

Pharmacological Evaluation of *Cannabis indica* For Their Aphrodisiac Potential

Research Article

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Abstract

Objective: The plant *Cannabis indica* is a very popular and well known plant across the globe since thousands of years. In India also it is even more popular as *bhanga* or *marijuana* from ancient time. A quite descriptions of this plant is written in various ancient books like the *granths*, *vedas*, *puranas*, *ayurvedic books*, *dravyaguna*, etc.. Number of medicinal properties have been described for different parts of the plant like flower buds (*Ganja*), Leaf (*Bhang*) & Leaf wax (*Charas*). All the parts of this plant have potent and complex psychotropic properties along with some additional therapeutic actions on different body systems. Here we have found some opportunities to evaluate herbal extracts of leaves for having aphrodisiac potential. **Method:** The aphrodisiac activity of *Cannabis indica* leaf extracts (Pet.ether, methanol & aqueous) were evaluated in male albino rats. Extracts were administered at a dose of 150mg/kg body weight. At an interval of 0,7,14,21 & 28 day several sexual behavior parameters were determined. At the end serum testosterone and FSH level were also determined. **Results:** This study also contributes to the pharmacology of aphrodisiacs as the plant *Cannabis indica* leaf extracts has significant effects on sexual physiology. It has shown effects on sexual behaviors as well as significant change in gonadotropin hormone levels in animal blood serum. **Conclusion:** This study provides a strong experimental evidence for its aphrodisiac use.

Key Words: Aphrodisiac, *Cannabis indica*, Herbal aphrodisiacs, Sexual behavior study.

Introduction

Infertility is one of the major health problems couple's lives; approximately 30% of couple's infertilities are due to male factors. Around 10-14% Indian population suffering from infertility. Nearly 27.5 million ones are trying to conceive suffering from infertility in India. WHO estimates that there are 60 -80 million infertile couples worldwide.(1) Loss of interest in sex or problems with sexual arousal makes most individuals experience and express discomfort in their sexual behavior. Such concerns or changes may arise from an illness or disability, medication or surgical procedure, changes accompanying the aging process(2), relationship difficulties(3) performance anxiety(4), or a combination of any of these factors. There are three basic types of sexual dysfunction; disorders of desire - takes the form of inadequate sexual desire (libido) in both sexes(5) disorders of excitement (or arousal) in men, impotence(6) disorders of orgasm, includes difficulty achieving orgasm in both men and women

but more common among men.(7) Libido refers to an individual's desire for sexual activity. This can also be called sex drive. Factors that affect libido include psychological factors, biological factors and social factors. Personality and the level of stress an individual is exposed to be internal psychological factors that can affect sexual desire(8).

Discovery of oral phosphodiesterase type 5(PDE5) inhibitors particularly *sildenafil* (*Viagra*), *tadalafil* (*Cialis*), and *vardenafil* (*Levitra*) has revolutionized treatment of male sexual disorder (MSD).(9) There are many herbal drugs that have been used by men with erectile dysfunction (ED) with varying degrees of success. Most potent herbal aphrodisiacs are available and have little or very little side effects.(10) The Ayurvedic system of medicine addresses the problem of sexual deficiencies by treatment with specialized therapy known as '*Rasayana therapy*'. A class of '*Rasayana*' or drugs also known as '*Vrishya*' or '*Vajikaran Rasayan*' has been prescribed debility, especially encountered old age. '*Vajakarna therapy*' includes aphrodisiac drugs for erectile dysfunction, reasons of infertility, spermatogenesis, semenogenesis, reproduction, methods of correcting defective semen and sexual satisfaction.(11)

According to traditional information and published text available from various sources a plant *Cannabis indica* leaves have some active phytochemical entity that we have been proved in our laboratory to exhibit good aphrodisiac potential.

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Cannabis indica Plant belongs to Family- Cannabaceae known as marijuana or bhang. This is a flowering plant that includes three species - *sativa*, *indica* and *ruderalis*. The plant is indigenous to central Asia and the Indian subcontinent. Cannabis has long been used for hemp fibre, for hemp oils, for medicinal purposes, and as a recreational drug. Cannabis plants produce a group of chemicals called cannabinoids, which produce mental and physical effects when consumed.(13)

Material and method

Plant material

The leaves of *Cannabis indica* were collected from Forest Research Institute, Dehradun, Uttarakhand, identified and authenticated by Prof. (Dr.) Rajiv Soni, Botanist & Centre Head, NIMAPC, Indore, M.P. with the Herbarium Reference No. NIMAPC/CI-161.

Preparation of extracts

Successive extraction of plant material was done with petroleum ether, methanol and water solvents in our laboratory. The method we used was simple maceration followed by sonication, filtration and evaporation of solvent to get dry extract.

Animals

Albino wistar rats were procured from IVRI Bareilly (U.P.) and acclimatized in the animal house facility of the department under ambient condition at room temperature (27°C ±2°C) with 12 hour day /night cycle. The animals were provided with pellet feed and free head access to drinking water *ad libitum*, except during experimentation. Ethical approval was obtained from Institutional Animal Ethical committee IAEC, Dept. of Biotechnology, Indian Institute of Technology(IIT), Roorkee. (BT/IAEC/2018/14).(14)

Pharmacological sexual behavioral study

The rats were randomly divided in to five groups of 6 animals each. Rats in Group 1 served as vehicle control and received 1ml of distilled water. Group 2 served as the standard drug control and received sildenafil citrate 5mg/kg body weight. Groups- 3, 4 and 5 were treated orally with the plant extracts (1ml) once daily at a dose of 150 mg/kg body weight respectively. six rats from each group were monitored for sexual behavior study after 1, 3 and 5 daily doses respectively.(15)

Preparation of female rats used in the behavioral study of male rats			Aphrodisiac study in male rats
48 Hours before Behavioral Study	6 hours before behavioral study	1 hours before behavioral study	Male behavioral study
Female rats were injected with estradiol benzoate (10 µg/100 gm) to bring the animal to estrous phase	Treated with progesterone (0.5 mg/100 gm) to bring rats to estrous phase	Checked for female responsiveness (vaginal smear test)	Male rats were exposed to estrous female for a period of 30 Minutes and observed for their sexual behavior patterns on days 0, 7, 14, 21 and 28 th of drug treatment

- **Group I (Control):** Normal animals treated with 1 ml/kg, p.o. of saline.
- **Group II (Standard drug):** Animals treated with Sildenafil citrate at a dose 5 mg/kg, s.c.
- **Group III:** Animals treated with petroleum ether extract (PEE) at a dose of 150mg/kg (*Cannabis indica*) p.o.
- **Group IV:** Animals treated with methanol extract (ME) at a dose of 150mg/kg (*Cannabis indica*) p.o.
- **Group V:** Animals treated with aqueous extract (AQE) at a dose of 150mg/kg (*Cannabis indica*) p.o.

Male albino rats were distributed into 5 groups consisting of six rats per group.

Evaluation of male sexual behavior

The animals will be divided into 5 groups each containing 6 animals (albino rats) and all the animals will be housed in individual cages and will be given food and water *ad libitum*. Sexual behaviour studies will be monitored in a separate room for 30 min. following the administration of standard drug & extracts and will be given 20 minutes adaptation period, after which a primed female was introduced into the study cage. On days 1, 7, 14, 21 and 28th sexual behavioural study were monitored. Experiment was performed in the same environment, during dark phase of the day cycle in a large cage (40x40x40cm) with a floor that

was similar to the home cages. The following male sexual behaviour patterns were recorded:

1. That is the number of mounts in a given period of time(30 min.) or number of mounts in a series.
2. That is the number of intromissions observed in 30 min interval.
3. That is the time interval between the introductions of female to the first mount by male.
4. That is the interval from the time of introduction of female to the first intromission by male.
5. That is the time interval b/w the first intromission and ejaculation time.

Attraction towards Female and Determination of Hesitation Time

A female rat was placed in a cage which has a barrier of 15cm separating male & female compartments which can be passed only by a motivated male rat. The hesitation time was recorded in seconds required by male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was given as 0 to 5 during an observation period of 15 minutes. A complete cross of the partition by male rat each time was given a score of 5 while an attempt to climb was given a score of 2 & disinterest to climb was rated as 0. The readings were recorded on 1, 7, 14, 21 and 28th days of drug treatment. This test is useful in

determining the willingness of a male rat to cross an aversive or obstructive position, thus indicating the intent of sexual attraction. Male rats of all the groups were subjected to experimentation & their scores for attraction as well as hesitation time were recorded.

Partner preference test

Partner preference test was used to evaluate the sexual motivation in no-contact conditions. The apparatus designed to have an open field arena (100cm X 50cm X 40cm) with two round cages made of wire mesh of 16cm diameter and 40 cm height diagonally placed at the opposite corners of the arena. We used one pair stimulus animals: a male in one cage and a receptive female in the other cage. The sexual incentive area for female and the social incentive area for male were established. The transmission of olfactory, visual and auditory cues was allowed while mating avoided.

Experimental males were placed for 5 min individually in the centre of arena. adaptation period given in the presence of stimulus animals and then tested for 10 min. The number of visits to male and female rats, as well as the time spent near each stimulus animal was recorded. The measure of sexual motivation was kept by a preference score, i.e the ratio between the time spent in sexual incentive area and the total time spent in two incentive areas. The readings were recorded on 0, 7, 14, 21 and 28th days of treatment.

Endocrine tests (Testosterone and FSH assay):

Blood samples were collected from above animals by retro orbital sinus route. Blood was then centrifuged for 15 min. to obtain serum. It was then stored in dry bottles and frozen for overnight before use. The serum testosterone & FSH level were quantitatively determined by using the direct human serum testosterone & FSH ELISA kit (Calbiotech) as the manufacturer’s protocol. The principle is the general antibody-antigen reaction based on enzyme linked immunosorbent assay as described. The serum testosterone & FSH concentration were then interpolated from a standard calibration curve.¹⁵

Statistical analysis

Statistical analysis was done by one way ANOVA followed by Dennett's t-test. The values here

are expressed in mean values ± S.E.M. where n=6. Notation * means p < 0.05, ** means (p < 0.01), ***means (p < 0.001); compared to vehicle control group.

Results

Sexual behavior study

Several female proceptive and male pre-copulatory behavior parameters were observed from the cage side when the extract treated male rats were introduced to the receptive female rats. The proceptive behavior displayed by the female rats included ear-wiggling characterized by a rapid antero-posterior to the male rats (darting) and a short jump with stiff legs followed by immobility and presentation (hopping). The male rats, upon introduction, responded with immediate advances towards the females and displayed pre-copulatory behavior such as chasing, anogenital sniffing which eventually cuminated into mounting. Lordosis was also displayed by the receptive female rats before, at the beginning and during the mounts. There was genital toileting after every mount that resulted in intromission. The extract produced no sedative effect on the male rats since none of the animals showed evidence of tiredness throughout the observatory period. Aphrodisiac studies in male rats were carried out for 28 days. The following observations were recorded in *Cannabis indica* leaf extract treated rats.

Effect on Body Weight

Regular oral administration of *Cannabis indica* leaf extracts in albino rats exhibited quite increase in their firmness and body weight as the results shown in table -5.

Effect on serum sex hormone level

Cannabis indica leaf extracts in albino rats exhibited a quite elevation of serum androgen and regulatory hormone level. The results as described in table-6 demonstrates a significant increase in blood testosterone and Follicle stimulating hormone(FSH) level.

Table-1: Effect of *Cannabis indica* leaf extracts on various sexual behavior frequencies in male albino rats

Test	Treatment group	Dose (mg/kg)	Score Mean ± SEM				
			0 day	7 day	14 day	21 day	28 day
Mount frequency	Vehicle control	1 ml	21.62±1.5	21.62±1.5	22.82±1.7	23.12±0.9	25.62±2.2
	Std. control	5 mg/kg	31.76±1.5***	35.58±2.1***	38.62±1.6***	43.23±1.8***	49.07±2.6***
	<i>CIPEE</i>	150	28.45±2.3***	29.35±2.8***	30.53±2.4***	35.55±3.3***	38.43±4.2***
	<i>CIME</i>	150	26.65±2.6***	27.24±2.5***	28.68±4.0***	33.32±1.4***	37.64±2.4***
	<i>CIAQE</i>	150	25.62±2.4**	26.45±3.6**	27.36±4.2**	32.36±5.2**	36.27±2.6**
Intromission frequency	Vehicle control	1 ml	7.06±0.52	7.90±0.43	8.36±0.46	8.27±0.48	8.66±0.98
	Std. control	5 mg/kg	9.25±0.73**	10.46±0.88***	11.00±0.25*	12.04±0.22***	13.1±0.3***
	<i>CIPEE</i>	150	9.38±0.42**	10.32±0.36***	11.26±0.48***	12.20±0.40***	13.15±0.3***
	<i>CIME</i>	150	8.82±0.72**	9.65±0.3**	11.05±0.35**	11.75±0.26**	12.75±0.37**
	<i>CIAQE</i>	150	8.25±0.43*	9.34±0.24**	10.53±0.54*	11.36±0.69*	12.42±0.82**

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Ejaculation frequency	Vehicle control	1 ml	2.25±0.18	2.56±0.39	2.70±0.29	3.21±0.20	2.14±0.27
	Std. control	5 mg/kg	2.18±0.08***	2.05±0.22***	2.82±0.35***	3.15±0.17***	3.37±0.48***
	<i>CIPEE</i>	150	2.02±0.51***	2.05±0.82***	2.67±0.43***	2.18±0.31***	2.33±0.24***
	<i>CIME</i>	150	2.22±0.23***	2.21±0.52***	2.89±0.72***	3.28±0.34***	3.45±0.63***
	<i>CIAQE</i>	150	2.28±0.12***	2.50±0.34***	2.86±0.35***	3.12±0.53***	2.16±0.26***

Full form for all the abbreviations denoted at the end.

Values are expressed in mean ± S.E.M. Where n=5), *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

Table-2: Effect of Cannabis indicaleaf extracts on various sexual behavior latencies in male albino rats

Test	Treatment group	Dose (mg/kg)	Score Mean ± SEM				
			0 day	7 day	14 day	21 day	28 day
Mount latency	Vehicle control	1 ml	62.71±1.4	64.43±2.3	67.31±0.17	65.23±0.3	64.26±1.1
	Std. control	5 mg/kg	64.89±2.3***	58.34±0.4**	54.33±1.2***	45.75±0.5***	36.56±1.3***
	<i>CIPEE</i>	150	60.24±2.6**	58.46±0.5**	55.22±0.6**	44.40±0.8**	37.34±1.4**
	<i>CIME</i>	150	61.21±6.3***	58.25±0.4***	57.24±1.3**	46.42±0.5**	38.23±1.5**
	<i>CIAQE</i>	150	62.03±3.5**	60.50±0.68**	58.72±0.6**	47.41±0.7**	42.24±0.44**
Intromission latency	Vehicle control	1 ml	165±3.9	170±5.1	164.4±8.5	171±4.3	166.8±3.5
	Std. control	5 mg/kg	189.6±4.1***	176±5.1***	150.2±5.2***	121±3.8***	119.5±6.7***
	<i>CIPEE</i>	150	153.6±2.5***	148±3.3***	142±3.4***	136±3.2**	118.2±1.2***
	<i>CIME</i>	150	158±2.4***	152±3***	144±3.2***	138±2.3***	120±2.4***
	<i>CIAQE</i>	150	162±4.7**	154±3.7***	147±2.2***	140±2.5***	123±3.6***
Ejaculation latency	Vehicle control	1 ml	7.86±1.1	6.37±0.75	8.66±0.37	8.20±0.28	8.55±0.22
	Std. control	5 mg/kg	8.85±0.8***	9.50±0.3***	10.33±0.5***	10.54±0.2***	10.75±0.2***
	<i>CIPEE</i>	150	8.54±0.5***	8.26±0.4**	9.45±0.5***	9.4±0.6**	10.5±0.4**
	<i>CIME</i>	150	8.26±0.4**	8.12±1.2**	9.24±0.3**	8.5±0.5**	9.6±1.5**
	<i>CIAQE</i>	150	8.62±0.6**	7.73±0.5*	9.12±0.4*	8.4±0.5*	9.4±0.4**
Postejaculatory pause	Vehicle control	1 ml	8.4±0.19	8.5±0.35	8.8±0.35	8.5±0.21	7.9±0.23
	Std. control	5 mg/kg	5.6±0.33***	5.7±0.40***	4.8±0.35***	4.4±0.39***	4.3±0.36***
	<i>CIPEE</i>	150	5.4±0.42***	5.5±0.21***	4.6±0.46***	5.1±0.25***	4.4±0.44***
	<i>CIME</i>	150	5.8±0.48***	6.10±0.32***	4.9±0.34***	5.6±0.43***	4.8±0.52***
	<i>CIAQE</i>	150	6.2±0.32**	6.62±0.3**	5.4±0.45**	6.2±0.45**	5.5±0.33**

Values are expressed in mean ± S.E.M. Where n=5), *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

Table-3: Effect of Cannabis indica leaf extracts on Hesitation time and Cumulative score for attraction towards female in male albino rats

Test	Treatment	Dose (mg/kg)	Score (Mean±SEM)			
			7	14	21	28
Hesitation time in sec.	Vehicle control	1 ml	340±12.25	343±22.5	332±22.2	341±13.4
	Std. control	5 mg/kg	219±5.22***	199±12.12***	172±12.10***	180±12.04***
	<i>CIPEE</i>	150	235±7.24***	186±5.2***	195.6±11.3***	193±8.2***
	<i>CIME</i>	150	246±12.2***	222±8.4***	218±10.3***	227±4.30***
	<i>CIAQE</i>	150	251±8.3***	228±9.8***	227±5.15***	232±7.41***
Attraction towards female	Vehicle control	1 ml	19.22±3.1	21.61±3.2	27±30.22	30.70±1.35
	Std. control	5 mg/kg	35.2±5.04***	38±3.4**	64.70±3.4***	84.23±1.3***
	<i>CIPEE</i>	150	34.14±4.3***	47±5.4**	59.21±3.1***	82.61±4.74***
	<i>CIME</i>	150	32.51±4.4***	35.4±1.6***	41.61±1.3***	68.61±5.0***
	<i>CIAQE</i>	150	27.21±4.3***	29.3±2.4***	35.24±2.8***	48.21±3.72***

Values are expressed in mean ± S.E.M. Where n=5), *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

Table -4: Effect of Cannabis indica extract on partner preference test in male albino rats

S. No.	Treatment	Dose (mg/kg)	Partner preference test (Mean±SEM)
			28 days
1	Control	1 ml	0.87±0.01
2	Standard	5 mg/kg	0.98±0.07***
3	<i>CIPEE</i>	150	0.97±0.02***
4	<i>CIME</i>	150	0.92±0.03**
5	<i>CIAQE</i>	150	0.89±0.01**

Values are expressed in mean±S.E.M. Where n=5), *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

Table-5: Effect of *Cannabis indica* extract on Body weight of albino rats

S. No.	Treatment	Dose (mg/kg)	Average Body Weight Of Rats In Grams (Mean±SEM)			
			7	14	21	28
1	Control	1 ml	312.22	318.27	324.73	327.08
2	Standard	5 mg/kg	308.72	314.33	318.43	322.48*
3	CIPEE	150	309.25	311.24	316.34	317.32
4	CIME	150	320.34*	322.16	323.22*	328.5*
5	CIAQE	150	318.43*	320.21	323.32*	325.24*

Values are expressed in mean±S.E.M. Where n=5), *p<0.05, **=p<0.01, ***p<0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett’s test.

Table-6: Effect of *Cannabis indica* extract on serum testosterone & FSH level in albino rats

Test	Treatment	Dose (mg/kg)	Serum hormone Levels (Mean±SD)
			28th day
Testosterone (ng/ml)	Vehicle control	1 ml	0.78
	Standard	5 mg/kg	0.85**
	CIPEE	150	0.88**
	CIME	150	0.83**
	CIAQE	150	0.84**
FSH (mIU/ml)	Vehicle control	1 ml	10.52
	Standard	5 mg/kg	11.45**
	CIPEE	150	12.40***
	CIME	150	12.20**
	CIAQE	150	11.13*

Values are expressed in mean ± S.E.M. Where n=5), *p<0.05, **=p<0.01, ***p<0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett’s test.

Discussion

Sexual behavior study

The present study clearly provides evidence for the ability of *Cannabis indica* leaf extract to enhance sexual behavior and performance in male albino rats. The experimental data reveals that oral administration of leaf extracts Pet. ether, methanol & aqueous (150mg/kg Body wt.), possess effective improvement in several copulatory behavior parameters, body firmness and blood androgen levels. Sildenafil citrate (5mg/kg) was used as a standard drug to compare our test results for the level of significance.

Petroleum ether extract produced a significant increase (P<0.001) as compared to vehicle control group in Mount frequency (MF), Intromission frequency (IF) & Ejaculation frequency (EF) on 0, 7, 14, 21 and 28th day in male Rats. Methanolic and aqueous extracts also produced significant increase (P<0.01) in these parameters. The expression of MF as libido, IF as potency and EF as duration of coitus considered. (Table-1)

Petroleum ether extract treated rats also showed significant decrease (P<0.001) as compared to vehicle control group in mount latency (ML), intromission latency (IL) and ejaculation latency (EL) from 0 day till 28th day in male rats. Methanolic and aqueous extracts also produced significant decrease (P<0.01) in these parameters. Similarly all the extracts exhibited decreasing effects (P<0.01 & 0.001) on Post ejaculatory Mount latency & hesitation time. (Table-2&3)

It has been observed that Petroleum ether extract treated rats showed significant increase (P<0.001) as compared to vehicle control group in attraction towards female from 0 day till 28th day of treatment. Methanolic and aqueous extracts also

produced significant increase (P<0.01) in these parameters. Corresponding significant decrease (P<0.001 as compared to vehicle control group) in cumulative score for Partner preference test was observed in all extracts in potency order of CIPEE > CIME > CIAQE. (Table-4)

Body weight

A significant increase in body weight of all *Cannabis indica* leaf extract treated animals was recorded. As the dose and duration was increasing, firmness of body was improving for some instance then it become stable. An increase of about 4.8% in group-1 (p<0.05), 4.54% in group-2 (p<0.05), 2.56% in group-3 (p<0.05), 2.6% in group-4 (p<0.05) & 2.2% in group-5 (p<0.05) was observed in 4 weeks. The body weight was constantly increasing. (Table-5)

Serum androgen levels in rats

Serum testosterone and FSH level was significantly increased in extract treated animals compared to vehicle control group animals. There was a regular increase in testosterone level b/w the readings at weekly intervals. In group 3,4&5 the level of significance was found to be p<0.01. (Table-6) (16-19)

Conclusion

In conclusion, the drug *Cannabis indica* leaf extracts demonstrated aphrodisiac properties *in-vivo* albino wistar rat models. Sexual behavior study parameters reveals and confirmed this drug extracts as a potent aphrodisiac aid. A significant increase in sexual activities and decrease in several latency periods proves it as well as the result outcomes of serum endocrine analysis also confirming its aphrodisiac potential. This

drug or its preparations would help peoples with sex related problems and performances.

Abbreviations

CIPEE- Cannabis indica petroleum ether extract

CIME- Cannabis indica Methanolic extract

CIAQE- Cannabis indica aqueous extract

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