In vivo Evaluation of Analgesic and Antipyretic activity of Aerial parts of Tabernaemontana divaricata in Experimental Animal models

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Running Title: Analgesic and Antipyretic activity of Tabernaemontana divaricata

Summary

The present study was designed to investigate the analgesic and antipyretic activity of methanol extract of aerial parts of Tabernaemontana divaricata in experimental animal models. The analgesic activity of T. divaricata at the doses of 100 and 200 mg/kg was assessed by various experimental models such as Acetic-acid and Hot-plate induced writhing and Hafner’s Tail-clip method, by using indomethacin (10mg/kg) pentazocine (10mg/kg) as standard drugs, while Yeast-induced hyperpyrexia was used to evaluate the anti-pyretic activity using parecetamol(150 mg/kg) as standard.

The methanol extract of T. divaricata (METD) at 200 mg/kg was found to be significantly (p < 0.001) reduced the Acetic-acid induced writhings in mice. Moreover in Hot-plate test, there was a significant dose dependent inhibition of pain (p < 0.01 & p < 0.001) at the doses of 100 and 200 mg/kg b.w. respectively. Also in the tail-clip test, METD caused a significant increase in the reaction time at both the doses used. METD significantly reversed the yeast induced hyperthermia in a dose dependent manner at different time of observation (1- 4hr).

The obtained results provide promising baseline information for the potential use of T. divaricata in the treatment of pain and pyrexia.

Key words: Analgesic, Antipyretic, methanol extract, T. divaricata,

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Introduction

Plant kingdom holds many species of plants containing the substance of medicinal values and most of them yet to be found. Natural products from higher plants have indeed offered some clinically useful drugs such as reserpine, vincristine, physostigmine, quinine, d-tubocurarine, ginseng, atropine, digoxin etc.[1]. In the developing world the trend has been changed from synthetic to natural herbal medicine because of its less toxicity and considered as a safest one. So nowadays, natural products are believed to be an important source of new chemical substance with potential therapeutic applications.

Tabernaemontana divaricata belongs to the family Apocynaceae, a common garden plant in tropical countries and also distributed in Coast forests of Bengal, Myanmar and mangrove forests of China and Japan[2]. It has been used as antioxidant, anti-infection, anti-cancer and analgesia as a folk medicine in India, Sri Lanka, Vietnam, Malaysia and Thailand. The plant grows up to a height of about 6-feet, bears attractive, white colored flowers and its juice posses a reliable anti-bacterial action in eye infection[3]. It has been used in Chinese, Ayurvedic and Thai as traditional medicine for the treatment of fever, pain and dysentery[3,4]. Alkaloids and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes are the major constituents present in Tabernaemontana divaricata[5].

In the present work, the methanol extract of aerial parts Tabernaemontana divaricata was evaluated for its analgesic and antipyretic activity at the doses of 100 and 200 mg/kg., b.w, using suitable experimental animal models.

Materials and Methods:

Plant material:

The plant was collected from the areas of Nagercoil and positively identified and authenticated by Prof. Mr. V Sivanandam, Dept. of Botany, Lekshmipuram College of Arts and Science, Neyoor, Kanyakumari District, Tamilnadu, India. The plant specimen was certified as Tabernaemontana divaricata of family Apocynaceae. The selected parts of the plant were then dried in shade at 21-30°C for 10 to 15 days.

Preparation of extract:

The shade dried powdered plant material was successfully extracted with methanol for 48 hrs at 55°C-65°C. The extract was filtered and concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness at room temperature. Then the extract was stored in the desiccators and used for subsequent experiments.

Animals:

Healthy Wister Albino mice (25-30g) and Wister Albino rats (150-225g) of either sex were used in the present study. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethics committee, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal. (Regd. No. JKKMMRFCP/IAEC/2010/002).
Analgesic activity:

The animals were randomly divided into four groups, comprising of six animals in each group. Group I served as saline control received normal saline (NaCl 0.9%). Group IV served as standard, received indomethacin and pentazocine 10mg/kg orally. Group II and III served as test, received Methanol extract of *Tabernaemontana divaricata* (METD) at the doses of 100 and 200 mg/kg, b.w, p.o, respectively.

(i) Acetic acid induced Writhing model

The mice were administered orally with NaCl 0.9%, METD (100 & 200 mg/kg) and standard drug indomethacin (10mg/kg). Thirty minutes after the treatment, the animals were given an intraperitoneal (i.p) injection of 0.6% v/v acetic acid in a volume of 10ml/kg to induce writhings. The number of writhings or stretches was counted for 15 minutes. A reduction in the writhing in tested groups compared to control group was considered as the presence of analgesia [6,7]. The percentage inhibition of writhing was also calculated using the formula:

\[
\text{\% Inhibition} = \frac{\text{Mean no. of writhes in control} - \text{Mean no. of writhes in test}}{\text{Mean no. of writhes in control}} \times 100
\]

(ii) Hot plate reaction time

Mice were placed on an Aluminium hot plate kept at a maximum temperature of about 55±0.5°C. The time(s) to discomfort (paw licking or jumping) was recorded as response latency. Only mice with a control response time of 4-10 sec were included in the study in order to prevent the paw damage. Prior to oral administration of normal saline, METD (100 and 200 mg/kg) and standard drug pentazocine (10 mg/kg), the reaction time of each mouse was done at 0 and 10 min interval. Then the response latency was measured at 0, 30, 60 and 120 min after drug administration, and the increase in reaction time of tested groups are compared with the control group [8,9].

(iii) Hafner’s Tail clip method

Swiss albino rats of either sex weighing 150-175 gm were divided into four groups of six animals each. All the rats were screened by applying a metal artery clip to the base of tail. The pressure exerted by the clip were so adjusted that it was just sufficient to make all control animals to respond. The animals that didn’t try to dislodge the clip within 10 seconds were rejected from this study. Each animal acted as its own control. The clip was applied 30, 60 and 120 min after the oral administration of normal saline, METD (100 & 200 mg/kg) and standard drug pentazocine (10 mg/kg). Change in the reaction time of treated groups was noted and compared with control [10].

Anti-Pyretic activity:

Male Swiss albino rats weighing 150-175 gm were divided into four groups of six animals each. All the animals were kept for overnight fasting with water *ad libithum* before the induction of pyrexia. On the next day 20% w/v of Bewer’s yeast suspension (10 ml/kg) was administered as subcutaneous (s.c) injection into the dorsal region of the animal. 24hrs after the injection, the rectal temperature of each animal was measured by using a digital tele-thermometer. Animals that failed to show an increase in temperature at least 0.7°C were rejected from the experiment. After the oral administration of vehicle, METD (100 mg/kg and 200 mg/kg) and standard drug parecetamol (150 mg/kg, b.w.), the rectal temperature was again measured at 1, 2, 3, and 4hr after the treatment [11].
Statistical Analysis:
All the results were expressed as mean ± standard error of mean. The data obtained were subjected to statistical analysis using one-way ANOVA and groups were compared by Tukey-Kramer multiple comparison test. Differences between groups were considered significant at P<0.05 level.

Results

Analgesic activity:

(i) Acetic acid induced Writhing
The results presented in Table 1, shows that both the doses of METD exhibited dose dependent inhibition of the control writhes significantly (p<0.01 & p<0.001) at a percentage inhibition of about 39.59 and 53.17 respectively, whereas the standard drug phenylbutazone also showed a significant inhibition (p<0.001) at a percentage of 58.48 when compared with control group.

Table no.1: Effect of *T. divericata* on acetic acid-induced writhing behavior in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Number of Writhings</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NaCl 9%)</td>
<td>–</td>
<td>63.54 ± 8.33</td>
<td>–</td>
</tr>
<tr>
<td>METD 100</td>
<td>100</td>
<td>38.38 ± 3.25**</td>
<td>39.59</td>
</tr>
<tr>
<td>METD 200</td>
<td>200</td>
<td>29.75 ± 2.40***</td>
<td>53.17</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>26.38 ± 2.20***</td>
<td>58.48</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; number of animals used (n=6) in each group. *** p < 0.001 when compared with control group.

(ii) Hot plate reaction time
As shown in Table 2, the METD produced at 200 mg/kg significantly (p<0.001) raised the pain threshold at different time of observation (0-120min) in comparison with control. pentazocine (10mg/kg), used as standard drug, also produced a significant analgesic effect.
Table no.2: Effect of *T. divericata* on Hot plate reaction time in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Hind paw lick latency in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0min</td>
</tr>
<tr>
<td>Control (NaCl 9%)</td>
<td>–</td>
<td>5.27±0.39</td>
</tr>
<tr>
<td>METD 100</td>
<td>5.13±0.46</td>
<td>7.45±0.50**</td>
</tr>
<tr>
<td>METD 200</td>
<td>5.03±0.54</td>
<td>8.68±0.62*</td>
</tr>
<tr>
<td>Pentazocine 10</td>
<td>5.18±0.43</td>
<td>9.82±0.67**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; number of animals used (n=6) in each group. *p<0.05, ** p < 0.01 and *** p < 0.001 when compared with control group.

(iii) Tail clip method

The effect of METD on tail clip is shown in the Table 3. The extract at 100 mg/kg showed a significant (p<0.01) inhibition of pain, and at 200 mg/kg, it is highly significant (p<0.001) when compared with the control. Standard drug, pentazocine (10 mg/kg) also showed a significant inhibition.

Table no.3: Effect of *T. divericata* on Haffner’s tail clip test in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30min</td>
</tr>
<tr>
<td>Control (NaCl 9%)</td>
<td>–</td>
<td>4.22±0.12</td>
</tr>
<tr>
<td>METD 100</td>
<td>4.18±0.04</td>
<td>5.80±0.3**</td>
</tr>
<tr>
<td>METD 200</td>
<td>4.08±0.09</td>
<td>7.80±0.4***</td>
</tr>
<tr>
<td>Pentazocine 10</td>
<td>4.50±0.07</td>
<td>8.70±0.6***</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; number of animals used (n=6) in each group. ** p < 0.01 and *** p < 0.001 when compared with control group.

Anti-Pyretic activity:

The s.c injection of yeast suspension markedly elevated the rectal temperature after 24 hr of administration. Treatment with METD at the doses of 100 and 200 mg/kg, bw, decreased the rectal temperature in a dose dependent manner. The effect was started from the first hour and maintained for 4hr. The yeast induced hyperthermia was a significantly reversed in both the doses at different time of observation. The anti-pyretic action of standard drug, paracetamol (150mg/kg) was observed to be highly significant (p<0.001). The results were shown in Table 4.
Table no.4: Effect of *T. divericata* on Yeast induced pyrexia in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Rectal temperature (°C) After Yeast injection</th>
<th>Rectal temperature (°C) After drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>37.1±0.05 39.8±0.04 39.8±0.02 39.9±0.05 39.8±0.06 39.7±0.08</td>
<td></td>
</tr>
<tr>
<td>METD 100</td>
<td>37.3±0.04</td>
<td>39.5±0.12* 39.1±0.07** 38.6±0.08** 38.1±0.06***</td>
<td></td>
</tr>
<tr>
<td>METD 200</td>
<td>37.4±0.07</td>
<td>40.0±0.09 39.7±0.02** 38.5±0.03*** 38.0±0.04** 37.6±0.03***</td>
<td></td>
</tr>
<tr>
<td>Parecetamol</td>
<td>150</td>
<td>37.0±0.02 39.9±0.02 38.9±0.03*** 38.1±0.06*** 37.8±0.04*** 37.2±0.03***</td>
<td></td>
</tr>
</tbody>
</table>

The result given are mean ± S.E.M.; number of animals used (n=6) in each group. * p < 0.05, ** p < 0.01 and *** p < 0.001 when compared with control group.

Discussion

The METD was evaluated for its analgesic activity in suitable experimental animal models. In order to distinguish between the central and peripheral analgesic action of METD, acetic-acid induced writhing model in mice was preferred to examine the effect. It is a reliable and affords rapid evaluation of peripheral type. Sensitizing of the nociceptive receptor to prostaglandins, leads to the constriction of the abdomen, and the animal reacts with a characteristic stretching behavior called writhing [12]. Intraperitoneal administration of acetic-acid induces the stretching by above mechanism. It was found that METD significantly inhibited the acetic-acid induced stretching in mice. This study reveals that the analgesic action produced by METD may be probably due to the inhibition of synthesis or action of prostaglandins.

Thermal and Mechanical stimulus parameters were used for Hot-plate and Tail-clip methods, respectively. An increase in reaction time is generally considered and important parameter of central and peripheral analgesic activity by non selective COX inhibition [13]. Results of both the tests indicating that METD showed a strong analgesic effect is most probably of opioid type, since the thermal and mechanical nociceptive stimuli are indicative of opioid type of analgesic effect. The presence of anti oxidative constituents such as terpenoids and flavonoids in plant has been shown to have diuretic, laxative, anti hypertensive, anti inflammatory and analgesic activity [14].

Pyrexia may be due to the infection or one of the sequels of tissue damage, inflammation, graft rejection, or other diseased states. Hypothalamus in the CNS delicate the balance between production and loss of heat and hypothalamus regulates the set point at which the body temperature is maintained. In fever this set point elevates and a drug like paracetamol or other Antipyretics resets the hypothalamus and does not influence in normal body temperature [15]. Yeast induced pyrexia is called pathogenic fever and its etiology includes the production of prostaglandins, which set the thermoregulatory center at minimum temperature [16]. The present study shows that METD possesses a significant antipyretic effect in yeast-induced pyrexia. So the inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol [17].
Conclusion

Based on the results obtained from the present study, it can be concluded that METD at the dose of 200 mg/kg, b.w. is found to be more potent and effective Analgesic and Anti-pyretic activity. More detailed studies are however necessary to identify the active principle(s) and its exact mechanism of action.

References