ANALGESIC STUDY OF METHANOLIC EXTRACTS OF LORANTHUS EUROPAEUS LEAF AND STEM

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ABSTRACT

In this study the analgesic potency of methanolic extracts of Loranthus europaeus leaf (200 & 400 mg/kg body weight) and stem (200 & 400 mg/kg body weight) was evaluated by applying acetic acid induced writhing and tail immersion method in mice. To determine the peripheral analgesic activity acetic acid induced writhing method was adopted, Diclofenac sodium (10 mg/kg body weight) served as standard. 1% acetic acid (0.1 ml/10 gm) was injected intraperitoneally after 30 minutes of the administration of the extracts, 5 minutes later writhing number of different groups were counted for next 15 minutes. All extracts yielded potent analgesic activities by achieving reduced writhing numbers compared with Control. Among the extracts the highest writhing inhibition (59.66%) was recorded for 400 mg/kg body weight of leaf extract. In tail immersion test to perform central analgesic efficacy, tail withdrawing times of different groups were observed after 0, 30, 60, 90 and 120 minutes of the administration of extracts. The popular analgesic Indomethacine (10 mg/kg body weight) served as standard. The extracts exerted analgesic efficiency by ensuring elongated tail withdrawing time compared with Control. One Way Anova followed by Dunnett’s multiple comparison test was performed to evaluate the statistical significance of the values. In both tests, experimental extracts achieved statistical significant P value (P<0.001) compared with Control. Thus the present study reports the analgesic potency of the leaf and stem extracts of Loranthus europaeus.

KEYWORDS: Loranthus europaeus, analgesic, writhing, acetic acid induced writhing method, tail immersion test.
INTRODUCTION

Pain is a defensive mechanism of the body having unpleasant sensory and emotional experience associated with real or potential tissue damage.\(^1\) Mediators like interleukin-1, tumor necrosis factor-TNF-α propagate the synthesis, release and action of autocoid prostaglandin E2 (PGE2) and F2α by endothelium and pericytes of brain capillaries which excite pain nerve endings. Increased prostaglandin levels within the peritoneal cavity increase capillary permeability and results in pain.\(^2\)

Pain becomes a concerning focus of global scientific research due to its implication ranges not only human but also animal diseases.\(^3\) Pain affects 40% of American peoples everyday including 50 million individuals with chronic pain.\(^4\) To manage pain Opioids and Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used. Despite of the widespread use, these are reported to have serious side effects. Multiple studies evinced that 40-45% patients on Opiate therapy experience constipation, 25% experience nausea, 75% suffer from sleep disorder breathing and this therapy associated with a 77% increased risk of cardiovascular events (eg, myocardial infarction, heart failure). Opioid overdoses may lead to life threatening side effects like respiratory depression, bradycardia, hypotension and long-term use of Opioids may cause dependency and addiction.\(^5\) Statistics showed that the use of NSAIDs increases the risk of significant GI complications (eg, bleeding, hospitalization, surgery) from 3.5 to 7.8 fold. Approximately 107000 patients are hospitalized yearly due to NSAID related GI complications and at least 16500 NSAID related deaths occur annually among arthritis patients alone in the United States.\(^6\) These are also believed to cause peptic ulcer and gastrointestinal bleeding.\(^4\)

To get rid of adverse effects of existing synthetic drugs people have a propensity to use traditional medicines. World Health Organization (WHO) estimated that more than 80% of world population rely on traditional medicines\(^7\) and the market is rapidly growing. Lot of medicinal plants like Aloe vera, Datura, ginger, turmeric, peppermint, cardamom and so forth have proven their potent analgesic properties. So study on natural plants is a beneficial way to find out potent and safe drugs that may replace current synthetic drugs of hazardous side effects.

\textit{Loranthus europaeus} (Family: Loranthaceae) is a deciduous, branching, yellowed berried mistletoe usually found on the branches of woody plants. Leaves are 4-6 cm long, opposite, simple, dark green with short petioles and blunt apex. The plant is a rich source of flavonoids,
alkaloids, terpenoids, polysaccharides\cite{8-9} and exhibited pronounced gene toxicity\cite{8}, cytotoxic\cite{10} and antioxidant\cite{9} properties. In this study the analgesic property of the methanolic extract of leaf and stem of *Loranthus europaeus* was evaluated.

**MATERIAL AND METHODS**

**Collection of plant**

The plant was collected from Chittagong, Bangladesh and it is identified by Dr. Shaikh Bokhtear Uddin, Associate Professor and Taxonomist, Department of Botany, University of Chittagong.

**Preparation of extracts**

The fresh parts (leaves, stems) of the plant were first washed with water to remove dirt and then cut into small pieces, sun dried for 3 days and finally dried at 45\(^\circ\)C for 48 hours in an electric oven. After complete drying, the leaves and stems were separately pulverized into coarse powders with the help of a grinding machine. The powders (200 g) were separately extracted with methanol (1L) and shaken for 7 days. Then solutions were individually filtered through cotton at first, then through filter paper. Then the filtrate solutions were concentrated at 40\(^\circ\)C with a rotary evaporator. The extracts were air dried to solid residues and dissolved in 2% tween solution.

**Experimental animals**

Young Swiss-albino mice of either sex, aged 7-8 weeks, weighing about 30-35 g were used for the experiment. The animals were purchased from the animal research branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Animals were kept in standard environmental conditions (12:12 h light and dark cycle at 25\(^\circ\)C temperature and 55-65% relative humidity) and fed ICDDR,B formulated rodent food and water.

**Chemicals and drugs**

Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Indomethacin from Sigma- Aldrich, Saline solution, Diclofenac sodium from Beximco Pharma Ltd. All the drugs and chemicals used were of analytical grade.

**Ethical approval**

All experiments regarding animals were performed by following the guidelines of the institutional animal ethical committee\cite{11} as well as the instructions of the International Centre
for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The ethical committee of Southeast University also observed and approved the experiment.

**Tests for analgesic activity**

**Acetic acid induced writhing method**

To perform the study six groups were created, each containing six mice (n = 6).

Group 1 (Control): Treated with 2% tween 80 in normal saline (0.1 ml/10 g of body weight p.o.).

Group 2 (Standard): Treated with diclofenac sodium (10 mg/kg of body weight p.o.).

Group 3 (LEL 200): Treated with 200 mg/kg body weight p.o. of leaf extract of the plant.

Group 4 (LES 200): Treated with 200 mg/kg body weight p.o. of stem extract of the plant.

Group 5 (LEL 400): Treated with 400 mg/kg body weight p.o. of leaf extract of the plant.

Group 6 (LES 400): Treated with 400 mg/kg body weight p.o. of stem extract of the plant.

After 30 minutes of the above treatment each mouse was administered 0.1 ml/10 g i.p. of 1% acetic acid. After 5 minutes of the induction of acetic acid, number of writhes or squirms (abdominal muscle contraction, stretching of hind limbs, trunk twisting) were recorded for 15 minutes.[12]

The percentage inhibition of writhing response was determined from the following equation.

\[
\text{Percentage inhibition of writhing response} = \left[\frac{(W_c - W_t)}{W_c}\right] \times 100.
\]

Where, \(W_c\) = mean value of number of writhing in the control group.

\(W_t\) = mean value of number of writhing in tests or standard group.

**Tail immersion test**

Total six groups of six mice (n=6) were designed for the test. The groups were treated as follows.

Group 1 (Control): Received 2% tween 80 in normal saline (0.1 ml/10 g body weight p.o.)

Group 2 (Standard): Received Indomethacine (10 mg/kg body weight p.o.)

Group 3 (LEL 200): Received 200 mg/kg body weight p.o. of leaf extract of the plant.

Group 4 (LES 200): Received 200 mg/kg body weight p.o. of stem extract of the plant.

Group 5 (LEL 400): Received 400 mg/kg body weight p.o. of leaf extract of the plant.

Group 6 (LES 400): Received 400 mg/kg body weight p.o. of stem extract of the plant.

The lower portion (3 cm) of the tail of each mouse was immersed in hot water (55± 0.5ºC). The withdrawal time (in seconds) of tail noted as the reaction time or tail flick latency was
recorded with the help of a stop watch at 0, 30, 60, 90 and 120 minutes after the administration of the above treatment.\textsuperscript{[3,13]}

Statistical analysis
The results were expressed as Mean ± Standard error of Mean (SEM). One Way Anova followed by Dunnett’s multiple comparison test using Graphpad prism version 6.05 was performed to determine the statistical significance.

RESULTS
Table 1: Effect of methanolic extracts of Loranthus europaeus (leaf and stem) on acetic acid induced writhing method compared with other groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Writhing number</th>
<th>% of Inhibition</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>88±2.39</td>
<td>--</td>
</tr>
<tr>
<td>Standard</td>
<td>23.50±1.23****</td>
<td>73.30</td>
</tr>
<tr>
<td>LEL 200</td>
<td>42±1.75***</td>
<td>52.27</td>
</tr>
<tr>
<td>LES 200</td>
<td>50±2.42***</td>
<td>43.18</td>
</tr>
<tr>
<td>LEL 400</td>
<td>35.50±1.34***</td>
<td>59.66</td>
</tr>
<tr>
<td>LES 400</td>
<td>44.17±1.97***</td>
<td>49.81</td>
</tr>
</tbody>
</table>

Results are presented as Mean±SEM, ***P<0.001 & ****P<0.0001 compared with Control group. One way ANOVA followed by Dunnett’s multiple comparison test was performed in Graphpad Prism version 6.05.

The analgesic activity of acetic acid induced writhing method was evaluated. The lower the writhing number the better the analgesic activity. All extracts achieved significant percentage of inhibition and also the potent statistical P value (P<0.001). Both the leaf extracts (200 and 400 mg/kg) yielded better analgesic activity than the stem extracts (200 and 400 mg/kg) of the plant. For stem extracts the inhibitions of writhing numbers were calculated as 43.18% and 49.81% for 200 and 400 mg/kg body weight respectively. Among the plant extracts, leaf of the plant at a dose of 400 mg/kg produced the highest inhibition (59.66%) whereas the standard group achieved 73.30% of inhibition.

Table 2: Effect of plant extracts (leaf, stem) on tail immersion test compared with other groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Average tail withdrawing time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>1.64±0.10</td>
</tr>
<tr>
<td>Standard</td>
<td>1.58±0.21</td>
</tr>
<tr>
<td>LEL 200</td>
<td>1.70±0.51</td>
</tr>
<tr>
<td>LES 200</td>
<td>1.62±0.12</td>
</tr>
<tr>
<td>LEL 400</td>
<td>1.66±0.86</td>
</tr>
<tr>
<td>LES 400</td>
<td>1.52±0.42</td>
</tr>
</tbody>
</table>
Results are presented as Mean±SEM, **P<0.01, ***P<0.001 & ****P<0.0001 compared with Control group. One way ANOVA followed by Dunnett’s multiple comparison test was performed in Graphpad Prism version 6.05.

In tail immersion test the level of analgesia was determined by means of the reaction time of each group. All the groups except the Control elongated the reaction time at every point (30, 60, 90 and 120 minutes) of the experiment. During this study all extracts along with the standard drug Indomethacin produced their highest reaction time at 120 minutes. Between leaf and stem extracts, leaf of the plant exhibited better analgesic activity than the stem by means of achieving longer reaction time. The P values (P<0.01, P<0.001) were achieved by all extracts at different time intervals (30, 60, 90 & 120 min) that prove the statistical significance of the analgesic activity of the plant. As expected, higher doses of the plant parts yielded longer reaction time, thus producing better analgesic activity. The highest reaction time for stem and leaf extracts was recorded as 3.32 and 4.13 respectively at a dose of 400 mg/kg, whereas the elongated reaction time for standard drug Indomethacin was recorded 8.02.

DISCUSSION
To evaluate the peripheral and central analgesic effect of the plant acetic acid induced writhing method and tail immersion test in albino mice were performed respectively. In acetic acid induced writhing method pain sensation is obtained by generating localized inflammatory response resulting in the release of free arachidonic acid from tissue phospholipid via cyclooxygenase and prostaglandin biosynthesis. The increased prostaglandin levels within the peritoneal cavity then cause pain by increasing capillary permeability.[14] This method was associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products which stimulates nociceptive neurons sensitive to NSAIDs.[15] In contrast tail immersion test is an established method for measuring the central analgesic effect of drugs through opioid receptors. Central pain mechanism includes the brain and spinal cord where the dorsal part of the spinal cord is abound with substance p, endogenous opioids, somatostatine and other inhibitory hormones that are the targets of pain and inflammation.[16] All extracts of the plant yielded promising analgesic activity in both tests. The extracts significantly decreased the writhing number in acetic acid induced writhing method and also prominently increased the reaction time in the
tail immersion test. Thus the study evinced the central and peripheral analgesic activity of the leaf and stem of *Loranthus europaeus*.

Previous researches suggest that phytochemicals like flavonoids, alkaloids, saponins, terpenoids are responsible for analgesic property.\[12,2,9\] For example, flavonoids are believed to target PGs involved in the late phase of acute inflammation and pain perception.\[17\] It may also increase the amount of endogenous serotonin or may interact with 5-HT\textsubscript{2A} and 5-HT\textsubscript{3} receptors that may be involved in the mechanism of central analgesic activity.\[13\] Moreover flavonoids are known to inhibit prostaglandin synthetase.\[18\] Besides flavonoids, terpenoids also reported to posses significant analgesic activities, whereas alkaloids may prevent inflammation through blocking the metabolic pathway of arachidonic acid.\[14\] The mistletoe plant *Loranthus europaeus* contains flavonoids, alkaloids, terpenoids, polysaccharides and also other phytochemicals.\[8-9\] So there is no surprise that the exhibited analgesic activity may be due to the presence of these phytochemicals. In all experiments leaf extracts produced a better effect than the stem, this might be the higher content of these phytochemicals in leaves than the stems of the plant.

**CONCLUSION**

The present study corroborates the potent analgesic activity of the leaf and stem of the mistletoe plant *Loranthus europaeus* and raises the possibility to develop a new promising natural drug. Further study is required to figure out the exact mechanism of action of the plant.

**REFERENCES**


