

## **Aphrodisiac Activity of *Crossandra infundibuliformis* (L.) on Ethanol Induced Testicular Toxicity in Male Rats**

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### **Summary**

According to Indian Systems of Medicine, *Crossandra infundibuliformis* (L.) belonging to the family (Acanthaceae), were used for treating male sexual disorders since ancient times. Aim of this study to evaluate the phytochemical constituents and the aphrodisiac potential of the petroleum ether extract of leaves of *Crossandra infundibuliformis* (L.) on ethanol induced testicular toxicity in albino wistar rats. Phytochemical screening revealed the presence of alkaloids and saponins. All the doses resulted in significant increase in mount frequency, intromission frequency and significantly prolonged the ejaculatory latency ( $P < 0.05$ ) and reduced mount and intromission latency ( $P < 0.05$ ). There was also a significant increase in serum testosterone concentrations in all the groups in a manner suggestive of dose-dependence ( $P < 0.05$ ). Results of this study concluded that the petroleum ether extract of *Crossandra infundibuliformis* (L.) increased the blood testosterone concentrations and this may be the mechanism responsible for its aphrodisiac effects and various masculine behaviors. It may be used to modify impaired sexual functions in animals, induced testicular toxicity in albino wistar rats.

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### **Introduction**

Erectile dysfunction is defined by the National Institutes of Health as the inability to attain or maintain an erection sufficient for satisfactory sexual performance <sup>[1]</sup>. It is a highly prevalent and often under treated condition. Although a wide range of risk factors contributes to the development of Erectile dysfunction, vasculogenic Erectile dysfunction is recognized as the most common organic etiology (70%), about (30%) of it related to diabetes mellitus <sup>[2]</sup>. Psychogenic causes (30%) include depression, performance anxiety, relationship problems and psychosocial stressors. Combination of organic and psychogenic factors is common <sup>[3]</sup>.

Plants have provided a source of inspiration for novel drug compounds and plant medicines have made large contributions to human health and well being. The plant-derived and herbal remedies continue to be a popular alternative for men and women seeking to improve their sexual life despite the availability of effective conventional medical treatments<sup>[4]</sup>. *Crossandra infundibuliformis* is an important horticultural plant and belongs to the family Acanthaceae.

In India, the plant is considered an aphrodisiac; it is ornamental and bears red flowers which have a high value in the Indian flower market. Micropropagation is being used extensively for the rapid clonal propagation of many fruits, nuts and ornamental trees<sup>[5]</sup>. Therefore, the present study was performed to verify the folklore claim of aphrodisiac activity of *Crossandra infundibuliformis* (L.) on ethanol induced testicular toxicity in albino wistar rats.

### Materials and Methods

**Plant collection:** The Plant material of *Crossandra infundibuliformis* (L.) was collected from Tirupati, Andhra Pradesh, in the month of August 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, West Tambaram, Chennai. The voucher specimen (PARC/2009/350) of the plant was deposited at the college, for further reference.

**Preparation of extracts:** The leaves of the plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (80gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of petroleum ether extract of *Crossandra infundibuliformis* (L.) was found to be 22.5 % w/w.

**Preliminary phytochemical screening:** The phytochemical examination of petroleum ether extract of leaves of *Crossandra infundibuliformis* (L.) was performed by the standard methods<sup>[6]</sup>.

**Animals used:** Twelve-week-old female (body weights around 150–200 gm) and male (body weights around 200–250 gm) albino rats of Wistar strain were used for the present study. They were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 01 / SVCP / 2009 - 2010).

**Experimental Design:** Healthy male albino rats showing brisk sexual activity were selected for the study. The male rats were divided in 5 groups each consist of 6 rats. The entire group was fed 6% ethanol for 30days in drinking water except group I. Group I received normal saline 10 ml/kg b.w per orally for a period of 30 days and served as control. Group II received ethanol 6% v/v in water feeding bottle as aqueous solution for period of 30days and served as negative control. Group III and IV received PECCI 200 and 400 mg/kg/p.o, daily for a period of 30 days respectively. Group V received Sildenafil citrate (5 mg/kg b.wt) daily for period of 30 days.

**Mounting behavior:** Mounting behavior was carried out by method of Agmo (1997)<sup>[7]</sup>. Healthy male albino rats and Female rats showing non-oestrus cycle were used for mating behaviour analysis. Female rats with maximum receptivity with male rats were selected for the experiment. The tests for sexual desire were carried out on 20<sup>th</sup> and 30<sup>th</sup> day after treatment of PECCI. All sexual behavior studies were carried out between 13:00 and 16:00 at room temperature 26 °C–28 °C. Sexual behavior studies were monitored in a separate room for 2 h following the administration and were given 20 min adaptation period, after which a female rat was placed in the same cage with the male in 1:1 ratio. The male and receptive female rats were introduced into

the mating cages, with 1:1 ratio. The mating behaviours were monitored, including: number of mounts without Intromission until ejaculation or mounting frequency (MF), number of intromission from the time of introduction of the female until ejaculation or intromission frequency (IF), the time interval between the introduction of the female time to the first mount by the male or mounting latency (ML), the interval from the time of introduction of the female 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). The values of the observed parameters for control, standard and PECCI treated groups were recorded.

**Hormonal analyses:** The blood was collected from retro orbital venous plexus of all animals at the 20<sup>th</sup> and 30<sup>th</sup> day of the experiment. Testosterone was estimated after separation of serum by using Radio Immunoassay (RIA) [8]. The RIA was carried out in diagnostic Endocrinology and clinical Biochemistry service, No. 56/64, First Avenue, Indra Nagar Adyar, Chennai-20.

**Statistical analysis:** The data were expressed as mean  $\pm$  standard error mean (S.E.M). The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

## **Results**

### **Phytochemical screening**

The results of preliminary phytochemical screening of petroleum ether extract of leaves of *Crossandra infundibuliformis (L.)*, revealed that presence of steroids, flavanoids and carbohydrates.

### **Male sexual behavior**

Increase in the sexual vigor of MF and IF (table 1) were observed in all dosed groups (namely 200 mg/kg and 400 mg/kg body weight) in a dose dependent manner that was statistically significant ( $P < 0.05$ ) when compared with the control. By the 30<sup>th</sup> day of the experimental period in the highest dosed group (400mg/kg), both MF and IF had increased to 2 times of their respective control values. In contrast, the mount latency (Figure 3) and intromission latency (Figure 4) decreased significantly with the doses and as the experimental period increased ( $P < 0.05$ ). There was also statistically significant prolongation of ejaculatory latency ( $P < 0.05$ ) following the administration of various doses of the plant stem extract.

### **Hormonal analysis**

Hormonal analysis revealed that the levels of testosterone increased gradually in all the experimental groups. Particularly on the 30th day, the levels of both hormones increased in a significant manner. However, the increase in testosterone (Groups III–IV) was lower when compared to the standard drug (Sildenafil citrate) group (Group V).

**Table 1:** Effect of *Crossandra infundibuliformis* (L.) on mating behavior in ethanol treated male rats

	Group I (Control-normal saline 10 ml/kg, p.o)		Group II (6% ethanol in drinking water)		Group III ( PEGI 200mg/kg)		Group IV (PEGI 400mg/kg)		Group V (Sildenafil citrate 5mg/kg,s.c)	
Mating behavior	20 <sup>th</sup> day	30 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
<b>ML</b>	13.16 ±0.46	12.05±0.88	14.25±0.93	13.80±0.71	11.01±0.19	9.05±0.70**	4.17±0.13*	5.25±2.01*	2.45±0.40**	2.17±0.42**
<b>IL</b>	12.01±0.38	11.02±1.31	13.27±0.53	14.25±1.11	6.25±0.49	6.01±0.13*	2.75±0.19*	2.10±2.03*	0.88±0.13**	1.92±0.24**
<b>EL</b>	2.71±1.40	2.65±1.24	238±1.43	217±2.08	280±1.05	295±2.10	396±3.45*	1216±4.08**	385±2.25**	1521±2.88**
<b>MF</b>	69.01±2.70	68.05±3.06	71.30±1.40	65±2.04	89.40±1.44	149.32±1.98**	91.30±1.23*	163±1.06**	134±2.14**	188.62±1.3**
<b>IF</b>	74.05±4.05	72.67±2.83	79.01±2.15	67±2.05	98.50±3.01	143±1.88**	97.04±1.35*	153±2.07**	131.44±1.07**	182±2.59**

**ML:** mounting latency, **IL:** intromission latency, **EL:** ejaculation latency, **MF:** mounting frequency and **IF:** intromission frequency.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test \*p<0.05; \*\* p<0.01.

Mating behaviour scores of various groups in 20th, 30th days of experiment. Values are expressed as mean ± SEM of six observations. Comparison between: Group I &II Vs group III, IV&V.

### **Discussions and Conclusions**

Erectile dysfunction (ED) is a common condition that may result from psychological, neurologic, hormonal, arterial or cavernosal impairment, or from a combination of these factors<sup>[9]</sup>. Ethanol in small amounts was shown to improve erection and to increase libido because of its vasodilator effect and suppression of anxiety, but in large amounts it can cause central sedation, decreased libido, and transient ED<sup>[10]</sup>.

The results of preliminary phytochemical screening of the petroleum ether extract of leaves of *Crossandra infundibuliformis L.* revealed that presence of alkaloids and saponins. The Present studies have implicated the saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property<sup>[11]</sup>.

Saponins present in the petroleum ether extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of leutinizing hormones (LH). This LH released normally by the pituitary gland helps to maintain testosterone levels; as LH increases, so does the testosterone<sup>[11]</sup>. The increase in testosterone seemed to have translated into the male sexual competence observed in this study. Furthermore, this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level.

PECI treated group's rats, such increase in the frequency of mount and intromission suggests that libido, sexual vigor and sexual performance were unimpaired<sup>[12]</sup>. The prolonged ejaculatory latency indicates enhancement of sexual function and suggests an aphrodisiac action. It has been documented previously that sexual behavior and erection are dependent on an androgen that may be acting both centrally and peripherally<sup>[13]</sup>. Testosterone supplementation has previously been shown to improve sexual function and libido<sup>[14]</sup>, in addition to the intensity of orgasm and ejaculations which might also be expected to improve<sup>[14]</sup>. The continued administration of the Peci for 30days at various doses which led to the significant increase in serum testosterone may be responsible for the marked effect on sexual behavior indices of the male rats. Increase in testosterone levels in the present study may thus account for the observed masculine behavior.

It was clear that the administration of Peci not only increases aphrodisiac activity but also enhances the spermatogenic potential, as the action may be in the hormonal level. With Group V (400mg/kg) showing best results, it was concluded that changes were dose dependent. The result was close to the effects produced by Sildenafil Citrate (Group VI), which was used as the standard reference drug in the experiment.

Moreover, research should aim at isolating the active principle(s) responsible for aphrodisiac activity and the mechanism by which the drug enhances sexual function. From the present investigation, we conclude that the petroleum ether extract of *Crossandra infundibuliformis L.* 400mg/kg body weight possesses potent aphrodisiac activity in ethanol treated male albino rats. This result is the scientific evidence in favour of the claims made in Indian Systems of Medicine that the Peci is clinically useful as sexual invigorator in males.

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