APRRODISIAC ACTIVITY OF VANDA TESSELLATA (ROXB.) HOOK. EX DON EXTRACT IN MALE MICE

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Objective: To study the effect of V. tessellata on the sexual behaviour of male mice and general toxicity, if any, in mice.

Methods: An aqueous suspension (2 g/kg, wet wt.) or extract (water or alcohol, 200 mg/kg) of root, flower or leaf of V. tessellata was administered (p.o.) to male mice and 1 hr, after administration their mounting behaviour was observed. The most active extract (alcohol extract of flower) was administered (50 or 200 mg/kg, p.o.) to different groups of male mice and their mounting behaviour, mating performance and reproductive performance were determined. The general short term toxicity of the alcohol extract in male mice was also determined.

Results: The flower and, to some extent, the root, but not the leaf of V. tessellata was found to stimulate the mounting behaviour of male mice. This activity was found in the alcohol extract of the flower. This extract (50 or 200 mg/kg) also increased mating performance in the mice. The pups fathered by the extract treated mice were found to be normal with an increasing trend in the male/female ratio of these pups. The alcohol extract was devoid of any conspicuous general toxicity.

Conclusion: The alcohol extract of V. tessellata flower stimulates the sexual behaviour of male mice.

SUMMARY

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KEY WORDS Sexual behaviour orchid mating performance phytomedicine vanda tessellata

INTRODUCTION

Although orchids are being cultivated and valued, mainly for ornamental purposes, some of them are used from time immemorial in traditional practices to treat various medical conditions. More than 13 species of orchids of traditional medical importance are reported in Kerala forests. One among them is V. tessellata (Roxb.) Hook. ex Don. This is an epiphytic orchid which is found in many parts of India (including Western Ghats), Sri Lanka and Burma. V. tessellata plants have been used in the indigenous medicine such as Ayurveda and local traditional medical practices. The leaf juice is used for the treatment of certain inflammatory conditions. It is also instilled into the ear as a remedy for otitis. The leaves in the form of a paste is applied to the body to bring down fever. The roots are used in rheumatism, nervous problems, bronchitis, dyspepsia and fever. Unani practitioners hold it to be laxative and tonic to the liver. It is also used to treat hiccough, piles, boils on the scalp, etc. This plant root is reported to contain an alkyl perulate and β-sitosterol - D-glucoside. The dried whole herb also contains long chain alkanes and alkanol sitosterol, resin, saponin, tannins, fatty acids, colouring agents, etc.

Medicinal orchids, in general, are not subjected to detailed pharmacological studies. Scientific studies on medicinal orchids can lead to the development of invaluable drugs to certain medical conditions. V. tessellata has not been evaluated in depth for its pharmacological properties, in spite of its traditional use in numerous medical conditions. Only the anti-inflammatory property of this plant has been studied. The steroidal fraction obtained from V. tessellata possessed significant anti-inflammatory activity against acute inflammation induced by carrageenan, serotonin and formaldehyde. The methanol extract of this plant root also showed remarkable anti-inflammatory activity against carrageenan - induced oedema in rodents.
The traditional use indicates that various parts of this plant are likely to have several pharmacological properties. Lawler reported that several Ayurvedic type preparations containing this plant (root or whole plant) were used as aphrodisiac and given for impotence and barrenness. Furthermore, one of the authors (Suresh Kumar P.K.) has come across the traditional use of this plant root for impotence in males in Amboori village in Thiruvananthapuram district. In view of these, in the present study, we have evaluated the effects of various parts of this plant on the male sexual behaviour and reproductive performance in mice. The active alcohol extract of the flower was also subjected to general short term toxicity studies in mice.

MATERIALS AND METHODS

Plant materials and preparation of extract: Flowered plants of *V. tessellata* were collected from wild in the month of November and December from roadside trees in Parassuvaikkal, Thiruvananthapuram, India. A voucher specimen (No: TBGT -19826) of the epiphytic plant with flower is kept in the herbarium of TBGRI (India). The fresh plant parts (leaf, root or flower) were cleaned and ground to prepare fresh plant suspension. A 10% suspension of the plant parts was prepared in water containing 1.0 % (w/v) gum acacia and used.

The flower was air dried at room temperature and to prepare water extract, 10 g of the dried flower powder was extracted with 100 ml of distilled water by stirring magnetically for 4 hrs at room temperature. The filtrate was dried by freeze drying. The yield of water extract was about 3.1 g from 10g of dried flower.

To obtain ethanol extract, the air dried flower was powdered and extracted with ethanol (100 ml/10g) with constant stirring for 4 hrs. The extract was filtered and the filtrate was evaporated to dryness at low temperature ( > 40 °C) under reduced pressure in a rotary evaporator. Approximately 2.8g of dried ethanol extract was obtained from 10g dried flower. The extracts were suspended in 5% Tween 80 and used for studying bio-activity (control animals received 5% Tween 80).

To determine the effect of heat, the ethanol extract was dispersed in 5% Tween 80 and placed in boiling water bath for 15 min. Then the extract was brought to room temperature and used.

Animals: Adult Swiss mice (25-35g) were used. They were fed with standard rodent pellet and water ad libitum and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70%)

Mounting behaviour: To quantify mounting behaviour, non - oestrous female mice were paired with males treated with single dose of the drug. Animals were observed for 3 hrs and their behaviours were scored as described. Males were placed individually in a clear aquarium. After 15 min acclimatization, a non-oestrous female was introduced into the arena. The number of mounts were recorded during a 15 min observation period at the start of 1st hr. Then the female was separated for 105 min. Again the female was introduced and the number of mounts was observed for 15 min as before (3rd hr). All experiments were performed from 09.00 to 12.00 hrs 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.

To determine the effects of different parts of the fresh plant on mounting, 4 groups were taken and each group contained 6 animals. The plant parts were suspended in 1% gum acacia in water. The first group received 1.0% gum acacia and served as control. Groups 2,3 and 4 were given suspension of leaf, flower and root respectively (2 g/kg; fresh wet weight).

Assessment of mating: Male mice were divided into 3 groups (6 animals in each group). One group served as control. The experimental groups received 50 and 200mg/kg of alcohol extract of *V. tessellata* flowers. The drug was administered in the evening (17.00-18.00 hrs) and each male was placed in a separate cage. After 1hr, five oestrous female were admitted into each cage and they were cohabitated overnight. The stage of the oestrous cycle was determined according to the criteria laid down by Ecksterin et al. The vaginal smear of each female mouse was examined under a microscope for the
Table 1. Effect of different parts of *V. tessellata* on sexual behaviour of male mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mounts / 15 min.</th>
<th>Group Number of mounts / 15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st hr</td>
<td>3rd hr</td>
</tr>
<tr>
<td>Control</td>
<td>2.8 ± 0.3</td>
<td>2.5 ± 0.20</td>
</tr>
<tr>
<td><em>V. tessellata</em> leaf</td>
<td>3.0 ± 0.32</td>
<td>2.7 ± 0.25</td>
</tr>
<tr>
<td>flower</td>
<td>11.0 ± 0.9**</td>
<td>9.6 ± 0.78**</td>
</tr>
<tr>
<td>root</td>
<td>5.5 ± 0.6*</td>
<td>5.4 ± 0.55 *</td>
</tr>
</tbody>
</table>

The drug was administered at a dose of 2g/kg (fresh weight) in 1.0 % gum acacia. Values are mean ± SD; n = 6. *P< 0.01; **P< 0.001 vs control. Values given in parenthesis represent percentage of control values.

Table 2. Effect of *V. tessellata* flower extracts on sexual behaviour of male mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mounts / 15 min.</th>
<th>Group Number of mounts / 15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st hr</td>
<td>3rd hr</td>
</tr>
<tr>
<td>Control</td>
<td>4.0 ± 0.51</td>
<td>3.8 ± 0.32</td>
</tr>
<tr>
<td><em>V. tessellata</em> flower water extract</td>
<td>4.2 ± 0.44</td>
<td>3.7 ± 0.40</td>
</tr>
<tr>
<td>Alcohol extract 200 mg/kg</td>
<td>7.8 ± 0.71*</td>
<td>7.6 ± 0.62*</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>18.4 ± 1.6 **</td>
<td>17.5 ± 1.3 **</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>19.2 ± 1.8 **</td>
<td>16.6 ± 1.4 **</td>
</tr>
</tbody>
</table>

Values are mean ± S.D; n = 6, in each group. * P < 0.01, ** P < 0.001 vs control. Values within parenthesis represent percentage of control values.

Effect on fertility: As in the above experiment, control and the treated males (50 as well as 200 mg/kg) were placed with oestrous females for overnight mating. However, in these experiments each male was co-habitated with one female, with proven fertility, not with five females. All the 6 females in each group were found to be sperm positive. These females were watched for pregnancy and birth of offspring. The litter size and number of male and female pups were recorded in each case.

Toxicity tests: To determine acute toxicity, if any, dose of 0, 0.5, 1.0 and 2 g/kg (p.o.) respectively of the alcohol extract of the flower suspended in 5% Tween 80 were given to 4 groups each containing 6 mice. The control mice received 5% Tween 80 in an identical manner. The mice were observed continuously for 1hr for any gross behavioural changes and deaths, if any, and intermittently for the next 6 hrs and then again at 24 hrs after dosing. The behaviour parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration.

To determine sub-acute toxicity, if any, groups of 6 animals each were administered (p.o.) daily 200 or 400 mg/kg alcohol extract of the flower (in 5% Tween 80) for 14 days. Control animals received 5% Tween 80 in an identical manner. Body weight, food and water intake, general behaviour, body temperature, and state of faecal droppings were monitored during the experiment. After treatment for 14 days animals were killed, blood was collected, internal organs were removed and weighed and observed for pathological changes. Blood haemoglobin level and serum glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) were determined. GPT and GOT activities were assayed using commercial kits (Span Diagnostic Private Ltd.) by the method of Reitman and Frankel.

Statistical treatment: The statistical comparison between control and treated groups was done with Student’s *t* test. Mean differences were considered significant at P<0.05.

RESULTS

Effect of *V. tessellata* on mounting behaviour of mice: The flower (2g/kg, wet weight; p.o.) treated male mice, 1hr after the treatment as well as 3 hrs after the treatment displayed excessive mounting behaviour as compared to control. This activity was found to a lesser extent in the root, whereas the leaf was inactive (Table 1).
When water and alcohol extracts of the flower were tested, it was found that the alcohol extract was active, but water extract was inactive (Table 2). The alcohol extract at a higher dose (200 mg/kg) 1 or 3 hrs after treatment increased the number of mount by 360% (Table 2) whereas at a lower dose (50 mg/kg) the increase was almost 100%. The activity of the extract was insensitive to heat. Keeping the extract in boiling water for 15 min did not influence the activity of the extract (Table 2).

**Effect of *V. tessellata* extract on mating of mice:** Administration of single dose of *V. tessellata* flower (alcohol extract) resulted in a dose dependent increase in the mating performance of the mice. Out of 6 control animals only one mated (inseminated) 2 females and the remaining 5 males mated 1 female each during the overnight experimental period whereas 50 mg/kg extract treated animals mated 2 females each except one which mated 3 females. In the 200 mg/kg drug treated group 4 animals mated 3 females each and 2 males mated 4 females each. The mean number of females (mean ± S.D) mated by one male in control, 50 mg/kg drug treated and 200 mg/kg drug treated groups were 1.17 ± 0.41, 1.83 ± 0.41 and 3.33 ± 0.52 respectively (P<0.001 control vs 200 mg/kg drug treated group).

**Effect of the drug on litter size and sex ratio of the pups:** The pups of the dams inseminated by the drug treated male mice were compared with those of control mice. All the females inseminated by the drug treated as well as control males became pregnant. The pups of control as well as experimental dams were born at full term with normal range of body weight.

The drug treatment did not influence the litter size. The average litter size of control, 50 mg/kg extract treated and 200 mg/kg extract treated groups were 9.3 ± 0.58, 10 ± 1.8 and 9.0 ± 1.1 (mean ± S.D) respectively. The sex ratio (m/f) of pups born to these groups were 0.76 ± 0.25 (control), 1.11 ± 0.73 (50 mg/kg, extract) and 1.50 ± 0.84 (200 mg/kg). Although there was an increase in the male/female ratio of the pups fathered by the drug treated mice, it fell short of statistical significance.

**General toxicity of the drug:** Alcohol extract of *V. tessellata* flower did not cause any mortality when a single dose was orally administered up to a concentration of 2g/kg body weight. At this dose, there was no gross behavioural changes.

Daily feeding for 14 days with *V. tessellata* alcohol extract (200 or 400 mg/kg) did not result in any conspicuous change in general behaviour of the animals or any other toxic symptoms. Body temperature, state of the stool and food and water intake (data not given) were also not influenced by the treatment. The body weight and weight of organs were not significantly influenced by the drug. Blood haemoglobin content and serum GPT and GOT levels were unchanged by the treatment (data not given).

**DISCUSSION**

The present investigation reveals that the alcohol extract of *V. tessellata* flower can remarkably enhance male sexual activity in normal mice. The effect of the drug on female sexual behaviour and fertility remains to be studied.

This plant root is used rarely in ethnomedical practices for impotency. Besides the use of its flower is not known in the ethnomedical practices. In the present study, the flower was found to be better than roots. The alcoholic extract of this flower is very promising for drug development. The drug may be of use for stimulating male sexual activity in cases where there are moderate sexual deficiencies.

In the present study, the alcohol extract of this drug was found to be devoid of any general conspicuous short term toxicity. Long term toxicity studies as well as systemic toxicity, if any, remain to be studied. However, since this drug is used in ethnomedical practices without any recorded toxicity, this plant is likely to be a safe drug.

Generally sexual behaviours are enhanced by elevated testosterone levels. Drug induced changes in neurotransmitter levels or their action in the cells could also change sexual behaviour. In this connection it should be remembered that on ethnomedical practices this herb is also considered as a nervous stimulant. Active investigations are in progress in this laboratory to explore the possible mechanisms of action.
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