

SPhysiological Efficiency of Sage Tea (*salvia officinalis*

L.)Administration on Fertility in Adult Female Rats

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الكفاءة الوظيفية لتناول شاي نبات المريمية (*salvia officinalis* L.)

في خصوبة أنثى الجرذان البالغة

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الخلاصة

نبات *Salvia officinalis* L. (الاسم الشائع المريمية) إحدى النباتات التي كانت تستخدم منذ العصور المصرية القديمة لزيادة خصوبة المرأة، وقد تم استخدامه تقليدياً لزيادة خصوبة على الأكثر من دون فهم. هدفت الدراسة الحالية تقصي الدور الوظيفي لشرب شاي نبات المريمية لتقييم كفاءة الخصوبة بوساطة بعض المعايير التكاثرية الانثوية خلال التجربة لمدة 28 يوم، لتشمل الهرمونات التكاثرية (هرمون محفز الجريب FSH والهرمون اللوتيني LH والاستروجين والبرجستيرون) ومؤشرات التكاثرية (مؤشرات الخصوبة والحمل وعبوشة ما بعد الولادة وعبوشة الجراء الفطيمة). إذ تم تقسيم أنثى الجرذان البالغة وعددها 36 جرذ عشوائياً إلى ثلاث مجموعات أحدها مجموعة سيطرة ومجموعتين معاملة، ففي التجربة الأولى (اعطيت الحيوانات فموياً جرعتين تقليديتين أحدهما مفردة 0.057 غم/كغم وزن جسم والآخرى مضاعفة 0.114 غم/كغم وزن جسم/يومياً من شاي نبات المريمية لمدة 28 يوم على التوالي). وأنجزت التجربة الثانية لتقييم مؤشرات التكاثر. أظهرت النتائج وجود زيادة معنوية عالية في مستويات هرمونات المصل لكل من FSH وLH والاستروجين والبرجستيرون لا تعتمد على الجرعة الفموية لشاي نبات المريمية. بينما أظهرت مؤشرات التكاثر وجود علاقة مع الجرعة الفموية المفردة لشاي نبات المريمية. يستنتج من النتائج الحالية بان شرب شاي المريمية ربما يحسن الخصوبة الانثوية للجرذان البالغة عن طريق محور تحت المهاد-النخامية-المبيض.

Abstract

Salvia officinalis L. (common name sage) is one of this which has been used since ancient Egyptian times for increasing women's fertility; it has been used traditionally to increase fertility have been rather poorly understood. The present work was aimed to investigate the physiological role of sage tea drinking in fertility capacity by some female reproductive parameters of rat during the experiment for 28 day to includes reproductive hormones (follicular stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone hormones) and reproductive indexes (fertility, gestation, post-natal viability and weaning viability indexes). Thirty six female rats were divided at random into three main groups'one control group and two treatment groups as first experiment (received orally by gavage single (0.057 g/kg B.W.) and double (0.114 g/kg B.W.) traditional doses/daily of sage tea drinking for 28 day respectively).

The second experiment was done to evaluate the reproductive indexes for female fertility. The results showed a higher significantly increase in serum FSH, LH, estrogen and progesterone hormones levels non dose dependent orally administration of sage tea. But, the reproductive indexes showed a single dose related orally administration of sage tea. Concludes from this present finding that sage tea drinking may be improves female fertility via of the hypothalamic-pituitary-ovarian axis in adult rats.

Introduction

Medicinal plants were considered in the past as the only form of health care readily available to the majority of human population (WHO, 2000). There is an increasing interest towards medicinal plants and their active ingredients drugs in the last years. Also, in traditional medicine a variety of plants had been used as sex stimulants (Islam *et al.*, 1991).

For centuries, people for a long time tried different types of herbs, these herbs may help in restrong fertility, some of them are; *Nigellasativa*, *Glycyrizza glabra* and *Tribulus terrestris* (Craig, 1999). Arabs have made use of herbal drugs to improve sexual performance and increase libido (Puri, 1971). In Egypt, the pollen grains of dates (*Phoenix dactylifera*) and seeds of hermala (*Peganum harmala*) are used to restore sexual potency (Amin *et al.*, 1969). Also in African tradition medicine, especially in Cameroon, *Zingiber officinale* and *Pentadiplan-dra brazzeana* are used as aphrodisiac and male sexual stimulation (Noumi *et al.*, 1998).

Salvia officinalis L. (common Sage), family *Lamiaceae* has been used since ancient Egyptian times for increasing women's fertility, and more. The Romans likely introduced it to Europe from Egypt as a medicinal herb (Watters, 1901). Today is cultivated all over the world and used widely in traditional medicine. Because of the wide range of traditional medicinal effects, sage enjoys the reputation of being a panacea: it has been used as sexual debility; for menstrual and menopausal problems; to treat nervous and mental conditions (Dweck, 2000). Many bioactive compounds present in this plant, such as phenolic compounds and terpenes. (Cuvelier, *et al.*, 1996; Wang, *et al.*, 1998; Lu, and Foo, 2001), which are thought to be the key for several therapeutic properties attributed to sage (Lima, 2006).

Fertility regulation with plant preparation has been reported in ancient literature of indigenous systems of medicine. A number of plant species have been tested for fertility

regulation years ago and were subsequently fortified by national and international agencies (Sinha, 1990; Dixit *et al.*, 1989; Puri, 1971). The usage of biologically active botanical substances or fertility-regulating agents of plant origin which are ecofriendly in approach and interfere with the natural patterns of reproduction becomes necessary (Dixit, 1992; Dehghan *et al.*, 2005). However, Woman's quality of life is often associated with a healthy hormonal balance throughout her reproductive and menopausal years. Disruptions in hormone balance can lead to menstrual disorders such as irregular bleeding and heavy bleeding, symptoms of premenstrual syndrome (PMS), as well as menopausal complaints later in life. There is a strong need for therapies to treat these problems, as it is estimated that over 30% of all women experience symptoms of PMS during their reproductive years (Lauritzen *et al.*, 1997). In addition, 3,500 women's enter their menopausal years every day in the U.S., the majority of who experience significant symptoms (Hudson, 1996). While Western medicine addresses these concerns with pharmaceuticals, often with undesirable side effects, herbs such as chaste berry (*Vitex agnuscastus*) and black cohosh (*Cimicifuga racemosa*) offer an option for women who would like a safe, natural approach to reducing menstrual cycle and menopausal discomforts (Tyler ,1994; Schulz *et al.*,1998).

Additionally, *Salvia officinalis*L. Consider phytoestrogen (Balacs, 1993).The estrogenic activity of sage has been demonstrated experimentally, and a team of investigators has shown the herb has strong effects in cases of oligomenorrhea and amenorrhea (Salbei, 2007).Therefore, can be used for hormone imbalances as premenstrual tension, irregular (or heavy menses), pelvic congestion fibroids, endometriosis and cysts. Herbs,

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which stimulate, regulate and promote a normal menstrual flow. They should never be used in pregnancy (Yardley, 2004). Therefore, the study aims to support the possibility of use this plant in herbal medicine as an alternative drug for improving the fertility of adult females.

Material and Methods

Preparation of plant material and sage tea drinking:

Dry sage plant (*Salvia officinalis* L.) was bought from the popular market in Al-Kut city/Wasit province, Iraq. A specimen has been classified at lush vegetation of the Iraqi National Council /the Ministry of Agriculture, Iraq. The sage extract was prepared as tea drinking routinely by pouring 300 mL of boiling water onto 4 g dried plant material and allowing to steep for 5 min (Lima, 2005).

Experimental animals:

Thirty six female albino strain rats, weighting (125-175 g) aged (2-3 months) were used for present work. Rats were obtained from the animal house of embryo and infertility research center in AL-Nahrain University. Animals maintained in rat cages in the animal house of College of Science/Wasit University. Experimental animals were given two week of acclimatized period in animal housing environment before the start of the experiments. During this period, the animals were maintained on natural light/dark cycle at $20 \pm 2^{\circ}\text{C}$ and fed with standard pellet diet and tap water ad libitum.

Study design:

First experiment:

The animals were divided at random into three main groups (12 female rats in each group). One control group and two experimental groups, the latter groups received orally sage tea at single and double doses/daily by gavage for 28 days respectively.

-First group: served as *control* - were administered orally by gavage with equal volume of distilled water (vehicle) for 28 days.

-Second group: served as *treatment group (A)* - were received orally traditional single dose/daily (0.057 g/kg B.W.) with an equal volume of sage tea for 28 days.

-Third group: served as *treatment group (B)* - were received orally traditional double dose/daily (0.114 g/kg B.W.) with an equal volume of sage tea for 28 days.

The experimental single and double doses were prepared traditionally per kg body weight.

Second experiment:

One day later of end orally treatment period with distilled water (vehicle) or sage tea, six female rats separated from above three groups of first experiment and then were housed independently to assess *reproductive indexes*. Nine sexually mature untreated males of

proven fertility were used in this experiment. Two female rats were housed independently of the estrous cycle with single a sexually mature untreated male of proven fertility. The presence of a copulation plug in the vagina was regarded as successful copulation and was considered the first day of pregnancy. For pregnancy confirmation, rats were examined by vaginal smear to determine the presence of diestrus stage cells during a 7 day-period after the finding of a vaginal plug.

The reproductive indexes:

The following reproductive indexes were calculated as follow: fertility index, defined as No pregnant females/No females with successful copulation $\times 100$; gestation index, defined as No of females with alive pups/ No of pregnant females $\times 100$; post-natal viability index, defined as No of pups alive on day 4 / No of alive pups $\times 100$; and weaning viability index, defined as No of pups alive at day 21/ No of pups alive at day 4 $\times 100$ (Ratnasooriya et al., 2003).

Blood sample collection and hormonal assay:

Collection of blood and separation of blood serum

After 24 hours from last orally dose of treatment period, the animal were sacrificed under ether anesthesia, the blood was collected from the animals in each group by cardiac puncture by used disposable syringe of 5ml, 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.

Hormonal assay

The serum reproductive hormonal assay of FSH, LH, estrogen E2 and progesterone were performed depends on kit assay procedure of ELISA kit (Germany), antibodies to Ag: detected by in which the sample to be analyzed were incubated in well of micro plate that are coated with Ag, antigen antibody binding occurs, after wash peroxidase Ag conjugate added

then ELISA enzyme substrate solution added, coloring change occurs, stop solution added then reading the absorbance at 450 nm by human reader system.

Statistical analysis:

The data of present works were made with using SPSS 16 (statistical software package) and analyzed by ANOVA (one way analysis). The results were expressed as mean \pm standard error of the mean (M \pm S.E.M.). Tukey HSD was used for comparisons between the experimental and control groups and paired-sample T test for comparison between treatment groups single (A) and double (B) doses for the evaluation of values and $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Hormonal assays:

The effects of orally administration of sage tea drinking on serum reproductive hormones were summarized in table (1). As shown there was a significantly ($P < 0.05$) increase in serum FSH, LH, estrogen and progesterone levels of after sage tea orally administration in both doses compared with control group. But these increased in hormone levels were not different significantly ($P > 0.05$) between (A) and (B) treatments during the experiment for 28 day.

1- Glycoprotein hormones: Follicle stimulating hormone (FSH) and Leutizing hormone (LH)

The glycoprotein FSH and LH was higher significantly ($P < 0.05$) increased after orally administration of sage tea in double dose (B) (0.62 ± 0.0161 and 0.60 ± 0.0167 mIU/ml) but different significantly ($P > 0.05$) with a single dose (A) group (0.308 ± 0.004 and 0.305 ± 0.002 mIU/ml) when compared with control group (0.52 ± 0.003 and 0.265 ± 0.002 mIU/ml) respectively. This is may be due to the phytoestrogen effects of sage tea on hypothalamus –pituitary gland axis. Its well consider as phytoestrogen (Balacs, 1993). Also, Whitehead and Lacey (2000) who show that phytoestrogen contain biological active compound which can effect on hypothalamus to release GnRH which to stimulate pituitary gland to secrete FSH and LH hormones. This finding in accordance with study of Hafez and Hafez (2000) who show that alcoholic and aqueous extracts of celery leaves when administration at two dose (500 and 1000 mg/kg) to the female mice lead to the significant increase ($p < 0.05$) for follicle stimulating hormone and Leutizing hormone because these leaves contain chemical compound induce gonadotropin releasing hormone (GnRH) from hypothalamus which stimulate pituitary gland to secretion FSH which stimulating follicle maturing in ovary and LH hormone which causes ovulation and form corpus luteal, these hormone induce the secretion of steroid hormone by negative feedback

mechanism between hypothalamus and pituitary gland to inhibit secretion of GnRH this lead to inhibit secretion of FSH and LH hormone from pituitary gland.

2- Ovarian steroid hormones: Estrogen and progesterone hormones

The statistical analysis obtain from the table (1) also has been shown a high significantly ($P < 0.05$) increase in serum steroid estrogen and progesterone levels in treated female rats with double dose of sage tea (9.933 ± 0.066 pg/ml and 26.01 ± 0.172 ng/ml) than to treated female rats with single dose of sage tea (9.7 ± 0.011 pg/ml and 25.31 ± 0.263 ng/ml) when to compared with control rats (8.616 ± 0.030 and 22.58 ± 0.430 ng/ml) respectively. This observation is similar to finding of Nagel (1998) who show that alcoholic and aqueous extracts of celery leaves contain phytoestrogen which increase estrogen level in administration mice serum. Similarly, Ethanolic extract of *Bupleurum marginatum* was found to have significant estrogenic activity as seen by the increased uterine weight and early opening and cornification of vagina in immature rats and histological features of the uterus (Sheel *et al.*, 1995). Moreover, Ganong (1995) who reported that phytoestrogen in their activation and chemical structure similar to the animal estrogen and progesterone by induce granulose cells in ovary to secretion progesterone and increase estrogen secretion from theca cells by positive feedback mechanism.

3- Reproductive indexes

In both single dose and double treated groups, the fertility index, the gestation index, the post-natal viability index were similar except the weaning viability index when compared to the control group. So, the orally administration of single dose of sage tea treated rats showed a positive indicator in all reproductive indexes compare with double dose treated rats (Table 2). This may be might to stimulate the sexual behaviors were supported by our findings by increase the estrogenic activity after orally administration of sage tea. However, no literatures available deal with reproductive indexes expect one study by Ruiz-Luna *et al.*, (2005) who used *Lepidium meyenii* (Maca) to investigate the its effects on several fertility parameters of female mice at reproductive age. Maca has been described to improve fertility since many centuries ago (Cobo, 1956).

In conclusion, the overall findings of the study support the traditional use of the sage tea drinking to enhance female fertility. This is might directly affect via of the hypothalamic-pituitary-ovarian axis.

Table 1: The effects of sage tea (*S. officinalis* L.) on serum reproductive hormones in female rats after 28 days

Parameter Group	FSH (mIU/ml)	LH (mIU/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)
Control group	0.52±0.003 ^a	0.265±0.002 ^a	8.616±0.030 ^a	22.58±0.430 ^a
Group A	0.60±0.0167 ^b	0.305±0.002 ^b	9.7±0.011 ^b	25.31±0.263 ^b
Group B	0.62±0.0161 ^b	0.308±0.004 ^b	9.933±0.066 ^b	26.01±0.172 ^b

Data = Mean ± S.E.M. (n= 6 rats in each group).

Control group: Received orally by gavage with equal volume of distilled water (vehicle) 28 days.

Group A: Received orally traditional single dose/daily with an equal volume of sage tea/daily for 28 days.

Group B: Received orally traditional twice dose/daily with an equal volume of sage tea/daily for 28 days.

Mean with different superscripts are different significantly ($p < 0.05$).

Table 2: The effects of sage tea (*S. officinalis* L.) on reproductive indexes in female rats after 28 days

Reproductive index (%) Group	fertility index	Gestation index	Post-natal viability index	Weaning viability index
Control group	83.30	100	100	96.80
Group A	100	100	100	100
Group B	100	100	100	90.00

Data = Mean (%) (n= 6 rats in each group).

Control group: Received orally by gavage with equal volume of distilled water (vehicle) 28 days.

Group A: Received orally traditional single dose/daily with an equal volume of sage tea/daily for 28 days.

Group B: Received orally traditional twice dose/daily with an equal volume of sage tea/daily for 28 days.

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