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The Effect of *Salvia fruticosa* Ingestion on Male Rat Sexual Behavior

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Abstract: Ingestion of *Salvia fruticosa* by adult Sprague-dawley rats at levels of 800 mg kg^{-1} body weight for 30 days was investigated for its effects on sexual behavior. *Salvia fruticosa* powder was orally administered to sexually active rats in a daily dose of 800 mg kg^{-1} body weight daily for 30 days. The sexual behavior was then monitored by trained observers on *Salvia fruticosa* treated rats and control rats that received vehicle. Testosterone serum level was measured in both treated and control rats. *Salvia fruticosa* ingestion significantly reduced the mount, intromission and ejaculation latencies. In addition, a significant increase in the copulatory efficacy was reported. Testosterone serum level was significantly decreased in *Salvia fruticosa* treated rats compared to controls. The present study concludes that continuous ingestion of *Salvia fruticosa* dried root for a period of thirty days improves the sexual motivation and performance in adult male rats. This effect can be ascribed to increased testosterone level.

Key words: *Salvia fruticosa*, sexual behavior, testosterone, rat, sexual motivation, Jordan

INTRODUCTION

Salvia fruticosa Mill. (Sf), formerly known as *Salvia triloba* L. (Lamiaceae) and commonly known as Greek Sf, is a native species of the Eastern Mediterranean basin. It has a long history of use as a culinary herb as well as in the treatment of various disorders. This herb (especially its leaves) has a folk reputation in the Eastern Mediterranean region for the treatment of various skin, blood and infectious ailments as well as ailments of the digestive, circulatory, respiratory,andosteomuscular systems (Carmona *et al*., 2005; Ali Shtayeh *et al*., 2000). It is also used as a hypoglycemic herb (Perfumi *et al*., 1991) and against inflammations, hepatitis and tuberculosis (Wichtl, 1994; Kaileh *et al*., 2007).

Twenty species of Salvia grow wildly in Jordan, this country is dominated by arid climate but with high biodiversity (Oran and Al-Eisawi ,1998). In Jordan *Salvia triloba* (Syn. *Salvia fruticosa*) is one of the most popular aromatic plants. It is used to improve the taste of tea (Lev and Amar, 2002).

The species is widespread in the Mediterranean basin as the climatic conditions favor its growth. Many studies on essential oil composition have reported 1, 8-cineole as the main component, followed by camphor R-thujone, thujone and caryophyllene. Besides the high content of oxygenated monoterpenes, the species is also known to contain biologically active sesquiterpenes and diterpenes (Pitarokili *et al*., 2003; Perry *et al*., 1996). Until now there is no information about the effects of this plant on the sexual desire and potency. Sexual impotence, recently known as erectile dysfunction, is defined as the inability to achieve or maintain a penile erection sufficient for sexual satisfaction (NIH, 1993). This problem is associated with adverse effects on the quality of life, specially family and social interrelationships (Laumann *et al*., 1999). The use of herbal medicine, enriched by information from plant research has always played a primary role in the treatment of this condition (Hollister, 1975). Based on the previous facts, the current study aims to investigate the effect of *Salvia fruticosa* on the sexual behavior of adult male rats. It tests the hypothesis that daily ingestion of dried *Salvia fruticosa* over a period of thirty days reduces the sexual behavior in adult male rats. Also, the serum testosterone level is assessed in those animals in order to elucidate the mechanism of action of this plant in influencing the sexual behavior.

MATERIALS AND METHODS

Experimental model: Adult male albino rats of sprague-dawley strain weighing about 300 g produced and raised in animal house unit in The Faculty of Medicine at Jordan University of Science and Technology (J.U.S.T.) were used in this study. All animal care procedures and treatments were conducted with the approval of the J.U.S.T. Committee on animal care and in accordance with the guidelines of the National Institute of Health on the use and care of laboratory animals (USA). Rats were allowed for three pre-experimental mating tests with sexually receptive females and those who achieved ejaculations in the three times within a period of <30 min

were chosen for this study. The chosen rats were divided randomly into two groups, control versus experimental and caged separately. Each group contained ten male rats. The animals were kept under controlled temperature of 21±1°C and 12 h light: 12 h darkness schedule (lights on 06.00 AM-18.00 PM). Food and water were available *ad libitum*. The rats were allowed for two weeks acclimatization period before starting the treatment.

Treatment: *Salvia fruticosa* plant was harvested from northern Jordan mountainous region during the spring (March-April). *Salvia fruticosa* were dried in an electrical oven at 35°C for 48 h. Dried roots were grinded into powder to facilitate its oral administration to the animals in a dose of 800 mg/kg/body weight as one single dose in the morning for thirty days. Each dose was solubilized in 4 mL distilled water before being administered by oral gavage. Control rats received the same volume of distilled water only. Female rats of the same strain were used in this experiment. Each female was brought into estrous by sequential subcutaneous injections of 30 μg estradiol benzoate (Intervet International B.V., Holland) and 1 mg progesterone (Schering AG, Germany) 48 and 4 h before testing, respectively. The females were screened with non-experimental males and the ones that show good sexual receptivity (solicitation and Lordosis in response to mounting) were used.

Sexual behavior test: The sexual behavior of male rats was monitored by two trained observers unaware of the experimental design in a sound-attenuated room according to the standard procedure (Agmo, 1997). The test was performed 24 h after the last administration. Single male rat was placed in a rectangular Plexiglas observation chamber (45×40×30 cm height) and allowed to acclimate for 5 min. Then, a sexually receptive female rat was introduced in the chamber. The following parameters of sexual behavior were measured as described by Agmo (1997).

Mount Latency (ML): Time from the introduction of the female until the first mount.

Intromission Latency (IL): Time from introduction of the female until the first intromission (vaginal penetration).

Ejaculation Latency (EL): Time from the first intromission until ejaculation.

Postejaculatory Interval (PEI): Time from ejaculation until the next intromission.

Mount Frequency (MF): Number of mounts preceding ejaculation.

Intromission Frequency (IF): Number of intromissions preceding ejaculation. Also, the following parameters were calculated on the basis of the above data.

Inter-Intromission Interval (III): Average interval between successive intromissions (calculated as ejaculation latency divided by intromission frequency).

Copulatory Efficacy (CE): A measure of intromissive success (calculated as intromission frequency divided by mount frequency + intromission frequency). Tests were normally ended immediately after the first post-ejaculatory intromission.

Testosterone assay: Four rats from each group, which were not submitted to mating tests, were used to analyze the serum testosterone level. Animals were euthanized by ethyl ether 24 h after the last dose.

Trunk blood was collected into centrifuge tubes and the serum was prepared by centrifugation (3000 rpm. for 30 min) and stored frozen (-20°C) until testosterone assay. The testosterone concentration was determined in triplicate using the Testosterone Enzyme Immunoassay test kit (BioCheck Inc., Foster City, California, USA) according to the manufacturer's instructions. The minimum detectable concentration of this assay was estimated to be 0.05 ng mL^{-1} and cross reactivity with other corticosteroids was minimal (<0.05%).

Statistical analysis: After applying the Levene's test to determine the homogeneity of variance, data were evaluated at 5 and 1% levels of significance by using t-test for independent samples. The data are presented as mean±Standard Deviation (SD). All statistical tests were performed using SPSS program (standard version 13.0, SPSS Inc., Illinois, USA).

RESULTS

Sexual behavior: The effect of treatment with *Salvia fruticosa* on the sexual behavior of adult male rats is shown in Table 1. Treatment with *Salvia fruticosa* induced significant ($p<0.01$) increase in mounting, intromission and ejaculation times in comparison with corresponding values of control rats. Also, a significant ($p<0.01$) decrease in the copulatory efficacy was detected in *Salvia fruticosa* treated rats compared with the controls. Although, there was no significant ($p>0.05$) difference in both postejaculatory and inter-intromission intervals between *Salvia fruticosa* treated and control rats.

Table 1: Effect of oral administration of *Salvia fruticosa* on the sexual behavior of adult male rats

Treatments	ML (sec)	MF	IL (sec)	IF	EL (sec)	PEI (sec)	CE	III (sec)
Vehicle	82.28±16.7	5.83±1.6	278.08±33.7	15.73±3.8	944.9±107.3	434.17±89.6	0.792±0.05	74.03±25.8
Salvia fruticosa (800 mg kg^{-1})	129.8±7.7**	9.4±1.2**	462.1±5.3**	21.0±1.6*	1427.0±84.8**	511.3±30.3	0.65±0.07**	97.33±16.2

Each value is representing the mean±standard deviation, obtained for 6 rats per group. *p<0.05 and **p<0.01 compared with vehicle-treated rats (t-test). ML = Mount Latency; MF = Mount Frequency; IL = Intromission Latency; IF = Intromission Frequency; EL = Ejaculatory Latency; PEI = Post-Ejaculatory Interval; CE = Copulatory Efficacy; III = Inter-Intromission Interval

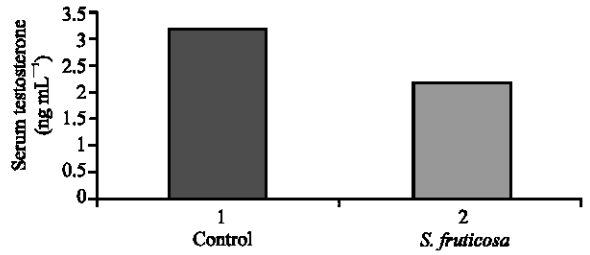

Fig. 1: Effect of oral administration of *Salvia fruticosa* on serum testosterone level in adult male rats compared to control rats. Each value is the mean±Standard Deviation (SD) obtained for four animals in each group. **p<0.01 (t-test)

Testosterone serum levels: Testosterone serum levels in *Salvia fruticosa* treated and control rats are illustrated in Fig. 1. Treatment with *Salvia fruticosa* significantly (p<0.01) decreased serum testosterone level in comparison with vehicle treated animals. Serum testosterone level in *Salvia fruticosa* t-treated rats was 2.16±0.630 ng mL^{-1}, while in control rats 3.182±0.683 ng mL^{-1}.

DISCUSSION

It is a common practice in Jordan to drink hot tea containing *Salvia fruticosa* leaves decoction in cold seasons. The cup (200 mL) of hot tea contains ~600-700 mg of *Salvia fruticosa* leaves extract. The average daily consumption by Jordanians 1-3 cups. Therefore, it dedicated to study the effects of *Salvia fruticosa* plant in reduce the male sexual behavior. The ingestion of the *Salvia fruticosa*, over a period of thirty days, significantly increased the mounting, intromission and ejaculation times in adult male rats. These parameters are considered to sexual motivation and libido (Beach, 1956). In addition, the copulatory efficacy is significantly decreased in these animals due to *Salvia fruticosa* treatment. Copulatory efficacy represents the efficiency of erection and penile orientation and is considered an indication to sexual potency and performance (Agmo, 1997). The depression in sexual desire and performance after ingestion of *Salvia fruticosa* could be ascribed to the significant decrease in serum testosterone level. Several previous studies have related the increase in testosterone level to the ability of different herbs in improving the sexual function (Gauthaman *et al*., 2002; Zanoli *et al*., 2008, 2009). The effects of testosterone on the sexual behavior and erectile function are well established. Testosterone has been found to enhance the sexual interest, add to the frequency of sexual acts and increase the incidence of nocturnal erections (Mulligan and Schmitt, 1993). These effects of testosterone are induced through multiple mechanisms that include central and peripheral pathways. Peripherally, testosterone has been found to enhance the erectile response of cavernous nerve and to increase blood flow into the sinuses of corpus cavernosum in the rat (Giuliano *et al*., 1993; Mills *et al*., 1998). The exact mechanism of how *Salvia fruticosa* boosts the testosterone level is still unclear. Al-hamood *et al*. (1998) have reported that the ingestion of *Salvia fruticosa* produce adverse effects on the fertility of male and female rats. However, future investigations are required at both cellular and intracellular levels to identify the exact mechanism of how *Salvia fruticosa* components decrease the testosterone level. One of the suggestions is that this plant may act through the hypothalamic pituitary-testicular pathway leading to this stimulatory effect.

CONCLUSION

This study concludes that *Salvia fruticosa* diet contains a compound or more in its structure that is/are able to reduce both sexual desire and performance in male rats. This depression could be mostly ascribed to decreased serum testosterone level. However, further studies that using the fractions obtained from the *Salvia fruticosa* by means of different solvents (methanol, acetone, chloroform, or water) are required and are in progress to isolate and identify the active ingredient (s) that is/are responsible for this effect.

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