

**ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY
OF ROOTS OF *ICHNOCARPUS FRUTESCENS***

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Summary

The effect of methanolic extract of *Ichnocarpus frutescens* (MEIF) was evaluated for its anti-inflammatory activity by using on carrageenan, and cotton pellet induced granuloma tests for its effect on acute and chronic phase inflammation models in rats, as well as analgesic activity in mice. Dose of 300 mg/kg MEIF and Indomethacin could block the writhing response by 57.54 % and 73.73 % ($p < 0.05$), respectively. It was also indicated that the MEIF showed significant ($p < 0.05$) antinociceptive action in hot plate reaction time method in mice. Maximum inhibition (54.63 %) was obtained at the dose of 100 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas indomethacin produced 57.65 % of inhibition. In the chronic model the MEIF 300 mg/kg, indomethacin and dexamethasone standard drug showed decreased formation of granuloma tissue by 22.64, 29.63 % and 34.84 % respectively. The results indicate the potent analgesic and anti-inflammatory effects significant ($p < 0.05$) and therapeutic efficacy of *Ichnocarpus frutescens* extract on animal models which are comparable with those of standard drugs such as Pentazocine, Indomethacin and Dexamethasone respectively.

Keywords: *Ichnocarpus frutescens*; Analgesic activity; anti-inflammatory effect; cotton pellet induced granuloma.

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Introduction

Ichnocarpus frutescens R. Br (Apocynaceae), commonly known as siamlata, is an evergreen, laticiferous, woody creeper with rusty red appearance, found almost throughout India. Leaves opposite, elliptic-oblong to broadly lanceolate, coriaceous, pubescent when young; flowers fragrant, greenish white or purplish, in axillary or terminal panicles of cymose clusters; follicles cylindrical, slender, usually two, divaricately placed; seeds long, slender, black, comose. The roots are reported to possess demulcent, alterative, tonic, diaphoretic and diuretic properties and are used in fevers, dyspepsia and skin troubles, usually in combination with bitters and aromatics. The root powder is administered with milk for diabetes, stone in the bladder and as blood purifier. A decoction of the shoots is used in fevers. Leaves are boiled in oil and applied in headaches and fevers; they are also applied to wounds between fingers. (1, 2). The whole plant is used as a tribal medicine in atrophy, bleeding gums, cough, simple fevers, liver disorder and dysentery. Stalk and leaves in decoction is used in the treatment of skin eruptions. A decoction of the roots of Colocynth, Anantamul, Sariva (Sanskrit) and Hedyotis biflora prepared in the usual way is administered with the addition of powdered long pepper bdellium in chronic skin diseases, syphilis, loss of sensation and hemiplegia (3). Studies on chemical constituents of the plant revealed the presence of phenylpropanoids, phenolic acids, coumarines, flavonoids, sterols and pentacyclic triterpenoids. (4, 5). Pharmacological investigations have demonstrated that *I. frutescense* possess hepatoprotective and antioxidant activity (6). There are no scientific papers reporting on the other pharmacological properties of this plant, the aim of this study were to evaluate the anti-inflammatory and analgesic activities of the methanolic extract of *Ichnocarpus frutescens* (MEIF).

Materials and Methods

Plant material

The roots of *Ichnocarpus frutescens* R. Br were collected during the month of June 2005 from Chennai, Tamilnadu, India. The plant material was taxonomically Identified and authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai, India. A voucher specimen (PARC/24/06) has been deposited in the Herbarium of the Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, India, for future reference. The roots of the plants were dried under controlled temperature, powdered and passed through a 40 mesh sieve and stored in an air tight container.

Extraction procedure

The powdered plant material was extracted using 95 % methanol and the solvent was completely removed by vacuum distillation to yield a reddish-brown residue (yield 5.4 %, w/w). This methanolic extract (MEIF) was examined chemically and was observed to contain flavonoids, terpenoids, and sterols. These constituents were confirmed using thin-layer chromatography (TLC). The extract was stored in a refrigerator and a weighed amount of MEIF was suspended in 2 % aqueous Tween 80 solution and used for the present study.

Carrageenan induced paw edema

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema according to the technique of (7). After 16h fast rats were divided into five groups of six each. Group I served as control group received Tween 80 (5 ml/kg) of 2% w/v, orally. Group II, III and IV animals received MEIF at a dose of 100, 200 and 300 mg/kg as a fine suspension in 2 % v/v aqueous Tween 80 solution orally. Group V was orally administered indomethacin at a dose of 10 mg/kg as a standard drug. After 1 h, 0.1 ml of 1 % w/v carrageenan suspension was injected subcutaneously in to the planter surface of the right hind paw. The paw volume was measured using a plethysmometer immediately and 3 h after carrageenan injection.

Cotton pellet induced granuloma

The rats were divided into five groups, each group consisting of six animals. After shaving off the fur, the animals were anaesthetized. Sterile pre-weighed cotton pellets (50 ± 1 mg) were implanted in the axilla region of each rat through a single needle incision (8). MEIF at a dose of 100, 200 and 300 mg/kg), positive controls (indomethacin 10 mg/kg) and vehicle control (2 % v/v aqueous Tween 80 solution, 5 ml/kg) were administered to the respective group of animals for seven consecutive days from the day of cotton pellet implantation. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the pellets was regarded as measure of granuloma formation.

Hot plate (Thermal) method

The hot plate test described by Turner (9) was used. The mice were first treated with different doses of *I. Frutescens* (100, 200 and 300 mg/kg p.o) after 1 h of extract administration they were placed on a hot plate maintained at 55 ± 1.0 °C. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time. Pentazocine (5 mg/kg s.c) was used as reference drug.

Acetic acid-induced writhing test

This test was done using the method described by Collier et al (10). The muscular contractions were induced in rats by intra peritoneal injection of 7 % solution of acetic acid (10 ml/kg). Immediately after administration of acetic acid, animals were placed in transparent cages, and the number of 'stretching' per animal was recorded during the following 15 min. methanol extract of *Ichnocarpus frutescens* were orally administrated in doses of (100, 200 and 300 mg/kg) and Indomethacin (10 mg/kg) were administered 30 min before the acetic acid injection.

Statistical Analysis

The results are presented as mean \pm SEM. "One-way ANOVA with Dunnett's post test was performed using Graph Pad Prism version 4.00 for Windows, Graph Pad Software, San Diego California USA, P values less than 0.05 were considered significance

Results

Inhibition of carrageenan induced paw edema

Intraplantar injection of carrageenan in the hind paw induced gradual increase in the edema paw volume in the control group. MEIF at doses of 100, 200 and 300 mg/kg significantly ($p < 0.01$) inhibited the edema formation of rat paw at 3 h after carrageenan challenge (Table 1). The reference drug, indomethacin at a dose of 10 mg/kg markedly reduced the paw edema.

Table1: Effect of MEIF on carrageenan-induced rat paw oedema

Treatment	Dose	% Increase in paw volume	% inhibition
Carrageenan control	-	61.89 ± 0.40	-
Indomethacin	10 mg/kg	26.27 ± 0.24 ^b	57.55
MEIF	100 mg/kg	28.07 ± 0.21 ^a	54.63
MEIF	200 mg/kg	29.68 ± 0.25 ^a	52.04
MEIF	300 mg/kg	30.70 ± 0.12 ^a	50.39

Each value represents the mean ± S.E.M., n = 6. ^a $P < 0.01$, ^b $p < 0.05$ compared with control, Dunnett's *t*-test after analysis of variance.

Inhibition of cotton pellet-induced granuloma

Animals treated with MEIF at a dose of 100, 200 and 300 mg/kg significantly ($p < 0.01$) inhibited the granuloma formation (Table 2). Indomethacin (10 mg/kg, p.o.), a reference drug elicited marked reduction in granuloma formation.

Table2: Effect of MEIF on cotton pellet-induced granuloma in rats

Treatment	Dose	Weight of granulation (mg)	% Inhibition
Control	-	91.01 ± 0.17	-
Dexamethasone	0.5mg/kg	62.94 ± 0.19	34.84
Indomethacin	10 mg/kg	68.59 ± 0.20	29.63
MEIF	100 mg/kg	81.96 ± 0.20	9.94
MEIF	200 mg/kg	74.49 ± 0.27	18.15
MEIF	300 mg/kg	70.40 ± 0.24	22.64

Each value represents the mean ± S.E.M., n = 6. $P < 0.01$ compared with control, Dunnett's *t*-test after analysis of variance.

Acetic acid-induced writhing test

Dose dependent antinociceptive effect was noted with the extract at the tested dose levels (Fig.1). Maximum percentage of inhibition of writhing responses exhibited by the MEIF extract at 300 mg/kg was 46.68 %, while the same at 200 and 100 mg/kg showed 44.58 % and 32.53 % reduction in acetic acid induced writhing responses respectively, which was comparable to that of standard indomethacin (10 mg/kg) that caused 73.73 % pain reduction.

Fig 1: Effect of MEIF on acetic acid-induced abdominal constrictions in mice

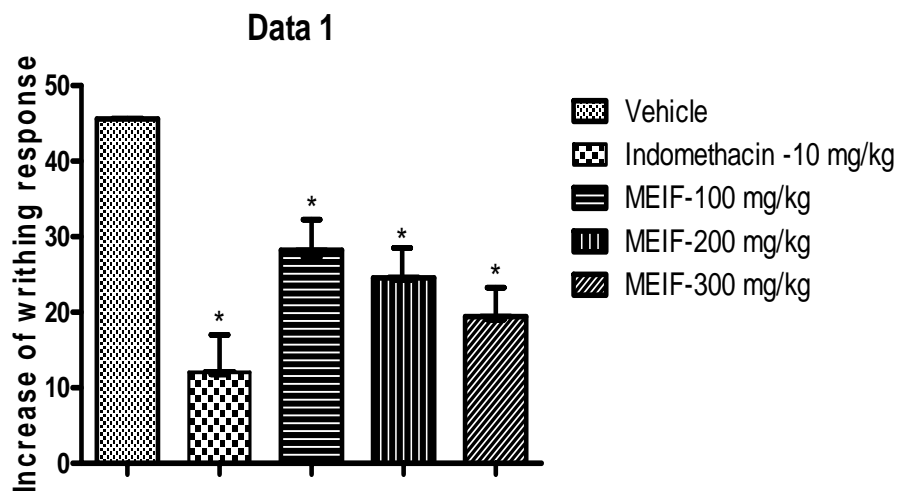


Fig 1: Effect of MEIF on writhing response test. Data are shown as Mean \pm S.E.M (n=6) P < 0.05 compared with control, Dunnett's t-test after analysis of variance.

Hot plate test

Fig.2 shows the results of the hot plate test. Three doses of extracts of *Ichnocarpus frutescens* increased the reaction time in a dose-dependent manner to the thermal stimulus. The highest nociception of thermal stimulus was exhibited at a higher dose of extracts 300 mg/kg of MEIF (62.78%), which is comparable to the Pentazocine (72.91 %)

Fig 2: Effect of MEIF on Hot plate (Thermal) induced pain responses in mice.

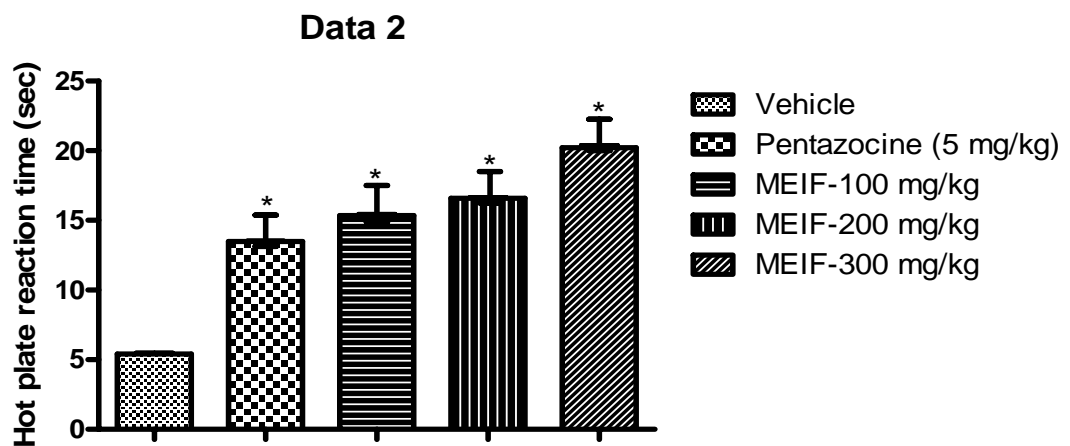


Fig 2: Central nervous system analgesic effect of MEIF in comparison with the Pentazocine standard the negative vehicle group. Each value represents the mean \pm S.E.M., $n = 6$. $P < 0.01$ compared with control, Dunnett's t-test after analysis of variance.

Discussion

MEIF significantly suppressed the carrageenan induced rat paw oedema 3 h after carrageenan challenge. Carrageenan induced rat paw oedema is commonly used as an experimental animal model for evaluation of the anti-inflammatory potential of natural products (7) and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenan, a more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents (11).

The cotton pellet granuloma bioassay is considered a model for studies on chronic inflammation and considered as a typical feature of established chronic inflammatory reaction (12). MEIF exhibited significant reduction in the granuloma formation in the cotton pellet-induced granuloma in rats. This reflected that MEIF may be effective in chronic inflammatory conditions. The result of present study indicates that crude fraction of methanol extract of *Ichnocarpus frutescens* possess significant anti-inflammatory activity on both acute and chronic inflammation.

Since acetic acid induced writhing can be considered a model of prostaglandin synthesis sensitive response (13), the enhanced analgesic effect of MEIF may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. The enhanced analgesic effect of MEIF in the hot plate test might again be due to the inhibitory action on prostaglandin synthesis. The validity of this test has been shown even in the presence of substantial impairment of motor performance, and the activity is supraspinally mediated (14); therefore MEIF may be exhibiting its analgesic effect by involving both peripheral and central nervous mechanisms. Anti-inflammatory activities of many plants have been attributed to their high sterol/ triterpenoid saponins (15). A survey of literature revealed that different pentacyclic triterpenoids (4) and flavonoids (5). Many naturally occurring triterpenoids exhibited a good anti-inflammatory activity have been isolated from various plants (16, 17). Therefore, the anti-inflammatory activity of methanolic extract of *I. frutescens* seems to be due to the high flavonoids and triterpenoids constituents. Though at this stage it is not possible to identify the exact phytochemical constituent(s) responsible for anti-inflammatory activities of *Ichnocarpus frutescens*, it may be assumed that the effects could be due chemicals present in the methanolic extract examined by qualitative test and these constituents were confirmed using thin-layer chromatography (TLC). The result of present study indicates that methanol extract of *Ichnocarpus frutescens* roots possess significant analgesic and anti-inflammatory activity on both acute and chronic inflammation. Further detailed investigation is underway to determine the exact phytoconstituents, which are responsible for the anti-inflammatory activity.

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