

## Aphrodisiac property of the elephant creeper *Argyrea nervosa*

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### Summary

Based on ethno-medical clues, the aphrodisiac property of *Argyrea nervosa* was studied in male mice. The root, flower and, to some extent, leaf (homogenate in 2% gum acacia) of the plant showed aphrodisiac activity as evidenced by an increase in mounting behavior of mice. When different extracts of the root were tested, the activity was found in the alcohol extract (200 mg/kg; p.o, single dose). The extract, 1 hr after administration, stimulated mounting behavior of male mice in a concentration-dependent manner. The root- or flower-treated male mice also exhibited a remarkable increase in mating performance. Further, the number of males was found to be more among the pups fathered by the herbal drug-treated mice compared to those by the control mice. Thus, the plant has promising potential to be developed into an effective medicine for stimulating male sexual activity with an influence on sex ratio favoring males.

**Key words:** Aphrodisiac, *Argyrea nervosa*, mounting behavior, mating frequency, sex ratio

### Introduction

*Argyrea nervosa* syn. *A. speciosa* Sweet (Family: Convolvulaceae) is used in the traditional systems of medicine as well as in local health traditions. It is found throughout India up to an altitude of 300 m (Srivastava et al., 1998). In the indigenous system of medicine, the plant is used to treat chronic ulcers, gonorrhoea, strangury and gleet. The root of this plant is used for rheumatism, gonorrhoea, chronic ulcer and diseases of nervous system. It is also used as a tonic, diuretic and aphrodisiac (Chopra et al., 1956; Srivastava et al., 1998). The research records of a notable traditional Ayurvedic practitioner in Kerala, India, the grandfather of one of us (K. Ravi) revealed that use of *A. nervosa* root by men would increase the chances of getting male offspring. Therefore, it was thought of interest to study the aphrodisiac property of this plant in mice, including evaluation of sex ratio of the offspring fathered by mice treated the herbal drug.

### Material and Methods

#### *Plant materials and preparation of water suspension of plant parts*

Fresh leaves, roots and flowers of *Argyrea nervosa* were collected from the wild at Thenmala, Thiruvananthapuram district, where this plant is found in the forest area. The plant was identified with the help of

taxonomists of Tropical Botanic Garden and Research Institute (TBGRI) and a voucher specimen was preserved in the institute herbarium. Fresh plant parts were cleaned and homogenized in 2% (W/V) gum acacia to obtain water suspension.

#### *Preparation of extracts*

##### Water extract

The roots were sliced, dried under shade and powdered. The powdered material was extracted with distilled water (5g/100 ml) with constant stirring for 4 hr and then filtered through a filter paper. The residue was again extracted as above with water. The combined filtrate was freeze-dried in a lyophilizer. The yield of water extract was determined (Subramoniam et al., 1998). (Since the heat sensitivity of the extract with reference to bio-activity is not known, the extraction was carried out at low temperature without using rigorous extraction procedures).

##### Alcohol extract

The alcohol extract of the powder of the leaves was prepared similarly using ethyl alcohol instead of distilled water. However, in this case the combined extract was evaporated to dryness in a rotary evaporator under reduced pressure at 40°C as described elsewhere

(Subramoniam et al., 1998). The yield of alcohol extract was determined.

#### Hexane extract

The hexane extract of the powder was prepared as above using n-hexane instead of alcohol. However, to ensure complete extraction, 2 g powder was extracted with 100 ml hexane and the process were repeated three times. The filtrates from the extractions were combined and dried in a rotary evaporator under reduced pressure at 40°C. The yield of hexane extract was determined.

#### Animal

Inbred Swiss albino mice (6-7 week old), reared in the animal house of TBGRI were used. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton, India Laboratories, Bangalore) and water *ad libitum*, as per the guidelines of Institute Animal Ethics Committee.

#### Mounting behavior

To quantify mounting behavior, non-estrus female mice were paired with males treated with a single dose or repeated doses of the plant drug (water suspension or extract). The control mice received the vehicle in an identical manner. The animals were observed for 3 hr and their mounting behavior was scored blindly as described earlier (Subramoniam et al., 1997). Half an hour after drug administration, the males were placed individually in a clean aquarium. After 15 min acclimation, a non-estrus female mouse was introduced into the arena. The animals remained paired for 3 hr. The number of mounts was recorded by two observers, who were uninformed about the drug treatment, during a 15 min observation period at the start of each hour. All experiments were performed from 09.00 to 12.00 hr on sunny days (room temperature 27-28°C). A mount was operationally defined as the male assuming the copulatory position but failing to achieve intromission. Intromission was defined as the male's penis entering the vagina in association with thrusting behavior.

#### Assessment of mating

Male mice were divided into different groups (6 mice in each group). One group served as control. Mice in experimental groups received concentrations of the extract of *Argeria nervosa* flower suspension in 2% gum acacia, as indicated, for 6 consecutive days. The control mice received 2% gum acacia in an identical manner. On the 6<sup>th</sup> day after the herbal drug administration each male

mouse was placed in a separate cage. In the evening (18.00 hr) five estrus females were admitted into each cage. Different stages of estrus cycle were determined according to the criteria laid down by Eckstein and Zuckerman. (1960). The next morning (07.00 to 08.00 hr) the vaginal smear of each female mouse was examined in a microscope for the presence of sperm. The number of sperm- positive females was recorded in each case.

#### Effect on fertility

As in the above-said mating experiment, control and herbal drug treated males were placed with estrus females for overnight mating. However, in these experiments each male was co-habited with one female, not with five females. Six sperm-positive females in each group were selected and watched for pregnancy and birth of offspring. The litter size and the number of male and female pups were recorded in each case.

#### Statistical treatment

Pair-wise statistical comparisons between control and treated groups were done with Students' *t* test. Mean differences were considered statistically significant if  $P < 0.5$ .

#### Results

##### Effect of *A. nervosa* on mounting behavior of mice

The effect of repeated oral administration (daily for 6 days) of relatively high doses of water suspension of different fresh parts of the plant [1g (wet weight)/kg] is given in Fig. 1. The root- as well as flower-treated males, one hr after final treatment, displayed excessive mounting behavior as compared to the control. The mounting of control as well as treated animals diminished rapidly with time so that by one hr after introducing into the aquarium the number of mounts decreased to more than 50 %. The number of mounts displayed by the flower- or root-treated animals was more than three times the values for the untreated controls during the observation period. However, the leaf-treated mice showed only marginal activity compared to root- and flower-treated ones (Fig. 1).

When the effect of a single dose of different extracts of *A. nervosa* root was tested, the ethanol extract, but not water and hexane extracts, was found to be effective (Table 1). The alcohol extract, at a dose of 200 mg/kg, caused about 400 % increases in the mounting compared to control (Table 1). The extract was sensitive to heat. Keeping the extract in boiling water for 15 min resulted in

loss of the activity (Table 1). The dose response of the alcohol extract is shown in Fig. 2. The herbal drug exerted the effect in a dose-dependent manner.

**Effect of the herbal drug on mating performance of mice**

Daily administration of *A. nervosa* root or flower suspension in water containing 2% gum acacia for six days to male mice resulted in remarkable increase in the mating performance of the mice (Fig. 3). The leaf suspension produced only moderate effect compared to root or flower. Out of the six control mice, no one mated (inseminated) more than two females during the overnight experimental period while two of the root-treated males mated four females each and four mated three females each; in the flower-treated group, one male mated four females and five males mated three females each. No male mated more than four females during the experimental period.

Fig. 1. Effect of repeated doses of *Argeria nervosa* (flower, root and leaf) on mounting behavior of male mice

Fresh (wet) plant parts (1 g/kg) were used. Values are Mean  $\pm$  SD, n = 3.

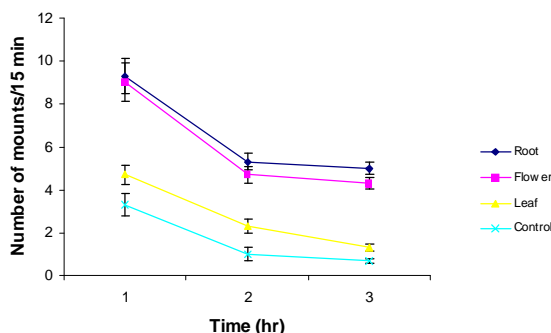


Table 1. Effect of a single dose of *Argeria nervosa* root extracts on mounting behavior of male mice

Group	Number of mounts/ 15 min		
	1 <sup>st</sup> hr	2nd hr	3rd hr
Control	3.0 $\pm$ 0.9	1.0 $\pm$ 0.4	0.5 $\pm$ 0.2
Water extract (200 mg/kg)	2.5 $\pm$ 1.1	1.0 $\pm$ 0.3	0.8 $\pm$ 0.3
Ethanol extract (200 mg/kg)	12.0 $\pm$ 3.1*	4.0 $\pm$ 1.2*	1.0 $\pm$ 0.1*
Heated ethanol extract (200 mg/kg)	2.0 $\pm$ 0.7	0.8 $\pm$ 0.2	0
Hexane extract (200 mg/kg)	3.0 $\pm$ 0.9	0.5 $\pm$ 0.4	0

Fig. 2. Effect of single administration of different doses of root (alcohol extract) on mounting behaviour of male mice.

Values are Mean  $\pm$  S.D. Values are significantly different from control values, P < 0.05.

(Mounting was observed for 15 min in the first hr)

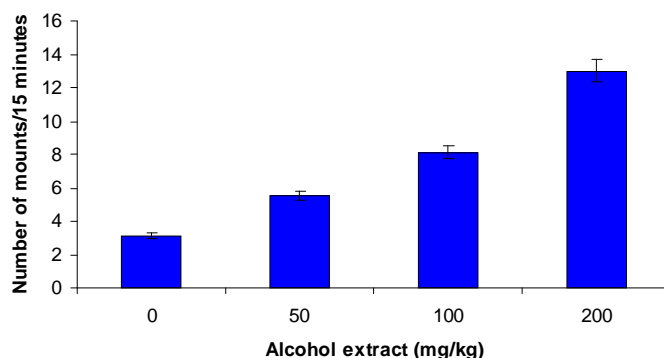
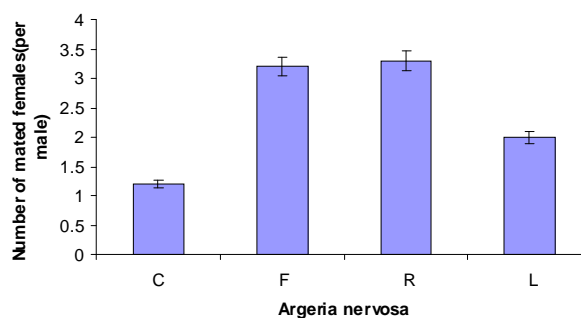


Fig.3. Effect of repeated administration of *A. nervosa* flower, root or leaf on the mating performance of male mice.

C, Control; F, Flower; R, Root; L, Leaf

1g (wet weight) plant part/kg was orally administered as a homogenate in water in each case. Values are Mean  $\pm$  S.D. n = 6. All values are significantly different from the control, P < 0.001 (compared to control).



**Effect of the herbal drug on litter size and sex ratio of pups**

The pups of dams inseminated by the root suspension-treated mice were compared with those of control mice (Table 2). The pups of control as well as experimental dams were born at full term with normal range of body weights. All the females inseminated by the herbal drug treated as well as control males became pregnant. Although there was a marginal decrease in the litter size of pups fathered by the herbal drug-treated mice, it fell short of statistical significance. Interestingly, male/female ratio of the pups was significantly increased in the herbal drug-treated group (Table 2).

Table 2. Effect of repeated administration of *Argeria nervosa* root to male mice on fertility

Groups	Litter size (Mean $\pm$ S.D)	Sex ratio [male/female] (Mean $\pm$ S.D)
Control (2% gum acacia)	12.4 $\pm$ 2.0	0.64 $\pm$ 0.4
<i>A. nervosa</i> root [1g (wet weight) /kg]	10.5 $\pm$ 2.2 (85)	0.96 $\pm$ 0.5* (150)

Repeated doses were given for six days. n = 4. \* P < 0.001. Values in parentheses represent % of respective control value

### Discussion

The present investigation reveals for the first time that the alcohol extract of *Argeria nervosa* can enhance male sexual activity in normal mice. The effect of the herbal drug on female sexual activity and fertility remains to be studied.

It is of interest to note that the herbal drug treatment altered sex ratio favoring males. This gives credibility to the ethno-medical belief that it could increase the chances to get male offspring. However, caution is required. Normally wide variation is observed in the sex ratio of mice. In each group 4 females were used in the present study. Although the observed results are statistically significant, we feel, some more experiments involving large number of animals in each group and different seasons are required to confirm this. Although aphrodisiac activity was reported in many plants, influence on sex ratio was not observed or confirmed (Subramoniam and Gayathri, 2001; Suresh et al., 2000).

*A. nervosa* root is used in traditional medicine over many generations without any known toxic symptoms. It is used in Ayurveda also (Akbarsha et al., 2003). However, the effect of overdoses and prolonged use, especially when used as extracts, fractions or purified compounds, are to be evaluated for toxicity, if any. Investigations are in progress in this laboratory to find the active principles involved in the observed sexual activities.

Sexual behaviors could be enhanced by elevated testosterone levels. Drug-induced changes in neurotransmitter levels, their actions in the cells or alterations in the signaling system such as nitric oxide signaling could also change sexual behavior. The alteration observed in the sex ratio could not be explained at this stage. Investigations are in progress in this laboratory to explore these possible mechanisms of actions.

The plant root and flower are attractive materials for developing safe and effective medicine for stimulating male sexual activity with likely influence on sex ratio. Such medicine, if successfully developed, will have socio-economic implications. Standardized phytomedicines with ensured efficacy and safety are relatively easy to develop compared to pure chemical entity drugs (Subramoniam, 2003).

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