Evaluation of Analgesic Activity of Ethyl Acetate Extract of Pergularia daemia Forsk. Roots

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ABSTRACT
Pergularia daemia Forsk. Syn. Daemiaextensa(Asclepiadaceae) commonly known with the name of “dustaputeega” in telugu is a perennial twining herb, growing widely along the road sides of Andhra Pradesh state in India. Traditionally the plant is used to treat jaundice and it is used as anthelmintic, laxative, anti-pyretic and expectorant, and is also used in infantile diarrhea. It is also used in treatment of malarial intermittent fevers. The present study was taken to evaluate the analgesic activity of Ethanolic extract of Pergularia daemia Forsk. Root. using eddy’s hot plate and heat conduction method. In eddy’s hot plate method the ethyl acetate extract showed significant analgesic activity at the doses of 200 mg/kg (p<0.05) and 400 mg/kg (p<0.01) as compared to control group, when analyzed statistically by Dunnet method. The result obtained show that the ethyl acetate root extracts of Pergularia daemia (Forsk.) Chiov, possesses significant analgesic activity which confirms the traditional claims of the plant mentioned in Ayurveda.

Key words: Pergularia daemia Forsk

INTRODUCTION
Pergularia daemia Forsk. Syn. Daemiaextensa(Asclepiadaceae) commonly known with the name of “dustaputeega” in telugu is a perennial twining herb, growing widely along the road sides of Andhra Pradesh state in India. Traditionally the plant is used to treat jaundice and it is used as anthelmintic, laxative, anti-pyretic and expectorant, and is also used in infantile diarrhea. It is also used in treatment of malarial intermittent fevers. Latex of this plant used for boils and snake sores 1. Entire plant used as an anthelmintic 2. It is also used in treatment of malarial intermittent fevers. Stem bark has been used to treat jaundice and it is used as anthelmintic, laxative, anti-pyretic and expectorant, and is also used in infantile diarrhea. It is also used in treatment of malarial intermittent fevers1. Latex of this plant used for toothache 3. Stem bark remedy for cold 4 and fever 4. Aerial parts of this plant reported the various pharmacological activities like hepato-protective 5, antifeed 5, anti-diabetic 6. Phytochemically the plant has been investigated for cardenolides, alkaloids, saponins 5, various terpenes and steroidal compounds 5. Aerial parts of the plant used for snake bite 9. Entire plant used as an anthelmintic 10, emmenagogue 11, emetic 12,13, antiseptic 14, emetic 15 and antivenin 16 and used to facilitate parturition 17, while used in Ayurvedic medicine for delayed childbirth 18, amenorrhea 19, asthma, snakebite, rheumatic swellings 13 and used to treat postpartum hemorrhage 18. Latex of this plant used for boils and sores 19. Dried leaf used as an emetic 20, antihemorrhagic 21 and used for bronchitis 20, amenorrhea, dysmenorrhea 10,22, asthma 12, healing cuts and wounds 23, while used to treat whooping cough 24 and to facilitate parturition 19. Fresh leaf used as fish poison 25, while leaf juice used for amenorrhea, dysmenorrhea, catarrhal infections, infantile diarrhea 12 and used reduce the body pain 24,26. Dried root used as an abortifacient 27, emetic, bronchitis 20 and used for cough, asthma and constipation 25, while fresh root used as an abortifacient 28,29 and used to treat gonorrhea 30. Shoots used to treat whooping cough 31. Stem bark has been used to treat malaria 32 and twig used as an antipyretic and appetizer The present study was taken to evaluate the analgesic activity of Ethyl acetate extract of Pergularia daemia Forsk. Root.

MATERIALS AND METHODS
Collection of plant materials: The roots of the Pergularia daemia were collected from the road sides of mangalagiri, Andhra Pradesh and it was authenticated by Department of Botany, AcharayaNagarjuna University, Guntur, Andhra Pradesh, India. Preparation of the extract: The shade dried roots 500 g were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60–80 °C) and then extracted with ethyl acetate using soxhlet apparatus at 50°C. extracts were concentrated under vacuum using rotary flash evaporator to get the residues. The percentage yields of ethyl acetate extract were found to be 23 ± 5%. Animals: Wistar albino rats of either sex, weighing 150–200 g were taken and maintained under standard laboratory conditions (temperature 25±2 °C, relative humidity 55±10% and 12-h light: 12-h darkcycle). Animals were allowed to take standard laboratory feed and ad libitum. The experiments were carried out after the prior approval of all experimental protocols by the institutional animal ethics committee.

Acute Toxicity studies: Acute toxicity study was performed for ethyl acetate extract as per OECD guidelines. Female albino wistar rats were used for acute toxicity study. The animals were kept fasting for overnight
Table: 1 Analgesic effect of ethyl acetate extract of *Pergularia daemia* Forsk root using Eddys Hot plate method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Basal Reaction (s)</th>
<th>Mean latency time with SEM in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control)</td>
<td>1% CMC</td>
<td>2 ml/kg</td>
<td>1.50 ± 0.22</td>
<td>1.50 ± 0.22 1.75 ± 0.25 1.67 ± 0.08 1.83 ± 0.31 3.23 ± 5.03 ± 0.26**</td>
</tr>
<tr>
<td>Group-2 (std)</td>
<td>Tramadol</td>
<td>4 mg/kg</td>
<td>1.83 ± 0.31</td>
<td>4.01 ± 1.03* 3.06 ± 0.26* 0.32* 0.26**</td>
</tr>
<tr>
<td>Group-3 (Test-1)</td>
<td>PDEAE</td>
<td>0.6 mg/kg</td>
<td>1.72 ± 0.03</td>
<td>2.32 ± 0.21 1.67 ± 0.21* 1.83 ± 0.31 3.12 ± 0.21</td>
</tr>
<tr>
<td>Group-4 (Test-2)</td>
<td>PDEAE</td>
<td>400 mg/kg</td>
<td>1.65 ± 0.37</td>
<td>0.72** 0.71** 0.31* 2.01 ± 0.26</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n=6. Data analyzed by One-way ANOVA followed by Dunnette’s test *P < 0.05, **P < 0.01

Note: PDEAE : ethyl acetate extract of *Pergularia daemia* Forsk Roots

with water *ad libitum*, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as a toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose, i.e., 2000 mg/kg. Two doses were selected for evaluation of analgesic activity, i.e., 200 mg/kg and 400 mg/kg.

Hot plate method in rats: The paws of mice and rats are very sensitive to heat temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stopwatch. Wistar albino rats weighing between 100-150 g were used for evaluation of analgesic activity; rats of either sex were divided into four different groups and six animals were taken for each group. Tramadol 4 mg/kg was administered as a standard drug. All the animals were kept overnight fasting prior to drug administration. Two doses of the test drug were given orally. After 30 minutes, the animals are placed on the hot plate and the observations were recorded and at the time interval of 60, 90, and 120 minutes. The results of Hot plate method in rats were tabulated in the following table.

Statistical analysis: The mean values±S.E.M. are calculated for each parameter using one-way analysis of variance (ANOVA) (Gennaro, 1995). It was carried out and the individual comparisons of the group mean values were done using Dunnet’s Procedure (1964).

**RESULTS AND DISCUSSION**

Pain is a condition which is regularly dealt with in daily clinical practice. Hence, any attempt to contribute an easily available analgesic drug from the available flora is always accepted without any reluctance. *Pergularia daemia Forsk. Roots* have been traditionally used by the tribals of middle Kerala to cure specific ailments. This attempt is to prove the efficacy of the plant extract as a potential analgesic drug and to demonstrate a positive result. Search for safe herbal remedies with potent antipyretic activity received momentum recently as the available antipyretic, such as paracetamol, aspirin, nimusulide etc have toxic effect to the various organs of the body [6]. Our investigation on the extracts showed the presence of triterpenoids and flavonoids in the ethanolic extract. According to these results, it may be hypothesized that flavonoids, which are present in the ethanolic extract, could be considered responsible for the analgesic activity. Acute toxicity studies did not reveal any toxic symptoms or death in any of the animals up to the dose of 3000 mg/kg body weight, were either extract.

The present study was carried out to evaluate the possibility of PDEAE in alleviating pain. The antinociceptive activity of the PDEAE was investigated using thermal-induced experimental nociception. The reaction time following the oral administration of different doses of PDEAE is presented in the table-1. PDEAE (400 mg/kg) produced a significant (*P < 0.01) increase in the mean reaction time throughout the observation period, i.e., at 30 min, 60 min, 90 min and 120 min, compared to the control and 30 mg/kg dose of PDEAE. The reference drug tramadol (4 mg/kg, i.p.) also caused significant (*P < 0.01) increase in the mean reaction time throughout the observation period, as compared to the control group. The percentage increase in the reaction time was dose-dependent and differed significantly among the groups of rats (*P<0.01) receiving different dose levels of the extract and tramadol.

From the results of the present study it can be inferred that Ethyl acetate extract of *Pergularia daemia Forsk. Roots* are an effective analgesic agent. While comparing with 200 mg/kg, 400 mg/kg body weight revealed higher effect than others.

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