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The Effects of Kolaviron (Methanolic Extract of *Garcinia kola* Seeds) on the Histoarchitectural Studies of the Hypothalamo-pituitary-gonadal Axis in Female Wistar Rats

Sunday Adelaja Ajayi

Kolaviron is known for its anti-inflammatory property whereas ovulation occurs through inflammatory process; this work therefore, determined its relationship to infertility. A total of thirty adult female Wistar rats were used for this experiment. The animals were randomly divided into three groups: A, B and C with ten animals in each group. Extracts were administered to the animals orally for 14 days. Group A (control) were given distilled water; Group B were given kolaviron at concentration of 200 mg kg^{-1} b.wt. once daily and Group C were given kolaviron at concentration of 100 mg kg^{-1} twice daily. At the end of the experimental period, the animals were anaesthesized with chloroform inhalation. The hypothalamus, anterior pituitary and ovary were removed, weighed and fixed in 10% formol calcium for Haematoxylin and Eosin staining, demonstration of Nissl substance in hypothalamus and demonstration of special stain using Aldehyde Fuschin Thionin for delta cells of anterior pituitary gland. The results showed that Relative brain weight ($F = 12.348$, $p<0.05$) and relative gonads weight ($F = 10.700$, $p<0.05$) were significantly difference across the groups. The histoarchitechture of the ovary showed that kolaviron ingestion by the female wistar rats revealed no growing follicles which were obvious in control group, the dump-bell shapes of the delta cells in the anterior pituitary gland were normal in group A but distorted in groups B and C while kolaviron administration showed no effect on the hypothalamus. In conclusion, kolaviron altered the histoarchitecture of hypothalamo-pituitary-gonadal axis which could cause infertility in female wistar rats.

Key words: Kolaviron, hypothalamus, anterior pituitary gland, ovary and infertility

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INTRODUCTION

Kolaviron is an extract of *Garcinia kola* seeds which has anti-inflammatory activity (Braide, 1993; Madubunyi, 1995). It was stated by Epsey (1980) that the process of ovulation is comparable to an inflammatory process. Anti-inflammatory drugs had been used in the past to block ovulation (Gaytan *et al*., 2002). Likewise, Freeman (1988) that the anti-inflammatory property of *Garcinia kola* seeds which gives kolaviron on extraction may be responsible for its ability to block ovulation when taken before the surge of luteinizing hormone. The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclo-oxygenase enzyme which is the mechanism exhibited for chemoprevention against many types of cancers (Liang *et al*., 1999). This showed that *Garcinia kola* may only cause abnormal follicle rupture but have no effect on the maturation of ovum (Gaytan *et al*., 2002). The objective of this study is to determine whether oral doses of kolaviron (a methanolic extract of *Garcinia kola* seeds) have any effect on the histoarchitecture of the hypothalamus, anterior pituitary and ovary in experimental female animals since alteration in any of the structures may results into infertility.

MATERIALS AND METHODS

A total of thirty adult female Wistar rats were used for this experiment. The animals were fed with standard rat pellet and given water liberally in the animal holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. The animals were randomly divided into three groups: A, B and C with ten animals in each group. Group A, were the control group that were given distilled water orally once daily for 14 days; Group B were given kolaviron orally at concentration of 200 mg kg^{-1} b.wt. once daily for 14 days; Group C were given kolaviron orally at concentration of 100 mg kg^{-1} twice daily for 14 days. At the end of the experimental period, the animals were anaesthesized with chloroform inhalation. The hypothalamus, anterior pituitary and ovary were removed, weighed and fixed in 10% formol calcium for Haematoxylin and Eosin staining, demonstration of Nissl substance in hypothalamus and demonstration of special stain using Aldehyde Fuschin Thionin for delta cells of anterior pituitary gland. All animals were handled in accordance with guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (NIH, 1985).

Extraction of kolaviron: Extraction of Kolaviron was achieved by the procedure previously described by Iwu (1985) and modified by Braide (1990). *Garcinia kola* seeds were peeled and air dried in the laboratory (25-28°C) and ground into powdered form. The powdered seeds were extracted with n-hexane, in a Soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol in a Soxhlet extractor. The extract was concentrated and diluted to twice its volume in distilled water and partitioned with chloroform. The concentrated chloroform fraction gave a yellow-brown solid known as kolaviron.

Histological analysis: The animals were anaesthesized with chloroform inhalation heads of the animals were removed, skulls opened with the aid of bone forceps and brains were removed, weighed using a Metler sensitive balance and fixed in 10% formal calcium immediately. Thereafter, the hypothalamus and pituitary gland were removed with the aid of a sharp scalpel blade and routinely processed for paraffin wax embedding. Then, 5 μm thick paraffin cross sections of the tissues were mounted on slides and stained using Haematoxylin and Eosin staining (Drury *et al*., 1967), Demonstration of Nissl substance using Thionin Method of Paget and Eccleston (1960) Demonstration of special stain using Aldehyde Fuschin Thionin for delta cells of anterior pituitary gland. The sections were examined under a LEICA research microscope LEICA DM750, Switzerland) with a digital camera attached (LEICA ICC50). Digital photographs of stained sections of the hypothalamus, pituitary and ovary were taken at various magnifications at the Department of Anatomy and Cell Biology, Obafemi Awolowo University Ile-Ife.

Statistical analysis of the weights of brain and gonad: The values were presented as mean± standard error of mean (M±SEM). Data were analyzed using one way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) using Statistical Package for Social Sciences (SPSS 17).

RESULTS

Relative brain and gonad weights: There was a significant ($p<0.05$) difference in relative brain and gonad weight across the group. There was a significant increase in the relative brain and gonad weight of the groups given 100 mg kg^{-1} b.wt. of kolaviron compare with control (group A) while there was a significant ($p<0.05$) decrease in the relative brain weight of the groups that received 200 mg kg^{-1} b.wt. when compared with the control (group A) (Table 1).

Table 1: Effect of kolaviron on the relative brain and gonad weight in female Wistar rats

	Group A	Group B	Group C
RBW	10.00±0.90	7.40±0.25*	11.80±0.37*#
RGW	4.80±0.58	3.40±0.24*	5.00±0.45#

Values are Mean±SEM of data from the animals, *Significant difference at $p<0.05$ when group A was compared with B and C, #Significant at $p<0.05$ when group B was compared with C

Histological findings

Haematoxylin and Eosin Staining of the ovaries: The histological findings showed that there were various follicular stages, grafian follicles and congestion of the stroma and intact blood vessels in group A while groups B and C showed no developing follicle and degenerated luteum with varicose blood vessels (Fig. 1).

Histological and histochemical studies of the anterior pituitary: Haematoxilin and Eosin were used to study the cells of the anterior pituitary gland while Aldehyde Fuhscin Thionin was used to analyse the delta cells and connective tissue fibres of the anterior pituitary gland. H and E and AFT showed that the cells of group A which was the control group were intact, group B showed that the round cells (delta cells) were degenerated and surrounding connective tissues were also disorganised. In group C, The round cells (delta cells) were degenerated and surrounding connective tissues appeared normal (Fig. 2, 3).

Histological and histochemical studies of the hypothalamus: Haematoxylin and Eosin and Nissl staining of the hypothalamus revealed that the cells of the hypothalamus in all the groups were intact. There was no evidence of distorted cells. This is shown in Fig. 4 and 5.

DISCUSSION

This study has shown that in adult female wistar rats, following ingestion of kolaviron for two weeks, there was marked distortion of the growing follicles in the ovaries, deformation of the delta cells of the anterior pituitary glands with no visible effects on the neural cells of the hypothalamus. The observed changes in the histoarchitectures of the anterior pituitary glands and ovaries could have considerable implications on the fertility status of female Wistar rats following the administration of kolaviron.

Estimation of relative brain and gonad weights as presented in Table 1 showed that there was a significant

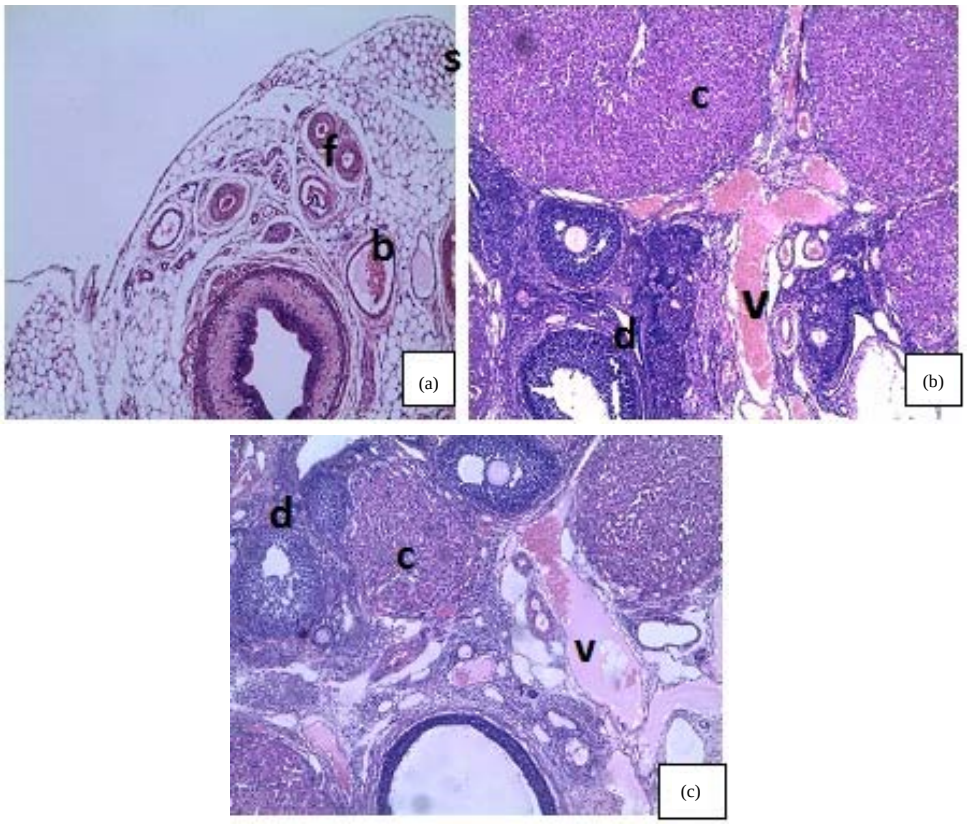

Fig. 1(a-c): Photomicrograph of transverse sections showing ovaries in groups (A-C) A contains F: Note the various follicular stages, b: Intact blood vessels and s: Well organised stroma, In (b) and (c), d: Degenerated follicles, c: Corpus albican, v: Vericose blood vessel. H and E. X100

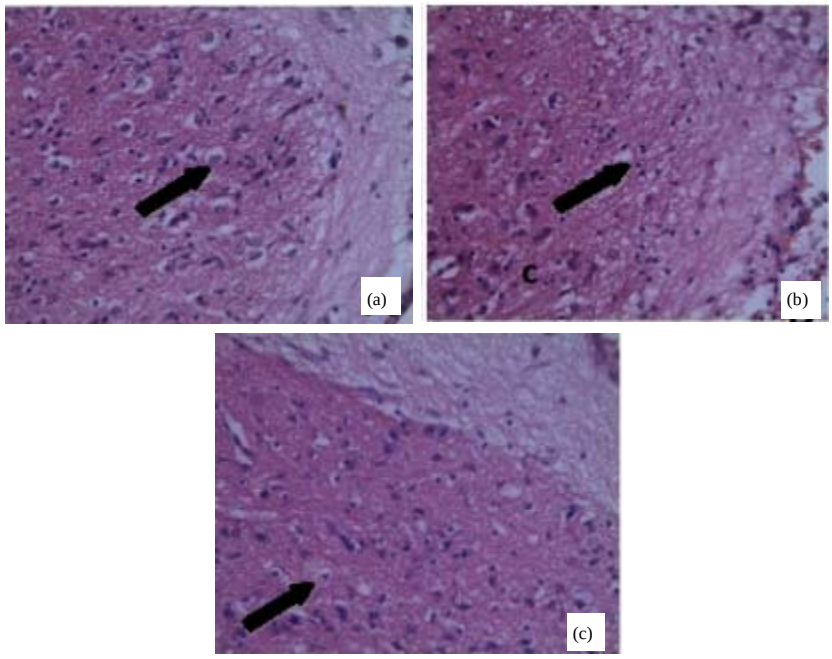

Fig. 2(a-c): Photomicrograph of longitudinal sections showing anterior pituitary cells in groups A-C , Cells (arrows) and surrounding connective tissues (CT) were normal and properly arranged in (a), in (b) and (c), some cells (arrow) appeared blurred and degenerated and surrounding connective tissues appeared normal and properly arranged H and E X400

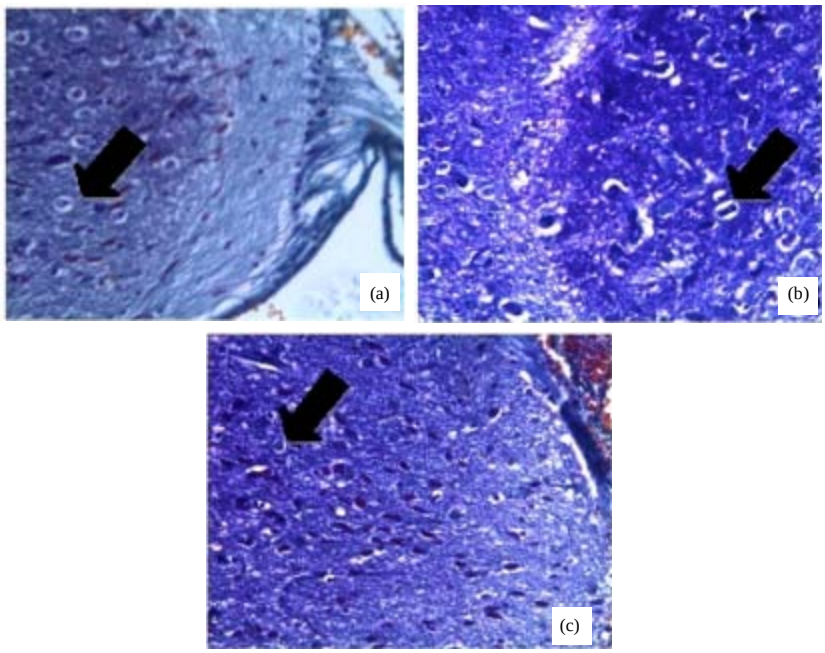

Fig. 3(a-c): Photomicrograph of longitudinal sections showing anterior pituitary cells in groups A-C, Cells (arrows) and surrounding connective tissues were normal and properly arranged in (a), in (b) and (c), the round cells (delta cells) were deformed (arrows) and surrounding connective tissues were also disorganised AFT X400

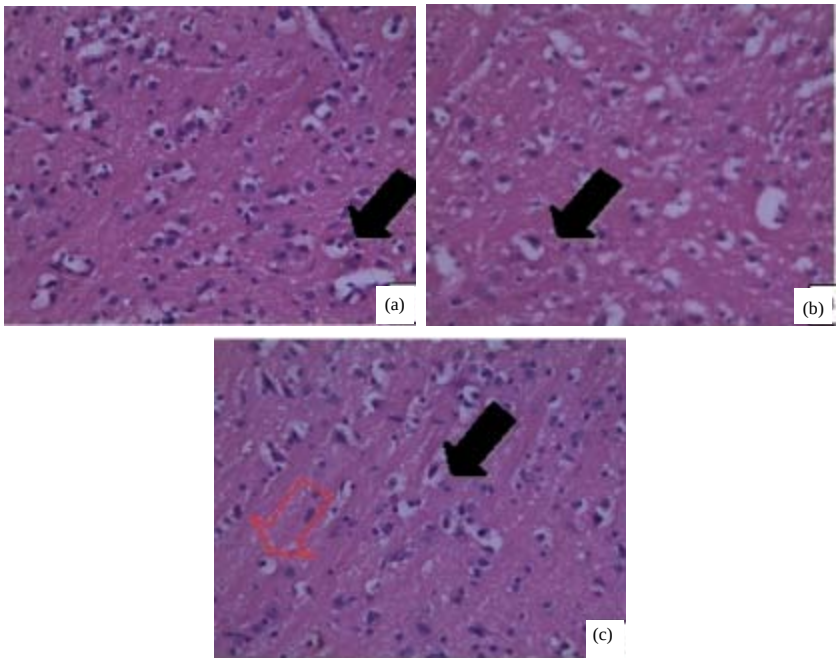

Fig. 4(a-c): Photomicrograph of longitudinal sections showing hypothalamus in groups (A-C), Normal neurons (black arrows), also note the presence of well stained numerous microglia (red arrows) in (a), (b) and (c) H and E X400

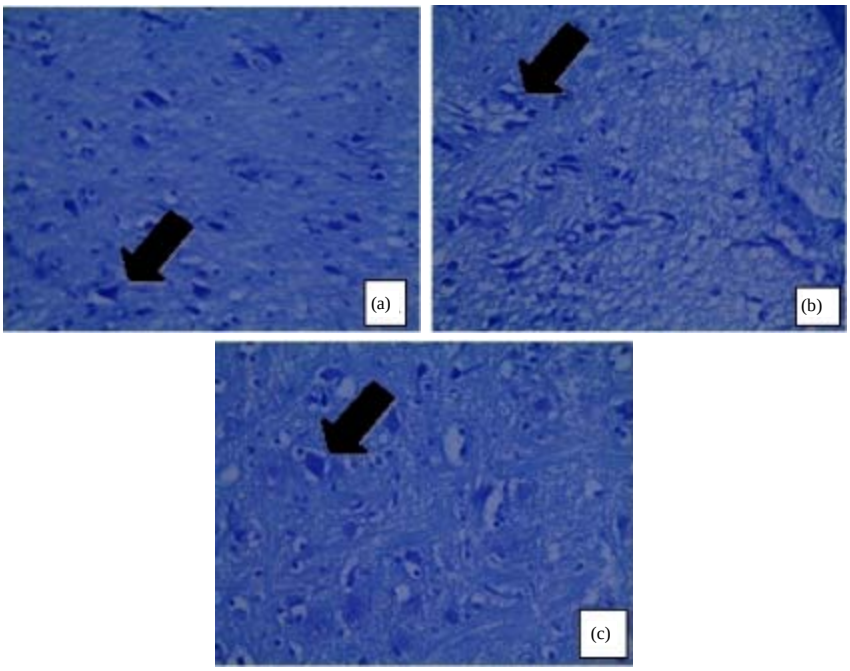

Fig. 5(a-c): Photomicrograph of longitudinal sections showing hypothalamus in groups (A-C), Normal neurons (arrow), also note the presence of well stained numerous microglia in (a), (b) and (c) Nissl. X400

decrease when relative brain and gonads weights of the control female wistar rats were compared with 200 mg kg^{-1} b.wt. of kolaviron treated female rats but significantly increase with 100 mg kg^{-1} b.wt. of kolaviron treated female rats. This opposes Uko *et al.* (2001) who reported that *G. kola* extract had no significant effects on the relative organ mass of the rats used in his study. The variation in relative brain and gonads weights could be as a result of the taste of kolaviron which altered the feeding pattern of the animals thereby having a reduced weight.

In Fig. 1, Group A showed the basic structure of the normal ovary of a mammal with the ovarian stroma well laid; the numerous follicles were seen with their various follicular stages, graafian follicles and corpus luteum. Groups B and C, had no developing follicle but old degenerated corpus luteum (corpus albican), the indication that the ovaries were not active thereby showing that Kolaviron prevented ovulation. This study supported the work of Iranloye and Owokunle (2008). Aldehyde thionin Fuschin which is a special stain that showed the features of the Delta cells which were numerous round shaped in group A while in B and C were few and deformed which would hinder the production of reproductive hormones Fig. 2 and 3.

The cells of hypothalamus were clearly seen in all the sections. Nissl cells and other supporting cells were not distorted by kolaviron administration as evidence in groups B and C which corroborated the works of Nwoha *et al.* (2007) that *Garcinia kola* diet provides slight neuroprotection to mice hippocampal neurons against neurotoxin and Adaramoye (2010) whom in his study recorded that kolaviron (biflavonoid from *Garcinia kola* seeds) had protective properties against g-radiation induced oxidative stress in brain of exposed rats. Therefore, deteriorating effects of kolaviron on the ovary and anterior pituitary gland had no relation to the hypothalamus since the neurons remained intact in all the groups.

CONCLUSION

It was concluded that kolaviron which was administered to the female wistar rats had no effects on the neurons of hypothalamus but altered the histoarchitectures of anterior pituitary glands and ovaries and this could cause infertility in adult female wistar rats.

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