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Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals

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Abstract

The alcoholic extract (50, 100 and 200 mg/kg, p.o) of *Clerodendron serratum* roots produced a significant antinociceptive, anti-inflammatory and antipyretic activities in animal models. The results support the traditional claims of *C. serratum* as a remedy for pain, inflammation and fever. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Clerodendron serratum (Linn.) Moon (Verbenaceae), known as 'Bharangi' in Ayurveda and 'Sirutekku' in Siddha system of medicine, is claimed to be useful in treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever (Nadkarni, 1954). There are no scientific studies in support of these traditional claims. Hence in the present study, an attempt has been made to investigate the antinociceptive, anti-inflammatory and antipyretic effects of *C. serratum* in experimental animals.

2. Materials and methods

2.1. Plant material

C. serratum roots (authenticated by Dr S. Usman Ali, Department of Pharmacognosy, Central Research Institute for Siddha, Government of

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India, Chennai) were collected during the month of October and voucher specimen (Pharm. No.14/96) has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chennai Medical College.

2.2. Preparation of alcoholic extract

Shade dried and coarsely powdered material (ca 4.5 kg) were extracted with 90% ethyl alcohol in an aspirator bottle at room temperature (72 h). Nearly 80% of the solvent was removed by distillation over boiling water-bath at atmospheric pressure and the remaining under reduced pressure. This extract (yield 6.67%) was dissolved in distilled water and used for animal experiments.

2.3. Animals

Albino mice (20–25 g), Wistar albino rats (150–200 g) and rabbits (1.5–2.0 kg) of either sex, maintained in the Animal Experimental Laboratory of Chennai Medical College at room temperature of 25 ± 2°C, relative humidity of 75 ± 5% and 12 h dark-light cycle. Food and water were given ad libitum.

2.4. Preliminary screening and acute toxicity studies

Mice were selected for this study (Turner, 1965). They were divided into eight groups each containing six animals. *C. serratum* was administered orally in varying doses (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.50 g/kg) to these animals. They were continuously observed for 2 h to detect changes in the autonomic or behavioural responses viz. alertness, spontaneous activity, irritability, pinna reflex, corneal reflex, urination, salivation, piloerection etc.

Any mortality during experimentation and the following 7 days was also recorded. A group of animals treated with the vehicle (distilled water) served as control. Based on the results of preliminary toxicity testing, the doses of 50, 100 and 200 mg/kg of *C. serratum* were chosen for further experiments.

Table 1
Effect of *Clerodendron serratum* on nociceptive responses in mice

Treatment (mg/kg)	Acetic acid induced writhing method	Hot plate method
	No. of writings	AUC (sq. cm)
Vehicle	30.30 ± 0.90	1.63 ± 0.43
Morphine sulphate (s.c) 5.0	1.00 ± 0.20**	32.60 ± 1.10**
C. serratum (p.o)		
50	19.67 ± 1.41**	7.90 ± 2.09*
100	13.50 ± 1.60**	11.80 ± 2.20**
200	11.80 ± 2.60**	15.50 ± 1.36**

Each value represents the mean ± SEM of six observations.
* $p<0.05$; ** $p<0.01$ compared to vehicle treatment.

2.5. Antinociceptive activity

This was investigated in mice by acetic acid induced writhing (Koster et al., 1959) and hot plate methods (Eddy and Leimbach, 1953)

2.5.1. Acetic acid induced writhing method

Three different groups of mice received 50, 100 and 200 mg/kg of *C. serratum* orally. Sixty minutes later 0.6% acetic acid (10 ml/kg) was injected (i.p.). The number of abdominal constrictions

Table 2
Effect of *Clerodendron serratum* on acute and chronic inflammation in rats

Treatment (mg/kg)	Acute study	Chronic study
	Vol. of paw edema (ml)	Wt. of cotton pellets (mg)
Vehicle	0.75 ± 0.07	50.18 ± 1.05
Phenylbutazone (p.o) 100	0.28 ± 0.10**	28.25 ± 1.30**
C. serratum (p.o)		
50	0.78 ± 0.05	41.25 ± 1.73**
100	0.44 ± 0.05**	39.25 ± 1.02**
200	0.40 ± 0.03**	36.75 ± 1.36**

Each value represents the mean ± SEM of six observations.
** $p<0.01$ compared to vehicle treatment.

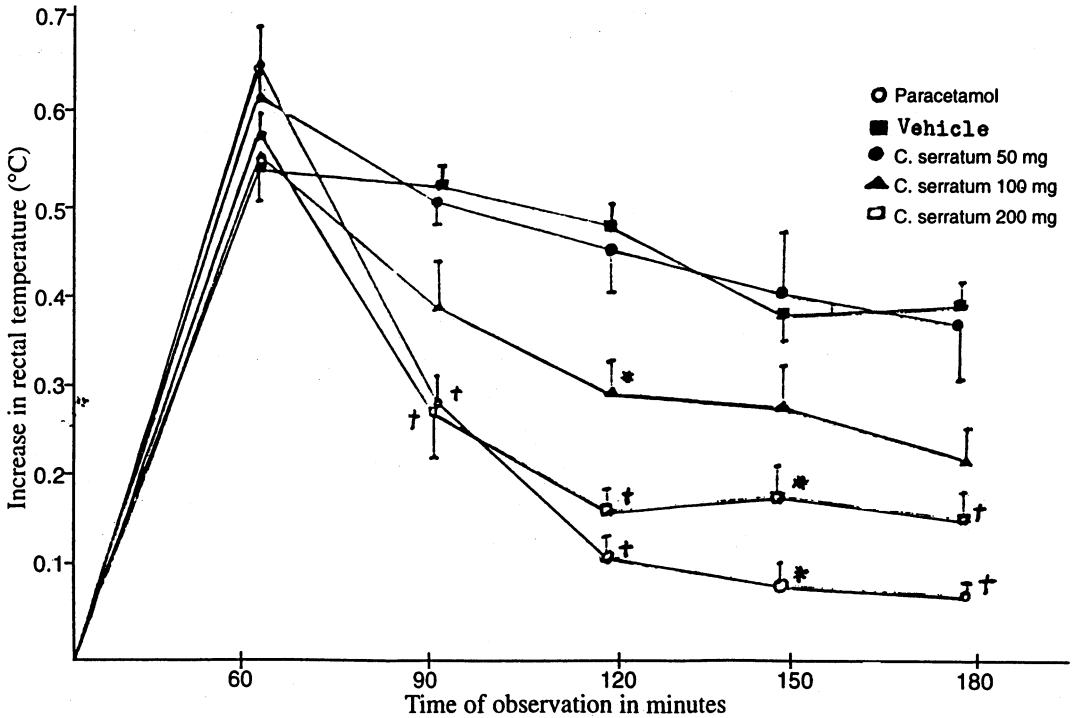

Fig. 1. Effect of *Clerodendron serratum* on TAB vaccine induced pyrexia in rabbits. * $p < 0.05$; † $p < 0.01$ compared to corresponding vehicle treatment. Each point represents the mean ± SEM increase in rectal temperature recorded in six animals.

during the following 15 min period were counted. A significant reduction in the number of abdominal constrictions compared to the control was considered as antinociceptive response. Morphine sulphate (5 mg/kg, s.c) was used for comparison.

2.5.2. Hot plate method

The animals were treated orally with different doses (50, 100 and 200 mg/kg) of *C. serratum*, and were placed on a hot plate maintained at 55 ± 0.5°C. The time taken to lick the hind-paw was taken as the reaction time. The reaction time was measured just prior to drug administration and later at 30 min intervals upto 3 h. Morphine sulphate was used as a standard drug (5 mg/kg, s.c).

The reaction time in each animal was plotted against the time of observation. The area under the time response curve was calculated as a measure of antinociceptive activity.

2.6. Anti-inflammatory activity

2.6.1. Acute inflammation

Anti-inflammatory activity was studied by carrageenin-induced paw edema method in rats (Winter et al., 1962). Carrageenin (1.0%) 0.1 ml was injected into the plantar surface of right hind-paw. The paw volume was measured 4 h later using a plethysmograph. The difference in volume between left and right hind paws was taken as a measure of edema. The test drug was administered orally in different doses (50, 100 and 200 mg/kg) 60 min prior to carrageenin injection.

2.6.2. Chronic inflammation

This was studied by cotton-pellet implantation method in rats (Winter and Porter, 1957). Under light ether-anaesthesia, sterile cotton-pellets weighing 10 mg were implanted subcutaneously in the arm-pits. The animals were treated orally with

different doses of *C. serratum* (50, 100 and 200 mg/kg) for 7 days. They were sacrificed on day 8, the cotton-pellets with adhering granulamatous tissues removed, dried at 50°C for 24 h and weighed. Standard anti-inflammatory agent phenylbutazone (100 mg/kg, p.o.) was used for comparison in both acute and chronic models.

2.7. Antipyretic activity

This was studied in rabbits (Brownlee, 1937). The animals were maintained in the laboratory for 24 h prior to the experiment. TAB vaccine 0.1 ml was injected into the marginal ear-vein and the rectal temperature was recorded every 15 min using 'Electrolab' 18-channel telethermometer. *C. serratum* was administered orally in doses of 50, 100 and 200 mg/kg, 60 min after TAB vaccine when there was significant pyrexia. Subsequently, the rectal temperature was recorded every 30 min upto 3 h. Paracetamol 100 mg/kg (p.o) was used for comparison.

2.8. Statistical analysis

The results were subjected to analysis of variance followed by Dunnett's *t*-test for multiple comparison.

3. Results

3.1. Preliminary screening and acute toxicity studies

C. serratum when administered in the dose range of 0.5–2.5 g/kg (p.o) to mice did not produce any significant change in the autonomic or behavioural responses during the observation period. The oral LD_{50} value in mice calculated graphically was found to be 1.8 g/kg.

3.2. Antinociceptive activity

In the acetic acid induced writhing method, *C. serratum* treatment similar to morphine, produced a significant reduction in the number of abdominal constrictions in mice. This reduction was dose related and was maximum with 200 mg/kg. (Table 1).

In the hot plate method, 100 and 200 mg/kg doses of *C. serratum* produced a significant increase in the area under curve (AUC, cm^2), similar to morphine. (Table 1).

3.3. Anti-inflammatory activity

In carrageenin-induced paw edema method (acute model), the standard anti-inflammatory agent (phenylbutazone) produced a significant reduction in the volume of paw edema in rats. Similarly, *C. serratum* in 100 and 200 mg/kg doses produced a significant reduction in the above parameters (Table 2).

In cotton-pellet granuloma method (chronic model), similar to phenylbutazone, *C. serratum* treatment reduced the weight of the cotton-pellets significantly compared to control animals (Table 2).

3.4. Antipyretic activity

Administration of TAB vaccine to rabbits produced a significant increase in rectal temperature at 60 min and was gradually decreasing after 120 min. Treatment with paracetamol (100 mg/kg) significantly reduced the rectal temperature. A dose dependent reduction in rectal temperature was observed during all periods of observation after administration with different doses of *C. serratum*. However, the reduction was significant only with 100 and 200 mg/kg doses (Fig. 1)

4. Discussion and Conclusion

Among several traditional claims, the usefulness of *C. serratum* in pain, inflammation and fever have been emphasised more in literature (Nadkarni, 1954). Hence, it was considered that investigations for these medicinal properties may give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to any systematic pharmacological screening so far.

The results of acute toxicity test indicated that *C. serratum* was fairly non-toxic. A significant reduction in acetic acid induced writhing indicated the potent antinociceptive activity of *C. serratum*. This has further been supported by the results of hot plate method where a significant increase in AUC was observed. However, the response was much less when compared to morphine. A potent anti-inflammatory effect for *C. serratum* was evidenced by the significant reduction in paw edema and cotton-pellet granuloma methods. However, the effect was less when compared to phenylbutazone. The reduction in pyrexia after *C. serratum* administration indicated the antipyretic activity of this plant. The response in higher doses was almost comparable to that of paracetamol.

Thus, the results of the present study confirmed the traditional claims suggested for *C. serratum*. The combination of antinociceptive, anti-inflammatory and antipyretic effect of *C. serratum* indicated a likelihood of intervention with prostaglandin synthesis, as prostaglandins have been established as a common mediator in all these responses. However, this possibility remains to be investigated in detail. Moreover, the active compounds responsible for these pharmacological actions also remain to be identified.

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