

Short Communication

Analgesic and Anti-inflammatory activity of leaves of *Rivea hypocrateriformis*

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The ethanolic extract of the leaves of *Rivea hypocrateriformis* obtained by soxhlet extraction method was evaluated for analgesic action by Radiant Heat Tail Flick method and was tested for anti-inflammatory action by carrageenan induced paw edema. The ethanolic extract in doses of 200 and 400 mg/kg of Body weight showed 64.83 and 100% inhibition of paw edema, respectively at the end of three hours compared to that of standard Ibuprofen (87.04%). In the Radiant Heat Tail Flick model, the ethanolic extract at high dose 400 mg/kg of Body weight increased the pain threshold significantly after 30 min, 1, 2 and 4 h of administration. The ethanolic extract showed dose-dependent action in all the experimental models. The results were analyzed for statistical significance using two-way ANOVA followed by Dunnett's test. A P value < 0.05 is considered significant.

Key words: Carrageenan induced paw edema, ethanolic extract *Rivea hypocrateriformis*, Tail flick method.

INTRODUCTION

The plant kingdom is abundant in species that act as anti-inflammatory to animal tissue. Whilst they are rarely as immediately powerful as the steroid drugs, they are very rarely as dangerous and potentially life threatening. In a well known study, 163 species of plants and fungi were tested to determine their anti-inflammatory activity. Due to adverse effects associated with the use of NSAIDs and other medications, there arise new scopes for Herbs and Herbal Formulation in treatment of inflammatory diseases (Derle et al., 2006).

In the present study, *Rivea hypocrateriformis* (vernacularly known as Phang) leaves (convolvulaceae) described as a new anti-inflammatory plant of this genus on the basis of folklore claims (Kirtikar and Basu, 1968; Sinha and Saxena, 2006). The leaves are ovate-orbicular, appressedly-silky-hairy beneath, rarely broad than long, shallow basal sinus, lateral nerves about 6 on either half and Petiole with a green gland on either side at the junction with the midrib. For boils, the latex (milky juice)

of the plant is applied and dressed.

Juice of leaves along with Babul twig and sugar is taken with cow's milk in rheumatic pain. The Juice of leaves is used for skin disease of hair scalp. The leaves are reported to contain isocoumarin, Desmethylbergenin hemihydrate 3,4,8,9,10-pentahydroxy-2-hydroxy-methyl-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-6 onehemihydrate, C₁₃H₁₄O₉·0.5H₂O (Zamarrud et al., 2006). Anti-implantation and pregnancy interruption efficacy of *R. hypocrateriformis* in the rat was reported. The purpose of this study was to evaluate whether ethanolic extract have anti-inflammatory activity on the basis of presence of flavanoid and coumarin in phytochemical screening of extract (Shivalingappa et al., 2001)

MATERIALS AND METHODS

Plant material

The plant was collected in the month of November to January from the field area around the Gandhinagar of Gujarat state. The collected plant was authenticated at Bioscience Department, Vallabh vidyanagar. The specimen of the same was deposited in the herbarium of K. J College of Pharmacy, Vadasma.

After collection, from all plant material, leaves were separated

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Table 1. Analgesic activity by radiant heat tail flick method.

Animal group	Drug dose (mg/Kg Body Weight)	Basal reaction time (s)	Tail flick latency in second at different time interval			
			0 h	30 min	2 h	4 h
Control Group	Vehicle	3	3	3	5	6
Test-1	200	3	5.0	8.72	9.2	11
Test-2	400	4	5.83	10.38	11.29	12
Std (Pentozocin)	20	3	12	12	12	12

and washed thrice with water to remove dust and debris stick on the surface. Then washed material was dried under shade.

Preparation of extracts

The dried raw material was coarsely powdered by a pulverizer. The alcoholic extract was obtained with 95% v/v alcohol for three days, using Soxhlet apparatus. The extracts were evaporated to dryness.

Animals

Albino rats of Wistar strain (250 - 300 g) were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 20^\circ\text{C}$; relative humidity 60 - 70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by institutional animal ethical committee ((Registration No.365/01/ab/CPCSEA).

Drugs

Carrageenan, Pentozocin (Ranbaxy), Ibuprofen (Biochem).

Acute Toxicity Study

In albino rats, the ethanolic extract of leaves of *Rivea hypocrateriformis*, was given orally by suspending the extract in Tween 80. The dose was given from 200 to 1000 mg/kg of body weight and animals were observed for 24 h period.

Analgesic activity (Vogel, 2002)

Tail flick method

The prescreened animals (reaction time: 3-4 sec) were divided into groups control, Standard and Test, 6 animals in each group. Pentozocin acted as the standard drug. The drugs were administered intraperitoneally. The tail flick latency was assessed by the analgesiometer (Inco., India). The strength of the current passing through the naked nicrome wire was kept at 2 - 3 amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 12 s to avoid tissue damage.

Anti-inflammatory study (Vogel, 2002)

Carrageenan induced paw edema

The animals were divided into groups as normal, control, Standard, Test -1 and Test - 2, 6 animals in each group. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0, 1, 3 and 5 h after the carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage anti-inflammatory activity was calculated. With Ibuprofen (20 mg/kg) as standard, ethanolic extract of plant at dose of 200 and 400 mg/kg was suspended in Tween 80 and given orally by feeding needle. % inhibition of paw edema is calculated by comparing the controls.

Statistical analysis

The results were analyzed for statistical significance using two-way ANOVA followed by Dunnett's test. A P value <0.05 is considered significant.

RESULTS

No adverse effect or mortality was detected in albino rats up to 1 gm/kg, orally of ethanolic extract of leaves of *R. hypocrateriformis* during 24 h observation period.

In the Radiant heat Tail Flick model, as summarized in Table 1, the test drug in high doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center. The results of anti-inflammatory activity are summarized in Table 2. The ethanolic extract of *R. hypocrateriformis* in doses of 200 and 400 mg/kg significantly suppressed carrageenan-induced paw edema in rats (Table 2).

DISCUSSION

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental

Table 2. Anti-inflammatory activity: % Inhibition of paw edema at different time interval.

Animal group	% Inhibition of paw edema at different time interval			
	0 h	1 h	3 h	5 h
Test-1 (200 mg/kg)	6.8	0.86	12.28	31.46
Test-2 (400 mg/kg)	6.87	10.86	18.94	33.33
Std (Ibuprofen)	2.11	5.65	16.49	25.46

model exhibits a high degree of reproducibility.

Carrageenan- induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas, the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h. The increase in the paw volume following carrageenan administration in the control (0.57 ± 0.14 ml) and ibuprofen treated group (0.21 ± 0.01 ml) corresponds with the findings of previous works. The ethanolic extract of *R. hypocrateriformis* produced dose-dependent and significant inhibition of carrageenan-induced paw edema. The inhibition was significant at the dose of 200 mg/kg (64.83%) and 400 mg/kg of body weight (100%) to that of the standard drug, ibuprofen (87.04%).

These data support the possible pharmacological activity, but the exact mechanism of action of anti-inflammatory activity was not performed yet. That is to be performed in future in support of relief from NSAIDs adverse effect.

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