Antinociceptive effect of the aqueous extract obtained from *Foeniculum vulgare* in mice: the role of histamine H₁ and H₂ receptors

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Summary

*Foeniculum vulgare* (fennel) has been widely used in traditional medicine for treatment of various diseases including pediatric colic. This study was designed to assess the antinociceptive effects of aqueous extract of *F. vulgare* on visceral pain and possible involvement of opioidergic, serotonergic, adrenergic and histaminergic systems. The results of this study showed that aqueous extract of *F. vulgare* (50, 100 and 200 mg/kg, IP) induces antinociceptive effects (P<0.001) and that the pretreatment with chlorpheniramine and cimetidine significantly attenuate this effect (from 71.9% to 21.6%, P<0.001 and from 71.4% to 35.9%, P=0.003, respectively). Furthermore, chlorpheniramine and cimetidine significantly decreased onset of first abdominal writhing (latency) in comparison with extract (P<0.05), however naloxone, cyproheptadine and phentolamine had no effect on antinociception and the latency induced by *F. vulgare*. The ED₅₀ value for antinociceptive effects of extract was 87.6 mg/kg. These results suggest that antinociceptive effects of *F. vulgare* are partially mediated by histamine H₁ and H₂ receptors.

Key words: *Foeniculum vulgare*, Antinociception, Histamine receptor, Writhing test

Introduction

Visceral pain (e.g., angina, colic, dyspepsia, pancreatitis, appendicitis, dysmenorrhea) caused by the activation of nociceptors in viscera constitutes a large portion of clinically treated pain. Visceral tissue injury and inflammation can activate nociceptive primary afferent fibers, which results in central sensitization or hyperexcitability of nociceptive neurons in the spinal cord dorsal horn and its consequence “hyperalgesia” (Giamberardino, 1999). Finding newly appropriate analgesics for the treatment of visceral pain is a major challenge for the pharmacy researchers, especially in terms of drug efficacy and side effects. For example, opioids present good efficacy but their use is limited to severe conditions because of well-described side effects including dependency, euphoria, sedation and constipation (Kuiken et al., 2005). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Blumenthal, 2000). Thus, study of plant species that have traditionally been used as pain killers should still be seen as a logical research strategy in the search for new analgesic drugs (Rang et al., 1998).

*Foeniculum vulgare* (FV) is a well-known umbelliferous plant native to Southern Europe and the Mediterranean area. For centuries, FV fruits have been used as traditional herbal medicine in Europe and China (Albert-Puleo, 1980). Previous studies have demonstrated some of the pharmacological effects of FV such as anti-inflammatory, antioxidant activity, carminative, diuretic, lactation stimulant and as dressings for wounds (Choi and Hwang, 2004). In addition, it has been reported that FV could be used in pediatric colic and some respiratory disorders due to its anti-
The filtrate was concentrated in a rotary evaporator and the extract stored at 4°C until use (Prashant et al., 2011). The plant extract yield (% w/w) was assessed as 18.9%.

Animals
Male albino NMRI mice weighing 25-30 g purchased from Pasteur Institute of Tehran were used in experiments. The animals were housed in a light-controlled room under a 12:12 h light-dark cycle (light on at 7:00 am) at 22±2°C. Food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were used only once. To reduce the experimental variations, all experiments were performed during the light phase of the cycle (10:00-17:00). All experimental procedures followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann, 1983) and were carried out according to a protocol approved by the local Animal Ethics Committee.

Experimental procedures
Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test), a chemical visceral pain model (Miranda et al., 2006). Mice were injected intraperitoneal (IP) with 10 ml/kg of 0.6% acetic acid solution after 30 min of IP injection of the extract (at doses of 50, 100 and 200 mg/kg). Indomethacin (5 mg/kg) was dissolved in vehicle and administered IP as the reference drug (Kozak et al., 1998). The control group received vehicle as negative control. The total number of writhing following the IP injection of acetic acid was recorded during 30 min, started immediately after the acetic acid administration. Antinociceptive activity was expressed as inhibition percent of the writhes using the following ratio:

\[
\text{Writhing pain score (\%)} = \frac{(\text{control mean} - \text{treated mean}) \times 100}{\text{control mean}}
\]

Dose-response curve was obtained for FV extract using groups of eight animals for a single dose and groups of 16 control animals with no doses. Least-squares linear regression analysis of the log dose-response curve allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) for the extract (Delporte et al., 2007). Furthermore, to reveal the antinociceptive mechanisms of FV, the possible involvement of opioidergic, serotonergic, noradrenergic and histamine receptor antagonists on FV-induced antinociception was examined. Animals were pretreated IP with either saline, opioidergic receptor antagonist (naloxone, 2 mg/kg), serotonergic receptor antagonist (cyproheptadine, 4 mg/kg), α-adrenergic receptor antagonist (phentolamine, 20 mg/kg), histamine H_{1}-receptor antagonist (chlorpheniramine, 10 mg/kg) or histamine H_{2}-receptor antagonist (cimetidine, 10 mg/kg), 15 min before the IP administration of vehicle or the most effective dose of FV (200 mg/kg). Mice were injected IP with 0.6% acetic acid after 30 min of the IP injection of the extract or vehicle and writhing test response was recorded during 30 min, started immediately after the acetic acid administration. Additionally, onset of the first abdominal writhing was recorded as latency. The time and dose of antagonists used were chosen based on the preliminary studies and the literature review (Leza et al., 1990; Girard et al., 2004; Zendehdel and Babapour, 2010;
Zendehdel et al., 2011). All drugs were purchased from Sigma-Aldrich Company (USA) and dissolved in 5% dimethyl sulfoxide (DMSO). The control group only received vehicle.

To study the acute toxicity of the extract, mice were divided into control and test groups (n = 8). The first group served as normal control. FV extract was administered IP to different groups at increasing doses of 400, 800, 1600, 3200 and 6400 mg/kg. After the injection of extracts, mice were allowed to have food and water ad libitum and all animals were monitored for possible mortality cases and behavioral changes for 72 h (Lorke, 1983).

Statistical analysis
The data were presented as the mean values ±SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) with Tukey’s post-hoc test. P-values less than 0.05 were considered as significance.

Results
Evaluation of antinociceptive effects of F. vulgare in writhing test
The results of this study showed that aqueous extract of FV at doses of 50, 100 and 200 mg/kg induced a significant reduction in pain response when compared to control group (P<0.001). Also, indomethacin significantly decreased the number of writhing as a reference drug (P<0.001). Furthermore, extract groups and indomethacin significantly delayed the latency when compared to control group (P<0.05) (Table 1). The ED₅₀ for antinociceptive effects of FV was 87.6 mg/kg.

Effects of cyproheptadine, phen tolamine and naloxone on the antinociceptive action of F. vulgare
Our data showed that the aqueous extract of FV (200 mg/kg) induced a significant reduction in pain response when compared to control group (P<0.001) while pretreatment with cyproheptadine, phentolamine and naloxone had no effects on the antinociceptive properties induced by the extract. Furthermore, FV significantly increased onset of first abdominal writhing compared with control group but none of the drugs had any effects on the latency time induced by the extract (Tables 2, 3 and 4).

Effects of chlorpheniramine and cimetidine on the antinociceptive action of F. vulgare
Intraperitoneal injection of FV extract (200 mg/kg) induced a significant reduction in pain response when compared to control group (P<0.001). Pretreatment with chlorpheniramine and cimetidine significantly attenuated the antinociceptive effects of the extract (from 71.9% to 21.6%, P<0.001 and from 71.4% to 35.9%, P=0.003, respectively). Furthermore, chlorpheniramine and cimetidine significantly attenuated latency time induced by the extract (Tables 5 and 6).

Acute toxicity
FV extract at doses of 400-6400 mg/kg given IP to the mice had no effects on the mice behavioral responses and had no mortalities during the monitoring period of 72 h after the administration. Therefore, it can be assumed that FV extract has a low toxicity profile.

Table 1: Effect of the aqueous extract of FV in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>153 ± 7</td>
<td>92.5 ± 2.75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>50</td>
<td>202 ± 15</td>
<td>47.5 ± 2.39</td>
<td>48.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>FV</td>
<td>100</td>
<td>220 ± 24</td>
<td>34.5 ± 3.68</td>
<td>62.7</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>238 ± 15</td>
<td>27.1 ± 2.68</td>
<td>70.7</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>213 ± 17</td>
<td>52.3 ± 1.83</td>
<td>43.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group
Table 2: Effect of cyproheptadine on FV-induced antinociception in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>160 ± 12</td>
<td>88.5 ± 4.75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>236 ± 24</td>
<td>30.8 ± 5.58</td>
<td>65.1</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>4</td>
<td>157 ± 15</td>
<td>91 ± 3.46</td>
<td>—</td>
<td>&gt;0.05 vs. control</td>
</tr>
<tr>
<td>Cyproheptadine + FV</td>
<td>4 + 200</td>
<td>244 ± 29</td>
<td>28 ± 4.11</td>
<td>68.3</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group

Table 3: Effect of phentolamine on FV-induced antinociception in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>155 ± 21</td>
<td>86.5 ± 7.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>244 ± 35</td>
<td>27.5 ± 3.9</td>
<td>68.2</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>2</td>
<td>163 ± 29</td>
<td>94 ± 11</td>
<td>—</td>
<td>&gt;0.05 vs. control</td>
</tr>
<tr>
<td>Phentolamine + FV</td>
<td>2 + 200</td>
<td>236 ± 38</td>
<td>29 ± 4</td>
<td>66.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group

Table 4: Effect of naloxone on FV-induced antinociception in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>159 ± 71</td>
<td>90.5 ± 2.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>240 ± 24</td>
<td>29.5 ± 3.68</td>
<td>67.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Naloxone</td>
<td>2</td>
<td>166 ± 43</td>
<td>89 ± 1.43</td>
<td>—</td>
<td>&gt;0.05 vs. control</td>
</tr>
<tr>
<td>Naloxone + FV</td>
<td>2 + 200</td>
<td>229 ± 14</td>
<td>32.17 ± 3.55</td>
<td>64.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group

Table 5: Effect of chlorpheniramine on FV-induced antinociception in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>145 ± 17</td>
<td>91 ± 6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>229 ± 34</td>
<td>25.5 ± 2.74</td>
<td>71.9</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>10</td>
<td>160 ± 16</td>
<td>87.67 ± 1.83</td>
<td>—</td>
<td>&gt;0.05 vs. control</td>
</tr>
<tr>
<td>Chlorpheniramine + FV</td>
<td>10 + 200</td>
<td>170 ± 20</td>
<td>71.33 ± 3.65</td>
<td>21.6</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group

Table 6: Effect of cimetidine on FV-induced antinociception in acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Latency (sec)</th>
<th>Writhing count (Mean±SEM)</th>
<th>Inhibition (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>163 ± 20</td>
<td>94.5 ± 8.75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FV</td>
<td>200</td>
<td>244 ± 24</td>
<td>27 ± 3.68</td>
<td>71.4</td>
<td>&lt;0.001 vs. control</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>10</td>
<td>152 ± 82</td>
<td>90.17 ± 2.27</td>
<td>—</td>
<td>&gt;0.05 vs. control</td>
</tr>
<tr>
<td>Cimetidine + FV</td>
<td>10 + 200</td>
<td>181 ± 23</td>
<td>60.5 ± 7.08</td>
<td>35.9</td>
<td>&lt;0.001 vs. control</td>
</tr>
</tbody>
</table>

Vehicle is 5% DMSO: control, FV: Foeniculum vulgare, and n = 8 for each group

Discussion

In the writhing test, acetic acid activates peripheral nociceptors on the sensory nerve fibers by the release of proinflammatory substances (Satyanarayana et al., 2004).
Visceral hyperalgesia is believed to arise as a consequence of a lowering in the threshold of “high threshold” receptors, activation of previously unresponsive receptors (peripheral sensitization) and subsequent neuroplastic changes in the central nervous system (CNS), in terms of increased central neuronal activity and excitability (central sensitization). These amplify the effects of pain-related stimuli coming from the affected visceral organs (Giamberardino, 1999).

The current study showed that the extract caused a significant and dose-dependent reduction of the nociception induced by acetic acid in writhing test in mice. This is the first report on the antinociceptive effect of FV in a visceral pain model. The findings are in agreement with the findings of Choi and Hwang (2004), providing evidence that oral administration of FV has an antinociceptive effect in thermal nociception test and that the effect of the plant extract is due to components such as anethole and flavonoids (quercetin and isoquercitrin). Previous studies have proved that anethole and flavonoids possess significant antioxidant, antinociceptive, antiinflammatory and gastroprotector activities in experimental models (Filho et al., 2008). In this study, indomethacin was used as a positive control. Drugs such as nonsteroidal anti-inflammatory drugs (indomethacin) attenuate the pain by the inhibition of cyclooxygenase in arachidonic acid pathways (Levine and Taiwo, 1994). Chanh et al. (1986) showed that isoquercitrin inhibits both the biosynthesis and the release of prostaglandin-like substances. Thus, the present experimental findings suggest that FV extract probably exerts its anti-inflammatory and antinociceptive effects primarily by inhibiting the release, synthesis and/or production of inflammatory cytokines and mediators, including prostaglandins and polypeptide kinins.

In the current study, pretreatment with chlorpheniramine and cimetidine significantly attenuated the extract-induced antinociception while naloxone, cyproheptadine and phenotamine had no effects. These results are consistent with our previous findings that reported H₁ and H₂ blockers antagonize the antinociceptive effect of Teucrium polium in mouse writhing test (Zendehdel et al., 2011). The involvement of histamine in inflammatory pain of chemicals (e.g. formalin-induced) is well documented. Parada et al. (2001) showed that the second phase of formalin response could be reduced by the inhibitory effect of sodium cromoglycate on histamine release. Olsen et al. (2002) reported a similar effect after pre-treatment with H₁-receptor antagonists. Peripheral histamine specifically activates and sensitizes itch-specific nociceptive C fibers (Schmelz et al., 1997), while it has emerged that central histamine plays an important role in antinociception (Robertson et al., 1988). The differences between findings in histamine and its antagonist activity are possibly associated with the type of experiment applied, species properties, the affected site by histamine and behavioral tests used in nociception studies. Briefly, central injection of histamine shows an analgesic effect in several paradigms including the tail-flick and hot-plate tests (Malmberg et al., 1994; Thoburn et al., 1994). Previously, evidence has demonstrated that systemic or central injection of histamine produces antinociception, which suggest an important role in the regulation of antinociception (Chung et al., 1984). Furthermore, it has been reported that the blockade of H₁ and H₂ receptors attenuate the antinociception induced by nefopam, decursinol and restraint (Girard et al., 2004). Both H₁ and H₂ receptor antagonists have been shown to block histamine-induced antinociception when applied intracerebroventricularly or into the periaqueductal gray (Thoburn et al., 1994). The broad functional overlap, as well as striking anatomical and molecular specificities characterizes these distinct sensations (Ikoma et al., 2006). Most convincing seems to be the evidence implicating histamine H₂ receptors in the periaqueductal gray in histamine mediated antinociception (Thoburn et al., 1994). However, H₁ receptors may be important in other areas such as the spinal cord (Suh et al., 1996). The current study results suggest that the extract may be effective on the inflammatory pain even at the CNS level because quercetin, as a component of FV,
can permeate across the blood-brain barrier (Ren et al., 2010). Filho et al. (1996) reported that quercetin as a flavonoid possesses an antinociceptive effect by acting through a central mechanism; therefore, flavonoids may be involved in the central and peripheral antinociceptive effect of the extract. In conclusion, the present study suggests that antinociceptive effects of FV are partially mediated by histamine H1 and H2 receptors; however, it is also possible that other mechanisms influence its antinociceptive effects.

**Acknowledgement**

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**References**


Delporte, C; Backhouse, N; Inostroza, V; Aguirre, MC; Peredo, N; Silva, X; Negrete, R and Miranda, HF (2007). Analgesic activity of *Ugni molinae* (murtilla) in mice models of acute pain. J. Ethnopharmacol., 112: 162-165.


Filho, VC; Santos, AS; Decampos, ROP; Miguel, OG; Yunes, RA; Ferrari, F; Messana, I and Calixto, JB (1996). Chemical and pharmacological studies of *Phyllatus caroliniensis* in mice. J. Pharm. Pharmacol., 48: 1231-1236.


Prashant, T; Bimlesh, K; Manoj, K; Mandeep, K; Jiban, D and Pardeep, S (2011). Comparative antihelmintic activity of aqueous and