Vol. 1/2 (2001) 111 - 115

JOURNAL OF NATURAL REMEDIES

Anti-inflammatory activity of *Rubia cordifolia* roots

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Received 11 December 2000; Revised and Accepted 27 April 2001

Abstract

Objective: To study the anti-inflammatory, analgesic, antipyretic and gastrolesive properties of petroleum ether extract of *Rubia cordifolia* roots. Materials and methods: Bioassay guided separation of petroleum ether extract was carried out to obtain a compound possessing anti-inflammatory activity. An active triterpene responsible for the anti-inflammatory activity was separated. The compound exhibited anti-inflammatory activity in the carrageenan-induced edema, cotton pellet granuloma and adjuvant-induced arthritis. The analgesic activity was studied using acetic acid induced writhing and radiant heat analgesiometer and antipyretic activity was assessed in yeast-induced hyperpyrexia in rats. The ulcerogenic potential was studied using pyloric ligated rats. Results: The compound possesses anti-inflammatory, analgesic and antipyretic activity and strong gastrolesive properties. Conclusion: The study justifies the use of *Rubia cordifolia* in the treatment of inflammation, pain and fever.

Key words: *Rubia cordifolia*, antiinflammatory activity

1. Introduction

Rubia cordifolia Linn (Rubiaceae) is an important crude drug commonly used in the indigenous system of medicine for the treatment of rheumatoid arthritis, inflammation and fever. This herb is not only mentioned in Ayurveda but is also one of the major ingredients of many marketed products [1, 2]. Since there is no systematic study confirming anti-inflammatory activity, we have investigated the anti-inflammatory, analgesic, antipyretic and ulcerogenic activity of the petroleum ether extract of the roots of this plant.

2. Materials and methods

2.1 Plant material

Dried roots of *Rubia cordifolia,* collected in the months of May-June, were obtained from Ayurveda Seva Sangh, Nashik. The identification and authentication was done at the Pharmacognosy division of our department.

2.2 Extraction

The powdered plant material (1.0 kg) was Soxhlet extracted with petroleum ether (60-80°C). The extract (3 g) was treated in succession with benzene, ethyl acetate and

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methanol (Yield, 1.8, 1.0 and 0.2 g respectively). These fractions were suspended in 0.5% gum acacia and tested for anti-inflammatory activity. The fractions exhibiting percentage inhibition of edema less than 20% were discarded.

The benzene fraction, which exhibited anti-inflammatory activity greater than 20% was separated by column chromatography with neutral alumina eluting with benzene:Ethylacetate (1:1) mixtures to afford a compound RCB1 (yield 0.75 g), having a triterpene nature [3].

2.3 Animals

Male Wistar rats, weighing 160-180 g, housed in standard laboratory conditions were used. They were fed with rodent commercial diet (Lipton, India Ltd.) and water *ad libitum.* Food was withdrawn 6 h before the test.

2.4 Assesment of anti-inflammatory activity

2.4.1 Carrageenan-induced rat paw edema

Male rats (n = 5) were treated orally with either vehicle (0.5% gum acacia, 4.0 ml/kg) or doses of extract/ fractions, or RCB1, ranging from 10-40 mg/kg, 60 min before an injection of 0.1 ml of 1.0% carrageenan [4] into the plantar tissue of the right hind paw. Reference group were treated with either ibuprofen (40 mg/kg p.o.) or hydrocortisone (10 mg/kg s.c.) 60min before carrageenan [5].

The contra-lateral hind paws were injected with 0.1 ml of saline as control. Paw volume was measured plethysmographically (UGO BASILE) at 0, 1 and 3 h after carrageenan. The percentage inhibition of paw edema was calculated.

2.4.2 Cotton pellet granuloma

Two sterile cotton pellets weighing 50 ± 1 mg were implanted subcutaneously in the axilla of rat under light ether anesthesia. The RCB1 (20mg/kg p.o.) was given once a day from day 1 to 7 of the experiment. On the 8th day, the rats were sacrificed by an overdose of ether and the cotton pellets surrounded by granuloma tissue were removed and were dried in oven (65°C) till constant weight [6].

The other groups treated orally with either vehicle of a standard drug ibuprofen (40 mg/kg) were also maintained simultaneously. The percentage inhibition of granuloma development, compared to the control group was calculated.

2.4.3 Adjuvant-induced polyarthritis

The arthritic syndrome was induced in rats by an injection of 0.1 ml of Freund's complete adjuvant into the subplantar region of right hind paw [7]. The test compound RCB1 (20mg/kg p.o.), ibuprofen (40 mg/kg p.o.), hydrocortisone (10mg/kg s.c.), or vehicle (4.0 ml/kg p.o.) were administered from day 1 to 30 of the experiment. Plethysmographic determination of paw volume was made every third day for both injected and contra-lateral foot.

The percentage inhibition of edema caused by different treatments was determined on the last day of the experiment using the equation,

Percent Inhibition = [1-(A-X) / (B-Y)] x 100

Where:

A = mean paw volume of drug treated rats on the particular day;

X = mean paw volume of drug treated rats immediately prior to adjuvant injection;

B = mean paw volume of adjuvant control rat on a particular day and

Y = mean paw volume of the adjuvant control rats (receiving adjuvant injection but no drug treatment) immediately prior to adjuvant administration.

2.5 Assessment of analgesic activity

2.5.1 Tail flick test

Five rats were used in each group. The possible analgesic activity of RCB1 (20 mg/kg) was studied 30 min after its oral administration.

The latency to tail flick was noted in each rat as described earlier [8]. Pentazocine (10mg/kg i.p.) was used as a standard analgesic.

2.5.2 Writhing test

Writhing syndrome was elicited in mice (n = 5) by intraperitoneal injection of 0.1ml of 0.6% acetic acid. The RCB1 (20mg/kg p.o.) was administered 30 min before acetic acid and the number of writhes displayed from 5 to 20 min after acetic acid were counted [9]. In this experiment ibuprofen (40mg/kg p.o.) served as a positive drug control.

2.6 Assessment of antipyretic activity

Antipyretic activity was determined in albino rats (n = 5) injected with 15 % Brewer's yeast in saline (1ml /100 g s.c. in nape of neck). Eighteen hours later the animals presenting an increase in rectal temperature (Aplab Instruments, India) of more than 1°C were randomly treated with either vehicle (0.1 ml/kg) or RCB1 (20 mg/kg) or ibuprofen (40 mg/kg) and rectal temperature was measured every hour for 2 h [10].

2.7 Assessment of ulcerogenic activity

Rats were treated orally with RCB1 (20 mg/kg) or ibuprofen (40 mg/kg) or vehicle (0.4 ml/100 g) every day at 1100 h for 4 consecutive days, the last being administered 45 min before subjecting the rats to pyloric ligation according to Sanyal *et al*., [11]. The degree of single ulceration (DSU) for each stomach was determined and scored according to Thuillier and Kulkarni [12].

Table 1.
Effect of some fractions of pet ether extract of roots of *R. cordifolia* on carrageenan-induced paw edema in rats.

Treatment	**Dose (mg/kg)**	**Paw volume (ml) at**		**Inhibition percent at 3 h.**
		0 h	**3 h**	
Vehicle	4 ml/kg	0.89 ± 0.02	2.45 ± 0.02	—
R.cordifolia	10	0.9 ± 0.03	1.91 ± 0.04*	35.25
Pet ether Ext.	20	0.85 ± 0.04	1.65 ± 0.04*	48.71
	40	0.91 ± 0.03	1.71 ± 0.02*	55.12
Benzene fraction				
	20	0.90 ± 0.03	1.96 ± 0.04*	33.75
	40	0.94 ± 0.03	1.81 ± 0.03*	45.60
Ethyl acetate fraction				
	20	0.85 ± 0.02	2.20 ± 0.04*	15.60
	40	0.91 ± 0.03	2.25 ± 0.04*	18.75
	100	0.95 ± 0.04	2.29 ± 0.04*	18.75
RCB1	10	0.9 ± 0.03	1.74 ± 0.03*	46.15
	20	0.9 ± 0.04	1.55 ± 0.06	58.33
	40	0.91 ± 0.03	1.50 ± 0.03*	62.17
Ibuprofen	40	0.89 ± 0.03	1.71 ± 0.02*	48.10
Hydrocortisone	10	0.92 ± 0.03	1.81 ± 0.02*	43.6

n = 5; The values are mean ± SEM; RCB1 = the compound obtained from benzene fraction using equal volumes of benzene and ethyl acetate as eluents; All drugs except hydrocortisone (s.c.) were given orally 1 h prior to carrageenan.

The approximate ED_{50} of RCB1 = 18 mg/kg

* The values were significantly different from the control at $P < 0.001$ (ANOVA, followed by Student's *t* - test). Fraction showing activity less than 20% was discarded.

Table 2.
Effect of RCB1 on adjuvant induced arthritis in rats.

Treatment	Dose mg/kg	Paw volume increase[a] ml	Edema inhibition %
Vehicle	—	2.14 ± 0.04	—
RCB1	20	1.04 ± 0.03*	51.4
Ibuprofen	40	1.14 ± 0.03*	46.70
Hydrocortisone	10	0.90 ± 0.04*	60.21

n = 5; values are mean ± SEM;
All drugs were administered orally, except hydrocortisone, which was administered subcutaneously;
[a] determined on 30th day; * P< 0.001 as compared to control (Student's *t* - test)

2.8 Behavioral and toxicity studies

Groups of 8 mice were treated with RCB1 (10,50,100, 500 and 1000 mg/kg) orally. The animals were observed for 24 h for any gross behavioral changes and mortality.

2.9 Chemical characterization

The Liebermann-Burchard test indicated that the compound RCB1 was triterpene.

2.9 Statistical Analysis

Statistical differences among control and treated groups were determined by analysis of variance followed by Student's *t* - test. Mann-Whitney U test was used for non-parametric analysis. Differences were considered significant at $P < 0.05$.

3. Results

Petroleum ether extract of *Rubia cordifolia* roots and its benzene soluble fraction showed significant anti-inflammatory at all doses (10-40 mg/kg p.o.) in carrageenan-induced paw edema in rats. The benzene fraction upon separation yielded a compound RCB1, triterenoid in nature, which showed potent anti-inflammatory activity in doses of 10-40 mg/kg p.o. (Table 1) and also exhibited significant anti-inflammatory activity in cotton pellet granuloma. RCB1, in a dose of 20mg/kg p.o., inhibited granuloma mass by 48.5% whereas ibuprofen (40mg/kg p.o.) reduced it by 45.1%.

The adjuvant- induced arthritis was also inhibited after oral administration of 20 mg/kg RCB1 (Table 2). The test compound also exhibited significant analgesic activity (Table 3).

In rats treated with test compound, (20 mg/kg p.o.) the yeast induced pyrexia was gradually reduced from 39.1 ± 0.05°C to 38.04 ± 0.04°C after 2 h of drug administration. Ibuprofen (40 mg/kg p.o.) reduced rectal temperature from 39.3± 0.07°C to 37.48± 0.13°C after 2 h.

The test compound (20 mg/kg p.o.) and ibuprofen (40 mg/kg p.o.) showed increased gastric irritation compared to the vehicle treated animals with ulcer index of 1.5 and 1, respectively ($P < 0.05$).

The test compound exhibited no change in the gross behaviour and all the animals treated orally with dose upto 1000 mg/kg survived after the observation period of 24 h. No change in the behavior was observed.

4. Discussion

The overall results of this study suggest a strong anti-inflammatory activity of the petroleum ether roots of *R. cordifolia* root and its

Table 3.
Analgesic activity of RCB1 in mice.

Treatment	Dose mg/kg	Latency to tail flick	Percentage analgesia	No. of writhes	Percentage analgesia
Vehicle	—	4.0 ± 0.31	—	59.16 ± 2.63	—
RCB1	20	6.62 ± 0.5*	23.81	26.8 ± 2.39#	54.6
Pentazocine	10	8.2 ± 0.6#	38.1	ND	ND
Ibuprofen	40	ND	ND	32.2 ± 1.74#	45.5

n = 5; ND - not done;
Values are mean ± SEM. * P < 0.01, # P < 0.001 as compared to control (Student's *t* - test).

constituent RCB1. The effectiveness of this compound against the primary phase of adjuvant arthritis and the carrageenan-induced edema indicates its ability to exert anti-inflammatory effect in the acute as well as the chronic phase of inflammatory process. Its activity on established polyarthritis indicate the inhibition of cell mediated mechanism of adjuvant arthritis.

The compound also exhibited analgesic activity in both the writhing and tail flick tests although the analgesic effect was stronger in earlier. Thus the results together with gastrolesive activity, higher than that of ibuprofen, suggest that the anti-inflammatory activity of RCB1 could be related to inhibition of both cyclo-oxygenase - I and cyclo-oxygenase - II enzyme [13].

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