

Anti-inflammatory activity of aerial parts and root extracts of *Croton bonplandianum* baill

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Abstract: The methanolic (95%) extracts of the aerial parts and root of *Croton bonplandianum* was evaluated for their anti-inflammatory activity against Carageenan-induced rat paw edema in albino rats. Both the extracts exhibited significant activity.

Index Terms: *Croton bonplandianum* Baill, Carageenan-induced rat paw edema, anti-inflammatory activity, toxicity study.

I. INTRODUCTION

The genus *Croton* belonging to the family Euphorbiaceae, comprises of 750 species and is habituated in tropical and sub-tropical regions. The present study is on *Croton bonplandianum* Baill (*Croton sparsiflorus* Morong) a shrub having high glabrescent stem is being grown as a weed abundantly in waste lands of India [1]. The vernacular names of the plant are “dog chilli” (English), Kala bhangra or ban tulasi (Hindi), galivana mokka or “kukka mirapa” (Telugu) and “relpoondu” (Tamil). Various medicinal properties like hypotensive, spasmolytic, antibacterial, antiseptic, and wound healing have been attributed to this plant [1 & 2]. The plant was found to have antimicrobial agents effective against multidrug- resistant microorganisms [3, 4] and anti-carcinogenic [2] activities. Various potential bioactive compounds like Rutin, crotosparine, dihydroporphines- crotosparinine, [5]; N-O-dimethylcrotosparine, N-methylcrotosparine, N-methylcrotosparinine, Beta-sitosterol [6], Phorbol derivative (I), Sparsiflorine, Crotoflorine [7], Isicrotosparinine, its N-Me derivatives, (+) – tetra-hydroglazievine and phorbol esters [8] Squalene, (9Z, 12Z)-octadeca-9, 12-dienoic acid, methyl 12-oxooctadec-9-enoate and phytol [9] were isolated from the leaves, aerial parts and seeds of *C. bonplandianum*.

Rural people of Andhra Pradesh use the latex of *Croton bonplandianum* for the treatment of arthritic pains where as the local people of coastal West Bengal use it for the treatment of cuts and wounds [2]. Literature survey indicated that there was no systematic anti-inflammatory activity done on the plant aerial parts and root extracts. Therefore, the plant was investigated for its anti-inflammatory activity as the folklore people use it as a remedy for rheumatism.

II. MATERIALS AND METHODS

The plant material for the study was collected from the road sides of Visakhapatnam district, Andhra Pradesh in the month of August 2006 and was authenticated by Prof. M. Venkaiah, Dept of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, INDIA. A voucher specimen (BGR-CB1) was deposited in the herbarium of Andhra University, Visakhapatnam for further reference. All the solvents and chemicals were of pure analytical grade and procured from Coastal enterprises Pvt. Ltd., Visakhapatnam. The carageenan was obtained from Merck Ltd., Ambernath.

Preparation of Extracts

Freshly collected plant materials were shade dried (1kg) and coarsely powdered. It was successively extracted with methanol (95%) in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure until a soft reddish brown mass was obtained (20g/kg of aerial parts and 15g/kg of root extracts). The dried extracts were preserved in a dessicator until further use. A weighed quantity of extract was suspended in 1% sodium carboxy methyl cellulose (CMC) solution for the experiment [10]. The phytochemical tests for the aerial parts extract were positive for the presence of steroids, triterpenoids, alkaloids, tannins and flavonoids. The phytochemical tests for the root extract were positive for the presence of steroids, alkaloids, triterpenoids and flavonoids [3, 4].

Acute toxicity studies [11]:

The rats of both the sex were acclimatized to the laboratory conditions for 10 days before commencement of the experiment. All animals were provided with standard pellet diet and water ad libitum. Wistar albino rats weighing between 200-250g were divided into groups of each ten with equal number of males and females. They were kept for overnight fasting. Three doses of the extract 100, 500 and 1000 mg/kg body weight, were given orally to three different groups. The animals were kept for observation for a period of 14 days. There were no signs of toxicity up to 1000 mg/kg body weight. The results were tabulated in Table 1.

Anti-Inflammatory Activity

Colony bred adult albino Wistar rats of either sex (supplied by B.N. Ghosh & Co., Calcutta) weighing 200g – 250g were selected. Animals were maintained at temperature $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity of $50 \pm 15\%$. A 12: 12 light: dark cycle was followed. All animals had free access to water ad libitum and standard pelleted laboratory animal diet (Ratan Brothers, India). The experimental protocol has been approved by the Institutional Animal Regulatory body of the Government (Regd. No. 516/01/A/CPCSEA).

The acute toxicity studies of methanolic extracts were carried out according to OECD guidelines. 1000mg/kg dose of both the extracts was found non-toxic in rats and was taken for further study.

The anti-inflammatory activity of the methanolic aerial parts and root extracts of *Croton bonplandianum* was determined in rats using carrageenan-induced paw oedema method [12]. The rats were divided into eight groups (I, II, III, IV, V, VI, VII, VIII), six in each group. Group I was treated with drug vehicle, 1% sodium CMC in saline (negative control); Group II was treated with non-steroidal anti-inflammatory drug ibuprofen (positive control); Group III, IV and V received methanolic aerial parts extract (200, 400 and 800mg/kg body weight) while the rest of the groups received methanolic root extract (200, 400, and 800 mg/kg body weight).

All the rats were weighed and the initial paw thickness of both hind paws of each rat was measured before induction of the oedema. The doses were administered orally as per kg body weight 1h prior to induction of edema. Paw edema induced in rats by injecting 0.1 ml of 1% carrageenan in normal saline into the sub-plantar surface of the right hind paw. The left paw was received the same volume of normal saline. The paw thickness was measured by using Zeitlin's Apparatus (constant loaded lever) [13]. The paw thickness was measured at regular intervals of one hour for a period of six hours after carrageenan injection. A significant reduction in the paw volumes of the test groups compared to drug vehicle-treated control animals were considered as anti-inflammatory response. The percentage inhibition of oedema was calculated using the following formula:

$$\text{Percentage increase in paw thickness} = \frac{(T_n - T_o)}{T_o} \times 100$$

Where,

T_n – paw thickness at time 't' h ($t = 1, 2, 3, \dots, n$)

T_o – paw thickness at time '0' h

$$\text{Therefore \% increase due to carrageenan} = (\% \text{ increase in right paw} - \% \text{ increase in left paw})$$

The percentage of maximal paw edema produced during 6h was calculated and plotted as line graph. The percentage of total paw edema as area under the time course curve (AUC) produced during 6h was also calculated and tabulated. Student's t-test was used to compare test and control group values and was considered as significant change. The difference between the treated and the control group would have arisen by chance is less than 5% of cases, is considered as statistically significant ($P < 0.05$); that arising in less than 1% of cases are highly significant ($P < 0.01$); while that arising in less than 0.1% of cases are very significant ($P < 0.001$).

III. RESULTS & DISCUSSION

The edema and percentage of rate of inhibition for each group were calculated. From the observed values, the percentage of maximal paw oedema produced during 6h was calculated and plotted as line graph. The percentage of total paw oedema as area under the time course curve (AUC) produced during 6h was also calculated and plotted as bar graph. The data obtained from the experiment were expressed as means \pm S.E.M, $N = 4$. Student's t-test was used to compare test and control group values. The differences in the test verses control values were considered statistically significant at $P \leq 0.05$. The *Croton bonplandianum* aerial parts and root extracts produced dose dependent inhibition of Carrageenan-induced rat paw edema and are tabulated in Table1 & 2. Root extract showed maximum anti-inflammatory activity.

Edema represents the early phase of inflammation in Carrageenan-induced paw edema and is the simplest and most widely used model for studying anti-inflammatory activity. The paw edema induced by the sub planar injection of Carrageenan in rats is biphasic, the first phase 1 hr. involves the release of serotonin and histamine while the second phase (over 1 hr.) is mediated by prostaglandins, the cyclooxygenase products and the continuity between the two phases is provided by kinins[14]. According to Vineger et. al., (1987) the development of the carrageenan induced edema derives from the release of cytoplasmic enzymes and inflammatory mediators like histamine and serotonin from mast cells and the increase of prostaglandin and thromboxanes via the catalytic activity of cyclooxygenase enzymes (COX-1 & COX-2) in the inflammatory area [15]. The macrophages in the carrageenan induced dermal tissue releases much interleukin-1 to induce accumulation of polymorphic nuclear cells (PMN) into the inflammatory area. The activated PMNs then release the lysozomal enzymes and activate oxygen, especially superoxide dismutase, to destroy connective tissue and induce paw swelling. The suppression of increase in paw oedema by ibuprofen is due to the inhibition of the enzyme arachidonate cyclooxygenase or COX [16]. Both extracts showed significant anti-inflammatory activity at 3 hr, against Carrageenan injection suggesting that the extracts predominantly inhibit the release of prostaglandins like substances. The percentage of inhibition of the total paw oedema shown by *C. bonplandianum* aerial parts and root extracts at a concentration of 200, 400 and 800 mg/kg was 62.05 ± 0.3 , 75.25 ± 0.12 , 86.49 ± 0.2 and 82.05 ± 0.3 , 91.95 ± 0.3 , 93.87 ± 0.34 respectively. The percentage of inhibition of the total paw edema by methanolic root extract (91.95 ± 0.3 for 400mg/kg and 93.87 ± 0.34 for 800mg/kg) was shown to be slightly more than the standard drug ibuprofen (89.02 ± 1.0).

In conclusion, the aerial parts and the roots of *Croton bonplandianum* showed significant anti-inflammatory activity which may be attributed to the presence of chemical constituents like steroids, triterpinoind, flavonoids and alkaloids.

Table 1 Toxicity studies of the methanol extracts of *C. bonplandianum* aerial parts and roots.

Treatment	Dose mg / kg b. wt.	No. of animals	No. of survives	No. of deaths	LD ₅₀ value
CBA	100	10	10	0	-
	500	10	10	0	-
	1000	10	10	0	>1000mg/kg b.wt.
CBR	100	10	10	0	-
	500	10	10	0	-
	1000	10	10	0	>1000mg/kg b.wt.

Table 2 Percentage inhibition of carrageenan-induced paw oedema in rats by prophylactic treatment with methanolic extract of *Croton bonplandianum* aerial parts and Ibuprofen.

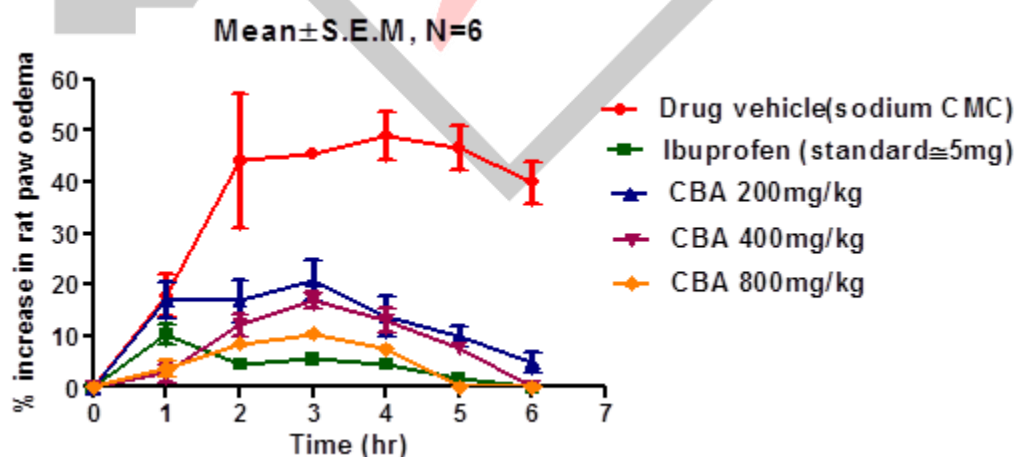
Significance: *P<0.05, **P<0.01, ***P<0.001.

Treatment	% Of Inhibition On The Maximal Paw Oedema	% Of Inhibition On The Total Paw Oedema (AUC)
Group 1 (Drug Vehicle)	0.0±5.2	0.0±0.9
Group 2(Ibuprofen 5mg)	81.6±1.8***	89.02±1.0***
Group 3(CBA 200mg)	56.0 ± 2.8***	62.05 ± 0.3***
Group 4(CBA 400mg)	64.26 ± 2.5***	75.25 ± 0.12***
Group 5(CBA 800mg)	78.53 ± 0.9***	86.49 ± 0.2***

Table 3 Percentage inhibition of carrageenan-induced paw oedema in rats by prophylactic treatment with methanolic extract of *Croton bonplandianum* root and Ibuprofen.

Significance: *P<0.05, **P<0.01, ***P<0.001.

Treatment	% Of Inhibition On The Maximal Paw Oedema	% Of Inhibition On The Total Paw Oedema (AUC)
Group 1 (Drug Vehicle)	0.0±5.2	0.0±0.9
Group 2 (Ibuprofen 5mg)	81.6±1.8***	89.02±1.0***
Group 6 (CBR 200mg)	60.9 ± 3.3***	82.05 ± 0.3***
Group 7 (CBR 400mg)	78.98 ± 3.7***	91.95 ± 0.3***
Group 8 (CBR 800mg)	89.66 ± 1.0***	93.87 ± 0.34***



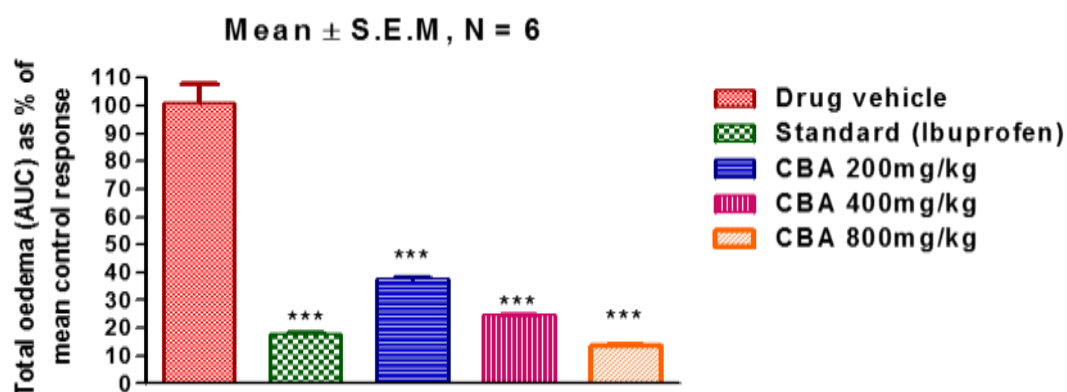


Figure 1 Effect of the crude methanolic extract of *Croton bonplandianum* (CBA) aerial parts (200, 400 and 800mg.kg⁻¹ body weight) and the standard drug Ibuprofen (2.5 x mol.kg⁻¹body weight) on A) the total paw edema and B) the maximal paw edema in carrageenan-induced paw edema.

Significance: *P<0.05,**P<0.01,***P<0.001; ns = not significant

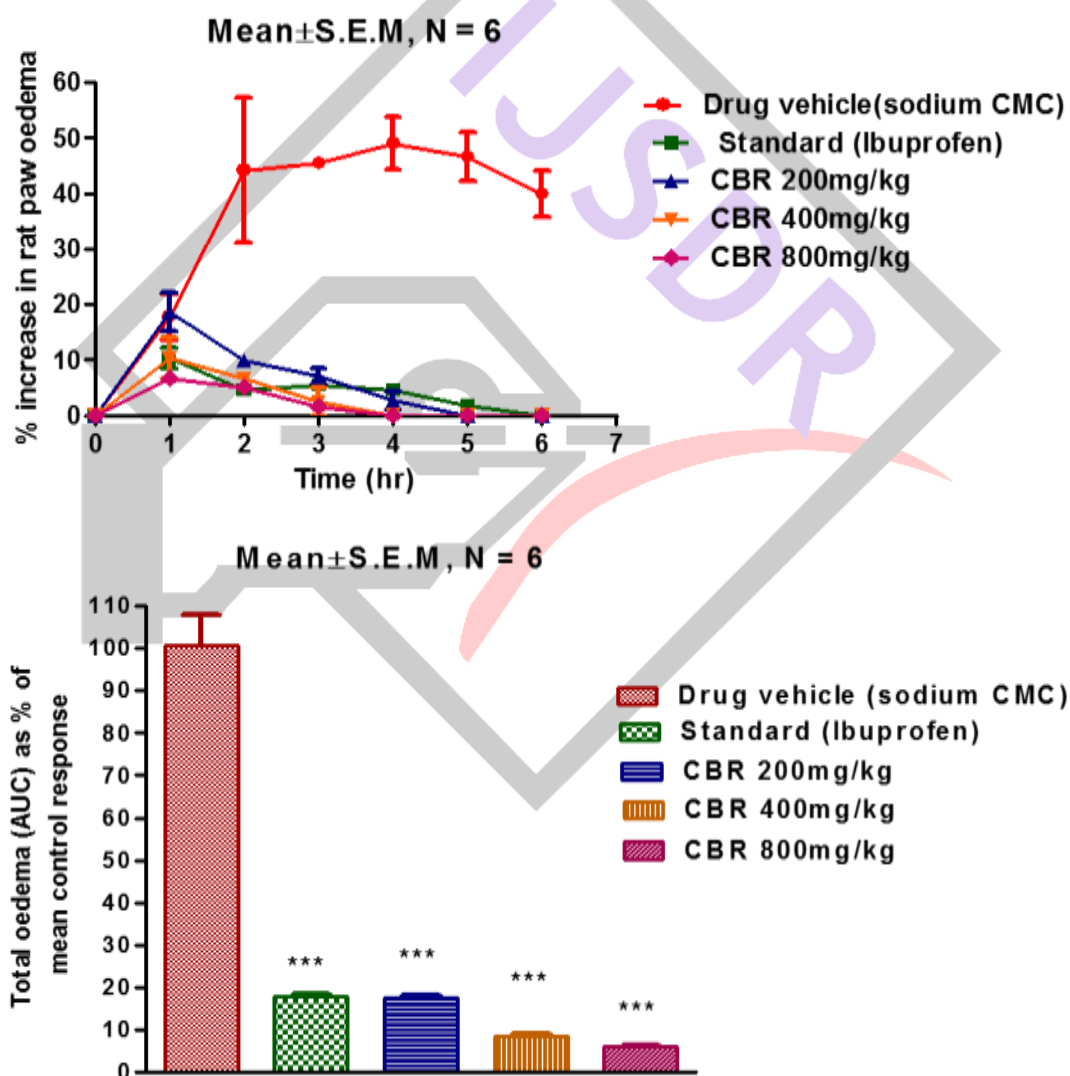


Figure 2 Effect of the crude methanolic extract of *Croton bonplandianum* root (CBR) (200, 400, 800mg.kg⁻¹ body weight) and the standard drug Ibuprofen (2.5 x 10⁻⁵mol.kg⁻¹ body weight) on A) the total paw edema and B) the maximal paw edema in carrageenan-induced rats.

Significance: *P<0.05,**P<0.01,***P<0.001; ns = not significant

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