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The effects of the ethanolic extract of *Ereromastax speciosa* leaf on the serum levels of leuteinizing hormone, follicle stimulating hormone, progesterone and estradiol in female pubertal rats

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ABSTRACT

Ereromastax speciosa is a medicinal plant that has been in use by some traditionalists in the region of calabar, cross river state and Akwa Ibom states of Nigeria in the treatment of reproductive issues in women. Claims have been made by the traditionalists on the efficacy of these leaves from the plant in the enhancing fertility in women of reproductive age, also its use in the treatment of internal heat. In this study, the effect of *Ereromastax speciosa* leaf extract on the serum levels of Luteinizing hormone(LH), Follicle stimulating hormone(FSH), Progesterone and Estradiol in two groups of female pubertal rats was carried out. 0.6mls of normal saline was administered to the female rats in group 1 which served as the control, while 0.6mls of the ethanolic extract of *E. Speciosa* was administered to female rats in group 2 by orogastric intubation for an experimental period of twenty one days. At the end of the experimental period, the serum of the sacrificed animals treated with extracts of *E. speciosa* and the controls were assayed for hormonal concentrations of LH,FSH, progesterone and estradiol. The results showed no significant difference in the serum levels of LH, FSH, Progesterone and Estradiol between the experimental groups and the control at $P<0.05$. This results suggests however that there is no toxic effect in the use of this herb on the female hormonal system, as well as its safety as a drug in the management of female infertility.

Key words: fertility, *Ereromastax speciosa*, LH, FSH, Progesterone, Estradiol.

INTRODUCTION

The medicinal plant *Ereromastax speciosa*, locally known as ikpo ikong amongst the efiks and ibbibios in akwa ibom and cross river state belong to the acanthaceae family. It is a tropical stout erect multibranched herb[1]. It is grown in the farmyards of most rural dwellers for medicinal and ornamental purposes.

Ereromastax speciosa leaves in combination with other herbs has been in use by traditionalists in the local regions of calabar and akwa ibom as a medicinal plant for women with fertility problems. Traditionalists by nature do not have a documented record of their herbal remedies, rather the knowledge on the use of these herbs are passed orally from previous generations [2].

Reports have shown that extracts from the herbs of *E. speciosa* has been employed in the treatment of dysentery, anaemia, diarrhoea[3] irregular, and urinary tract infection[4].

Reports have shown that women are increasingly using herbs amongst other things to combat the negative effect of industrial pollutants on fertility[5]. In Nigeria, a survey conducted on 1200 pregnant women demonstrated that 12% used native herbs [6]. Reports have also shown that a number of health workers who in spite of being agents of orthodox medicine still resort to the use of herbs in treating children[7].

The most common causes of female infertility are hormonal, these are commonly associated with ovulation, polycystic ovarian syndrome, premature ovarian failure, damage to the fallopian tube or uterus, or problems with the cervix. Endocrine disorders result from excessive production of hormones or insufficient production of one or more hormones or the lack of the tissues response to normal circulating hormones [8]. The female reproductive cycle functions primarily by the interplay between the luteinizing hormone, follicle stimulating hormone, progesterone and estradiol. Also, the integrity of the female reproductive organs can be assayed by the serum levels of these hormones.

Reports have shown that some medicinal herbs have an effect on the female reproductive hormonal system and these herbs are used to enhance fertility,

Vitex agnus-castus (Verbenaceae), (Chasteberry or chaste-tree), is used for the treatment and management of female reproductive disorders including premenstrual problems (PMS), menopausal symptoms, and insufficient milk production [9].

Xylopi aethiopica (African Pepper) the leaves and roots encourage fertility, and has been used for the ease of child birth. It is recommended as a post partum tonic for women [10].

MATERIALS AND METHODS

Sample collection and preparation

Ereromastax speciosa leaves were identified and authenticated in the herbarium unit of the department of botany faculty of sciences in the university of calabar, and the forestry department cross river state. Thereafter, the fresh leaves were harvested from a traditional farm at akai effa calabar municipality. The fresh leaves were thoroughly washed and allowed to dry under shade. The dried leaves were blended into fine powder using a Q-link electrical blender model QBL-18L40. Two hundred and forty one (241.0g) blended *E. speciosa* leaves were soaked in 2500mls of 98% ethanol as extracting solvent and allowed to stand for 72 hours at room temperature. The mixture was filtered using whatman No 1 filter paper to obtain a homogenous filtrate. The extract was concentrated in vacuo at a low temperature of (37-40°C) using a rotary evaporator (Model RE52A, China). The concentrates yielded 28.3g.

Animal grouping and experimentation

Young female albino rats of weighing between 180 and 210 grams were purchased from the animal house department of agricultural sciences university of calabar, and kept in the animal unit of the Biochemistry department, faculty of basic medical sciences university of calabar, calabar. The rats were housed in groups of six animals per cage and maintained for one week prior to the experiment to conditions of light (14 hours light and 10 hours dark), and ambient temperature of 28±1°C. The animals were housed in wooden cages with wire-mesh top. The animal facility was well ventilated animals were fed with pelletized feed (Agro Feeds Ltd., Calabar) and allowed tap water ad libitum throughout the experimental period.

At the end of the 21 day experimental period, feed was withdrawn from the rats but they had free access to water. They were then anaesthetized under chloroform vapors and sacrificed. Whole blood was collected via cardiac puncture using sterile needles and syringes into plain tubes and allowed to clot for two hours to clot. The clotted blood was centrifuged at 3000rpm for 10 mins for serum collection meant for biochemical assay.

Biochemical assay

The method employed microwell Enzyme -Linked Immunosorbent Assay(ELISA) using analytical grade reagent

Estimation of serum luteinizing hormone

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of LH-Enzyme reagent was added to all the wells. The microplate was swirled for 20-30 seconds and covered, this mixture was allowed to incubate for 60 minutes at room

temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve

Estimation of serum follicle stimulating hormone(FSH)

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of FSH-Enzyme reagent was added to all the wells. The microplate was swirled for 20-30 seconds and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins

The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve

Determination of serum progesterone levels

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum was pipetted into the assigned wells. 0.050ml of progesterone Enzyme reagent was added to all the wells. The microplate was swirled for 20 seconds to mix, 0.050ml progesterone biotin reagent was added to all the wells, the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins

The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Determination of serum estradiol levels

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum reference was pipetted into the assigned wells. 0.050ml of estradiol Biotin reagent was added to all the wells. The microplate was swirled for 20 seconds to mix, the mixture was incubated at room temperature for 30mins, 0.050ml estradiol enzyme reagent was added to all the wells, the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 90 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Statistical Analysis

Follicle stimulating hormone, luteinizing hormone, progesterone and estradiol measurements are presented as mean $\pm$ SE. One way Analysis of Variance (ANOVA) and the LSD post hoc test were used to analyse the data ($P < 0.05$).

RESULTS

Table 1: Animal grouping and experimentation

Grouping	No. of rats	Treatment	Dosage
1	6	Normal saline	0.6mls
2	6	e. speciosa extract	0.6mls

Table 2: Comparison of serum reproductive hormone concentrations between different experimental groups of female pubertal rats

	FSH(μ /l)	LH(μ /l)	E2(Pg/ml)	PRO(Pg/ml)
Group 1	0.18 ± 0.06	1.13 ± 0.57	60.00 ± 15.28	10.47 4.53
Group 2	0.77 ± 0.39	0.00 ± 0.00	69.33 ± 40.83	13.13 ± 0.98

Values are expressed as mean $\pm$ SEM, $P < 0.05$

DISCUSSION

In this study, the effect of the extract of *Ereromastax speciosa* on the serum levels of FSH, LH, Progesterone and Estradiol for 21 days showed no significant difference in all the experimental groups compared to the control ($P < 0.05$).

This shows the safety in the use of this plant in managing infertility, as well as the possibility of the extract to mediate its effect by maintaining a proper balance in the serum levels of the reproductive hormones.

This study demonstrates that the effects of the administration of the extracts of *E. speciosa*, leaves on the serum levels of follicle stimulating hormone (FSH) in pubertal rats for 21 days did not show any significant difference in the experimental groups of rats when compared to their control groups. FSH is required for the progressive growth of the ovarian follicles at various stages from the primary to the mature graffian follicular stage. This finding suggests the nourishing effect of the plant extracts on the endocrine system by maintaining the FSH levels thus, allowing the proper functioning of the endocrine system in the pubertal rats.

The LH / FSH ratio is balanced for proper endocrine functioning. Suggesting the effects of the extracts of *E. speciosa*, on hormonal balancing of the female LH and FSH levels. Evidence from previous experiments have shown that the *Lepeidium Meyenii* (Maca herb) is a fertility enhancer. Maca produces a natural balance in the female hormonal system thus, regulating female hormonal imbalances[9].

The normal ratio of LH/ FSH for premenopausal women is approximately 1[11].

Progesterone is responsible for maintaining the uterus during the luteal phase of menstruation and thus preparing it for implantation of the fertilized egg.

The serum progesterone levels in this experiment suggests the nourishing effect of the plant extract on the uterus of the pubertal rats. Similar reports in the Dong Quai herb has been shown to have uterine nourishing and toning effect[12].

The results in this study, on the effects of the extracts of *Ereromastax speciosa* leaves on the serum levels of estradiol (E_2) in pubertal female rats for 21 days suggests the positive effects of the extract on the endocrine system in female pubertal rats.

Estradiol is mainly synthesized by the granulosa and theca cells of the ovaries. Serum estradiol level is principally used to monitor the induction of ovulation and differential diagnosis of amenorrhea [13, 14]. Estradiol is responsible for the maturation of the sexual organs and development of secondary female sexual characteristics

CONCLUSION

The findings in this study indicates the probable potentials of the extract, *eromastax speciosa* in the management of female infertility.

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