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*Original Article*

# Evaluation of Analgesic Potential of *Solanum trilobatum* Roots

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**Abstract**

In the present work, the antinociceptive action was assayed in several experimental models in mice: writhing, formalin and hot plate tests. The crude alkaloid fraction (25, 50, 100 mg/kg) and in a dose-dependent manner significantly reduced the nociception by acetic acid intraperitoneal injection ($P < 0.001$). In the formalin test, the extract (50 and 100 mg/kg) also significantly reduced the painful stimulus in both phases of the test ($P < 0.001$). Treatment with the extract (25, 50, 100 mg/kg) when given i.p. or pentazocine (5 mg/kg, s.c.) produced a significant increase of the reaction time in hot plate test. These result showed that the alkaloid extract of *Solanum trilobatum* contains active analgesic principles acting both centrally and peripherally.

**Keywords:** Analgesic; 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 (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 whole plant (3).

Pharmacological investigations have demonstrated that *S. trilobatum* possess antioxidant activity (4), and hepatoprotective activity (5). In the preliminary study, the methanol extract of *S. trilobatum* (MEST) root exhibited significant analgesic activity. Therefore, the present study has been planed to investigate the analgesic activity of crude alkaloids fraction (CAF) of methanol extract of *S. trilobatum* root in different experimental models.

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## Experimental

*Plant material*

The roots of *S. trilobatum* (Solanaceae) were collected during the month of June 2005 from Thirukovilur, 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.

*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).

*Preparation of extract*

The suspension of dried and finely ground powder of *S. trilobatum* root (5 kg) in toluene: water:concentrated HCl (3:2:1) was refluxed while stirring for 5 h. The reaction mixture was subsequently alkalinized with 40% NaOH and refluxed again with 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% aqueous acetic acid by stirring twice for 1 h at room temperature. The aqueous acid extract was separated from the toluene layer and then alkalinized with 25% aqueous ammonia. The mixture was briefly heated, then cooled at room temperature. The crude alkaloids which precipitated were filtered, washed with water, dried over anhydrous sodium sulphate and evaporated to yield crude alkaloids (4.1 g). CAF was stored in desiccators and used for further experiment after suspending in aqueous Tween 80 solution (2 % v/v).

*Acute toxicity study*

Acute toxicity study was performed as per OECD-423 guidelines (6). Swiss Albino mice of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout the period. The fasted mice were divided into different groups of six animals each. CAF 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.

*Hot plate (Thermal) method*

The hot plate test described by Turner et al. (7) was used. The mice were first treated with different doses of CAF (25, 50 and 100 mg/kg) after 1 h of extract administration they were placed on a hot plate maintained at 55 ± 1°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. (8). The muscular contractions were induced in rats by i.p. injection of 7% solution of acetic acid (10 mL/kg). Immediately after administration of acetic acid, animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min. Methanol extract of CAF were orally administrated in doses of (25, 50 and 100 mg/kg) and indomethacin (10 mg/kg) were administered 30 min before the acetic acid injection.

*Formalin-induced pain in mice*

The method described by Tjolsen et al. (9) was used. Animals were injected subplantarly with 20 µl of 2.5% formalin into the dorsal right hind paw of mice. CAF (50 mg/kg or 100 mg/kg) or indomethacin (10 mg/kg) or vehicle control was orally administered to different groups of rat. Drug pre-treatment was given orally 1 h prior to formalin injection. One mice of each group was observed simultaneously from 0 to 30 min following formalin injection. The time (seconds) that spent licking (or) biting the injected paw, indicative of pain was monitored. The first period (earlier or neurogenic phase) was recorded 0-5 min after formalin injection and the second period (later or inflammatory phase) was recorded 15-30 min after the injection.

*Statistical analysis*

The results are presented as mean ± SEM. One

**Table 1.** Effect of CAF on hot plate (Thermal) induced pain responses in mice

| Treatment | Dose (mg/kg) | Hot plate reaction time (s) | Inhibition (%) |
|---|---|---|---|
| Control (Saline) | 0.5 mL | 5.01 ± 0.06 | - |
| CAF (25 mg/kg) | 25 mg/kg | 12.68 ± 0.23* | 60.48 |
| CAF (50 mg/kg) | 50 mg/kg | 14.90 ± 0.21* | 66.37 |
| CAF (100 mg/kg) | 100 mg/kg | 17.93 ± 0.22* | 72.05 |
| Pentazocine (5 mg/kg) | 5.0 | 20.73 ± 0.23* | 75.83 |

Each value represents the mean ± S.E.M., n = 6. *P < 0.001 compared with control, Dunnett's *t*-test after analysis of variance.

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

*Hot plate method*

The hot plate (Thermal) induced test was performed in order to determine whether the analgesic activity of extract was caused by central or peripheral mechanisms. In hot plate (Thermal) induced method, CAF at dose of 50 and 100 mg/kg showed significant (P < 0.001) latency time to heat stimulus (Table 1). Pentazocine (5 mg/kg) was used as positive control. As expected, pentazocine produced analgesia and induced an increase in time latency of pain.

*Acetic acid-induced writhing test*

Dose dependent antinociceptive effect was noted with the extract at the tested dose levels. Maximum percentage of inhibition of writhing responses exhibited by the CAF at 100 mg/kg was 65.63%, while the same at 25 and 50 mg/kg showed 46.36% and 57.68% reduction in acetic acid induced writhing responses, respectively, which was comparable to that of standard indomethacin (10 mg/kg) that caused 73.82% pain reduction.

*Formalin-induced pain in mice*

Using a classical pain model, CAF and indomethacin were evaluated in the formalin-induced pain in mouse. The effect of CAF on the time spent licking the injected paw during the early phase (0-5 min) of the formalin test is shown in Table 3. CAF significantly (P < 0.05) decreased the time mice spent licking hind paw during the early phase. At 100 mg/kg CAF exhibited inhibition of 10.90% in the formalin-induced licking during the first phase (Table 3). CAF (100 mg/kg) also inhibited (57.67%) the late phase (15-30 min) of the formalin-induced licking (Table 3). Indomethacin had no effect on the first phase, but it produced a reduction (59.94%) of the second phase at 10 mg/kg.

## Discussion

The present study assessed the antinociceptive effects of the CAF of *S. trilobatum* roots. The methods for investigating anti-nociception were selected such that both centrally and peripherally mediated effects were investigated. Indeed, the acetic acid-induced abdominal constriction is believed to show the involvement of peripheral mechanisms whereas the hot plate test is believed to show that of central mechanisms (8). The formalin test is used to investigate both peripheral and central mechanisms (9). The extract of *S.*

**Table 2.** Effect of CAF on acetic acid-induced abdominal constrictions in mice

| Treatment | No. of abdominal constrictions | Inhibition (%) |
|---|---|---|
| Control (CMC, 10 mL/kg) | 45.92 ± 0.22 | - |
| Indomethacin (10 mg/kg) | 12.02 ± 0.14* | 73.82 |
| CAF (25 mg/kg) | 24.63 ± 0.13* | 46.36 |
| CAF (50 mg/kg) | 19.43 ± 0.12* | 57.68 |
| CAF (100 mg/kg) | 15.78 ± 0.23 * | 65.63 |

Data are shown as mean ± S.E.M., n = 6. *P < 0.001 significantly different from control; Dunnet's *t*-test after analysis of variance.

**Table 3.** Effect of CAF on formalin-induced licking in mouse

| Treatment | Licking time (s) (0-5 min) | Inhibition (%) | Licking time (s) (15-30 min) | Inhibition (%) |
|---|---|---|---|---|
| Control (CMC, 10 mL/kg) | 43.10 ± 0.32 | - | 174.2 ± 0.40 | - |
| Indomethacin (10 mg/kg) | 39.97 ± 0.42 | 7.26 | 69.77 ± 0.39 | 59.94 |
| CAF (50 mg/kg) | 35.03 ± 0.32 | 18.72[a] | 77.73 ± 0.38 | 55.37[a] |
| CAF (100 mg/kg) | 38.40 ± 0.36 | 10.90[b] | 73.73 ± 0.29 | 57.67[a] |

Data are shown as mean ± S.E.M., n = 6. [a]$P < 0.001$, [b]$P < 0.05$, significantly different from control; Dunnet's *t*-test after analysis of variance.

*trilobatum* was shown to possess antinociceptive activity evident in all the nociceptive models signifying the presence of both central and peripherally mediated activities.

Since acetic acid induced writhing can be considered a model of prostaglandin synthesis sensitive response (10), the enhanced analgesic effect of CAF may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. However, an important disadvantage of this model is that other classes of drugs can reveal other effects, such as adrenergic antagonists and muscle relaxants (11), favouring possible false positive results. Due to its lack of specificity it is usual to analyse positive results in the writhing test together with the results of other tests. For this reason the formalin test was selected to continue this investigation, since it is more specific and it is possible to identify two distinct phases of nociception. Our results showed that the time spent in licking the injured paw was significantly reduced by i.p. administration of CAF extract in both phases. In this test, the early phase is thought to be produced by direct activation of nociception neurons by formalin, whereas the late phase reflects pain generated in acutely injured tissue (13). The biphasic control of pain, which reflects these different pathological processes, allows the elucidation of the possible mechanism involved in analgesia (9). Centrally acting drugs, such as opioids, inhibit both phases of pain by equally (12) involving the effect produced by prostaglandins released at this level in response to inflammation (13) and by endogenous opioids through their action on the central nervous system (9) demonstrated that the late phase in formalin test depends on an inflammatory reaction in peripheral tissue. Peripheral acting drugs such as acetylsalicylic acid (13) which block prostaglandin synthesis reduce nociception only in the late phase. In fact, the antinociceptive effects of CAF of *S. trilobatum* suggest an involvement at both central and peripheral levels. In the hot plate test, a central model that has selectivity for opioid-derived analgesics (8), i.p. injections of crude alkaloid fraction exerts a potent antinociceptive action confirming the central activity of this extract.

In conclusion, our results show that the CAF of *S. trilobatum* possess a central and peripheral antinociceptive activity, which act partly through an opioid mediated mechanism. These preliminary reports lend to support to the use of this plant in folk medicine for analgesic (1), mainly because of low toxicity. Further, work is needed to clarify the exact active constituents responsible for antinociceptive action and their mechanism of action.

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