

Research Article

Effect of *Englerina drummondii* Balle ex Polhill & Wiens on Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Oestrogen, Progesterone on MSG Induced Alterations in Reproductive Parameters in Female Rats

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Abstract: Hormones are chemical substances that aid reproductive process and must be in normal proportion for fertility to take place. Hormones are important for both men and female reproductive process. Herbal medicine is been used across the globe to improve reproductive process. The aim of this study finds out the effect of *Englerina drummondii* Balle ex Polhill & Wiens on follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, progesterone on MSG induced alterations in reproductive parameters in rats. The study involves a total of 45 female wistar rats weighing between 160g to 180g. The animals were randomly picked into 9 groups with 5 animals per group. Administration was as follows: Group 1(control) 5ml/kg of water, group 2 (MSG 800mg/kg), group 3 extract (100 mg/kg), group 4 extract (200 mg/kg), group 5 extract (400 mg/kg), group 6 extract (100 mg/kg + MSG 800 mg/kg), group 7 (200 mg/kg + MSG 800mg/kg), group 8 (400mg/kg + MSG 800mg/kg) and group 9 (letrozole 0.6mg/kg + MSG 800mg/kg). Administration of extract was done through oral gavage for 28 days, thereafter, the animals were sacrificed on the 29th days, then blood samples were collected for hormonal analysis. The results shows that extract of *Englerina drummondii* Balle ex Polhill & Wiens leaves significantly increased the serum level of gonadotropic hormones (FSH, LH) and significantly decreased the serum level of sex hormones (progesterone and estradiol). The statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 23.

Keywords: Hydroalcohol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, progesterone.

Introduction

Hormones are chemical substances that aid reproductive process and must be in normal proportion for fertility to take place. Hormones are important for both men and female reproductive process. Herbal medicine is been used across the globe to improve reproductive process. Both gonadotropic hormones (FSH, LH) and sex hormones (progesterone, oestrogen) are secreted by the anterior pituitary gland and ovary respectively. However, these hormones are control by the hypothalamus.

The plant *Englerina drummondii Balle ex Polhill & Wiens* is a species of a commonly known plant called mistletoe that belongs to a large family called Loranthaceae. *Englerina drummondii Balle ex Polhill & Wiens*. This plant's leaves are green in colour and has fruits and grow on other plants as parasite [1]. This plant was obtained from the forest in Khana Local Government Area of Ogoniland, from avocado tree. It is commonly called Atabe by the people of Khana in Ogoniland [2]. The plant *Englerina drummondii Balle ex Polhill & Wiens* is a species of a commonly known plant called mistletoe and it is a parasitic plant. Mistletoe is utilized medicinally for several centuries in treating and curing ailments like cancer, menopause symptoms, sterility, nervous tension, hypertension, asthma, diabetes, and headache [3].

Several plants have been used across the globe by traditionalists for the treatment of various diseases without proper documentation especially in Africa. Phytomedicine involves the use of various plant's parts such as leaves, stems, seeds, fruits, barks and roots to treat certain disease at home [2].

In phytomedicine, mistletoe leaves are employed in potentiating labour and its extract are known to display oxytocic functions [4] on uterine muscle [5]. *Englerina drummondii Balle ex Polhill & Wiens* (mistletoe) is use by the traditionalist to supress or stop bleeding and it is combined with *African cucumba* for the treatment of fibroid, but no scientific documentation [2]. *Englerina drummondii Balle ex Polhill & Wiens* phytochemical constituents include: tannin, flavonoid, saponin, glycoside, alkaloid, polyphenols, steroids, phenols, glycoside, steroids, phytate and carbohydrates [2].

Monosodium Glutamate (MSG) is a known substance synthesized from L-glutamic acid and used as a flavour enhancer in foods. Monosodium Glutamate (MSG) is a substance commonly used as food additives and as a flavor enhancer [6]. MSG causes increase in the serum estrogen and progesterone levels in adult female rats [7]. MSG suppresses the female reproductive function in rat possibly by impairing the functions of ovary and uterus [8].

LH motivates ovaries to create oestradiol thus their determination is crucial when investigating sterility [9]. The MSG mechanism of action is to activate enzyme aromatase that catalyzes the conversion of testosterone to estradiol, thus increases estradiol synthesis [10]. MSG has noted to cause oligozoospermia and increase abnormal sperm morphology in a dose dependent manner in male Wistar rats [11].

Materials and Method

Collection, Identification and Preparation of Plant materials

Englerina drummondii Balle ex Polhill & Wiens (mistletoe) leaves were obtained during the raining season from a forest in Khana Local Government Area, Rivers State. The plant was introduced to me (researcher) by Prof B.A. Ekeke (Prof of Silviculture and Forestry) of the Forestry Department, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria. It was identified and authenticated by Dr. Ekeke Chimezie, Department of Plant Science and Biotechnology, University of Port Harcourt with the Herbarium Number: UPH/V/1468. Plant extract was carried out according to the method described by Handa et al. [12]. Based on the study, LD₅₀ of mistletoe as determined (Matthew, et al. [13], is 0.4g/kg (400mg) of body weight was used.

Ethical committee of University of Port Harcourt approved the research with the Ref number: UPH/CEREMAD/REC/MM71/019.

Experimental Animals and Management

Young female wistar rats were obtained from the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were housed in cages and maintained under natural environmental condition. These animals were fed with normal standard diets.

Study design

Forty-five (45) animals were randomly selected and grouped into nine (9) groups with five rats per group. Their initial weight was taken. Group 1 (control), received 5ml/kg of distil water + feed, group 2, received mono sodium glutamate (MSG) 800mg/kg + feed, group 3, received extract 100mg/kg + feed, group 4, received extract 200mg/kg + feed, group 5, received extract 400mg/kg + feed, group 6, received extract 100mg/kg + MSG 800mg/kg + feed, group 7, received extract 200mg/kg + MSG 800mg/kg + feed, group 8, received extract 400mg/kg + MSG 800mg/kg + feed, group 9, received letrozole 0.6mg/kg + MSG 800mg/kg + feed.

Administration of extracts was done for 28 days and on 29thday, the animals were sacrificed and blood samples were collected.

Blood Collection

Animals were anaesthetized with Chloroform soaked in cotton wool and placed in a desiccator and 5ml of blood samples collected through cardiac puncture with syringe and shared into the plane bottles. The blood was allowed for 900 seconds and then centrifuged for 900 seconds. Thereafter, the serum was collected and transferred into another bottle and stored in a freezer for hormonal analysis.

Analysis of Sample

Blood was used for hormonal analysis as described by Bolon et al. [14].

Statistical Analysis

Data are presented as mean \pm SEM and were analysed using a one-way Analysis of Variance (ANOVA). $P < 0.05$ was declared as significant statistically.

Results

Follicle-stimulating hormone: When control group (group 1) is compared with the treated groups: extract 100mg/kg, extract 400mg/kg, extract 200mg/kg + MSG 800mg/kg and letrozole 0.6mg/kg + MSG800mg/kg, it shows a significant increase in FSH and when MSG group only (group 2) is compared with the treated groups extract 100mg/kg, extract 400mg/kg, extract 100mg/kg + MSG 800mg/kg, extract 200mg/kg + MSG 800mg/kg, and letrozole 0.6mg/k + MSG 800mg/kg, also shows a significance increased in FSH (Table 2).

Luteinizing hormone: When the control (group 1) is compared with groups treated with extract 100mg/kg, extract 100mg/kg + MSG 800mg/kg and extract 200mg/kg + MSG 800mg/kg, it shows a significant increase in LH whereas when MSG treated group only (group 2) is compared with groups extract 100mg/kg and extract 200mg/kg + MSG 800mg/kg, there is a significance increased in LH (Table 1).

Progesterone: When the control group is compared with groups treated with MSG 800mg/kg only, extract 100mg/kg, extract 200mg/kg, extract 400mg/kg, extract 100mg/kg + MSG 800mg/kg, extract 200mg/kg + MSG 800mg/kg, extract 400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg, there is a significance decreased in progesterone and when MSG 800mg/kg treated group (group 2) is compared with control and the group treated with extract 100mg/kg + MSG 800mg/kg, there is a significance increased in progesterone and when compared with groups extract 200mg/kg + MSG 800mg/kg, extract 400mg/kg + MSG 800mg/kg ,and letrozole 0.6mg/kg + MSG 800mg/kg, it shows a significance decreased in progesterone (Table 1).

Estradiol: When the control group is compared with the treated groups MSG 800mg/kg only, extract 100mg/kg, extract 200mg/kg, extract 400mg/kg, extract 100mg/kg + MSG 800mg/kg, extract 200mg/kg + MSG 800mg/kg, and extract 400mg/kg + MSG 800mg/kg, it shows a significance increased while the group with letrozole 0.6mg/kg + MSG 800mg/kg shows a significance decreased in estradiol. Similarly, when the MSG 800mg/kg treated only is compared with control and the group treated with extract 400mg/kg + MSG 800mg/kg, there is a significance increased and a significance decreased in group extract 100mg/kg, in estradiol (Table 1).

Table 1. Effect of *Englerina drummondii* Balle ex Polhill & Wiens on follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, progesterone on MSG induced alterations in reproductive parameters in rats

Groups	FSH (mIU/L) Mean ± SEM	LH (mIU/L) Mean ± SEM	Progesterone (ng/ml) Mean ± SEM	Estradiol (pg/ml) Mean ± SEM
Normal control	9.74±0.27	5.29±0.70	12.06±0.95	1.82±0.03
MSG 800mg/kg	9.12±0.64	7.00±1.45	^a 9.68±1.17	^a 1.3920±0.01
Extract 100mg/kg	^{ab} 14.82±0.64	^{ab} 10.82±2.09	^a 7.68±0.93	^{ab} 1.06±0.02
Extract 200mg/kg	7.6260±1.07	7.67±0.68	^a 9.74±0.63	^a 1.44±0.11
Extract 400mg/kg	^{ab} 17.43±1.68	6.85±0.52	^a 7.69±0.55	^a 1.49±0.04
MSG + Extract 100mg/kg	^b 12.26±0.57	^a 8.79±0.93	^b 12.43±0.25	^a 1.45±0.01
MSG + Extract 200mg/kg	^{ab} 22.35±0.99	^{ab} 21.32±1.25	^{ab} 4.11±0.83	^a 1.51±0.02
MSG + Extract 400mg/kg	11.26±1.35	4.34±0.25	^{ab} 6.00±0.72	^{ab} 3.57±0.19
MSG + Letrozole 0.6mg/kg	^{ab} 12.81±0.86	4.76±0.98	^{ab} 5.54±0.54	^a 1.44±0.09
a = p < 0.05 when compared with normal control b = p < 0.05 when compared with the MSG (800mg) only treated group FSH = Follicle stimulating hormone; LH = Luteinizing hormone; MSG = Mono sodium glutamate				

Discussion

The study revealed increased in gonadotropic hormones (FSH and LH) and decreased in sex hormones (progesterone and estrogen). FSH enhances the maturation of gonads, gametogenesis and steroidogenesis [15]. The study suggest that the significance increased in both FSH and LH may be due to gonadal failure, resulting in loss of negative feed-back mechanism of estradiol on the hypothalamus and pituitary. FSH is subject to oestrogen feed-back mechanism from the gonads through the hypothalamus-pituitary-gonadal axis. The increased serum level of FSH may be due to low frequencies released of GnRH. This study agreed with previous study by Stamatiades and Kaiser [16] which said GnRH released, occurs in a pulsatile manner, with decrease frequencies stimulating more FSH production and increase frequencies stimulating more LH production. It could also be that the extract and letrozole might have interfered with the gonads (ovaries) or anterior pituitary gland leading to increase level of FSH and this is in consonant with earlier study by [17] which revealed that elevated levels of FSH are associated with unresponsive gonads or hyperfunctioning pituitary adenomas.

Progesterone and oestrogen are steroid hormone produced by the gonads, placenta and adrenal glands. It is responsible for preparing the endometrium for uterine implantation of the fertilized ovum and maintenance of pregnancy. The study suggests that MSG may have interfered at the level of the ovary. This is because both progesterone and oestrogen are secreted by the ovary and from the study, these two hormones (progesterone and oestrogen are significantly decreased and this may

have negative feed-back mechanism at the level of hypothalamus or pituitary, leading to the significant rise in both FSH and LH. However, it could be that interference may be at the level of the ovary, and this could be the reason why the proestrus, estrus and diestrus stages of the estrous cycle are significantly decreased.

Study by Zia et al. [7] revealed that MSG causes increase in the serum estrogen and progesterone levels in adult female rats and diltiazem prevents this effect. Also, study by Obochi et al. [18] revealed that Monosodium Glutamate (MSG) alone increased total protein, cholesterol and estradiol (estrogen), this in turn, induced alterations in reproductive parameters in rats and when treated with extracts of garlic, it reduces the effects that might have been induced by the MSG alone. This study differs from studies by Zia et al. and Obochi et al. [7, 18] in that the serum levels of progesterone and estrogen is significantly decreased in the group treated with MSG only. However, this could be due to increase in the dosage of MSG to 800mg/kg as against 0.08mg/kg and 100mg/kg in the studies by Zia et al. and Obochi et al. respectively [7, 18].

The increased level of LH could be as a result of the extract which may alter the toxic effect of the MSG. This study agreed with previous study by Ofem et al. [19] which suggest that the leave extract of mistletoe contain potent substances that increases the serum levels of FSH, LH and testosterone. The mistletoe extract and letrozole when administered to the rats may have cushion the effect that may have caused by the MSG. Letrozole prevents androgens from converting into estrogen and when estrogen is blocked, the pituitary gland receive information asking it to produce FSH that enhance the production of egg by the ovary (<https://www.todayparent.com> 2018). This agreed with the study that revealed increase in FSH in the group treated with letrozole + MSG and decrease in serum levels of estrogen.

The MSG mechanism of action is to activate enzyme aromatase that catalyzes the conversion of testosterone to estradiol, thus increases estradiol synthesis [10]. However, treatments with *Englerina drummondii* Balle ex Polhill & Wiens (mistletoe) extracts may have interfered with the action of MSG thus, reducing the serum levels of estrogen. Mechanism of action of the *Englerina drummondii* Balle ex Polhill & Wiens extracts is unknown but believed to have similar mechanism of action as letrozole, thus decrease the effect of MSG. The decrease in serum estrogen in wistar rats treated with extract of *Englerina drummondii* Balle ex Polhill & Wiens may suggest decrease in the negative feedback effect of estrogen on hypothalamus–pituitary axis, thereby increases FSH which enhances follicular growth and development leading to fertility.

Conclusion

Administration of *Englerina drummondii* Balle ex Polhill & Wiens leaves extracts in female rats causes significant increases in follicle stimulating hormones (FSH), luteinizing hormone (LH) while progesterone and estradiol are significantly decreased. MSG induced alterations in reproductive parameters in the female rats possibly by impairing the functions of ovary thereby causing significantly decreased in estrogen and progesterone and this resulted in increased in the release of FSH and LH.

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Declarations

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