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Effect of Aqueous Leaves Extract of *Datura alba ness* (Thorn Apple) on Gonadotropic Hormones (FSH, LH) and Prolactin Following Administration of MSG and Clomiphene Citrate in Female Wistar Rats

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Abstract

Phytomedicine has become popular among and majority of women depends on herbal medicine when seeking healthcare. Several women opted for herbal medicine when looking for fertility and most the women that hormonal imbalance depends on herbal medicine for correction. Hormones is substance that must be required and must be in the right values for reproduction to occur. The aim is this study is to ascertain the Effect of aqueous leave extract of *D. alba ness* (Thorn Apple) on gonadotropic hormones (FSH, LH) Treated with monosodium glutamate (MSG) and clomiphene citrate in female wistar rats. The animals were randomly selected into 7 with five animals per group. Each group received the following: group 1 10mls/kg of distil water, group 2 low dose of extract (1000mg/kg), group 3 high dose of extract(2000mg/kg), group 4 MSG 800mg/kg, group 5 clomid 50mg/kg, group 6 MSG + high dose of extract and group 7 clomid+ high dose of extract. Administration of extract was done for 21 days, and on the 22nd day, the animals were sacrificed and blood samples were collected for hormonal analysis. The results revealed significant decreased in the serum levels of follicle stimulating hormones (FSH) in all the treatment groups (extract 1000mg/kg, extract 2000mg/kg, MSG 800mg/kg, MSG 800mg/kg + extract 2000mg/kg, and clomid 50mg/kg + extract 2000mg/kg) when compared with control and significant increase in serum levels FSH in clomid 50mg/kg treated group. Luteinizing hormone (LH) serum levels significantly decreased in all the treatment groups when compared with the control group. LH levels in the clomid group significantly increased when compared with other treatment groups. The statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 23.

Keywords: Female, Aqueous, Leave, Extract, Gonadotropic, Hormones, MSG.

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 (Gbaranor *et al.*, 2021). In female mammals, FSH stimulates ovarian follicle growth and maturation, as well as E2 synthesis by granulosa cells, whereas LH stimulates androgen production by theca cells and ovulation of the dominant follicle(s) (Ofem *et al.*, 2014). There are many hormonal disorders that cause infertility. Hypothyroidism, hyperprolactinemia (high male hormone levels) and luteal phase defect (low progesterone) are a few examples of these disorders. Hormonal disorders are a major cause of infertility in women. The inability of women at ovulation and regulation of hormone levels leads to too high or too low production of hormones and following factors may cause hormonal disorders: gland problems such as thyroid gland, pituitary gland and hypothalamus gland problems **(**Meneses and Holland, 2014).

Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that blocks the negative feedback of estrogen on the pituitary gland. Through this interaction, increase levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are released by the anterior pituitary (Chehab *et al.*, 2015; Usadi and Merriam 2015). Clomiphene citrate have many adverse effects, such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath. Clomiphene citrate has also been

shown to cause ovarian and uterine abnormalities (Nagao and Yoshimura, 2001).

MSG suppresses the female reproductive function in rat possibly by impairing the functions of ovary and uterus (Mondal *et al.*, 2017). MSGs are known to trigger changes on reproductive factors like reduced serum levels for progesterone (El-Beltagy and Elghaweet, 2016). MSGs also triggers changes on reproductive factors by triggering atretic follicles, fragment oocyte, vascular mobbing, and vacuolated stroma in ovary and endometrial glans loss and reduced endometrial thickness (El-Beltagy and Elghaweet, 2016).

Phytomedicine is a crucial part or aspect of medicine globally that is providing needed alternatives for treating ill-patients. Several plants have been used across the globe by traditionalists for the treatment of various diseases without proper documentation especially in Africa (Gbaranor *et al*., 2021d) and also, herbal medicine is being used across the globe to improve reproductive process (Gbaranor *et al.*, 2021c). Most people in the rural areas and some in the urban areas depends on herbal medicine each time they have medical issues (Gbaranor *et al.*, 2021b). World Health Organization's (WHO) report, revealed that 60% of the World's populations depend on herbal and traditional medicine, and 85% of the World's developing countries use traditional medicine in caring for diseases (Shuaib, M *et al.*, 2023). In countries where infertility care is difficult to access, women turn to herbal medicines to achieve parenthood (Kaadaaga *et al.*,), plants are the basis for traditional medicine in developing countries all over the world. This is because these plants contain certain elements or compound known as phytochemicals that are active in carrying out these functions.

MATERIALS AND METHOD

Collection and Identification of Plant materials

Datura alba ness (Thorn apple) leaves were obtained from a forest in Omuma Local Government Area, Rivers State. The plant was identified and authenticated at Faculty of Pharmacy, Department of Pharmacognosis and Natural Medicine, University of Uyo, Uyo, Akwa Ibom State, by a Taxonomist. Mrs Emmanuella G. Udoma, with Herbarium Number: UUPH /74(C).

Preparation of Extract

The procedure described by Yakubu *et al.*, (2005) was employed with a modification. Then the fresh leaves were washed to remove sand and other particles and thereafter, air dry at room temperature for about 2. Mechanically, the dried leaves were pulverised and about 100g of dried powder leaves were soaked in 600mls of aqueous solvent and allow it to stand for 72hrs with a continuous agitation morning and evening. Then, it was filter to obtain the clear solution which was mounted on a water bath for drying at a temperature range of 50*C in order not.

Acute Toxicity Test (LD_{50} Determination)

The LD50 of the extract was determined using the method described by Lorke (1983).

Phase 1: This phase requires nine animals (mice). The nine animals were randomly selected and grouped into three groups of three animals per group. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality will occur.

Phase 2: This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (2600, 3900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality. However, there was no mortality and 5000mg/kg was taken as the LD_{50} of the study. Thereafter, the different dosage of the extract was determined using 20% for low, and 40% for high respectively. Therefore, the dosage was determined and was given as 1000mg/kg as low and 2000mg/kg as high

Ethical Consideration

This study was approved by the ethical committee of Faculty of Basic Medical Sciences, University of Uyo with the ethical number: UU_FBMSREC_2023_002

Experimental Animal

Females wistar rats weighing between 130-180g were used for the research. Female rats were sourced from animal house, Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Uyo. The animals were kept in cages and maintained at their natural condition The animals were weighed before commencement of administration and after administration. They were kept clean in a disinfected cage with saw dust as their beddings in animal house and were allowed to acclimatize for two weeks in their new environment, with free access to food and water.

Study Design

Female rats (35) were used for this study. They were selected randomly and group into 7 groups with 5 rats/group. Administration of extracts was done for 21 days and on 22nd day, the animals were sacrificed and blood samples were collected and reproductive parameters were evaluated.

Group 1. Negative control received 5mls of distil water + feed
Group 2. Received low dose of extract + feed
Group 3. Received high dose of extract + feed
Group 4. Received MSG only + feed

Group 5 Received clomid only + feed
Group 6 MSG + high dose of extract
Group 7 Clomid + High dose of extract

Blood Collection

Animals were anaesthetized with Ketamin 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*., (1997).

Statistical Analysis

The data was analyzed using Statistical Package for Social Science software (SPSS) version 23, USA. One-way analysis of variance (ANOVA) and Post hoc test was used to determine the mean difference and statistically significant values at $P<0.05$. The results that were obtained was expressed as Mean and Standard Error (Mean ± SEM).

RESULTS

The mean values were: 0.81 ± 0.04, 0.35 ± 0.03, 0.590 ± 0.03, 0.32 ± 0.31, 1.18 ± 0.04, 0.54 ± 0.031, 0.55 ± 0.03 (m/u/ml). There was a significant decrease in follicle stimulating hormone (FSH) levels in the groups treated with extracts 1000 mg/kg, 2000 mg/kg, MSG 800 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with group given 10 ml/kg of distilled water. FSH levels in clomid 50 mg/kg treated group was significantly higher when compared with control group. There was a significant increase in extract 2000 mg/kg group, clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with extract 1000 mg/kg group. FSH levels in MSG 800 mg/kg is significantly lower when compared with extract 2000 mg/kg. There was a significant increase in FSH levels in clomid 50 mg/kg group when compared with extract extract 2000 mg/kg and MSG 800 mg/kg treated group. FSH levels in the clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg was significantly higher when compared with MSG 800 mg/kg. There was a significant decrease in FSH levels in MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with clomid 50 mg/kg (Figure 1.1).

The mean values were: 1.82 ± 0.03, 0.85 ± 0.03, 0.97 ± 0.02, 0.75 ± 0.03, 1.14 ± 0.03, 0.72 ± 0.03, 0.88 ± 0.04 (m/µ/ml). There was a significant decrease in the luteinizing hormone (LH) levels in all the treated groups when compared with control. LH levels in MSG 800 mg/kg is significantly decrease when compared with extract 2000 mg/kg. There was a significant increase in LH levels in clomid 50 mg/kg group when compared with the groups extract 1000 mg/kg, extract 2000 mg/kg and MSG 800 mg/kg. LH levels in the group MSG 800 mg/k and MSG 800 mg/kg + extract 2000 mg/kg was significantly decrease when compared with extract 2000 mg/kg while significantly higher in the clomid 50 mg/kg when compared with extract 2000 mg/kg. Clomid 50 mg/kg significantly increased LH levels when compared with MSG 800 mg/kg. LH levels significantly decreased in MSG 800 mg/kg + extract 2000 mg/kg group but significantly increased in clomid 50 mg/kg + extract 2000 mg/kg when compared with clomid 50 mg/kg. Clomid 50 mg/kg + extract 2000 mg/kg significantly increased LH levels when compared with MSG 800 mg/kg + extract 2000 mg/kg (Figure 1.2).

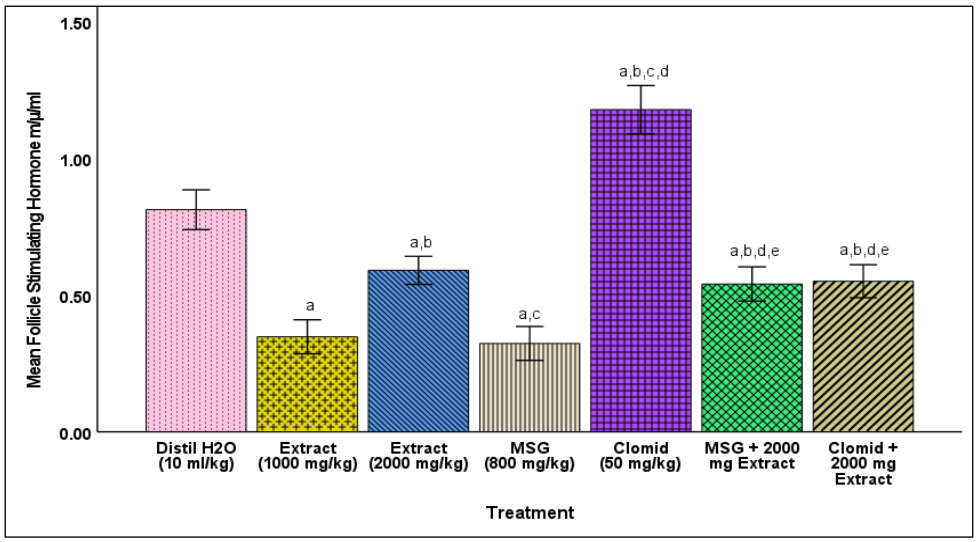

Figure 1.1: Comparison of follicle stimulating hormone in control, extract 1000 mg/kg, extract 2000 mg/kg, MSG 800 mg/kg, clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg

a = p < 0.05 when comparing with control group
b = p < 0.05 when comparing with extract 1000 mg/kg (low dose) group
c = p < 0.05 when comparing with extract 2000 mg/kg (high dose) group
d = p < 0.05 when comparing with MSG 800 mg/kg group
e = p < 0.05 when comparing with clomid 50 mg/kg group
f = p < 0.05 when comparing with MSG 800 mg/kg + extract 2000 mg/kg group

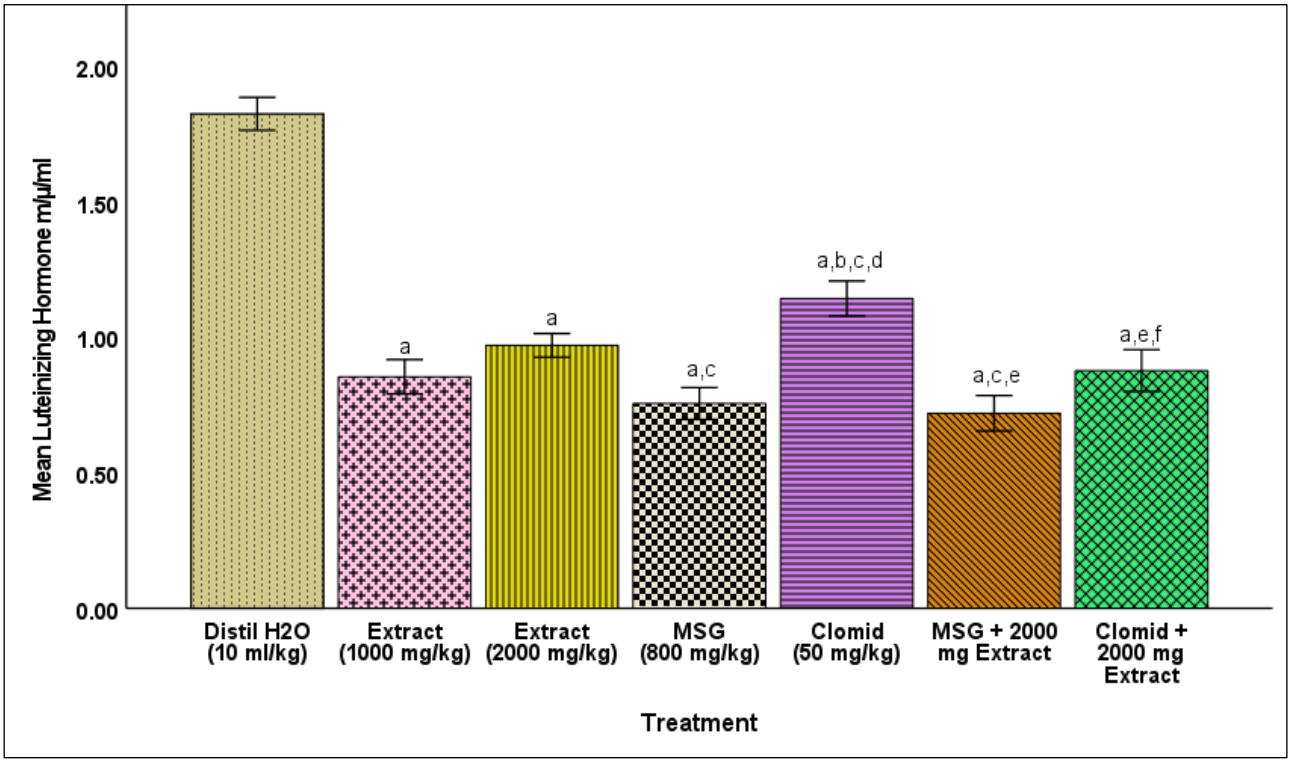

Figure 1.2: Comparison of luteinizing hormone in control, extract 1000 mg/kg, extract 2000 mg/kg, MSG 800 mg/kg, clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg

a = p < 0.05 when comparing with control group
b = p < 0.05 when comparing with extract 1000 mg/kg (low dose) group
c = p < 0.05 when comparing with extract 2000 mg/kg (high dose) group
d = p < 0.05 when comparing with MSG 800 mg/kg group
e = p < 0.05 when comparing with clomid 50 mg/kg group
f = p < 0.05 when comparing with MSG 800 mg/kg + extract 2000 mg/kg group

DISCUSSION

There is remarkable decrease in the serum levels of follicle stimulating hormone in the following treated groups extract 1000 mg/kg, extract 2000 mg/kg, MSG 800 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with the control while clomid while the group treated with clomid only was significantly increased when compared with control. This increase in follicle stimulating hormone due to clomid could be that the negative feedback effect caused by estrogen has been blocked by clomid resulting in increased FSH. This agreed with previous study that revealed estrogen is thought to stimulate pituitary gonadotropin release by excluding estradiol from hypothalamic receptor sites (Avery *et al.*, 1990; Akin, 1993; Abdul *et al.*, 1996). This interaction neutralizes the normal negative feedback control of estrogen and results in enhanced secretion of LH-RH, FSH-RH. Again, the serum levels of FSH is higher in the group extract 2000 mg/kg when compared with extract 1000 mg/kg and this increase could be due to dose dependent. The increased may also be that the extract stimulates the secretion of follicle-stimulating hormone (FSH), which triggers the ovary to produce one or more eggs. The levels of FSH in MSG treated group is significantly reduced when compared with the group extract 2000 mg/kg. This decreased in FSH in the MSG only group could be credited to the reactive oxygen species (ROS) produced by the monosodium glutamate (MSG). Monosodium glutamate has the ability to damage nerve cells of the hypothalamus and this could alter the neural control of reproductive hormone secretion through the hypothalamic-pituitary-gonadal regulatory axis and this alteration in reproductive hormone secretion may cause adverse effect on the reproductive capacity of the animals (Igwebuike *et al.*, 2011). MSG also suppresses the female reproductive

function in rat possibly by impairing the functions of ovary and uterus (Mondal *et al.*, 2017). Nonetheless, FSH increased essentially in the MSG 800 mg/kg + extract 2000 mg/kg when compared to the MSG 800 mg/kg only group and this could be credited to the phytochemicals present in the leaves extract of *D. alba ness* that may suppress the action of MSG. Subsequently, the diminished levels of follicle stimulating hormone (FSH) observed in the MSG alone treated group could be credited to an inhibitory impact on the hypothalamus-pituitary-ovarian-axis which was enhanced when co-treated with *D. alba ness* leaves extract. There was no significant difference in the levels of FSH among the groups co-administered with MSG plus extract and clomid plus extract respectively.

LH is responsible for ovulation, necessary for formation of corpus luteum and activates the secretory functions of corpus luteum. It also causes maturation of vesicular follicle into graafian follicle along with follicle-stimulating hormone (Comprehensive textbook). The investigations discovered that, the serum levels of luteinizing hormone in all the treated groups were significantly decreased when compared with control group. Results from the current examination uncovered that the administration of MSG caused a remarkable decrease in the serum levels of luteinizing hormone (LH) in the MSG 800 mg/kg treated group when compared with the extract 2000 mg/kg treated group. This could be that MSG triggers changes in the reproductive factors as earlier reported by El-Beltagy and Elghaweet, (2016). However, there is essential increased in the serum levels of LH in clomid 50 mg/kg treated group when compared with extract 1000 mg/kg, extract 2000 mg/kg and MSG 800 mg/kg treated groups respectively. Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that blocks the negative feedback of estrogen on the pituitary gland. Through this interaction, increase levels of luteinizing hormone (LH) released by the anterior pituitary (Chehab *et al.*, 2015; Usadi and Merriam 2015). Also, the serum levels of LH essentially decreased in the treated groups MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with clomid 50 mg/kg only treated group and this shows that clomid could promote LH production and this could enhance reproductive function.

CONCLUSION

The findings revealed significant decreased in the serum levels of follicle stimulating hormones (FSH) in all the treatment groups and significant increase in serum levels FSH in clomid 50mg/kg treated group when compared with control and this decreased in the serum levels of FSH may be due to the bioactive substance present in the *Datura alba ness* leaves extract. Luteinizing hormone (LH) serum levels significantly decreased in all the treated groups when compared with the control group. LH levels in the clomid group significantly increased when compared with other treatment groups.

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