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EFFECT OF ETHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* (RAM TULSI) ON SEXUAL BEHAVIOUR IN MALE MICE

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ABSTRACT: The leaves of *Ocimum gratissimum* Linn. (Labiatae) have been used in Ayurvedic medicine since ancient times for the treatment of male sexual disorders. The present study investigated the effect ethanolic extract of ram tulsi on general mating behaviour, libido, potency along with its likely gastric ulceration and adverse effects on sexually normal male albino mice. The suspension of the extract was administered orally at the dose of 100, 250, and 500 mg / kg body weight, to different groups of male rats (n = 6) once a day for seven days. The female Swiss Albino mice involved in mating were made receptive by hormonal treatment. The general mating behaviour, libido and potency were determined and compared with the standard reference drug sildenafil citrate. The probable gastric ulceration and adverse effects of the extract were also evaluated. Oral administration of the extract significantly increased the Mounting Frequency, Intromission Frequency; Intromission Latency, Erections as well as aggregate of penile reflexes and caused significant reduction in the Mounting Latency and Post Ejaculatory Interval. The most appreciable effect of the extract was observed at the dose of 500 mg/kg. The test drug was also found to be devoid of any conspicuous gastric ulceration and adverse effects. The results indicated that the ethanolic extract of *Ocimum gratissimum* produced a significant and sustained increase in the sexual activity of normal male mice, without any conspicuous gastric ulceration and adverse effects. Thus, the resultant aphrodisiac effectivity of the extract lends support to the claims for its traditional usage in sexual disorders.

Key words: *OCIMUM GRATISSIMUM* (RAM TULSI)

INTRODUCTION

Ocimum gratissimum Linn. (Labiatae) is a shrubby, perennial 0.2-1.2 meter in height, much branched, woody, below stems and branches sub quadrangular. Flowers were in simple or branched rather short racemes, in tolerably close whorls, rachis quadrangular, softly pubescent; bracts sessile, longer than the calyx, softly pubescent. Calyx 3 mm long in flower, becoming twice as long as in fruit, pubescent and glandular; upper lip rounded, veined, scarcely mucronate, curved upwards in fruit, longer than lower; lower lip strongly nerved, the two central teeth short subulate, the lateral teeth shorter and broader, lanceolate. Leaves are 6.3-12.5 cm by 3.8-1.8 cm in size along with elliptic lanceolate shape. Leaf has acute apex, coarsely crenate serrate margin with more or less pubescent. Corolla 4 mm long, pale greenish yellow, pubescent outside; upper lip 3 mm broad with 4 rounded teeth; upper filaments with graded tooth at the base. This plant is distributed throughout India and often cultivated. It is popularly known as Ram tulsi in Hindi. The plant has pungent taste with some characteristic flavour [1]. Eugenol isolated from leaf oil was found as chief chemical constituent along with beta caryophylene, germacrene, alpha copaene, humutene, beta elemene, beta bourbonene, gamma murolene, identified as minor component in oil by GC-MS spectroscopy method [2]. A new sequiterpene gratissimine is also isolated from leaf [3]. Antileishmanial activity of Eugenol-rich essential oil is also reported from *Ocimum gratissimum* [4]. The leaf is also found useful in various pharmacological activities as antibacterial, [5]

antifungal, [6] hypoglycemic, [7] increase sexual behavioral activities in mice, [8] antidiarrhoeal [9], and analgesic [10].

EXPERIMENTAL

Plant material and extraction

The leaves of *Ocimum gratissimum* Linn. (Labiatae) collected in and around Bhopal were identified in Department of Pharmacy, Barkatullah University, Bhopal. A voucher specimen (No BUPH/4041B) was deposited in the department. The plant was morphologically examined for shape of leaves, apex, base, margin etc. Powder (# 60) of the dried leaf was used for continuous soxhlet extraction with petroleum ether (60-80^0 C), benzene and ethanol respectively. Before starting of animal studies permission of Internal animal ethics committee of Department of Pharmacy, Barkatullah University was obtained by meeting on date 26/12/2006 Ref No BUPH/ IAEC / 7865. The Pharmacy Department of Barkatullah University is also approved for animal experimentation by (Letter No .444/01/C/CPSCEA date July 2001).

Chemicals used

Sildenafil citrate was purchased from Zydus Cadila, (Ahmadabad, India). Other drugs used were ethinyl oestradiol (Infar Limited, Calcutta, India), progesterone (Sun Pharmaceutical Industries Limited, Mumbai, India) and 5% xylocane ointment (Astra IDL Limited, Bangalore, India)

Animals

Twelve weeks old male and female Swiss Albino mice strain weighing 30–35 g were used for the study. They were housed singly in separate standard cages and maintained under standard laboratory conditions (temperature 24–28°C, relative humidity 60–70%, 12 h light-dark cycle) with free access to solid pellet diet (Gold Mohar, Lipton-India) and water *ad libitum* throughout the study except during the experiment. The ethical committee of the Department for animal cares and use approved the study design. Separate animals were used for each pharmacological screening method.

Drug preparation

Since ram tulsi leaves in ayurvedic medicine is orally administered, therefore, the ethanolic extract of ram tulsi was suspended in distilled water using Tween 80 (1%) for oral administration. Sildenafil citrate and ethinyl oestradiol were also suspended in distilled water using Tween 80 (1%) separately, for oral use. Progesterone was dissolved in olive oil for subcutaneous injection. All the drug solutions were prepared just before administration.

Mating behaviour test:

The test was carried out by the methods of Dewsbury and Davis Jr [11]and Szechtman et al [12], modified by Amin et al [13]. Healthy and sexually experienced male Swiss Albino mice (30–35 g) that were showing brisk sexual activity were selected for the study. They were divided into six groups of six animals each and kept singly in separate cages during the experiment. Group I represented the control group, which received 10 ml/kg of distilled water orally. Groups II–IV received suspension of the ethanolic extract of *Ocimum gratissimum* orally at the doses of 100, 250 and 500 mg/kg body weight, respectively, daily for 7 days at 18:00 h. Group V served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg body weight, 1 h prior to the commencement of the experiment. Group VI was fed with alcohol 6% in drinking feed bottle with normal diet. Since the male animals should not be tested in unfamiliar circumstances, the animals were brought to the laboratory and exposed to dim light (in 1 w fluorescent tube in a laboratory of 14' × 14') at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat) by the Ratna Soorya et al method [14] (as the female mice allow mating only during the estrus phase). They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 7th day after commencement of the treatment of the male animals. The experiment was conducted at 20:00 h in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals with one female to one male. The observation for mating behaviour was immediately commenced and continued for first two mating series. The test was terminated if the male failed to evince sexual interest. The occurrence of events and phases of mating were called out to be recorded on audiocassette as soon as they appeared. Their disappearance was also called out and recorded. Later, the frequencies and phases were determined from cassette transcriptions: number of mounts before ejaculation or Mounting Frequency (MF), number of intromission before ejaculation or Intromission Frequency (IF), time from the introduction of female into the cage of the male up to the first mount or Mounting Latency (ML), time from the introduction of the female up to the first intromission by the male or Intromission Latency (IL), time from the first intromission of a series up to the ejaculation or Ejaculatory Latency (EL), and time from the first coition up to the next intromission by the male or Post Ejaculatory Interval (PEI). In the second mating series only the EL was recorded. The values for the observed parameters of the control, test and standard animals were statistically analyzed by using one-way analysis of variance (ANOVA) method.

Test for libido

The test was carried out by the method of Davidson [15], modified by Amin et al. Sexually experienced male Swiss Albino mice were divided into six groups of six animals each and kept singly in separate cages during the experiment. Group I represented the

control group, which received 10 ml/kg of distilled water orally. Groups II–IV received suspension of the extract orally at the doses of 100, 250 and 500 mg/kg body weight, respectively, once a day in the evening (18:00 h) for 7 days. Group V served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg body weight, 1 h prior to the commencement of the experiment. Group VI was fade with alcohol 6% in drinking feed bottle with normal diet. The female mice were made receptive by hormonal treatment and all the animals were accustomed to the testing condition as previously mentioned in mating behaviour test. The animals were observed for the Mounting Frequency (MF) on the evening of 7th day at 20:00 h. retracting the sheath exposed the penis and 5% xylocaine ointment was applied 30, 15 and 5 min before starting observations. Each animal was placed individually in a cage and the receptive female mice were placed in the same cage. The number of mountings was noted. The animals were also observed for intromission and ejaculation. The MF in control, test and standard animals was statistically analyzed by employing one-way analysis of variance (ANOVA) method.

Test for potency

The effect of the test drug was studied according to the methods described by Hart and Haugen [16] and Hart [17], modified by Amin et al. The male mice were divided into 6 groups of six animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups II–IV received suspension of the test drug orally at the doses of 100, 250 and 500 mg/kg body weight, respectively, daily for 7 days. Group V received a suspension of sildenafil citrate orally at the dose of 5 mg/kg body weight, 1 h before the commencement of the experiment. Group VI fed with 6% alcohol mixed drinking water with normal diet. On the 8th day, placing the animal on its back in a glass cylinder partial restraint carried out the test for penile reflexes. The perpetual sheath was pushed behind the glans by means of thumb and index finger and held in this manner for a period of 15 min. Such stimulation elicits a cluster of genital reflexes. The following components were recorded: Erections (E). The frequency of these parameters observed in control, test and standard groups was statistically analyzed by using one-way analysis of variance (ANOVA) method.

Test for ulcerogenecity

The male mice (25–35g) were divided into four groups of six animals each. Group I represented the control group, which received 10 ml/kg of distilled water. Groups II–IV received suspension of the extract orally at the doses of 100, 250 and 500 mg/kg body weight, respectively, daily for 7 days. After the treatment, on 8th day, all animals were killed and the stomach was then incised along the grater curvature and washed carefully with physiological saline. Any gastric lesions were observed immediately using a magnifying glass. The number of erosions per stomach was assessed for severity, according to score of Cioli et al [18] as (0) absence of lesion, vasodilation or up to 3 pinpoint ulcers, (1) for more than 3 pinpoint ulcers, (2) from 1 to 5 small ulcers (< 2 mm); (3) more than 5 small ulcers (< 2 mm); (4) 1 or more giant ulcers.

All treated mice were observed at least once daily for any overt sign of toxicity (salivation, rhinorrhoea, lachrymation, ptosis, writhing, convulsions and tremors), stress (erection of fur and exophalmia) and changes in behaviour (such as spontaneous movements in cage, climbing, cleaning of face). In addition food and water intake were noted.

RESULT AND DISCUSSION

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated no significant increase in Mounting frequency as shown in the group treated with test drug 250 mg dose (37.80 ± 0.84) and in the group treated with 500 mg (51.4 ± 1.14) when compared to the control (23.60 ± 1.14). However, this activity was found maximum in the group treated with the standard drug (51.80 ± 1.30).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated a significant increase in Intromission frequency in the group treated with test drug 250 mg dose (52.12 ± 1.99) and in the group treated with 500 mg (65.15 ± 2.65) when compared to the control (16.33 ±1.78). However, this activity was found maximum in the group treated with the standard drug (67.01 ± 2.55).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated a significant increase in Mounting latency as shown in the group treated with test drug 250 mg dose (81.00 ± 6.89) and in the group treated with 500 mg (98.00 ± 2.55) when compared to the control (75.00 ± 6.24). However, this activity was found maximum in the group treated with the standard drug (100.00 ± 6.15).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated a significant increase in Intromission latency as shown in the group treated with test drug 250 mg dose (211.16 ± 5.46) and in the group treated with 500 mg (232.00 ± 15.86) when compared to the control (132.50 ± 4.53). However, this activity was found maximum in the group treated with the standard drug (243.33 ± 4.85).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated a significant increase in Ejaculatory latency as shown in the group treated with test drug 250 mg dose (78.83 ± 0.89) and in the group treated with 500 mg (112.32 ± 0.10) when compared to the control (58.63 ± 0.47). However, this

activity as found maximum in the group treated with the standard drug (100.85 ± 3.25).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated a significant increase in Post ejaculatory index as shown in the group treated with test drug 250 mg dose (41.00 ± 1.30) and in the group treated with 500 mg (54.00 ± 1.30) when compared to the control (56.00 ± 1.23). However, this activity was found maximum in the group treated with the standard drug (71.00 ± 1.20).

The test drugs ethanolic extract of leaves of *Ocimum gratissimum* clearly indicated that significant increase in Erections as shown in the group treated with test drug 250 mg dose (9.57 ± 0.20) and in the group treated with 500 mg (12.02 ± 0.08) as shown compared to control (4.19 ± 0.10). However, this activity was found maximum in the group treated with the standard drug (12.05 ± 0.06). (Table No 2)

Treatment with dried ethanolic extract of *Ocimum gratissimum* leaves remarkably influenced the behavior of the treated animals, which were more attracted towards female. Post-ejaculatory index PEI indicates the involvement of nitrous oxide (NO) based mechanism. An increase in PEI was observed in treated groups ethanol extract of *Ocimum gratissimum* leaves treated animals were superior in this regard ($P<0.01$) compared to the control group while testosterone treated animals were the next to follow ($P<0.05$). Percentage of ejaculating animals was also increased after treatment when compared to the control group animals. The mount intromission and post-ejaculatory latency (PEL) were significantly reduced in extract 250 mg treated groups. Mount latency time that is an indicator of physical exhaustion during a sexual act was reduced by 36% in *Ocimum gratissimum* leaves treated group as compared to control group. Intromission (IL) and PEL time was reduced by 36% in *Ocimum gratissimum* leaves treated group ($P<0.05$) whereas only 17% reduction was observed in testosterone treated group. Reduced IL and PEL have been correlated with invigoration of endocrine system thereby, resulting in enhanced sexual performance and motivation.

SUMMARY AND CONCLUSION

The present results indicated that the ethanolic extract of leaves of *Ocimum gratissimum* significantly increased the sexual behaviors of normal male albino mice without any gastric ulceration and adverse effects. Also provided scientific evidence in favour of the claims made in ayurvedic medicine that the *Ocimum gratissimum* is clinically useful as sexual invigorator in males. It can be administered in suitable formulation in 250 mg and 500 mg dose levels safely.

TABLE NO 1 Protocol for evaluation of Sexual behaviour activity of leaves of ***OCIMUM*** *GRATISSIMUM*

S NO.	DRUG CODE	DOSE	ANIMAL AVERAGE WEIGHT	DOSE ADMINISTERED
1	Control	Normal diet and only vehicle	30 gm-35 gm	OD for 7 days
2	RT/OG	3 mg in 2 ml	30 gm-35 gm	OD for 7 days
3	RT/OG	7.5 mg in 2 ml	30 gm-35 gm	OD for 7 days
4	RT/OG	10 mg in 2 ml	30 gm-35 gm	OD for 7 days
5	Standard	Sildenafil citrate orally at the dose of 5 mg/kg	30 gm-35 gm	OD for 7 days
6	Alcohol	6% in feeding bottle of water	30 gm-35 gm	OD for 7 days

Table No 2 Effects of Ethanolic Extract of *Ocimum gratissimum* (SEXUAL BEHAVIOURS)

GROUPS	MF (In No)	IF (In No)	ML (In Second)	IL (In No)	EL (In Second)	PEI (Seconds)	E (In No)
I) CONTROL	23.60±1. 14	16.33 ± 1.78	75.00± 6.24	132.5 ±4.53	58.63 ± 0.47	56.00 ±1.23	4.19 ±0.10
II) 100 mg	25.4±1.14	46.67 ± 2.39	70.00±3.25	145.5 ± 5.24	59.75 ±0.94	27.00±1.22	7.76 ±0.19
III) 250 mg	37.80± 0.84	52.12±1.99	81.00±6.89	211.16 ±5.46	78.83 ± 0.89	41.00 ± 1.30	9.57 ±0.20
IV) 500 mg	51.4± 1.14*	65.15±2.65*	98.00± 2.55*	232.00 ±15.86*	112.32 ± 0.10*	54.00± 1.30*	12.02 ±0.08*
V) STANDARD	51.80± 1.30	67.01 ± 2.55	100.00± 6.15	243.33 ±4.85	100.85±3.25	71.00 ± 1.20	12.05 ±0.06
VI) Alcohol	13.44±1. 14	8.43 ± 1.78	35.00± 8.14	82.5 ±2.43	48.23 ± 2.47	26.00 ± 3.85	2.59 ±5.50

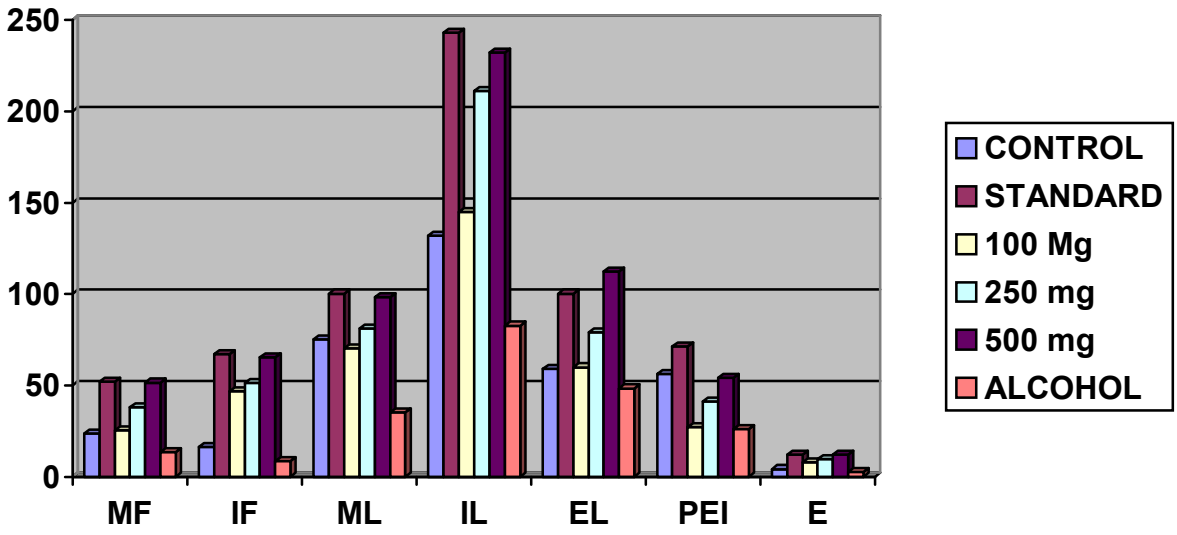
Values are mean ±SEM of six animals
Statistical significance: *= $p<0.01$ comparison was done with their respective control group

MF Mounting frequency
IF Intromission frequency
ML Mounting latency
IL Intromission latency
EL Ejaculatory latency
PEI Penile ejaculatory index
E Erections

Table No 3 Effect of on orientational activities towards female, towards environment & towards self on 7-day administration in normal mice and treated mice

Group (Dose\kg b. wt)	**Towards female (60 minutes)**		**Towards environment (60 minutes)**	**Towards self (60 minutes)**	
	No. of licking	No. of anogenital sniffing	***No. of climbing***	No. of genital grooming	No. of non-genital grooming
I) Control	3.16 ±0.30	3.83 ±0.47	16.33 ±0.95	7.66 ±0.55	14.33 ±0.73
II) 100 mg DOSE (RT/OG)	3.66 ±0.42	3.50 ±0.22	15.33 ±0.33	7.33 ±1.11	12.66 ±0.88
II) 250 mg DOSE (RT/OG)	9.33 ±0.40	4.50 ±0.88	19.16 ±1.19	9.33 ±1.62	17.66 ±1.62
II) 500 mg DOSE (RT/OG)	6.66 [a] ±0.80	7.33 [a] ±0.42	24.83 [a] ±1.06	12.66 [a] ±1.11	24.33 [a] ±2.15
IV) Standard	5.6 ±0.16	6.50 ±0.22	23.33 ±0.33	12.66 ±0.47	22.16±0.55
VI) Alcohol	1.16 ± 1.22	1.10 ±4.19	6.52 ±4.25	2.19 ±9.55	4.23 ±5.37

Values are mean ±SEM of six animals
Statistical significance: a= $p<0.01$ comparison was done with their respective control group



Graph showing sexual behaviour of ethanolic extract of *Ocimum gratissimum*

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