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DISTORTIONS IN REPRODUCTIVE FUNCTIONS OF AQUEOUS BITTER KOLA (*GARCINIA KOLA*) SEED EXTRACT ADMINISTRATION ON WISTAR RATS

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ABSTRACT

This study investigated the changes in female reproductive hormones (hormonal profile) and relative ovarian weights due to administration of aqueous extract of bitter kola (*Garcinia kola*) on female Wistar rats. Thirty (30) adult wistar rats of between 150 g – 200 g were procured, acclimatized and randomly selected into five (5) groups of six (6) rats each. Group A (Control) received standard rat diet and water *ad libitum*, while group B rats were fed with 100 mg/kg body weight of aqueous extract of *Garcinia kola.* Groups C, D and E received 200 mg/kg, 300 mg/kg and 400 mg/kg body weight of aqueous extracts of *Garcinia kola* respectively. After twenty-one (21) days of administration of test substance, the rats were sacrificed via cervical dislocation and blood samples collected and assayed for serum hormonal levels [Follicle Stimulating Hormone - FSH, Luteinizing Hormone - LH, Oestrogen and Progesterone]. Study also determined the comparative changes in body / ovarian weights of animals before and after administration of test substance, following which the Statistical Package for Social Sciences (SPSS, version 20) was used to conduct statistical test (student t-test) on obtained data; at p-values ≤ 0.05 as significant level, study observed a significant increase in body with increasing dose of *G. kola* administration. *G. kola* also caused an increase in serum hormonal levels of female wistar rats, promoting oogenesis and maturation of the ovarian follicle. Further studies aimed at corroborating these results should be carried out.

KEYWORDS: *Gacinia kola*, FSH, LH, Estrogen, Progesterone.

INTRODUCTION

In Africa, *Garcinia kola* seed is commonly recommended in folklore medicine for the treatment of diabetes and its associated complications.[1,2] *Garcinia kola* seed administration significantly ameliorate hyperglycemia mediated damage by decreasing the blood glucose level, enhancement of the antioxidant system, inhibition of lipid peroxidation, and improving the architecture of the kidney, liver, and testes in STZ-induced diabetic rats.[1] In addition, *G. kola* seed intervention has been found to restore the kidney and liver function biomarkers, the sperm characteristics as well as the plasma levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, triiodothyronine (T_3), and thyroxine (T_4) to normal in STZ-induced diabetic rats.[1, 2]

Extracts of the plant have been traditionally used for ailments such as laryngitis, liver diseases and cough.[3] The seeds are used to prevent or relieve colic, cure head or chest colds and relieve cough (Iwu, 1993). The seed also has anti-inflammatory, antimicrobial, antidiabetic and antiviral as well as antiulcer properties.[4] Various extracts of *Garcinia kola* have been found to elicit a number of biochemical properties including hepato-protection, antidiabetic properties and antigenotoxic potentials.

Studies involving bioassay-guided fractionation of *Garcinia kola* seed have yielded complex mixtures of phenolic compounds, triterpenes and benzophenones.[5,6] Iwu *et al.,* (1990) isolated cycloartenol and its 24-methylene derivatives from the petroleum spirit extract of the seeds. The ethyl acetate-soluble fraction of the acetone extract contains biflavanones with ether soluble fractions. Recently, one of the major components of *Garcinia* biflavonoid was isolated from the roots of *Garcinia kola*. It showed inhibitory effects against methicilin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE). Terashima *et*

al., (2002), also reported two new chromanols, garcinoic acid and garcinal, together with δ-tocotrienol isolated from *Garcinia kola* seeds.[6]

The acclaimed health effects of *G. kola* seed against liver and reproductive disorders in traditional medicine and its proven ability to suppress oxidative stress in different experimental models of organ toxicity increases curiosity into its effect sleep deprived induced stress on the hypothalamic-pituitary-gonadal axis-hypothalamus (HPGA) axis in wistar rats. These affirm the fact that it could have effect on the hormones of the hypothalamic-pituitary-gonadal axis. Considering the anti-oxidant capacity of *G. Kola*, it becomes necessary to determine its effects on the female reproductive system.

The principal organs of the human female reproductive system are quit intricate; the most important of which are the ovaries, fallopian tubes, uterus, and the vagina.[7,8] The female hormonal system, like that of the male, consists of three hierarchies of hormones, as follows.

1. A hypothalamic releasing hormone, gonadotropin-releasing hormone (GnRH).
2. The anterior pituitary sex hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which are secreted in response to the release of GnRH from the hypothalamus.
3. The ovarian hormones, oestrogen and progesterone, which are secreted by the ovaries in response to the two female sex hormones from the anterior pituitary gland. These various hormones are not secreted in constant amounts throughout the female monthly sexual cycle; they are secreted at drastically differing rates during different parts of the cycle.

Aim of Study

This study aimed at investigating the changes in hormonal profile / reproductive functions of aqueous bitter kola (*garcinia kola*) seed extract administration on female wistar rats. Specifically, study;

i. Examined the effects different doses of *G. Kola* on the ovarian weights
ii. Determined the changes in serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) due to administration of *G. Kola* to female wistar rats.
iii. Investigated the changes in serum Oestrogen and Progesterone levels of *G. Kola* fed, female Wistar rats.

MATERIALS AND METHOD

Study Design

Thirty (30) adult female wistar rats of an average weight of about between 160g - 200g were procured and acclimatized for two (2) weeks. The rats were then maintained in the Animal Holding of the Department of Public and Community Health, College of Health Sciences, Novena University, Ogume, Delta State and randomly selected into five (5) groups of six (6) rats each as follows.

Group A (Control): Received standard rat diet and water *ad libitum.*
Group B: Received 100 mg/kg body weight of aqueous seed extract of *Gacinia Kola*.
Group C: Received 200 mg/kg body weight of aqueous seed extract of *Gacinia Kola* (Olaleye *et al.*, 2006, 2014).
Group D: Received 300 mg/kg body weight of aqueous seed extract of *Gacinia Kola* (Obi and Nwoha, 2014).
Group E: Received 400 mg/kg body weight of aqueous seed extract of *Gacinia Kola* (Onasanwo and Rotu, 2014).

The treatment materials were administered twice daily for a period of two-week via oral gavage. The weights of animals were measured weekly and administration lasted for 14 days.

Preparation of *G. kola* Extract

About 8.2kg of powdered samples (blended *Garcinia kola*) was weighed into a glass container and 5 liters of solvent (pure n-hexane) was added stirred at intervals of 2 hours and was left to stand for 72 hours. The defatting process was repeated by adding another 2 liters of pure n-hexane to the plant shaft for another 72hours. This was done to properly remove the fat present in the *Garcinia kola*. The solvent (n-hexane) containing the crude fat was collected. The solvent (n-hexane) containing the crude fat collected after 72 hours was added together and concentrated using a rotary evaporator after being filtered, it was then set at 40˚C and was further concentrated in a vacuum oven at temperature of 40˚C and pressure of 600mm Hg.

The *Garcinia kola* shaft (that is, defatted seeds) was spread and air-dried for 5 hours so as to remove the traces of n-hexane used. The defatted, dried marc was then repacked into a glass container and 5liters of solvent (water) was added stirred at intervals of 2 hours and was left to stand for 72 hours. The process was repeated by adding another 5 liters of distilled water to the plant shaft for another 72hours. The solvent (pure water) containing the crude aqueous extract was collected after 72 hours was concentrated using a rotary evaporator after being filtered it was set at 40˚c and was further concentrated in a vacuum oven at temperature of 40˚c and pressure of 600mm Hg.

The crude extract was made into solution with methanol and equal volume of water was added. It was done in batches 200ml of this mixture (methanol/water) was added 200ml of chloroform and transferred into a separating funnel of 500ml and was carefully shaken and allowed to stay for 30minutes for proper partitioning of the chloroform and mixture (methanol/water) layer. This process was repeated four times for proper extraction of kolaviron (active component of *G. Kola*) with the aid of chloroform. The chloroform fraction was collected and concentrated using a rotary evaporator, it was set at 40˚C. The crude chloroform fraction was further concentrated in a vacuum oven at 40˚c in the pressure of

600mmHg as to properly remove any trace of solvent (chloroform).
Percentage yield was calculated as follows;

$$\% \text{ yield} = \frac{\text{weight of extract} \times 100}{\text{weight of plant sample used}}$$

Preparation of Stock Solutions from *G. Kola* Extract
After weighing 2g of *G. Kola* with electronic balance, the substance was then homogenized in pestle and mortar using 10ml of distilled water, and then filtered with Wattmann filter paper. This gave a 200mg/ml stock solution. Graded doses of *G. Kola* [high, medium, low and very low] were estimated from previously established lethal dose (192mg/kg). About 1g, 2g, 3g and 0.4g were dissolved in 100 ml, 200ml, 300ml and 400ml of distilled water to make the aforementioned stock solutions respectively. The body weights of the animals were then taken and the dose of test drugs in millilitre to be administered was calculated.

Administration of *G. kola* Extract Stocks
The rats in the treatment groups received calculated doses of G. kola stocks (aforementioned) per kilogram body weight per day for a duration of two weeks. This was achieved through the use of orogastric tube, while the control rats received equal volume of distilled water through the same route and for the same period.

Sacrifice of Animals
Animals were sacrificed through cervical dislocation on twenty–second day of the experiment. The abdomino-pelvic cavity was quickly opened to expose the ovary for extraction, following which it was weighed and recorded using mettler Toledo weighing balance

Blood Sample Collection
At the end of the twenty first (21) day, the animals were euthanized by cervical decapitation and blood samples were collected from the superior vena cava. The sample was centrifuged at 3000rpm for 15minutes and the sera collected were stored frozen. The animals were dissected and the testes were removed, cleared of adherent tissues and weighed immediately using an electronic weighing balance. Of each animal euthanized, one Ovary was used for histological studies while the other Ovary was homogenized in 100mM Phosphate buffer (pH 7.4), centrifuged at 3000rpm for 15minutes.

Dissection of the Ovary and Uterus
Following the sacrifice of rats by cervical dislocation, the abdomino-pelvic cavity was opened up and the ovary and uterus were dissected out, dried and weighed. The organs were quickly fixed in 10% formal saline for routine histological studies after Hematoxylin and Eosin staining.

Biochemical Techniques
Preparation of Sample
The different serum samples from the experimental animals were collected using a 2ml syringe. For each group, the obtained serum was centrifuged at 3500rpm for 5 minutes with the aid of a centrifuge. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated till needed for the assays.

Immunoassay of Hormones
Serum samples from the experimental animals were assayed for serum concentration of follicle stimulating hormone (FSH), luteinizing hormone (LH) Estrogen and Progesterone using Enzyme-Linked Immunosorbent Assay (ELISA). The hormonal assay was done at the Capitol Hill Hospital and Laboratory, Airport road, Warri, Delta State. Nigeria. ELISA kit was used for the immunoassay. Basically, the procedure for running the assays was the same.

Morphometric Techniques
The animals' body weights were measured before and after administration of test substance using the weighing balance. The body and ovary weights were measured using Mettler Toledo weighing balance as at the time of sacrifice of the experimental animals.

Statistical Analysis
The results were calculated using mean and standard error of means (SEM) respectively. The results from the various assays were analyzed using one way ANOVA test and for significant level of ($p < 0.05$) as taken below variables. The graphical summary of the observation on the selected oxidative stress parameters on the serum were presented.

RESULTS

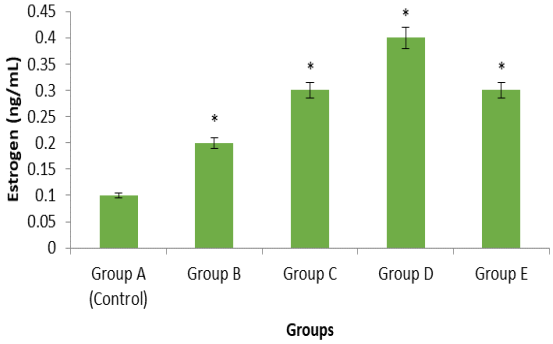

Figure I: Comparative Effects of Graded Doses of G. Kola Extract on Serum Oestrogen Levels.

Figure I above shows the changes in Oestrogen levels of administration of graded doses of G. Kola extract to female Wistar rats. From the figure, a statistically significant ($p < 0.05$) increase is seen in Oestrogen levels with increased regimen of test extract. It was further observed that G. Kola extract attenuated the Serum Oestrogen levels of female wistar rats in a dose dependent manner, though significantly increased ($p < 0.05$) upon comparison with control group. * = $p < 0.05$ compared with control rats;

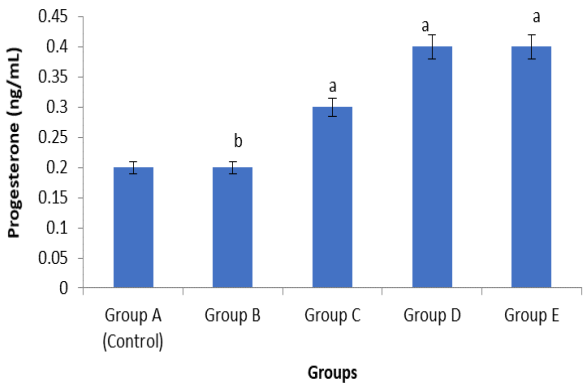

Figure II: Comparative Effects of Graded Doses of G. Kola Extract on Serum Progesterone Levels.

Figure II above shows the changes in Progesterone levels in administration of graded doses of G. Kola extract to female Wistar rats. Here, a statistically significant ($p < 0.05$) increase is seen in Progesterone levels across groups except the low dose treated group (Group B) with increased doses of test extract. This is suggestive that G. Kola extract caused a significant increase ($p < 0.05$) in Serum Progesterone levels of female wistar rats in a dose dependent manner a = statistically significant increase, b = statistically insignificant increase at $p < 0.05$ compared to control group

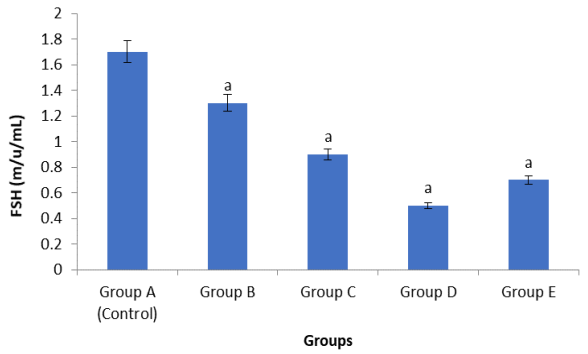

Figure III: Comparative Effects of Graded Doses of G. Kola Extract on Serum Follicle Stimulating Hormone (FSH) Levels.

Figure III above shows the changes in Follicle Stimulating Hormone (FSH) levels in administration of graded doses of G. Kola extract to female Wistar rats, a statistically significant ($p < 0.05$) decrease is seen in FSH levels across groups. This decrease was however attenuated on administration of high dose extract to wistar rats as seen in group E. a = statistically significant decrease at $p < 0.05$ compared to control group.

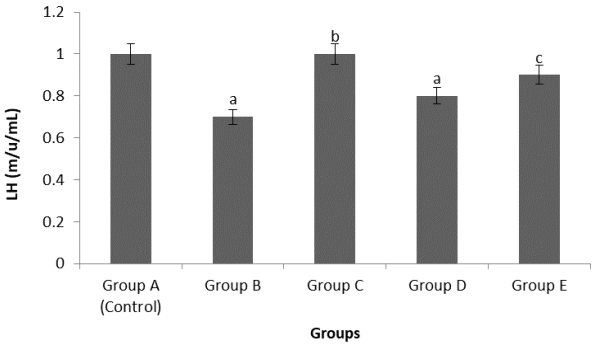

Figure IV: Comparative Effects of Graded Doses of G. Kola Extract on Serum Luteinizing Hormone (LH) Levels

Data from figure IV (above) of this study shows changes in serum levels of Luteinizing Hormone (LH) with statistically insignificant, but progressive increase (with graded doses) in G. Kola administration to female Wistar rats ($p < 0.05$). This decrease was reversed following administration of G. Kola in a dose dependent manner when compared to control despite the increase. a = significant decrease ($p < 0.05$), b = insignificant increase, c =

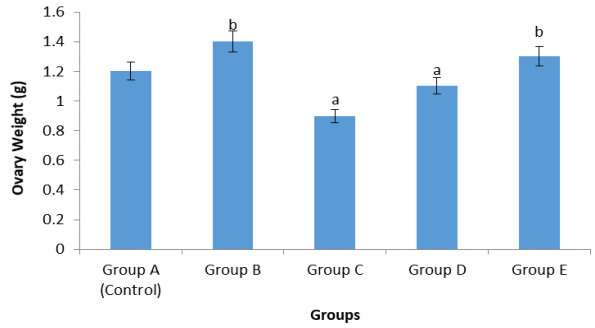

Figure V: Comparative Effects of Graded Doses of G. Kola on Ovarian Weights

Figure V shows the changes in ovarian weights in female wistar rats fed with graded doses of G. Kola aqueous extract. Here, ovarian weight was significantly ($p < 0.05$) decreased in groups C and D rats when compared to normal rats (control). a = significant decrease, b = significant increase

DISCUSSION

Results from this study reveals a reduction in the ovary weight of female wistar rats fed with aqueous extract of *G. Kola* when compared to control group. In this study, relative organ weight of the ovary (figure V.) was observed. Here, control group had 1.41% increment, Group B; 1.25%, Group C; 1.34% and Groups D and E; 1.47% and 1.44% respectively. There were also mild alterations in relative organ and body weights with increase doses; but not significant ($p < 0.05$) when compared in between groups.

Both acute and chronic *G. Kola* administration have been found to be intricately associated with changes in sleep patterns and cognitive deficits both in preclinical as well clinical studies, resulting in weight loss.[9] This finding concurs with the results from figure 4.1 and 4.6 of the current study.

In this study, we also examined female hormonal parameters such as serum Oestrogen, Progesterone, FSH and LH levels. A statistically significant increase ($p < 0.05$) was found in most of the female sex hormones with increased dosage of *G. kola* compared to those of the control group. There were however insignificant decreases in Oestrogen and Progesterone levels in low and medium dose groups. Though the precise mechanism responsible for these differences between treatment groups is not known, however, as mentioned above, sleep deprivation due to *G. kola* administration may have induced stress as an intrinsic part of many injurious health problems with endocrinologic, immunologic and metabolic consequences. Therefore, it is expected that unknown factors that affected sleep duration due to *G. kola* administration may have contributed to the differences in changes in female sex hormone across treatment groups.[10]

The changes in sex hormones due to stress hormone (Cortisol) concentrations in sleep deprived animals fed with *G. kola* had been studied. [11] From available records, female sex hormone levels were observed to have significantly increased as compared with the control group. This single finding implicates that G. kola mediates its effect on the male and female reproductive system, possibly via sleep deprivation and elevation of stress hormone levels. These results are in agreement with those of numerous studies that established changes in steroid hormone levels in sleep deprived animals.[11,12] Furthermore, it has been shown that stress induced by chronic *G. kola* administration can exhibit much higher glucocorticoid potency on female sex hormones explaining the possible mechanism of action of G. kola as observed in this study.

Interestingly, the stimulation of the female gonads by gonadotropin-releasing hormone (GnRH) causes release of FSH and LH from the pituitary for transport through blood to their sites of action. More so, FSH is known to stimulate the maturation of Graafian follicle, while LH synthesizes estrogen from testosterone by aromatization. From current study, the declining Estrogen levels at higher doses as observed in this study maybe due to inhibitory effects of *G. kola* on pituitary gonadal axis and direct toxic effect on follicular and theca cells in the female Wistar rats. According to Amah *et al.*, (2012) and Ladan *et al.*, (2013) such suppression could ultimately result in variations in the estrous cycle as well as other hormone interaction and secretion.

Physiologically, the pituitary secretion of gonadotropins is influenced by hypothalamic GnRH pulse frequency and amplitude (Gill *et al.*, 2002). It is well known from animal and human models that low-dose estrogen helps to define the gonadotropin secretory profile in women by inhibiting hypothalamic secretion of GnRH (Welt *et al.*, 2003). Hence, a significant decrease ($p < 0.05$) in the oestrogen in a dose dependent manner of *G. kola* may likely result in spontaneous abortions.

Research has shown that the hormonal activity of the anterior lobe is controlled by chemical messengers sent from the hypothalamus through tiny blood vessels to the anterior lobe (William, 2006). In the 1950s, the British neurologist Geoffrey Harris discovered that cutting the blood supply from the hypothalamus to the pituitary impaired the function of the pituitary. In 1964, chemical agents called releasing factors were found in the hypothalamus; these substances, it was learned, affect the secretion of growth hormone, a thyroid-stimulating hormone called thyrotropin, and the gonadotropic hormones involving the testes and ovaries.[13] In 1969 the American endocrinologist Roger Guillemin and colleagues isolated and characterized thyrotropin-releasing factor, which stimulates the secretion of thyroid-stimulating hormone from the pituitary. In the next few years his group and that of the American physiologist Andrew Victor Schally isolated the luteinizing hormone-releasing factor, which stimulates secretion of both LH and FSH, and somatostatin, which inhibits release of growth hormone. For this work, which proved that the brain and the endocrine system are linked, they shared the Nobel Prize in physiology or medicine in 1977.

The presence of the releasing factors in the hypothalamus helped to explain the action of the female sex hormones, oestrogen and progesterone, and their synthetic versions contained in oral contraceptives, or birth-control pills. During a woman's normal monthly cycle, several hormonal changes are needed for the ovary to produce an egg cell for possible fertilization. When the oestrogen level in the body declines, the follicle-releasing factor (FRF) flows to the pituitary and stimulates the secretion of the follicle-stimulating hormone. Through a similar feedback principle, the declining level of progesterone causes a release of luteal-releasing factor (LRF), which stimulates secretion of the luteinizing hormone. The ripening follicle in the ovary then produces oestrogen, and the high level of that hormone influences the hypothalamus to shut down temporarily the production of FSH. Increased progesterone feedback to the hypothalamus shuts down LH production by the pituitary. The daily doses of synthetic oestrogen and progesterone in oral contraceptives, or injections of the actual hormones, inhibit the normal reproductive activity of the ovaries by mimicking the effect of these hormones on the hypothalamus.[13]

Though the exact mechanism underlying the alterations and/or interactions of FSH and LH is not fully

understood; however, several related pathways have been revealed that circulating levels of FSH and LH stimulate or inhibit the hypothalamus-pituitary-gonadal (HPG) axis leading to decreased decrease and / or increased Oestrogen and progesterone secretion (Breen, 2006). Additionally, declines in their level of production may be associated with activation of the hypothalamus-pituitary-adrenal (HPA) axis inducing elevations in corticosteroid.[14] Consequently with some degree of stress, Oestrogen and Progesterone levels decrease via inhibition of the HPG axis caused by elevated levels of serum FSH and/or LH under regulation by the hypothalamus and pituitary gland.

CONCLUSION

Present study demonstrated that prolonged administration of aqueous extract of *G. kola* on wistar rats at variable doses greatly increased oxidative stress marker activities and influenced serum Oestrogen, Progesterone, luteinizing hormone and follicle stimulating hormone. The administration of *G. kola* extracts therefore possess as a good source of antioxidant activities to reduce the possible damage due to tissues.

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