PRELIMINARY APHRODISIAC ACTIVITY OF HYBANTHUS ENNEASPERMUS IN RATS

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Abstract

Orally administered ethanol (300 mg/kg) and aqueous (300 mg/kg) extracts of *Hybanthus enneaspermus (L)* F. Muell were evaluated for its aphrodisiac activity in sexually inactive male rats both in a single dose regimen and in a chronic regimen as a daily dose for 28 days. Mount and intromission latency and number of mounts, intromissions and ejaculations were the parameters used for assessing sexual arousal and performance. Following a single dose administration, the aqueous extract produced a decrease in the mounting and intromission latency, with an increase in the ejaculatory and intromission frequency. In the chronic model, both the alcohol and aqueous extracts increased the number of mounts, ejaculations and intromissions with decrease in the mounting and intromission latency. Treatment with aqueous extract also elevated the testosterone levels in sexually inactive male rats. The findings suggest that *H. ennaespermus* may exert aphrodisiac activity in sexually inactive male rats.

Keywords: *Hybanthus enneaspermus*; Aphrodisiac activity; Sexual performance; Sexual arousal, L-dopa; Testosterone.

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Introduction

Hybanthus enneaspermus (L) F.Muell (*Violaceae*), (1) is a herb distributed chiefly in Bunderkhand, Agra, Bengal, Madras, Gujarat, Karnataka, Sri Lanka, tropical Asia, Africa, and Australia (2). Known as "Purusharatna" (gem for men) in Sanskrit, the plant is used as a diuretic, in impotency, as a demulcent (3), and for scorpion-sting (4). The plant is also attributed for its antimicrobial and antiplasmodial action (5,6). Various phytoconstituents viz. dipeptide alkaloids, aurantiamide acetate, isoarborinol and β-sitosterol have been isolated from different parts of this plant (7,8,9).Preliminary phytochemical screening of aqueous and alcoholic extracts of *Hybanthus enneaspermus* revealed the presence of alkaloids, carbohydrates, glycosides, tannins, phenols and flavonoids (10).

The plant is documented in the folklore medicine of South India for its aphrodisiac and stimulant activity. It has been widely used by the Nakkala, Irula, Sugali or Lambadi and Yerukala tribes of Chittor District (Andhra Pradesh, India) for this activity (11). The present study was hence undertaken to investigate the folklore claims of the aphrodisiac properties of *H. enneaspermus*.

Materials and Methods

The plant *H. enneaspermus* was collected in the fields of Chickbalapur, Kolar dist, Karnataka, India in the month of June 2001 and identified by the taxonomist Dr. Gopalkrishna Bhat, Poorna Prajna College Udupi, Karnataka. A voucher specimen number PP509 has been deposited in the Dept. of Pharmacognosy, College of Pharmaceutical Sciences, Manipal.

The alcohol extract was prepared by exhaustive extraction of the shade-dried powdered drug (1 kg) with 95% ethanol using a Soxhlet apparatus. The extract was concentrated *in vacuo* to a syrupy consistency (12%). For the preparation of aqueous extract, the shade dried powdered drug (1 kg) was macerated with chloroform water for 48 h with constant stirring. The liquid extract was concentrated *in vacuo* to a syrupy consistency (18%).

Experimental animals

Healthy Wister adult male and female albino rats between 2-3 months of age and weighing about 150-200 g were used for the study. Housed in polypropylene cages (2 rats of the same sex per cage), maintained under standard conditions (12 h light and 12 h dark cycle; 25± 30 C; 35-60% humidity), the animals were fed with standard rat pellet diet (Hindustan lever Ltd., Bombay, India) and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance (IAEC/KMC/47/2001-2002).

Acute toxicity

Healthy adult albino rats of either sex, starved overnight were divided into six groups (n=6) and were orally fed with the alcohol and aqueous extracts of *H. enneaspermus* in increasing doses i.e. 30, 100, 300, 600, 1000 and 3000 mg/kg body weight (12). The rats were observed continuously for 2 h for behavioral, neurological and autonomic profile. Further at the end of 24 and 72 h the rats were observed for any lethality or death . One-tenth of the maximum dose (3000mg/kg) at which no side effects were observed was selected for the evaluation of aphrodisiac activity. The dose of reference standard L-dopa was selected based on previous studies (13).

Selection of sexually inactive males

Sexually inactive male rats were screened and selected by mating a male with a receptive female rat daily for two consecutive weeks. The parameters to evaluate male sexual behavior recorded during the 30 minute period of mating included latency (time) of first mount and number of mounts; latency of first intromission and number of intromissions; latency of ejaculation (time from intromission to ejaculation) and number of ejaculations and post ejaculatory pause (time from ejaculation to next mount) (14). Rats which showed a significant reduction in mount latency and decreased numbers of ejaculations were considered to be sexually inactive. The study was carried out at dusk between 20.00 to 22.00 hrs under a zero watt red bulb in a sound proof room.

Aphrodisiac activity

The selected sexually inactive male rats were divided into six randomized groups of six animals each. In the single dose regimen, three groups of rats were orally treated with a single dose of reference standard L-dopa (100 mg/kg), alcohol extract (300 mg/kg) and aqueous extract (300 mg/kg) respectively. In the chronic regimen the remaining three groups were administered L-dopa (100 mg/kg), alcohol extract (300 mg/kg) and aqueous extract (300 mg/kg) daily for 28 consecutive days.

Assessment of sexual behavior in rats

Ovarectomized female rats were treated with 50 μg/kg of diethylstilbestrol subcutaneously, 48 h before, and 500 μg/kg progesterone 6 h before the study to induce the estrous phase for receptivity, which was confirmed by a vaginal smear. The receptive female rats were then mated with sexually inactive male rats to assess the sexual behavior, which was evaluated on the basis of parameters mentioned previously (14) 2 h after treatment in the single dose group and once weekly in the chronic regimen.

Estimation of testosterone

After completion of 28 days of treatment, blood samples were collected from the retro-orbital sinus in heparinised tubes. Samples were centrifuged and supernatant plasma was separated. Testosterone was measured in heparinised plasma by the use of the pathozyme testosterone ELISA kit. Samples, standards and controls were dispensed into appropriate wells, followed by testosterone HRP (Horseradish peroxidase) reagent and anti-testosterone reagent. These were incubated at 37^0 C for 90 minutes. Distilled water and substrate solution were added into wells, gently mixed and incubated for 20 minutes. The absorbance was read immediately at 450 nm using a microplate reader in an autoanalyser.

Statistical analysis

The statistical analysis of data was carried out using paired ‘t’ test for single dose treatment and One way ANOVA followed by post hoc Scheffe’s test for chronic treatment using SPSS computer package.

Results

In acute toxicity studies, the extracts of *H. enneaspermus* produced no toxic effects or mortality in rats even at a dose of 3000 mg/kg. Table 1 depicts the sexual behavior of inactive male rats.

Table 1: Effect on sexual behavior in sexually active and inactive male rats

Parameter	Sexually active males (n=6; Mean ± SEM)	Sexually inactive males (n=6; Mean ± SEM)
Mount latency (s)	5.2 ± 0.7	141.1±14.91[a]
No. of mounts	7.2±0.41	4.5±0.73[c]
Intromission latency (s)	8.9±1.05	119.66±23.28[b]
No. of intromissions	12.80±1.04	33.55±5.6[c]
Ejaculation latency (s)	213.8±16.72	407.7±56.01[c]
No. of ejaculations	1.6±0.16	0.88±0.17[c]
Post ejaculatory pause (s)	352.70±14.18	441.38±19.92[c]

[a] $p<0.0001$ compared to sexually active group; [b] $p<0.001$ compared to sexually active group; [c] $p<0.01$ compared to sexually active group

In the single dose regimen, L-dopa and the aqueous extract of *H. enneaspermus* significantly increased the ejaculatory frequency indicating the facilitation of sexual performance but failed to produce any significant change in mount latency (Table 2)

Table 2: Effect of single dose oral administration of drugs on sexual behavior in sexually inactive male rats.

Parameters	L-Dopa (100 mg/kg)		Alcoholic extract (300 mg/kg)		Aqueous extract (300 mg/kg)	
	Before	After	Before	After	Before	After
Mount latency (s)	74.83±17.54	50.83±27.3	53.00±15.95	29.00±1.52	59.83±17.72	48.5±8.62
No. of mounts	5.16±1.35	3.5±0.67	6.0±1.29	10.83±1.95	5.0±1.59	11.33±1.99[c]
Intromission latency (s)	109.5±41.67	17.66±4.83[c]	1255.5±38.6	25.0±2.51[c]	98.16±30.0	32.16±4.6[c]
No. of intromissions	32.16±7.5	17.83±2.18	41.66±9.63	52.83±3.36	34.16±6.69	56.0±7.36[c]
Ejaculation latency (s)	769.16±100.16	402.83±27.6	728.50±112.64	117.41±47.93[a,d]	639.66±147.82	451.83±59.23
No. of ejaculations	1.16±0.16	1.83±0.1[b]	1.50±0.22	2.66±0.33	1.16±0.16	2.67±0[a]
Post ejaculatory pause (s)	441.66±20.02	423.5±40.21	417.66±27.23	413.0±39.23	527.33±58.88	486.33±46.63

[a] $p<0.005$ compared to before treatment; [b] $p<0.01$ compared to before treatment; [c] $p<0.05$ compared to before treatment; [d] $p<0.01$ compared to L-Dopa (ANOVA). values are Mean ± SEM (n=6).

In the chronic regimen the effect of L-dopa, alcohol and aqueous extracts of *H. enneaspermus* on sexual behavior was assessed on the 7th, 14th, 21st and 28th day. At the end of the first week, all treated groups showed a significant decrease in mount latency and an increase in ejaculatory frequency thereby indicating an enhancement in libido and sexual performance (When compared before treatment).However no change was observed in the other parameters of sexual behavior. Similar findings were observed on the 14th day of assessment, whilst on the 21st day, a significant decrease in the intromission latency was found in addition to the above said parameters.

Table 3 depicts the sexual behavior of inactive males on the 28th day of post treatment. In the aqueous extract treated group, the significant decrease observed in mount, intromission and ejaculatory latency suggested improved sexual motivation whilst the significant increase observed in the number of mounts and ejaculations were indicative of facilitation of sexual performance. In addition, sexually inactive male rats treated with the aqueous extract showed a significant rise in the serum testosterone level (5.8 $\pm$ 1.04 ng/dl) as compared to their base line level(Sexually inactive male 2.63$\pm$0.32 ng/dl). L-dopa (3.56$\pm$0.39) failed to produce any significant change in serum testosterone level.

Parameters	Inactive	L-Dopa	Alcoholic extract	Aqueous extract
Mount latency (s)	141.1±14.91	4.83±2.4[a]	0.833±0.083[a]	2.0±1.36[a]
No. of mounts	4.5±0.73	4.5±1.4	0.66±0.066[b]	12.0±1.0[c]
Intromission latency (s)	119.66±23.8	23.66±13.3[b]	17.0±4.5[c]	62.33±7.81
No. of intromissions	33.55±5.6	29.66±4.18	42.66±5.75	58.83±3.7[b]
Ejaculation latency (s)	407.7±56.1	509.0±92.14	292.0±35.78	38.33±5.84[c]
No. of ejaculations	0.88±0.17	1.5±0.2	3.0±0.25[a]	3.33±0.21[a]
Post ejaculatory pause (s)	441.38±19.92	481.3±17.31	296.0±17.31[c]	371.0±57.48

Table 3 : Effect of L-Dopa and extracts of *Hybanthus enneaspermus* on sexual behavior in sexually inactive males on 28th day.

[a] $p<0.0001$ compared to before treatment; [b] $p<0.005$ compared to before treatment

[c] $p<0.001$ compared to before treatment; values are Mean±SEM (n=6)

Discussion

In our study, both the alcoholic and aqueous extracts of *H. enneaspermus* were found to be safe and devoid of side effects up to a dose level of 3000 mg/kg body weight. The terms libido, sexual motivation and sexual performance are normally used in aphrodisiac studies. (Burses et al, 1983) Libido is defined as sexual arousal and is measured in terms of mount and intromission latencies. These measures are confounded by the erectile process (potency) necessary for successful execution of copulatory patterns (15).

In single dose therapy, while the alcohol extract treated group exhibited enhancement in the sexual performance but no effect on libido, similar to L-dopa treated group, the aqueous extract treated group displayed increase in sexual performance as well as libido. In the chronic treatment regimen, extracts were found to enhance both sexual performance and arousal, with the sexual arousal produced by *H. enneaspermus* comparable to that of L-dopa. Sexual performances of animals in the aqueous extract treated group were more significant than that of the L-dopa and the alcohol treated groups.

Aphrodisiac agents act by increasing the testosterone levels or by altering central neurotransmitters like dopamine (13,16). Our study showed an increase in testosterone levels in the aqueous extract treated group, which probably suggests one of the above mechanisms. A further detailed study of its effect in gonadotropins and central neurotransmitters has to be elucidated.

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