ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES OF Heliotropium indicum Linn. IN EXPERIMENTAL ANIMAL MODELS.

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Summary

Chloroform extract of Heliotropium indicum was investigated for anti-inflammatory and anti-nociceptive activities in experimental animal models. Anti-inflammatory activity was evaluated with carrageenan induced paw edema model in albino wistar rats of both sexes, and compared to a positive control drug, Diclofenac sodium. The extract was given (ip) in a concentration of 50, 100 & 150 mg/kg b.w. before carrageenan injection. The extract of H. indicum with a concentration of 150 mg/kg b.w. showed maximum (80.0%) inhibition on carrageenan induced rat paw edema. Anti-nociceptive activity was evaluated with hot plate model in male Swiss albino mice, and compared to a control drug, Pentazocine. The extract was given (ip) in a concentration of 50, 100 & 150 mg/kg b.w., 30 minutes before estimating the discomfort reaction (paw licking and jumping) which was recorded as response latency. The extract of H. indicum with a concentration of 150 mg/kg b.w. showed maximum (82.79%) anti-nociception in the hot-plate test.

Keywords: Heliotropium indicum, anti-nociceptive, anti-inflammatory, hot plate, carrageenan.

Short title: Anti-inflammatory and anti-nociceptive activities of Heliotropium indicum Linn.
Introduction

Herbal products derived from plant extracts are more progressively being used to treat a broad range of clinical diseases. Despite the minimal understanding about their mode of action, there is an increasing interest in the pharmacological evaluation of various plants used in the long-established Indian traditional systems of medicine. Thus, we carried out the present investigation to evaluate the anti-nociceptive and anti-inflammatory action of Heliotropium indicum in mice and rats respectively.

H. indicum, commonly known as Indian Turnsole, is a herb with pale violet flowers belonging to the family Boraginaceae \[^{[1,2]}\]. It is distributed in the tropical and temperate regions of the world, and found throughout India. The whole plant is claimed to possess medicinal properties. The leaves are used for the treatment of ophthalmic disorders, erysipelas and pharyngodynia \[^{[1,3]}\]. The roots are used as astringent, expectorant and febrifuge. The aqueous extract of leaves was proved to be active against Schwart's leukaemia \[^{[4]}\]. On the basis of these common uses of this plant in conventional folk medicine and its above reported activities in the literature, we have evaluated the anti-nociceptive and anti-inflammatory action of the chloroform extract of H. indicum in mice and rats respectively.

Materials and Methods

Collection of plant materials

Fresh leaves of H. indicum were collected from Nagercoil in Kanyakumari district of Tamilnadu state, India. It was identified by the Department of Botany, The American College (Madurai, India) and a voucher specimen (KM 02/2005) is maintained in the laboratories of the Division of Pharmacology, KM College of Pharmacy (Madurai, India).

Chloroform extract

The leaves were collected, washed thoroughly and dried in shade. The dried leaves of *H. indicum* were reduced to a fine powder with a mechanical grinder. About 700 g of dry powder was extracted with chloroform using soxhlet apparatus. Freshly prepared *H. indicum* extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol [5]. The extract was concentrated to dryness using a rotary evaporator attached to a vacuum pump and stored at a temperature of -4°C until use. The yield was 7 g, in a dark brownish green residue form.

Animals

Albino rats of Wistar strain (150 - 200 g) of both sexes and male Swiss albino mice (20 -25 g), were used to study the anti-inflammatory and anti-nociceptive activities, respectively. The animals were maintained under controlled room temperature (27°C ± 2) and relative humidity (60 - 70%) in a 12 h light-dark cycle, with free access to food and water ad libitum. The experimental procedures were carried out in strict compliance with the guidelines of the Institutional Animal Ethics Committee. Food was withdrawn 12 hrs before and during the experimental hours.

Anti-inflammatory Study

The animals were divided into 5 groups, viz., Group 1 [Control (5ml of normal Saline, 0.9%)], Group 2 [Diclofenac sodium (30 mg/kg)], Group 3 [*H. indicum* (50 mg/kg)], Group 4 [*H. indicum* (100 mg/kg)] and Group 5 [*H. indicum* (150 mg/kg)]. Antiinflammatory activity was measured using carrageenan induced rat paw oedema assay [6,7]. Groups of 6 rats were given a dose of the extract (plant extract was dissolved in sterile distilled water and administered intra peritoneally as mentioned above). The anti-inflammatory activity was expressed as percent inhibition of paw oedema and compared to control group. Diclofenac sodium (Courtesy: The Madras Pharmaceuticals,

Chennai.) 30mg/kg body weight was used as the standard drug.

Hot Plate Method

The animals were divided into 5 groups: Group 1 [Control (0.5ml of normal Saline, 0.9%)], Group 2 [Pentazocine (0.156 mg/kg)], Group 3 [H. indicum (50 mg/kg)], Group 4 [H. indicum (100 mg/kg)] and Group 5 [H. indicum (150 mg/kg)]. Pentazocine (Courtesy: Ranbaxy Laboratories.) at an intraperitoneal dose of 0.156 mg/kg, was used as the standard drug. The test was performed using hot plate to measure the response latency [8]. The hot plate was maintained at 55°C and the animals were placed into the Perspex cylinder on the heated surface and the time (in sec) to discomfort reaction (paw licking and jumping) was recorded as response latency. Normally animals showed response in 4 - 6 seconds. A cut off period of 15 sec was observed to avoid damage to the paws.

Statistical Analysis

Statistical analysis of the experiment results was conducted by ANOVA, including post hoc Dunnett tests. All data are expressed as the mean ± standard error of the mean (SEM). p < 0.001 was considered significant.

Results

Effect on carrageenan-induced paw edema

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti-inflammatory activity of the chloroform extract of H. indicum was evaluated by carrageenan-induced rat paw oedema method [6,7] and the result is shown in Table 1. The extract was tested at 3 different dose levels.
**Table I**: Effect of *Heliotropium indicum* Linn. on carrageenan induced rat paw edema.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg, ip)</th>
<th>Increase in paw volume (mean ± SEM) in ml</th>
<th>% Edema inhibition relative to control at 3rd hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (5ml of normal Saline, 0.9%)</td>
<td>0.503 ± 0.033</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac sodium (30 mg/kg)</td>
<td>0.050 ± 0.027†</td>
<td>63.16</td>
</tr>
<tr>
<td>3</td>
<td><em>H. indicum</em> (50 mg/kg)</td>
<td>0.285 ± 0.001†</td>
<td>65.40</td>
</tr>
<tr>
<td>4</td>
<td><em>H. indicum</em> (100 mg/kg)</td>
<td>0.233 ± 0.052†</td>
<td>72.70</td>
</tr>
<tr>
<td>5</td>
<td><em>H. indicum</em> (150 mg/kg)</td>
<td>0.133 ± 0.014†</td>
<td>80.00</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.
n = 6 in each group.
† *p* < 0.001, significantly different from control.

The results showed that the chloroform extract with a dose of 150 mg/kg b.w showed 80% of inhibition on carrageenan induced rat paw edema at third hour. This result indicated that chloroform extract with a dose of 150 mg/kg b.w showed maximum anti-inflammatory activity as compared to the reference drug Diclofenac sodium, which showed only 63.16% inhibition. Chloroform extract with a dose 50 mg/kg b.wt produced 65.4% of inhibition and is also high as compared to the reference drug. The extract with a dose of 100 mg/kg b.wt produced 72.7% of inhibition and is higher as compared to the reference drug. The development of edema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances.[9]
Significantly high (80.0%) anti-inflammatory activity of chloroform extract (150 mg/kg b.wt) of *H. indicum* may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The present result indicates the efficacy of chloroform extract (150 mg/kg b.wt) of *H. indicum* as an efficient therapeutic agent in acute anti-inflammatory conditions.

**Effect on hot plate test**

The hot plate method is one of the most common tests for evaluating the anti-nociceptive effect of drugs/compounds in rodents [10]. Administration of *H. indicum* extract showed significant anti-nociceptive activity in the hot-plate test.

**Table II:** Effect of *Heliotropium indicum* Linn. on hot-plate reaction time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Reaction time at 30 min after drug treatment (sec)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (0.5ml of normal Saline, 0.9%)</td>
<td>2.96 ± 0.782</td>
<td>47.83</td>
</tr>
<tr>
<td>2</td>
<td>Pentazocine (0.156 mg/kg)</td>
<td>6.26 ± 0.896‡</td>
<td>100.00</td>
</tr>
<tr>
<td>3</td>
<td><em>H. indicum</em> (50 mg/kg)</td>
<td>4.82 ± 0.725‡</td>
<td>77.87</td>
</tr>
<tr>
<td>4</td>
<td><em>H. indicum</em> (100 mg/kg)</td>
<td>5.02 ± 0.842‡</td>
<td>81.09</td>
</tr>
<tr>
<td>5</td>
<td><em>H. indicum</em> (150 mg/kg)</td>
<td>5.13 ± 0.714‡</td>
<td>82.79</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n = 6 in each group. ‡ *p* < 0.001, significantly different from control.
The antinociception by pentazocine, a mixed agonist-antagonist, and the hot plate test revealed these results positive and the mechanism of *H. indicum* antinociception might involve opioid receptors.

**Discussion**

Bioactive Pyrrolizidine alkaloids such as acetyl indicine, Indicine-N-oxide and esters of retronecine from extract of *H. indicum* were reported [11]. Many pharmacological activities of Pyrrolizidine alkaloids were evaluated in earlier studies [12]. On preliminary phytochemical screening the chloroform extract of *H. indicum* was found to contain alkaloid compounds. These phytocompounds may be present in the crude extract of *H. indicum* that may account for the anti-nociceptive and anti-inflammatory activities. The results from the present study strongly indicate that the chloroform extract of *H. indicum* possesses anti-nociceptive and anti-inflammatory activities. Further studies may reveal the exact mechanism of action responsible for the anti-nociceptive and anti-inflammatory activities of *H. indicum*.

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**References**


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