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Research Article

Anti-Inflammatory and Antipyretic Activities of *Hygrophila spinosa* T. Anders Leaves *(Acanthaceae)*

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Abstract

Purpose: *Hygrophila spinosa T. Anders (Acanthaceae) is commonly used in the traditional system of medicine for the treatment of inflammation, pain, jaundice, rheumatism, arthritis, anaemia, etc. In the present study, we investigated the anti-inflammatory and antipyretic activities of the petroleum ether, chloroform, alcoholic and aqueous extracts of the leaf of this plant.*

Methods: *The anti-inflammatory activity of the various extracts was studied based on their effects on carrageenan-induced paw oedema in rats while antipyretic activity was evaluated on the basis of their effect on Brewer's yeast-induced pyrexia in rats. The extracts were screened for alkaloids, steroids, proteins, flavonoids, saponins, mucilage, carbohydrates, organic acids, fats and oils.*

Results: *Preliminary phytochemical screening revealed the presence of alkaloids, steroids, proteins, flavonoids, fats and oils, tannins, mucilage and organic acids in the leaves of H. spinosa. Chloroform and alcoholic extracts of leaves of H. spinosa produced significant ($p < 0.05$ and $p < 0.01$) anti-inflammatory and antipyretic activities in a dose-dependent manner. On the other hand, petroleum ether and aqueous extracts did not show significant anti-inflammatory and antipyretic activities. The maximum anti-inflammatory activities produced by chloroform and alcoholic extracts (400 mg/kg) were 33.7% and 47.5%, respectively. These two extracts also reduced elevated body temperature in rats at 200 and 400 mg/kg body weight doses throughout the observation period of 6 h.*

Conclusion: *Chloroform and alcoholic extracts of H. spinosa leaves have anti-inflammatory and antipyretic activities.*

Keywords: *Hygrophila spinosa, anti-inflammatory activity, antipyretic activity, leaf extracts*

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Introduction

Hygrophila spinosa T. Anders (Acanthaceae) is commonly found in water-logged areas throughout India[1]. The plant is used as a diuretic and for the treatment of rheumatism, jaundice, inflammation, pain, hepatic obstruction, gout, bacterial infection etc[2-6]. The aerial parts of the plant are reported to contain lupeol, stigmasterol and butelin while the seeds mainly contain fatty acids[7]. Its root contains an alkaloid named hygrosterol[8] while its flower contains apigenin 7-o-glucuronide[9]. However, no data were found regarding the pharmacological and phytochemical evaluation of the leaves of the plant. The aim of the present study is to investigate the anti-inflammatory and antipyretic properties of the petroleum ether, chloroform, alcoholic and aqueous extracts of the leaves of *H. spinosa*.

Material and Methods

Drugs and reagents

Tween 80 (Lobachem, India), Indomethacin (MicroLab, India), carrageenan (Sigma), Brewer's yeast (Tetragon Chemie), and paracetamol (GlaxoSmithKline) were used in the study.

Plant material

The leaves of *H. spinosa* were collected from Berhampur, Orissa, India. The plant was identified by Dr. N. K. Dhal, Scientist, Regional Research Laboratory, Bhubaneswar, India and a voucher specimen (no. 9999) was preserved for further references.

Preparation of extracts

The leaves were washed thoroughly, dried under a shade and pulverized. The coarse powder was extracted successively with petroleum ether, chloroform and alcohol using a soxhlet apparatus. Finally, the aqueous extract was prepared by decoction. The extracts were dried using a rotary vacuum evaporator and stored in a desiccator until further use.

Animals

Wistar rats of both sexes, weighing 150 – 200 g were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Royal College of Pharmacy and Health Sciences Berhampur, Orissa, India.

Anti-inflammatory activity

The anti-inflammatory activity of the extracts was determined according to the method of Borgi *et al*[10] and Vogel *et al*[11]. The rats were divided into ten groups of six rats each. The control group received 1% (v/v) Tween 80 in water, p.o. at a dose of 10 ml/kg. The positive control group was treated orally with the standard drug, indomethacin (10mg/kg). Different extracts were administered to the other groups in doses of 200 and 400 mg/kg as shown in Table 1. All the suspensions were administered 30 min before the induction of oedema by administering 0.1 ml of 1% w/v carrageenan in saline[12,13]. The degree of paw oedema of all the groups was measured using a plethysmometer at 30, 60, 120, 180 and 240 min after the administration of carrageenan to each group.

Antipyretic activity

Animals were selected for the experiment after confirmation of approximate constant rectal temperature for 7 days. The antipyretic activity of the extracts was evaluated based on Brewer's yeast-induced pyrexia in rats[14,15]. Pyrexia was induced by subcutaneous injection of 10 ml/kg of 15% w/v Brewer's yeast suspension below the nape of the neck. The rectal temperature of each rat was measured at time, 0 h, using a telethermometer and before injection of the yeast. At 18 h following yeast injection, the different groups were treated with the vehicle, extracts (200 and 400 mg/kg) and standard

drug, paracetamol (150 mg/kg). The rectal temperature was then recorded over a period of 6 h.

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis of the data were carried out using Student's t-test and results were considered significant when $p < 0.05$.

Results

Anti-inflammatory activity

The chloroform and alcoholic extracts of *H. spinosa* produced significant ($p < 0.05$) anti-inflammatory activity, while petroleum ether and aqueous extracts did not. Significant reduction of paw oedema was observed 30 min and 3 h after carrageenan injection, for alcoholic and chloroform extracts, respectively. The reduction in carrageenan-induced paw oedema by 400 mg/kg of chloroform and alcoholic extracts after 4 h was 43.7 and 47.5%, respectively, while oedema reduction by the standard drug, indomethacin (10 mg/kg) was 53.7% (see Table 1).

Antipyretic activity

Chloroform and alcoholic extracts produced significant antipyretic activity ($p < 0.05$), but petroleum ether and aqueous extracts did not. Chloroform extract significantly decreased the elevated rectal temperature 3 h after the administration of a dose of 400 mg/kg only, while the alcoholic extract reduced the hyperthermia at both 200 and 400 mg/kg doses 1 h after administration. The initial and final rectal temperatures in the groups treated with chloroform extract (400 mg/kg), alcoholic extract (400 mg/kg) and paracetamol (150 mg/kg) were 38.03 ± 0.16 and 37.41 ± 0.26, 38.55 ± 0.14 and 37.81 ± 0.19, and 38.70 ± 0.15 and 37.87 ± 0.18 ^{0}C, respectively. Paracetamol and alcoholic extract showed significant antipyretic activity throughout the test period of 6 h (see Table 2).

Discussion

Carrageenan-induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic[16]. The first phase (1-2 hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin[17,18]. The results of this study indicate that the chloroform and alcoholic extracts of *H. spinosa* significantly reduced carrageenan-induced paw oedema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis.

Usually most anti-inflammatory and analgesic drugs possess antipyretic activity. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus[19]. Therefore, the antipyretic activity of chloroform and alcoholic extracts of *H. spinosa* is probably by inhibition of prostaglandin synthesis in hypothalamus.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids in the chloroform extract, as well as alkaloids, flavonoids, tannins and steroids in the alcoholic extract of the leaves of *H. spinosa*[20]. The anti-inflammatory and antipyretic potentials of alkaloids, steroids and flavonoids have been reported in various studies[21-23]. Therefore, the anti-inflammatory and antipyretic activities of the chloroform and aqueous extracts may be due to the presence of alkaloids, sterols and flavonoids.

Conclusion

The results of the present study indicate the anti-inflammatory and antipyretic activities of

Table 1: Effect of extracts of *H. spinosa* leaf on paw oedema induced by carrageenan in rats

Treatment	Dose (mg/kg)	Increase in paw volume (in ml) after various times				
		30 min	1 h	2 h	3 h	4 h
Control	-	0.26 ± 0.02	0.40 ± 0.04	0.73 ± 0.04	0.88 ± 0.05	0.80 ± 0.05
Indomethacin	10	0.18 ± 0.04*	0.23 ± 0.02**	0.35 ± 0.02**	0.43 ± 0.05**	0.37 ± 0.03**
HSPE	200	0.28 ± 0.06	0.45 ± 0.07	0.68 ± 0.07	0.86 ± 0.07	0.71 ± 0.08
	400	0.25 ± 0.05	0.42 ± 0.05	0.66 ± 0.07	0.78 ± 0.05	0.75 ± 0.04
HSCH	200	0.23 ± 0.04	0.32 ± 0.04	0.55 ± 0.06	0.63 ± 0.04*	0.55 ± 0.05*
	400	0.22 ± 0.01	0.28 ± 0.05	0.48 ± 0.09	0.52 ± 0.04**	0.45 ± 0.07*
HSAL	200	0.22 ± 0.01	0.30 ± 0.04	0.52 ± 0.06*	0.62 ± 0.06**	0.53 ± 0.05*
	400	0.18 ± 0.01**	0.25 ± 0.02*	0.42 ± 0.05*	0.48 ± 0.05**	0.42 ± 0.06**
HSW	200	0.27 ± 0.04	0.38 ± 0.07	0.68 ± 0.08	0.87 ± 0.08	0.83 ± 0.09
	400	0.28 ± 0.03	0.42 ± 0.04	0.72 ± 0.07	0.88 ± 0.07	0.78 ± 0.08

*Values are expressed as mean ± S.E.M. (n = 6); * p < 0.05, ** p < 0.01 vs. control*
HSPE, petroleum ether extract; HSCH, chloroform extract; HSAL, alcoholic extract; HSW, aqueous extract

Table 2: Effect of extracts of *H. spinosa* leaf on Brewer's yeast-induced pyrexia in rats

Treatment	Dose (mg/kg)	Rectal temperature in ^{0}C at various times (h)					
		- 18 h	0 h	1 h	3 h	5 h	6 h
Control	-	37.33 ± 0.08	38.08 ± 0.11	38.30 ± 0.09	38.26 ± 0.06	38.25 ± 0.09	38.28 ± 0.06
HSPE	200	37.52 ± 0.10	38.20 ± 0.16	38.43 ± 0.16	38.31 ± 0.16	38.25 ± 0.16	38.20 ± 0.12
	400	37.20 ± 0.12	38.00 ± 0.08	38.01 ± 0.09	38.01 ± 0.09	38.05 ± 0.05	38.01 ± 0.06
HSCH	200	37.82 ± 0.09	38.63 ± 0.11	38.48 ± 0.06	38.41 ± 0.06	38.38 ± 0.09	38.28 ± 0.12
	400	37.35 ± 0.16	38.03 ± 0.16	37.73 ± 0.23	37.61 ± 0.21*	37.63 ± .21**	37.41 ± 0.26*
HSAL	200	37.92 ± 0.11	38.78 ± 0.03	38.51 ± .05**	38.38 ± .09**	38.16 ± .14**	38.10 ± .14**
	400	37.72 ± 0.19	38.55 ± 0.14	38.15 ± 0.19*	38.11 ± 0.20*	37.88 ± .22**	37.81 ± .19**
HSW	200	37.30 ± 0.12	38.03 ± 0.14	38.08 ± 0.14	38.03 ± 0.16	38.00 ± 0.14	37.95 ± 0.15
	400	37.55 ± 0.09	38.28 ± 0.19	38.26 ± 0.17	38.18 ± 0.14	38.05 ± 0.16	38.01 ± 0.16
Paracetamol	150	37.85 ± 0.17	38.70 ± 0.15	38.43 ± 0.14*	38.25 ± .12**	38.00 ± .15**	37.87 ± .18**

*Values are expressed as mean ± S.E.M. (n = 6); * p < 0.05, ** p < 0.01 compared with 0 h of the same group*
HSPE, petroleum ether extract; HSCH, chloroform extract; HSAL, alcoholic extract; HSW, aqueous extract

the leaves of *H. spinosa*. However, further investigations are required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

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