen, PROG- Progesterone, PRL- Prolactin, GK- *Garcinia kola*

Table 2: Effect of ethanol extract of the seeds of *Garcinia kola* on cell count of the pituitary gland sections

S/N	GROUPS(n=5)	ACIDOPHILS	BASOPHILS
1	GROUP A (CONTROL)	18.80±1.31	57.80±4.77
2	GROUP B (GK30mg/kg)	4.30±0.95*	13.75±4.85*
3	GROUP C (GK100mg/kg)	8.75±0.5*	63.25±5.39
4	GROUP D (GK1000mg/kg)	2.50±1.04*	23.25±3.15*
5	GROUPE (Bromocriptine2.5mg)	5.50±0.87*	56.75±4.71

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

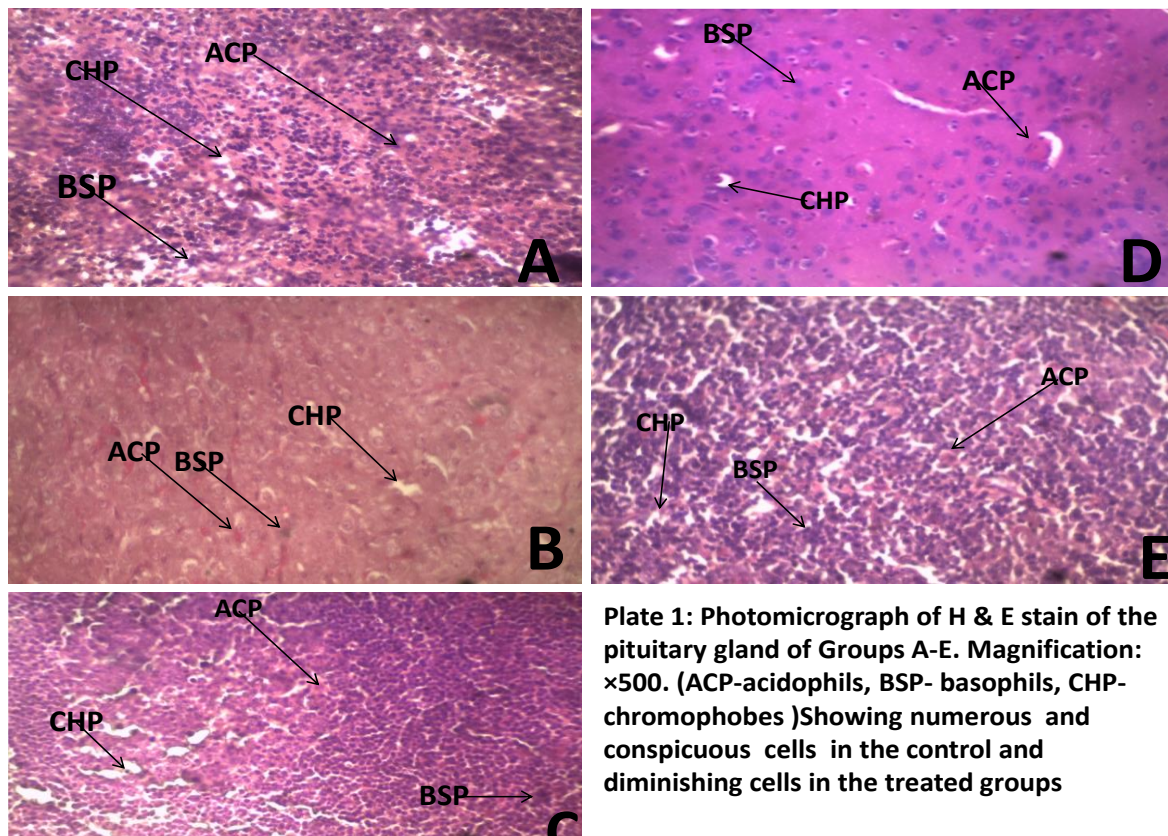


Plate 1: Photomicrograph of H & E stain of the pituitary gland of Groups A-E. Magnification: x500. (ACP-acidophils, BSP- basophils, CHP- chromophobes) Showing numerous and conspicuous cells in the control and diminishing cells in the treated groups

4. Discussion

The hormones progesterone, estrogen and prolactin are essential female hormones. The level of progesterone and estrogen the body produces during pregnancy determines the amount of prolactin which helps in milk production. The amount of progesterone and estrogen present in the

blood decreases towards the completion of gestation while prolactin level increases [15]. However, the result of the current investigation shows a significant increase in estrogen and progesterone level in group B (30mg/kg) and D (1000mg/kg) when compared with the CTRL group. This result is in agreement with previous study that the seed of

Garcinia kola caused a decrease in serum level of the gonadotropins (FSH and LH) and prolactin, while remarkably increasing the serum level of estradiol and progesterone [16]. *Garcinia kola* seed acts on prolactin via negative feed-back mechanism by increasing progesterone and estrogen concentration in the females [16]. This increase now acts through negative feed-back mechanism to reduce the prolactin level produced in the wistar rat, hence suppressing lactation just like the bromocriptine which acts as an anti-lactating agent [17].

Acidophilic cells (α - cells, lactotrophs) of the anterior pituitary gland are responsible for the production of the prolactin hormone during lactation [18]. Hence, they enlarge during pregnancy. Presence of acidophils in the anterior pituitary gland indicates production of prolactin [19], this agrees with the result seen in the control group. There was a marked decrease in acidophils in the treated groups especially the high dose group. Hence it can be deduced that the reduction in acidophilic cells led to the reduction in the level of prolactin production across the groups when compared to the control group which had large amounts of acidophils. The decline in the number of acidophils was also observed in the bromocriptine group. This result agrees with the previous study by [9] that bromocriptine reduces the quantity of milk produced during lactation and could also be used for the treatment of hyperprolactinaemia. Hence, it can be deduced from this result that the seed of *Garcinia kola* could reduce the quantity of breast milk by decreasing the number of the acidophils. [20] Had reported lactation suppression with synthetic ergot alkaloids such as Bromocriptine used in the current study. This has also been reported by [6], they showed significant serum prolactin depression following administration of bromocriptine. Hence this effect observed in the groups treated with the extract of *Garcinia kola* seeds could be traced to the presence of alkaloids in the extract as revealed by the phytochemical analysis.

5. Conclusion

This study has shown that *Garcinia kola* seed is able alter the hormonal function and also interfere with lactation by reducing the acidophilic cells of the anterior pituitary gland.

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