EVALUATION OF THE ANTINOCICEPTIVE ACTIVITY OF AMARANTHUS HYBRIDUS LINN. ROOT EXTRACTS

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Abstract: The work evaluated the antinociceptive activity of Amaranthus hybridus Linn. root extracts using the central and peripheral antinociceptive experimental animal models. The oral administration of ethanol and aqueous root extracts (100 and 200 mg/kg) produced significant (p < 0.01) and dose dependent results compared to their respective controls. The aqueous extract (200 mg/kg) produced more inhibition of abdominal writhes in mice than the other test extracts. Both the test extracts significantly (p < 0.01) and dose dependent increase the hot plate pain threshold in mice but the aqueous extract (200 mg/kg) was exhibiting more increase in pain threshold than the other test doses of extracts. Dose dependent and significant (p < 0.01) reduction of painful sensation in mice tail immersion test due to oral administration of test doses was also observed. Oral acute toxicity study indicated the non toxic nature of root extracts. The present investigation revealed that ethanol and aqueous root extracts of A. hybridus Linn. possess significant and dose dependent central and peripheral antinociceptive activity justifying its traditional use in treating conditions associated with painful conditions.

Keywords: Amaranthus hybridus Linn., antinociceptive, hot plate test, tail-immersion test, acetic acid induced abdominal writhes

Amaranthus hybridus Linn. (Amaranthaceae) commonly known as Smooth pigweed is an erect branched annual herb distributed throughout tropical and temperate regions of India as a common weed in the fields and wastelands (1, 2). The plant has been used in treating dysentery, ulcers and hemorrhage of the bowel due to its astringent property (3). Leaves possess antibacterial effect (4), cleansing effect and also help to reduce tissue swelling (5). The basis for the traditional use of this herb in the management of painful related conditions is yet to be scientifically verified. The present investigation was envisaged to ascertain antinociceptive activity of ethanol and aqueous extracts from the roots of A. hybridus Linn. and to evaluate its possible mechanism of action utilizing various pain models such as acetic acid-induced writhing, hot-plate and tail-immersion tests.

MATERIALS AND METHODS

Plant material

The roots of A. hybridus were collected from the campus of Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India in July 2008. Identification and botanical authentication of plant was carried out at National Institute of Science Communication and Information Resources, New Delhi by Dr. H. B. Singh, where a voucher specimen was lodged (reference no.: NIS-CAIR/RHMD/CONSULT/2008-09/1044/75).

Preparation of root extracts

The roots of A. hybridus were shade dried for 15 days and grounded into powder by hammer mill. The selected plant part (300 g) was extracted with ethanol and distilled water successively using Soxhlet apparatus for 16 h. The respective solvents were evaporated to dryness in a rotary evaporator. The yield of the ethanol and aqueous extract was 3.8 and 3.1 g/100 g of crude drug, respectively. The dried weight of each extract was used to determine the concentration in mg/mL and was stored in air-tight containers at 4°C till required. The fresh test samples of dried extracts were prepared with 1% (w/v) carboxymethylcellulose (CMC) before each pharmacological study.

Phytochemical screening

The preliminary phytochemical screening of the ethanol and aqueous crude extracts of A.
hybridus Linn. roots was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols. High performance thin layer chromatography (HPTLC) fingerprinting of both extracts was also performed on Reprostar with a digital camera (CAMAG, Switzerland) (6, 7).

Experimental animals

The study was conducted on mature albino mice, five to six weeks of age, weighing 30 ± 5 g, which were housed in colony cages (six mice per cage) at an ambient temperature of 25 ± 2°C with 12 h light and 12 h dark cycle. The mice were fed normal diets purchased commercially from vendors and had free access to water. The animals were allowed to acclimatize to the laboratory environment for 14 days. Animals described as fasting had been deprived of food for at least 18 h but were allowed free access to drinking water. The Institutional animal ethical committee (Guru Jambheshwar University of Science and Technology, Haryana, India) approved the experimental protocol and care of laboratory animals were taken as per the guidelines of CPCSEA, Ministry of Forest and Environment, Government of India (Registration no. 0436).

Acute toxicity study

Healthy adult mice of either sex were treated with graded dose of A. hybridus Linn. extracts (50, 100, 200, 400 mg/kg/day). The mice were observed continuously for 8 h daily for behavioral, neurological and autonomic profile for 30 days to study any possible toxic effects (8, 9).

Acetic acid-induced writhing response

Swiss albino mice were divided into six groups. The study was carried out according to the method previously described (10). Swiss albino mice were injected intraperitoneally with 0.6% acetic acid at a dose of 10 mL/kg. Selected doses (100 mg/kg and 200 mg/kg) of ethanol and aqueous extracts of root and 1% CMC as control vehicle were administered orally to animals 1 h prior to the acetic acid injection. Indomethacin (10 mg/kg) was administered intraperitoneally (i.p.) to mice. The number of writhes was counted for 20 min after a latency period of 5 min. The response consisted of abdominal wall contractions, pelvic rotation, followed by hind limb stretches. The percentage analgesic activity was calculated as percentage analgesic activity

\[
\frac{N_c - N_t}{N_c} \times 100\% 
\]

where \(N_c\) is the average number of stretches of the control group and \(N_t\) is the average number of stretches of the test drug group (11–14).

Hot plate method

The hot plate test was used to measure the response latencies. This test was performed according to the method previously described (15). In this experiment the hot plate was maintained at 55 ± 1°C to observe the pain responses of mice. The response consists of paw licking and jumping of Swiss albino mice. Before determination, each mouse was first habituated to the hot-plate test twice. The latent time before the occurrence of the pain response was recorded as an analgesic parameter. The untreated mice exhibiting latency time greater than 30 s or less than 5 s were excluded. The latency time was determined before and 30 min, 1, 2, 3 and 4 h after administration of ethanol and aqueous root extracts (100 mg/kg and 200 mg/kg), 1% CMC as control vehicle by oral route and pentazocine (10 mg/kg, i.p.). If the reaction time was more than 30 s, the latency was recorded as 30 s.

Table 1. Effect of the A. hybridus Linn. root ethanol and aqueous extracts on the writhing method in mice.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg)</th>
<th>No. of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>42.66 ± 4.12</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>6.50 ± 2.11**</td>
<td>84.76</td>
</tr>
<tr>
<td>Aqueous root extract</td>
<td>100</td>
<td>18.16 ± 2.75**</td>
<td>57.43</td>
</tr>
<tr>
<td>Aqueous root extract</td>
<td>200</td>
<td>10.50 ± 2.69**</td>
<td>75.39</td>
</tr>
<tr>
<td>Ethanol root extract</td>
<td>100</td>
<td>27.50 ± 4.64*</td>
<td>35.54</td>
</tr>
<tr>
<td>Ethanol root extract</td>
<td>200</td>
<td>21.50 ± 4.71**</td>
<td>49.60</td>
</tr>
</tbody>
</table>

Values expressed as the mean ± SEM. **p < 0.01 and *p < 0.05 vs. control group, Student’s t-test (n = 6 per group). Inhibition of pain is expressed as a percentage.
Table 2. Effect of *A. hybridus* Linn. root ethanolic and aqueous extracts on the hot plate test in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>5.75 ± 0.21</td>
<td>5.38 ± 0.39</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10</td>
<td>6.67 ± 0.36</td>
</tr>
<tr>
<td>Aqueous root extract</td>
<td>100</td>
<td>6.10 ± 0.31</td>
</tr>
<tr>
<td>Aqueous root extract</td>
<td>200</td>
<td>6.25 ± 0.28</td>
</tr>
<tr>
<td>Ethanol root extract</td>
<td>100</td>
<td>6.25 ± 0.28</td>
</tr>
<tr>
<td>Ethanol root extract</td>
<td>200</td>
<td>5.80 ± 0.22</td>
</tr>
</tbody>
</table>

Values expressed as the mean ± SEM, **p < 0.01 and *p < 0.05 vs. control group, Student’s *t*-test (n = 6 per group).

Table 3. Effect of the *A. hybridus* Linn. root ethanol and aqueous extracts on the tail immersion test in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>3.50 ± 0.11</td>
<td>3.52 ± 0.12</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10</td>
<td>3.46 ± 0.18</td>
</tr>
<tr>
<td>Aqueous root extract</td>
<td>100</td>
<td>3.40 ± 0.13</td>
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<tr>
<td>Aqueous root extract</td>
<td>200</td>
<td>3.81 ± 0.16</td>
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<tr>
<td>Ethanol root extract</td>
<td>100</td>
<td>3.17 ± 0.10</td>
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<tr>
<td>Ethanol root extract</td>
<td>200</td>
<td>3.21 ± 0.06</td>
</tr>
</tbody>
</table>

Values expressed as the mean ± SEM, **p < 0.01 and *p < 0.05 vs. control group, Student’s *t*-test (n = 6 per group).
Tail immersion method

Swiss albino mice were administered orally with control vehicle i.e., 1% CMC (10 mL/kg) and selected doses (100 mg/kg and 200 mg/kg) of ethanol and aqueous extracts of *A. hybridus* Linn. roots while pentazocine (10 mg/kg) as standard was given *i.p.* Mice were inserted in a conoid paper receptacle with its tail protruding. The protruding tail of animals was immersed in hot water maintained at 55.0 ± 1.0°C to prevent tissue damage. The time taken to withdraw the tail was noted as reaction time, which was recorded by stopwatch. A cut off time of 10 s was maintained at 55.0 ± 1.0°C to prevent tissue damage. The reaction time was measured before and 30 min, 1, 2, 4 and 6 h after the treatment, respectively (11, 13, 16).

**Statistical analysis**

All the values of biochemical estimation were expressed as the mean ± standard error of the mean (SEM) and analyzed with one-way analysis of variance (ANOVA) followed by post hoc Dunnett’s *t*-test. Differences between groups were considered significant at p < 0.05 levels.

**RESULTS**

The results of acetic acid-induced writhing test shown in Table 1 demonstrate that the ethanol and aqueous roots extracts of *Amaranthus hybridus* Linn. restrained the writhing reflex induced by 0.6 % acetic acid at a dose of 10 mL/kg. The significant and dose dependent protective effects were observed as 35.54% (p < 0.05) and 49.60% (p < 0.01) for ethanol extract 100 and 200 mg/kg *p.o.*, respectively. The aqueous extract showed 57.43% (p < 0.01), 75.39 % (p < 0.01) inhibitions with 100 and 200 mg/kg *p.o.* doses, respectively. Indomethacin (10 mg/kg) significantly (p < 0.01) inhibited the writhing response with an inhibition percentage of 84.76.

Table 2 revealed that the oral administration of ethanol and aqueous extracts (100, 200 mg/kg) significantly and dose dependently increases the latency time in mice compared to 1% CMC (10 mg/kg) treated mice. Pentazocine (10 mg/kg, *i.p.*) significantly produced maximum latency time of 18.72 s (p < 0.01) after 2 h of administration compared to CMC treated mice. The antinociceptive activity of the aqueous root extract of *A. hybridus* Linn. at the dose of 200 mg/kg was 15.15 s.

Oral administration of ethanol and aqueous root extract significantly reduced the painful sensation due to tail immersion of Swiss albino mice in warm water after a latency period of 2 h (Table 3). The ethanol root extract 200 mg/kg produced significant analgesia (p < 0.05) but aqueous root extract produced higher effect (p < 0.01) with lower dose 100 mg/kg. Aqueous root extract (200 mg/kg) was found to be more comparable to the standard drug pentazocine.

The preliminary phytochemical screening of freshly prepared root extracts revealed the presence of tannins, carbohydrates, saponins, flavonoids and alkaloids. The presence of flavonoids like rutin and quercetin was confirmed by HPTLC fingerprinting (results not shown). The treatment of normal mice with graded dose of *A. hybridus* Linn. root extracts (up to a dosage of 400 mg/kg body weight/day) for 30 days revealed the non-toxic nature of root extracts. There was no change in general behavior or appearance such as restlessness, laxative, coma, respiratory distress, hair loss, loss in body weight etc. Toxicity study showed no mortality till the end of the experiment.

**DISCUSSION**

The antinociceptive effect of ethanol and aqueous root extracts of *Amaranthus hybridus* Linn was investigated in this study. The activity was demonstrated on basis of chemical nociception in the test models of acetic acid-induced writhing and on thermal nociception in hot plate test and tail immersion test. The acetic acid-induced abdominal constriction is believed to show the involvement of peripheral mechanisms, whereas the hot plate and tail immersion are believed to show that of central mechanisms (17). The study indicated that both tested extracts possessed peripheral and central antinociceptive effect.

The results showed that extract of *Amaranthus hybridus* Linn. produced consistent and dose-dependent inhibition of acetic acid-induced visceral nociceptive response in mice. In writhing response, acetic acid causes pain by inducing release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGF2 alpha in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs (14, 17). Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of test extract, probably like indomethacin, is linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor.

The hot plate and tail immersion methods elucidated the centrally acting antinociceptive effects of the ethanol and aqueous extracts (18). The hot plate is a specific central antinociceptive test in...
which opioid agents exert their analgesic effects via supra spinal and spinal receptors (19). The tail immersion test indicated that the pharmacological actions were mediated by µ opioid receptors rather than κ and δ receptors (20). The effect of the extracts on these pain models indicate that it might be centrally acting.

Therefore, it is concluded that A. hybridus Linn. ethanol and aqueous extracts possess significant peripheral and central antinociceptive effects in laboratory animals. The results provided experimental evidence for traditional use of this plant in some painful conditions and also suggest the presence of biologically active principles, which may be worth of further investigation and elucidation.

REFERENCES


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