

ORIGINAL RESEARCH ARTICLES

ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIPYRETIC EFFECTS OF *CROTON CAUDATUS* LEAVES

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(Received 03 September 2012) (Accepted 26 November 2012)

ABSTRACT

Croton caudatus (Euphorbiaceae) is claimed to be useful in treatment of sprains, arthritis and liver disorders in traditional system of medicine. In the present investigation antinociceptive, anti-inflammatory and antipyretic effects of the 80% aqueous ethanolic extract from leaves of *C. caudatus* have been evaluated. The ethanolic extract reduced the nociception induced by acetic acid at dose of 100, 200 and 400 mg/kg significantly ($P < 0.001$) and in dose dependent manner. In the hot plate test, the extract significantly increased the latency time of jump. The naloxone reversed the antinociception of the extract in hot plate test, indicating that *C. caudatus* ethanolic extract has morphinomimetic properties. The extract significantly decreased the rectal temperature in yeast-induced pyrexia in rats at highest tested dose. The ethanolic extract at a dose of 400 mg/kg produced 47% protection in carrageenan-induced edema. Steroids and/or triterpenoids, flavonoids and their glycosides are the major constituents of ethanolic extract of *C. caudatus*.

Keywords: *Croton caudatus*, Antinociceptive activity, Anti-inflammatory activity, Antipyretic activity, Ethanolic extract, Naloxone.

INTRODUCTION

Plant derived natural products play an important role nowadays to cure various disease conditions. Plant species are persistently being investigated for the identification of novel therapeutic agents based on traditional system of medicine. The Indian traditional system of medicine, especially Ayurveda, has put forward a number of therapeutic claims on plant drugs. However, it is important to conduct thorough investigation of as many traditionally used medicinal plants as possible with reference to modern system of medicines^{1,2}.

Croton caudatus Geisel. (Family: Euphorbiaceae) is a large scandent shrub. The stem bark of *Croton caudatus* is reported to contain triterpenoids: taraxerone, taraxerol and taraxeryl acetate, nor diterpenes viz crotoncaudin, teuvidin³, isocrotoncaudin⁴ and 5 α -stigmastane-3,6-dione⁵. Pradhan⁶ has reported the presence of dotriocotanol, β -amyirin and β -sitosterol in stem. Traditionally, the leaves are claimed as a cure for sprains, liver disorders, febrifuge and malaria^{7,8}. The leaf paste is applied topically for the treatment of arthritis and to treat paralysis⁹. To the best of our knowledge no pharmacological work was carried out on the leaves of *C. caudatus*. Present study was conducted to verify the claim and evaluate the *in-vivo* antinociceptive, anti-inflammatory and antipyretic properties of the leaves of *C. caudatus* by preparing 80% aqueous ethyl alcohol extract and screening resultant extract for the above said activities to track the activity.

MATERIALS AND METHODS

Plant material

C. caudatus leaves were collected in Guntur, Andhra Pradesh, India and authenticated by Dr. Radha,

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Taxonomist, Osmania University, Hyderabad, India. A voucher specimen (CC-03-09) is being maintained in G. Pulla Reddy College of Pharmacy, Hyderabad, India. The leaves were allowed to shade dry.

Extraction

Extraction of the dried leaves (500 g) was performed with 80% aqueous ethyl alcohol by maceration process for 5 days. The solvent was removed under reduced pressure using rotary evaporator, yielding 11% of dry extract. The preliminary phytochemical analysis was performed for the presence of alkaloids, terpenoids, steroids and their glycosides, cardiac glycosides, phenols, coumarins, flavanoids and their glycosides using standard procedures^{10,11}.

Chemicals

All the chemicals used were of analytical grade. The standard aspirin (USV Ltd, Mumbai), buprenorphin (Neon Laboratories Ltd, Mumbai), diazepam (Piramal Healthcare Ltd, Mumbai), naloxone (Samarth Pharma Ltd, Mumbai), brewer's yeast (Blue bird, Hyderabad), paracetamol (Cipla Ltd, Mumbai), carrageenan (SDH Fine Camp, Mumbai) were purchased locally. Ibuprofen was a gift sample from IOL Chemicals Ltd, Hyderabad, India.

Animals

Male Wistar rats, 10-12 weeks old (150-200 g) and Swiss Albino mice of either sex, 8-10 weeks old (22-26 g) were used in experiments. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum* during quarantine period. The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee approved all the procedures for investigations.

Acute toxicity studies

To determine the acute toxicity, a single oral administration of the aqueous ethanolic extract of

C. caudatus in different doses (500, 1000 and 2000 mg/kg) were administered to different groups of mice¹². Control group received the vehicle (CMC). The animals were observed continuously for initial 2 h, intermittently for 6 h, and then at 24 h, 48 and 72 h following drug administration for death and abnormality in behavioural changes.

Pharmacological Screening

Antinociceptive activity

Writhing test

The Siegmund *et al.*,¹³ technique modified by Koster *et al.*,¹⁴ was adapted to assess the antinociceptive activity in the pre-screened mice. Overnight fasted mice were divided into seven groups of six animals each. Group I served as control, received CMC; Group II-IV animals were treated with ethanolic extract of *C. caudatus* at an oral dose of 100, 200 and 400 mg/kg, respectively. Group V served as positive control and animals received aspirin (100 mg/kg).

An attempt was made to investigate the participation of opioid system in antinociceptive effect of this plant. A separate group of mice were pre-treated with non-selective opioid receptor antagonist, naloxone (5 mg/kg, *i.p*) which was injected 15 min before oral administration of the extract (400 mg/kg) and acetyl salicylic acid (100 mg/kg) to group VI & VII animals, respectively. After 30 min of extract/drug administration, all animals were given an *i.p* injection of 0.6% acetic acid (0.1 ml/10 g) and number of writhes produced in animals was recorded for 30 min.

Hot-plate method

The method of Eddy and Leimbach¹⁵ and Hosseinzabeh *et al.*,¹⁶ was employed in pre-screened mice. The temperature of hot plate (Eddy's hot plate, Dolphin, Mumbai) was maintained at 55±0.2°C. Animals were placed into the perspex square on the heated surface and the time between placement and licking and jumping was recorded as latency. Overnight fasted mice were divided into seven groups of 10 animals each. Control animals were treated with CMC (Group I), while buprenorphin (1 mg/kg, *p.o*) was

Table I: Effect of *C. caudatus* on acetic acid-induced abdominal writhing test

Group	Treatment	No. of Writhings	% Inhibition
I	Control	145.0 ± 0.45	-
II	Ethanol Extract 100 mg/kg	108.7 ± 0.33 ^a	25
III	Ethanol Extract 200 mg/kg	80.00 ± 0.37 ^a	44.82
IV	Ethanol Extract 400 mg/kg	67.83 ± 0.40 ^a	53.22
V	Aspirin 100 mg/kg	40.00 ± 0.45 ^a	72.41
VI	Ethanol extract 400mg/kg + Naloxone 5mg/kg	66.67 ± 0.49 ^a	54.02
VII	Aspirin 100mg/kg + Naloxone 5mg/kg	40.50 ± 0.43 ^a	72.06

¹Values are Mean ± SEM; n=6; ^aP<0.001, when compared with control

Table II: Effect of ethanolic extract of *C. caudatus* on hot plate test in mice

Group	Treatment (mg/kg)	Latency time (sec)					
		0 min	30 min	60 min	90 min	120 min	180 min
I	Control	3.00±0.36	3.00±0.36	2.83±0.30	3.17±0.30	3.50±0.43	3.33±0.42
II	Ethanolic Ext-100	3.33±0.33	4.00±0.36	4.67±0.49 ^a	5.00±0.36 ^b	5.67±0.56 ^a	5.50±0.22 ^b
III	Ethanolic Ext-200	2.83±0.30	4.33±0.33 ^a	6.00±1.06 ^a	5.67±0.56 ^b	6.17±0.48 ^b	6.17±0.30 ^c
IV	Ethanolic Ext-400	3.16±0.30	5.00±0.58 ^a	6.50±0.76 ^b	6.00±0.36 ^c	6.50±0.43 ^c	6.67±0.42 ^c
V	Buprenorphine-1	3.50±0.43	8.00±0.77 ^c	10.83±0.60 ^c	13.67±1.11 ^c	11.33±1.33 ^c	10.83±0.75 ^c
VI	Ethanolic ext-400 + Naloxone 5	3.50±0.22	4.00±0.45	4.50±0.22 ^b	4.83±0.54 ^a	5.83±0.30 ^b	5.17±0.54 ^a
VII	Buprenorphine-1 + Naloxone -5	3.33±0.21	3.50±0.22	4.33±0.33 ^b	4.50±0.67	4.83±0.30 ^a	5.17±0.30 ^b

Values are Mean ± SEM; n=10; ^aP<0.05, ^bP<0.01 and ^cP<0.001, when compared with control

Table III: Effect of ethanolic extract of *C. caudatus* on carrageenan induced rat paw edema

Groups	Treatment (mg/kg)	Mean paw edema (ml)			
		0 h	1 h	3 h	5 h
I	Control	0.65 ± 0.048	1.15 ± 0.048	1.68 ± 0.119	1.95 ± 0.055
II	Ethanolic Ext - 100	0.65 ± 0.063	0.87 ± 0.028	1.30 ± 0.037	1.47 ± 0.029 ^c
III	Ethanolic Ext - 200	0.57 ± 0.01	0.92 ± 0.021	1.12 ± 0.064 ^a	1.25 ± 0.048 ^c
IV	Ethanolic Ext - 400	0.55 ± 0.032	0.79 ± 0.066	1 ± 0.017 ^b	1.03 ± 0.056 ^c
V	Ibuprofen -100	0.48 ± 0.064	0.73 ± 0.064	0.76 ± 0.038 ^c	0.86 ± 0.046 ^c

Values are Mean ± SEM; n=6; ^aP<0.05, ^bP<0.01 and ^cP<0.001, when compared with control

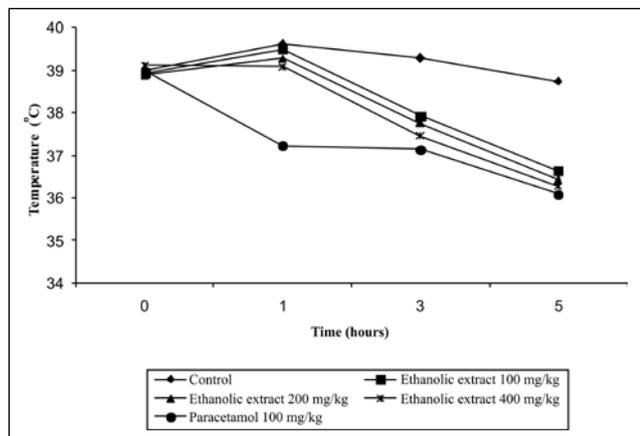


Fig. 1: Effect of ethanolic extract of *C. caudatus* on Brewer's yeast induced pyrexia

used as positive control (Group V), aqueous ethanolic extract of *C. caudatus* was administered orally at a dose of 100, 200 and 400 mg/kg respectively in CMC suspension (Group II-IV). The opioid receptor antagonist naloxone (5 mg/kg, *i.p*) was administered 15 min prior to oral administration of ethanolic extract (400 mg/kg, group VI) and buprenorphin (1 mg/kg, group VII). All the substances were administered 30 min before the experiment. The reaction time was recorded before and at 30, 60, 90, 120 and 180 min after substance administration. The latency period of 20 sec was defined as complete analysis and measurement was terminated to avoid injury.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the carrageenan-induced edema in rat paw according to the technique of Winter *et al*¹⁷. Overnight fasted rats were divided into five groups of six animals each. Group I served as control received CMC suspension orally. Group II-IV animals were treated with aqueous ethanolic extract at a dose of 100, 200 and 400 mg/kg respectively as a fine suspension of CMC, orally. Group V was orally administered with ibuprofen at a dose of 100 mg/kg, as a standard drug. An hour after administration, edema was induced in the animals by injecting 0.1ml of 1% w/V carrageenan in normal saline into the plantar aponeurosis of right hind paw. The measurement of the paw volume was carried out by means of plethysmograph, immediately and 1, 3 and 5 h after carrageenan injection.

Antipyretic activity

The antipyretic activity was studied by employing the technique of yeast-induced pyrexia in Wistar rats. Initial rectal temperature was recorded using Tele clinical thermometer (Digital Tele Thermometer, Dolphin). Pyrexia was induced by subcutaneous injection of a 15% Brewer's yeast suspension (10 ml/kg) as described by Burn¹⁸. Eighteen hours later pyrexia was assessed and those animals that do not show a minimum rise of 1.5°C were discarded. The selected pyretic animals were divided into five groups consisting of six animals in each. Group I animals served as control was given the vehicle. The group II-IV animals were administered with *C. Caudatus* extract at a dose of 100, 200 and 400 mg/kg respectively in CMC suspension, orally. The fifth group was administered with paracetamol (100 mg/kg). The rectal temperature was recorded just prior to and at 1, 3 and 5 h after administration of substances.

RESULTS

Preliminary phytochemical analysis of aqueous ethanolic extract revealed the presence of steroids and/or triterpenoids, flavanoids and their glycosides, phenolic compounds and the absence of cardiac glycoside and coumarins. In toxicity studies, no mortality and abnormal behavioural changes were observed with acute toxicity test dose of 2000 mg/kg b.w. in mice. Further the pharmacological studies were carried out at an oral dose of 100, 200 and 400 mg/kg.

Antinociceptive activity

The analgesic activity of *C. Caudatus* leaves extract was evaluated using acetic acid-induced writhing test and hot plate test. Table I illustrates the results of acetic acid induced writhing test. Oral administration of the extract at different dose levels elicited significant ($P < 0.001$) antinociceptive activity in acetic acid induced writhing test by decreasing the number of writhes in comparison to control mice. The analgesic activity was dose dependent. Among the three tests dose levels, the mice received

ethanolic extract at a dose of 400 mg/kg produced higher protection (53.22% inhibition). The activity produced by acetyl salicylic acid (72% inhibition) was high when compared to activity produced by ethanolic extract of *C. caudatus*. The administration of naloxone along with *C. caudatus* (400 mg/kg) and aspirin has not altered the significant antinociceptive effects. These results indicated that naloxone has no effect on antinociceptive properties of *C. caudatus* and acetyl salicylic acid.

The results of hot plate test are shown in Table II. Oral administration of ethanolic extract of *C. caudatus* at all dose levels, resulted in significant prolongation of the latency time in the hot plate test. The ethanolic extract at a dose of 400 mg/kg has demonstrated superior analgesic activity compared to the test doses of 100 and 200 mg/kg. However, the ethanolic extract produced the antinociceptive activity from 30 min after treatment. These effects reached their peak at 120 min after administration. Buprenorphin significantly ($P < 0.001$) increased the response latency period of mice with maximum effect obtained at 90 min after treatment. The activity of ethanolic extract is not comparable with buprenorphin during any course of time. The administration of opioid receptor antagonist naloxone significantly reversed the antinociceptive effects of ethanolic extract of *C. caudatus* (400 mg/kg) and buprenorphin from 30 min. These reversed effects were observed throughout the experiment.

Anti-inflammatory activity

Induction of acute inflammation in control rats resulted in prominent increase in paw thickness. From Table III, it is obvious that all extracts had shown significant anti-inflammatory activity. The ethanolic extract at a dose of 400 mg/kg produced maximum percent protection of 47% inhibition, whereas animals received a test dose of 100 and 200 mg/kg elicited 25 and 36% inhibition of inflammation at fifth hour. Ibuprofen produced significant ($P < 0.001$) protection of 56% at a dose of 100 mg/kg. The anti-inflammatory effect of *C. caudatus* is not comparable with the reference anti-inflammatory agent used in this study.

Antipyretic activity

The results of antipyretic activity are presented in Fig. 1. The basal body temperature of rats was elevated by 1.5°C with the subcutaneous administration of 15% Brewer's yeast in Wistar rats. Pyretic control animals maintained a mean body temperature of 39.29°C during the course of experiment. The reduction in the rectal temperature was observed from 1 h after treatment in all three test doses and the activity was highly significant at 3 h after extract administration. The animals received 400 mg/kg of ethanolic extract demonstrated maximum reduction in body temperature at 3 h and the activity is comparable with the reference standard paracetamol (100 mg/kg).

DISCUSSION

The results of the present study may help to establish the scientific basis for the utilisation of *C. caudatus* leaves for the treatment of pain and inflammations. Findings of this study demonstrated that the ethanolic extract of *C. caudatus* has got significant antinociceptive properties. The ethanolic extract significantly inhibited acetic acid-induced writhings in mice which are related to increase in peritoneal fluid levels of PGE_2 and PGF_2 ^{19, 20}. In addition, pre-treatment with non-selective opioid receptor antagonist, naloxone has not antagonised the *C. caudatus* (400 mg/kg) and acetyl salicylic acid induced analgesic activity in writhing test. The hot plate test was employed to verify if *C. caudatus* ethanolic extract could show central nociceptive effect, as the test is a specific central nociceptive test²¹. The *C. caudatus* ethanolic extract also significantly increased the latency time for jumping in the hot plate test. This effect largely depends on endogenous opioids. This effect was reversed by an opioid receptor antagonist naloxone pre-treatment in buprenorphin and extract (400 mg/kg) treated animals. These results suggest that antinociceptive effect of *C. caudatus* might involve the opioid receptors and the central analgesic properties, indicate that the *C. caudatus* ethanolic extract appear to have morphinomimetic activity.

The findings of this study also demonstrated that aqueous ethanolic extract of *C. caudatus* has got significant anti-inflammatory activity in carrageenan-induced paw edema. Carrageenan injected into the rat paw provokes a local acute inflammatory reaction that is a suitable criteria for evaluation of anti-inflammatory agents¹⁷. The aqueous ethanolic extract significantly decreased the rectal temperature of hyperthermic rats, bringing down the elevated temperature to almost normal. The efficacy of this dose (400 mg/kg) of ethanolic extract is comparable to the antipyretic activity of paracetamol.

Phytochemical analysis of ethanolic extract of *C. caudatus* indicated that the presence of steroids and/or triterpenoids, flavanoids and their glycosides are the major constituents, which may be responsible for the observed pharmacological activities of this species^{22,23,24}. But the exact mechanism of action is not known and has to be established in various models.

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