Research Paper

Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models

A. Chakraborty, R. K. B. Devi, S. Rita, Kh. Sharatchandra, Th. I. Singh

Department of Pharmacology, Regional Institute of Medical Sciences, Lamphelpat, Imphal - 795004. India.

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Correspondence to:
A. Chakraborty
E-mail:
dr_ananya@yahoo.com

ABSTRACT

Objective: To evaluate the antiinflammatory and analgesic activities of the aqueous extract of *Spilanthes acmella* (SPA) in experimental animal models.

Material and Methods: SPA was evaluated for antiinflammatory action by carrageenan-induced rat paw edema. The analgesic activity was tested by acetic acid-induced writhing response in albino mice and tail flick method in albino rats.

Results: The aqueous extract of SPA in doses of 100, 200 and 400 mg/kg showed 52.6, 54.4 and 56.1% inhibition of paw edema respectively at the end of three hours and the percentage of protection from writhing was 46.9, 51.0 and 65.6 respectively. In the tail flick model, the aqueous extract of SPA in the above doses increased the pain threshold significantly after 30 min, 1, 2 and 4 h of administration. SPA showed dose-dependent action in all the experimental models.

Conclusion: The present study indicates that SPA has significant antiinflammatory and analgesic properties.

KEY WORDS: Carrageenan, writhing, tail flick

Introduction

Spilanthes acmella [SPA] (Bengali-Akarkara, Assamese-Pirazha, Manipuri-Maanja-lei, Telegu-Maratitige) is an indigenous herb belonging to the family Compositae.[1] It is grown as an annual herb throughout the tropics. It has conical small yellow flowers. The whole plant is claimed to possess medicinal properties. The flowers are chewed to relieve toothache and the crushed plant used in rheumatism.[2,3] The leaves are also eaten raw or as a vegetable by many tribes of India. SPA is generally known as toothache plant.[4] However, no scientific data are available to validate the folklore claim. Therefore, this study was undertaken to evaluate the a) antiinflammatory potential of the aqueous extract of SPA on carrageenan-induced rat paw edema and b) analgesic activity using acetic acid-induced writhing test in albino mice and tail flick response in albino rats.

Material and Methods

Preparation of the extract

Fresh aerial parts of SPA were collected from the campus of the R.I.M.S., Imphal, identified and authenticated. The plant parts were cleaned, dried under shade and powdered by a mechanical grinder. Sixty grams of the pulverized plant was extracted with distilled water using a soxhlet apparatus. The yield was 13.5% in powder form. The extract of SPA was administered as a suspension in 2% gum acacia to the animals.

Phytochemical studies

Freshly prepared SPA extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol.[5]

Animals

Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2^{0}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 the institutional animal ethics committee.

Drugs

The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), acetic acid (Ranbaxy Laboratories Ltd., Punjab), aspirin (Vikash Pharma, Mumbai), pethidine (Bengal Immunity, Kolkata).

Acute toxicity study

No adverse effect or mortality was detected in albino rats up to 3 gm/kg, *p.o.* of SPA during the 24 h observation period.

Antiinflammatory study

The animals were divided into groups as shown in Table 1. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia 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' and '3' hours after the carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage antiinflammatory activity was calculated. Aspirin 100 mg/kg, *p.o.* suspended in 2% gum acacia was used as the standard drug.

Acetic acid-induced writhing test

The prescreened animals were divided into groups as shown in Table 1. Aspirin in doses of 50, 100 and 150 mg/kg, suspended in 2% gum acacia was used as the standard drug. The drugs were autoclaved at 121°C for 30 min[6] (the compounds were assumed to be heat stable) and administered subcutaneously. Writhing was induced 30 min later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water.[7] The number of writhes was counted for 30 min immediately after the acetic acid injection. The percentage protection was calculated.

Tail flick method

The prescreened animals (reaction time:3-4 sec) were divided into groups as shown in Table 2. Pethidine 5 mg/kg 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 constant at 6 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

Table 1

Effects of the aqueous extract of *Spilanthes acmella* (SPA) on carrageenan-induced rat paw edema and acetic acid-induced writhing response in mice

Group	*Carrageenan-induced rat paw edema*			*Acetic acid-induced writhing response in mice*		
	Dose (mg/kg, *p.o.*)	Increase in paw volume (mean ± SEM) in ml	% inhibition of paw edema	Dose (mg/kg, *s.c.*)	No. of writhing movements	% of protection
N/saline	10 ml/kg	0.57 ± 0.14	–	8 ml/kg	80.30 ± 0.95	–
SPA	100	0.27 ± 0.06*	52.6	100	42.67 ± 4.18†	46.9
SPA	200	0.26 ± 0.03*	54.4	200	39.33 ± 6.46†	51.0
SPA	400	0.25 ± 0.03*	56.1	400	27.66 ± 3.48*	65.6
Aspirin	–	–	–	50	30.33 ± 2.49†	62.3
Aspirin	100	0.21 ± 0.01†	63.1	100	16.33 ± 6.70†	79.7
Aspirin	–	–	–	150	16.00 ± 5.27†	80.1
One-way ANOVA		F 4.80			22.78	
		df 4,25			6, 35	
		P *<0.01*			*<0.001*	

n = 6 in each group. **P<0.01*, †*P<0.001* compared to control

Table 2

Analgesic activity of the aqueous extract of *Spilanthes acmella* (SPA) on tail flick response in rats

Group	*Drug dose mg/kg, i.p.*	*Predrug (mean ± SEM) reaction time (in sec)*	*Reaction time in sec (mean ± SEM)*			
			30 min	1 h	2 h	4 h
D. Water	1 ml/kg	3.3 ± 0.21	4.25 ± 0.4	4.16 ± 0.3	4.30 ± 0.3	4.10 ± 0.5
SPA	100	3.6 ± 0.21	6.16 ± 0.5*	7.90 ± 0.9*	7.10 ± 0.7*	7.50 ± 0.8*
SPA	200	3.7 ± 0.25	8.20 ± 0.7*	8.0 ± 0.7*	8.00 ± 0.7*	8.80 ± 0.8*
SPA	400	3.6 ± 0.23	8.00 ± 0.8*	9.83 ± 0.2*	9.10 ± 0.5*	9.00 ± 0.6*
Pethidine	5	3.8 ± 0.17	9.16 ± 0.5*	9.33 ± 0.3*	9.30 ± 0.4*	8.00 ± 0.9*
One-way ANOVA		F 0.74	11.40	15.0	12.72	8.01
		P *>0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*

n = 6 in each group; df = 4,25. **P<0.01* compared to control

tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage.[8]

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A *P value <0.05* was considered significant.

Results

The results of the animal experiments are shown in Tables 1 and 2. In the acute inflammation model, the aqueous extract of SPA in doses of 100, 200 and 400 mg/kg, *p.o.* produced dose-dependent inhibition of paw edema (Υ=0.87). The test and the standard drugs produced significant inhibition of paw edema as compared to the control.

The aqueous extract of SPA (100, 200 and 400 mg/kg, *s.c.*) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner (Υ=0.99). The standard drug, aspirin, in increasing doses produced increased inhibition of writhing movements. The results were found to be highly significant (*P<0.001*) in comparison to the control.

In the tail flick model, there was no significant difference in the mean predrug reaction time between the different groups. Thirty min after drug administration, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. The test drug produced a dose-dependent increase in the reaction time (Υ=0.94) at various time intervals of observation. Preliminary phytochemical analysis of the aqueous extract of SPA revealed the presence of flavonoid compounds.

Discussion

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing antiinflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[9] 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.[10] The increase in the paw volume following carrageenan administration in the control (0.57 ± 0.14 ml) and aspirin treated group (0.21 ± 0.01 ml) corresponds with the findings of previous workers.[11,12] The SPA extract produced dose-dependent and significant inhibition of carrageenan-induced paw edema (Υ=0.87). The inhibition was however, less than that of the standard drug, aspirin.

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The number of writhing movements during a 30 min observation in the control group was 80.33 ± 0.95 which corresponds with the findings of other workers.[13,14] In the tail flick model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center.

The results of the present study suggest that the aqueous extract of SPA in doses of 100, 200 and 400 mg/kg significantly suppressed carrageenan-induced paw edema in rats and demonstrated significant analgesic activity in acetic acid-induced writhing and tail flick models. However, the analgesic activity of SPA was found to be more significant on the acetic acid-induced model *(P<0.001)* than the tail flick model *(P<0.01)* and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

On preliminary phytochemical screening the aqueous extract of SPA was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception.[15] Hence, the presence of flavonoids may be contributory to the antiinflammatory and analgesic activities of aqueous SPA. Further studies may reveal the exact mechanisms of action responsible for the analgesic and antiinflammatory activities of SPA.

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