

**EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *CADABA TRIFOLIATA* (ROXB) WT. & ARN**

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ABSTRACT

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. Inflammation involves 2 basic processes early inflammatory response later followed by healing. There are two types of inflammation, Acute and Chronic. Acute inflammation is of short duration and represents the early body reaction, resolves quickly and is usually followed by healing. Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at same time. The anti-inflammatory activity of new substances can be evaluated by using various pre clinical screening method. The aim of the study includes

preliminary phytochemical screening of methanol extract of *Cadaba trifoliata* and its possible anti-inflammatory activity in experimental models.

KEYWORDS: Inflammation, carrageenan, formalin, *Cadaba trifoliata*.

INTRODUCTION

Cadaba trifoliata (roxb) Wt. & Arn, is a shrub, grows up to eight meter in height. It belongs to the family Capparaceae, found throughout India in dry land, also in plain land. Leaves are lanceolate, oblanceolate, oblong, palmate compound, arranged alternately; leaves surfaces are rough; deep and tap roots; stems hard wooded. In the traditional medicine, decoction of leaves of this is given in combination with castor oil and turmeric for the treatment of amenorrhoea and dysmenorrhoea. The plant is also used as scrofula, purgative, as poultice to

boils to remove suppuration^[1] anti rheumatic, anthelmintic, anti phlogistic, anti syphilic, antibacterial^[2,3] etc., However, *C. trifoliata* has not yet been explored scientifically for the analgesic and anti inflammatory properties. This encourages us to perform the present study, which includes preliminary phytochemical screening of methanol extract of *Cadaba trifoliata* and its possible anti-inflammatory activity in experimental models.

MATERIALS AND METHODS

Chemicals

Tween 80 (Alpha Labchem, Mumbai, India), indomethacin (Micro Labs, Mumbai, India), pentazocine (Ranbaxy, New Delhi, India), carrageenan (Sigma Chemicals, UAS), diclofenac sodium (Apex Labs, Chennai), animal feed (Hindustan liver, Mumbai, India.) were used in the study. All other chemicals used were of analytical grade.

Plant collection and authentication

Fresh aerial parts of *Cadaba trifoliata* were collected during the month of March 2008 from Western Ghats, Tamilnadu, India and authenticated by Dr.V.Chelladurai, Govt. Research officer, Botany C.C.R.A.S. Govt. of India, (Retired), Tirunelveli, Tamilnadu, India. A voucher specimen (CT/2007/001) was deposited in our laboratory for future reference.

Preparation of the extract

The collected aerial parts of *Cadaba trifoliata* was washed in running tap water, shade dried, powdered by mechanical grinder, sieved number 22 mesh and about 1 kg of powder was extracted exhaustively with methanol (150 ml×3), using a Soxhlet^[4] apparatus at a temperature range from 40-50°C for 18h. The yield was found to be 11.5% w/w. The extract was subjected to preliminary phytochemical analysis^[5] to identify the nature of phytoconstituents present in the extract. The extract was made in to suspension using 1% v/v aqueous Tween 80 during experiment.

Acute toxicity study

Acute oral toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines^[6], revised draft guidelines no. 423. The LD50 value was >2000 mg/kg.

Animals

Male wistar rats (150-180 gm) and albino mice (25-30 mg) were procured from central animal house of our institute and maintained under the environmental conditions (22-28±1°C, 60-70% relative humidity and 12 h dark/light cycle) and fed with standard rat feed and water *ad libitum*. The experimental procedure was approved by the Institutional Animal Ethics Committee (Approval no. 509/02/C/CPCSEA).

Estimation of anti inflammatory activity

Carrageenan- induced paw edema

The animals were segregated into four groups of six each and treated with any of the following. 1.0% aqueous (v/v) tween 80 (10 ml/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*), MCT (200 mg/kg, *p.o.*) and MCT (400 mg/kg, *p.o.*). After 30 min of drug administration, an acute hind paw edema was induced by single dose sub plantar injection of 0.1 ml of 1% w/v carrageenan in normal saline.^[7,8] The degrees of paw oedema of all the groups were measured by mercury displacement method using plethysmometer. The observation was made before and up to 4 hours after carrageenan injection at an interval of 1 hour. The percentage inhibition was calculated using the following formula.^[9] % inhibition = $1 - (V_t/V_c) \times 100$. Where V_t represent paw volume of MCT/indomethacine, V_c represent paw volume of tween 80 treated group.

Formalin induced paw edema model

The animals were treated as similar to carrageenan induced paw edema model, except that in place of carrageenan, formalin^[10] (20 µl of freshly prepared 2% formalin) was used as edematogenic agent. The treatment was continued for 6 consecutive days.

Statistical analysis

Experimental data were expressed as mean±SEM. The statistical significance was determined by one-way ANOVA followed by Dunnett's test using Graph Pad In Stat v.3.0.10.0 (GraphPad Software, Lajolla, CA, U.S.A) and the *P* value less than 0.01 were considered significant.

RESULTS

Phytochemical Analysis

Different chemical tests on methanol extract of aerial parts of *Cadaba trifoliata* showed the presence of alkaloids, steroids, flavonoids and tannins.

Anti-inflammatory activity

MCT exhibited significant ($p < 0.01$) reduction of hind paw edema on carrageenan induced paw edema model (table 4). The percentage inhibition of paw edema was found to be 4.30% (1/2h), 29.8% (1h), 38.2% (2h), 56.5% (3h) and 57.2% (4h) at 200 mg/kg and 8.60% (1/2h), 33.8% (1h), 45.4% (2h), 61.1% (3h) and 61.2% (4h) at 400 mg/kg, whereas indomethacin exhibited 12.8%, 44.6%, 51.6%, 67.2% and 67.5% inhibition at 1, 2, 3 and 4th hour respectively. MCT also reduced significantly the formalin induced paw edema by 55.8% and 67.6% at 200 and 400 mg/kg respectively after 6 days. The activity was comparable to the 79.5% shown by the indomethacin.

Table 1. Effect of methanol extracts of *Cadaba trifoliata* on carrageenan induced rat hind paw oedema.

Drugs and dose	Paw volume in ml (mean \pm S.E.M)				
	30 min	60 min	120 min	180 min	240 min
Tween 80 10ml/kg	0.47 \pm 0.17	0.74 \pm 0.17	0.97 \pm 0.14	1.31 \pm 0.05	1.26 \pm 0.25
Indomethacin 10 mg/kg	0.41 \pm 0.27 (12.8)	0.41 \pm 0.27 (44.6)	0.47 \pm 0.41 (51.6)	0.43 \pm 0.65 (67.2)	0.41 \pm 0.72 (67.5)
MCT 200 mg/kg	0.45 \pm 0.25 (4.30)	0.52 \pm 0.25 (29.8)	0.60 \pm 0.34 (38.2)	0.57 \pm 0.56 (56.5)	0.54 \pm 0.15 (57.2)
MCT 400 mg/kg	0.43 \pm 0.62 (8.60)	0.49 \pm 0.62 (33.8)	0.53 \pm 0.59 (45.4)	0.51 \pm 0.97 (61.1)	0.49 \pm 0.76 (61.2)

n = 6, $p < 0.01$ vs. control (Tween 80), values in parenthesis represents % inhibition of paw edema.

Table 2. Effect of methanol extracts of *Cadaba trifoliata* on formalin induced paw edema.

Treatment and dose	Paw volume in ml		Increase in paw volume	% inhibition
	Paw volume before treatment	Paw volume 6 days after treatment		
Tween 80 (control) 10 ml/kg	0.45 \pm 0.11	0.79 \pm 0.65	0.34 \pm 0.54	-
Indomethacin 10mg/kg	0.40 \pm 0.17	0.47 \pm 0.73	0.07 \pm 0.56	79.5
MCT 200mg/kg	0.43 \pm 0.16	0.58 \pm 0.36	0.15 \pm 0.20	55.8
MCT 400mg/kg	0.41 \pm 0.27	0.52 \pm 0.61	0.11 \pm 0.34	67.6

n = 6, mean \pm SEM, $p < 0.01$ vs. control.

DISCUSSION

Carrageenan is an established acute inflammatory model, which causes the release of histamine, serotonin, prostaglandin, bradykinin and lysosomes^[11, 12, 13] by biphasic mode^[14], resulting the formation of edema. Formalin is a chronic inflammatory model, the edema

formation by formalin results from the release of some neurogenic compounds and tissue mediated response.^[15]

The findings of the present study revealed that aerial parts of *Cadaba trifoliata* possess significant acute and chronic anti-inflammatory property. Most of the non steroidal anti inflammatory drug produced the activity by inhibiting prostaglandin synthesis. The reference drug, indomethacine is also a non steroidal anti inflammatory drug, which acts by inhibiting prostaglandin synthesis, anti inflammatory observed in the present study, possibly, involves the suppression or interference in the formation of prostaglandins and MCT may also have some inhibitory effect on an enzymes cyclooxygenase I and cyclooxygenase II, which are known to play a major role in prostaglandin synthesis.^[16, 17]

Flavonoids^[18, 19], tannins^[20], alkaloids and steroids^[21,22] have been reported in earlier studies for analgesic, anti-inflammatory and antipyretic properties. Therefore, in conclusion, the presence of alkaloids, phytosterols, tannins and flavonoids are responsible for the anti-inflammatory activity of methanol extract of *Cadaba trifoliata*. Since, formalin induced edema closely resembles human arthritis^[23], the present study support its folklore use as anti rheumatic. Further studies are in progress in our lab for the isolation of active constituent responsible for the activity.

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