

Hormonal studies of some herbs medicine extract (*Salvia Officinalis*) and its effect on total body weights and some reproductive organs in the mature female mice

دراسة هرمونية لتأثير نبات الميرمية على وزن الجسم الكلي وبعض الاعضاء التناسلية في اناث الفئران الناضجة

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Abstract:

Present investigation was designed to show the influence of quotation of *Salvia officinalis* on some reproductive organs for female mice. Three groups of this animals (6each) treated orally with quotation of the herb in two different doses (30 and 60 mg/kg) respectively for four weeks. The present study showed significant differences ($P \leq 0.05$) in body weight for both groups as compared to control group, also significant differences ($P \leq 0.05$) in ovary and uterus only in the group treated with high dose as compared to control group. However significant differences ($P \leq 0.05$) found in reproductive hormone (FSH, LH) for both groups treated with *Salvia officinalis* as compared to control group. Histological study showed a remarkable increase in number of ovarian follicles, increase growth and development of oocyte and zona granulosa for groups treated with *Salvia officinalis* as compared to control group.

الخلاصة:

هدفت الدراسة الحالية الى تقصي تأثير المستخلص المائي لأوراق لنبات الميرمية على الجهاز التكاثري لإنات الفئران البالغة. اخذت ثلاثة مجاميع (6 فئران لكل مجموعة) جرعت فمويا المجموعة الاولى بماء الحنفية واعتبرت مجموعة سيطرة لأغراض المقارنة بينما جرعت المجموعة الثانية والثالثة بالمستخلص المائي لنبات الميرمية بتركيز (30 و 60 mg/kg) على التوالي عن طريق الفم لمدة اربعة اسابيع. اظهرت النتائج فروقات معنوية ($P \leq 0.05$) بالنسبة لوزن الجسم الكلي لكلا المجموعتين المعاملة بالمستخلص المائي مقارنة مع مجموعة السيطرة، كما اظهرت النتائج وجود فروقات معنوية ($P \leq 0.05$) بالنسبة لوزن المبيض والرحم فقط في المجموعة المعاملة بالتركيز العالي من المستخلص المائي مقارنة مع مجموعة السيطرة. بينت النتائج وجود فروق معنوية ($P \leq 0.05$) في الهرمونات التكاثرية (FSH, LH) لكلا المجموعتين المعاملة بالمستخلص المائي لنبات الميرمية *Salvia officinalis* مقارنة مع مجموعة السيطرة. اوضحت الدراسة النسجية زيادة في النمو والتطور للحويصلات المبيضية والبويضة لكلا المجاميع المعاملة بالمستخلص المائي للنبات مقارنة مع مجموعة السيطرة.

Introduction

Salvia officinalis it is a medical herb available in nature, it has been used since ancient Egyptian times for growing productiveness of the women, this herb has many biological activity such as antiviral, antioxidant, antifungal and antimicrobial activity, also many study showed this herb has antimutagenic activity in many mammalian [1]. Sage has a long history with culinary and medicinal use [2]. So, it is usually used for management of asthma, flatulent dyspepsia, pharyngitis, uvulitis, stomatitis, gingivitis, glossitis angina, cough [3, 4]. Also the oil of *S. officinalis* has essential activity as Anti-microbial against *Staphilicocos aureous* and *Candida albicans* [5]. German Commission recommended interior use of sage for extreme perspiration and dyspeptic signs, and the exterior use for infection of mucous membranes of throat and mouth [6]. Sage is reputed to be one of the richest sources of potent antioxidant and employed to treat symptoms of menopause [7]. The herb has strong effects in cases of oligomenorrhea and amenorrhea [8]. It can treated menopausal symptoms, premenstrual stiffness and imbalance hormone because it have estrogenic component, while certainly not be used in pregnancy [9]. The aim of the study to revealed the effect of sage on some hormones and reproductive organ weight in female mice.

Material and Method

Mature animals (n=18), with age 9-10 weeks and (19-21) g body weight were attained from the faculty of Pharmacy. They were acclimatized at room in temperature (22-24°C). Experimental animals were given two week for acclimatized period in animal housing environment before the beginning treatment. During this period, animals fed with standard pellet diet and tap water.

Study design

The animals were divided randomly into three main groups each one contain (n=6). The first group (A) used as control and received equal volume of tap water and other two as experimental groups (B,C) treated with 30 and 60 mg/kg of sage respectively. All groups received material by gavage for four weeks.

Preparation aqueous extract for herb

Water extract of *herb* was done by soaked 100 mg of powder dry of herb leaves in 100 ml of sterilized boiling water for (20 min) with incessant moving. The crude mixture was filtrated and kept in sterile dark bottles at 4 °C until used, this extract was added to the water supplement of the mice after calculated daily water drinking (100 ml/day/mouse) (10). The doses Calculation according to the body weight.

Ovaries and uterus (reproductive organs) weight

At the end of experiments, the animal were sacrificed to open abdomen and remove both ovaries and uterus these organs were washed in water twice then dried and weighted by using electronic balance [22].

Blood sample collection

The blood sample collection after 24 hours from the last dose, before killing the animal (anesthetized with chloroform) by cardiac puncture used disposable syringe of 3ml their blood samples were collected in a centrifuge tubes for maximum coagulation and separation of serum, after centrifuged at 3000 rpm/10minutes, serum was isolated, frozen at (-20°C) and then processed for hormonal assays [22].

Hormonal assay

Prepared kit was used for hormones assayed depends on ELISA kit, antibodies to Ag then binding occurs, after that peroxidase Ag wash. Added conjugate, substrate solution of ELISA enzyme adding after this added color change finally stop reaction solution added. Hormone then read at 450 nm.

Histological study

Ordinary histological sections were prepared from ovary organ to study any histological changes that may be occurred in the experimental groups after administration with aqueous extract of *Salvia officinalis*. The mice sacrificed and organs were processed according to [11].

Statistical analysis:

Data were analysed' using SAS View 512+ Software (Abacus Concept, Inc. Calabasas, CA, USA). Differences among groups were measured using one way analysis of variance (ANOVA) followed by the least significant differences, statistically significant at (P≤0.05) [12].

Result:

Table (1) shows the effects of doses from aqueous extract, which revealed that both doses are significantly different (P≤0.05) from the control group in their effect on the total body weights, whereas ovary and uterus weight did not show any significant difference between the low and high dose, on other hand the result revealed significant different (P≤0.05) in the ovary and uterus weights only at high dose as compared with the control group. The results of table (2) shows that significant different (P≤0.05) in the FSH and LH hormone for both group treated with different doses from aqueous extract of *Salvia officinalis* as compared with the control group, while no any significant different between low and high dose in FSH and LH hormone. The histological examination of the ovary section (Figure 1) showed the normal histological appearance from ovarian follicles, (Figure2) demonstrating a distinct zonal granulosa nearby the oocyte and compact theca follicular and increase the presence of primordial follicles, while in the figure (3) showed increase growth and development of oocyte and zona granulosa.

Table (1): The effects of different doses of aqueous extract from *Salvia officinalis* on total body weight and reproductive organs

Parameter	Control	30mg/kg	60mg/kg	LSD*
Total body weight (g)	20.33 ±0.82 B	23.50±1.37 A**	24.67±1.51 A	3.0
Ovary weight (mg)	0.77±0.18 B	0.94±0.09 AB	1.09±0.22 A	0.30
Uterus weight (mg)	1.69±0.51 B	1.93±0.53 AB	2.52±0.37 A	0.80

**Different letter refer to significant difference, LSD (least significant differences). Similar letters refer to not any significant difference.

Table (2): The effects of different doses of aqueous extract from *Salvia officinalis* on female reproductive hormone

Hormone (mIU/ml)	Control	30mg/kg	60mg/kg	LSD*
FSH	1.94 ±0.66 B	3.46±0.28 A**	3.82±0.34 A	1.50
LH	1.97±0.26 B	2.64±0.32 A	2.79±0.16 A	0.60

**Different letter refer to significant difference, LSD (least significant differences). Similar letters refer to not any significant difference.

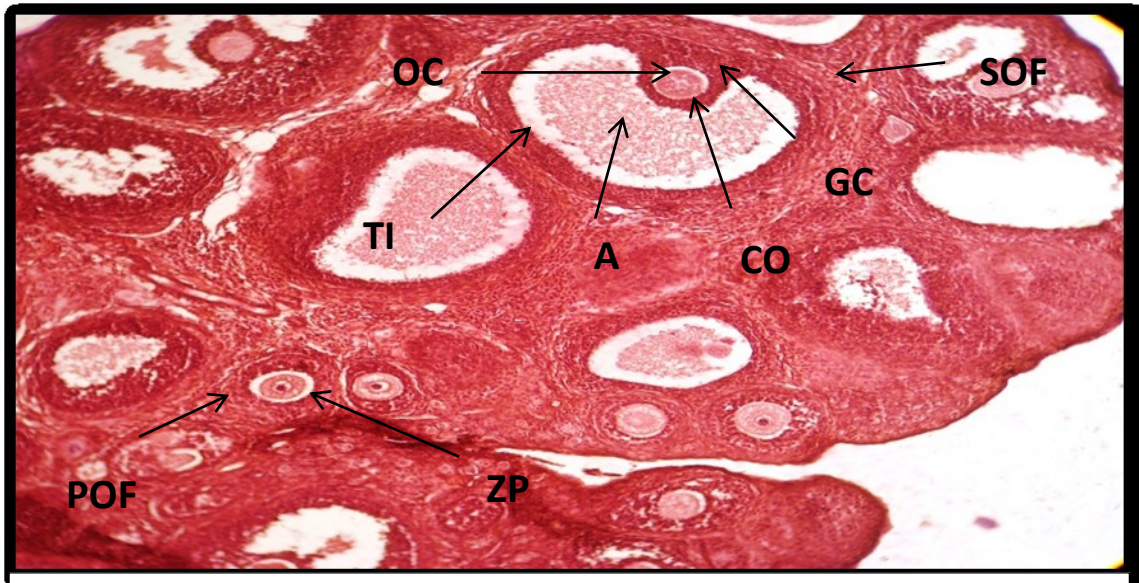


Figure (1): Cross histological section of the ovary from intact mature female mice showing secondary ovarian follicles (SOF), Oocyte (OC), Theca interna (TI), Antrum (A), Cumulus Oopherus (CO), granulosa cells (GC), Primary ovarian follicles (POF) and zona pellucida (ZP) (H&E.stain, X400).

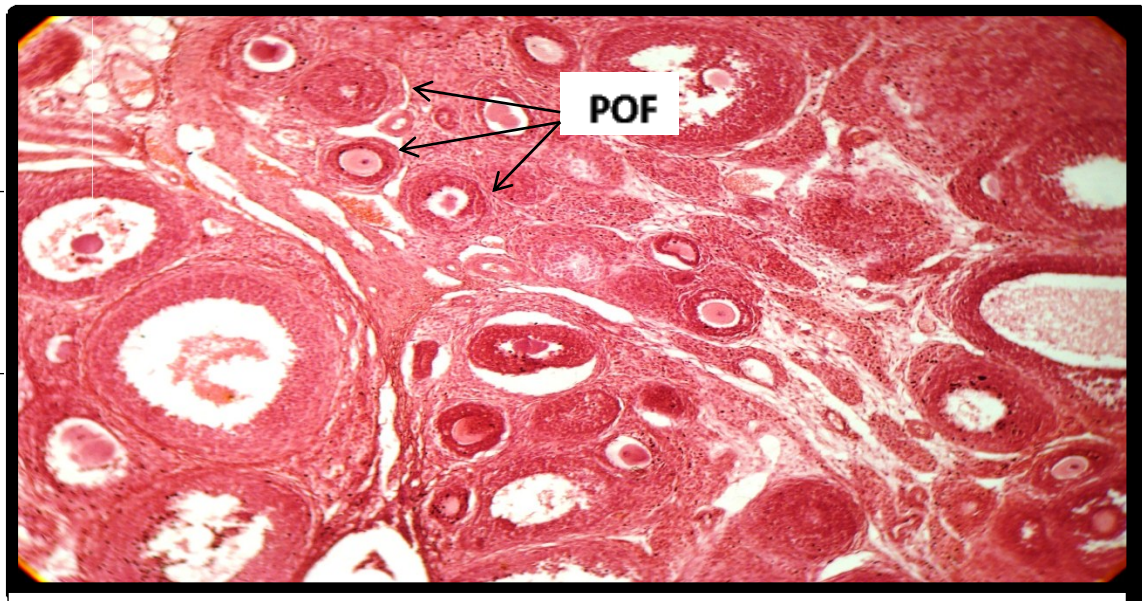


Figure (2): Cross histological section of the ovary from intact mature female mice treated with 30 mg/kg from *salvia officinalis* showed increase granulosa surrounding the oocyte and increase the presence of primary ovarian follicles (POF) (H&E.stain, X200).

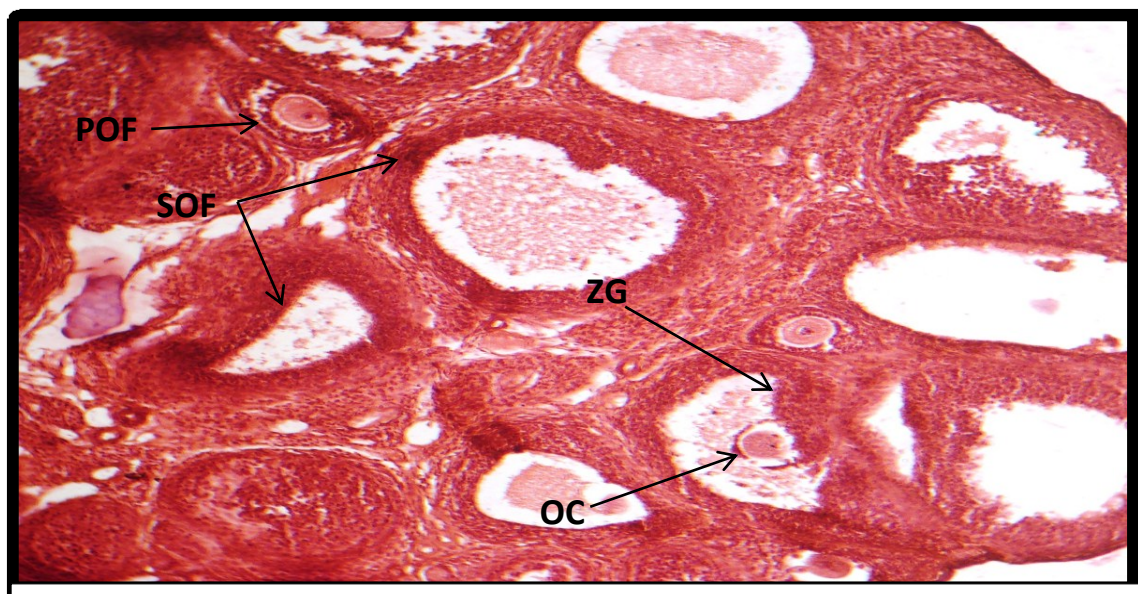


Figure (3): Cross histological section of the ovary from intact mature female mice 60 mg/kg from *salvia officinalis* showed increase growth and development of oocyte (OC) and zona granulosa (ZG) for secondary ovarian follicles (SOF) (H&E.stain,

Discussion:

According to our results of female mice for both groups treated with different doses of *salvia officinalis* show significant increase in the total body weight as compared to the Control group this increase can be attributed to the fact that sage herbs it's known to be good stimulus for appetite also treated disorders in the digestive and promote weight gain [13, 14]. The sage plant as also have hypoglycemia effect, which this effect can, that causes pituitary gland activated excretion of (GH) [15, 16]. Growth hormone (GH) responsible for increase muscle mass, increase cellular uptake of amino acids then synthesis protein and regulate lipolysis [16]. While the present study disagreement with what found by [17] demonstrated that no any significant differences in the body weight of mature female rat treated with extract of *salvia officinalis*. The present study in agreement with what found by [18]. The present study also showed significant increase in the weight of reproductive organs (uters and ovaries) for both groups treated with *salvia officinalis* this increase can attributed to the influence of these herbs on estrogen hormone, which is very important for increase thickness of endometrial by increase the number and highest of epithelial cells, as well as increase in endometrial glands diameters all this causes increase the blood flow and fluid content [19,20]. However another studies showed *salvia officinalis* contain steroid and isoflavonoid which is considered as phytoestrogens which have direct influence on CNS convinces activation of cell division and estrus then growth of the genital tract of animals [21, 16]. Present study in agreement with what found by [22] who showed increase in the total body weight and reproductive organs (both ovary and uterus) in the mature female mice treated with different doses of aqueous extract of *salvia officinalis*. The present data showed significant increase serum luteinize hormone (LH) and serum follicle stimulated hormone (FSH) level after oral administration of groups with different doses of aqueous extract of *salvia officinalis*, this increase can be attributed to biological active compound (steroid and isoflavonoids) found in this herbs which consider phytoestrogen and can effect on hypothalamus to release GnRH which to stimulate pituitary gland to secrete FSH and LH hormones [14, 23]. The present study in agreement with [24] who found increase secretion of FSH and LH hormones in mice after treated with of aqueous extract of *salvia officinalis*. While the present study disagreement with what found by [22] who showed significant increase in LH hormone while found significant decrease in FSH hormone of female mice after aqueous extract of

salvia officinalis treated. Also the present study disagreement with what found by [25] who demonstrated no any significant differences in the level of hormone LH and FSH after treated female rat with *salvia officinalis*. Histological lesion in the reproductive organs such as ovaries, containing the growth in the number of the graafian follicle due to influence some elements found in herb such as estrogen, which causes activation hormone excretion like LH [22].

Conclusion

In conclusion, the present study demonstrates that widespread effect of that herb on reproductive hormone which causes activated, as well as increases in body weight and reproductive organs.

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