Evaluation of antidiarrheal and antinociceptive activity of methanolic extract of Alstonia scholaris Linn. on mice models

Mohammad Shahadat Hossain*, Ziku Chandra Dey, Md. Imdadul Hoque, Md. Saddam Hossain Bhuiyan, Hasan Al Banna

Abstract

Alstonia scholaris is an indigenous medicinal plant of Bangladesh. The leaves have been used traditionally as folk remedies for the treatment of many diseases including diarrhea, dysentery, and malaria and snake bites. The ripe fruits of the plant are used in syphilis and epilepsy. It is also used as a tonic, anti-periodic, and anthelmintic. However, there was no study on whole plant extract of Alstonia scholaris. The present study designed to investigate the anti-nociceptive and anti-diarrheal activities of Alstonia scholaris on animal models at different doses such as 200 mg/kg and 400 mg/kg. Various methods also employed for investigating these activities such as castor-oil induced diarrhea, castor-oil induced enteropooling and gastrointestinal motility test, and acetic acid induced writhing test, tail immersion and hot plate methods. The diarrheal episode was inhibited by 50.79% and 57.14% for methanol extract at the doses of 200 and 400 mg/kg respectively. The extract significantly (p<0.05, p<0.01) lessened the intestinal volume (0.50 ± 0.04 ml for 200 mg/kg) and (0.47 ± 0.02 ml for 400mg/kg) for methanolic extract compared to control (0.65±0.03 ml) in castor-oil induced enteropooling and also decreased intestinal transit (55.58 – 61.12%) for methanolic extract comparable with standard (loperamide 5 mg/kg). The methanol extract of Alstonia scholaris significantly (P< 0.05 and P< 0.01) reduced the number of writhing, increased latency to flick tail in tail immersion method and elevated the mean basal reaction time in hot plate method. The results of methanolic extract showed highly significant but dose dependent anti-diarrheal and anti-nociceptive activity, which supports its use in traditional herbal medicine.

Keywords: Alstonia scholaris, Antidiarrheal, Antinociceptive, Castor oil, Intestinal transit, Acetic acid.

Introduction

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of pain and arthritic conditions. Pain is an unpleasant sensation localized to a part of the body. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distention, or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system.1

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause.2 Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency. Now-a-days, opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side effects and low potency.3 In case of morphine, acute morphine poisoning, hypotension, drug dependence, etc.

Diarrheal disease is the second leading cause of death in less than five children in world wide. According to latest data every year diarrhea kills around 760 000 children under five. There are nearly 1.7 billion cases of diarrhea disease year, globally. 4 Worldwide, diarrhea accounts for more than 5-8 million deaths in infants and small children less than 5 years each year.
According to World Health Organization (WHO) estimation for the year 1998, there were about 7.1 million deaths due to diarrhea. In Bangladesh, one third of the total child death burden is due to diarrhea. WHO organized a Diarrhea Control Program where they emphasized use of traditional medicines to combat the episodes of diarrhea. Diarrhea appeared by several mechanism such as increasing the gut motility, along with increased secretion of ions and a decrease in the absorption of fluid, and thus a loss of electrolytes, particularly Na and water. Synthetic drugs as well as conventional treatments are being failed to fulfill their objectives, due to their toxic and adverse side effects. For this reason it becomes necessary to search other alternatives such as plants. For these consequences herbal medicine has made a comeback to improve the fulfillment of our present and future health needs.

Considering the importance of plants as a vital source of medicine even today in the present study a plant Alstonia scholaris Linn, which is popularly known as “Saptaparni” or the “Devil tree” is one of the most versatile medicinal plants having a wide spectrum of biological activity. It is a common tree, growing up to 3.0 meter in height, distributed throughout the sub-Himalayan belt, West Bengal, Bihar, peninsular India and Southeast Asia. It is a beautiful foliage tree with a large canopy, and because of this, it has become a popular ornamental tree in the landscapes and gardens in the warm and temperate regions of Florida, Texas, and California in the United States. Leaves are 4-7 in a whorl, coriaceous, bluntly acuminate, dark green above and pale beneath. Bark is rough, tessellated corky grey to grey white and contains whorled branches. Greenish white flowers in unambrellately branched manner. They are 7-10 mm long, white, cream or green. The phytochemical constituents of Alstonia scholaris have been extensively investigated. Alkaloids, flavonoids, reducing sugars, steroids, saponins and tannins were documented as the chief chemical constituents. The bark of Alstonia scholaris is useful in malarial fevers, abdominal disorders, dyspepsia and in skin diseases. The bark is bitter, astringent, digestive, laxative, antihelmintic, antipyretic, stomachic, cardio-tonic and Tonic. The bark extract has been reported to possess anti-plasmodial, immuno-stimulant, anticancer effect and is also hepatoprotective. Bark is also used as febrifuge, depurative and galactogogue. It is effective in leprosy, skin diseases, puritis, chronic and foul ulcers, asthma, bronchitis, agalactia and debility. In folklore medicine, milky juice is applied on wounds, ulcers and rheumatic pains; mixed with oil and dropped into ear, it relieves ear ache. Leaves used in beriberi, dropsy and congested liver. Latex applied to sores, ulcers, tumors and rheumatic swellings. The ripe fruits of the plant are used in syphilis and epilepsy. It is also used as a tonic, antiperiodic, and anthelmintic. The plant has hepatoprotective activity on liver injury. The aqueous extract of Alstonia scholaris significantly reduced elevated blood glucose level in streptozotocin (STZ) diabetic rats without showing any hypoglycemic effect in normal rats. Ethyl acetate (EA) fraction from ethanolic extract of Alstonia scholaris leaves possesses anti-anxiety and anti-depressant activities.

**Materials and Methods**

**Plant material**

The plant samples were collected from the forests of Chittagong and Chittagong Hill Tracts in May 2010. The plant was identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, University of Chittagong and a voucher specimen SBU 1121. Dt. 06.03.2009. CTGUH, has been deposited in the Department of Botany, University of Chittagong, Chittagong, Bangladesh. All parts of the plants were thoroughly washed with water and dried in a shade at room temperature for 7 days; after that they were dried in an oven at 40° C for the next 2 days to facilitate grinding.

**Preparation of extract**

The dried parts of plant were powdered coarsely and about 500 g of powdered material was separately macerated in methanol (2 L) at room temperature for seven days accompanying occasional shaking and stirring. The whole mixture was then filtered and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get the dried extracts.

**Animals**

White albino mice (Swiss-web star strain, 20-35 g body weight [b. wt.]) bred in the animal house of Jahangir Nagar University, Bangladesh was used for the experiments. The procedures in this study for animal handling were performed in accordance with the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day-night cycle. All animal experiments were carried out according to the guidelines of Institutional Moreover; the experiments were conducted in an isolated and noiseless condition. The test animals were divided into several groups for different dose such as 200 mg/kg of 400 mg/kg etc. The animals were acclimatized to laboratory condition for one week prior to experimentation.

**Chemicals**

Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh), nalbuphine (Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh), acetic acid (MERCK, Mumbai, India), Loperamide hydrochloride 99.71% (Square Pharmaceuticals Ltd., Bangladesh), castor oil (WELL’s Heath Care, Spain), normal saline solution, 0.9% NaCl (Popular Pharmaceuticals Ltd., Bangladesh) and charcoal meal (10% activated charcoal in 5% gum acacia) were used.

**Antidiarrheal activity**

**Castor oil induced diarrhea**

This experiment was carried out by the method described by Awouters et al. The experimental mice were kept fasting condition for 18 hours. Four groups of mice were taken for this experiment. Group I treated as control (saline 2 ml/kg body weight orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i. p.) and Group III-IV received methanolic...
extract (200 and 400 mg/kg b. wt. i. p.). Then 1 h later, castor oil (0.4 ml/mice) was administered orally. The mice were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

**Castor oil induced enteropooling**

Intraluminal fluid accumulation was determined by the method of Robert et al. 18 h fasted mice were divided into four groups of five animals each. Group I treated as control (saline 2 ml/kg b. wt. orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p.) and Group III-IV received methanolic extract (200 and 400 mg/kg b. wt. i.p. respectively) before administration of castor oil (1 hr later) in all mice orally to induce diarrhea. Then 1 h later, the mice were sacrificed by overdose of chloral hydrate anesthesia and the small intestine was ligated both at the pyloric sphincter and at the ileocecal junctions and dissected out. The small intestine was weighed. The intestinal contents were collected by milking into a graduated tube and the volume was measured. The intestines were weighed and the differences between full and empty intestines were calculated.

**Gastrointestinal motility test**

The experiment employed the method described by the method described by Mascolo et al. Mice were fasted for 18 h and divided into four groups of five animals each. At first, castor oil was administered orally to these animals to induce diarrhea. One hour later, Group I received saline (2 ml/kg body weight orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p.) and Group III-IV received methanolic extract (200 and 400 mg/kg b. wt. i.p.). One hour after, i. p. administration of treatments, mice received 0.5 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, the animals were sacrificed by overdose of chloroform anesthesia and the distance traveled by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of the total distance of the intestine.

**Anti-nociceptive activity**

**Acetic acid induced writhing test**

Mice were divided into four groups of either sex containing five of each. Group I treated as control group (received saline 2 ml/kg body weight orally), Group II treated as standard (received diclofenac sodium 10 mg/kg b. w.), Group III and Group IV treated with methanol extract (200 and 400 mg/kg) orally 30 min before acetic acid injection.

Immediately after administering acetic acid, mouse were observed and the number of writhing or stretches were counted for 20 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The percent inhibition (% analgesic activity) was calculated by

\[
\% \text{ inhibition} = \frac{(A-B)}{A} \times 100
\]

Where, A= Average number of writhing of the control group.

**Tail immersion method**

Mice were divided into four groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b. w.) as control, group II received the standard drug nalbuphine (5 mg/kg b.w.) subcutaneously, group III and IV received 200 and 400 mg/kg of extract (p. o.) respectively. The latency of mice tail withdrawal from hot water was noted as the basal reaction time. The lower 3 cm portion of the tail of mice was dipped in a water bath maintaining at temperature of 55 ± 0.5°C. The reaction time was noted at 0, 30, 60, and 90 min. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animals.

**Hot plate method**

The anti-nociceptive activity of the extract was measured by hot-plate method. Mice in groups of four each were treated with vehicle (normal saline), Diclofenac sodium (10 mg/kg, i.p.) and Alstonia scholaris (200 and 400 mg/kg, p. o.). The mice were placed on a hot plate maintained at 56±0.5°C. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of +20s was followed to avoid any thermal injury to the paws. The reaction time were recorded before and after +0, +15, +30, +45 and +60 min following administration of test or standard drug.

**Results**

**Anti-diarrheal activity**

**Castor oil induced diarrhea**

In the castor oil-induced diarrhea experiment, the methanolic extract of *Alstonia scholaris* produced a marked anti-diarrheal effect in the mice, as shown in Table 1. At doses of 200 and 400 mg/kg, the methanolic extracts produced very significant inhibition of (p < 0.01, p < 0.001) defecation in compare to control. The total number of wet feces produced upon administration of castor oil decreased (6.20± 0.37 at 200 mg/kg and 5.40± 0.51 at 400 mg/kg) in dose dependent fashion while loperamide decreased 4.60 ± 0.51 percentage of defecation at the dose of 5 mg/kg.

**Castor oil induced enteropooling**

Castor oil caused accumulation of water and electrolytes in intestinal loop treatment with the *Alstonia scholaris* extract (200 and 400 mg/kg) produced moderately significant (p < 0.05, p < 0.01) and dose-dependent reduction in intestinal weight and volume (Table 2). The intestinal volume was decreased by 23.08% and 27.69% for methanolic extracts at doses 200 and 400 mg/kg respectively. On the other hand, the standard drug, loperamide (5 mg/kg), also significantly inhibited (p < 0.001) intestinal fluid accumulation (36.92%).

**Gastrointestinal motility test**

In this method, the methanolic extract of *Alstonia scholaris* was also significantly lessened the gastrointestinal distance (23.42 ± 2.29 cm for 200 mg/kg and 20.52 ± 1.68 cm for 400 mg/kg for methanolic extract) traveled by the charcoal meal in
the mice gastrointestinal tract compared with the control (40.67 ± 0.88 cm) group (Table 3). Loperamide (5 mg/kg) produced a marked (72.15%) decrease in the propulsion of charcoal meal through gastrointestinal tract.

**Analgesic Activity**

**Acetic acid induced writhing test**

Table 4 shows the effects of the extract on acetic acid induced writhing in mice. Oral administration of the extract moderately (p < 0.01) inhibited writhing response induced by acetic acid which was comparable to the reference drug. Inhibition of writhing by methanolic extract was dose dependent (such as 24.45% and 39.65% inhibition for the dose of 200 mg/kg and 400 mg/kg respectively) and significant effect was found in dose of 400 mg/kg.

**Tail Immersion Test**

There was a significant increased of the tail withdrawal reflex time following administration of the extract at dose of 200 mg/kg and 400 mg/kg. The result was statistically significant (P < 0.05, P < 0.01) and was comparable to the reference drug Nalbuphine (Table 5). The highest nociceptive inhibition was exhibited by *Alstonia scholaris* (200 mg/kg and 400 mg/kg) at 90 min.

**Hot plate method**

*Alstonia scholaris* (200 and 400 mg/kg) significantly (P < 0.05, P < 0.01) elevated the mean basal reaction time as compared to control group. The highest nociceptive inhibition was exhibited by *Alstonia scholaris* (400 mg/kg) at 60 min. The maximum nociception inhibition by Diclofenac was observed at 45 min, 60 min. *Alstonia scholaris* (200 mg/kg) produce significant inhibition of nociception was observed at 60 min as compared to control group. The observations are given in Table 6, please see supporting information.

**Discussion**

Different people of multifarious regions use some medicinal plants as part of their traditional therapy. As the plant is established as promising medicinal plant more in-depth investigation is need to explore the other pharmacological effects by investigating the unexplored parts of the plants. During the primary investigation of phytochemicals available in the extract, we found that methanolic extract of *Alstonia scholaris* possess some chemical compounds like alkaloid, tannin, flavonoids, steroids, saponin etc. So, we envisioned to explore anti-diarrheal and anti-nociceptive activities. The methanolic extract of *Alstonia scholaris* showed the maximum significant anti-diarrheal (p < 0.001) effect at the dose of 200 mg/kg and 400 mg/kg in compare to standard drug loperamide (5 mg/kg) and also possessed 22.08% and 27.27% inhibitions of defecation respectively in the cast of Castrol oil induced diarrhea. There are some mechanism are available to explain the diarrheal effect of castor oil include inhibition of intestinal Na⁺ K⁺ ATPase activity, thus reducing normal fluid absorption, 29 activation of adenylate cyclase or mucosal cAMP-mediated active secretion, 30 stimulation of prostaglandin formation and platelet activating factor. However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hyper-secretory response. On the other hand, in enteropooling method, the extract depicted significant (p < 0.01) effect at the dose of 400 mg/kg and also reduced the volume of intra-luminal contents respectively. These effects, which have direct consequences to reduced water and electrolytes secretion into the small intestine, suggest that the extract may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hyper-secretation. Hyper-motility characterizes diarrhea where the secretory component is not the causal factor. Pre-treatment with the extract suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which significantly indicates that the fruits peel extract may be able to reduce the frequency of stooling in diarrheal conditions such as 55.58% and 61.12% inhibited by methanolic extract at the dose of 200 mg/kg and 400 mg/kg respectively. All these findings strongly suggested that the methanolic crude extract should have anti-diarrheal activity and as per our best of knowledge which was never been explored before. On the other hand, in acetic acid induced writhing test the methanolic extract of *Alstonia scholaris* L. showed significant (p < 0.01) inhibition such as 24.45% and 36.95% at the dose of 200 mg/kg and 400 mg/kg respectively, Table 4. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels. In hot plate method, the methanolic extract of *Alstonia scholaris* L. at a dose of 200 mg/kg & 400 mg/kg body weight showed significant antinociceptive activity. The results were found to be statistically significant Fig. 2. In tail immersion method, the extend of activity shown by the crude extracts are less than that of the standard drug nalbuphine but many folds more than that of the control group, which justifies its activity. The results were found to be statistically significant, Fig. 1. This tail immersion method was used to evaluate the central mechanism of analgesic activity. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. This peels extract inhibited both Narcotic analgesics inhibit both peripheral and central mechanism of pain.

Above observations suggest that the extract in graded doses reduce diarrhea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enteropooling and inhibit both peripheral and central mechanism of pain. Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, flavonoids, sterol and/or triterpenes and anti-nociceptive properties of medicinal plants due to alkaloid, flavonoid, steroids, glycoside etc. Hence, tannins, alkaloids, steroids, saponin and glycoside may be responsible for the mechanism of action of extract of *Alstonia scholaris* against diarrheal and nociception.

**Conclusion**

Along with wide range of traditional uses such as treating diarrhea, as a tonic etc., In present studies, *Alstonia scholaris* (methanol extract) showed dose dependent anti-diarrheal activity at the dose of 200 mg/kg and the inhibition rate was found at 50.79% ; whether at the dose of 400 mg/kg, the inhibition rate increased up to 57.14%. It also depicted significant success rate (P<0.05 and P<0.01). From the above point of view, it can be concluded that further investigation of *Alstonia scholaris* plant extract will help to develop noble anti-diarrheal drug and pain killer based on natural resources.
Table 1: Effect of methanolic extract of *Alstonia scholaris* on castor oil induced diarrhea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total number of feces</th>
<th>% Inhibition of defecation</th>
<th>Total number of diarrheal feces</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil + Saline (2 mL/kg p.o)</td>
<td>15.4 ± 0.51</td>
<td>---</td>
<td>12.60 ± 0.51</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil + Loperamide (5 mg/kg i.p)</td>
<td>8.20 ± 0.37***</td>
<td>46.75</td>
<td>4.60 ± 0.51***</td>
<td>63.49</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil + Extract (200 mg/kg i.p)</td>
<td>12.00 ± 0.89**</td>
<td>22.08</td>
<td>6.20 ± 0.37***</td>
<td>50.79</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil + Extract (400 mg/kg i.p)</td>
<td>11.20 ± 0.58***</td>
<td>27.27</td>
<td>5.40 ± 0.51***</td>
<td>57.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.001 when compared with control group.

Table 2: Effect of methanolic extract of *Alstonia scholaris* on castor oil induced enteropooling in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Weight of intestinal content (g)</th>
<th>Volume of intestinal content (mL)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil + Saline (2 mL/kg p.o)</td>
<td>1.69 ± 0.09</td>
<td>0.65 ± 0.13</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil + Loperamide (5 mg/kg i.p)</td>
<td>1.57 ± 0.08</td>
<td>0.40 ± 0.08</td>
<td>37.75</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil + Extract (200 mg/kg i.p)</td>
<td>1.67 ± 0.11</td>
<td>0.49 ± 0.10</td>
<td>25.41</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil + Extract (400 mg/kg i.p)</td>
<td>1.59 ± 0.10</td>
<td>0.44 ± 0.04</td>
<td>32.14**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.001 when compared with control group.

Table 3: Effect of methanolic extract of *Alstonia scholaris* on small intestinal transit in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total length of intestine (cm)</th>
<th>Distance traveled by marker (cm)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Castor oil + Saline (2 mL/kg p.o)</td>
<td>54.09 ± 1.62</td>
<td>40.83 ± 0.78</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>Castor oil + Loperamide (5 mg/kg i.p)</td>
<td>53.16 ± 1.04</td>
<td>14.21 ± 0.57**</td>
<td>73.26</td>
</tr>
<tr>
<td>III</td>
<td>Castor oil + Extract (200 mg/kg i.p)</td>
<td>49.20 ± 0.97</td>
<td>27.72 ± 0.88*</td>
<td>43.67</td>
</tr>
<tr>
<td>IV</td>
<td>Castor oil + Extract (400 mg/kg i.p)</td>
<td>50.06 ± 0.95</td>
<td>22.81 ± 1.23**</td>
<td>54.45</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.001 when compared with control group.

Table 4: Effect of methanolic extract of *Alstonia scholaris* on Acetic acid induced Writhing test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>No. of Writhing</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline water(0.9% Nacl solution)</td>
<td>10 mg/kg, p.o</td>
<td>60 ± 2.89</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Diclofenc Na</td>
<td>10 mg/kg, i.p</td>
<td>31.67 ± 2.03***</td>
<td>47.22</td>
</tr>
<tr>
<td>A. scholaris</td>
<td>Methanolic extract</td>
<td>200 mg/kg p.o</td>
<td>45.33 ± 2.33*</td>
<td>24.45</td>
</tr>
<tr>
<td>A. scholaris</td>
<td>Methanolic extract</td>
<td>400 mg/kg p.o</td>
<td>37.83 ± 3.06**</td>
<td>36.95</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.01, *** p < 0.001 when compared with control group.
Table 5: Effect of methanolic extract of *Alstonia scholaris* on Tail immersion test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>0 min</th>
<th>30min</th>
<th>60min</th>
<th>90min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline water (0.9% NaCl solution)</td>
<td>10 mg/kg, p. o.</td>
<td>3.56 ±0.52</td>
<td>3.86 ±0.17</td>
<td>3.63 ±0.30</td>
<td>3.97 ±0.22</td>
</tr>
<tr>
<td>Positive</td>
<td>Nalbuphine</td>
<td>10 mg/kg, i. p.</td>
<td>3.94 ±0.41</td>
<td>5.15 ±0.57</td>
<td>7.62±0.82*</td>
<td>10.67 ±0.41***</td>
</tr>
<tr>
<td><em>B. scholaris</em></td>
<td>Methanolic extract</td>
<td>200 mg/kg p.o</td>
<td>3.38 ±0.48</td>
<td>4.34±0.48</td>
<td>4.84 ±0.84</td>
<td>5.11 ±0.90</td>
</tr>
<tr>
<td><em>B. scholaris</em></td>
<td>Methanolic extract</td>
<td>400 mg/kg p.o</td>
<td>3.54 ±0.36</td>
<td>4.95±0.95</td>
<td>6.03±0.90</td>
<td>6.16 ±0.88</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.01, *** p < 0.001 when compared with control group.

Table 6: Effect of methanolic extract of *Alstonia scholaris* on Hot plate test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline water(0.9% NaCl solution)</td>
<td>10 mg/kg, p.o</td>
<td>6.47 ±0.61</td>
<td>6.04 ±0.30</td>
<td>6.60 ±0.30</td>
<td>7.44 ±0.54</td>
<td>6.64±1.50</td>
</tr>
<tr>
<td>Positive</td>
<td>Diclofenc Na</td>
<td>10 mg/kg, i.p</td>
<td>8.82 ±1.78</td>
<td>11.37±1.61*</td>
<td>13.97±0.85**</td>
<td>14.90±1.02**</td>
<td>16.23±1.66**</td>
</tr>
<tr>
<td><em>A. scholaris</em></td>
<td>Methanolic extract</td>
<td>200 mg/kg p.o</td>
<td>6.72±0.87</td>
<td>7.48±0.60</td>
<td>8.03±1.16</td>
<td>8.72±1.16</td>
<td>10.04±1.16</td>
</tr>
<tr>
<td><em>A. scholaris</em></td>
<td>Methanolic extract</td>
<td>400 mg/kg p.o</td>
<td>7.33±0.52</td>
<td>8.32±0.63</td>
<td>10.78±0.50</td>
<td>11.72±1.33</td>
<td>12.26±1.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. (n = 5). * p < 0.05, ** p < 0.01, *** p < 0.001 when compared with control group.

Figure 1: Effect of methanolic extract of *Alstonia scholaris* on Tail immersion method
Figure 2: Effect of methanolic extract of *Alstonia scholaris* on hot plate method

References


