

Original Article

## Aphrodisiac activity and curative effects of ethanolic extracts of *Ipomoea carnea* against sexual behavior on prolonged immobilization-induced stress in rodents

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### ARTICLE INFO

Received 24 Oct 2014

Revised 12 Nov 2014

Accepted 25 Nov 2014

### Keywords:

- Aphrodisiac activity
- *Ipomoea carnea* leaves
- Ethanolic extract
- Mice
- Rats

### ABSTRACT

The present study was designed to investigate the aphrodisiac activity of the ethanolic extract of *Ipomoea carnea* leaves. Aphrodisiacs can be categorized according to their mode of action into three groups: substances that increase libido (i.e., sexual desire, arousal), substances that increase sexual potency (i.e., effectiveness of erection) and substances that increase sexual pleasure. Therefore, the search for a better tolerated aphrodisiac agent appears to be a necessity. The aphrodisiac activity was evaluated in various experimental animal models like Effect on fertility in mice, Effect on sperm properties in mice and Sexual behaviour on prolonged immobilization stress in rats. The ethanolic extract of *Ipomoea carnea* leaves even up to the dose level of 2000mg/kg. It has not produced any lethal effect. In Effect on fertility model, ethanolic extract of *Ipomoea carnea* leaves only 200 & 400 mg/kg dose treated groups but not 100 mg/kg dose had shown a significant increase in litter size but no effect on M/F ratio. Ethanolic extract of *Ipomoea carnea* leaves was tested for its effect on sperm properties with different dose levels and all doses (100,200 & 400 mg/kg) have shown a significant increase in spermatogenic activity with scant intertubular spaces between the tubules. Sexual behaviour in prolonged immobilization stress induced model, a significant increase in number of mounts and thrusting and decrease in mounting latency were recorded with 200 & 400 mg/kg treated doses only but not with 100 mg/kg treated group. The present investigation revealed that the ethanolic extract of *Ipomoea carnea* leaves was found to possess aphrodisiac activity.

### 1. INTRODUCTION

Erectile dysfunction is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse. It has been estimated to affect 20 million to 30 million men in the United States. Result from psychological, neurologic, hormonal, arterial or cavernosal impairment or

from a combination of these factors [1]. The underlying causes for sexual disorders may be psychological, psychiatric, organic, interpersonal or related to pharmacological factors and treatment should be provided accordingly, further 49% of 40 years age group and 67% of 70 year age group men suffer from sexual dysfunction disorders.

In recent years, there has been phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in the official books of Ayurveda and Siddha reported to possess aphrodisiac activity.

Review of various published works it has been revealed that a good number of plant based drugs i.e., Aalooka (Tuber), Badara (Root, Bark, Fruit), Dhanya (Seed), Dugdika (Whole plant), Kapikachchu (Root, Seed, Hairs), Kokilaksha (Root, Seed), Musali (Root), Rushabha (Tuber), Talamuli (Root), Kottaikaranthai (Root, Leaf, Flower, Seed), Mullangi (Root, Leaf, Seed), Neermuli (Flower, Seed) [2] reported with aphrodisiac activity.

Apart from the above mentioned individual herbal drugs, there are certain polyherbal formulations launched by the various companies in market like “Tentex Forte, Tentex Royal (Himalaya Drug Company Bangalore), Vita-Ex-Gold (Baidyanath) is also recommended for aphrodisiac activity. Recently scientific interest in the pharmacology of sexual behaviour has been given impetus by the discovery of drugs (indigenous) that can stimulate or inhibit such behaviour [3-5]. Ayurveda, an ancient Indian system of healing, described various plants for the treatment of sexual disorders particularly like erectile dysfunction and SHRUSHTI, a Herbal Pharma Industry in Bangalore, has come out with a aphrodisiac formulation consisting of plant ingredients of *Bacopa monnieri*, *Asparagus adscendens*, *Astercantha longifolia*, *Asparagus recemosus*, *Mucuna pruriens* and *Withania somnifera*.

The plant *Ipomoea carnea* is a large, diffuse or struggling shrub with milky juice, leaf ovate cordate, entire, acuminate, flower large campanulate, pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes; Fruits glabrous capsule; Seed silky, belonging to family Convolvulaceae [6-8]. It is well distributed in India and found particularly in Chhattisgarh and Madhya Pradesh [9-11]. The plant is commonly known as Besharam, Behaya and used for skin troubles successfully. The milky juice of Beshram is used for the treatment of leucoderma [12]. The juice is collected and applied externally on affected parts, anti-inflammatory. It is used to decrease the teratogenic effect resulting from cyclophosphamide [13]. Aqueous extract of *Ipomoea carnea* shows neuromuscular blocking activity [14]. It used as purgative and cathartic [15]. The leaves of *Ipomoea carnea* contain 1-3 flavonol glycosides and Ergine (D-Lysergic acid amide) [16]. Polyhydroxylated alkaloids were isolated from the leaves, flowers and seeds [17]. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine, 2-epilentiginosine, calystegines B (1), B (2), B (3) and C (1) and N-methyl-trans-4-hydroxy-l-proline and beta sitosterol [18, 19]. To the best of our knowledge there was lack of scientific reports available in support of its traditional claim of hepatoprotective potential. So far, there has been only one research reported on hepatoprotective effect against carbon tetrachloride [20,21] induced liver damage in rats has been investigated. So the present study was aimed to assess the aphrodisiac activity of this *Ipomoea carnea* in different models of experimental animals, mice and rats.

## 2. MATERIAL AND METHODS

### 2.1. Drugs and chemicals

Tentex Forte (Himalaya Drug Company, Bangalore).

### 2.2. Plant material

The plant *Ipomoea carnea* is widely distributed throughout India. The plant herbarium specimen was identified and authenticated by Mr. P.G. Diwakar, Joint Director, Botanical Survey of India, Western circle-7, Koregaon Road, Pune-1 on dated 11/01/2011, and Voucher No. RASICA4. The leaves of *Ipomoea carnea* were washed thoroughly in tap water, shade dried and powdered. Powdered material was subjected to extraction in a Soxhlet apparatus at 60-70°C for 6 h continuously in 50% distilled ethanol. The extracted material was evaporated to dryness under reduced pressure (40-45°C). The yield of the material was 12.63 g %. The extract obtained was further subjected to pharmacological investigation.

### 2.3. Animals

Healthy albino Wistar rats of age between 10-15 weeks of either sex were used after approval of the institutional ethics committee. They were kept in departmental animal house in well cross ventilated room at 22 ± 2°C with light and dark cycles of 12 h for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee (Reg. No-346/CPCSEA).

### 2.4. Acute toxicity study

Acute toxicity study was performed according to OECD guidelines No. 420. Swiss albino mice of either sex were divided into six groups with six animals each. Aqueous extracts of *Ipomoea carnea* leaves were studied for acute toxicity at doses different dose levels of 5, 50, 300, 500 and 2000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days [21].

### 2.5. Models for aphrodisiac activity

*Effect on fertility in mice* [22, 23]:

*Experimental Procedure:*

Adult swiss albino male mice of (25-35g) each consisting of 6 animals was divided in to following groups

Group I: Normal control (Gum acacia 10ml/kg, p.o)

Group II: Standard drug (Tentex Forte 171 mg/kg, p.o)

Group III: Ethanolic extracts of *Ipomoea carnea* (100 mg/kg, p.o)

Group IV: Ethanolic extracts of *Ipomoea carnea* (200 mg/kg, p.o)

Group V: Ethanolic extracts of *Ipomoea carnea* (400 mg/kg, p.o)

In the evening (17.00 to 18.00) different groups of mice were treated as mentioned above and then each male mouse was placed in separate cage. After one hr, one oestrous female with proven fertility was admitted into each cage and cohabitated overnight. Later these females were watched for pregnancy and birth of off springs. With the litter size and number of male and female pups were recorded in each group. Similarly aphrodisiac activity of standard drug was also evaluated.

#### Effect on Sperm Properties in mice [24]:

##### Experimental Procedure:

Adult swiss albino male mice of (25-35g) each consisting of 6 animals were divided in to following groups

Group I: Normal control (Gum acacia 10ml/kg, p.o)

Group II: Standard drug (Tentex Forte 171 mg/kg, p.o)

Group III: Ethanolic extracts of *Ipomoea carnea* (100 mg/kg, p.o)

Group IV: Ethanolic extracts of *Ipomoea carnea* (200 mg/kg, p.o)

Group V: Ethanolic extracts of *Ipomoea carnea* (400 mg/kg, p.o)

Animals were administered with vehicle/standard drug/ formulation for a period of daily once for 30 days and at the end of treatment period animals were sacrificed by overdose of ether anesthesia. Histopathological studies of testis were done by fixing the testes in Bouin's fluid and passed through ascending series of ethanol and then through xylene, and embedded in paraffin wax. Tissues were sectioned at 5 mm and stained with haematoxyline and eosin.

#### Sexual behavior on prolonged immobilization-induced stress in rats [25]:

##### Experimental Procedure:

Adult albino rats of (150-200 g) each consisting of 6 animals were divided in to following groups.

Group I: Normal control (Gum acacia 10 ml/kg, p.o)

Group II: Stress Control

Group III: Standard drug (Tentex Forte 171 mg/kg, p.o)

Group IV: Ethanolic extracts of *Ipomoea carnea* (100 mg/kg, p.o)

Group V: Ethanolic extracts of *Ipomoea carnea* (200 mg/kg, p.o)

Group VI: Ethanolic extracts of *Ipomoea carnea* (400 mg/kg, p.o)

Different groups of prepubertal (40 days of age) male albino rats were housed under controlled environmental conditions and had free access to laboratory chow food and tap water. In the morning time animals were treated as mentioned above. Immobilization Stress was induced by wrapping the animals in wire mesh daily 3 h for a day during the light period, starting at 8:00 A.M., for 15 days. Control animals for adaptation were left undisturbed in their cages. The males were placed in the observation 2 h after the beginning of the dark phase and 10

min before the females dropped into the cage. The latency, number of mounts and thrusting were recorded in red light of 40-watt capacity simultaneously by two investigators with light provided. In the mount behavior the male places his forepaws on the female without pelvic movements, while in the thrusting behavior it executes repeated deep pelvic thrusts.

### 3. STATISTICAL ANALYSIS

All values are expressed as mean  $\pm$  SEM from 6 animals and results are subjected for statistical analysis using one-way ANOVA (analysis of variance) followed by Post hoc test (Dennett's 't' test).  $P < 0.05$  will be considered as statistically significant.

### 4. RESULTS

#### 4.1 Determination of acute toxicity (LD<sub>50</sub>)

The ethanolic extracts of *Ipomoea carnea* when administered orally to different groups of mice with different dose levels even upto the dose level of 2000mg/kg dose did not produced any mortality.

#### 4.2 Aphrodisiac activity

A. Effect of ethanolic extracts of *Ipomoea carnea* on fertility in mice:

When compared to control group Tentex Forte and ethanolic extracts of *Ipomoea carnea* with 200 and 400mg/kg doses but not 100mg/kg treated groups have shown an increase in the litter size. Similarly when compared to control group Tentex Forte (171mg/kg) and ethanolic extracts of *Ipomoea carnea* (100, 200 and 400mg/kg) treated groups does not exhibit a significant increase in M/F ratio.

**Table 1.** Effect of Ethanolic extracts of *Ipomoea carnea* on litter size in female mice (Fertility model)

S. No.	Group	Dose (mg/kg)	Mean $\pm$ SEM
1	Control	10ml	4.500 $\pm$ 1.455
2	Standard	171mg	11.333** $\pm$ 0.3333
3	Ethanolic extracts of <i>Ipomoea carnea</i>	100 mg	6.667ns $\pm$ 1.358
4	Ethanolic extracts of <i>Ipomoea carnea</i>	200 mg	9.167** $\pm$ 0.3073
5	Ethanolic extracts of <i>Ipomoea carnea</i>	400 mg	10.167** $\pm$ 0.3073
One way ANOVA		F	8.954
		df	29

n=6 in each group.

Significance at \* $P < 0.05$ , \*\*  $P < 0.01$  & ns-not significance Vs control.

**Table 2.** Effect of ethanolic extracts of *Ipomoea carnea* on M/F ratio in female mice (Fertility model)

S. No.	Group	Dose (mg/kg)	Mean ± SEM
1	Control	10ml	0.7350 ± 0.2631
2	Standard	7gm	1.302 ± 0.1371
3	Ethanolic extracts of <i>Ipomoea carnea</i>	100	0.8983± 0.2368
4	Ethanolic extracts of <i>Ipomoea carnea</i>	200	1.160 ± 0.1221
5	Ethanolic extracts of <i>Ipomoea carnea</i>	400	1.108 ± 0.1534
One way ANOVA		F Df	1.374 29

n=6 in each group

Significance at \* $P < 0.05$ , \*\*  $P < 0.01$  & ns-not significance Vs control

### B. Effect of ethanolic extracts of *Ipomoea carnea* on sperm properties in mice

When compared to the control animals histopathology of the testis in Standard (Tentex Forte 171mg/kg) and ethanolic extracts of *Ipomoea carnea* 400mg/kg treated groups has showed an outstanding increase in spermatogenic activity, with scant intertubular spaces where as low and medium dose of Ethanolic extracts of *Ipomoea carnea* has shown mild and moderate increase in the spermatogenic activity, with scant intertubular spaces between the tubules.

**Table 3.** Effect of ethanolic extracts of *Ipomoea carnea* on mounting latency, number of mounts and thrusting in Stress induced altered sexual behavior in rats

S. No.	Group	Dose mg/kg	Mounting Latency in sec/30 min mean ± SEM	Number of Mounts mean± SEM	Thrusting mean ± SEM
1	Control	10 ml	925.83 ± 34.169	9.833 ± 0.6009	6.667 ± 0.4216
2	Standard Tentex Forte	1gm	59.167** ± 3.301	33.167 ** ± 2.272	31.333** ± 2.186
3	Ethanolic extracts of <i>Ipomoea carnea</i>	100	965.83ns ± 5.764	14.167ns ± 1.302	11.167ns ± 0.6009
4	Ethanolic extracts of <i>Ipomoea carnea</i>	200	204.50** ± 20.013	22.000** ± 1.506	20.000** ± 1.342
5	Ethanolic extracts of <i>Ipomoea carnea</i>	400	73.667** ± 5.136	31.500** ± 0.7638	29.000** ± 1.265
One way ANOVA		F df			704.35 35

n=6 in each group

Significance at \* $P < 0.05$ , \*\*  $P < 0.01$  & ns-not significance Vs control

### C. Effect of Ethanolic extracts of *Ipomoea carnea* on Sexual behavior in prolonged immobilization induced stress in rats

When compared to stress control animals, Tentex Forte (1gm/kg) and ethanolic extracts of *Ipomoea carnea* (200, 400 mg/kg) treated groups have shown a significant increase in the number of mounts, thrusts and decrease in the mounting latency. But low dose of ethanolic extracts of *Ipomoea carnea* 100 mg/kg did not exhibited a significant effect on number of mounts, thrusting and mounting latency.

## 5. DISCUSSION

Erectile dysfunction is more prevalent in males and so, it is more conventional to focus on male sexual difficulties. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems. Further it may result from psychological, neurologic, hormonal, arterial or cavernosal impairment or from a combination of these factors. The underlying causes for sexual disorders may be psychological, psychiatric, organic, interpersonal or related to pharmacological factors and treatment should be provided accordingly. Generally, a prevalence of about 10% cases occurs across all ages and sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age. As men advances with age, the absolute number of Leydig cells decreases by about 40%, and the vigour of pulsatile leutenizing hormone release is dampened. In association with these events, free testosterone level also declines by approximately 1.2% per year. All these contributed in no small measure to prevalence of sexual dysfunction in the aged males [26].

Male sexual behavior is regulated by a range of redundant mechanism involving several neuropeptides like oxytocin and galanin, with inhibitory activity and neurotransmitters (mainly dopamine, serotonin, noradrenaline and NO). The stimulatory effect of oxytocin on male sexual behavior is proportionately greater in sexually sluggish than in sexually potent animals. Low non-stereotypy-inducing doses of direct or indirect dopaminergic drugs improve the copulatory performance of sluggish/impotent males, while a further improvement of the sexual behavior of vigorous copulators is not always clearly apparent. Finally, facilitation of central noradrenergic transmission, either by alpha 2-adrenoreceptors blockade or by stimulation of beta 2-adrenoreceptors, while having either no effect or a worsening effect in sexually potent animals, improves copulatory behavior in sexually sluggish animals [27].

Many central neurotransmitters and neuropeptides are involved in the control of male sexual behavior. Increased brain noradrenergic and dopaminergic activities may improve parameters of copulatory activity, indicating their facilitatory role in the process. The effects of serotonin and dopamine on male copulatory behavior seem to occur by interaction with testosterone. A proper androgenic status is also necessary for a normal sexual performance, the deleterious effects of castration being reversed by hormonal replacement; Moreover,

chronic treatment of prepubertal rats with testosterone can precipitate the onset of first mount, thrusting and ejaculation, probably by stimulation of sexual arousal. It is possible that increased plasma testosterone concentration, in addition to the higher catecholamine and serotonin levels expected to occur after prolonged stress, might account for the enhanced sexual performance described at the onset of puberty. As a result of hypothalamic-pituitary-adrenal axis activation, prolonged stress may inhibit the male reproductive functions through a depression of the hypothalamic pituitary- testicular axis. Chronic intermittent immobilization-induced stress caused a significant decrease in plasma LH of both pubertal and adult rats, whereas plasma testosterone was lower than control levels in adult stressed rats but was more than twofold higher in pubertal animals, suggesting that prolonged stress probably acts in a different way on the gonadal axis during distinct phases of sexual development. Since adrenergic innervation seems to play a pivotal role in testicular steroidogenesis around the onset of puberty, it is we proposed that sympathetic over stimulation might explain the increased testosterone levels observed in pubertal stressed rats. Prolonged immobilization caused no significant change in plasma FSH but induced a significant delay in testicular maturation, in addition to a decrease in spermatid production and sperm density in both pubertal and adult animals [28].

Testosterone supplementation improves sexual function and libido, in addition to the intensity of orgasm and ejaculation which is likely to improve. Testosterone in the blood exists in three different forms namely: free, albumin-bound and sex-hormone binding globulin (SHBG). While it is generally considered that SHBG bound testosterone is not available for uptake by tissues, opinion is mixed as to whether the biologically active testosterone is restricted to the small quantity of the hormone that is free (2%) or includes the larger amount of albumin-bound hormone (20–80%). However, investigations suggest that both free and albumin-bound testosterone is biologically available. Generally, elevated testosterone level also enhances the sexual behavior in humans. Therefore, an increase in testicular and serum free testosterone concentration can confirm aphrodisiac potential inherent in the plant extract. Generally a sexual behavior is enhanced by elevated testosterone levels, and Drug induced changes in neurotransmitter levels or their action in the cells could also changes sexual behavior.

The purpose of the present study is to determine whether prolonged immobilization- induced stress from prepuberty interferes with the onset of sexual behavior at puberty and with fertility during adulthood. The exposure to stress decrease male sexual activity and cause longer latencies with decrease in number of mounts and thrusting. In order to evaluate whether the ethanolic extracts of *Ipomoea carnea* has effect on litter size and M/F ratio, the experiment fertility in mice model was selected and ethanolic extracts of *Ipomoea carnea* has shown an increase in the litter size but no effect on M/F ratio. Further whether the ethanolic extracts of *Ipomoea carnea* has modifying the effect of mounting latency, mounts and thrusting in stress induced animals in prolonged immobilization stress model ,was sexual

behavior in rats to assess I sever stress. The above all functional and histological parameters noted and photomicrography of testicular tissue clearly depicts that the ethanolic extracts of *Ipomoea carnea* possessed aphrodisiac activity.

## 6. CONCLUSION

It is interesting to note that during acute toxicity study, the ethanolic extracts of *Ipomoea carnea* was non-toxic and has not produced any lethal effect even upto the dose level of 2000mg/kg in mice. When compared to control even after prolonged immobilization induced stress, ethanolic extracts of *Ipomoea carnea* has shown an significant effect which were indicated by increase in litter size in Fertility model, increase in spermatogenic activity and Sperm properties in mice model and increase in thrusting, number of mounts and decrease in mounting latency in Sexual behavior model in rats.

The ethanolic extracts of *Ipomoea carnea* has not shown any influence on m/f ratio in Fertility model in mice. Thus the results concluded that the ethanolic extracts of *Ipomoea carnea*, has a definitely aphrodisiac activity. In order to understand exact mechanism of aphrodisiac effect of ethanolic extracts of *Ipomoea carnea* it is necessary to perform assays for neuronal nitric oxide synthase and androgen receptor protein in different models of experimental animals.

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