Phytochemical Screening and Evaluation of Analgesic Activity of Solanum surattense

Tabassum Quibria¹, Biplab Kumar Das¹,², Tahrim Hasan¹,³, Md. Azim Uddin¹ and Md. Morshedul Alam¹, ⁴*

Received 28 November, 2013; Published online 14 June 2014

© The author(s) 2014. Published with open access at www.uscip.us

Abstract

The leaves of the medicinal plant Solanum surattense (Family- Solanaceae) were extracted in 95% ethanol to evaluate centrally acting analgesic potential at two different doses, (250 and 500 mg/kg body weight). Three different test methodologies; hot plate test, acetic acid induced writhing test and formalin test were employed to assess the analgesic activity. A significant analgesic activity of the extract was observed in mice; 39.34% and 60.47% (P<0.05 and P<0.001) protection against thermal-induced pain, 33.98% and 49.59% (P<0.001), protection against acetic acid-induced writhing and 40.00% and 67.80% (P<0.05 and P<0.001) protection against formalin induced pain. The crude extract of the plant was found to have significant (p<0.05) analgesic activity at the dose of 250 and 500 mg/kg that suggests the presence of active principles in the extract responsible for analgesic activity.

Keywords: Solanum surattense, Analgesic activity, Peripheral pain, Mice, Ethanol extract

1. Introduction

Due to having adverse side effects, like gastric lesions, caused by Non-steroidal anti-inflammatory drugs (NSAIDs) and tolerance and dependence induced by opiates, the use of these drugs, as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this seeking process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and
According to World Health Organization (WHO) still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001).

Accordingly, we have investigated the *Solanum surratense* (local name-Kantakari) for its analgesic property. This plant is also known as Thorny Nightshade, Yellow Berried Nightshade, Thai eggplant is a medicinal plant in the family Solanaceae. This plant has versatile medicinal properties such as anti-inflammatory, Anthelmintic, Anti-arthritic, Anti-asthmatic, Anti-bilious, Anti-catarhral, Anti-epileptic, Anti-gonorrhoeal, Anti-gout, Anti-hysteria, Anti-leprotic, Anti-migraine, Anti-phlegmatic, Anti-spasmodic, Anti-sterility, Anti-syphilitic, Anti-tussive, Appetizer, Bitter, Blood Purifier, Cathartic, Diuretic, Exhine, Expectorant, Febrifuge, Hair Tonic, Hepatoprotective, Lithontriptic, Purgative, Sialagogue, Stomachic (Ghani, 2003).

The aim of the present work was to evaluate the analgesic activity to support the pharmacological effects and phytochemical investigation of this plant as well. Many scientists have reported the medicinal value of various plants in different perspectives (Morshed et al., 2011a, 2011b; Parvin et al., 2013; Shadli et al., 2014). Although numerous studies have shown the medicinal values of this plant, there still remains ample scope for further in depth research. So far, for the first time an attempt was taken to investigate the analgesic effect of *Solanum surratense* by using 95% ethanol extract of the leaves of the plant. Accordingly, we disclose herein the analgesic effect of the leaves of *Solanum surratense* to further establish the scientific basis of the traditional uses of this plant.

2. Materials and Methods

2.1 Plant material

For this present investigation, *Solanum surattense* was collected from an abandoned area in Ashulia, Dhaka Bangladesh in mid November and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 35470 for *Solanum surattense* was deposited. Approximately 5 kg of the plant was collected and washed with tap water. Then they were dried in shaded place instead of direct sunlight because drying under direct sunlight might have resulted in the evaporation of the active components. The collected plant parts were dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was then sieved and the granular portion was grinded again. The grinded portion was sieved once again and the entire powder was then weighed using an analytical balance. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.2 Preparation of extract

To prepare the extract, 317gm of powdered material was taken in a clean, glass beaker and soaked in 1 liter of 95% ethanol. The container with its contents was sealed with aluminum foil and kept for a period of 5 days accompanying occasional shaking and stirring. The whole mixture then underwent a series of filtrations and it was done in four steps:

1. First it was filtered with a piece of clean, white, pure cotton material. After the filtrate was collected, the residue on the cloth was squeezed to collect the remaining extract
2. Then the filtrate was filtered using absorbent cotton
3. The same step as repeated using fresh cotton
4. Finally the filtrate was filtered through Whatman filter paper.
The filtrate (ethanol extract) obtained was evaporated using rotary evaporator to remove the ethanol. Small amounts were placed in the evaporator. It rendered a gummy concentrate of greenish black color. The gummy concentrate was designated as crude extract of ethanol. It was left for air-drying for a few hours after which it was placed in a refrigerator in a closed container for further use and protection.

2.3 Animals
Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

2.4 Phytochemical screening
Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch’s test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer’s reagent, Hager’s reagent and Dagendorff’s reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

2.5 Analgesic activity

2.5.1 Hot Plate Test Method
Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of six mice in each group for control, positive control (standard) and 2 test samples group respectively. Each group received a particular treatment i.e. control (distilled water 10ml/kg, p.o.), positive control (Diclofenac Sodium, 10 mg/kg, p.o.) and the test sample (ethanolic extract of Solanum surattense: 250 mg/kg, p.o. & 500 mg/kg, p.o. respectively).

A 600 ml test beaker was placed on thermostat hot plate (Gallenkamp thermostat Cat No: HL054). The temperature was regulated to 50° ± 1°C. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. A cut off period of 20 seconds (Franzotti et al., 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120, 180 and 240 minutes after oral administration of the samples (Eddy et al., 1953; Kulkarni, 1999; Toma et al., 2003).

2.5.2 Acetic Acid Induced Writhing Method
The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice (Ahmed et al., 2004). In this method, acetic acid is administered intraperitoneally to the
experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study Diclofenac Sodium was used to serve the purpose. The plant extract was administered orally in two different doses (250 and 500 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.6% v/v acetic acid solution (0.1ml/10g). Animals were kept individually under glass jar for observation. Each mouse of all groups were observed individually for counting the number of writhing they made in 10 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. The animal did not always accomplish full writhing, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing.

Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Ketorolac (10 mg/kg) was used as a reference substance (positive control). The percentage inhibition of writhing was calculated as follows:

\[
\% \text{ Inhibition} = (1 - \frac{VT}{VC}) \times 100
\]

\( VT = \) number of writhing motions in drug-treated mice.

\( VC = \) number of writhing motions in the control group of mice

2.5.3 Formic Acid Induced method

Twenty microliters of 0.5% formalin was injected subcutaneously into the right hind paw of the mice. The number of licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 minutes after formalin injection (early phase) and 20–30 minutes after formalin injection (late phase). Ethanolic extract of *Solanum surattense* (250 and 500 mg/kg, p.o.) was administered 30 minutes before formalin injection. Diclofenac Sodium (10 mg/kg, p.o.) was administered 30 min before formalin injection. The control group received the same volume of distilled water by oral administration. The percentage inhibition of writhing was calculated as follows:

\[
\% \text{ Inhibition} = (1 - \frac{VT}{VC}) \times 100
\]

\( VT = \) number of writhing motions in drug-treated mice.

\( VC = \) number of writhing motions in the control group of mice

2.6 Statistical Analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by analysis of variance (ANOVA) followed by Dunnet’s multiple comparisons. The results obtained were compared with the vehicle control group. The p<0.05, 0.001 were considered to be statistically significant. All the statistical tests were carried out using SPSS statistical software.

3. Results

3.1 Phytochemical Screening

Phytochemical screening of the prepared extracts was conducted to separate the various phytochemical components that dissolved in the 95% ethanol. The results of phytochemical screening are given in Table 1. Phytochemical analysis of the plant extracts revealed the presence of
alkaloids, carbohydrates, tanins, gum, reducing sugars and terpenoids in the ethanolic extract of Solanum surattense.

**Table 1** Results of chemical group test of the ethanolic extracts of Solanum surattense.

<table>
<thead>
<tr>
<th>Plant in Extract</th>
<th>Tanin</th>
<th>Saponin</th>
<th>Flavonoid</th>
<th>Gum &amp; Carbohydrate</th>
<th>Alkaloid</th>
<th>Reducing Sugar</th>
<th>Terpenoid</th>
<th>Steroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. surattense</td>
<td>+++</td>
<td>--</td>
<td>--</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

High = +++; Absence = --.

3.2 Analgesic study

3.2.1 Hot plate method

Results of hot plate test are presented in Table 2 and Figure 1 for the crude extract of Solanum surattense. The extract was found to exhibit a dose dependent increase in latency time when compared with control. At 180 minutes, the percent inhibition of two different doses (250 and 500 mg/kg body weight) was 39.34% & 60.47%. The results were found to be statistically significant (p<0.001).

**Table 2** Analgesic effect of the ethanolic extract of Solanum surattense using the hot plate method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (n=6)</th>
<th>Positive control (n=6)</th>
<th>EESS (250 mg/kg) (n=6)</th>
<th>EESS (500 mg/kg) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 H</td>
<td>15.33±1.67</td>
<td>12.03±1.66*</td>
<td>12.15±1.87*</td>
<td>11.41±1.43**</td>
</tr>
<tr>
<td>½ H</td>
<td>11.78±2.23</td>
<td>13.46±1.41*</td>
<td>13.88±1.23*</td>
<td>13.48±1.45*</td>
</tr>
<tr>
<td>1 H</td>
<td>9.65±2.39</td>
<td>15.78±1.31***</td>
<td>14.98±1.14***</td>
<td>15.26±1.26***</td>
</tr>
<tr>
<td>2 H</td>
<td>8.18±2.34</td>
<td>17.05±1.37***</td>
<td>16.36±0.83***</td>
<td>17.13±1.03***</td>
</tr>
<tr>
<td>3 H</td>
<td>6.90±1.68</td>
<td>18.33±1.18***</td>
<td>16.93±0.76***</td>
<td>18.31±0.99***</td>
</tr>
<tr>
<td>4 H</td>
<td>5.75±1.11</td>
<td>15.43±1.95***</td>
<td>15.33±1.00***</td>
<td>17.31±1.12***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM (n=6 animals per group). *P<0.05, ***P<0.001 vs. control; EESS = Ethanol extract of Solanum surattense.

**Fig. 1.** Bar diagram showing the percentage inhibition of analgesic effect of the ethanolic extract of Solanum surattense using the hot plate method.
3.2.2 Acetic acid-induced writhing test

Next we checked the effects of the *Solanum surattense* extract on acetic acid-induced writhing in mice. Both doses of the extract showed significant reduction (p<0.001) of writhing induced by the acetic acid after oral administration in a dose dependant manner. After oral administration of two different doses (250 and 500 mg/kg body weight), the percent inhibition was 33.98% & 49.59%. The reference drug diclofenac sodium was found more potent than the plant extract at all of the dose levels (Table 3).

**Table 3** Analgesic effect of the ethanolic extract of *Solanum surattense* using the acetic acid induced writhing method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of writhings (5-15) Min</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.66±8.57</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Diclofenac)</td>
<td>18.83±2.85***</td>
<td>52.3</td>
</tr>
<tr>
<td>EESS (250 mg/kg)</td>
<td>27.50±4.50**</td>
<td>37.3</td>
</tr>
<tr>
<td>EESS (500 mg/kg)</td>
<td>21.00±6.22***</td>
<td>56.4</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM (n=6 animals per group). ***P< 0.001 vs. control. EESS = Ethanol extract of *Solanum surattense*.

3.2.3 Formalin-induced method

Finally, we checked at which stage of pain our plant extract works more effectively. For this study, we performed formalin-induced pain method. We found that the ethanolic extract of *S. surattense* demonstrated a dose-dependent relationship in late phase of the formalin-induced pain. In the early phase, there were no significant inhibition at the doses of 250 and 500mg/kg of the extract compared to the control group. In the late phase, the doses of 250mg, 500mg/kg of EESS and diclofenac sodium (10 mg/kg) significantly reduced (p<0.001) the nociception with percentage inhibition of 71.13%, 40.00% and 67.80% respectively when compared with the control (Table 4 & Figure 2).

**Table 4** Effect of *Solanum surattense* ethanol extract on formalin-induced pain in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Standard (Diclofenac sodium)</th>
<th>EESS (250 mg/kg)</th>
<th>EESS (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early phase (0-5 Min)</td>
<td>24.66±1.50</td>
<td>12.66±1.21**</td>
<td>17.83±1.16**</td>
<td>13.50±1.51*</td>
</tr>
<tr>
<td>Late phase (20-30 Min)</td>
<td>15.00±2.75</td>
<td>4.33±0.81***</td>
<td>9.00±0.89**</td>
<td>4.83±2.31**</td>
</tr>
</tbody>
</table>
Values were expressed as mean ± SEM (n=6 animals per group). ***P< 0.001 vs. control. EESS = Ethanol extract of *Solanum surattense*.

**Fig. 2.** Bar diagram showing the percentage inhibition of analgesic effect of the ethanolic extract of *Solanum surattense* using the formalin-induced method.

### 4. Discussion

The hot plate method is considered to be selective for the drugs acting centrally. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity (Sabina *et al*., 2009). It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Ibironke and Ajiboye, 2007). Therefore, the ethanolic extracts of the plants must have a central activity. On the other hand, narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain (Elisabethsky *et al*., 1995; Pal *et al*., 1999).

Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed *et al*., 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte *et al*., 1988). In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria *et al*., 2008). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent, reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte *et al*., 1988; Ferdous *et al*., 2008). Flavonoids have been reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana *et al*. 2001; Rao *et al*., 1998). Thus, the significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways. The abdominal writhing induced by acetic acid was also reported to be less selective (Collier *et al*., 1968) and proposed to act indirectly.
by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003). Besides these, acetic acid induced writhing in mice attributed visceral pain that finds much more attention of screening analgesic drugs (Hasan et al., 2010). In our study, the crude extracts of the plants showed significant (p<0.001) analgesic action compared to the reference drug diclofenac sodium.

There are also reports on the role of tannins in anti-nociceptive activity (Vanu et al., 2006). Besides alkaloids are well known for their ability to inhibit pain perception (Uche et al., 2008). The extracts of the plants and diclofenac sodium (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. The plant extract of *Solanum surattense* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism (Sabina et al., 2009).

It has been shown in the current studies that the early phase of formalin induced pain reflects the direct effect of formalin on nociceptors, whereas the late phase reflects that inflammatory pain appeared to be dependent on prostaglandin synthesis (Shibata et al., 1989; Tjølsen et al., 1992). Our results showed that the ethanolic extract of *Solanum surattense* exerted significant inhibitory effect on nociceptive response of the late phase of the inflammatory pain model in the formalin test. The formalin test may be a more useful model of clinical pain in which the late phase was dependent on peripheral inflammation and changes in central processing (Tjølsen et al., 1992). The histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase of the formalin test (Tjølsen et al., 1992). There is strong evidence that peripheral inflammatory procedure is involved in the late phase. The inhibitory effect of the extract on nociceptive response in the late phases of formalin test suggested that the anti-nociceptive effect of the extract could be due to its peripheral action.

Concerning to the phytochemical constituents, the preliminary qualitative phytochemical screening showed the presence of alkaloids, tannins, gums, reducing sugars, alkaloids and terpenoids in the ethanolic extract of *Solanum sutattense*. Therefore, it is assumed that tannins and alkaloids present in the extract are responsible for the observed analgesic activity, which has been reported as an anti-analgesic constituent (Vanu et al., 2006; Uche et al., 2008).

### 5. Conclusion

Finally, we demonstrate that the ethanolic extracts of *Solanum surattense* are endowed with both central and peripheral analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect. Further studies are needed, including toxicity evaluation and purification of active analgesic constituents from *Solanum surattense* extracts looking toward a pharmaceutical use.
Acknowledgement

We would like to show our gratitude to all of the stuffs of the Department of Pharmacy, North South University, Bashundhara, Dhaka, Bangladesh.

References


http://dx.doi.org/10.1211/0022357991772312

http://dx.doi.org/10.9734/BJPR/2013/4865


http://dx.doi.org/10.1016/S0378-8741(98)00048-8


http://dx.doi.org/10.1016/0304-3959(89)90222-4

http://dx.doi.org/10.1016/0304-3959(92)90003-T

http://dx.doi.org/10.1016/S0378-8741(02)00334-3


http://dx.doi.org/10.1248/bph.29.693

http://dx.doi.org/10.1007/s11418-007-0224-x

http://dx.doi.org/10.1016/0304-3959(83)90201-4