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Original Article

Evaluation of Anti-inflammatory and Analgesic Activity of Root Extract of *Solanum Trilobatum* Linn

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Abstract

Methanolic extract of *Solanum trilobatum* Linn. (MEST) belonging to the family of Solanacea was evaluated by hot plate and acetic acid induced writhing methods to assess its analgesic activity. The extract was also evaluated for its anti-inflammatory activity by subjecting into 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. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. 300 mg/kg doses of MEST and indomethacin could block the writhing response by 46.68% and 73.73% ($p<0.05$), respectively. It was also indicated that the MEST showed significant ($p<0.001$) antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug pentazocine treated controls, suggesting the central activity of MEST. Maximum inhibition (56.71%) was obtained at a dose of 100 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas indomethacin (standard drug) produced 57.65% of inhibition. In the chronic model (cotton pellet induced granuloma) the MEST 300 mg/kg, indomethacin and dexamethasone standard drug showed decreased formation of granuloma tissue by 21.48%, 29.63% and 34.84%, respectively. The results indicate the potent analgesic and anti-inflammatory effects and therapeutic efficacy of *Solanum trilobatum* extract on animal models which are comparable with those of standard drugs such as pentazocine, indomethacin and dexamethasone, respectively.

Keywords: *Solanum trilobatum*; Analgesic activity; Anti-inflammatory effect; Animal model.

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Introduction

Solanum trilobatum Linn. (Solanacea) 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 has been administered to cases of acute and chronic bronchitis (1). Roots, berries and flowers are used for cough (2). Previous reports indicate that some chemical constituent, such as solasodine and β-solamarine have been isolated from the whole plant (3)

Pharmacological investigations have demonstrated that *S. trilobatum* possess antioxidant activity (4), hepatoprotective activity

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(5) and anti-inflammatory activity (6). In the preliminary study, the crude methanolic extract of *Solanum trilobatum* (MEST) root exhibited significant anti-inflammatory activity on carrageenan induced rat paw oedema. Therefore, the present study has been planned to investigate the anti-inflammatory activity and analgesic activity of methanolic extract of *Solanum trilobatum* root in different experimental models of acute and chronic inflammation.

Experimental

Plant material

The roots of *Solanum 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 air-dried powdered roots of *Solanum trilobatum* were defatted with petroleum ether (60-80 °C) to remove low polar compounds. The defatted material was further extracted with methanol at ambient temperature. The methanolic extract was filtered and concentrated to a syrupy mass (Yield 6.2% w/w) under reduced pressure at 50-55 °C. The methanolic extract (MEST) was examined chemically and was observed to contain alkaloids, saponins and sterols. These constituents were confirmed using thin layer chromatography (TLC).The extract was stored in a refrigerator and a weighed amount of MEST was suspended in 2% aqueous Tween 80 solution and used for the present study.

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 a 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 (7). Swiss Albino mice of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout. The fasted mice were divided into different groups of six animals each. MEST was administered orally at a dose of 5 mg/kg. The control animals received a similar volume of 2% (v/v) aqueous Tween 80 solution. Mortality in each group was observed for 7 days. As no mortality was observed, the procedure was repeated at doses 50, 300and 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.* (8). After 16h of fast the rats were divided into five groups of six each. Group I served as control group and received Tween 80 (5 ml/kg) of 2% w/v, orally. Group II, III and IV animals received MEST at a dose of 100, 200 and 300 mg/kg as a fine suspension in 2% v/v aqueous Tween 80 solution orally. Indomethacin was administered drug to Group V orally at a dose of 10 mg/Kg. After 1 h, 0.1 ml of 1% w/v Carrageenan suspension was injected subcutaneously in to the plantar 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 using ketamine. Sterile pre-weighed cotton pellets (50±1 mg) were implanted in the axillary region of each rat through a single needle incision (9). MEST (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

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Table 1. Effect of (Methanolic Extract of Solanum trilobatum linn) MEST 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
MEST	100 mg/kg	26.79±0.18[b]	56.71
MEST	200 mg/kg	28.07±0.21[a]	54.64
MEST	300 mg/kg	30.52±0.15[a]	50.68

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.

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 a measure of granuloma formation.

Hot plate (Thermal) method

The hot plate test described by Turner (10) was used. The mice were first treated with different doses of *S. trilobatum* (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. A cut-off period of 15 s was considered as maximal latency to avoid injury to the paws. 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 a reference drug.

Acetic acid-induced writhing test

This test was done using the method described by Collier *et al.* (11). Muscle contractions were induced in rats by intra peritoneal injection of 7% solution of acetic acid (10 ml/kg). Immediately after administration of acetic acid, the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min. Methanolic extract of *solanum trilobatum* was administrated orally at doses of (100, 200 and 300 mg/kg) and indomethacin (10 mg/kg) was administered 30 min before the acetic acid injection.

Statistical Analysis

The results are presented as mean±SEM. 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 significant.

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. MEST at doses of 100, 200 and 300 mg/kg significantly ($p<0.01$) inhibited edema formation in rat paw 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

MEST at doses of 100, 200 and 300 mg/kg

Table 2. Effect of MEST 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
MEST	100 mg/kg	83.59 ± 0.19*	8.15
MEST	200 mg/kg	78.70 ± 0.21*	13.52
MEST	300 mg/ kg	71.46 ± 0.15*	21.48

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

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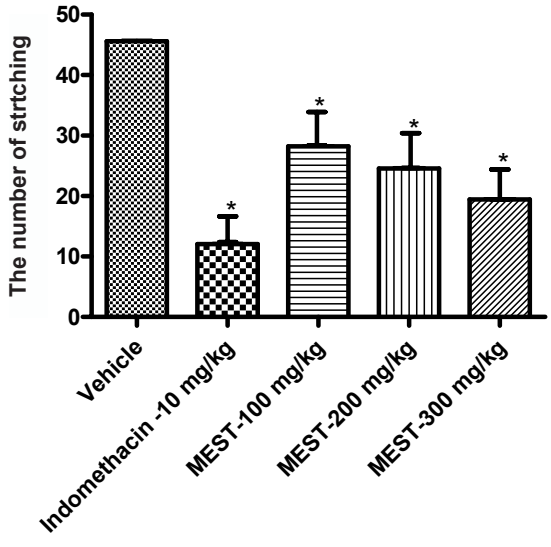

Figure 1. Effect of MEST on writhing response test. Each value represents the mean±S.E.M., n=6. P<0.05 compared with control, Dunnett's t-test after analysis of variance.

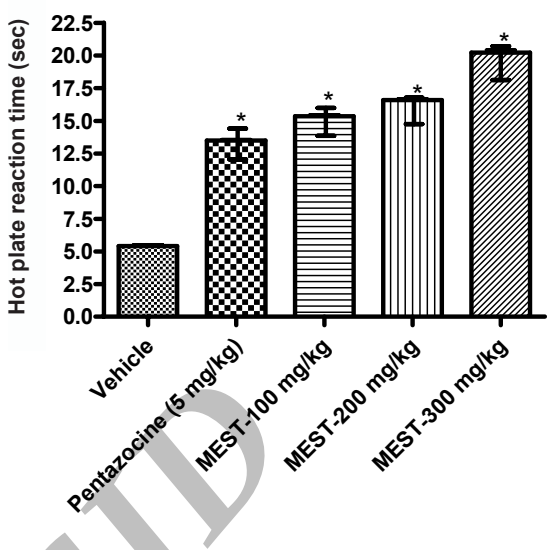

Figure 2. Central nervous system analgesic effect of MEST in comparison with the pentazocine standard the negative vehile group.Each value represents the mean±S.E.M., n=6. P<0.001 compared with control, Dunnett's t-test after analysis of variance.

significantly ($p<0.01$) inhibited granuloma formation (Table 2). Indomethacin (10 mg/kg, p.o.), a reference drug, elicited marked reduction in granuloma formation.

Acetic acid-induced writhing test

Dose dependent antinociceptive effect was noted with the extract at the tested dose levels (Figure 1). Maximum percentage of inhibition of writhing responses exhibited by the MEST 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.

Hot plate test

Figure 2 shows the results of the hot plate test. Three doses of extracts of solanum trilobatum increased the reaction time in a dose-dependent ($p<0.001$) manner to the thermal stimulus. The highest nociperception of thermal stimulus was exhibited at a higher dose (300 mg/kg) of MEST (62.78%), which is comparable to that of pentazocine (72.91%).

Discussion

MEST 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 (8) and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after administration of carrageenan. A more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome.

The cotton pellet granuloma bioassay is considered as a model for studies of chronic inflammation and is considered as a typical feature of established chronic inflammatory reaction (13). MEST exhibited significant reduction of granuloma formation in rats in the cotton pellet-induced granuloma. This means that MEST may be effective in chronic inflammatory conditions. The result of the present study

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indicates that crude fractions of methanol extract of *Solanum trilobatum* 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 (14), the enhanced analgesic effect of MEST may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. The enhanced analgesic effect of MEST 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 (15); therefore MEST 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 (16). Previously reported solasodine alkaloid present in this plant has significant anti-inflammatory activity (6).Though it is not possible at this stage to identify the exact phytochemical constituent(s) responsible for anti-inflammatory activities of *Solanum trilobatum*, 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 the present study indicates that methanolic extract of *Solanum trilobatum* 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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References

(1) Nadkarni KM. *Indian Materia Medica.* Vol. 1, 3rd ed. Bombay, Popular Prakasan Pvt. Ltd. (1976) 1153-1154

(2) Anonymous. *The Wealth of India-A Dictionary of Indian Raw Materials & Industrial Products.* Vol. IX (Rh-So), New Delhi, Publication and Information Directorate, Council of Scientific and Industrial Research (1972) 395-396

(3) Purushothaman KK, Balakrishana K, Sarada A and Bhema Rao R. Extraction of β-solamarine from *solanum trilobatum* Linn. *Indian Drugs* (1987) 24: 214-215

(4) Sini H and Devi KS. Antioxidant activities of the chloroform extract of *Solanum trilobatum*. *Pharm. Biol.* (2004) 42: 462-466

(5) Shahjahan M, Sabitha KE, Mallika Devi R and Shyamala CS. Effect of medicinal plants on tumourogenesis. *Ind. J. Med. Res.* (2004) 123: 23-27

(6) Emmanuel S, Ignacimuthu S, Perumalsamy R and Amalraj T. Anti-inflammatory activity of *Solanum trilobatum*. *Fitoterapia* (2006) 77: 611-612

(7) Ecobichon DJ. *The Basis of Toxicology Testing.* New York, CRC Press (1997) 43-86

(8) Winter CA, Risley EA and Nuss GW. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* (1962) 111: 544- 547

(9) Winter CA and Porter CC. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities in hydrocortisone esters. *J. Am. Pharm. Assos.* (1957) 46: 515-519

(10) Turner RA. *Screening Methods in Pharmacology.* New York, Academic Press (1965) 63

(11) Collier HOJ, Dinneen LC, Johnson CA and Scheider C. The abdominal contraction response and its suppression by antinociceptive drugs in the mouse. *Br. J. Pharmacol. Chemother.* (1968) 32: 295-310

(12) Spector WG. The granulomatous inflammatory exudates. *Int. Rev. Expt. Path.* (1969) 8: 1-55

(13) Ochi T, Motoyama Y and Goto T. The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models. *Eur. J. Pharmacol.* (2000) 391: 49-54

(14) Hough LB, Nalwalk JW, Leurs R, Menge WM and Timmerman H. Antinociceptive activity of impentamine, a histamine congener, after CNS administration. *Life Sci.* (1999) 64: PL79-PL86

(15) Gupta MB, Bhalla TN, Gupta GP, Mitra CR and Bhargava KP. Anti-inflammatory activity of natural products: I. Triterpenoids. *Eur. J. Pharmacol.* (1969) 6: 67-70

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