Anxiolytic activity of Indian *Abies pindrow* Royle leaves in rodents: An experimental study


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Putative anxiolytic activity of ethanolic extract of Indian *A. pindrow* Royle leaf was investigated in rats using various experimental paradigms of anxiety viz. open field exploratory behaviour, elevated plus maze (EPM) and elevated zero maze (EZM) tests. Pilot studies indicated that single dose administration of extract had little to no acute behavioural effects, hence the extract was administered orally at different dose levels once daily for three consecutive days, while lorazepam (LR) (0.5 mg/kg, ip) was administered acutely. Ethanolic extract of *A. pindrow* (AP) leaves (50 and 100 mg/kg, po) showed significant anxiolytic effects on all the paradigms of anxiety. The results indicate that AP and LR induced a significant increase in open field ambulation and slight increase in rearings and activity in center, whereas grooming and faecal droppings remain unchanged. In EPM, significant augmentation of open arm entries, and time spent on open arms was noted in AP treated rats. In EZM test, significant increase in time spent on open arms and entries in open arms was observed, whereas slight increase in head dips and stretched attend postures was also observed. The AP extract showed consistent and significant anxiolytic activity in all the tests. The effects induced by ethanolic extract of AP were less marked than those of lorazepam were.

Indian *Abies pindrow* syn. *Abies webbiana* (Family: Pinaceae) is commonly known as Talispatra in Hindi. It grows in the humid forest of Himalayas. Leaves of the plant have been a useful Ayurvedic remedy for fever, inflammatory conditions, bronchitis, asthma, expectorant and as carminative etc. *A. pindrow* leaves are known to posses anti-inflammatory, analgesic, barbiturate hypnosis potentiating, anti-ulcerogenic activities in rats, antistress activity in mice and hypotensive activity in dogs. No systematic investigations have been carried out to elucidate the neuropharmacological properties of *A. pindrow* though some reports indicate that *A. pindrow* has antistress-adaptogenic activity. The present study has, therefore, been undertaken to assess the effect of ethanolic extract of *A. pindrow* leaves on various behavioural models of anxiety in rats.

Materials and Methods

**Plant material**—Shade-dried leaves of *A. pindrow* were collected from Regional Research Center for Ayurveda, Jammu-Tawi, India. The specimen voucher has been preserved. Dried powdered (2.9 kg) leaf was extracted with ethanol. The extract was concentrated under steam bath to a final yield of 60 g (2.1% w/w).

**Animals**—Adult Charles Foster albino rats (150 ± 10 g), of either sex, were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, and were randomly distributed into different experimental groups. The rats were housed in groups of 6 in polypropylene cages at an ambient temp. of 25° ± 1° C and 45-55 % RH, with a 12:12 hr light/dark cycle. The animals had free access to standard pellet chow (Brooke Bond-Lipton, India) and tap water given through drinking bottles. Experiments were conducted between 0900 and 1400 hrs.

**Drug treatments**—Ethanolic extract of the *A. pindrow* (AP) leaves was dissolved in 3% Tween-80 prior to oral administration. AP extract was administered orally by using orogastric cannula in the doses of 50 and 100 mg/kg once daily for 3 consecutive days. Lorazepam (0.5 mg/kg, ip) was used as the standard anxiolytic agent and was administered to one group of rats 30 min. before experiments for comparison. Control rats were treated with the vehicle (3% Tween 80). Experiments
were conducted on day 3rd, one hour after the last drug administration.

Behavioural testing:

1. Open-field test (OFT)\textsuperscript{7} — The open-field apparatus was made of plywood and consisted of squares (61×61 cm). The entire apparatus was painted black except for 6 mm thick white lines, which divided the floor into 16 squares. Open-field was lighted by a 40W bulb focussing onto the field from a height of about 100 cm. The entire room, except the open-field, was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioural aspects were noted:

(a) Ambulation: this was measured in terms of the number of squares crossed by the animal;
(b) Rearings: number of times the animal stood on its hind limbs;
(c) Self grooming: number of times the animal groomed facial region, and licked/washed/scratched various parts of its body;
(d) Activity in centre: number of central squares crossed by the animal; and,
(e) Faecal droppings: number of faecal droppings excreted during the period.

2. Elevated plus-maze test (EPM)\textsuperscript{8} — The maze had two opposite arms, 50×10 cm, crossed with two enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10×10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly-lit room and elevated 50 cm above the floor. Naive rats were placed individually in centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and enclosed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the rat were in the arm. Observations were made by a neutral 'blind' observer.

3. Elevated zero-maze test (EZM)\textsuperscript{9} — The rats treated with AP extract showed anxiolysis in terms of significant increase in time spent in open arms, entries in open arms and number of head dips on elevated zero maze. However, the response stretched attend postures remain unchanged. LR also caused more anxiolysis in comparison to AP extract (Table 2).

Statistical analysis — The data are expressed as means ±SD for each treatment group. The data obtained from each response measures were subjected to Kruskal-Wallis one way analysis of variance (ANOVA) and inter-group comparisons were made by Mann-Whitney U test for only those responses which yielded significant treatment effects in the ANOVA test\textsuperscript{10}.

Results

Open-field exploratory behaviour — Rats treated with both the doses of AP extract showed dose dependent significant increase in open field ambulation, rearings, self-groomings and activity in centre in comparison to vehicle treated rats, evincing significant anxiolytic activity of AP. However, the open-field faecal droppings remain unchanged. Lorazepam (LR) also induced significant anxiolytic activity and the effects were found to be more than that of AP extract (Table 1).

Elevated plus maze behaviour — AP treated rats exhibited dose dependent significant increase in time spent and entries made in open arms and significant decrease in time spent/entries in enclosed arms in comparison to control rats. These results also indicate significant anxiolysis in rats by AP extract. LR caused more anxiolysis in comparison to AP extract (Table 2).

Elevated zero maze behaviour — The rats treated with AP extract showed anxiolysis in terms of significant increase in time spent in open arms, entries in open arms and number of head dips on elevated zero maze. However, the response stretched attend postures remain unchanged. LR also caused significant anxiolytic activity and the effects were comparable to that of AP extract (Table 3).
Discussion
The question of reliability and validity is of prime importance in establishing experimental paradigms of practical predictable value. These factors assume further importance when animal models of human behaviour, and its perturbations are being used. The paradigms used in the present study have been subjected to thorough critical appraisal and validated as animal models of anxiety\textsuperscript{11,14}. Thus, in the open-field and similar tests, when the animals are taken from their home cage, and placed in a novel environment, they express their anxiety and fear by decrease in ambulation, rearings, and other exploratory behaviours. Likewise, the elevated plus and zero maze tests are based on the principle that exposure of the maze leads to an approach conflict which is considerably stronger than that evoked by exposure to the enclosed part of the maze\textsuperscript{15}. All these behaviours are increased by anxiogenic agents and attenuated by anxiolytics under identical experimental conditions.

The findings of the present study indicate that ethanolic extract of AP treatment caused significant dose related anxiolysis in rats tested on all the behavioural paradigms viz. open field exploratory behaviour, elevated plus maze behaviour and elevated zero maze behaviour tests. However, the anxiolytic activity of AP was found to be less marked than that of the common benzodiazepine anxiolytic agent lorazepam.

Recently AP has been reported to exhibit antistress--adaptogenic activity\textsuperscript{5} besides having hypoglycemic, anti-inflammatory, analgesic and hypnosis potentiating activities\textsuperscript{6}. Ethanolic extract of AP leaves have also been shown to inhibit cold restraint stress induced ulcers in rats\textsuperscript{6}. Observed anxiolytic activity of AP leaf extract in the present study may be attributed to the antistress activity of AP.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambulation</th>
<th>Rearings</th>
<th>Self-groomings</th>
<th>Activity in centre</th>
<th>Faecal droppings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>42.67±3.32</td>
<td>7.33±2.42</td>
<td>6.50±2.07</td>
<td>1.00±0.63</td>
<td>4.50±1.51</td>
</tr>
<tr>
<td>AP (50 mg/kg)</td>
<td>66.00±7.40</td>
<td>8.83±2.92</td>
<td>7.67±1.96</td>
<td>2.67±1.03</td>
<td>3.00±0.89</td>
</tr>
<tr>
<td>AP (100 mg/kg)</td>
<td>76.17±6.27</td>
<td>9.66±2.34</td>
<td>9.00±1.41</td>
<td>3.83±1.33</td>
<td>1.83±1.60</td>
</tr>
<tr>
<td>LR (0.5 mg/kg)</td>
<td>10.33±4.37</td>
<td>12.83±1.47</td>
<td>10.67±4.67</td>
<td>4.67±1.82</td>
<td>1.67±0.13</td>
</tr>
</tbody>
</table>

Superscripts a,b,c indicate statistical significance respectively in comparison to vehicle, AP (50 mg/kg) and AP (100 mg/kg) treatments. a and a, denote \textit{P}<0.05 and <0.01 respectively.

Table 2—Effect of ethanolic extract of Indian \textit{A. pindrow} on elevated plus maze behaviour in rats. Abbreviations are same as in Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time spent on (sec)</th>
<th>Entries on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enclosed arms</td>
<td>Open arms</td>
</tr>
<tr>
<td>Vehicle</td>
<td>180.15±8.58</td>
<td>80.70±9.36</td>
</tr>
<tr>
<td>AP (50 mg/kg)</td>
<td>147.56±5.65</td>
<td>105.25±8.56</td>
</tr>
<tr>
<td>AP (100 mg/kg)</td>
<td>160.41±6.55</td>
<td>120.67±7.26</td>
</tr>
<tr>
<td>LR (0.5 mg/kg)</td>
<td>142.50±3.89</td>
<td>126.75±3.73</td>
</tr>
</tbody>
</table>

Superscripts a,b,c indicate statistical significance respectively in comparison to vehicle, AP (50 mg/kg) and AP (100 mg/kg) treatments. a and a, denote \textit{P}<0.05 and <0.01 respectively.

Table 3—Effect of ethanolic extract of Indian \textit{A. pindrow} on the elevated zero maze behaviour in rats. Abbreviations are same as in Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time spent on open arms (sec)</th>
<th>Head dips</th>
<th>Stretched attend postures</th>
<th>Entries in open arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>48.91±5.38</td>
<td>9.17±2.64</td>
<td>4.17±1.72</td>
<td>4.00±1.67</td>
</tr>
<tr>
<td>AP (50 mg/kg)</td>
<td>64.18±6.60</td>
<td>11.33±2.50</td>
<td>3.17±1.47</td>
<td>7.17±1.60</td>
</tr>
<tr>
<td>AP (100 mg/kg)</td>
<td>70.25±5.55</td>
<td>12.83±3.49</td>
<td>2.67±0.52</td>
<td>9.00±1.41</td>
</tr>
<tr>
<td>LR (0.5 mg/kg)</td>
<td>71.58±4.41</td>
<td>13.00±1.41</td>
<td>3.50±0.15</td>
<td>11.00±2.37</td>
</tr>
</tbody>
</table>

Superscripts a,b indicate statistical significance respectively in comparison to vehicle and AP (100 mg/kg) treatments. a and a, denote \textit{P}<0.01.
reported earlier. Ethanol extract of AP leaves are reported to contain various types of glycosides, terpenoids and flavonoids. Benzodiazepines are known to posses both antistress and anxiolytic properties. The available literature on the possible effects of AP leaf extract on neural mechanisms are scanty, as such at present it is difficult to explain the observed anxiolytic effect of AP. Therefore, further investigations are warranted to elucidate the role of possible neurotransmitters underlying anxiolytic effect of AP leaves keeping in view the presence of various chemically active substances in this extract.

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References
1 Chopra R N, Nayar S L & Chopra I C, Glossary of Indian medicinal plants (CSIR, New Delhi) 1956,201.
7 Bronstein P M, Open-field behaviour of the rat as a function of age: Cross-sectional and longitudinal investigations, J Comp Physiol Psychol, 80 (1972) 335.
12 File S E, How good is social interaction as a test of anxiety? in Selected models of anxiety, depression and psychosis, edited by P Simon, P Soubrie & D Wildlocher (Karger, Basel) 1988, 151.