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EFFECT OF SALVIA OFFICINALIS DECOCTION ON THE HORMONE PROFILE OF NORMAL AND LETROZOLE TREATED ADULT FEMALE RABBITS

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ABSTRACT

This study evaluated the effect of the *Salvia officinalis* leaf extract on serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in adult female rabbits. Ten adult female rabbits (4-6 months) randomly assigned to two groups of 5 rabbits each were used for the work. For the first group, their levels of reproductive hormones were determined before and after daily oral administration of *Salvia officinalis* decoction (30 ml) for 28 days before being allowed to stay untreated for a further 28 days to re-measure the levels of the hormones. The levels of FSH and LH of rabbits in the second group were also determined before and after induction of hormonal disturbance with Letrozole (1.0 mg/kg) daily for 21 days. Thereafter, the rabbits were administered *Salvia officinalis* leaf extract (30 ml) daily for 28 days after which the serum concentrations of the hormones were determined again. Results obtained showed that treatment with *Salvia officinalis* leaf extract significantly increased the levels of follicle stimulating hormone (1.62±0.27) and luteinizing hormone (1.37±0.20) in group one when compared with basal values of 0.82±0.38 and 0.38±0.24 respectively ($p<0.05$) and dropped significantly to 1.16±0.19 and 0.79±0.39 respectively following stoppage of treatment. In the induced group, the significantly elevated FSH (1.53±0.16) and LH (1.56±0.43) values due to letrozol administration were also lowered to 0.97±0.16 and 0.96±0.18 respectively following treatment with *Salvia officinalis*. We therefore conclude that *Salvia officinalis* decoction may promote fertility in females via enhancement of FSH and LH levels and may reverse hormone changes due to letrozole due to restoration of negative feed-back mechanism.

KEYWORDS: Follicle Stimulating Hormone, Letrozol, Luteinising Hormone, Rabbits, *Salvia officinalis.*

1.0 INTRODUCTION

The roles of reproductive hormones in the reproductive process are well established (Orieke *et al*., 2019), even as information on infertility in humans (the inability to conceive after 1 year of unprotected intercourse) and its relationship with disturbances in systemic concentration of female sex hormones is well documented (Olooto *et al*., 2012; Roupa *et al*., 2009; Ombelet *et al*., 2008). Currently, existing global data show that about 25% of couples may experience an episode of either primary or secondary infertility during their reproductive life due to changes in hormone levels (Burtis *et al*., 2008). Primary infertility refers to cases with no previous successful pregnancies, while in secondary infertility previous conceptions have at one time or the other being achieved. Both types of infertility generally have common causes and have also been linked to hormonal dysfunction due to problems affecting the hypothalamic-pituitary-gonadal axis (Benksim *et al*., 2018; Al-Turki, 2015). Measurement of serum peptide and steroid sex hormones is therefore an essential practice in the diagnosis of infertility and the search for effecting management strategies (Bulun and Adashi, 2007; Lo and Lamb, 2004; Hall, 2004). The manufactured medicines currently used in the treatment of infertility are very expensive in addition to their side effects, so many people resort to using alternative therapies such as herbal medicine. Among the herbs used traditionally in treating infertility are *Salvia officinalis*.

Salvia officinalis L. is an evergreen perennial shrub with characteristic flavor and aromatic smell found in the regions of the Mediterranean basin and southeastern Europe, although today it may be found in virtually all parts of the world (Ghorbani and Esmaeilizadeh, 2017; Koşar *et al*., 2010). Extract prepared from the plant have been used over the years for various medicinal and pharmaceutical purposes (Russo *et al*., 2013; Veličković *et al*., 2003; Pedro *et al*., 2016). Phytochemical evaluation of the plant showed significant amounts of compounds, aromatic oils, vitamins and essential minerals known for their roles in fighting disease and promoting women's health (Grdiša *et al*., 2015). Antioxidant agents like phenolic acids and flavonoids

and phytoestrogens which act on the hypothalamus to stimulate secretion of FSH and LH from the pituitary gland due to the liberation of GnRH have all been isolated from *Salvia officinalis* (Ayat *et al*., 2009; Deans and Simpson, 2000). Its ability to promote memory, prevent Alzheimer's disease has been linked to its serotonin content even as the anti-cancer and anti-diarrheal effects are well documented (Lu, Y. and Foo, 2001; Hamidpour *et al*., 2014). In ethno-medicine, *Salvia officinalis* is used lower blood sugar in diabetics, treat bleeding and infections affecting the gums, manage cerebral palsy and weakness of nerves, expel gases from the intestine and to treat inflammations (Hamidpour *et al*., 2014; Khalil and Li, 2011). Extract from *Salvia officinalis* has also been used to treat colds, asthma and allergies (Miura *et al*., 2001).

Results of many studies have indicate that *Salvia officinalis* L. promotes fertility in women and does so by enhancing ovarian (Abdallah *et al*., 2010) and uterine functions (Beigi Boroujeni et al., 2015; Kbyeh, 2015).

In this current study, the effect of *Salvia officinalis* decoction on reproductive hormones, particularly FSH and LH, in female rabbits was evaluated.

2.0 MATERIALS AND METHOD

2.1. Purchase of *Salvia officinalis* and preparation of extract in solution

Dried *Salvia officinalis* L was bought from a herbal market in Brak-AL Shati, Libya and was taken to the Laboratory where extract in solution was prepared from it. To achieve this, 4 grams of the dried plant material was boiled in 500 ml of water for 5 minutes, allowed to cool before being filtered to obtain the filtrate containing the extract. This extract in solution was transferred into a sterile container and preserved in a refrigerator until needed.

2.2. Experimental animals

Ten female rabbits of the Chinchilla specie of average body weight 1.04 ± 0.28 kg and 5-6 months old were used for the study. The animals were assigned to 2 separate plastic cages labeled groups 1 and 2 with suitable lighting conditions, fed two meals of bread, date and clover daily and allowed access to water *ad libitum*. Prior to commencement of experiments, the animals were allowed a period of one week of stay in their various cages to help them adapt to their environment. All experiments were carried out in Physiology laboratory of the Department of Medical Lab Technology, Sebha University, Libya. International guidelines for care and used of laboratory animals were strictly adhered to. Animals were also fasted 12 hours before commencement of experiment. The rabbits were treated according to the schedule below:

Group 1: Administered 30 ml of the extract daily for 28 days after measurement of basal FSH and LH values before being allowed to stay for another 28 days without treatment. FSH and LH concentrations were re-measured after the period.

Group 2: Administered Letrozole (1 mg/kg body weight) for 21 days after measurement of basal FSH and LH values before being treated with the extract (30 ml daily) for 28 days. The concentrations of FSH and LH were re-measured after the period.

All treatments were via the oral route. At the end of the experiment, about 5 ml of blood was collected from the ear vein of each rabbit into plain bottles and was allowed a period of one and half hours to clot and retract before being centrifuged at 3000 rpm for 10 minutes, to obtain clear serum which was used for the analysis.

2.3. Hormone (FSH and LH) assays

The serum LH and FSH were estimated by cobase e411 Immunoassay System. Immunoassay reactions utilizing electrochemiluminescence (ECL) . ECL is a process in which highly reactive species are generated from stable precursors at the surface of an electrode. These highly reactive species react with one another, producing light. The development of ECL immunoassays is based on the use of a ruthenium (II)-tris(bipyridyl)[Ru(bpy)] complex and tripropylamine (TPA). The final chemiluminescent product is formed during the detection step. The chemiluminescent reactions that lead to the emission of light from the ruthenium complex are initiated electrically by applying voltage to the immunological complexes that are attached to the streptavidin-coated microparticles.

2.4. Statistical analysis

The results were expressed as mean ± SD, while significant differences between data were determined by one-way analysis of variance (ANOVA). Level of significance was set at $p < 0.05$.

3.0 RESULTS

3.1 Effect of *Salvia officinalis* on the hormone profile of normal rabbits

Treatment with *Salvia officinalis* significantly increased the concentrations of FSH and LH when compared with basal values ($p<0.05$). This observed increase in hormone level in the female rabbits reversed and tilted towards basal values after 28 days of terminating treatment (Table 1).

Table 1: Effect of *Salvia officinalis* extract on FSH and LH values of rabbits.

Group	Treatment	Follicle Stimulating Hormone (ng/ml)	Luteinising Hormone (ng/ml)
1	A	0.82±0.38 [a]	0.38±0.24 [a]
1	B	1.62±0.27 [b]	1.37±0.20 [b]
1	C	1.16±0.19 [c]	0.79±0.39 [a]

Values represent the mean ± SD for N =5. Values in the same column marked * are not significantly different from each other (p< 0.05). A = basal value, B = value after 28 days oral treatment with Salvia officinalis, C = value after 28 days of stoppage of treatment.

3.2 Effect of *Salvia officinalis* on letrozole induced hormonal changes in rabbits

While the effects of letrozole on FSH and LH concentrations of the rabbits was similar to that of *Salvia officinalis*, having caused significant rise in the values of both parameters (p<0.05), 28 days administration of the extract to the same rabbits after stoppage of letrozole treatment did not cause further increase in the values of these parameters, instead decline back to almost basal values were observed (Table 2).

Table 2: Effect of *Salvia officinalis* on letrozole induced hormonal changes in rabbits.

Group	Treatment	Follicle Stimulating Hormone (ng/ml)	Luteinising Hormone (ng/ml)
2	A	0.82±0.50 [a]	0.96±0.19 [a]
2	B	1.53±0.16 [b]	1.56±0.43 [b]
2	C	0.97±0.16 [a]	0.96±0.18 [a]

Values represent the mean ± SD for N =5. Values in the same column with the same alphabet are not significantly different from each other (p< 0.05). A = basal value, B = value after 21 days oral treatment with Letrazol, C = value after 28 days of treatment with Salvia officinalis.

4.0 DISCUSSION

The results of this study has shown that *Salvia officinalis* extract increased the levels of FSH and LH in female rabbits following 28 days oral administration, suggesting that the extract may contain active principles with FSH and LH enhancing potentials in females. The fact that the observed activity declined following stoppage in the administration of the extract further suggests that the increase the serum levels of these hormones in the rabbits was actually an activity attributable to the administered *Salvia officinalis* extract. This finding is consistent with that of Al-bediry and Al-Maamori (2005). This activity of the extract may be due to its reported phytoestrogens content (Ayat *et al*., 2009; Deans and Simpson, 2000). Phytoestrogens stimulate the release of GnRH from the hypothalamus which in turn stimulates the secretion of FSH and LH by the anterior pituitary gland (Al-bediry and Al-maamori, 2013). This function of phytoestrogens may also enhance the development of the female genital tract in addition to increasing the length of menstrual cycle, alleviating symptoms of menopause and to a large extent protect against the development of endometrial cancer (Burton and Wells, 2002). The fact that phytoestrogens are predominatly flavonoids (Carlos and Michael, 2012) and that flavonoids were significantly found in *Salvia officinalis* (Ahmad and Mahdi, 2017) further supports the effect of *Salvia officinalis* in this study. Phytoestrogens usually produces physiological and developmental effects by triggering estrogenic activity via estrogen receptor signaling pathways (Al-Fatlawi and Al-Gbouni, 2018; Carlos and Michael, 2012). The observed increase in FSH concentration following treatment with *Salvia officinalis* decoction further suggests that the plant has the potential to improve reproductive processes I females. FSH enhances sexual development via initiating processes involved in oogenesis and adequate development of reproductive organs (Orieke et al., 2019). The decline observed in the levels of FSH and LH in the rabbits following stoppage of the administration of the extract may be attributed to a reversal of this estrogenic activity due to withdrawal of treatment.

Letrozol, a non-steroidal aromatase inhibitor, caused decreased the levels of estrogen in the rabbits and by that stimulates the release of more FSH and LH due to absence of negative feed-back of estrogen (Rad *et al*., 2016). This negative feed-back was however restored following oral administration of *Salvia officinalis*, hence the decrease in the levels of these hormones. This decrease in the FSH and LH level may be due to the effect of phytoestrogens that are naturally present in *Salvia officinalis* (Dadfar and Bamdad, 2019).

CONCLUSION

Since results of this study indicate that *Salvia officinalis* decoction increased the concentrations of FSH and LH in the female rabbits and attenuated letrozole induced in the levels of these hormones, we therefore conclude that the herbal preparation may enhance fertility in females and may further be of values in correcting problems associated with hormone changes due to letrozole use due to its ability to effectively restore the negative feed-back mechanism needed for the sustenance of the levels of these hormones.

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