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

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Abstract

Reproductive process cannot be complete without hormone and if these hormones are not in the right proportion, then reproductive process could fail. Hormones are chemical substances produced by the endocrine gland and its physiological action is exerted on a target organ different from the secretion's site. The aim is this study is to evaluate Effect of aqueous leave extract of *D. alba ness* (Thorn Apple) on gonadotropic hormones (FSH, LH) and prolactin following administration of MSG and clomid citrate in male wistar rats. The study involves 35 male wistar rats weighing between 130g to 160g. 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 through oral gavage for 21 days, thereafter, the animals were sacrificed on the 22nd day, and blood samples were collected for hormonal analysis. The results showed significant increase in serum levels of FSH and LH in the MSG group and decreased in FHS levels in all others treated groups when compared with control. The serum levels of FSH and LH rises when extract was co-administered with MSG. The serum levels of LH slightly increased when extract was co-administered with clomid serum levels of FSH do not increased when extract was co-administered with clomid. The statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 23.

Keywords: Male, Aqueous, follicle-stimulating hormone (FSH), luteinizing hormone (LH), clomiphene citrate.

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 being 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 controled by the hypothalamus (Gbaranor *et al*, 2021). Follicle stimulating hormone (FSH) and luteinizing hormone (LH) play essential roles in the control of mammalian reproductive function (Ofem *et al*, 2014). Hormones are one of the determining factors for reproduction to occur and this must be in the right proportion (Gbaranor *et al*, 2021).

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). It has been stated by the World Health Organization, that approximately 80% of the universal population use herbal and/or folk medicines as a source of therapeutic agents (Gupta *et al*, 2003; Alagesaboopathi 2014). Again, World Health Organization's (WHO) report, revealed that 60% of the World's populations depend on herbal and traditional medicine.

Monosodium Glutamate (MSG) is a substance commonly used as food additives and as a flavor enhancer (Husarova and Ostatnikova, 2013). MSG causes increase in the serum estrogen and progesterone levels in adult female rats (Zia *et al*, 2014). MSG suppresses the female reproductive function in rat possibly by impairing the functions of ovary and uterus

(Monda *et al*, 2017). LH motivates ovaries to create oestradiol thus their determination is crucial when investigating sterility (Fischbach F, Dunning). The MSG mechanism of action is to activate enzyme aromatase that catalyzes the conversion of testosterone to estradiol, thus increases estradiol synthesis (Eweka *et al*, 2010). MSG has been noted to cause oligozoospermia and increase abnormal sperm morphology in a dose dependent manner in male Wistar rats (Onakewhor *et al*, 1998). 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).

Clomiphene citrate treatment in intact male rats was found to inhibit their reproduction (Brown and Chakraborty, 1988). Clomiphene citrate has also been found to increase serum level of LH and FSH in male rats which stimulate testosterone and sperm production by the testes (Weissenberg, 1992; Fertilitext, unpublished data, 1997). clomiphene citrate exerts its effect on the initiation and maintenance of spermatogenesis by raising the endogenous serum FSH, LH and testosterone levels (Sorbie and Perez-Marrero 1984). CC competes with estradiol at the hypothalamic receptor level, specifically located on the anterior hypothalamus and hypophysis, which blocks the negative feedback mechanism and results in increased GnRH (Herzog *et al*, 2020). Clomid stimulate pituitary gonadotropin release by excluding estradiol from hypothalamic receptor sites and this interaction inhibits the normal negative feedback control of estrogen and results in the secretion of LH and FSH (Avery *et al*, 1990; Akin 1993; Abdul *et al*, 1996).

MATERIALS AND METHOD

Collection and Identification of Plant materials

Fresh leaves of *Datura alba ness* (Thorn apple) were obtained in May, 2023 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

Fresh leaves of *Datura alba ness* (Thorn apple) was obtained from Omuma Local Government Area of Rivers State. 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 dried at room temperature for about 2 weeks. 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 filtered 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

A total of 35 males wistar rats weighing between 130-180g were used for the study. Male 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.

Experimental Design

35 male rats were used for this study. They were randomly selected and group into 7 groups with 5 rats per 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: 1.28 ± 0.02 (10ml/kg of distil water), 0.43 ± 0.23 (extract 1000 mg/kg), 0.43 ± 0.02 (extract 2000 mg/kg), 1.41 ± 0.02 (MSG 800 mg/kg), 0.39 ± 0.02(clomid 50 mg/kg), 1. 00 ± 0.02(MSG 800 mg/kg + extract 2000 mg/kg) and 0.39 ± 0.02 (clomid 50 mg/kg + extract 2000). There was a significant decrease in follicle stimulating hormone (FSH) levels in the groups treated with extract1000 mg/kg extract 2000 mg/kg, clomid 50 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 while MSG 800 mg/kg significantly increased FSH when compared with the control group. FSH levels was significantly higher in the groups treated with MSG 800 mg/kg and MSG 800 mg/kg + extract 2000 mg/kg when compared with group treated with extract 1000 mg/kg. MSG 800 mg/kg and MSG 800 mg/kg + extract 2000 mg/kg also significantly increased FSH levels when compared with extract 2000 mg/kg. FSH levels was significantly lower in the groups treated with clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg when compared with MSG 800 mg/kg. MSG 800 mg/kg + extract 2000 mg/kg significantly increased FSH levels when compared with clomid 50 mg/kg (Figure 1.1). The mean values were: 0.79 ± 0.05 (group 1), 0.64 ± 0.03 (group 2), 0.64 ± 0.03 (group 3), 1.66 ± 0.57 (group 4), 0.57 ± 0.47(group 5), 1.33 ± 0.04 (group 6) and 0.94 ± 0.03 (group 7). The LH levels significantly increase in MSG 800 mg/kg and MSG 800 mg/kg + extract 2000 mg/kg groups when compared with control. The serum levels of LH in MSG 800 mg/kg treated group is significantly increase when compared with extract 1000 mg/kg and extract 2000 mg/kg. No significant difference in LH levels between extract 1000 mg/kg and extract 2000 mg/kg. LH levels in the group treated with clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg extract 2000 mg/kg was significantly decreased when compared with MSG 2000 mg/kg. MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg significantly increased LH levels when compared with clomid 50 mg/kg. LH levels was significantly lower in the groups treated with clomid 50 mg/kg + extract when compared with MSG 800 mg/kg + extract 2000 mg/kg (Figure 1.2).

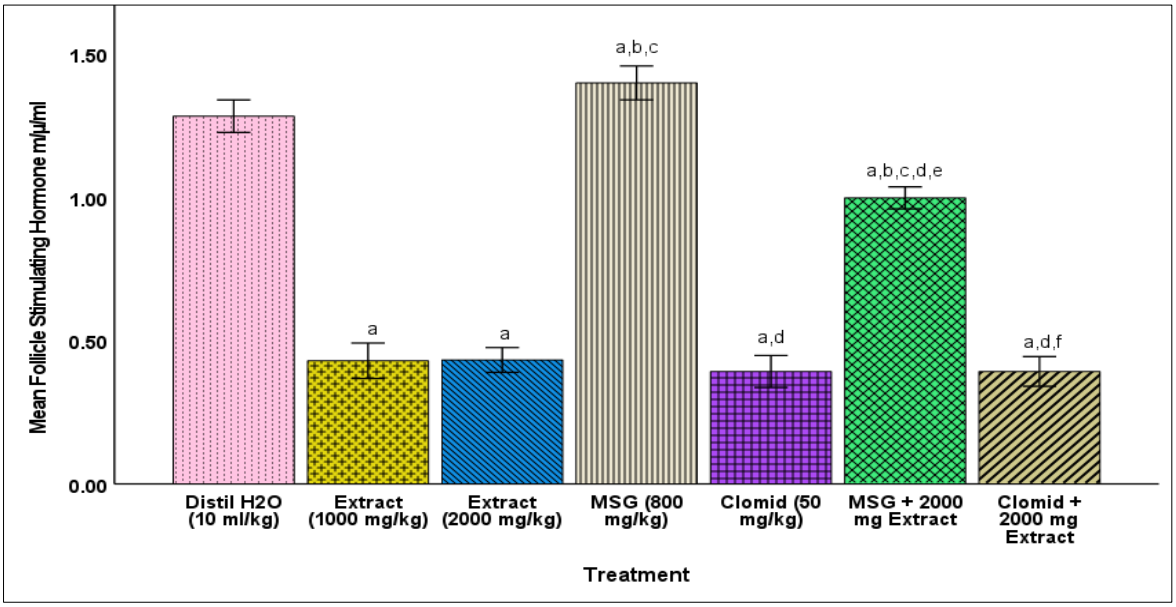

Figure 1.1: Comparison of follicle stimulating hormone levels in control, extract 1000 mg/kg, extract 2000 mg/kg, MSG 800 mg/kg, clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg.

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

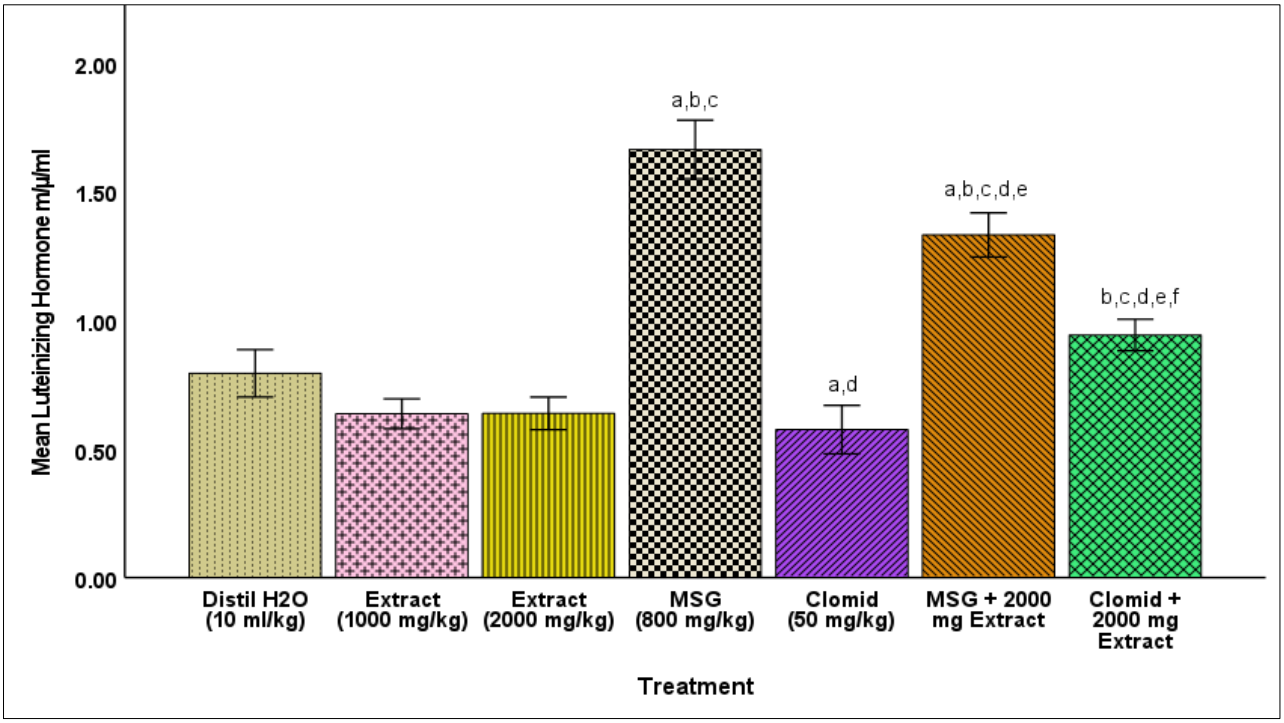

Figure 1.2: Comparison of luteinizing hormone levels in control, extract 1000 mg/kg, extract 2000 mg/kg, MSG 800 mg/kg, clomid 50 mg/kg, MSG 800 mg/kg + extract 2000 mg/kg and clomid 50 mg/kg + extract 2000 mg/kg.

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

Reproduction is one of the processes of life and thus provide avenue for continuity in the family. When reproduction failed to occur, it resulted in infertility which is an important health issue that if not treated may result to separation among homes across the globe. Reproduction or reproductive process cannot take place without reproductive hormones and these hormones must be in the right proportion for reproduction to occur. Reproductive hormones are crucial to the process of fertility in humans and animals. When these hormones are not in the right proportion, it brings several pathological conditions. Physiologically, these hormones promote development and fertility.

Follicle stimulating hormone (FSH) is one the reproductive hormone that enhances follicular growth. It is also one of the gonadotropic hormones produced by the gonadotrophs' cells of the anterior pituitary gland. The gonadotropes carryout an important function in the hypothalamo-pituitary-gonadal axis (hypothalamo-pituitary-ovary axis and the hypothalamo-pituitary-testis axis). Follicle stimulating hormone is regulated by the gonadotropin-releasing hormone (GnRH). Gonadotropin releasing hormone is secreted from arcuate nucleus of the hypothalamus. Follicle stimulating hormone secretion is regulated by feedback inhibition through gonadal hormones such as estrogen, testosterone, activin, inhibin, and follistatin. Testosterone a male hormone is responsible for the provision of negative feedback signal to inhibit gonadotropin secretion from anterior pituitary. In this study, the serum levels of follicle stimulating hormone (FSH) is significantly lower in the groups treated with extract 1000 mg/kg, extract 2000 mg/kg, clomid 50 mg/kg, monosodium glutamate (MSG) 800 mg/kg + extract 2000 mg/kg and clomid 50 mg + extract 2000 mg/kg when compared with the control group while the group treated with MSG 800 mg/kg alone has its serum follicle stimulating hormone significantly higher when compared with the control group. Interestingly, the group co-administered with MSG 800 mg/kg + extract 2000 mg/kg has its serum levels of follicle stimulating hormone significantly decreased when compared with

the control group. When MSG 800 mg/kg was administered alone, the serum levels of follicle stimulating hormone was higher than the control group which received distil water. However, when the MSG 800 mg/kg was co-administered with extract 2000 mg/kg, the serum levels of follicle stimulating hormones significantly decreased when compared with the MSG 800 mg/kg group. This significantly decreased in the serum levels of follicle stimulating hormone in the group treated with MSG 800 mg/kg + extract 2000 mg/kg may be due to the presence of bioactive substance in the *D. alba ness* leaves extract. This extract tends to interfere with the MSG thus, reducing the serum levels of FSH, but the mechanism at which the extract interfere with the substance (MSG) is not known. The extract may interfere with hypothalamus thus preventing the release of gonadotropin releasing hormone and this will in turn prevent the anterior pituitary gland from secreting FSH. Also, the group treated with MSG 800 mg/kg alone has its follicle stimulating hormone significantly increased when compared with extract 1000 mg/kg and extract 2000 mg/kg. This showed that the extract actually brought the serum levels of FSH down. Previous study shows that 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). This could be that monosodium glutamate affects the cells of the hypothalamus that regulate the secretion of the follicle stimulating hormone through the hypothalamic-pituitary-gonadal regulatory axis, resulting in increase in the serum levels of follicle stimulating hormone in the group treated with MSG 800 mg/kg. This study agreed with previous studies by Nayanatara *et al*, (2008) and Igwebuike (2011) that revealed that MSGs are implicated or suspected in male sterility by triggering testicular haemorrhage. Again, extract (1000 mg/kg and 2000 mg/kg) treated groups has its follicle stimulating hormone significantly decreased when compared with control group. This could be the extract contain active substance that lower the serum FSH. This plant could serve to correct hormonal imbalance. That is, people with high serum levels of FSH could benefit. The study revealed that the group treated with clomiphene citrate (clomid) 50 mg/kg only also has its follicle stimulating hormone significantly decrease when compared with control. This decrease could that clomid affect FSH by interfering with the hypothalamus cells. The serum levels of follicle stimulating hormone in the group treated with clomid 50 mg/kg + extract 2000 mg/kg significantly decreased when compared with the control group. This could be that the both the extract and clomid interferes with the function of the hormonal gland located in the hypothalamus which causes decreased levels of FSH.

Luteinizing hormone is a hormone produced by the anterior pituitary gland and is also one of the gonadotropic hormone. In male, the luteinizing hormone play a vital in stimulating the Leydig cells to secrete the hormone called testosterone. The study revealed that the treated group with MSG 800 mg/kg alone significantly has increased in luteinizing hormone levels when compared with the control group, extract 1000 mg/kg and extract 2000 mg/kg. This increase in serum levels of LH could be due to interference at the hyopthalmic level or at the gonad's level by the MSG. This agreed with previous studies by Nayanatara *et al*, (2008) and Igwebuike (2011) that revealed that MSGs are implicated or suspected in male sterility by triggering testicular haemorrhage. Also the group treated with clomid 50 mg/kg alone has its luteinizing hormone significantly reduced and this decrease may be due to dose dependent. The study showed that the serum levels of luteinizing hormone significantly increased in the group treated with MSG 800 mg/kg + extract 2000 mg/kg when compared with all the treated groups, including control exception of the group the treated with MSG 800 mg/kg alone. This could be that the bioactive components of the *D. alba ness* extract may interfere with the mechanism of action in MSG and as such resulting in increased serum levels of luteinizing hormone. Again, in the group treated with clomid 50 mg/kg, the serum levels of luteinizing hormone are significantly decreased when compared with the control group. This decrease in the circulating concentration of luteinizing hormone caused by treatment with exogenous clomid may suppresse gonadotropin releasing hormone (GnRH). However, when clomid 50 mg/kg + extract 2000 mg/kg co-administered, its resulted in significant increase in the serum levels of luteinizing hormone when compared with the control group. This increased in serum levels of luteinizing hormone in the group combined with clomid and extract could be that the bioactive substance in the extract has a physiological action that has synergetic effect with clomid, thus inducing the increase in the hormone. The extract improves the serum levels of luteinizing hormones when co-admistered with clomid and MSG. This could be that the extract contains bioactive substance that regulate the secretion of luteinizing hormone. Clomid is known to enhance testosterone secretion through the pulses release of gonadotropin releasing hormone (GnRH) from the hypothalamus and thus cause the stimulation of the anterior pituitary gland to produce luteinizing hormone. The luteinizing hormone stimulate the Leydig cells to produce testosterone.

Both follicle stimulating hormone and luteinizing hormone are produced by the gonadotrophs cells of the anterior pituitary gland and both of them physiologically affects the growth, development and maturation of the gonads. They also enhance the secretion of sex hormone and pubertal maturation. It is interested to know that both serum levels of follicle stimulating hormone (FSH) and luteinizing hormone

(LH) significantly increased when extract is administered in combination with monosodium glutamate (MSG). This could be that the extract interferes with the action of MSG leading to the serum rise in both FSH and LH respectively.

CONCLUSION

The follicle stimulating hormone (FSH) and luteinizing hormone (LH) serum levels increases with monosodium glutamate (MSG) and decreases when extract was co-administered with MSG and this tends to lower reproductive hormones in male. Follicle stimulating hormone and luteinizing hormone, serum levels in clomid treated group also decreases but increases luteinizing hormone serum levels when clomid is co-administered with extract.

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