Antinociceptive activity of methanol extract of leaves of *Solanum sisymbriifolium* in heat and chemical-induced pain

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**ABSTRACT**

*Solanum sisymbriifolium* Lamk. (Solanaceae) is a perennial prickly herb traditionally used in folk medicine to treat different ailments. This study evaluated the methanol extract of *S. sisymbriifolium* leaf (MSS) (100, 200, and 400 mg/kg; p.o) for analgesic activity using heat- and chemical-induced pain models such as hot plate, tail immersion, formalin-induced licking and acetic acid-induced writhing. Interaction with the opioid receptor system was verified using naloxone to antagonize the effect of MSS, if any. MSS demonstrated significant and dose-dependent antinociceptive activity, though moderate, in both hot plate and tail immersion tests (p < 0.05). Naloxone reversed the antinociceptive effect in both tests. MSS produced profound antinociceptive effect in formalin test and acetic acid-induced writhing at 400 mg/kg dose. These results imply that the antinociceptive effect of MSS is mainly due to its central effect and peripheral effects also contribute to some extent. These results provide preliminary evidence of antinociceptive activity of *S. sisymbriifolium* leaves and the potential of the plant in treating different painful conditions.

**INTRODUCTION**

*Solanum sisymbriifolium* Lamk. (Solanaceae) is a densely prickly perennial herb commonly known as sticky nightshade. The plant is traditionally used in the treatment of stomachache, remitting fever, hysteria (Uddin, 2016), respiratory tract infections, central nervous system (CNS) disorders and diarrhea (Ibarrola et al., 2000; Ferro et al., 2005). This plant is also used as diuretic, antihypertensive (Gonzales Torrez, 1992), emenagogue and fertility regulator (Martinez-Crovetto, 1981). Pharmacological investigations of different parts of *S. sisymbriifolium* have reported hypotensive (Ibarrola et al., 2000), analgesic (Shilpi et al., 2005), neuropharmacological and anti-diarrheal activity (Apu et al., 2013). Phytochemical analyses confirmed isolation of a number of pharmacologically active compounds from the plant including solamine, solasodine and solasodine (Mazumdar, 1984), neolignan, designated as sisymbriofolin, and carpesterol together with β-sitosterol and its β-D-glucoside, cuscohygrine, (Evans and Somanabandhu, 1980; Ferro et al., 2005), solacaprine, solamarine, and β-solamarine (Ferro et al., 2005) and isonuataigenin-3-O-β-solatriose (Ibarrola et al., 2000). Solasodine, a major alkaloid, isolated from the plant has demonstrated variety of biological activities including anti-inflammatory (Kusano et al., 1987), cytotoxic (Nakamura et al., 1996), hepatoprotective (Lin et al., 1988), antinociceptive (Basu and Lahiri, 1977; Pandurangan et al., 2010), anti-inflammatory (Pandurangan et al., 2011), anticonvulsant, CNS depressant (Chauhan et al., 2011) and antiatherosclerotic activity (Dixit et al., 1992). Solasodine is effective in skin tumor treatment (Cham et al., 1987; Cham et al., 1991). Nuatigenosido isolated from this plant has been reported to have cardiovascular effect (Ibarrola et al., 2006). The use of this plant in folk medicine in different ailments and analgesic report of whole plant extract by Shilpi et al. (2005) in acetic acid-induced writhing motivated this study to evaluate the methanol extract of *S. sisymbriifolium* (MSS) leaf in different peripheral and central pain models in mice to find out whether the leaf is the major contributor in the effect as well the possible pathways involved in the analgesic effect.

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MATERIALS AND METHODS

Plant material and extraction
The leaves of *S. sisymbriifolium* were collected from Natore of Rajshahi Division, Bangladesh in January, 2011. Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium identified the samples (DACB: 35424). Powdered dried leaves (58 g) were extracted with 350 ml of methanol by Soxhlet apparatus at 50°C for 36 h. Filtration was done using a sterile cotton filter, then the solvent was removed by rotary evaporator and 9.02 gm extract (Yield 15.55%) was obtained. This extract was used for the phytochemical screening and animal studies.

Chemicals and drugs
Methanol (Merck, Germany), morphine sulphate (Gonoshasthaya Pharmaceuticals Ltd.), naloxone (Sigma, USA), diclofenac sodium (Square Pharmaceuticals Ltd.), formalin (Merck, Germany) and acetic acid (Merck, Germany).

Animals
Swiss albino male mice weighing 20-25 gm were used in the study (Animal Resources Branch of the International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b)). The mice were housed in cages in group and had access to food and water ad libitum. Room temperature of the animal house was 25 ± 2°C and relative humidity was 55-60%. 12 h light and 12 h dark cycle were maintained during the 14 day acclimatization period and throughout the experimental period. The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences developed *Ethical Principles and Guidelines for Scientific Experiments on Animals* (1995) were followed for the animal welfare.

Drugs and treatments
Morphine sulphate, used as a positive standard, at the dose of 5 mg/kg body weight (b.w.) was injected intraperitoneally (i.p.) 15 min prior to hot plate, tail immersion and formalin test while diclofenac sodium was injected (i.p.) at the dose of 10 mg/kg dose in acetic acid-induced writhing test. Vehicle controlled group was gavaged 0.9% saline water (vehicle) at the dose of 10 ml/kg. MSS was administered by oral gavage to mice at three different doses i.e., 100, 200, and 400 mg/kg b.w. I.P. injection of naloxone at 2 mg/kg dose was given 15 min before administering morphine or MSS in appropriate route to anticipate any possible involvement of MSS-opioid receptor interaction.

Phytochemical screening
Following standard qualitative phytochemical procedure described by Ghani (2003) alkaloids, carbohydrates, flavonoids, glucosides, glycosides, proteins, reducing sugars, saponins and steroids were tested in MSS.

Acute toxicity test
Three different mice groups (n = 5) received MSS orally at 500, 1000 and 2000 mg/kg dose. Animals were closely observed for the next 72 h for any abnormality in behavior, any allergic response, morbidity and mortality (Walker et al., 2008).

Antinociceptive tests

Hot plate test
The test was performed following previously described method (Imam et al., 2012). Mice were fasted overnight with water given *ad libitum*. The animals were treated with morphine or MSS or vehicle and were placed on hot plate maintained at a temperature of 55 ± 1°C. To avoid any tissue damage in the paw of the mice 20 s was considered as cut off period. The response to the thermal stimuli as forepaw licking, withdrawal of the paw(s) or jumping was documented at 30, 60, 90, and 120 min after treatment.

Tail immersion test
Mice were pretreated with morphine or MSS and ~2 cm of the tail was immersed in warm water maintained at 52 ± 1°C. The latency period at 30, 60, 90, and 120 min after treatment were documented (Toma et al., 2003). In this test, 20s was considered as cut off period to avoid tail tissue damage.

Formalin test
Formalin (20 µl of 1.35% formalin) was injected into the sub-plantar region of the right hind paw of mice 30 min after MSS treatment and 15 min after injection of morphine. Paw licking was considered as nociceptive response and was documented at 0-5 min (early phase) and 15-25 min (late phase) after formalin injection (Coelho et al., 2005).

Acetic acid-induced writhing test
Morphine or MSS was administered in mice and then 0.7% acetic acid was injected i.p. after 15 and 30 min respectively (10 ml/kg) to induce writhing. The number of writhing was documented for 10 min starting after 5 min post treatment (Vogel, 2007).

Statistical analysis
The results are presented as mean ± standard error of mean (SEM). The statistical analyses of the results were performed using ANOVA (SPSS 11.5). Dunnett’s or Bonferroni’s test was performed as post-hoc test as appropriate. Differences between groups were considered significant at a level of *p* < 0.05.

RESULTS

Phytochemical screening
Preliminary phytochemical screening of MSS detected the presence of alkaloids, glycosides, carbohydrates and saponins.

Acute toxicity
Oral administration of MSS up to 2000 mg/kg did not show any mortality, allergic manifestations or abnormality in behavior during the 72 h observation period in mice.
Hot Plate test
MSS at 200 and 400 mg/kg significantly increased the latency to the thermal stimulus (p<0.01) dose dependently. MSS at 400 mg/kg dose produced strong antinociception. The effect of morphine at 5 mg/kg dose was highly significant (p<0.001). The effects of morphine and MSS were reversed by naloxone (Table 1).

Tail immersion test
The antinociceptive activity of MSS and standard drug morphine on the tail immersion test are given in Table 1. MSS at all three doses showed significant increase in latency and at 400 mg/kg dose showed highest increase in latency (<0.001) which was reversed by naloxone. The effect of morphine was highly significant (p<0.001) and strongest among the treatment groups.

Formalin test
MSS at all doses (100, 200, and 400 mg/kg) reduced both early and late phase of paw licking induced by formalin (Table 2). However, the reduction in licking was significant (p <0.05) only for morphine and MSS at 400 mg/kg doses.

Acetic acid-induced writhing
Oral administration of MSS at 100, 200, and 400 mg/kg doses reduced acetic acid-induced writhing compared to the control group (Table 2). However, the reduction was significant only at 400 mg/kg dose (p < 0.05) and interestingly the effect was much stronger than that of diclofenac sodium.

Table 1: The antinociceptive effect of S. sisymbriifolium leaf, morphine, and reversal effect of naloxone in hot plate and tail immersion test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>2.20 ± 0.07</td>
<td>2.36 ± 0.06</td>
<td>2.37 ± 0.14</td>
<td>1.99 ± 0.05</td>
<td>2.59 ± 0.09</td>
</tr>
<tr>
<td>Morphine</td>
<td>5 (i.p.)</td>
<td>11.63 ± 0.13***</td>
<td>8.56 ± 0.22***</td>
<td>9.41 ± 0.20***</td>
<td>8.05 ± 0.17***</td>
<td>4.56±0.18*</td>
</tr>
<tr>
<td>MSS</td>
<td>100</td>
<td>1.95 ± 0.13</td>
<td>3.15 ± 0.19</td>
<td>3.37 ± 0.21</td>
<td>2.53 ± 0.12</td>
<td>2.02 ± 0.23</td>
</tr>
<tr>
<td>MSS</td>
<td>200</td>
<td>2.17 ± 0.12</td>
<td>4.30 ± 0.16</td>
<td>4.32 ± 0.12</td>
<td>4.05 ± 0.23</td>
<td>3.83 ± 0.04</td>
</tr>
<tr>
<td>MSS</td>
<td>400</td>
<td>3.26 ± 0.16*</td>
<td>4.44 ± 0.26**</td>
<td>4.54 ± 0.25**</td>
<td>5.16 ± 0.21***</td>
<td>4.05±0.22*</td>
</tr>
<tr>
<td>NLX + MSS</td>
<td>2 (i.p.)</td>
<td>1.95 ± 0.16</td>
<td>2.62 ± 0.11</td>
<td>2.85 ± 0.11</td>
<td>3.54 ± 0.14</td>
<td>2.00 ± 0.09</td>
</tr>
<tr>
<td>NLX + MSS</td>
<td>2 + 5</td>
<td>3.54 ± 0.19a</td>
<td>4.23 ± 0.14a</td>
<td>4.06 ± 0.22a</td>
<td>3.06 ± 0.22a</td>
<td>2.79 ± 0.13a</td>
</tr>
<tr>
<td>NLX + MSS</td>
<td>2 + 100</td>
<td>2.60 ± 0.06</td>
<td>2.10 ± 0.15</td>
<td>2.74 ± 0.21</td>
<td>3.60 ± 0.18b</td>
<td>2.68 ± 0.17</td>
</tr>
<tr>
<td>NLX + MSS</td>
<td>2 + 200</td>
<td>2.43 ± 0.16</td>
<td>2.18 ± 0.16c</td>
<td>2.39 ± 0.20b</td>
<td>2.19 ± 0.16c</td>
<td>2.34 ± 0.17</td>
</tr>
<tr>
<td>NLX + MSS</td>
<td>2 + 400</td>
<td>2.49 ± 0.19</td>
<td>2.86 ± 0.21d</td>
<td>2.53 ± 0.18e</td>
<td>3.58 ± 0.21d</td>
<td>2.63 ± 0.20d</td>
</tr>
</tbody>
</table>

Each value is presented as the mean ± SEM (n = 5). MSS = Methanol extract of S. sisymbriifolium leaves; NLX = Naloxone. * p <0.05; ** p <0.01; *** p <0.001 compared with the control group (Dunnett’s test). + p <0.001 compared with the morphine group; † p <0.05 compared with the MSS 100 group; ‡ p <0.05 compared with the MSS 200 group; § p <0.01 compared with the MSS 400 group (Bonferroni’s test).

Table 2: The antinociceptive effect of S. sisymbriifolium leaf extract and morphine in formalin-induced paw licking and acetic acid-induced writhing test in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Early phase (0-5 min)</th>
<th>% Inhibition</th>
<th>Late phase (15-30 min)</th>
<th>% Inhibition</th>
<th>Number of writhing</th>
<th>Acetic acid-induced writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>163.67 ± 9.30</td>
<td>-</td>
<td>153.67 ± 12.1</td>
<td>-</td>
<td>28.5 ± 1.29</td>
<td>-</td>
</tr>
<tr>
<td>Morphine</td>
<td>5</td>
<td>50.00 ± 3.28*</td>
<td>69.45</td>
<td>0.00 ± 0.00*</td>
<td>100.00</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>10</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>6.3 ± 1.27*</td>
<td>77.89</td>
</tr>
<tr>
<td>MSS</td>
<td>100</td>
<td>143.00 ± 6.64</td>
<td>12.63</td>
<td>139.00 ± 7.88</td>
<td>9.55</td>
<td>21.2 ± 0.93</td>
<td>25.61</td>
</tr>
<tr>
<td>MSS</td>
<td>200</td>
<td>138.67 ± 6.80</td>
<td>15.28</td>
<td>110.00 ± 6.03</td>
<td>28.12</td>
<td>16.9 ± 0.98</td>
<td>40.70</td>
</tr>
<tr>
<td>MSS</td>
<td>400</td>
<td>102.00 ± 6.70*</td>
<td>37.68</td>
<td>60.30 ± 8.26*</td>
<td>2.7 ± 0.77*</td>
<td>90.53</td>
<td></td>
</tr>
</tbody>
</table>

Each value is presented as the mean ± SEM (n = 5). MSS = Methanol extract of S. sisymbriifolium leaves; NT = not tested. * p <0.05 compared with the control group (Dunnett’s test).
DISCUSSION

The results of the present study indicate moderate but significant antinociceptive activity of MSS in particular at 400 mg/kg dose. The effect was more profound in heat-induced pain models. It is believed that hot plate method demonstrates the supraspinal reflex mediated by μ₁ and μ₂-opioid receptors whereas the tail immersion test monitors the spinal reflex involving μ₂ and δ-opioid receptors (Arslan and Bektas, 2010). These methods are, therefore, useful for screening molecules effective in spinal and supraspinal region. The increase in latency in these two tests by MSS indicates the modulation of central nervous system pain signaling. This hypothesis is further supported by the reversal of antinociception of MSS at all doses by naloxone in both hot plate and tail immersion test. Reversal of antinociceptive effect of MSS by naloxone raises the possibility of involvement of opioid receptor mediated effect at the spinal and supraspinal level which is predominant in case of morphine in both tests. Formalin-induced paw licking is a well-accepted chemical induced pain model that works in two divergent phases; early phase neurogenic pain due to direct stimulation of the sensory afferent fibers followed by inflammatory pain due to action of different inflammatory mediators (Parada et al., 2001; Le Bars et al., 2001). The effect of formalin can be consistently inhibited by typical analgesic and anti-inflammatory drugs like indomethacin, diclofenac sodium and morphine (Tjolens et al., 1992). The results of our present study have shown that MSS reduced the number of paw licking significantly in neurogenic and inflammatory pain phases (p<0.05). Acetic acid-induced release of prostaglandins, serotonin, bradykinin, histamine, TNF (some common endogenous mediators) in the peripheral tissue fluid is associated with the development of pain and writhing (Ikeda et al., 2001). The profound antinociceptive effect of MSS at 400 mg/kg dose in acetic acid-induced writhing test, therefore, implies that MSS may be involved in the inhibition of inflammatory mediators like cyclooxygenase, lipoxygenase and others which results in the interruption of signal transduction in primary afferent nociceptors. Solasodine which have already been shown antinociceptive, anti-inflammatory and CNS depressant activity may play important role in MSS’s antinociceptive effect along with other phytochemicals (Basu and Lahiri, 1997; Pandurangan et al., 2010; Pandurangan et al., 2011; Chauhan et al., 2011). The results of the present study underpin the preliminary basis of traditional use of S. sisymbriifolium in some painful conditions. It also presents the possibility of inhibition of inflammatory mediators as well as modulation of opioid system by compounds present in MSS. However, further research with isolated compounds is required to better understand the principal chemical entity/entities that contribute to the effect, the mechanisms involved and optimization of the preparation to be used.

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REFERENCES


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