Antinociceptive and Relaxant Effects of Aqueous Extract of the Aerial Part of Ziziphora clinopodioides

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ABSTRACT

Background and Aim: Plants from Iranian biomes, such as Ziziphora clinopodioides has been used as natural medicines by local populations in the treatment of several diseases such as asthma and stomachache and hypertension. In this study we investigated the antinociceptive and spasmodic effects of aerial parts of aqueous extract of Ziziphora clinopodioides (APAEZC).

Methods: The extract was injected to male mice 15 min before the onset of experiments intraperitoneally. Writhing and hot-plate tests were applied to study the analgesic effect of APAEZC and compared with that of diclofenac sodium (30 mg/kg, i.p.) or morphine (8 mg/kg, i.p). For evaluating spasmodic effects of the extract, 5 cm of smooth muscle from ileum of rats were removed and set up for recording the isotonic contractions. Control contractions were obtained by adding acetylcholine (10^4M) to each tissue preparation and the spasmodic action of different APAEZC concentrations (0.1, 0.2 and 0.3%) on maximum contraction induced by acetylcholine were evaluated 5 min later. Results: APAEZC exhibited a significant (P<0.05) antinociceptive effect in both chronic and acute pain in all doses in mice and also showed a significant spasmodic effect at dose 0.2 (P<0.05) and dose 0.3% (P<0.01).

Conclusion: The findings of this study indicated that APAEZC have analgesic and relaxant effects. These results support the traditional claim of Ziziphora clinopodioides as an antinociceptive and antispasmodic therapeutic agent.

Key words: Ziziphora clinopodioides, Pain, Spasmodic, Hot plate, Writhing test.

INTRODUCTION

In humans, pain has injurious effects on sleep, cognitive abilities such as learning,[1] attention,[2] and the capacity for work.[3] Pain is caused following tissue or peripheral nerve damage or injuries to different parts of the central nervous system in humans and animals. Although there are different effective analgesic drugs and widely used, but these drugs have side-effects and making theoretical use problematic.[4] In the recent years, tendency to herbal medicine has been increased and people have recognized and used of many cultivated or wild plants. Plant products have less toxic effects than synthetic ones and are a good source for novel therapeutic agents.[5,6] Ziziphora clinopodioides (Mountains’ Kakoty) from the genus of Ziziphora and family of Lamiaceae is a traditional medicinal plant, which is a semi-perennial shrub-like plant that grows on low hills, grassland and arid slopes and is widely distributed in China, Mongolia, Turkey, Iran, Kazakhstan and Kyrgyzstan.[6] It is mainly used for the treatment of heart disease, high blood pressure, asthma hyperhidrosis, palpitation insomnia, edema, cough, bronchitis, lung abscess and other diseases in these traditional medicines.[7] In Iranian traditional medicine, it is used as sedative, gastric pain, stomachache carminative and the dried aerial parts of this plant are used in cooking and other dairy products as additive and giving aroma to food.[8] Different studies showed that the main constituents of the plant essential oil were pulegone, neomenthol, menthone, 1, 8-cineole, thymol, carvacrol, Linalool and piperitone.[9] New studies demonstrated that Ziziphora clinopodioides has antibacterial and antioxidant activities.[10] In a research, Ghafari et al. showed the anti-inflammatory effect of Ziziphora clinopodioides.[10] They found that the essential oil of this plant inhibits acetif acid toxic reactions in the rats bowl and this is due to the inhibition of oxidative stress of cellular texture. The present study was performed for examining the folkloric claims of traditional regarding the analgesic and relaxant effects of this medicinal plant.

MATERIALS AND METHODS

Extract Preparation

The plant (aerial parts) used for the present study was collected locally in the Ghochan (in north Khorasan province-Iran). Plant was identified by Dr. Abbas Zarezadeh in the Department of Botany in Agricultural Research Center (Yazd Iran). The powder (400 g) was extracted exhaustively in a Soxhlet apparatus with distilled water (180 ml). The extract was filtered using filter paper and then

concentrated in vacuo at 40°C using a Rotary evaporator. The residues obtained were stored until used.

**Animals**

For evaluation of acute and chronic antinociceptive effect of extract, 60 Swiss male albino mice (25-30g) were used. We also used 20 rats for determination of spasmolytic effect of the extract. All animals were normal and healthy and kept in automatically controlled temperature conditions (23 ± 2°C), in 12 h light-dark cycles, with standard food for rodents and tap water "ad libitum". 48 hr prior to the experiments, the animals were kept in the laboratory and 8 hr prior to the experiment, they were deprived of food. All the experimental animals were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. All experimental protocols were approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences.

**Drugs and Treatments**

The drugs used were acetylcholine iodide (from the Sigma Chemical Company, St. Louis, USA), stored at -4°C and diluted to the desired concentrations in 0.9% saline just before use. Glucose, NaCl, KCl, CaCl$_2$, H$_2$O, MgCl$_2$, NaHCO$_3$, NaH$_2$PO$_4$ was acquired from Merck KGA, Darmstadt, Germany. The standard drug morphine sulphate (8 mg/kg) used in hot plate test and diclofenac sodium (30 mg/kg) in writhing and licking tests were administered intraperitoneally 15 min before the experiments while the animals in control group received vehicle orally (normal saline). APAEZC was intraperitoneally administered to the test animals 30 min before the experiments at the doses of 100, 200 and 300 mg/kg body weight in both the chemical-induced and heat-induced pain models.

**Hot-plate Test**

The hot-plate test was carried out according to the method previously described.\[1\] Briefly, before the initial of experiment, mice were habituated to a Plexiglas cylinder for 5 min. In these experiments, the hot-plate apparatus was maintained at 54±0.1°C. Animals were placed into an acrylic cylinder (20 cm in diameter) on the heated surface and the time (in seconds) between placement and licking of their hind paws or jumping (whichever occurred first), was recorded as the response latency (reaction time). Each mouse served as its own control. A 45-s cut-off was used to prevent tissue damage. After baseline behavior tests, mice were immediately administered with drugs. The animals were intraperitoneally (i.p.) received vehicle (saline, 10 ml/kg), the extract (100, 200 and 300 mg/kg) and morphine (8mg/kg) 15 min before the test. The reaction time of each mouse was again evaluated at 15, 30, 45 and 60 min after treatment. Antinociception was expressed by mean percentage maximum possible effect (%MPE). The %MPE was calculated using the following formula:

\[
\text{% MPE} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off} - \text{Control latency}} \times 100
\]

**Acetic Acid-induced Writhing Test**

The abdominal constriction test described by Bagheri et al.\[12\] was used to measure the analgesic activity of the extract. Male mice pre-treated with extract (100, 200 and 300mg/kg) or diclofenac (30 mg/kg). 15 min later, all mice were treated with intraperitoneal injection of 0.6% acetic acid to cause a typical stretching response. Five min after acetic acid injection, mice were kept in individual cages and writhing or stretching of each mouse was counted for a period of 30 min by a blinded individual. The analgesic effect was measured by calculating the mean reduction in the number of abdominal constrictions for each drug as compared to saline control. Percentage inhibition of writhing was calculated by using the following formula:

\[
\text{% Inhibition} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean number of writhes (control)}} \times 100
\]

**Assessment of Spasmolytic Effect**

In this study 0.1, 0.2 and 0.3% extract was examined for their spasmolytic action. Experiments were performed as described previously reported by Bagheri et al.\[13\]. Briefly, adult male Albino rats were sacrificed by cervical dislocation. Segments of ileum (5 cm in length), were excised, flushed their contents and trimmed them of mesentery. The specimens were conserved in Tyrode’s solution until the onset of experimental procedure. The tissue sample was fixed at the bottom of the internal chamber of an organ bath containing 50 ml Tyrode’s solution in the axis of its longitudinal muscle and its opposite end was tightly tied to the isotonic transducer lever with a piece of trade, the chamber was maintained at 37°C and bubbled with 95% O$_2$ and 5% CO$_2$. Isotonic responses were recorded using an isotonic Transducer (T2) and an oscillograph recording system (the bioscience 400 Series Washington oscillograph). Then, it was allowed to stabilize for 15 min prior to the addition of drug and washed out in 30 min intervals by a fresh Tyrode’s solution. Control contractions were obtained by adding acetylcholine (10-4M) to each tissue preparation and the spasmolytic action of different extract (0.1, 0.2 and 0.3%) on maximum contraction induced by acetylcholine were evaluated 5 min later. Tissue specimens then were washed out by Tyrode’s solution and incubated in the separate sets of experimental solutions at least for 20 min.

**Statistical Analysis of Data**

Results were expressed as mean ± standard error (S.E.M) and statistically assessed by Two-way ANOVA, followed by post hoc Tukey’s test using Graph pad prism version 5. A value of $P<0.05$ was considered as significant.

**RESULTS**

**Hot-plate Test**

Latency responses for animals in different groups are shown in Table 1. The latencies for time 0 (base line latency) were statically analyzed by Two-way ANOVA, followed by post hoc Tukey’s test using Graph pad prism version 5. A value of $P<0.05$ was considered as significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>9.7±2.1</td>
<td>10.3±3.2</td>
<td>11.6±1.6</td>
<td>11.4±2.8</td>
<td>11.1±2.5</td>
</tr>
<tr>
<td>APAEZC</td>
<td>100</td>
<td>8.2±1.6</td>
<td>12.3±3.9</td>
<td>11.3±2.6</td>
<td>10.3±2.4</td>
<td>9.3±2.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8.6±0.9</td>
<td>14.1±3.2</td>
<td>11.9±2.5</td>
<td>10.9±2.8</td>
<td>9.1±3.1</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>9.1±1.5</td>
<td>15.7±3.4</td>
<td>13.6±2.9</td>
<td>11.7±1.7</td>
<td>10.4±2.1</td>
</tr>
<tr>
<td>Morphine</td>
<td>8</td>
<td>9.5±1.6</td>
<td>17.9±3.4</td>
<td>15.3±4.2</td>
<td>13.7±3.7</td>
<td>12.8±4.8</td>
</tr>
</tbody>
</table>

The analysis of latency times of different groups. The baseline time of each group is considered as control and other times compare with baseline. *$P<0.05$, **, $P<0.01$
effect was observed 15 min after drug administration. As shown in Figure 1, the %MPE at 15 min post treatment time point for all doses of extract was significantly greater than that of the control group.

**Acetic Acid-Induced Writhing Test**

The effect of extract on acetic acid induced writhing is presented in Table 2. All doses of the extract reduced acetic acid-induced writhing significantly. These results showed that with increasing the extract concentration, the number of writhing was decreased. The smallest dose of the extract showed relatively moderate analgesic activity with 34% (P<0.05) inhibition of acetic acid-induced writhing compared to controls and the extract 200 and 300 showed 39.5% and 58.4% inhibition and lower to that of the standard drugs.

**Spasmolytic Effect of Extract on Ileum Contraction Induced by Acetylcholine**

In this study, we investigated the relaxant effect of the extract in three concentrations (0.1%, 0.2% and 0.3%). Our findings showed that the extract in concentrations of 0.2 and 0.3 significantly reduced acetylcholine (10−4M) induced contractions the percentage of contraction of extract 200 and 300 showed 39.5% and 58.4% inhibition and lower to that of the standard drugs.

**DISCUSSION**

Our findings indicated that the extract reduces the number of acetic acid induced writhes in a dose-dependent manner comparable to that of the sodium diclofenac. The analgesic effect of the extract may be due either to its action on visceral receptors sensitive to acetic acid or to the inhibition of production/action of prostaglandins.[14] In the other hands, hot-plate test is one of the most common tests for monitoring the phasic nociceptive responses to a noxious heat stimulus of high intensity.[15]

**CONCLUSION**

In conclusion, the results of the present study showed an analgesic and relaxant effects for the extract. However, the exact mechanism of these effects remains to be clarified and further investigations including chronic.

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**Figure 1:** The percentage of Maximum Possible Effect (%MPE) of different treatments on acute pain inhibition at different time points in hot plate test (n=6).

**Figure 2:** Spasmolytic effect of APAEZC on acetylcholine-induced contractions in the isolated rat’s ileum. *indicates the significant difference (p<0.05) and ** indicates the significant difference (p<0.01) as compared to the control group.
toxicity studies and activity-guided fractionation must be performed in order to assess the active compounds of the extract.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We certify that no actual or potential conflict of interest related to this article exists.

ABBREVIATIONS

APAECZ: Aerial part of aqueous extract of Ziziphora clinopodioides.

REFERENCES
