

Evaluation of analgesic, Anti-Pyretic And Anti-Inflammatory activities of *Andropogon Muricatus* roots extract

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Received on: 15-04-2010; Revised on: 12-05-2010; Accepted on: 13-06-2010

**ABSTRACT**

The present study was planned to evaluate the analgesic, antipyretic and anti-inflammatory activities of ethanolic roots extract of *Andropogon muricatus* (AMEE) in albino rats following oral administration. The results showed that the ethanolic extract significantly reduce the acetic acid induce writhing in analgesic model. Its effects on antipyretic activity were also appreciable it significantly reduces fever at higher doses within 2 hrs. on yeast induce hyperthermia in rats. Edema induced by carrageenan was also significantly reduced within 1 to 7 hr. post dosing at all the dose levels used.

**Key words:** Analgesic, Antipyretic, Anti-inflammatory, *Andropogon muricatus*, AMEE.

**INTRODUCTION**

Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus [1]. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persistent long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection). With many pathological conditions, tissue injury is the immediate cause of the pain, and this results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation [2].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states [3]. It is the body’s natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of proinflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF-α), which increase the synthesis of prostaglandin E2 (PGE2) near hypothalamic area and thereby trigger the hypothalamic-pituitary axis to elevate the body temperature [4]. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis [5, 6]. These synthetic agents irreversibly inhibit COX-2 with high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles.

*Andropogon muricatus* Retz. (Family Poaceae.) commonly known as Khas in Hindi and found thought northern to southern India. Roots are refrigerant, febrifuge, diaphoretic, stimulant, stomachic, analgesic, anti-inflamatory and emmenagogue, used in stranguary, colic, flatulence, obstinate vomiting; paste used as a cooling application in fevers [7, 8].

A bibliographic survey showed that there are no reports on the analgesic and anti-pyretic and anti-inflammatory activity of *Andropogon muricatus*. This prompted us to investigate the said pharmacological activities of titled plant in experimental models of algesia, pyrexia and inflammation.

**MATERIALS AND METHODS**

**Plant material:**

The roots of *Andropogon muricatus* were collected from local market of Etawah (Uttar Pradesh) India in the month of May. The plant was identified at Pharmacognosy Department of Sir Madanlal Institute of Pharmacy, Etawah (UP), India. The voucher specimen of *Andropogon muricatus* roots (API-101) has been preserved in our herbarium for further collection and reference.

**Preparation of extracts:**

The roots were washed thoroughly, dried under a shade and pulverized. The coarse powder was extracted with ethanol (80 %) using a soxhlet apparatus to obtain *Andropogon muricatus* ethanolic extract (AMEE). The extracts were dried using a rotary vacuum evaporator and stored in a desiccators until further use.

**Phytochemical screening**

The ethanol extract of *Andropogon muricatus* were subjected to phytochemical tests [9, 10] to identify the nature of chemical constituents present in the plant material.

**Drugs and reagents**

Indomethacin (Sigma Lab, India), Carrageenan (Sigma-Aldrich), Brewer’s yeasts (Loba Chem, Mumbai) and Paracetamol (Glaxo Smith Kline) were used in the study. The chemicals used in present research programme were of analytical grade and procured from local sources.

**Animals**

Wister albino rats of either sex weighing about 150-200 gm and adult albino mice of either sex weighing 25-30 gm were employed for this study and were procured from institute animal house. The animals were maintained under standard laboratory conditions. Animals were allowed to take standard labora-
Acute toxicity Studies (LD<sub>50</sub>)

Acute toxicity study of ethanol extract from roots of <i>Andropogon muricatus</i> was performed in albino rats according to OECD guidelines<sup>[11]</sup>. The animals were kept fasting for overnight providing only water, after administration of ethanol extract orally at doses of 5– 2000 mg / kg. Animals were then allowed free access to food and water and observed a period of 48 hrs for signs of acute toxicity. The number of deaths within this period was recorded.

EXPERIMENTAL PROTOCOL

**Analgesic activity**

Acetic acid-induced writhing response in rats:

To evaluate the analgesic effects of the plant extract, the method described by Koster <i>et al</i>.<sup>[12]</sup> was used with slight modifications. Different groups of five rats each received orally normal saline solution (2ml/kg) (i.e. control), indomethacin (10mg/kg), or plant extract (100, 150, 250mg/kg). Thirty minutes later, 0.7% acetic acid (10ml/kg) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an anti-nociceptive.

**Antipyretic activity**

Brewer’s yeast induce hyperthermia in rats

Antipyretic activity was measured by slightly modifying the method described by Adams <i>et al</i>.<sup>[13]</sup>. Rats were fasted overnight with water <i>ad libitum</i> before the experiments. Pyrexia was induced by subcutaneously injecting 20% w/v brewer’s yeast suspension (10ml/kg) into the animal’s dorsum region. 17 hr after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7°C were used for experiments. AMEE (100,150 mg/kg and 250 mg/kg b.w.), Paracetamol (200mg/kg b.w.) or vehicle was administered orally and the temperature was measured at 0, 1, 3, and 7 hr after treatment.

The percentage of inhibition of abdominal contractions for the extract treated groups was compared with control group.

\[
\% \text{ inhibition} = \frac{\text{Control} - \text{test/Control}}{\text{Control}} \times 100
\]

**Anti-inflammatory activity**

Carrageenan induced hind paw edema in rats

Paw edema was produced in rats by carrageenan following the methods of Winter <i>et al</i>.<sup>[14]</sup> Male rats weighing 100–120 g were divided into groups of five animals. A volume of 0.05 ml of 1% carrageenan in normal saline solution (NSS) in 0.2M carbonate buffer was injected intra-dermally into the plantar side of the right hind paw of the rat. Test drugs and vehicle were given 1 h prior to carrageenan injection. Paw volumes were measured using a plethysmometer at 1, 3 and 5 hr after carrageenan, injection. Results obtained were compared with those obtained from their control groups, which received vehicle only.

The percentage of inhibition was calculated by using the formula,

\[
\% \text{ inhibition} = \frac{\text{Vc} - \text{Vs}}{\text{Vc}} \times 100
\]

Where, Vc= Average paw volume of control;
Vs = Average paw volume of test

**Statistical treatment**

The results were subjected to two way ANOVA followed by dunnet’s test. The data is deemed to be statistically significant if *p<0.05. (Significant) **p<0.01(highly significant)

**RESULTS**

**Phytochemical screening**

Preliminary phytochemical screening of ethanolic extract from roots of <i>Andropogon muricatus</i> showed positive results for saponins, flavonoids, tannins, alkaloids, and glycosides etc. (Table:1)

**Phytochemical Screening**

Table: 1Phytochemical screening

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Phytoconstituents</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

\(+ = \text{Positive}; - = \text{Negative}\)

**Acute toxicity test (LD<sub>50</sub>)**

Oral administration of graded doses (100, 150 and 250mg/kg p.o.) of the ethanolic extract of <i>Andropogon muricatus</i> to rats did not produce any significant changes in behaviour, breathing, cutaneous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 72hr of administering the extract to the animals.

**Analgesic activity**

Acetic acid-induced writhing in rats:

The ethanolic extract of <i>Andropogon muricatus</i> and indomethacin induced significant decrease in the number of writhes when compared to the control (Table 2). The extract at 250mg/kg and indomethacin at 10mg/kg exhibited higher anti-nociceptive power i.e. 63 and 70% respectively indicating that the extract has comparable antinociceptive than the reference drug used in this study.

**Analgesic activity**

Table: 2-Writhing Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>No. of writhes</th>
<th>Inhibition of Writhing Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml</td>
<td>45.8±1.86</td>
<td>……………</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>13.57±1.10**</td>
<td>70</td>
</tr>
<tr>
<td>AMEE 100</td>
<td>33.87±1.82*</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>AMEE 150</td>
<td>23.03±1.53*</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>AMEE 250</td>
<td>17.13±1.46**</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{Values are expressed as mean ± S.E.M. (n = 5); * p= 0.05(significant) **p=0.01 (highly significant) vs. control. AMEE- Andropogon muricatus ethanolic extract}\)

**Brewer’s yeast-induced pyrexia in rats**

There was significant reduction in rectal temperature of rats by AMEE shown in Table 3.
Antipyretic activity

Table: 3 - Brewer's yeast induced pyrexia in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Rectal temperature in °C at time (hr.)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
<td>1 hr</td>
<td>3 hr</td>
</tr>
<tr>
<td>Control</td>
<td>5ml</td>
<td>37.12±0.21</td>
<td>37.30±0.11</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>200</td>
<td>37.30±0.10</td>
<td>35.42±0.03**</td>
</tr>
<tr>
<td>AMEE 100</td>
<td>37.88±0.42</td>
<td>36.82±0.18*</td>
<td>36.67±0.20*</td>
</tr>
<tr>
<td>AMEE 150</td>
<td>37.53±0.12</td>
<td>36.10±0.10*</td>
<td>35.45±0.3*</td>
</tr>
<tr>
<td>AMEE 250</td>
<td>37.43±0.32</td>
<td>36.00±0.10*</td>
<td>35.95±0.06**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n = 5); * p = 0.05 (significant); ** p=0.01 (highly significant) vs. control. AMEE: Andropogon muricatus ethanolic extract.

Carrageenan-induced paw oedema:

The ethanolic extract of Andropogon muricatus roots (AMEE) significantly decreased the paw edema induced by carrageenan in rats at all dose level (100, 150 and 250) but reduction at the dose of 250 mg/kg was comparable to indomethacin (10 mg/kg) Table 4.

Anti-inflammatory activity

Table: 4 Carrageenan –induced paw edema

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Paw volume increase (ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1hr</td>
<td>3hr</td>
<td>5hr</td>
</tr>
<tr>
<td>Control</td>
<td>5ml</td>
<td>0.37±0.01</td>
<td>0.79±0.03</td>
</tr>
<tr>
<td>Indomet</td>
<td>10</td>
<td>0.13±0.12**</td>
<td>0.20±0.06*</td>
</tr>
<tr>
<td>AMEE 100</td>
<td>0.32±0.05</td>
<td>0.39±0.01*</td>
<td>0.41±0.01*</td>
</tr>
<tr>
<td>AMEE 150</td>
<td>0.28±0.02</td>
<td>0.35±0.01*</td>
<td>0.40±0.04**</td>
</tr>
<tr>
<td>AMEE 250</td>
<td>0.17±0.01*</td>
<td>0.31±0.02**</td>
<td>0.31±0.02**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n = 5); * p = 0.05 (significant); ** p=0.01 (highly significant) vs. control. AMEE: Andropogon muricatus ethanolic extract.

DISCUSSION

The data presented here suggests that the AMEE possesses analgesic antipyrctic and anti-inflammatory activities. The extract at the doses tested was shown to possess anti-nociceptive activity evident by the abdominal constriction response induced by acetic acid to evaluate peripherally acting analgesics [19]. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal contractions response [18]. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2a in peritoneal fluids [17], as well as lipoxygenase products [18]. The significant reduction in acetic acid-induced writhes by AMEE suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Carrageenan induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic. The first phase (1-2 hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin [19,20]. The results of this study indicate that the ethanolic extract of Andropogon muricatus significantly reduced carrageenan induced paw oedema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis.

Usually most anti-inflammatory and analgesic drugs possess anti- pyretic activity. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus [21]. Therefore, the antipyretic activity ethanolic extract of Andropogon muricatus is probably by inhibition of prostaglandin synthesis in hypothalamus. The analgesic, antipyretic and anti-inflammatory activities of ethanolic extract may be due to the presence of alkaloids, sterols and flavonoids.

The results of the present study indicate the analgesic, antipyretic and anti-inflammatory activities of the roots of Andropogon muricatus. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

ACKNOWLEDGEMENT

Authors thank Mr. Vivek Yadav, Chairmen and Dr. U. K.Sharma, Director, Sir Madanlal Group of Institution, Etawah, for providing necessary facilities for the present research work

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