Research Article

EVALUATION OF ANTI-PYRETIC AND ANALGESIC ACTIVITY OF MARSILEA MINUTA LINN.

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ABSTRACT

Pyrexia and analgesia are associated with several pathological conditions. Synthetic drugs available for the treatment of these conditions cause multiple unwanted effects. The main objective of the present work is to find out the good pharmacological activities in herbal source. The current study was aimed to investigate the antipyretic and analgesic properties of ethanolic leaf extract of Marsilea minuta (EEMM) in rats. EEMM was employed to assess antipyretic activity in Brewer’s yeast-induced pyrexia in rats and the analgesic activity was studied using tail-immersion method and Eddy’s hot-plate device maintained at 55°C in rats. Dried powder plant materials were subjected to successive solvent extraction taking Petroleum ether, Benzene, Chloroform, Ethanol and triple distilled water. EEMM at a dose of 200 mg/kg and 400 mg/kg p.o were subjected to antipyretic and analgesic activity. The EEMM had significant dose dependent antipyretic and analgesic properties at 200 and 400 mg/kg p.o. The results obtained revealed significant (p < 0.05) antipyretic activity at 400 mg/kg dose tested as compared with standard. Analgesic activity was evaluated using tail-immersion method and Eddy’s hot plate method. The analgesic activity was observed maximum at 400 mg/kg (significance p < 0.001). Paracetamol (150 mg/kg p.o) and Diclofenac sodium (9 mg/kg i.p) was taken as standard drug. The results of the present study demonstrate that EEMM possess significant antipyretic and analgesic activity. This study provides evidences for the antipyretic and analgesic activity of Marsilea minuta Linn which could partly contribute to its ethno medical use.

Keywords: Anti-pyretic activity; Analgesic activity; Marsilea minuta Linn; Aspirin; pyrexia

INTRODUCTION

Over the centuries, phytopharmaceuticals have been utilized by different communities of the world. From ancient era herbs are said to be “God’s gift” to man. They were used for the treatment of various diseases and for increasing longevity of human’s life. Herbal remedy has become most popular and promising remedy in today’s era. Fever and pain are very regularly observed in today’s stressful and unhealthy lifestyle. Allopathic medicines are giving promising action but they are having more side effects and moreover they are also costly. So there is a need to search herbal actives which promises better action. Marsilea minuta Linn (Marsileaceae) is usually found at the edges of ponds and irrigation channels and as a weed in wet rice fields and it is found throughout India. Marsilea minuta Linn has traditional medicinal value. Traditionally the plant is used to stop nose bleeding, treat indigestion, used in kidney infection; as diuretic, anti- toxic, in hepatitis, in diabetes etc. Marsilea minuta Linn was reported for anti-fertility activity, in-vitro antibacterial activity, anti inflammatory activity, sedative and anticonvulsant activity, anti-inflammatory and analgesic activity, antidepressant activity, adaptogenic and antiglucose activity and hypcholesterolemic activities. According to chemical moieties present in plant, it is proposed that Marsilea minuta Linn has been beneficial in fever and pain symptoms. Owing to such usefulness of this plant an attempt was made to evaluate antipyretic and analgesic activity in the present study.

MATERIALS AND METHOD

Collection and Authentication of Plant

The plant Marsilea minuta Linn was collected in the month of December 2014, from rice fields of Villupuram, Tamilnadu, India. The plant was identified and authenticated by Prof. P. Jayaraman, Plant anatomy research centre, Chennai, Tamil Nadu, India who authenticated the plant from available literature and the voucher specimen NO. is PARC/2011/ 865 and the same one is being maintained in the laboratory for future reference.

Preparation of Plant Extract

Marsilea minuta Linn leaves where directly collected washed with water and dried in shade. Then it was subjected to pulverization and the powder was passed through the sieve No. 60 for powder and it was stored in an air tight container. The powdered leaves of Marsilea minuta Linn was successfully extracted with ethanol using soxhlet apparatus for 2 days. The obtained extract was concentrated by distillation stored in a desiccator and used for subsequent experiments.

Animals

Swiss albino rats weighing 150-240 g and albino mice weighing (15-18 g) were used in the present study. Animals were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35 %) in 12 h light and 12 h dark cycle respectively for 2 week before and during the experiments. Animals were supplied ad libitum with standard rodent pellet diet and had

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free access to water. The temperature was maintained at 25 ± 2°C. The animals were deprived of food for 24 h before the test. All the animals were acclimatized to the laboratory conditions prior to experimentation. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 99/PO/C/6/CPCSEA)

**Acute Toxicity Studies**

Acute toxicity study was carried out for the fraction of EEMM according to the Organization for economic co-operation and development (OECD) 420 guidelines (OECD, 2001). Swiss albino mice of either sex (18-22 g weight) were used for acute oral toxicity study. The fractions were administered to different groups in doses ranging from 100-2000 mg/kg p.o. They were observed for signs of toxicity and mortality for 72 h. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice up to 2000 mg/kg, p.o during the 72 h observation period. Based on the results obtained from this study, the dose was fixed to be 200 mg/kg and 400 mg/kg for dose dependent study.

**Experimental Design**

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

- **Group I:** Animals served as control (2% gum acacia sol.)
- **Group II:** Animals were treated with Brewer’s yeast via subcutaneous injection (10 ml/kg).
- **Group III:** Animals were treated with Brewer’s yeast (10 ml/kg s.c) and the standard drug Paracetamol (150 mg/kg p.o)
- **Group IV:** Animals were treated with yeast (10 ml/kg) and EEMM (200 mg/kg p.o)
- **Group V:** Animals were treated with yeast (10 ml/kg) and EEMM (400 mg/kg p.o)

**Antipyretic Activity**

**Yeast Induced Pyrexia Method**

Albino rats were divided into five groups each containing six rats. Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer’s yeast (10 ml/kg) in distilled water. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer (SK-1250 MC, Sato keiryoki Mfg.) to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 19 h after yeast injection and animals that showed an increase of 0.3-0.5°C in rectal temperature were selected. The EEMM 200 mg/kg, 400 mg/kg p.o and Paracetamol 150 mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments. The temperature was measured at 1st, 2nd and 3rd h after drug administration.

**Analgesic Activity**

**Eddy’s Hot Plate Method**

Albino mice were divided into four groups each containing six mice. Animals were divided in to control, standard Diclofenac sodium (9 mg/kg i.p) and test groups of EEMM 200 mg/kg, 400 mg/kg p.o respectively. The animals were individually placed on the hot plate maintained at 55°C, one hr after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response. Whichever appeared first, the cut off time for the reaction was 15 sec and it is observed for every half an hour up to 2 h.

**Tail Immersion Method**

Albino mice were divided into control, standard Diclofenac sodium (9 mg/kg i.p) a groups of EEMM 200 mg/kg, 400 mg/kg p.o respectively. Each groups containing six mice and prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55-56°C. Any animal failing to withdraw its tail within 5 sec is rejected from the study. The selected animals were then divided into four groups of six mice each.

**Experimental Design**

- **Group I:** Control group animals received 2 ml/kg of 1% NaCMC p.o
- **Group II:** Standard group animals received Diclofenac sodium at a dose of 9 mg/kg i.p
- **Group III:** Received EEMM 200 mg/kg in 1% NaCMC p.o
- **Group IV:** Received EEMM 400 mg/kg in 1% NaCMC p.o

After administration of the above scheduled drugs, the reaction time was measured in sec at 0 min (before drug challenge), 15, 30 and 60 min.

**Statistical Significance**

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet’s multiple comparisons. The results obtained were compared with the vehicle control group. The p < 0.05 were considered to be statistically significant.

**RESULTS**

Natural herbs have been used for medicinal purposes in many nations and continue to be a remedy for number of ailments even with the uprising in antibiotics and other synthetic medicines. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed drugs due to their reliable effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. Since these drugs have more toxic effect to the various organs of the body, searching of herbal remedies with potent antipyretic and analgesic activity received momentum recently. The present study has been carried out to investigate the antipyretic and analgesic properties of ethanolic leaf extract of Marsilea minuta Linn by Brewer’s yeast-induced pyrexia in rats and the analgesic activity was studied using tail-immersion and Eddy’s hot-plate methods.

**Acute Toxicity**

EEMM was found safe at all test doses (200, 400 and 2000 mg/kg p.o). During 72 h assessment time, test animals were found normal.

**Anti-pyretic Activity**

Administration of ethanolic leaf extract of Marsilea minuta Linn (200 and 400 mg/kg p.o) produced a significant (p < 0.05) antipyretic activity compared to control. However, the extent of temperature regulation in rats treated with ethanolic leaf extract of Marsilea minuta Linn 400 mg/kg showed lesser than paracetamol (150 mg/kg body weight p.o) treated group. In time course, rats treated with Marsilea minuta Linn 400 mg/kg and paracetamol (150 mg/kg body weight p.o) elicited maximum temperature regulation at 6 h after administration of respected drugs in pyrexia induced rats. Results are given in Table 1.

**Analgesic Activity**

**Eddy’s Hot Plate**

Eddy’s Hot-plate test was assayed to characterize the analgesic activity of the EEMM. The results presented in Table 2 shows that...
significant (P < 0.0001) dose dependent effect was observed in EEMM treated (200 and 400 mg/kg p.o) mice comparatively lesser than Diclofenac sodium (9 mg/kg i.p). The oral administration of the EEMM at doses 200 mg/kg body weight p.o and 400 mg/kg body weight p.o significantly raised the pain threshold at different time of observation (0- 120 min) in comparison with control. Diclofenac sodium (9 mg/kg i.p) used as standard drug, also produced a significant analgesic effect during all the observation times when compared with control values (P < 0.0001). Results are given in Table 2.

Tail Immersion
Tail immersion method was assayed to characterize the analgesic activity of the ethanolic leaf extract of Marsilea minuta Linn at doses 200 mg/kg body weight p.o and 400 mg/kg p.o. The results in Table 3 shows that significant (P < 0.0001) dose dependent effect was observed in EEMM treated (200 and 400 mg/kg p.o) mice comparatively lesser than Diclofenac sodium (9 mg/kg i.p.). Diclofenac sodium (9 mg/kg i.p.) used as standard drug, also produced a significant analgesic effect when compared with control values (P < 0.0001). Results are given in Table 3.

Table 1: Results of Effect of EEMM on yeast-induced pyrexia in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial rectal temperature (°C)</th>
<th>Rectal temperature in (°C) after 19 H of yeast administration</th>
<th>% Reduction in rectal temperature in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 H</td>
<td>2 H</td>
</tr>
<tr>
<td>Group I</td>
<td>37.6 ± 0.43</td>
<td>39.57 ± 0.28</td>
<td>39.20 ± 0.13</td>
</tr>
<tr>
<td>Group II</td>
<td>38.50 ± 0.09</td>
<td>40.16 ± 0.17</td>
<td>40.02 ± 0.32</td>
</tr>
<tr>
<td>Group III</td>
<td>38.61 ± 0.14</td>
<td>40.11 ± 0.31</td>
<td>39.61 ± 0.32*</td>
</tr>
<tr>
<td>Group IV</td>
<td>38.77 ± 0.54</td>
<td>40.16 ± 0.28</td>
<td>39.88 ± 0.18</td>
</tr>
<tr>
<td>Group V</td>
<td>38.50 ± 0.09</td>
<td>40.16 ± 0.17</td>
<td>39.61 ± 0.32*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM (n = 6), the data was analyzed by ANOVA followed by Dunnett’s test. * P < 0.05 significant compared to control

Table 2: Results of Effect of EEMM on Analgesic Activity (Eddy’s Hot Plate Method) in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.22 ± 0.02</td>
<td>3.23 ± 0.04</td>
<td>3.31 ± 0.04</td>
<td>3.28 ± 0.10</td>
<td>3.25 ± 0.12</td>
</tr>
<tr>
<td>Group II</td>
<td>3.20 ± 0.01</td>
<td>5.60 ± 0.03</td>
<td>5.85 ± 0.03</td>
<td>5.79 ± 0.08</td>
<td>5.71 ± 0.00</td>
</tr>
<tr>
<td>Group III</td>
<td>3.22 ± 0.28</td>
<td>3.58 ± 0.18</td>
<td>3.95± ± 0.23</td>
<td>3.91** ± 0.48</td>
<td>3.85 ** ± 0.97</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.25 ± 0.27</td>
<td>4.82± ± 0.22</td>
<td>5.58 ± 0.72</td>
<td>5.49 ** ± 0.76</td>
<td>5.41** ± 0.27</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett’s test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01, ***P < 0.001

Table 3: Results of Effect of EEMM on Analgesic Activity (Tail immersion method) in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reaction Time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group I</td>
<td>2.33 ± 0.33</td>
</tr>
<tr>
<td>Group II</td>
<td>2.33 ± 0.42</td>
</tr>
<tr>
<td>Group III</td>
<td>2.00 ± 0.36</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.16 ± 0.60</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett’s test. Asterisks indicated statistically significant values from control. *p < 0.05, **p < 0.01

DISCUSSION

Attempts were made to investigate the antipyretic and analgesic properties of EEMM belonging to family of Marsileaceae. Infection, tissue damage, inflammation, graft rejection, or other diseased states causes fever. Antipyretics are the agents that reduce the elevated body temperature. Equilibrium between production and loss of heat is very much necessary to regulate the body temperature and the hypothalamus regulates and maintains the body temperature. In fever hypothalamus elevates the body temperature and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast induced pyrexia is pathogenic fever which results from the production of prostaglandins.18 The present results shows that EEMM possesses a significant antipyretic effect as like paracetamol (standard drug) in yeast-induced elevation of body temperature in rat model indicating the inhibition of prostaglandin synthesis could be again as like the mechanism of paracetamol19 Perhaps, various mediators are there behind the etiology of fever. Anti-pyresis could be achieved by inhibiting any of these mediators.20 The data represents the analgesic potential of EEMM. The ethanolic extract at the doses tested was shown to possess analgesic activity evident in all the pain models, representing it possesses central mediated activities. The Eddy’s hot-plate and tail-immersion tests are useful in revealing centrally mediated analgesic response, which focuses mainly on changes above the spinal cord level21 The significant increase in pain threshold produced by EEMM in these models suggest involvement of central pain pathways. Pain is centrally controlled via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems.22-26 The analgesic effect produced by the EEMM may be through central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of PGs, LTs, and other endogenous substances that plays fundamental role in inflammation and pain.

CONCLUSION

The results obtained in this study indicate that EEMM (Marsilea minuta L.) possesses potent antipyretic and analgesic properties against different stimuli, which are mediated via central inhibitory mechanisms. This could provide a rationale for the use of this plant in fever, pain and inflammatory disorders in folk medicine. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.
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