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Effect of Methanolic Extract of Seeds of *Lepidium sativum* Linn. on Proceptive and Receptive Behaviors of Female Rats



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ABSTRACT

Sexual behavior in female rat has been divided into proceptivity and receptivity. These behaviors serve to attract males and allow females to control the initiation and rate of copulation. This study examined the effect of methanolic extract of seeds of *Lepidium sativum* Linn. (MELS) on proceptive and receptive behaviors of female rats. Ovariectomized female wistar rats were divided into three groups (n=6). Group 1, 2 and 3 received distilled water (10 ml/kg), 200 and 400 mg/kg of MELS respectively for 21 days, orally. On 11th and 21st day, each female subject was tested in estrous phase for their sexual behavior in copulatory test. Behavioral estrus was induced by subcutaneous administration of 25 µg estradiol benzoate 48 h prior to behavioral testing and 500 µg of progesterone 5 h before testing. As a measure of proceptivity, the number of hops, darts, ear wiggling and solicitations made by MELS treated female rats were significantly increased when compared against control estrous females. Lordosis quotient, as a measure of receptivity was unaffected by any of the doses of MELS. The results suggest the possible use of MELS to treat various sexual desire/interest disorders and sexual arousal disorders in women, since psychological arousal in women could be very close to proceptivity in female rats.

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INRODUCTION

A great deal is known about the sexual behavior of the female rat which includes several categories of response patterns^{1, 2}. Sexual behavior in female rat has been divided into appetitive, precopulatory, and consummatory responses, which contain earlier denotations of sexual attractivity, proceptivity, and receptivity. These behaviors serve to attract males and allow females to control the initiation and rate of copulation³. Proceptivity can be defined as those behaviors that were suggested to represent the efforts of the female to arouse the sexual interest on the part of their sexual mates and to solicit attention and approach of the male^{4, 5}. In rats, these behavioral components include dartings, hoppings, ear wiggling and solicitation^{2, 4, 6, 7}. Proceptive behaviors have been shown to be dependent on the action of progesterone (P)¹. Receptive behaviors, on the other hand, are those that facilitate the act of copulation. Female rats, for example, exhibit displays of lordosis, a supraspinal reflex characterized by a crouching, sway-backed posture, with the tail flicked to the side to facilitate the male mounting and intromission^{1, 8}. Receptivity can also be defined by the frequency with which the female exhibits the lordosis response when mounted by a male⁹. Lordosis is the final element of female rat sexual behavior. Before the female can receive the tactile stimulation provided by the mounting male, which is necessary for activating the lordosis circuit, she needs to have detected a male, approached him, and eventually activated his mounting behavior. About 90% of sexual interactions are initiated by the female while the male initiates only about 3% of such interactions¹⁰. The lordotic response is stimulated in a dose-dependent manner by treatment of ovariectomized (OVX) rats with estradiol benzoate (EB)² and is further facilitated to maximal levels by progesterone (P)³. Thus, a female rat needs both estrogen and progesterone to display the full complement of sexually motivated precopulatory behavior which are key determinants of sexual encounters^{1, 10}.

Arousal in women may be separated into genital arousal (potency) and psychological arousal (libido, motivation)¹¹. Among these, psychological arousal in women could be very close to proceptivity⁷. Hence, the study of proceptive behavior is likely relevant to preclinically investigate the potential of compounds for the treatment of various sexual disorders¹². The basic neural and behavioral mechanisms controlling sexual desire or motivation are similar in rodents and in humans and thus a valid reliable model of rodent sexual motivation would be of great utility for studying the behavioral and neurobiological basis of sexual motivation^{1, 13}. Also, a large literature is associated with the use of rodents for the assessment of sexual behavior¹⁴.

Lepidium sativum L. is an edible herb and a member of the Cruciferae (Brassicaceae) family. It is commonly cultivated throughout the temperate regions of India and Pakistan¹⁵. Also, it is a cool season annual plant, cultivated as salad throughout India. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. It can be grown in all elevations the whole year around. However, the best crop is obtained in winter season. Seeds are sown in the plains from September to February and on the hills, from March to September¹⁶. The seeds are reddish in colour, oblong, somewhat angular and curved slightly on one side with rugous surface. Near the point of attachment there is a white scar, from which a small channel extends to 1/3 the length of the seeds. Seeds are odorless and taste is pungent and mucilaginous¹⁷.

Lepidium sativum seeds contain an alkaloid, glucotropaeolin, sinapin, sinapic acid, mucilaginous matter and uric acid¹⁸. Moreover, a few phenolic constituents, such as, sinapic acid and sinapin were isolated from its seed extract¹⁹. Steroidal, phenolic, alcoholic and terpene types of component were reported to be present in methanolic extract of seeds of *Lepidium sativum* Linn²⁰. Yadav et al. revealed the presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids and amino acids from the ethanolic extract of *Lepidium sativum* L. seeds²¹. *L. sativum* seeds contain volatile essential aromatic oils, fatty oils, carbohydrates, proteins, fatty acids, vitamins (β -carotene, riboflavin, ascorbic acid and niacin), flavonoids and isothiocyanate glycosides²².

Lepidium sativum seeds are scientifically documented for their prokinetic and laxative effects¹⁹, bronchial asthma²³, antihypertensive and diuretic²⁴, inflammatory joint diseases and osteoarthritis²⁵, hepatoprotective²⁶, anti-inflammatory, antipyretic and analgesic²⁷, antidiarrheal and spasmolytic activities²⁸ and hypoglycaemic activity²⁹. However, its role in various sexual behaviors in female rats has not been studied. In light of this, the present investigation was carried out to study the effect of methanolic extract of seeds of *Lepidium sativum* L. on various proceptive and receptive behaviors of female rats.

MATERIALS AND METHODS

Plant material

Dried seeds of *Lepidium sativum* L. were obtained from the local market of Nadiad, district Kheda, Gujarat, India and authenticated by the Botanist Dr.Yogesh T. Jasrai, Department of Botany, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India and a

voucher specimen (DDU/FOP/11-12/F-02) was deposited in the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India.

Preparation of the extract

The collected dried seeds were washed, shade dried and pulverised with mechanical grinder for size reduction followed by sieving to obtain coarse powder. The powdered material was defatted with sufficient quantity of petroleum ether and then air dried. The defatted material (10 gm) was then extracted with 100 ml of methanol for 48-hours using cold maceration process in a conical flask stoppered with glass corks. The mixture was stirred every 12-hours using a sterile glass rod. After 48-hours, the extract was filtered off using sterile filter papers (Whatman No. 1) into a clean conical flask. The filtered extract was transferred to evaporating disc and subjected to water bath evaporation, where the alcoholic solvent was evaporated to produce semisolid extract. The yield of the extract was 15% w/w.

Chemicals and drugs

Estradiol benzoate AR and progesterone AR were purchased from the Rajesh Chemicals, Mumbai. Ketamine injection (Themis Medicare Limited, Bhel, Haridwar, Uttarakhand), Xylazine hydrochloride (Xylaxin, Indian immunological Ltd, Andhra Pradesh), sesame oil (Research Lab Fine Chem. Industry, Mumbai), propylene glycol (Loba chemie Pvt. Ltd, Mumbai) were purchased from the local market.

Animals

Swiss male albino mice (18-22 gm) and wistar rats of either sex (150-200 gm) were used. They were maintained at $25 \pm 2^\circ\text{C}$ and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). The animals had free access to food (Amrut feed, Chakan oil mills, India) and water *ad libitum* throughout study. Institutional Animal Ethics Committee approved the protocol. All the experiments were carried out between 9:00- 16:00 hour.

Acute toxicity test

Acute toxicity study was performed in healthy adult male albino mice (18-22 gm) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development (OECD). Methanolic extract of seeds of *Lepidium sativum* L. (MELS) was administered at the doses of 175, 550 and 2000 mg/kg in mice for oral toxicity study. Mice

were then observed for incidence of mortality or any sign of toxicity up to 24 hours after oral administration. The dosing schedule of OECD (guideline 425) was followed. Only one mouse received a dose at a particular time. First animal received a dose of 175 mg/kg/p.o. Animal was observed for 03 hours after dosing for any toxicity signs, survival or death. If the first animal died or appeared moribund, the second animal received a lower dose. The dose progression or reduction factor was 3.2 times of the previous dose. If no mortality was observed in the first animal then the second animal received a higher dose. Dosing of the next animal was continued depending on the outcome of the previously dosed animal for a fixed time interval (03 hours). The test was stopped when one of the stopping criteria was met:

- 05 reversals occur in any 06 consecutive animals tested.
- 03 consecutive animals died at one dose level.

Survived animals were observed for outcomes for a period of 24 hours (AOT425 Guidelines).

Surgery (Ovariectomy)

All the female rats were ovariectomized (OVX) 30 days prior to starting of the experiment using standard aseptic surgical techniques under deep anaesthesia. Females were anaesthetized with ketamine (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/Kg, i.p.). All females received at least one week of postoperative care prior to initiation of experiment³⁰.

Induction of behavioral estrus

For induction of behavioral estrus, OVX female rats were subcutaneously (SC) administered with 25 µg estradiol benzoate (EB; in 0.1 ml sesame oil) 48 h prior to behavioral testing and 500 µg of progesterone (P; in 0.1 ml propylene glycol) 5 h before testing³¹.

Selection of male rats for inclusion in the study

To make sexually experienced, male rats were given four training sessions (twice a week for 2 weeks) with receptive females for the period of 30 min. Only males displaying at least two ejaculations during the four training test sessions were included in the study³¹.

Statistical analysis

The results are expressed as mean ± SEM (n = 6). Comparison between the control and MELS treated groups were made by one-way analysis of variance (ANOVA) followed by Dunnett post hoc test. P<0.05 was considered to be statistically significant.

Experimental protocol

OVX female rats were divided into 03 groups with 06 rats in each group. They were orally treated as follows for the period of 21 days.

Group 1 received distilled water (10 ml / kg),

Group 2 received MELS 200 mg/kg,

Group 3 received MELS 400 mg/kg.

On 11th and 21st day, 01 hour after the respective treatments, each female rat were tested in estrus phase (i.e. estradiol and progesterone treatment) for their sexual behavior in copulatory test for the period of 30 minutes wherein 10 sexually experienced adult male rats were used as copulatory partners. These same 10 partners were paired with each of the experimental female groups, thus controlling potential difference in male responsiveness. During 30 minutes test period, various female proceptive (i.e. hops, darts, ear wiggings and solicitations) and receptive (i.e. lordosis and lordosis quotient) behaviors along with rejection responses like boxing, running away and kicking were observed.

Measures of proceptivity

1. Dart: It corresponds to a run of several steps, typically 3-5 steps, abruptly terminated by assumption of crouching posture⁴.
2. Hop: It is the short lip or jump with stiff legs followed by immobility in which female is landing on all four paws, followed by assumption of crouching posture^{4, 25}. The distance covered by one hop by the hind feet is approximately equivalent to the whole length of the extended body⁶.
3. Ear wiggling: Rapid lateral shaking of the head causing the appearance of distinctive anteroposterior ear vibrations^{2, 32}.
4. Solicitation: It corresponds to headwise orientation of female towards the male followed by abrupt run away⁷.

Measure of receptivity

1. Lordosis quotient: A lordosis quotient can be computed by dividing the number of lordosis (dorsiflexion of back in response to mounting) with total number of mounts and multiplying this ratio with 100⁴.

RESULTS

Acute oral toxicity test

All mice were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of 2000 mg/kg. From this data and pilot study reports; two different doses 200, and 400 mg/kg were selected for further study.

Hops, Darts and Ear wiggling

MELS at 200 and 400 mg/kg doses significantly ($p < 0.01$) increased the hops of female rats as compared to the control estrous females after 11 days and 21 days of treatment. Numbers of darts in female rats were significantly ($p < 0.01$) increased by MELS 200 and 400 mg/kg after 21 days of treatment. However, after 11 days of treatment, only MELS 400 mg/kg dose significantly ($p < 0.01$) increased the number of darts in female rats when compared against control estrous female rats. Ear wiggings in MELS 400 mg/kg treated female rats were significantly ($p < 0.01$) increased as compared to the control estrous females, regardless of the treatment period (Figure 1).

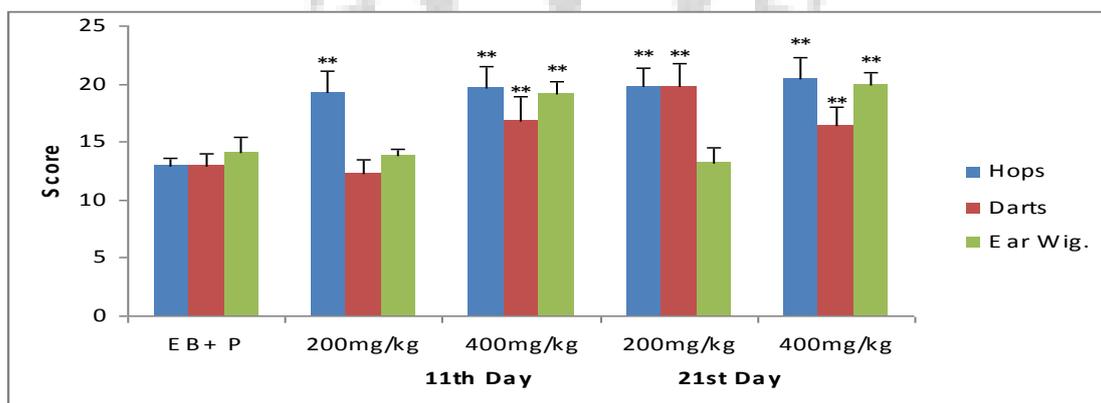


Figure 1: Effect of MELS on hops, darts and ear wiggling of estrous females

Results are expressed as mean \pm SEM ($n = 6$). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnett's test * $p < 0.05$, ** $p < 0.01$.

Solicitation and Lordosis

The number of solicitations made by estrous females were 02.33 ± 0.49 while the number of lordosis posture assumed by estrous female rats during entire 30 minute of copulatory period were 6.66 ± 0.49 . After 21 days of treatment period, 400 mg/kg dose of MELS significantly ($p < 0.05$) increased the solicitations made by estrous females. On the other hand, lordosis behavior, as a marker of receptivity was significantly ($p < 0.05$) increased in female rats treated

with 200 mg/kg dose of MELS, after 11 and 21 days of treatment period. However MELS 400 mg/kg dose was not found to be significant in this regard, irrespective of the treatment period (Table 1).

Table 1: Effect of MELS extract on solicitation and lordosis of estrous females

Treatment (mg/kg)		Solicitations	Lordosis
Estrous phase	EB+P	02.33±0.49	6.66±0.49
After 11 days treatment	MELS-200	02.66±0.61	14.16±1.97 *
	MELS-400	04.00±0.57	10.33±1.38
After 21 days treatment	MELS-200	03.00±0.57	15.50±2.57 **
	MELS-400	04.83±0.70 *	12.33±1.47

Results are expressed as mean ± SEM (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnett's test *p<0.05, **p<0.01.

Lordosis quotient and Rejection

Table 2: Effect of MELS extract on lordosis quotient and rejection responses of estrous females

Treatment (mg/kg)		Lordosis quotient (%)	Rejections
Estrous phase	EB+P	81.78±2.11	2.16±0.65
After 11 days treatment	MELS-200	73.99±1.91	3.16±0.47
	MELS-400	71.08±2.32*	2.83±0.47
After 21 days treatment	MELS-200	81.51±1.54	2.66±0.49
	MELS-400	81.25±3.24	1.66±0.21

Results are expressed as mean ± SEM (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnett's test *p<0.05, **p<0.01.

The lordosis quotient of EB+P primed control female rats was 81.78±2.11. None of the doses of MELS was effective in increasing lordosis quotient of female subjects when compared against estrous female rats, regardless of the treatment period. Rejection responses of control estrous females towards male rat were found to be 2.16±0.65. 400 mg/kg dose of MELS non-significantly reduced various rejection responses made by female subjects after 21 days of treatment period (Table 2).

DISCUSSION

In the present experiment, MELS enhanced appetitive proceptive sexual behaviors selectively in OVX rats primed with EB+P. In females primed with EB+P, MELS increased hops, darts, ear wiggling and solicitations, precopulatory behaviors that females use in close proximity to males to arouse them³³. The increment in the solicitations is an indicative of increased libido in female rats as solicitation is nothing but the request, the female subjects made to attract the male rats for the copulation. Hence MELS significantly increased various female rat proceptive behaviors. Also, rejection responses like boxing, turning, running away from the male, rolling over and kicking, which are considered as negative behaviors¹², were non significantly decreased in female rats treated with MELS 400 mg/kg dose after 21 days of treatment period. These findings further support the role of MELS in increasing sexual excitement in female rats. Lordosis quotient is the most important indicator of female receptivity. In contrast to the proceptivity, with MELS, no effect on lordosis quotient was found in OVX rats primed with EB+P. However, lordosis behavior, as a marker of receptivity was significantly ($p < 0.05$) increased in female rats treated with 200 mg/kg dose of MELS, after 11 and 21 days of treatment period.

Female rat sexual behavior is tightly linked to ovulation and the synchronous timing for both events is controlled by the hypothalamic–pituitary–gonadal (HPG) axis with estrogen as the ultimate conductor. Unlike primates, normally cycling female rats show sexual receptivity only during the proestrous (ovulatory) portion of the reproductive cycle. Removal of the ovaries leads to immediate cessation of sexual behavior which can be restored by treatment with estrogen and progesterone³⁴. Thus in this study, various sexual behaviors were studied in OVX female rats primed with EB and P. Also, ovariectomizing the female rats was important, otherwise they would become pregnant and hence useless for use during the study. The study was essentially conducted between 9.00 a.m to 5.00 p.m to reduce any other variations.

According to epidemiological studies, the most common complaint in women seeking treatment for sexual dysfunction is hypoactive sexual desire disorder³⁵. This set of symptoms has been reported in approximately 30% of women in population based studies, and is associated with wide variety of medical and psychological causes³⁶. Considering that sexual desire in women is homologous to proceptive sexual motivation in animals¹³, or to appetitive aspects of sexual activity in female rats³⁷, the present results support the use of MELS as a

promising pharmacological agent for the treatment of women with hypoactive sexual desire disorders.

With regard to the mechanism of the test drug, it is difficult to interpret the mechanism involved in potentiation of proceptive behaviors (psychological arousal). The probable mechanism of increased proceptivity could be due to changes in neurotransmitter levels or their action at cellular levels could alter sexual proceptivity. Further, phytochemical investigation of the extract indicated that it contains steroids, saponins, glycosides, alkaloids, flavonoids, tannins and carbohydrates. Thus, the resultant potentiation of proceptivity by seeds of *Lepidium sativum* might also be attributed to the aforementioned phytoconstituents. However, study needs further investigation to know the exact mechanism underlying enhanced appetitive proceptive sexual behaviors.

CONCLUSION

To conclude, seeds of *Lepidium sativum* significantly increased various proceptive behaviors in female rats suggesting its possible use to treat various sexual interest and sexual desire disorders in females.

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