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

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Summary

Choloroform 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^{o}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 intraperitonial 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.

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

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 odema 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 chlroform 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.

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

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*.

Acknowledgements

This work was supported by a grant from the Suresh Kare Indaco Foundation, Mumbai, India. We are grateful to the staff & management of Glorigin Research, Bangalore, India, for the technical guidance and for assistance in collection of the investigational plant material. We are also thankful to Prof. M Nagarajan, KM College of Phrmacy, Madurai, for providing the facilities for the research work.

References

1. Chadha YK. The wealth of India. A dictionary of Indian raw materials industrial products. New Delhi. Council of Scientific And Industrial Research. 1991: 5, 28.

2. Warrier PK, Indian medicinal plants. Madras. Orient Longman Ltd. 1995: 3,136.
3. Reddy JS, Rao PR, Reddy MS. Wound healing effects of Heliotropium indicum, Plumbago zeylanicum and Acalypha indica in rats. J Ethnopharmacol. 2002; 79(2, suppl): 249-251.
4. Karnick CR. Pharmacopoeial standards of herbal plants, 1st ed. Delhi. Sri Satguru Publications. 1994: 179.
5. Kokate CK. Practical Pharmacognosy, 4th ed. Delhi. Vallabh Prakashan, 1994: 107-111.
6. Adeyemi OO, Okpo SO, Ogunti OO. Analgesic and anti-inflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia. 2002; 73: 375-380.
7. Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drug. Proc Soc Exp Biol Med. 1962; 111: 544-547.
8. Eddy NB, Lembach D. Synthetic analgesic II Dithienylamines. J Pharmacol Exp Ther. 1953; 707: 385-393.
9. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan in rats. J. Pharmacol. Exp. Ther. 1969; 166: 96-103.
10. Carter RB. Differentiating analgesic and non-analgesic drug activities on a rat hot plate: Effect of behavioral end-point. Pain. 1991; 47: 211-220.
11. Mattock AC. Minor alkaloids of Heliotropium indicum Linn. J Chem Soc. 1961; 329-331.
12. Jose R. Trigo. The chemistry of antipredesor defence by secondary compounds in Neotropical Lepideptera: facts, perspectives and caveats. J Braz Chem Soc. 2000; 11(6, suppl): 551-561.

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