ANALGESIC ACTIVITY OF METHANOL EXTRACT AND ITS FRACTIONS OF *STREBLUS ASPER* (LOUR.) ROOTS

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**ABSTRACT**

The roots of *Streblus asper* (Lour) (Family: Moraceae), a quite available medicinal plant of Bangladesh, has been investigated for the screening of its analgesic activity by peripheral mechanism of pain reduction. The powdered roots were extracted with methanol at room temperature and partitioned with petroleum ether, carbon tetrachloride, dichloromethane and ethyl acetate by modified Kupchan partition method. In the screening of peripheral analgesic activity by acetic acid induced writhing method, the ethyl acetate and petroleum ether fraction at doses 400mg/kg and 200mg/kg body weight induced extremely significant (p<0.001) decrease in the number of writhes. The crude methanolic extract and other fractions also induced significant (p<0.05) decrease. The roots of *Streblus asper* can be considered as a potential candidate for analgesic products.

**Keywords:** *S. asper*, Analgesic, Acetic Acid, Writhing, Swiss-albino mice.

**INTRODUCTION**

As nature gives us problems, so it gives the solution. Before civilization, when there were no hospitals and no man-made industries to cause pollution, there were diseases, and those diseases were cured by nature itself through the medicinal plants. Over the centuries, man has learnt to synthesize medicine, but some plants possess so wide range of pharmacological activities, that it is too difficult to synthesize the constituents in laboratory. Right screening of plants for finding a proper lead is required, focusing on bioactivities [1-6]. An unpleasant sensory and emotional experience related with actual or potential tissue damage can be termed as pain. Many medicinal plants have reputation of effective remedies in traditional
analgesia, but there is lack of scientific data [7]. Consequently, analgesic drugs derived from plant origin are getting more importance with time [8,9].

Bangladesh has a lot of medicinal plants which have been used traditionally for management of pain. One of them is *Streblus asper* (Family: Moraceae) which is a shrub or small evergreen tree. The various plant parts are used in folk medicines for the treatment of different diseases such as filariasis, leprosy, toothache, diarrhea, and cancer [10]. Also, infusion and decoction of the bark are used in fever, diarrhea and dysentery, extract of the bark produces macrofilaricidal activity and adrenaline-like action on myocardium in rodents [11], latex possesses astringent and antiseptic properties, and used as sedative in neuralgia treatment, leaves are used as galactagogue, seeds are useful in epilepsy, piles and diarrhea, paste of seeds is used topically in leucoderma, roots are used in the treatment of epilepsy, some ulcers, sinuses and as antidote to snakebite [12], paste of roots is applied to boils and inflammatory swellings, fruits are used in eye complaints. Kamloside, indroside, strebloside, strophalloside, cannodimemoside, strephanolloside, asperoside, 16-O-acetyl-glucogitomethoside, glucokamloside, glucogitodimethoside, sarmethoside and glucostrebloside have been isolated from the root bark of the plant. From the stem bark, α-amyrin, α-amyrin acetate, lupeol acetate, β-sitosterol, lupeol and diol, strebloside and mansonin have been isolated [13]. Very limited pharmacological investigation has been done on the roots of this plant. The present study was designed to observe analgesic activities of the crude extracts and their different partitioning fractions of the root of *Streblus asper* Lour. and to rationalize the traditional uses of the plant parts for pain reduction.

**MATERIALS AND METHODS**

**Drugs and chemicals**

Acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), Diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh), Normal saline solution (Beximco Infusion Ltd., Bangladesh), and DMSO were used in this investigation.

**Plant materials**

The roots of *Streblus asper* were collected and identified from Bangladesh National Herbarium, Dhaka (Accession no. DACB-38760). The roots (after cutting into small pieces) were sun dried for some days. Then the pieces were dried in oven for 24 hours at low temperature to achieve better grinding. After drying, with the help of high capacity grinding machine, the roots were ground in coarse powder in the Phytochemical Research Laboratory,
University of Dhaka. About 1 kg of the powdered material was soaked in 4 liters of methanol. Cotton plug and aluminium foil were used to seal the container and its content and kept for 15 days with occasional shaking and stirring. Then the mixture was filtered through cotton and Whatman No.1 filter paper. The filtrate was concentrated with a Heidolph rotary evaporation at 39°C and then air dried to get solid remainder. The weight of the crude methanol extract obtained from the powdered root was 25 gm. 5 gm of dried methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water and was dissolved completely. This was the mother solution which was partitioned off successively by petroleum ether, carbon tetrachloride, dichloromethane and ethyl acetate. The filtrates obtained were evaporated by using a rotary evaporator to give a thick concentrate.

Animals
Swiss-albino mice of both sex that are aged 4-5 weeks and weighing 25-30 gm were collected from the animal house of the Department of Pharmacy, Jahangirnagar University, Bangladesh. They were kept in standard environmental condition (at 24.0±1 °C temperature, 55–65 % relative humidity and 12h light/12h dark cycle). The animals were fed with pelletized mice feed supplied from ICDDR,B (International Centre for Diarrhoeal Diseases and Research, Bangladesh) and water ad libitum. The Institutional Ethical Review Committee approved the procedures and animal suffering were minimized.

Acetic acid-induced writhing method for peripheral analgesic assay
The peripheral analgesic activity of the samples was evaluated in mice (n=5/group) using acetic acid induced writhing method [14,15,16]. Intraperitoneal acetic acid administration produces pain which will cause successive writhing. Analgesic agent administered earlier will reduce the writhing. In this experiment, the plant extracts (400 mg/kg and 200mg/kg body weight), diclofenac sodium (positive control) and vehicles were administered orally to the overnight fast mice. Thirty minutes later intraperitoneal administration of 0.7 % v/v acetic acid solution (0.15 ml/10 g body weight) was conducted. Then the animals were placed on an observation table and the writhing number was counted.

Statistical analysis
Values were expressed as mean ± standard deviation and statistical comparison were done by using t-test. The p<0.05 value was considered as statistically significant and p<0.001 value as extremely significant.
RESULTS AND DISCUSSION

The analgesic activity of *Streblus asper* roots were screened by the current experiment. The data obtained from the experiment and statistical significance has been summarized in Table I. Peripheral analgesic action of the crude methanolic extracts and its different solvent soluble fractions were monitored here by acetic acid-induced writhing method. Mice model was injected with acetic acid intraperitoneally to cause abdominal writhing. The tested plant extracts were able to reduce the writhing significantly (P<0.05) at the doses 400 mg/kg and 200 mg/kg body weight after oral administration (Table I). The highest activity was shown by Petroleum ether fraction of root extracts having the percent inhibition of 93% compared to the control saline acetic acid-induced mice. However, all extracts inhibited acetic acid-induced writhing effectively. The reference analgesic drug diclofenac sodium was effective to show analgesia with 58 % inhibition.

Table I  Analgesic Activity of Methanolic Root Extract and Its Different Fractions of *Streblus asper* (Lour.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Oral Dose (mg/kg body weight)</th>
<th>Number of Writhing&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of Inhibition of Writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>0.15 ml/10 gm of body weight</td>
<td>20 ± 1.304</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac Sodium**</td>
<td>50</td>
<td>8.4 ± 0.927</td>
<td>58</td>
</tr>
<tr>
<td>ME 1*</td>
<td>400</td>
<td>10.2 ± 2.153</td>
<td>49</td>
</tr>
<tr>
<td>ME 2*</td>
<td>200</td>
<td>10.4 ± 2.482</td>
<td>48</td>
</tr>
<tr>
<td>PEF 1**</td>
<td>400</td>
<td>1.4 ± 1.166</td>
<td>93</td>
</tr>
<tr>
<td>PEF 2**</td>
<td>200</td>
<td>4.4 ± 1.913</td>
<td>78</td>
</tr>
<tr>
<td>CTF 1**</td>
<td>400</td>
<td>6.8 ± 1.463</td>
<td>66</td>
</tr>
<tr>
<td>CTF 2*</td>
<td>200</td>
<td>8.4 ± 2.111</td>
<td>58</td>
</tr>
<tr>
<td>DMF 1*</td>
<td>400</td>
<td>7.4 ± 2.182</td>
<td>63</td>
</tr>
<tr>
<td>DMF 2*</td>
<td>200</td>
<td>11 ± 2.4</td>
<td>45</td>
</tr>
<tr>
<td>EAF 1**</td>
<td>400</td>
<td>2.6 ± 1.4</td>
<td>87</td>
</tr>
<tr>
<td>EAF 2*</td>
<td>200</td>
<td>9.2 ± 1.393</td>
<td>54</td>
</tr>
</tbody>
</table>

<sup>a</sup> values represent mean ± SEM (n=5).

**p<0.001 (One-way ANOVA and Dunnett’s t test, significantly different from control.
*p<0.05(One-way ANOVA and Dunnett’s t test, significantly different from control. ME: Crude Methanolic Extract; PEF: pet ether fraction of methanolic extract; CTF: carbon tetrachloride fraction; DMF: dichloromethane fraction; EAF: ethyl acetate fraction of methanolic extract of roots of the plant.

The methanolic extract and different fractions of root of *Streblus asper* at the dose of 400 mg/kg and 200 mg/kg body weight induce a significant analgesia compared to the control
group. The petroleum ether fraction show extremely significant analgesic property at doses 400mg/kg and 200mg/kg body weight in peripheral analgesic activity assay having the writhing inhibition percentage 93% and 78% respectively. Ethyl acetate fraction shows extremely significant analgesic property at dose 400mg/kg having 87% inhibition percentage. Carbon tetrachloride fraction and dichloromethane fraction show very significant peripheral analgesic activity having writhing inhibition percentage 66% and 63% respectively. Biogenesis of prostaglandin E2 and prostaglandin F2α (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway) take place following inflammation [17]. Presence of prostanoids and sympathetic neuronal mediator have been reported in mice treated with acetic acid. Released prostaglandins, mainly prostacyclin (PGI2) and to a lesser extent prostaglandin-E have been held responsible for pain sensation. The analgesic property of the crude extract and different partitionates might be due to the possible interfering with the prostaglandin biosynthesis and other autacoids by inhibiting cyclooxygenase enzymes or in the binding of prostanoids to the receptors. Also, the inhibition of neuronal mediator production might be a cause. The standard drug Diclofenac sodium causes analgesia by inhibiting cyclooxygenase enzymes and thereby inhibiting prostaglandin synthesis [18,19,20]. It can be assumed from the data presented, that the extracts of roots of Streblus asper possess significant analgesic property.

CONCLUSION
We screened the root part of a very available medicinal plant of Bangladesh for finding peripheral analgesic property as it is used traditionally in toothache. Acetic acid-induced writhing method was used for the study. The crude methanolic extract, petroleum ether soluble fraction, ethyl acetate soluble fraction, carbon tetrachloride soluble fraction and dichloromethane soluble fraction, all were found effective for the management of pain in swiss-albino mice. Further investigation is required for finding the mechanism of this action.

REFERENCES


