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# Analgesic Activity of Aqueous Extract of Solanum Xanthocarpum Berries (SXB) in Animal Models

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**ABSTRACT**

**Background and aim:** Traditional medicines can be considered a reliable source of new drugs as they are safe, dependable, and cost-effective compared to synthetic drugs. Drugs that can alter pain sensitivity are known as analgesics, and many herbal products have this property. The purpose of this study was to see how effective an aqueous extract of Solanum xanthocarpum berries (SXB) is as an analgesic in animal models.

**Materials and methods:** Analgesic activity was assessed using the tail-flick method (for central action) in rats and the acetic acid-induced writhing test (for peripheral action) in mice. Three doses of the plant extract (500, 1000, and 1500 mg/kg) prepared by dissolving the drugs in 2% gum acacia were used. The tail-flick method and the acetic acid-induced writhing test used standard pethidine 5 mg/kg and 100 mg/kg aspirin, respectively. The vehicle served as a controlled drug.

**Results:** In the acetic acid-induced writhing test ($p<0.001$), the plant extract showed significant analgesic activity, while no analgesic activity was found in the tail-flick method.

**Conclusion:** The aqueous extract of Solanum xanthocarpum berries exerts its analgesic activity through peripheral pain mechanism though it does not have any central action.

## 1. Introduction

Pain is an unpleasant sensory and emotional experience resulting in tissue damage.[1] The perception of a noxious stimulus is a subjective experience different from pain as it includes a strong emotional component. The amount of pain a stimulus produces is determined by factors other than the stimulus itself. Pain is considered to be a symptom of some underlying medical condition. Drugs that can alter sensitivity or reduce pain are called analgesics, and many herbal products have this property.[2] Analgesics such as morphine, aspirin, and nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat severe pain, such as cancer. However, studies have shown physical dependency, addiction, and tolerance with opiates, whereas gastrointestinal disorders with NSAIDs.[3] Modern drugs or conventional medicines bring adverse effects, sometimes more dangerous than the disease itself.[4] Traditional herbal medicines are natural plant-derived substances with minimal or no industrial processing that traditional healers use to treat various illnesses in local practice. Widespread interest has grown recently to develop drugs derived from plants. This interest primarily originates from the belief that traditional medicine is safe and dependable compared to the costly commercially available drugs with adverse effects. Promoting traditional medicine's use of compound formulations of plant medicines in their natural or semi-processed form for medical disorders can help to increase the production of potent, safe, and cost-effective drugs of plant origin.[5] The study of plants for various medicinal properties should be a significant feature for the scientific validity of locals' folklore claims about the plants' utility and a new potential source of herbal drugs.[6]

Solanum xanthocarpum, locally known as Leipung-khanga, belonging to the family Solanaceae, is a widely distributed perennial herb with a woody base commonly found in the North-Eastern states of India. Flowers are purple; the fruit type is berry globose measuring about 6-8 mm in diameter, green in color becoming reddish yellow when ripe.[7] Berry juice is commonly used to treat sore throats, coughs, and asthma. Carminative properties of the stem, flowers, and fruits are employed to treat burning sensations in the feet accompanied by watery vesicular eruptions.[8] The reaction of an animal to a mildly painful stimulus, usually mechanical or thermal, is often used to measure nociception in animal models of analgesic drugs. Although animals cannot verbally communicate, they exhibit the same motor behavior and physiological response as a human response to pain.[9] As a result, the current study evaluated the analgesic activity of aqueous extract of Solanum xanthocarpum berries (SXB) in suitable animal models, considering its use in various ailments locals.

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## 2. Materials and methods

***Plant material***

The fresh ripe berries of Solanum xanthocarpum were collected from around the Imphal area and were identified and authenticated by Professor and Head of Botany department, DM College of Science, Imphal.

***Plant extract preparation***

The berries were cleaned, dried in the shade, powered by the grinder, and stored in an airtight container to be used later. Eighty grams of powdered berries using a Soxhlet apparatus were extracted with distilled water. The resulting brownish polar extract was evaporated, shade dried, scraped out, weighed, and stored in a glazed porcelain jar for future use.[10] The percentage yield was 23.25%, and the extract thus obtained was used for the analgesic study.

***Toxicity studies***

The Organization for Economic Cooperation and Development (OECD) guidelines 423 were used for acute toxicity testing.[11] Toxicity testing was done by administrating aqueous extract of Solanum xanthocarpum at doses 100, 200, 400, 800, 1600, and 3000 g/kg per oral to groups of mice each group consisting of 10 mice, and mortality was observed after 24 hours.

***Animals***

After receiving approval from the Institutional Animal Ethics Committee (IAEC), Wistar albino rats of 100-200 gm and Wistar albino mice of 25-30 gm (both of either sex, non-pregnant) were obtained from the Central Animal House, RIMS, Imphal. The animals were maintained at a temperature of 24-280°C, with a relative humidity of 50–55% and a 12-hour light/dark cycle (6–10 hours light, 18–6 hours dark). The animals were allowed free access to water and fed standard animal feed. Before the experiment, all animals were given a seven-day acclimatization period in the laboratory.

***Analgesic activity***

***1. Tail flick method***

The tail-flick method described by D'Armor and Smith was used with slight modifications.[12] Healthy albino rats of the Wistar strain, either sex, weighing between 100-200gms, were collected from the RIMS Central Animal House, and the prescreened animals with a reaction time of less than six seconds were used in the study. During the experiment, the animals were fasted overnight but given free access to water ad libitum. They were divided into five groups, each with six animals. Group I was given 2 % gum acacia in distilled water p.o as a control. Group II, III, and IV were the test groups 1, 2, and 3 that received an aqueous extract of SXB 500, 1000, and 1500 mg/kg p.o. Group V received the standard drug pethidine at a 5 mg/kg p.o. The test drug was p.o administered after being suspended in distilled water with % gum acacia. The volume of the drugs was kept constant at 10 ml/kilogram of body weight of the animals. An analgesiometer was used to measure the animals' tail-flick latencies (reaction time). The current flowing was maintained at a constant 6 Amps through the naked nichrome wire. The distance between the heat source and the tail skin was selected to be 1.5 cm. The radiant heat application site was measured from the root of each rat's tail and fixed at 2.5 cm. The time it took the animals to withdraw (flick) their tail from the hot wire was used to measure reaction time. The reaction time cutoff was fixed at 10 seconds to avoid tissue damage. After the drug was administered, reaction time was measured at 1 hour, 2 hours, and 3 hours. After each time interval, the average values of reaction time were calculated.

***2. Acetic acid-induced writhing test***

The acetic acid-induced writhing test was carried out with the help of Witkin et al.'s method with slight modifications.[13] Wistar strain albino mice of either sex weighing between 25-30gms were taken from the RIMS Central Animal House. The animals were screened, and those who failed to exhibit writhing within 10 minutes were discarded. The writhing movement was described as abdominal constriction and the hind limb extension. The prescreened animals (a total of 30) were fasted overnight but had free access to water throughout the experiment. The animals were divided into five groups, each with six animals. Group I was the control group who received 2% gum acacia in distilled water p.o. Group II, III, and IV were the test groups 1, 2, and 3 that received an aqueous extract of SXB 500, 1000, and 1500 mg/kg p.o. The standard drug aspirin was given to Group V at 100 mg/kg p.o. The drugs were supplied p.o after being suspended in distilled water using % gum acacia. The volume of the drugs was kept constant at 25 ml/kilogram of body weight of the animals. Writhing was induced in each mouse 60 minutes later by injecting 10ml/kg body weight of % acetic acid in distilled water intraperitoneally. After 5 minutes of injection, the number of writhing was counted for 20 minutes. The following formula was used to calculate the percentage of protection at each dose level:

$$\text{Percentage protection} = 100 - \left(\frac{No.\ of\ writhes\ in\ the\ treated\ group}{No.\ of\ writhes\ in\ control\ group}\right) \text{x } 100$$

***Statistical analysis***

The data were statistically analyzed using a one-way ANOVA followed by a Dunnet's 't-test to determine whether there was a significant difference between groups. Significant was defined as a p-value of less than 0.05.

## 3. Results

***Acute toxicity***

In the doses tested, the aqueous extract of Solanum xanthocarpum berries was safe. After 24 hours, there was no mortality up to a dose of 3000mg/kg per oral.

**Table 1. Analgesic activity of the SXB aqueous extract in albino rats on the tail-flick test.**

| Group | Drug dose (mg/kg) | Pre-drug reaction time in seconds (Mean±SEM) | Reaction time in seconds (Mean±SEM) | | | P-value |
|---|---|---|---|---|---|---|
| | | | 60 mins | 120 mins | 180 mins | |
| Group I (Control) | 10 ml/kg | 3.71±0.14 | 3.83±0.17† | 3.88±0.20† | 3.80±0.25† | †p<0.001 (significant when compared to standard. |

| | | | | | | |
|---|---|---|---|---|---|---|
| Group II (Test 1) | 500 mg/kg | 3.76±0.11 | 3.86±0.21$^{*\#\dagger}$ | 3.83±0.27$^{*\#\dagger}$ | 3.77±0.22$^{*\#\dagger}$ | $^{*}$p>0.05 (not significant compared to the pre-drug reaction time).<br>$^{\#}$p>0.05 (not significant when compared to control)<br>$^{\dagger}$p<0.001 (significant when compared to standard). |
| Group III (Test 2) | 1000 mg/kg | 3.86±0.16 | 3.91±0.22$^{*\#\dagger}$ | 3.88±0.24$^{*\#\dagger}$ | 3.70±0.19$^{*\#\dagger}$ | $^{*}$p>0.05 (not significant compared to the pre-drug reaction time).<br>$^{\#}$p>0.05 (not significant when compared to control)<br>$^{\dagger}$p<0.001 (significant when compared to standard). |
| Group IV (Test 3) | 1500 mg/kg | 3.75±0.20 | 3.88±0.26$^{*\#\dagger}$ | 3.85±0.25$^{*\#\dagger}$ | 3.81±0.20$^{*\#\dagger}$ | $^{*}$p>0.05 (not significant compared to the pre-drug reaction time).<br>$^{\#}$p>0.05 (not significant when compared to control)<br>$^{\dagger}$p<0.001 (significant when compared to standard). |
| Group V (Standard) | 5 mg/kg | 3.81±0.25 | 8.9±0.30$^{**\#\#}$ | 8.76±0.38$^{**\#\#}$ | 8.43±0.41$^{**\#\#}$ | $^{**}$p<0.001 (significant when compared to the pre-drug reaction time).<br>$^{\#\#}$p<0.01 (significant when compared to control). |

The pre-drug reaction times of the different groups did not significantly differ (p>0.05). There was no significant (p>0.05) increase in the control and test groups' reaction time compared to the pre-drug reaction time at any subsequent recordings made during the experiment. At 60, 120, and 180 minutes after i.p. injection, the mean reaction time in seconds for pethidine (5mg/kg) was 8.9±0.30 (p<0.001), 8.76±0.38 (p<0.001) and 8.43±0.41 (p<0.001) respectively.

**Table 2. An acetic acid-induced writhing test revealed the analgesic activity of the SXB aqueous extract in albino mice.**

| Group | Drug dose (mg/kg), per oral. | No. of writhing movement (Mean±SEM) | Percentage of protection | P-value |
|---|---|---|---|---|
| Group I (Control) | 25 ml/kg | 49.24±3.42$^{\#}$ | ----- | $^{\#}$p<0.001 as compared to the standard. |
| Group II (Test 1) | 500 mg/kg | 28.38±3.75$^{*\#}$ | 42.36 | $^{*}$p<0.01as compared to the control.<br>$^{\#}$p<0.001 as compared to the standard. |
| Group III (Test 2) | 1000 mg/kg | 24.26±3.36$^{**\#\#}$ | 50.73 | $^{**}$p<0.001 as compared to the control.<br>$^{\#\#}$p<0.01 as compared to the standard. |
| Group IV (Test 3) | 1500 mg/kg | 19.67±2.22$^{**\#\#\#}$ | 60.05 | $^{**}$p<0.001 as compared to the control.<br>$^{\#\#\#}$p<0.05 as compared to the standard. |
| Group V (Standard) | 100 mg/kg | 13.34±2.28$^{**}$ | 72.90 | $^{**}$p<0.001 as compared to the control. |

n=6 in each group, $^{*}$p<0.01, $^{**}$p<0.001 as compared to the control.

Writhing movements were inhibited 72.90 % by the standard drug aspirin at a dose of 100mg/kg. Compared to the control group, the number of writhing was significantly reduced in the test and standard groups.

## 4. Discussion

Pain relievers that act on the peripheral or central neural systems to relieve pain selectively without altering consciousness are known as analgesics.[14] Centrally acting analgesics act by modifying the physiological response to pain as well as raising the pain threshold. On the other hand, Peripherally acting analgesics act by inhibiting the generation of impulses at the pain chemoreceptor site.[15] Pain-state models using thermal stimuli, such as the tail-flick test, and chemical stimuli, such as the acetic acid-induced writhing test, were used to screen for analgesic activity in this study. The tail-flick test is used to detect centrally acting analgesics, whereas the acetic acid-induced writhing test detects analgesics that act centrally and peripherally.[16]

After 60, 120, and 180 minutes of administration, the test drug produced no significant increase ($p>0.05$) in the pain threshold in the tail-flick model at doses of 500, 1000, and 1500 mg/kg. At 60, 120, and 180 minutes, the standard drug pethidine (5 mg/kg) significantly increased the pain threshold ($p<0.001$). The finding of the standard drug pethidine is similar to the study done by Chakraborty A et al.[17] Analgesics that contain opioids are more effective at blocking mechanically induced pain.[18] The standard drug pethidine exerts its action through the μ receptors indicating narcotic involvement.[19] As the test drug did not produce any significant increase in the pain threshold, it can be suggested that the aqueous extract of Solanum xanthocarpum berries do not have centrally acting analgesic property. The findings of our study revealed that an aqueous extract of Solanum xanthocarpum berries reduced the number of writhes significantly in a dose-dependent manner. When given p.o, the aqueous extract of SXB at doses of 500, 1000, and 1500 mg/kg reduced writhing movement by 42.36 %, 50.73 %, and 60.05 %, respectively, whereas the standard drug aspirin inhibited writhing movement by 72.90 %. During a 20-minute observation period, the number of writhing movements in the control group was 2.46 per minute, consistent with the finding of Hazare et al.[20] The percentage inhibition and several writhing movements found by the common drug aspirin are similar to the findings of Banerjee S et al.[21] Various endogenous chemical pain mediators like prostaglandin E2 (PG), substance P, serotonin, histamine, bradykinin are released by acetic acid, which further causes stimulation of nociceptive neurons.[12, 23, 24] Prostaglandins and kinins appear to play an essential role in the pain process in peripheral tissues, and writhing induced by chemical substances injected intraperitoneally is thought to be the consequence of prostaglandin sensitization of chemo-sensitive nociceptors.[20, 25] The extract of Solanum xanthocarpum berries caused inhibition of this pain, which is thought to be due to inhibition of release of prostaglandins, the mechanism of which is almost similar to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDS). A study done by Rahman et al. on methanolic extract of aerial parts of Solanum xanthocarpum showed significant antinociceptive activity in mice when evaluated by the acetic acid-induced writhing method.[26] Solanum xanthocarpum contains alkaloids, phenols, flavonoids, glycosides, sterols, saponins, carbohydrates, fatty acids, and amino acids.[27] The presence of alkaloids and flavonoids can be responsible for analgesic activity.[28] The different constituents of flavonoids have inhibited neuropathic and inflammatory pain. The effect is brought about by inhibition of the peroxidase active site if COX 1, COX 2, and lipoxygenase resulting in inhibition of prostaglandins, thromboxane, and leukotriene products, respectively.[29]

## 5. Conclusion

The aqueous extract of Solanum xanthocarpum berries demonstrated significant analgesic activity through peripheral pain mechanisms. However, further studies with the plant are required to evaluate the dose-dependent analgesic activity and determine the active principle responsible for the exact mechanism of analgesic activity.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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