



Evaluation of anti-inflammatory activity of *Solanum trilobatum* roots

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SUMMARY

This study evaluated the anti-inflammatory potential of the crude alkaloidal fraction (CAF) of methanol extract of *Solanum trilobatum* Linn. (Solanaceae) root in animal models of inflammation. Crude alkaloidal fraction at doses of 25, 50 and 100 mg/kg significantly ($p < 0.01$) reduced carrageenan induced rat paw volume at 3 h after carrageenan challenge as compared to control group of animals. CAF (25, 50 and 100 mg/kg) significantly ($p < 0.01$) and dose dependently suppressed cotton pellet induced granuloma formation. Topical application of CAF (1, 5 and 10 mg/ear) markedly inhibited multiple application of TPA in mice. CAF elicited pronounced inhibitory effects on formaldehyde and adjuvant induced arthritis in rats. These results indicate that CAF of methanol extract of the *Solanum trilobatum* has anti-inflammatory activity in acute and chronic inflammation.

Key words: Anti-inflammatory activity; carrageenan induced rat paw; cotton pellet induced granuloma; tetradecanoyl phorbol acetate; formaldehyde induced arthritis; adjuvant induced arthritis; crude alkaloidal fraction; *Solanum trilobatum*

INTRODUCTION

Solanum trilobatum Linn. (Solanaceae) is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka in moist places. This plant is well known in Ayurveda and Siddha system as 'Alarka' and 'Tuduvelai', respectively. The Siddha system of medicine uses a ghee prepared from this plant for treatment of tuberculosis. The decoction of entire plant is has been administered to cases of acute and chronic bronchitis (Nadkarni, 1976). Roots, berries and flowers are used for cough (Anonymous, 1972). Previous reports indicate that

some chemical constituent, such as solasodine and β -solamarine have been isolated from whole plant (Purushothaman *et al.*, 1987).

Pharmacological investigations have demonstrated that *S. trilobatum* possess antioxidant activity (Sini and Devi, 2004), hepatoprotective activity (Shahjahan *et al.*, 2004), anti-inflammatory activity (Emmanuel *et al.*, 2006), anti cancer (Venugopal *et al.*, 2007) and antimicrobial activity (Swapnalatha and Kannabiran, 2007). In the preliminary study, the crude alkaloid fraction of methanol extract of *S. trilobatum* (MEST) root exhibited significant anti-inflammatory activity on carrageenan induced rat paw oedema. Therefore, the present study has been planed to investigate the anti-inflammatory activity of crude alkaloids fraction of methanol extract of *S. trilobatum* root in different experimental models of acute and chronic

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inflammation.

MATERIAL AND METHODS

Plant material

The roots of *S. trilobatum* (Solanaceae) were collected during the month of June 2005 from Tirukovilur, 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/23/06) has been deposited in the Herbarium of the Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, India, for future reference.

Preparation of extract

The suspension of dried, finely ground powder of *S. trilobatum* root (5 kg) in toluene: water and con. HCl (3 : 2 : 1), was refluxed under stirring for 5 h. The reaction mixture was subsequently alkalinized with 40% aq. NaOH and refluxed again under stirring for 2 h. Following phase separation, the upper, pale-yellow toluene layer was separated out, and the remaining dark brown aqueous mixture was extracted twelve times with 100 ml portions of toluene. The combined toluene extracts were clarified with charcoal, and then concentrated in vacuum to small volume. The concentrated toluene extracts were extracted with equal volume of 25% aq. Acetic acid by stirring twice for 1 h at room temperature. The aqueous acid extract was separated off from the toluene layer and the alkalinized with 25% aqueous ammonia. The mixture was briefly heated, then cooled at room temp. The crude alkaloids which precipitated were filtered off, washed with water dried over anhydrous sodium sulphate and evaporated to yield crude alkaloids (4.1 g).

Phytochemical study of crude alkaloidal fraction

Phytochemical evaluation was undertaken to ascertain the qualitative chemical composition of

crude alkaloidal fraction of *S. trilobatum* using commonly employed and readily performed chromatographic technique (TLC) to identify the major phytoconstituents. The crude alkaloidal fraction was examined separately on silica gel G plates using the solvent mixture: CHCl₃-MeOH-1% aq. NH₃ (2 : 2 : 1). The spots were revealed by saturated solution of ceric sulphate in con. H₂SO₄ (Stahl, 1969). The crude alkaloidal fraction gave the picture revealing the presence of three spots having the following R_f values and colors: 24 (pink), 56 (yellow) and 75 (blackish).

Animals

Albino (Wister) rats 180 - 200 g of either sex and albino mice (20 - 25 g) were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum*. The animals were acclimatized for period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 711/02/A/CPESEA).

Acute toxicity study

Acute toxicity study was performed as per OECD-423 guidelines (Ecobiochon, 1997). Swiss Albino rats of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout. The fasted rats were divided into different groups of six animals each. CFA was administered orally at a dose of 5 mg/kg. Mortality in each group was observed for 7 days. The mortality was not observed, the procedure was repeated at doses 50, 300 and 1000 mg/kg.

Carrageenan induced paw edema

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema according to the technique of (Winter *et al.*, 1962). After 16 h 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 CFA at a dose of 25, 50 and

100 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 into the planter surface of the right hind paw. The paw volume was measured using a plethysmometer immediately and 3 h after carrageenan injection (Perianayagam *et al.*, 2008).

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 (Winter and Porter, 1957). CAF (25, 50 and 100 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; 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 (Perianayagam *et al.*, 2008).

Mice ear inflammation induced by multiple topical applications of TPA

Chronic inflammation was induced by topical application on alternate days of 20 µl of tetradecanoyl phorbol acetate (TPA) (2 mg/ear × 5 times) to both the inner and outer surface of the ear of each mouse (Stanley *et al.*, 1991). CAF (0.5, 1 and 2 mg/ear) and dexamethasone (0.05 mg/ear) were applied topically twice daily for 4 days in the morning immediately after TPA application and 6 h later. On the last day, the compounds were applied only in the morning and ear thickness was

measured 4 h after the last TPA application (Perianayagam *et al.*, 2008).

Induction of formaldehyde-induced arthritis

Experimental arthritis was produced by subcutaneous injection of 0.1 ml (2% w/v in normal saline) formaldehyde solution into the plantar tissue of the right hind paw on the first and third day (Brownelee, 1950). Different groups were administered (25, 50 and 100 mg/kg) or aspirin (100 mg/kg) or vehicle control (5 ml/kg) for 7 days. The paw volume was measured below the ankle joint using a digital vernier caliper. The mean increase in the paw diameter of each group was measured prior to the injection of formaldehyde and once a day, every day after 1 h of treatment up to the 7th day of treatment. On day 3 of the treatment, the paw volume was measured before the injection of formaldehyde (Perianayagam *et al.*, 2008).

Induction of adjuvant induced arthritis

Arthritis syndrome was induced by subcutaneous injection of 0.1 ml complete Freund's adjuvant (CFA) into the plantar tissue of the right hind paw of each rat (Newbould, 1963). The complete Freund's adjuvant injected rats challenged with CAF (25, 50 and 100 mg/kg) or indomethacin (4 mg/kg) or vehicle control (5 ml/kg) administered orally, starting at 8 days after complete-Freund's adjuvant injection for 14 consecutive days. The linear cross-section immediately below the ankle joint was measured at 2 h, 8, 12, 16, and 24 days post-injection of CFA. The percentage increase in paw thickness was calculated (Perianayagam *et al.*, 2008).

Statistical Analysis

The results are presented as mean ± S.E.M. One way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons were used for statistical evaluation. *P* values less than 0.05 were considered significance

RESULTS

Acute toxicity study

There were no overt signs and symptoms of toxicity observed even at dose of 1000 mg/kg treated rats.

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. CAF of *S. trilobatum* at doses of 25, 50 and 100 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.

Inhibition of cotton pellet-induced granuloma

Animals treated with CAF at doses of 25, 50 and 100 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.

Inhibition of mice ear inflammation induced by multiple topical applications of TPA

Topical application of tetradecanoyl phorbol acetate

Table 3. Effects of crude alkaloid fraction of MEST on chronic inflammation induced by TPA in mice

Treatment	Dose (mg/kg)	% Increase in ear thickness
Control	-	35.29 ± 0.08
Dexamethasone (Standard)	0.05	25.23 ± 0.07*
CAF	0.5	30.64 ± 0.13*
CAF	1	23.49 ± 0.09*
CAF	2	22.57 ± 0.08*

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

to mouse produced a short inflammatory response. CAF (1, 5 and 10 mg/ear) exhibited a significant ($p < 0.01$) inflammation reduction in prolonged inflammation induced by multiple application of TPA to mouse ear (Table 3). The reference drug, dexamethasone demonstrated pronounced reduction of inflammation.

Inhibition of formaldehyde induced arthritis

CAF administered orally at doses of 25, 50 and 100 mg/kg for 7 consecutive days produced significant ($p < 0.01$) inhibition of the formaldehyde induced increase in paw thickness, when compared to

Table 1. Effects of crude alkaloid fraction of MEST on carrageenan induced rat paw oedema

Treatment	Dose	% Increase in paw volume	% inhibition
Carrageenan control	5 ml/kg	61.89 ± 0.40	-
Indomethacin	10 mg/kg	26.27 ± 0.24*	57.55
CAF	25 mg/kg	34.35 ± 0.13*	44.49
CAF	50 mg/kg	26.79 ± 0.18*	56.71
CAF	100 mg/kg	30.52 ± 0.15*	50.68

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

Table 2. Effects of crude alkaloid fraction of MEST on cotton pellet induced granuloma in rats

Treatment	Dose	Weight of granulation (mg)	% Inhibition
Control(Tween 80) 2% w/v	5 ml/kg	91.01 ± 0.20	-
Indomethacin	10 mg/kg	65.19 ± 0.08*	28.37
CAF	25 mg/kg	80.76 ± 0.13*	11.26
CAF	50 mg/kg	76.46 ± 0.15*	15.98
CAF	100 mg/kg	70.39 ± 0.08*	22.65

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

Table 4. Effects of crude alkaloid fraction of MEST on formaldehyde induced paw oedema in rats

Group	Dose (mg/kg)	% Oedema rate						
		1 day	2 day	3 day	4 day	5 day	6 day	7 day
Control	-	45.32 ± 0.06	33.58 ± 0.09	30.71 ± 0.12	40.21 ± 0.12	38.49 ± 0.06	29.57 ± 0.10	15.51 ± 0.11
Aspirin	100	25.73 ± 0.15* (43.22)	20.75 ± 0.16* (38.20)	22.70 ± 0.08* (26.08)	19.60 ± 0.14* (51.25)	16.74 ± 0.15* (56.50)	15.65 ± 0.16* (47.07)	9.58 ± 0.16* (38.23)
CAF	25	32.69 ± 0.17* (27.86)	30.52 ± 0.21* (9.11)	26.48 ± 0.23* (13.77)	28.25 ± 0.10* (28.99)	22.55 ± 0.14* (41.41)	19.27 ± 0.13* (34.83)	12.48 ± 0.11* (19.53)
CAF	50	28.54 ± 0.12* (37.02)	29.11 ± 0.18* (40.28)	24.71 ± 0.11* (19.53)	21.43 ± 0.15* (46.70)	18.16 ± 0.12* (52.81)	18.96 ± 0.42* (35.88)	8.73 ± 1.41* (43.71)
CAF	100	26.23 ± 0.21* (42.12)	25.20 ± 0.15* (24.95)	22.12 ± 0.15* (27.97)	19.80 ± 0.21* (50.75)	16.94 ± 0.25* (55.98)	13.86 ± 0.26* (53.12)	9.75 ± 0.21* (37.13)

Each value in parenthesis indicates the percentage inhibition rate.

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

Table 5. Effects of Crude alkaloid fraction of MEST on complete Freund's adjuvant (CFA) induced paw oedema in rats

Group	Dose (mg/kg)	% Oedema rate				
		8 day	12 day	16 day	20 day	24 day
Control	-	60.85 ± 0.21	66.12 ± 0.22	62.88 ± 0.11	60.75 ± 0.15	56.76 ± 0.17
Indomethacin	4	50.63 ± 0.17* (20.18)	52.70 ± 0.15* (20.29)	41.55 ± 0.18* (33.92)	37.73 ± 0.14* (37.89)	31.24 ± 0.26* (44.96)
CAF	25	53.18 ± 0.22* (12.60)	54.72 ± 0.14* (17.24)	43.17 ± 0.15* (31.34)	41.43 ± 0.17* (31.80)	35.57 ± 0.12* (37.33)
CAF	50	54.55 ± 0.11* (10.35)	61.55 ± 5.02* (6.91)	48.18 ± 0.24* (23.37)	48.44 ± 0.20* (20.26)	39.30 ± 0.21* (30.76)
CAF	100	52.41 ± 0.09* (13.87)	55.12 ± 0.24* (16.63)	43.95 ± 0.27* (30.10)	41.92 ± 0.19* (30.99)	35.82 ± 0.25* (36.89)

Each value in parenthesis indicates the percentage inhibition rate.

*Oedema value was measured 2 h after the drug administration, which was performed at 8 days after the CFA injection. Each value represents the mean ± S.E.M., n = 6. **p* < 0.01 compared with control, Dunnett's *t*-test after analysis of variances.

the control group of animals (Table 4). The reference drug, aspirin (100 mg/kg) caused marked reduction of formaldehyde induced increase in paw thickness.

Inhibition of adjuvant induced arthritis

CAF (25, 50 and 100 mg/kg) elicited significant (*p* < 0.01) and dose dependent inhibition of adjuvant induced increase in paw thickness when compared to the control group animals (Table 5). The reference drug, indomethacin at 4 mg/kg profoundly inhibited the adjuvant induced arthritis.

DISCUSSION

CAF 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 (Winter *et al.*, 1962) 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 (Castro *et al.*, 1968).

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 (Spector, 1969). CAF exhibited significant reduction in the granuloma formation in the cotton pellet-induced granuloma in rats. This reflected that CAF may be effective in chronic inflammatory conditions.

Topical application of phorbol ester provides skin inflammation model which is more suitable for evaluation for topical and systemic anti-inflammatory agents. It has been established that TPA produces its inflammatory effect by activation of protein kinase C with subsequent cytosolic phospholipase A₂ stimulation, arachidonic acid mobilization and biosynthesis of prostaglandin and leukotrienes (Nishizuka, 1988). The chronic inflammation elicited using repeated application of TPA is inhibited principally by corticosteroids such as dexamethasone (Stanley *et al.*, 1991) Repeated topical application of CAF significantly inhibited ear oedema induced by TPA in mice.

Treatment of CAF for 7 days, there was significant inhibition of formalin- induced rat paw oedema. The formalin injection into rat paw produces localized inflammation and pain. The formalin induced rat paw oedema is one of the most suitable tests to evaluate anti-proliferative activity and to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritics (Greenwald, 1991). In case of cotton pellet induced granuloma, there was significant reduction in granular tissue formation. This result is in confirmation with the anti-proliferate activity exhibited in formalin induced paw oedema in rat.

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation, hyper proliferation of the synovial lining and cartilages destruction (Schiff, 1997). In this study, Complete Freund's Adjuvant-induced a significant increase

in rat paw thickness related to the pathogenesis of the disease. The arthritic rats treated with CAF demonstrated significant reduction in paw thickness.

According to the literature, the crude alkaloidal fractions of *S. trilobatum* whole plants chemical analysis have revealed solasodine and β -solamarine (Purushothaman *et al.*, 1987). Recent studies showed that solasodine, sobatum and methanol extract of *Solanum trilobatum* leaves exhibited significant anti-inflammatory activity against carageenan induced rat paw oedema (Emmanuel *et al.*, 2006). In our phytochemical studies, the crude alkaloidal fraction showed three spots on thin layer chromatography.

The result of present study indicates that crude alkaloidal fraction of methanol extract of *S. trilobatum* root possess significant anti-inflammatory activity on both acute and chronic inflammation. These preliminary reports lend to support to the use of this plant in folk medicine for inflammation (Nadkarni, 1976), mainly because of low toxicity. Further, work is needed to clarify the exact active constituents responsible for anti-inflammatory action and their mechanism of action.

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