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Original Research Article

Changes in some female reproductive parameters of Albino wistar rats by hydroethanol leaf extract of *fleurya aestuans*

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ABSTRACT

Background: This study investigated the effects of hydro-ethanolic leaf extract of Fleurya aestuans (FA) on some female reproductive hormones in female albino wistar rats.
Methods: Thirty-two (32) rats weighing 120 to 200g were grouped into four of eight rats each. After two weeks of acclimatization, group 1 served as the control group. Groups 2, 3 and 4 served as the test groups and were orally administered with (75, 150 and 300) mg/kg body weight of hydro-ethanolic leaf extract of Fleurya aestuans (FA) respectively, for 42 days. The animals were allowed access to water and feeds ad libitum. At the end of treatment, the animals were properly sedated and blood samples were collected via cardiac puncture. The blood samples were centrifuged and serum obtained was used to determine the concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone using the ELISA method. Also, the ovaries of each rat were harvested and fixed for histological studies. Raw data were analyzed using SPSS version 20.0.
Results: The hydro-ethanolic leaf extract of F. aestuans significantly ($P<0.05$) increased serum concentrations of FSH and LH in all test groups. Whereas, estrogen and progesterone increased significantly ($P<0.05$) in test Groups 2 (75mg/kg) and 3 (150mg/kg) but were significantly ($P<0.05$) decreased in test Group 4 (300mg/kg). Histological examination of the ovaries showed only primary follicles in Groups 1 and 4, while, Graafian follicle and corpus luteum were seen in groups 2 and 3. This suggests that the hydro-ethanolic leaf extract of Fleurya aestuans contains possible biologically active properties that may be potent in enhancing serum concentrations of FSH, LH, estrogen and progesterone but may be ineffective at a higher dose.
Conclusions: The current study showed that hydro-ethanolic leaf extract of Fleurya aestuans promotes fertility in females and suggests that caution be taken in the quantity of this extract consumed if to be considered for fertility enhancement purposes in females.

Keywords: *Fleurya aestuans*, Hydro-ethanolic, Graafian follicles, Oestrogen, Progesterone, Reproductive hormones

INTRODUCTION

Biologically active substances derived from medicinal plants which have the ability to influence endocrine and reproductive processes in animals and humans have received a great deal of interest due to their profitable and harmful effects[1]. Most of these bio-active molecules possess fertility boosting potentials through their effects on the hypothalamic-pituitary gonodal axis.[2]

Some examples of such plants include life root (*Senecio aureus*), wild carrot (*Daucus carota*), fruit extract of *Abelmoscus esculentu*[3] and wild yam (*Dioscorea villosa*) which are used traditionally to improve fertility.[3,4]

Fleurya aestuans (Family – Urticaceae), commonly found growing in shaded areas and waste places is one of the traditional plants with such bio-active molecules and therapeutic agents.[5] The plant is called West Indian wood

nettle in English, "Ohuahihara" in Igbo, "Fiyafiya" in Yoruba, "Picapica" in Spanish and "Huo yan sang ye ma" in Chinese.[6] Based on our ethno-medicinal survey, *Fleurya aestuans* is widely used by Nigerian and African traditional healer to treat anaemia, infertility and reproductive dysfunctions in women. In Rivers state for example, fresh leaves of *Fleurya aestuans* soaked in water or alcohol is prescribed by herbalist to cure infertility in females and as blood tonic. In Benin City, the leaf of this plant is crushed in a mortar with native chalk and applied over the lower abdomen of pregnant women for proper development of the foetus.[7] According to a study, leaf of *Fleurya aestuans* is used as a hemostatic on cuts and wounds, and as a remedy for snake poison in Nigeria[8]. *F. aestuans* soaked in water is administered to women in labour in order to deliver the placenta after child birth, while the root of the plant cooked in water is taken to stop excessive menstrual bleeding in Cameroon.[9] In Gabon, the boiled leaves of *F. aestuans* are eaten with peanuts as a therapy for stomach-ache in pregnant women.[10]

The use of *Fleurya aestuans* in the treatment of female infertility is however without any remarkable scientific proof. Thus, this study therefore aims at providing scientific information on the effects of hydro-ethanolic leaf extract of *Fleurya aestuans* on female reproductive hormones using albino wistar rats as models.

METHODS

Preparation of plant material and extraction

The preparation of the extract was carried out at the Department of Pharmacognosy and Natural Medicine, University of Port Harcourt, Nigeria. Fresh leaves of *Fleurya aestuans* were rinsed in clean water to remove dirt and dried at room temperature (26^{o} C) for a period of three (3) weeks. The dried leaves were milled to fine powder using manual grinding machine (Modelcorene, A.5 lander YCIA S.A) and 780g of the plant was obtained. The weighed quantity of the plant was dissolved in 400ml of Water-Ethanol mixture (25:75% v/v BDH) for 72 hours in an Extraction Jar. During this period it was well macerated (shaken) to enable it absorb the solvent. After which it was filtered using a Whatman No 1 filter paper to separate the filtrate from the residue. The filter paper was folded into four portions and put in the funnel and placed into 1,000ml beaker, the filtrate containing the extract was carefully poured into the funnel which filtered into the beaker through the filter paper.

After obtaining the filtrate, it was then poured into an evaporating dish which was thereafter dried on a steam bath at a temperature of 45^{o} C. The drying was monitored until it turned into a Paste form. The yield of the crude ethanolic extract of *F. aestuans* leaves obtained weighed 75.3g and was stored in a refrigerator (Haier Thermocool) at 4^{o}C for use during the experiment.

Preparation of the animal model and experimental design

A total of 32 female albino wistar rats weighing 120 to 200 grams were acquired from the animal farm of the Faculty of Basic Medical Sciences, University of Port Harcourt. They were allowed adaptation for two weeks in the animal house of the Department of Human Physiology, University of Port Harcourt. They were then weighed using a digital scale with accuracy of 0.001 gram, and randomly divided into 5 groups of 8 rats each. Three groups 2, 3 and 4) served as the test groups and were orally administered with (75, 150 and 300) mg/kg of hydro-ethanolic extract of *Fleurya aestuans respectively* for 42 days. Group 1 served as the control and rats here were allowed free access to water and normal rat chow.

The extract was administered to the rats using an oral gavage, once daily (9am-10am), throughout the period of the study. At the expiration of administration period, the rats were weighed, and five (5) were taken from each group. Five milliliter (5ml) blood sample was collected by cardiac puncture into Plain bottles and EDTA bottles for hormonal assay by ELISA. The hormonal assay was done at the Department of Chemical pathology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria[11]. Also, the ovaries of the rats were harvested and fixed in 10% formalin for histological studies.

Data analysis

Data obtained from the study were analyzed using the SPSS software version 20.0. One way analysis of variance (ANOVA) was used to analyze the means and significant differences. Comparisons between the groups were made using least significant difference (LSD) post Hoc tool. Differences at $P<0.05$ (95% confidence interval) were taken to be statistically significant.

RESULTS

The results in table one show that the FSH level of the entire test groups (2, 3 and 4) significantly increased ($P<0.05$), in a dose-dependent fashion. The result on LH changes indicated that only groups 3 and 4 had remarkable increases ($P<0.05$). The outcome on progesterone revealed that just group 2 animals had significantly increased ($P<0.05$) levels, whereas that of group 4 appreciably decreased ($P<0.05$). The animals in groups 2 and 3 showed remarkable increases in estrogen levels, but a significant reduction in that of group 4.

Considering the histological investigation on the influence of hydro-ethanol leaf extract of FA on rats ovaries, majorly primary follicles were seen on the photomicrographs of groups 1 and 4 animals. However, for groups 2 and 3 as seen in figures 2 and 3 respectively, both had Graafian (i.e. matured) follicles and corpus luteum were mainly visible on their ovaries.

Table 1: The FSH level of the entire test groups (2, 3 and 4).

Groups	FSH (miu/ml ± sem)	LH (miu/ml ± sem)	Progesterone (ng/ml ± sem)	Estrogen (Pg/ml ± sem)
Group 1 (control)	4.60 ± 0.25	7.40 ± 0.25	36.60 ± 1.81	126.0 ± 8.72
Group 2 (75mg/kg)	6.40 ± 0.25*	7.80 ± 0.37	41.80 ± 0.92*	222.0 ± 12.81*
Group 3 (150mg/kg)	8.20 ± 0.20*	10.80 ± 0.20*	39.80 ± 0.58	194.0 ± 11.23*
Group 4 (300mg/kg)	9.60 ± 0.25*	10.60 ± 0.25*	20.40 ± 1.44*	40.60 ± 2.32*

All results obtained from the study were presented in tables and expressed as mean plus/minus standard error of mean (M±S.E.M) as above; *Represents significant difference at P<0.05 with respect to group 1 animals.

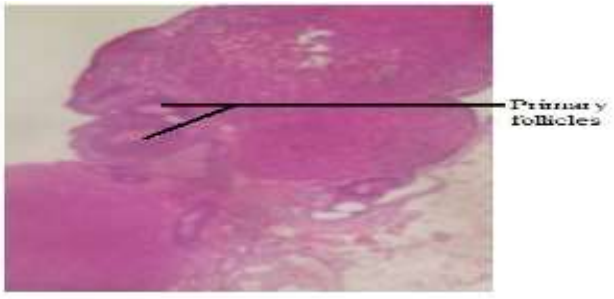

Figure 1: Photomicrograph of ovary of the control animals (group 1) (x 400 H & E).

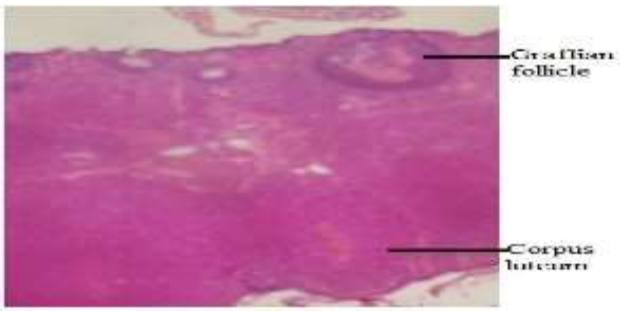

Figure 2: Photomicrograph of ovary of test group 2 animals after 42 day's oral administration with 75mg/kg of FA (x 400 H & E).

DISCUSSION

Estrogen and progesterone in females are produced mostly in the gonads (ovaries) under the influence of FSH and LH secreted by the anterior pituitary gland. The secretions of sex hormones are known to be regulated by hypothalamic GnRH which acts on the pituitary gland, and thus regulate the secretion of gonadotropins which in turn influence the secretions of gonadal hormones.[12]

The significant (P<0.05) increase in the serum concentration of luteinizing hormone (LH) and follicle stimulating hormone (FSH) seen in the test groups when compared with the control in this study shows that the extract of FA possibly contain some phyto-estrogen properties. According to[13], phyto-estrogens are plant-derived natural compounds similar to estrogens in terms of structural and functional performance and therefore have both estrogenic and anti-estrogenic effects. He submitted further that, phyto-estrogens influence the hypothalamic-pituitary-gonadal axis as well as the external genitalia.[13] This possible phyto-estrogens component of the plant extract may have some stimulatory role in the release of GnRH (gonadotropin releasing hormone), aromatase and 17-α hydroxylase enzyme which are necessary in estrogen and progesterone production, thus, increasing the secretion of gonadotropins (FSH and LH), and then the release of estrogens and progesterone.[14] High doses of dietary equol (that is a metabolite of isoflavone) have been reported to increase gonadotropin level.[15] Thus, the finding of this study on the increased levels of the gonadotropins and estrogen is in line with earlier established thoughts. Since phyto-estrogens stimulate aromatase and increase conversion of testosterone to estrogen, they possess the ability to function competitively and bind to estrogen receptors, and elevate estrogen and progesterone levels.[16] It therefore implies that the hydroethanol extract of FA (with the possible constituents of phyto-estrogens) in this study, may have mimicked the functions of GnRH which would have probably led to the increase level of the gonadotropins.[12]

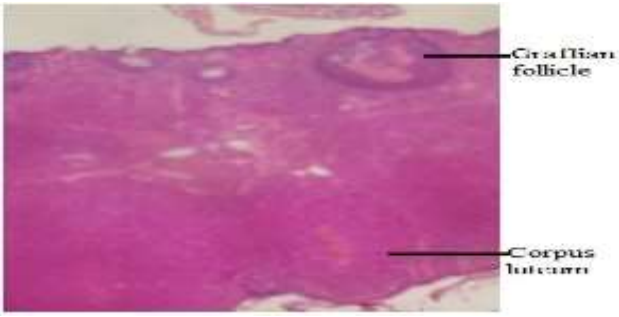

Figure 3: Photomicrograph of ovary of test group 3 animals after 42 day's oral administration with 150mg/kg of FA (x 400 H & E).

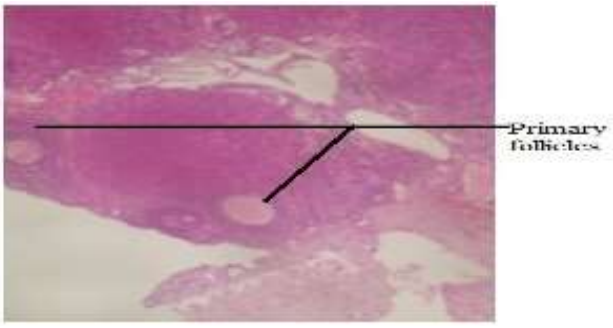

Figure 4: Photomicrograph of ovary of test group 4 animals after 42 day's oral administration with 300mg/kg of FA (x 400 H & E).

Furthermore, higher doses of a plant extract containing phyto-estrogen properties can result in a clear decline in sex hormones, due to its possible ability of modulating the secretion of these hormones by stimulating the gonadotrophs of the anterior pituitary gland.[12,17] Agreeing to a report by Adlercreutz et al phyto-estrogens present in FA extract could elicit production and binding of globulin to liver steroidal hormone, and with increasing productive levels of binding globulin to liver steroidal hormone; a reduction in estrogen and progesterone is expected in group 4.[18] A decline in the serum concentration of estrogen and progesterone may be ascribed to a diminished aromatase activity or substrate supplementation during estrogen and progesterone synthesis.[19] This is in consonance with the result of the present study. Reduction in estrogen and progesterone levels observed in group 4 may obstruct ovulation, preparation of the reproductive tract for zygote implantation, and the successive maintenance of the pregnancy state.

The findings of this study therefore have vital inferences for female fertility and contraceptive development at moderate and higher doses. Plant products as fertility and contraceptive agents will be more tolerable for economic reasons and side effects that are less than chemical agents.

The slides in figures 1 to 4 showed that *F. aestuans* extract could act as a stimulant, causing the progression of folliculogenesis to the stage of graffian (mature) follicle as seen in Groups 2 and 3. This means that test Group 2 and 3 did not have developmental arrest secondary to the administration of the plant extract. However, in Group 4, the plant extract appeared to have possibly acted as a repressing agent, blocking the progression of folliculogenesis to the stage of mature follicles in a way that only primary follicles were seen in the cortical areas of the ovary. This implies that there was a developmental arrest in Group 4.

The progression of folliculogenesis to the stage of graafian follicle and the formation of corpus luteum in Group 2 and 3 correlates with the increase in the levels of FSH of the present study. Thus, the outcome of the present study's histological investigation corroborates with the earlier position of Trisomboon et al. that very high levels of phyto-estrogens can play an inhibitory role on the gonadotrophs of the anterior pituitary gland.[17]

Follicle stimulating hormone (FSH) activates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells.[20] Hence, the probable significant increase in the serum levels of FSH by FA extracts might have influenced the process of folliculogenesis as well as the maturation of the follicle.

Similarly, the inhibition of folliculogenesis and developmental arrest likely caused by the plant extract in Group 4 is in line with the low plasma level of estrogen observed in the present study. Estrogen in combination with follicle stimulating hormone stimulates granulosa cell proliferation during follicular development, this agrees with the present study.[21] However, due to the low level of estrogens in group 4 of the present study, an inhibition of folliculogenesis and developmental arrest is expected.

CONCLUSION

Moderate doses of the hydro-ethanol leaf extract of FA have shown positive tendencies in boosting female fertility. However, higher doses of this extract in this study have indicated to have anti-fertility effect. The current study therefore suggests that caution be taken in the dose of hydro-ethanol extract of FA considered for fertility enhancement purposes in females. Further studies are recommended on both hydro-ethanol and other extracts of FA to help characterize the actual phyto-estrogen, its mechanism of action and safe dose for fertility boosting in females.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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