Evaluation of Analgesic Activities of 80% Methanol Leaf Extract of Solanum incanum L. (Solanaceae) in Mice

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ABSTRACT

Solanum incanum Linnaeus is traditionally used for treatment of pain and other ailments. But there is no scientific evidence on its analgesic activity to-date. Thus the aim of this study was to evaluate the analgesic activities of 80% methanol leaf extract of S.incanum in mice. After extraction of the crude using 80% methanol, S.incanum extract was evaluated for analgesic activity in hot plate test and acetic-acid induced writhing test. Mice were randomly assigned to different groups and treated with 100, 200 and 400 mg/kg doses of the extract and reference control groups (morphine 5 mg/kg and Aspirin 150 mg/kg) and negative control were treated with 2% tween 80. In the hot-plate method, all doses of the extract and the standard drug of morphine prolonged the reaction time significantly (p<0.05, or p<0.01 or p<0.001) as compared to negative control throughout the observation period. Prolongation of reaction time produced by 100mg/kg of the extract was lower (p<0.01) compared to morphine, 200mg/kg, and 400mg/kg at 90 and 120 min. However, middle and higher dose exerts comparable result at 30, 60 and 90 and 120 min in relation to the standard drug. In addition 80% methanol extract of S.incanum showed a significant protection (p<0.05) against acetic acid induced writhing compared to negative control. The extract produced a significant analgesic activity with 55.6, 38.2 and 44.8% inhibition of number of writhing at 100, 200 and 400 mg/kg dose levels, respectively. In conclusion, this study clearly suggests that 80% methanol leaf extract of S. incanum is endowed with central and peripheral analgesic activity. Hence, the findings collectively uphold the traditional use of the plant for pain treatment.

Keywords: Analgesic, S.incanum, Hot plate, Acetic acid

1. INTRODUCTION

Pain could be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.1,2 The intensity of pain is difficult to measure and an individual's perception of pain depends on the individual's emotional state, circumstances under which the pain was acquired, and whether it is perceived as a threatening signal.3 Pain is an enormous problem globally and estimates suggest that 20% of adults suffer from pain globally and 10% are newly diagnosed with chronic pain each year. According to a 2014 study on the global burden of chronic pain, at least 10% of the world's population is affected by a chronic pain condition and every year, an additional 1 in 10 people develops chronic pain.4 The lifetime prevalence for LBP among Africans (47%) was also found to be considerably higher than the estimates (38.9%) reported and the lifetime, annual and point prevalence of LBP was estimated to be higher among African adults compared to African children and adolescents.5 Although there is no study on the prevalence of pain in Ethiopia, a population based urban study has shown that prevalence of primary headache disorders was found to be 21.6% and that for migraine was 10%.6

Currently, the drugs used to relieve pain are either narcotics such as opioids or nonnarcotic such as salicylates. However, use of NSAIDs is associated with a number of adverse effects such as gastric irritation and gastric ulcer, alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition, which may result in increased bleeding.7 The issue of tolerance and dependence induced by opiates is also a major challenge in the use of narcotics for pain management. Moreover, despite the progress that has made in past in the development of pain therapy there is a need for effective and potent analgesic since about 40% of the respondents suffering from chronic pain are not satisfied with the treatment.8 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 herbal medicines are deemed to be safe, have good efficacy, are culturally accepted and have lesser side effects than the synthetic drugs. Because of potential side effects and inefficiency of chemical and synthetic drugs, application of complementary medications, particularly medicinal plants, for pain management is increasing. Some of herbs and medicinal plants proved to be scientifically used for the treatment: Aloe vera, Cordia verbenaceae, Curcuma longa, Curcuma longa, Kalanchoe pinnata, Schinus terebinthifolius.

The experimental plant Solanum incanum L is traditionally used for pain management in Ethiopian folklore medicine. Solanum incanum Linnaeus or Sodom/bitter apple (English), Embouy (Amharic), Hiddi (Afan Oromo) is a perennial, wild shrub like herb that grows up to 1.8 m in height that belongs to Solanaceae family which grows in many regions of Africa, Middle East and Far East Asia. In Africa the whole plant herb is used as a folklore remedy for sore throat, angina, stomach-ache, colic, headache, relieve of painful menstruation, liver problems and pain caused by onchocerciasis, pleurisy, pneumonia and rheumatism. The plant parts are also widely used to alleviate skin problems, such as infections, whitlow, ringworm, burns, sores, rashes, wounds, warts, carbuncles, ulcers, inflammations and benign tumors. Alternatively, the roots are chewed or its infusions applied externally on scarifications. Leaves parts are also used for washing painful areas, while in some cases they are burnt and the ash mixed with fat for use as an ointment. In addition, fresh leaves juice traditionally are used in Ethiopia for their wound healing activity. Moreover, there is evidence that the root part of S.incanum is endowed with analgesic and wound healing activities in animal models. Phytochemical studies indicate whole part of the herb contains substances such as steroidal alkaloids, glycolalkaloids, antioxidants (flavonoids and chlorogenic), and saponins which are thought to have analgesic activity in different studies. Thus the main aim of this study is to evaluate analgesic activities of 80% methanol leaf extract of Solanum incanum L in mice.

2. MATERIALS AND METHODS

2.1. Chemicals, drugs and reagents

Distilled water (DW), Morphine (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia), absolute methanol (Indenta chemicals, India) and Glacial acetic acid (Sigma – Aldrich laborkenchemikalien, Germany), Aspirin (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia) obtained from the respective vendors were used in the experiment.

2.2. Plant material

Fresh leaves of Solanum incanum was collected from Bishoftu town, Oromia region which is 60 km away from Addis Ababa, Ethiopia in January 2019. The plant material was then wrap with plastic shit and transport to Pharmacology laboratory at the department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University. Identification and authentication of the plant material was done by taxonomist and Voucher specimen (Collection Number BA/001) at national herbarium, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia. The collected plant material was identified to eliminate any dead material and then dried at room temperature under shaded area without exposing to direct sunlight. The dried plant materials were then broken in to small pieces using mortar and pestle and then prepared for extraction.

2.3. Experimental animals

The experiment was performed using Swiss albino mice of either sex weighing (20-30g) obtained from animal house of Addis Ababa University, College of Health Sciences, School of Pharmacy. The animals were maintained under standard laboratory condition (room temperature with 12:12 h-light–dark cycle) and were provided with the standard animal feed and water ad libitum. Animals were also acclimatized to the laboratory conditions for 7 days before the commencement of the actual experiment. All animals in this study were handled in accordance with the internationally accepted standard guidelines for use of animals (OECD, 2008) and the protocol was approved by the School of Pharmacy Ethics committee.

2.4. Extraction

The 80% methanol extract was prepared using maceration technique by soaking 150 g dry powder of Sincanum leaves with 80% methanol solution (1.8 L) in Erlenmeyer flask for 72 h at room temperature. The mixtures were filtered through gauze and then by Whatman No. 1 filter paper. The mark was re-extracted successively two times for a total of 6 days. The filtrates were combined and the solvent was removed by evaporation using a rotary evaporator (Buchi labortechnik, Switzerland) at 40 °C under reduced pressure. The remaining solvent was then removed in a lyophilizer (Delvac, India) to obtain a complete dry powdered residue. The resulting dry extract was weighed and the total yield was found to be 22%. The dried extracts were reconstituted with 2% tween 80 for oral administration.

2.5. Acute toxicity test

Acute toxicity test was performed based on the limit test recommendations of OECD Guideline 425 (OECD, 2008). On day one, a single female mouse was fasted for 3-4 h and given 2000 mg/kg of the extract orally. The mouse was then kept under strict observation for any physical or behavioral changes within 24 h, with special attention during the first 4 h. Following the results from the first mouse, other four female mice were fasted for 3-4 h and administered a single dose of 2000 mg/kg. They were then observed in the same manner. The observation continued for further 14 days for any signs of toxicity. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern.

2.6. Grouping and dosing of animals

Mice were randomly divided into five groups of six mice per group. Group I served as negative control and received vehicle (2% of Tween 80, P.O., 0.1 ml/10 g). Group II served as positive control and treated with standard drugs: morphine (5mg/kg, I.P.) for hot plate test and (4 mg/kg, S.C.) Aspirin (30 mg/kg, P.O.) for writhing test. Group III-V were treated with the extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively. Doses were selected based on acute toxicity study. From the acute toxicity studies as per OECD (2008) guidelines, after having considered the safety of the plant, 1/10th of the maximum dose (2000 mg/kg) was considered as a middle dose. Half and double of the middle dose were considered as the low and high dose, respectively. Dose of Morphine (5 mg/kg) and Aspirin (30mg/kg) was selected based on previous studies. The test doses were prepared freshly on the day of the experiment. Animals were treated with the vehicle or extract/standard 1 h before commencement of the experiments.
2.7. Tests for Analgesic Activity

2.7.1. Hot Plate Test

For the hot plate test, following the administration according to the grouping, mice were placed on a hot plate set at 55 ± 0.5 °C. The latency of nociceptive response like when the mice licking their fore- and hind paws or jumping or flicking were recorded at different times after oral administration of the extract or control (5 mg/kg morphine or vehicle (2% Tween 80). Latency time or reaction time (in seconds) on the hot plate for each mouse was determined at time 0 before and intervals of 30, 60, 90 and 120 minutes after treatment with the respective agent. A cut of period of 15 seconds was given to avoid damage to the paw. Percentage latency (% Latency) and Maximum Possible Analgesia (% MPA), was calculated for each group as shown below.

\[ \% \text{Latency} = \frac{\text{Mean latency time (control group} - \text{test group})}{\text{Mean latency for control group}} \times 100\% \]

Percentage Maximum Possible Analgesia (% MPA) obtained by using this formula:

\[ \% \text{MPA} = \frac{\text{Latency test} - \text{Latency pre drug}}{\text{Cut off} - \text{Latency pre drug}} \times 100\% \]

2.7.2. Acetic acid Induced Writhing Test

This test was performed as described by Koster et al, 1959 cited by Chattopadhyay et al., 2012. Mice were treated as described under grouping and dosing and one hour following treatment, mice received I.P. injection of 0.6% v/v acetic acid solution at a dose of 10 ml/kg. Five minutes after administration of acetic acid, the number of writhes or stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) was counted for 15 min. A reduction in the number of writhes as compared to the control group was considered as evidence for the presence of analgesia, expressed as percent inhibition of writhing, which calculated according to the following formula:

\[ \% \text{Inhibition} = \frac{\text{Mean no. of writhes (control} - \text{test})}{\text{Mean no. of writhes in control}} \times 100\% \]

2.8. Preliminary phytochemical screening

The hydro-alcoholic extract of *S. incanum* leaves was screened according to Trease and Evans (1996), cited by Musa et al., 2009, for its secondary metabolites according to standard procedures.

2.9. Statistical Analysis

Results was expressed as mean ± standard error of mean (SEM) of responses. The results were analyzed statistically using SPSS Software Ver.20. The statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey post Hoc test. The value, p<0.05 was considered as statistically significant.

### 3. RESULT

#### 3.1. Acute toxicity study

The acute toxicity study indicated that the leaf extract of *S. incanum* at a dose of 2000 mg/kg caused no mortality within the first 24 h and for the next 14 days. Physical and behavioral observations of the experimental mice also revealed no visible overt signs of acute toxicity like skin and fur, eyes and mucus membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. The data suggest that LD50 of the leaf extract is more than 2000 mg/kg.

#### 3.2. Hot plate test

The effect of 80% methanolic extract of *S. incanum* on the hot plate test is presented in Table 1. Accordingly, all doses of the extract and the standard drug of morphine produced significant central analgesic effect by increasing the latency time as compared with negative control. The increase in latency was significantly higher (p< 0.01) for extract and morphine as compared with the negative control in all times of latency measurement. However, there was no significant difference in latency period between the extract and the standard drug. In addition, there was no significant difference in latency between different doses of the extract.

Table 1: Effect of 80% methanol leaf extract of *S. incanum* on the hot plate test

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Latency time (sec) ±S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Min</td>
</tr>
<tr>
<td>Neg. con</td>
<td>5.67±1.542</td>
</tr>
<tr>
<td>MOR (5mg/kg)</td>
<td>6.33±1.856</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>4.17±0.601</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>4.33±3.333</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>7.17±1.579</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M (n = 6); 1=p<0.05, 2=p<0.01, 3=p<0.001; a: relative to control MOR - morphine 5 mg/kg; data in parenthesis show percentage increase in latency of licking relative to time zero within individual group.

As shown in table 2 the extract at a dose of 200 and 400 mg/kg has resulted in 143.9% and 174.08 % increase in latency at 120 and 60 min respectively compared to the negative control. In addition, the middle dose of the extract has resulted in percent increase in latency of 143.9% at 120 min. Furthermore, the positive control morphine has caused significantly different analgesia effect at 30 min resulting in 161.12% of increase in latency but less than the higher dose of the extract at 60 min (174.08%).
The trend time was observed at 30 min. The extract.

In addition, Fig 1 depicts percentage increase in reaction time across the observation period. Time to peak activity was longer for the extract (60 min) than for the standard drug (30 min). 100 mg/kg of the extract protection waned across time. By contrast, the change for 200 mg/kg somewhat increases with increase in observation time except for the 120 min time. The trend with 400 mg/kg was different, showing both increase and decrease in latency at different intervals.

Data represent mean ± S.E.M and percent inhibition of number of writhing with respect to the negative control, (n = 6); 1=p<0.05, 2=p<0.01, 3=p<0.001; a

### Table 2: The Percent latency (% latency) effects of *S.incanum* 80% methanol extract

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Percent Latency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Neg. CON</td>
<td></td>
</tr>
<tr>
<td>MOR</td>
<td>161.12</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>148.2</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>116.05</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>119.14</td>
</tr>
</tbody>
</table>

### Table 3: Effect of 80% methanol leaf extract of *S. incanum* on the hot plate test in acetic acid induced writhing test model

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>%Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg. CON</td>
<td>63.40±3.187</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>23.50±4.945</td>
<td>62.9</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>28.17±6.041</td>
<td>55.6</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>39.17±5.322</td>
<td>38.2</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>35.00±6.623</td>
<td>44.8</td>
</tr>
</tbody>
</table>

4. **DISCUSSION**

The presented study revealed that the 80 % extract of *S.incanum* have analgesic activity in hot plate and acetic acid induced writhing tests. Hot plate tests are the most common tests of central nociceptive activity that are based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated activity. They were selected for this study because of several advantages including sensitivity to strong analgesics, limited tissue damage, accuracy of results and they are also less time consuming. In the hot plate method, a plate heated at a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinally integrated responses. As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or Paracetamol.

The peak increase in the latency time was observed at 30 min of interval in the hot plate test for the 100 mg/kg of the extract and the standard drug Morphine in the present study. However the middle and the higher dose of the extract have shown peak latency at 90 and 60 min interval respectively. This difference could be attributed the time lag between drug entering the central compartment and distribution into the target site or formation of an active metabolites that are endowed with analgesic activity. The lower dose have shown time dependent decrease in its analgesic activity which could be explained by the decrease in the concentration of the active compounds in the brain with time but the 200 and 400 mg/kg doses have relatively long lasting analgetic effect throughout the time intervals.

Similar studies have also shown comparable analgesic activity of different plant extracts in the hot plate model. For example, according to Abiye et al, 2019, evaluation of the extract of *H. revolutum* leaves in the hot plate test showed significant central analgesic effect. This was demonstrated by increase in latency time or increase in threshold of pain by thermal stimuli. In addition, a study done by Nasrin et al, 2017 showed that *Solanum sisymbriifolium* was have got moderate but significant antinociceptive activity of particularly at higher dose (400 mg/kg). The effect was more profound in heat-induced pain models than chemical induced model showing the central antinociceptive activity of the plant. Moreover, study done by Hajhashemi et al, 2010...
The effect of the extract hot plate method provides a confirmation of its central effect. *S. incanum* showed a central anti nociceptive activity by increasing the latency to discomfort and may act like centrally active drugs, by activating the periaqueductal grey matter to release endogenous peptides (i.e., endorphin or enkephalin). These endogenous peptides descend to spinal cord and function as inhibitors of the pain impulse transmission at the synapse in the dorsal horn.  

Acetic acid-induced abdominal constriction method was employed to evaluate peripheral antinociceptive activity because it is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods. Although, acetic acid-induced writhing is a highly sensitive and useful test for analgesic drug development but not a selective pain test as it gives false positive result with sedatives, muscle relaxants. Intraperitoneal injection of acetic acid produces pain through activation of chemoceptive nociceptors or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin.  

The present results showed that the 80% methanolic extract of *S. incanum* induced analgesic effect against the writhing syndrome indicating its peripheral effect. Prolongation of reaction time produced by 200, and 400mg/kg of the extract was significantly lower compared to Aspirin at all-time points. But 100 mg/kg of has comparable effects with Aspirin. The *S. incanum* extract at all test doses significantly reduced the writhing response while the lower dose was superior in its action compared to the other doses. This may be explained by the difference in the pharmacokinetics of constituents of the plant extract. So, the higher dose has more central distribution and has greater central effect as seen in the hot plate test but the lower dose has greater peripheral effect. Similar study by Manjyot *et al.* who has evaluated the extracts derived from leaves of *S. melongena* Linn. exhibited significant analgesic activity in albino rats by inhibiting acetic acid induced writhing, which is a model of visceral pain.  

In peripheral tissues, prostaglandins and kinines would seem to play an important role in the pain process and writhing induced by chemical substances injected intraperitoneally is said to be the consequence of sensitization of the chemoceptive nociceptors by prostaglandins. This response is characterized by abdominal contractions accompanied by movements of the hind paws particularly. The writhing response is due to sensitization of chemoceptive nociceptors by prostaglandins and in particular PGE2 and PGF2 as well as lipooxygenase products. These results suggest that the pain killing effect of this plant may be by the prostaglandins synthesis inhibition. This test also confirms the peripheral action of Aspirin.  

The analgesic effect of 80% methanolic extracts of *S. incanum* may be attributed to the presence of different phytoconstituents. Preliminary phytochemical screening in this study have shown the have indicated that the leaves of *S. incanum* have different secondary metabolites like terpenoids, alkaloids, flavonoids, saponins, tannins and phenolic compounds which was almost similar with a study done by Manal *et al.*, 2017. The leaves of *S. incanum* were extracted with 80% methanol using maceration technique. Methanol is a universal, safe and effective solvent in extracting polar and less polar secondary metabolites. It is a preferable solvent in extracting semi-polar compounds (e.g., phenolic acids, flavonoids, and alkaloids) from different parts of medicinal plants. It is also a best solvent to get the highest phenolic and flavonoid contents, followed by 80% ethanol, 80% acetone and distilled water.  

The secondary metabolites obtained in this plant extract have been reported to have different extents of analgesic activities. For instance, alkaloids exert their analgesic action by interfering with the CNS neurotransmitter activity. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. Flavonoids may also increase the amount of endogenous serotonin or may interact with 5-HT2A and 5-HT3 receptors, which may be involved in the mechanism of central analgesic activity. Flavonoids are also found to target the prostaglandins, which are involved in the late phase of acute inflammation and pain. Thus, in the 80% methanol leaf extract of *S. incanum*, flavonoids could contribute to suppression of activation and sensitization of peripheral chemo-sensitive nociceptors by acetic acid and both alkaloids and flavonoids could contribute for the central effect in the hotplate test.

5. CONCLUSION

In conclusion, the results obtained in the present study clearly suggest that 80% Methanol leaf extract of *S. incanum* is endowed with significant central and peripheral analgesic activity as shown by increase in latency in hot plate test and protection against acetic acid induced writhing tests. Hence, the findings collectively uphold the traditional use of the plant for wound treatment.

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Disclosure of Conflict of Interest: The authors have not declared any conflict of interests.

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Aqueous Extract from Leaves


