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The Effect of Sage Tea Compounds on the Reproductive Organs of Mature Female Mice

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Abstract

In Iraq and other Arab countries, *salvia officinalis*, or sage, is widely available and it is used to make a well-liked herbal tea that women with reproductive disorders can sip on a daily basis. The goal of this study include examine the probability usage of this herb in female hormonal disturbance as estrogen replacement choice via determination of the primary characteristics of ovarian and uterine tissues in adult female mice administered sage extract through histological and physiological analysis. The experiment included 36 mature female mice divided into three main groups (12 female mice in each group) were given equal amount of distilled water to control group while single and double dose of saga tea (0.057 g/ kg B.W. and 0.114 g/ kg B.W. respectively to group 2 and 3) for duration of 28 days. Following a course of treatment, six female mice were chosen from each group anesthetized and have blood samples taken for a test on reproductive hormones. ovarian and uterine sections were prepared for histological examination according to the routine microscopically technique at 5µm thickness then stained in H&E stain and examined to detect the number and diameter of ovarian follicles also detect the variation in uterine layers thickness via ocular micrometer. Due to the reproductive hormone assays, groups with sage extract treatment showed an increased in serum levels of progesterone, FSH, LH, and E2. However, histological analysis of ovarian and uterine sections showed a significant increase ($P>0.05$) in the diameter of the ovary as well as in the thickness of the endometrium and myometrium. In addition, numbers and diameters of ovarian follicles (primary, secondary, Graff follicles, and the corpus luteum also indicated a significant increase ($P>0.05$)compared with control group. neither group's 2 nor 3 treated sage extract showed dose-dependency.

Keywords: Reproductive organs, ovarian hormones, Sage tea, female mice, *salvia officinalis*.

تأثير مركبات شاي الميرمية على الأعضاء التناسلية في إناث الفئران الناضجة

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الخلاصة

في العراق والدول العربية الأخرى، تتوفر المريمية على نطاق واسع وتستخدم لصنع شاي الأعشاب الذي يمكن للنساء اللاتي يعانين من اضطرابات إنجابية احتساءه يوميًا. يتضمن الهدف من هذه الدراسة فحص او بيان احتمالية استخدام هذه العشبة في الاضطرابات الهرمونية الأنثوية كبديل للإستروجين من خلال تحديد الخصائص الأولية لأنسجة المبيض والرحم في إناث الفئران البالغة التي تم تجريعها مستخلص الميرمية من خلال التحليل النسيجي والفسيولوجي. تضمنت التجربة استخدام ستة وثلاثون فأرا ناضجا تم تقسيمهم الى ثلاث مجاميع رئيسية (تتضمن اثنا عشر فأرًا في كل مجموعة) تم تجريعهم كميات متساوية من الماء المقطر لمجموعة السيطرة وجرعتين (فردية مزدوجة) من مستخلص شاي الميرمية عن طريق الفم (0.057 جم/كجم من وزن الجسم و0.114 جم/كجم من وزن الجسم على التوالي) لمدة 28 يومًا. بعد انتهاء دورة العلاج، تم اختيار ستة فئران من كل مجموعة لتخديرها وأخذ عينات من الدم لأختبار الهرمونات التكاثرية (الهرمون المحفز للجريبات المبيضيه والهرمون المحفز للجسم الاصفر) . تم تحضير مقاطع من المبيض والرحم للفحص النسيجي وفق التقنية المجهرية الروتينية بسمك 5 مايكرومتر ثم تلوينه بصبغة هيماتوكسيلين- ايوسين وفحصه لمعرفة عدد وقطر الجريبات المبيضية بالإضافة إلى كشف الاختلاف في سمك طبقات الرحم عن طريق الميكرومتر العيني. وفقا للفحوصات الهرمونية ، فان المجموعات التي خضعت للعلاج بمستخلص الميرمية زيادة في مستويات البروجسترون، الاستروجين وايضا هرمونات الهرمون المحفز للجريبات المبيضيه والهرمون المحفز للجسم الاصفر في الدم. ومع ذلك، كما وأظهر التحليل النسيجي لمقاطع المبيض والرحم زيادة معنوية ($P<0.05$) في أقطار المبايض وكذلك سمك بطانة وعضل الرحم. بالإضافة إلى ذلك، أشارت أعداد وأقطار جريبات المبيض (الأولية والثانوية وحويصلات غراف والجسم الأصفر أيضًا إلى زيادة كبيرة ($P < 0.05$) مقارنة بمجموعة السيطرة، كما ولم يظهر مستخلص الميرمية المستخدم في المجموعة 2 أو 3 اي اختلاف اعتمادا على الجرعة.

الكلمات المفتاحية: الاعضاء التناسلية ,هرمونات المبيض, شاي الميرمية , اناث الفئران .

1. Introduction

A significant health and social issue that impacts a large number of people worldwide is infertility [1]. A year of regular, unprotected intercourse followed by the inability to generate a clinical pregnancy is the hallmark of infertility [2]. Worldwide, about 48 million couples struggle with infertility. In 50% of cases, male infertility is the cause; however it can come from one or both couples [3]. Varieties of plants have been used as sex stimulants in traditional medicines[4]. One of the most of these herbal medicines family is Salvia officinalis L. Since ancient Egypt, this plant, often known as common sage, has been utilized for a variety of purposes, including boosting female fertility [5].

According to Alrezaki et al. [6], *S. officinalis* has been suggested for a variety of gynecological disorders and has been labeled in numerous ethno pharmacological reports. As a result, this herb famous as “Maramia” in many Arabs countries likes Iraq and Saudi Arabia. Because of its widespread use in both medicinal and culinary preparations, it is referred to as the "queen of herbs" [7]. A lot of research has been done on the plant, and reports on its biological activities are abundant [8]–[11]. *Salvia officinalis L*. is widely used in traditional medicine, nonetheless very little is known about its effects on female reproductive tissues. So the main goal of this study was to examine the probability usage of this herb in female hormonal disturbance in estrogen replacement therapy.

2. Materials and methods

2.1 Plant material and extraction

Salvia officinalis L., a dry sage plant, was purchased from a well-known market vendor in Al-Kut city, in Wasit province\ Iraq. The plant was classified due to the State Board for Seed Testing and Certification in addition to Iraqi Ministry of Agriculture, order number: 479. The sage tea drinking was made according to Lima's [12]. method, which involved boiling four grams of dry sage aerial parts in three hundred milliliters of distilled water, letting it cool for five to ten minutes, filtering it, and then giving the mice the filtrate, which is the sage tea, orally. 0.8 milliliters of the prepared sage tea were administered orally via stomach tube.

2.2 Animal treatment

This study has been approved in the animal house in College of Education for Pure Sciences/Wasit University. This study was performed using 36 virgin female and 9 male of proven fertility mice weighting about (100-210 g) aged between (3-4 months), provided with water and libitum. The females were dispersed into 3 groups, 12 mice in every group.

Group 1 (control group), mice in this group were fed (1 mL) of distilled water for 28 days.

Group 2 given orally a standard single dose/daily (0.057 g/kg B.W.) of sage tea at a consistent volume (4.285 ml/kg B.W.) for 28 days, depending on the mice body weight.

Group 3 was given the conventional double dose/daily (0.114 g/kg B.W.) of sage tea orally, with the amount (8.570 ml/kg B.W.) varying according to the mice body weight over the course of 28 days.

for reproductive index measurements, 6 female mice were housed separately after receiving treatments of either distilled water or sage tea. Single, sexually mature, untreated male mice who had demonstrated fertility was kept apart from two female mice in a cage apart from the estrous cycle. The first day of pregnancy was defined as the appearance of a copulation plug in the vagina, indicating effective copulation. Mice were tested by vaginal smear to identify the existence of diestrus stage cells seven days following the discovery of a vaginal

plug in order to confirm pregnancy. Followed by reproductive indices that determined according to Ratnasooriya et al[13].

2.3 Hormonal Assay

To obtain plasma, centrifugation of collected blood samples for 10 minutes at 3000 rpm. Enzyme linked immunosorbent assays (ELISAs) were used to measure the levels of estradiol, progesterone and testosterone in accordance with the manufacturer's instructions.

2.4 Histological preparations and Histo-Physiological Examinations of Female Reproductive Organs

The animals received treatment for 28 days before being weighed and sacrificed. Their ovaries and uterus were removed, weighed, and then preserved for 24 hours in neutral buffered formalin (NBF). According to Munro [14]; 5 µm thickness sections of uterine and ovarian tissues routinely processed then stained with (H&E) routines stain, finally microscopically examination to determine some of histological examinations which included:

a. Ovaries Diameters measurement

After calibrating with a stage micrometer, the ovary diameter was determined using an ocular micrometer. 40x objective lens were used for examined and compared with the control group.

b. Endometrial and Myometrial Measurement

After calibrating with a stage micrometer, the uterine endometrial thickness was determined using an ocular micrometer.

c. Ovarain follicles and Corpora Lutea Counting

Each slide's corpora lutea and various follicle counts were computed. After calibrating with a stage micrometer, an ocular micrometer was used, and 10X and 40X ocular lenses were used for the examination.

2.5 Statistical Analysis

The ANOVA method was used to analyze the data in this work. A paired-sample T test was also performed using SPSS 16 (a statistical software program) to compare treatment groups (2) and (3). Statistics were deemed significant if $P<0.05$. Mean ± standard error was used to express each result.

3. Results and Discussion

3.1 Serum Reproductive Hormones

FSH serum glycoprotein in control groups was (0.50±0.002) while (0.263±0.002) of LH. The values of these two glycoproteins were significantly ($P>0.05$) higher after oral administration of sage tea drinking in double dose to reach 0.63±0.0163 and 0.309±0. While in single dose reached to 0.61±0.0168 and 306±0.003 (Table1). However, over an experimental duration, there was no significant difference in these glycoprotein between (2) and (3) treatments group.

Table 1- Effects of *Salvia* extract on serum reproductive hormones

Groups	FSH (mIU/ml)	LH (mIU/ml)
Group 1 (Control)	0.50±0.002	0.263±0.002
Group 2	0.61±0.0168	0.306±0.003
Group 3	0.63±0.0163	0.309±0.006

3.2 Ovarian hormones

Due to statistical analysis; there was significant increase (P>0.05) in the levels of the steroid hormones progesterone and estrogen in the serum. The levels of these hormones were 9.9±0.068b and 27.02±0.173 ng/ml in the treated female mice treated with a double dose of sage tea, compared to 9.8±0.012 and 26.32±0.264b ng/ml in the treated female mice treated with a single dose of sage tea. The control mice serum steroid hormone levels were 8.615±0.031 and 21.57±0.429 a ng/ml, respectively (Table 2).

Table -2: Effect of *Salvia* extracts on serum reproductive hormones

Groups	*Estrogen (pg/ml)*	*Progesterone (ng/ml)*
Group 1 (Control)	8.615±0.031	21.57±0.429
Group 2	9.8±0.012	26.32±0.264
Group 3	9.9±0.068	27.02±0.173

3.3 Histological Results

1. Ovaries Diameters

When compared to the ovarian diameter of control female mice (3516.33±1.21 µm), the histological sections of the ovaries in the oral treatment group with both single and double doses of sage tea revealed a significantly significant increase (p<0.05) in ovarian diameter that reached a highly value (3898.16±2.62 µm) for the double dose and (3889.32±1.03) for the single dose. However, there was no significant difference (P<0.05) in the increase in ovarian diameters between groups 2 and 3 (table 3).

2. Endometrial and myometrium thickness

Table 3 lists the endometrial and myometrial thickness after consuming sage tea in double and single doses orally. The statistical analysis showed that the endometrial and myometrial thickness of the uterus increased significantly after sage tea treatment, in comparison to the control group as showed in table3. Nevertheless, over the course of the 28-day experiment, there was no significant alteration ($P<0.05$) in the increased endometrial and myometrial thickness in either group (2) or (3) (Table 3).

Table - 3: Effect of *Salvia* extract on uterine layer thickness and ovarian diameter

Groups	*Ovarian diameter (µm)*	*Endometrial thickness (µm)*	*Myometrium thickness (µm)*
Group 1 (Control)	3516.33±1.21	467.5±0.68	223±0.56
Group 2	3889.32±1.03	514.66±1.18	236±0.84
Group 3	3898.16±2.62	509.5±1.87	237.34±1.38

3- Ovarian follicles and Corpora Lutea Counting

Due to the results from (Tables 4) and figures (4) to (9), there was non-dose-dependent after sage tea treatment but significant differences ($p<0.05$) in the numbers and diameters of ovarian follicles, and corpus lutea. In comparison to the control group (6±0.36, 4.9±0.20, 3.2±0.19 and 5.30±0.2), there was significantly increasing ($p<0.05$) in the numbers of primary, secondary, Graafian's follicles and corpus lutea, reaching (8.4±0.21, 8±0.50, 5.8 and 8.8±0.25) for the double group and (8.6±0.28, 6.8±0.23, 6.3±0.23 and 7.80±0.28) for the single group. Additionally, when compared to control female mice (50±0.50, 140.0±0.50, 230±0.30 and 660.10±0.30µm respectively), also there was significantly increasing ($p<0.05$) noticeable in the diameters of primary, secondary and Graafian's in both treated groups after oral administration of sage tea. These values were (58±1.00, 155.2±1.60, 250.98±0.73 and 686.80±1.58µm) for the double dose group and (57. 98±1.60, 150.0±2.0, 250.98±0.73 and 668.30±0.30µm) for the single dose group table 4.

Table – 4: Effect of Salvia extract on ovarian follicles number and Corpora Lutea

Parameters / *Groups*	*Primary follicles*		*Secondary follicles*		*Graafian follicles*		*Corpora Lutea*	
	Numbers (N)	Diameters (D)	Numbers (N)	Diameters (D)	Numbers (N)	Diameters (D)	Numbers (N)	*Diameters (D)*
Control	6±0.36	50±0.50	4.9±0.20	140.0±0.50	3.2±0.19	230±0.30	5.30±0.2	*660.10±0.30*
Group 2	8.6±0.28	57. 98±1.60	6.8±0.23	150.0±2.0	6.3±0.23	250.19±0.40	**7.80±0.28**	*668.30±0.30*
Group 3	8.4±0.21	58±1.00	8±0.50	155.2±1.60	5.8±0.30	250.98±0.73	**8.8±0.25**	*686.80±1.58*

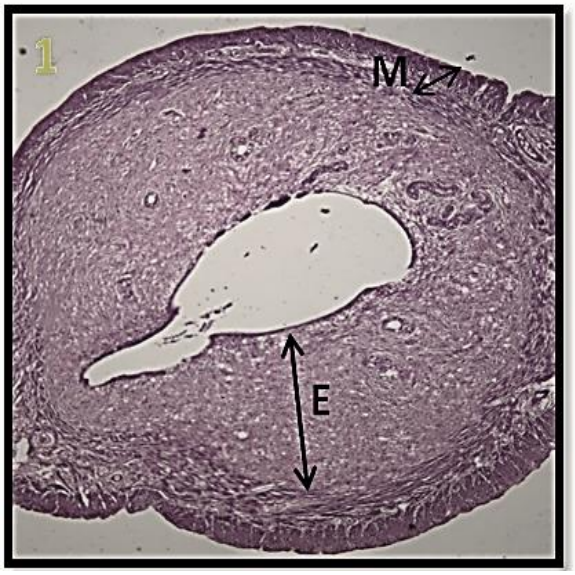

Figure - 1 microscopic section of uterus in control group mice shows endometrial thickness (E) and myometrial thickness (M). H&E stain, 40 x.

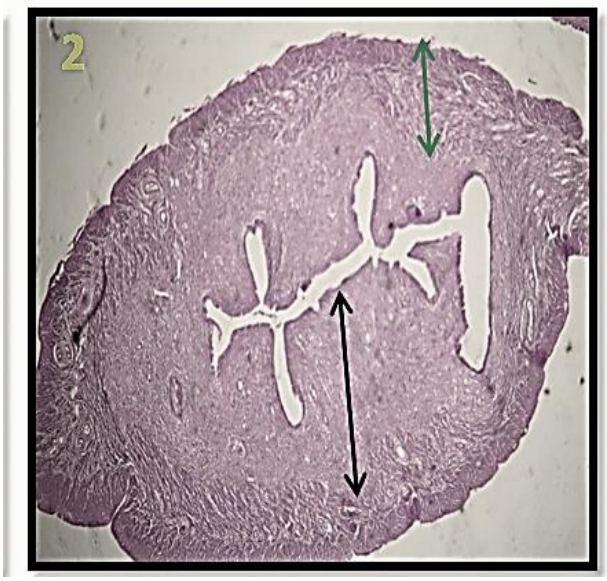

Figure -2 microscopic section of uterus in mice consumed single dose of sage extract showed an increase in endometrial thickness (black arrow) and myometrium (green arrow). H&E stain, 40 x.

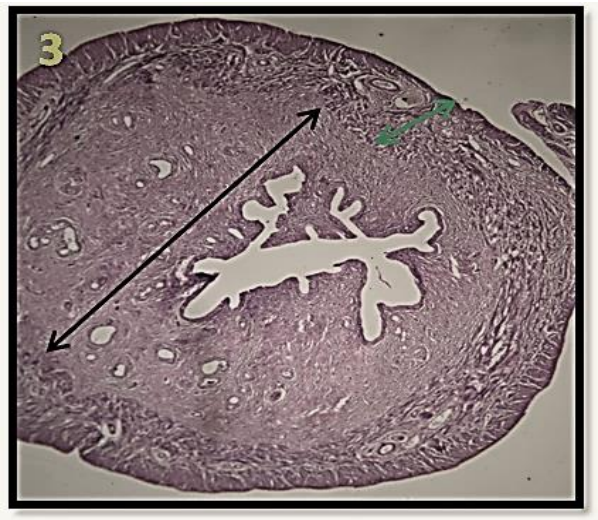

Figure – 3 microscopic section of uterus mice consumed double dose of sage extract showed an increase in endometrial thickness (black arrow) and myometrium (green arrow). H&E stain, 40 x.

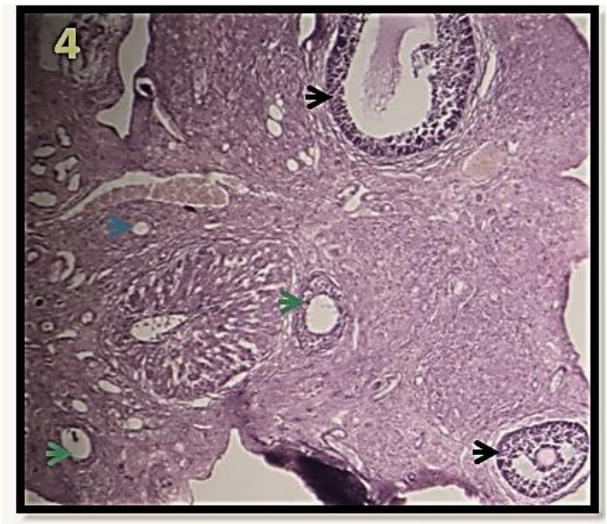

Figure -4 microscopic section of uterus mice consumed orally distilled water showed primary follicle (blue arrow), secondary follicle (green arrow) and Graffian follicle (black arrow). (H&E stain, 100x

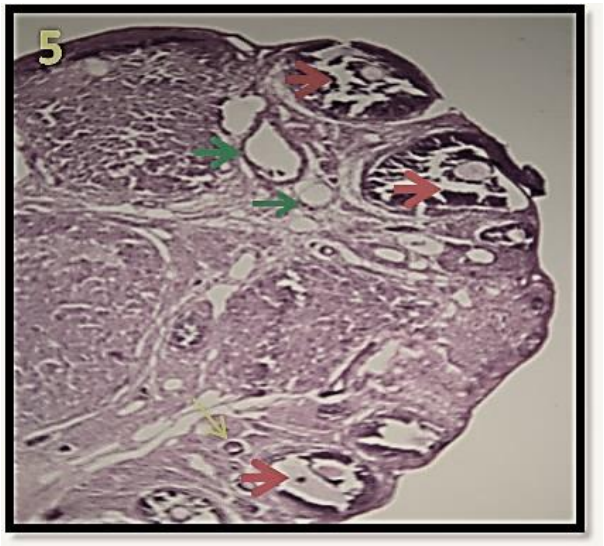

Figure -5 microscopic section of uterus mice single dose of sage extract showed primary follicle (yellow arrow), secondary follicle (green arrow) and Graffian follicle (red arrow). H&E stain, 100x.

Figure -6 microscopic section of uterus mice double dose sage extract showed primary follicle (black arrow), secondary follicle (yellow arrow) and Graffian follicle (green arrow). H&E stain, 100x.

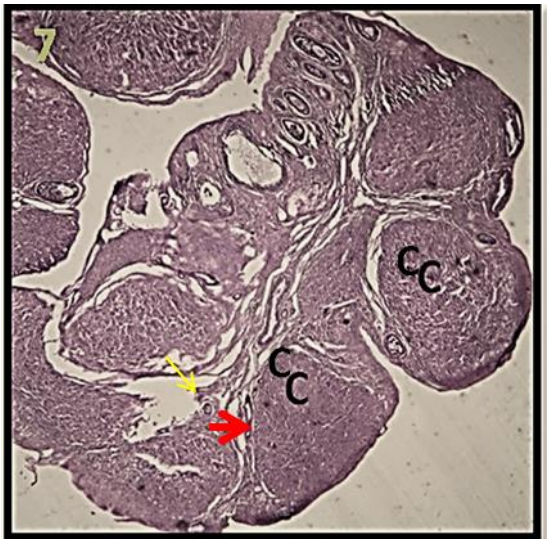

Figure -7 Microscopic section of uterus mice consumed orally distilled water showed Corpora Lutea (C). H& E stain, 40x.

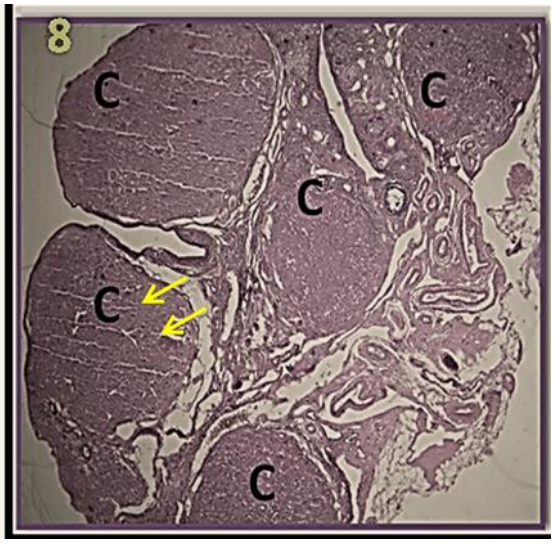

Figure -8 Microscopic section of uterus mice consumed single dose sage extract showed Corpora Lutea (C). H& E stain, 40x.

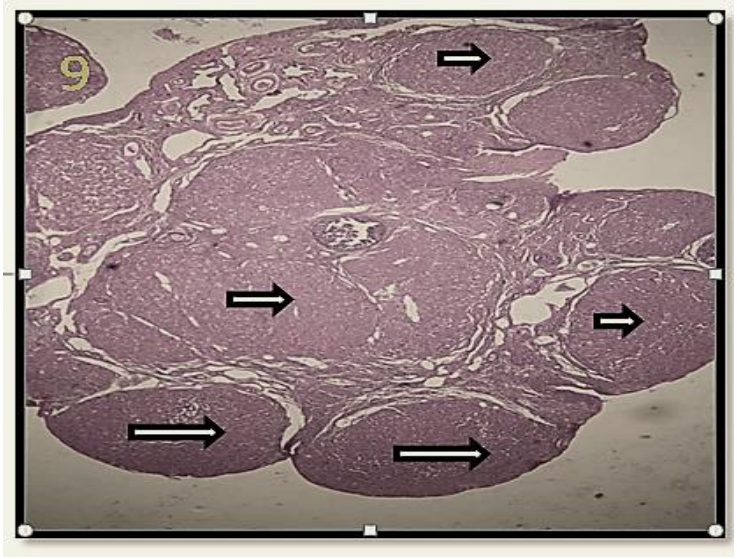

Figure -9 Microscopic section of uterus mice consumed single dose sage extract showed an increase in Corpora Lutea (arrows). H& E stain, 40x.

Due to the hormonal results, both serum FSH and LH values were significantly higher in double dose and single dose of sage's tea administration in comparison to this glycoprotein in control group. However, over the course of the 28-day experiment, there was no significant difference in the increase in glycoprotein FSH and LH between treatments (A) and (B). The explanation for these results may be due to the effect of the high phytochemical phenolic content of *Salvia* extract on reproductive function. This proposed was accepted with Maggini *et al*. [15]; Elgayed *et al*. [10] (when he refers to the effective herbal approach of *Saliva* to be appropriate for the long-term treatment of menopausal symptoms.

Phytoestrogen properties of herb used on the hypothalamus-pituitary glands axis may be the cause of these results due to the fact that this herb is widely acts as phytoestrogen [8]. Furthermore, Whitehead and Lacey [16]. discovered that phytoestrogens possess biologically active compounds that may stimulate the secretion of FSH and LH hormones from pituitary gland by releasing gonadotropin releasing hormone (GnRH) from the hypothalamus. This result is consistent with two studies on celery extract by Hafez and Hafez [17]. and Al-Gorbawi's [18] which demonstrated that administration of alcoholic and aqueous extracts of celery leaves as in *Salvia* caused an important increasing in ovarian hormone as these leaves have a chemical compound that induced releasing of (GnRH), which stimulates FSH secretion, stimulating of maturing ovarian follicle, which lead to ovulation and forms corpus luteal. These hormones also cause steroid hormone secretion by pituitary gland via negative feedback mechanism of hypothalamus -pituitary gland, which prevents the secretion of GnRH. Al-Gorbawi's [18]. research also supports this observation, indicating that oral administration of alcoholic and aqueous extracts of celery leaves in female mice caused noticeable increase in the hormones LH and FSH.

Furthermore, these results are consistent with Elgayed et al. [10] who study the reproductive function of this herb on uterine mice which propose possible estrogenic possessions through induced proliferative changes in the uterine of treated mice. And suggest that *Saliva* extract can be used as a hormonal replacement for women during menopause. Finally, due to the literature, decreasing risk of breast cancer among postmenopausal women is related with flavonol consumption which found in Saga's extract [8]. Also *Salvia* comprises other bioactive components that display a valuable role in improving quality of life during the menopause [9].

4. Conclusions

Sage extract recommended for treating female infertility disorders and hormonal replacement for women during menopause, sage extract works best when taken in large doses because it may stimulate steroidogenesis and folliculogenesis, which can improve uterine and ovarian function due to the high effectiveness of these herb contents of phytoestrogen components.

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