ANALGESIC ACTIVITY OF VARIOUS EXTRACTS OF LEAVES OF
MURRAYA KOENIGII (L) SPRENG

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ABSTRACT

The present study was undertaken to investigate the analgesic activity of various extracts of *Murraya koenigii* (L.) Spreng. Three types of extracts (petroleum ether extracts, ethanol extracts and water extracts) were prepared from leaves of *Murraya koenigii* Spreng. Two methods i.e. tail flick and writhing tests were used to study analgesic activity. Pentazocine (10 mg/kg) and aspirin is used as standard drugs. In this work, petroleum ether extract, methanol extract and water extracts were examined at doses of 200 mg/kg and 400 mg/kg. Treatment of rats with all the extracts showed significant analgesic activity. Petroleum ether extract at the dose of 200 mg/kg and 400 mg/kg has significant analgesic activity as compared to ethanol and water extracts with percentage of inhibition 36.33% and 38.18% after 3h treatment by using tail flick method. The mean number of writhing of *Murraya koenigii* Spreng was found to be $101.41 \pm 1.20$ and $82.6 \pm 1.31$ with percentage of inhibition $13.32\%$ and $29.40\%$ at the doses of $200$ mg/kg and $400$ mg/kg of petroleum ether extract after 1h treatment by writhing method.

These results indicate that the extracts of leaves of *Murraya koenigii* Spreng showed significant analgesic activity through both tests.

KEYWORDS: Analgesic activity, aspirin, rat, tail flick test, writhing test.

INTRODUCTION

*Murraya koenigii* (L.) Spreng belongs to the family Rutaceae, commonly known as curry-leaf tree. It is found almost everywhere in the Indian subcontinent, it shares aromatic nature, deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin
smooth grey or brown bark and dense shady crown.\[1\] Most part of plant is covered with fine down and has a strong peculiar smell. \textit{M. koenigii} is genus of tree, native to tropical Asia from Himalaya foothill’s of India to Srilanka eastward through Myanmar, Indonesia, Southern China and Hainan. The \textit{M. koenigii} is having grey color bark. Leaves are bipinnately compound, 15-30 cm long each bearing 11-25 leaflets alternate on rachis. Margins irregularly creatate, petioles 2-3 mm long, flowers are bisexual, white, funnel shaped sweetly scented, regular with average diameter of fully opened flower being in average 1.12 cm inflorescence. Fruits are ovoid to subglobose, wrinkled or rough with glands. It is having the size of 2.5 cm long and 0.3 cm in diameter and gets purplish black when ripen. Fruits are generally biseeded. Seeds generally occur in spinach green color, 11 mm long, 8 mm in diameter and weighs up to 445 mg.\[2\] The pulp of the fruit contains 64.9 per cent moisture.

Analgesic is a drug that relieves pain and in popular speech it is known as a ‘pain-killer’.\[3\] Pain is of two types - slow and acute pain. Drugs that relieve pain due to multiple causes and increase the threshold to pain are termed as analgesics e.g. aspirin, nimusulide. Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (acetaminophen), the nonsteroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others. The leaves and roots are bitter, acrid, cooling, anthelmintic, analgesic, it cures piles, allays heat of the body, thirst, inflammation and itching. It is also useful in leucoderma and blood disorders. An infusion of the toasted leaves in used to stop vomiting. The plant is credited with tonic and stomachic property.\[4\] The branches of \textit{Murraya koenigii} are very popular for cleaning the teeth as datum.\[1\] Many of the antibiotic and other synthetic drugs have shown sensitization reactions and other undesirable side effects and there is a feeling that herbal drugs are comparatively safer. So, the present study was undertaken to investigate the analgesic activity of various extracts of \textit{Murraya koenigii} (L.) Spreng.

**MATERIAL AND METHODS**

The study was carried out on rats using either sex, maintained under standard laboratory conditions. Petroleum ether (40° to 60° C), ethanol (95%), acetone, acacia gum (2%), pentazocine (10mg/kg) etc. and leaves from \textit{Murraya koenigii} Spreng were used as material in study.
Animals
Albino Wistar rats of the either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions on wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The experimental protocol was approved by the Institutional Animal Ethics Committee and the laboratory animals were taken care of as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India.

Preparation of extract
Leaves of Murraya koenigii (L.) Spreng (500g) were taken and dried under the shade. The material was packed in Soxhlet apparatus and extracted with petroleum ether for 20 days. The material was taken out from Soxhlet apparatus and air dried. The liquid which was obtained distilled off for the extra solvent and concentrated on water bath (yield 37.6%). The material which was previously extracted with petroleum ether was taken and extracted with ethanol for 15 days. The material was taken out of Soxhlet apparatus and air dried. The liquid was distilled off for the extra solvent and concentrated on water bath (yield 15.2%). The material was again dipped in distilled water for 5 days, filtered and extracted. The total filtered liquid was taken and concentrated on water bath (yield 22.4 %). The extract at the different doses of 100, 200 and 400 mg/kg was suspended in gum acacia (2.0%) and administered orally.

Tail flick method
A modification (Clark et al.) [5] of the method originally described by Pizziketti et al. [6] was employed. Rats were closely restrained in a wire mesh cage and the lower halves of their tails were dipped in a beaker of cold water (0-1 °C). Analgesia was assessed with a tail flick apparatus (Analgesiometer). Baseline latency (reaction time) was observed prior to drug (extract) treatment and at 1, 2 and 3 hr after the drug (extract) administration.

Writhing test
The method described by Koster et al. [7] was used. Mice were made to writhe by intraperitoneal injection of 0.6% v/v aqueous acetic acid (10.0 ml/kg). Test substances were administered 90 min before injection of acetic acid. Animals were kept under observation immediately after acetic acid injection and a number of writhes were recorded after 1 h treatment.
Statistical analysis
Group results are expressed as mean ± SEM. One-way ANOVA followed by either Dunnett's or Tukey-Kramer, post hoc tests of significance was applied for multiple comparisons amongst different groups. \( P < 0.05 \) was regarded as statistically significant.

RESULTS AND DISCUSSION
All the extracts (petroleum ether, ethanol and water extracts) were examined at doses of 200 mg/kg and 400 mg/kg. It was found that all the extracts showed analgesic activity. In tail flick model, the mean retention time of petroleum ether, ethanol and water extract at the doses of (400 mg/kg) for *M. koenigii* was found to be 6.41 ± 0.15, 7.13 ± 0.11, 7.78 ± 0.04, 7.29 ± 0.09 and 7.35 ± 0.11 with percentage inhibition of 13.41, 21.04, 36.95, 35.42 and 38.18%; 6.86 ± 0.11, 7.18 ± 0.15, 7.83 ± 0.09, 7.33 ± 0.11 and 7.37 ± 0.14 with % of inhibition i.e. 7.04, 20.48, 36.54, 35.07 and 38.01% and 6.80 ± 0.11, 7.67 ± 0.09, 9.06 ± 0.15, 8.58 ± 0.19 and 8.06 ± 0.13 with percentage of inhibition i.e. 7.85, 15.06, 26.58, 24.01 and 32.21% after 0h, 1/2h, 1h, 2h and 3h of treatment, respectively (Table 1). The analgesic activity of petroleum ether at the dose of 400 mg/kg was comparable with the standard i.e. pentazocine at a dose of 10 mg/kg as shown in the table 1.

In acetic acid induced writhing model, petroleum ether extracts at 400 mg/kg showed significant analgesic activity than the petroleum ether extracts at 200 mg/kg. The mean number of writhing of leaves of *Murraya koenigii* was found to be 101.41 ± 1.20 and 82.6 ± 1.31 with percentage of inhibition 13.32% and 29.40% at the doses of 200 and 400 mg/kg of petroleum ether extract after 1h treatment, respectively. This analgesic activity of petroleum ether at the dose of 400 mg/kg was comparable with the standard i.e. aspirin at a dose of 100 mg/kg as shown in the table 2. The mean number of writhing of ethanol extract of leaves of *M. koenigii* was found to be 114.6 ± 1.14 and 102 ± 1.19 with percentage of inhibition i.e. 2.05% and 12.82% at the doses of 200 and 400 mg/kg after 1h treatment, respectively. Likewise the mean number of writhing of water extract was found to be 98.6 ± 1.35 and 96 ± 1.26 with percentage inhibition of 15.72% and 17.94% at the same doses after 1h treatment (Table 2).

Pain is an unpleasant sensation, with a large subjective component. It is often accompanied by depression and a feeling of hopelessness. Petroleum ether, ethanol and water extract were examined for analgesic activity at the doses of 200 and 400 mg/kg doses. After comparison of
all the three extracts for analgesic activity, it was found that maximum activity was shown by petroleum ether extract followed by ethanol and water extract. Thus the observation in the present study are in close agreement to the previous worker as it is concluded that the aqueous and alcoholic extracts of *Hibiscus rosa sinensis* possess significant analgesic activity in Wistar albino rats. The formalin pain test is very useful for evaluating the mechanism of pain and analgesia. Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this model, while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase. Significant antidepressant activity was reported from various leaves extracts of *Murraya koenigii* Spreng.

Table: 1 Analgesic effect of petroleum ether, ethanol and water extract of leaves of *Murraya koenigii* Spreng on rats using tail flick method.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>0hr.</th>
<th>1hr.</th>
<th>2hr.</th>
<th>3hr.</th>
<th>4hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>7.38±0.13</td>
<td>9.03±0.15</td>
<td>12.34±0.10</td>
<td>11.29±0.16</td>
<td>11.89±0.15</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>10</td>
<td>7.10 ± 0.16 (3.79%)</td>
<td>7.54 ± 0.14 (16.50%)</td>
<td>7.43 ± 0.12 (39.78%)</td>
<td>7.36 ± 0.07 (34.80%)</td>
<td>7.46 ± 0.09 (37.25%)</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract</td>
<td>200</td>
<td>6.76 ± 0.08 (8.40%)</td>
<td>7.30 ± 0.11 (19.15%)</td>
<td>9.06 ± 0.13 (26.58%)</td>
<td>8.21 ± 0.09 (27.28%)</td>
<td>7.59 ± 0.12 (36.16%)</td>
</tr>
<tr>
<td>4</td>
<td>Petroleum ether extract</td>
<td>400</td>
<td>6.41 ± 0.15 (13.14%)</td>
<td>7.13 ± 0.11 (21.04%)</td>
<td>7.78 ± 0.09 (36.95%)</td>
<td>7.29 ± 0.04 (35.42%)</td>
<td>7.35 ± 0.11 (38.18%)</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol extract</td>
<td>200</td>
<td>7.21 ± 0.07 (2.30%)</td>
<td>7.51 ± 0.09 (20.80%)</td>
<td>8.97 ± 0.13 (27.30%)</td>
<td>10.56±0.11 (6.46%)</td>
<td>9.51 ± 0.05 (19.34%)</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol extract</td>
<td>400</td>
<td>6.86 ± 0.11 (7.04%)</td>
<td>7.18 ± 0.15 (20.48%)</td>
<td>7.83 ± 0.09 (36.54%)</td>
<td>7.33 ± 0.14 (35.07%)</td>
<td>7.37± 0.11 (38.01%)</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol extract</td>
<td>200</td>
<td>7.36 ± 0.15 (0.27%)</td>
<td>8.78 ± 0.12 (32.76%)</td>
<td>12.05±0.15 (2.35%)</td>
<td>11.21±0.11 (0.71%)</td>
<td>10.19±0.14 (14.29%)</td>
</tr>
<tr>
<td>6</td>
<td>Water extract</td>
<td>400</td>
<td>6.80 ± 0.11 (7.85%)</td>
<td>7.67 ± 0.09 (15.06%)</td>
<td>9.06 ± 0.15 (26.58%)</td>
<td>8.58 ± 0.19 (24.01%)</td>
<td>8.06 ± 0.13 (32.21%)</td>
</tr>
</tbody>
</table>

Results are expressed as standard error mean of three observations. Value in parentheses shows percentage inhibition.
Table: 2 Analgesic effect of petroleum ether, ethanol and water extract of leaves of *Murraya koenigii* Spreng on rats using writhing test.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>Number of Writhing 1 hr</th>
<th>Percentage of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>117.0 ± 1.23</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard (Aspirin)</td>
<td>100</td>
<td>81 ± 1.46</td>
<td>30.76%</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether</td>
<td>200</td>
<td>101.41 ± 1.20</td>
<td>13.32%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>82.6 ± 1.31</td>
<td>29.40%</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol extract</td>
<td>200</td>
<td>98.6 ± 1.36</td>
<td>15.72%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>96 ± 1.25</td>
<td>17.94%</td>
</tr>
<tr>
<td>5</td>
<td>Water extract</td>
<td>200</td>
<td>114.6 ± 1.14</td>
<td>2.05%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>102 ± 1.10</td>
<td>12.82%</td>
</tr>
</tbody>
</table>

Results are expressed as standard error mean of three observations.

CONCLUSION

The results obtained in this study indicate that the extracts possesses analgesic properties which are mediated via peripheral and central inhibitory mechanisms. Finally, this study provides a rationale for the use of *Murraya koenigii* (L.) Spreng in pain in folk medicine.

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