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Analgesic and anti-inflammatory activities of leaf extract of *Hiptage benghalensis* (L.) Kurz

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ABSTRACT

In present work, the methanolic extract of leaves of *Hiptage benghalensis* (MEHB*)* was screened for the analgesic (using hot plate test and acetic acid-induced writhing test in mice) and anti-inflammatory (using rat paw edema test) activity at the doses of 200 and 400 mg/kg body weight. A significant ($p<0.0005$) analgesic effect was observed with 200 mg/kg and 400 mg/kg in both tests. The maximum anti-inflammatory response was produced at 3 hr and 2 hr with MEHB doses of 200 and 400 mg/kg of respectively. These results suggest that the methanolic extract of *Hiptage benghalensis* has exhibited significant analgesic and anti-inflammatory effects, which were comparable with standard drugs.

Key words: *Hiptage benghalensis*; analgesic; anti-inflammatory; writings; Carrageenan.

INTRODUCTION

Hiptage benghalensis (L.) Kurz (synonym: *Banisteria benghalensis* and *Hiptage madablota* Gaertn*)* is a herb of the family Malpighiaceae. It is distributed throughout India, Srilanka and the Andaman Islands, Bangladesh and Myanmar to southern China[1]. The plant is large, evergreen, climbing shrub with brownish bark peeling off in flakes; young parts silky. Leaves opposite, coriaceolate to elliptic-oblong, apex acute to acuminate, base rounded to cuneate and the margins are entire. In central India, flowering occurs mainly between February and April, sometimes also in October; fruiting occurs mainly from April to June. In ayurveda the leaves and bark are considered vulnerary; the leaves are highly regarded for treating skin diseases. The leaf juice possesses insecticidal properties and is used an external application for scabies. The plant is also used in the treatment of chronic rheumatism and asthma[1]. This study has been undertaken to evaluate the analgesic and anti inflammatory activity of the methanolic extract of *Hiptage benghalensis* using hot plate and rat paw edema method.

MATERIAL AND METHODS

Plant Material

The leaves of *H. benghalensis* were collected from Thirupathi hills, Andhra Pradesh, India. It was authenticated by Prof. V. Raju, Dept of Botany, Kakatiya University, Warangal, India.

Preparation of extract

The leaves were cut into small pieces and shed dry and then ground into coarse powder for the maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50-55 ^{0}C and stored in vaccum desiccator. The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Drugs and chemicals

The drugs and chemicals used were carrageenan and acetic acid (SD fine chemicals Limited, Mumbai), gum acacia and diclofenac sodium (Dr. Reddy's Labs, Hyderabad), Pentazocine (Pure Pharma Ltd., Mumbai) and methanol (Merck, Mumbai).

Phytochemical screening

The methanolic extract was screened for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates[2].

Animals

Albino mice (25-30g) and Wister rats (175-250 g) of either sex were selected and maintained under standard husbandry conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Screening for analgesic activity

Hot- Plate Test

The hot plate test was used to measure analgesic activity by the method described by Eddy and Leimbark (3) with minor modifications. In this experiment, the hot plate was maintained at 55 ± 0.5^0 C. All animals were selected 24 hr prior to experimentation and the animals were selected on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 2-15 sec respectively. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 20 sec. Pentazocine 10 mg/kg was administered intraperitoneally as a reference standard. 30 min after administration of vehicle (2% gum acacia) / methanolic extract (200 and 400 mg/kg) /standard drug animals were placed individually on to the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recorded as the reaction time or latency of the pain response.

Writhing Test

Abdominal construction induced by intraperitoneal injection of acetic acid was carried out according to the procedures described previously (4)**.** The leaf extract of *H. benghalensis* was tested at 200 and 400 mg/kg. Diclofenac sodium, a reference anti-inflammatory and analgesic compound, was used at 20 mg/kg. The extract and reference drug were administered orally 30 min before the administration of 0.7% acetic acid in a volume of 10mg/kg i.p. Control animals received 2% of gum acacia under the same experimental condition. Immediately after injection of the acetic acid, each animal was isolated in an individual cage and the normal of construction was cumulatively counted for a period of 20 min, beginning 3 min after acetic acid injection. The number of writhings and stretching was recorded and the % was calculated using the following ratio:

% of protection= (Control mean-Treated mean)/ Control mean*100

Screening for anti-inflammatory activity by rat paw edema method

The normal paw volumes of all the rats were measured initially and were divided into four groups each consists of six animals treated orally with the vehicle as control (2% gum acacia), standard diclofenac sodium (20 mg/kg) and methanolic extract (200 and 400 mg/kg) respectively. Carrageenan (0.1 ml of a 1% suspension in saline) was injected sub plantar region of the right hind paw of each rat. The vehicle, drug and extract were administered 30 min prior to the injection of Carrageenan. The paw volumes of all the rats were recorded at 1, 2, 3 and 4 hr after Carrageenan treatment by using plethysmometer[5]. A significant reduction in the paw volume compared

to vehicle treated control animals was considered a inflammatory response.

% Inhibition= [(V_T –V_0) control -(V_T –V_0) treated groups] / (V_T –V_0) control *100

V_0 = paw volume of the rat before administration of Carrageenan

V_T = paw volume of the rat after administration of Carrageenan at different time intervals

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the methanolic extract of *H. benghalensis* **(**MEHB**)** reveals the presence of steroids, terpenoids, carbohydrates and glycosides.

Analgesic studies

In this study, we have demonstrated the

Table 1: Effect of methanolic extract from *H. benghalensis* on the hot plate test in mice

S. No	Group	Dose (mg/kg)	Reaction time after administration of control/ standard/extract in sec			
			0 min	60 min	120 min	240 min
1.	Control		2.17 ± 0.75	2.33 ± 0.52	2.17 ± 0.41	1.67 ± 0.52
2.	Pentazocine	10	2.83 ± 0.75	6.83 ± 0.75[b]	6.33 ± 1.63[b]	2.33 ± 0.52[a]
3.	*H. benghalensis*	200	2.67 ± 0.82	7.83 ± 1.60[b]	6.67 ± 0.82 [b]	1.83 ± 0.75
4.	*H. benghalensis*	400	2.83 ± 0.75	9.00 ± 0.89[b]	9.33 ± 0.82[b]	2.50 ± 0.55[a]

Values are in mean ± SD; (n =6), a= $p < 0.05$, b= $p < 0.0005$ Vs Control.

Table 2: Effect of methanolic extract from *H. benghalensis* on acetic acid induced writhing test in mice

S. No	Group	Dose (mg/kg)	No. of writhes	% inhibition
1.	Control		79.5 ± 5.96	-
2.	Diclofenac	20	17.17 ± 3.46 [a]	78.46 ± 3.57
3.	*H. benghalensis*	200	40.5 ± 3.39 [a]	48.64 ± 7.53
4.	*H. benghalensis*	400	29.83 ± 1.83 [a]	62.28 ± 3.79

Values are in mean ± SD; (n =6), a= $p < 0.00001$ Vs Control.

Table 3: Effect of methanolic extract from *H. benghalensis* on the paw edema test in rats

S. No	Group	Dose (mg/kg)	Paw edema volume after			
			1 hr	2 hr	3 hr	4 hr
1.	Control		0.18 ± 0.02	0.20 ± 0.03	0.22 ± 0.03	0.18 ± 0.02
2.	Diclofenac Sodium	20	0.14 ± 0.01[a]	0.13 ± 0.02[b]	0.12 ± 0.02 [b]	0.12 ± 0.01[b]
3.	*H. benghalensis*	200	0.17 ± 0.03[a]	0.14 ± 0.02[b]	0.13±0.02 [b]	0.13 ± 0.02---[b]
4.	*H. benghalensis*	400	0.15 ± 0.02	0.13 ± 0.02[b]	0.12 ± 0.01 [b]	0.13 ± 0.01[b]

Values are in mean ± SD; (n =6), a= $p < 0.05$, b=$p<0.0005$ Vs Control.

effect of MEHB (200 and 400 mg/kg; p.o.) on hot plate test and acetic acid induced writhing in mice. The results of hot plate test and acetic acid induced writhing test were shown in table 1 and 2. The MEHB (200 and 400 mg/kg) showed the significant increase in reaction time and reduction in the number of writhes induced by acetic acid in a dose dependent manner which were comparable with reference compounds, diclofenac and pentazocine respectively. A significant ($p<0.0005$) analgesic effect to the thermal stimulus was observed at 60 min with 200and 400 mg/kg of *H. benghalensis* which is comparable to the effect of standard pentazocine.

The mouse writhing assay is useful test to evaluate mild analgesic agents. Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, PGs, bradykinin and substance P which stimulate pain nerve endings. Inhibition of acetic acid-induced writing in mice suggests that the analgesic effect of the extract may be peripherally mediated via the inhibition of the synthesis and release of prostaglandins[4]. Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb in mice. This is due to nociceptive property of acetic acid[6]. Therefore, the MEHB might inhibit the synthesis and /or release of these endogenous substances.

Anti-inflammatory activity

Carrageenan induced paw edema test provides a skin inflammation model suitable for evaluation of topical and systemic anti-inflammatory agents. The results of MEHB against Carrageenan induced paw edema is shown in table 3. There was a dose dependent inhibitory activity in Carrageenan induced paw inflammation at all assessment times. Diclofenac sodium, a COX-inhibitor at the dose of 20 mg/kg, p.o. signifinatly reduced the paw edema. This indicates action against release of histamine, serotonin and kinins in early phase, while later phases are suspected to be arachidonate metabolites producing an edema dependent on mobilization of neutrophils[7].

In conclusion, the methanolic extract of *H. benghalensis* have exhibited a significant analgesic and anti inflammatory activity.

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