Study of antinociceptive and anti-inflammatory activities of certain Iranian medicinal plants

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Abstract

Aim: Four medicinal plants of Trigonella foenum-graecum, Zhumeria majdae, Achillea wilhelmi and Viola tricolor are traditionally used in Iran as analgesic and for treatment of inflammatory disorders. At the present study, the antinociceptive and anti-inflammatory effects of these plants have been studied.

Methods: The antinociceptive and anti-inflammatory activity of methanol extracts of tested plants were evaluated using hot-plate and carrageenan-induced edema methods respectively. The plant extracts were studied by i.p administration at three doses of 100, 200 and 400mg/kg.

Results: In the hot-plate test, the extracts of T. foenum-graecum (100 mg/kg) and Z. majdae (200 and 400mg/kg) significantly increased the tolerance to pain in female albino mice in comparison to control. The administration of T. foenum-graecum at doses of 100 and 200mg/kg and V. tricolor (400mg/kg) significantly reduced the paw edema in male rat which measured in all the times of observation after carrageenan administration in comparison to control and reference (Ibuprofen, 400mg/kg).

Conclusions: The present work comparatively demonstrated considerable antinociceptive and anti-inflammatory effect of all of the tested plants especially T. foenum-graecum. The results here confirm traditional uses of T. foenum-graecum both as analgesic or anti inflammatory agents.

INTRODUCTION

In Iranian traditional medicine, many of the plants have been suggested as anti-inflammatory and to treat bruise, pain and rheumatism [1, 2]. Regarding wide range of undesired side effects of available anti-inflammatory and analgesic drugs, many studies are being directed to find compounds with lesser side effects. The present study was conducted to verify and compare the antinociceptive and anti-inflammatory activity of methanol extracts of four most common used medicinal plants.

Fenugreek (Trigonella foenum-graecum L.; Fabaceae) is a plant whose seeds and leaves have several pharmacological effects such as hypoglycemia, hypcholesterolemia, antioxidant and appetite stimulation [3-6]. This plant under the name of “Shanbalileh”, in Iranian traditional medicine has been used as hypoglycemic and antirheumatism [2].

Mohrkhosh (Zhumeria majdae Resh. f.& Wendelbo : Lamiaeaceae) has a limited geographical range in bandar-abbas in Hormozgan province in the southeastern of Iran [7]. Plant leaves have been used for many years as
treatment of stomachaches, antiseptic, carminative especially in infants and for treatment of painful menstruation [8]. Antibacterial and antioxidant activity of the essential oil of Z.majdae has been previously reported [9].

Achillea, a genus of Asteraceae family, has long been used in traditional medicine. Top flowering of the plant is used as antiflatulence, spasmyloytic, antinociceptive and diuretic. Aerial parts of A. wilhelmsii K.Koch contains volatile oils, flavonoids, terpenoids, alkaloids, saponins and sesquiterpen lactones [10]. We have reported immune-stimulating effects of this plant [11].

Viola tricolor L., Violaceae commonly known as violet is used in folk medicine for various purposes such as anti inflammatory agent especially in common cold; it is externally used for mild seborrheic complains and various skin conditions such as eczema [1]. Modern pharmacological studies have demonstrated that violet has antimicrobial and cytotoxic activity and is useful for treatment of mild-to-moderate atopic dermatitis [12]. Chemical studies on V. tricolor have shown the presence of flavonoids, anthocyanins, coumarins, tannins, saponins, carotenoids and phenolic acids [13].

**MATERIALS AND METHODS**

**Plant material**

The dried seeds of the fenugreek were prepared from the local market and the other plants were gathered in June-2006 from Kerman and scientifically approved by Dr. Mirtajaldini, Department of Botany, Bahonar University, Kerman, Iran. A voucher specimen of the gathered plants was deposited in the herbarium of Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. Information of the tested plants has given in the Table 1.

**Preparation of plant extract**

500g of dried plant was extracted with methanol using percolation method [14]. The obtained extracts were dried under vacuum to give viscose mass. The yield of the extraction is given in Table 1. The extracts were suspended in 0.1% DMSO/normal saline (v/v) for the in vivo tests.

**Drugs and reagents**

Ibuprofen tablet (Iran, Hakim Pharmaceutical Co. Ltd., Iran); Dexamethasone ampule (Sina-Daru Pharmaceutical Co. Ltd., Iran); Carboxymethylcellulose-sodium (CMC-Na, Iran); Carrageenan (type I, Sigma Chemical Co., St. Louis, MO, U.S.A.) and Hot plate (LE-7406, PANLAB, Spain) were used in the present study.

**Animals**

Female albino mice weighing 20–25 g and male rats weighing 250-300 g, were employed for antinociceptive and anti inflammatory tests respectively. The animals were obtained from the Neuroscience Research Center, Kerman University of Medical Sciences. They were housed in a room temperature 22 ± 2 °C at 12-h light:12-h dark cycle and had free access to food and water except during the time of experiments. Groups of 6 animals each were used in all tests. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for once experiment only. This study complied with current ethical regulations on animal research (National Research Council of USA, 1996) and related rules of our school and all animals used in the experiment received humane care (NO, EC/KNRC/85-2).

**Table 1. Extraction results and additional information of 4 tested plants used for studying their antinociceptive and anti inflammatory activities**

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family</th>
<th>Common/folk name</th>
<th>Part used</th>
<th>Voucher No.</th>
<th>Yield of extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trigonella foenum-graecum L.</td>
<td>Fabaceae</td>
<td>Fenugreek/ Shanbalileh</td>
<td>Seeds</td>
<td>-</td>
<td>23.5%</td>
</tr>
<tr>
<td>Zhumeria majdae Resh. f.&amp; Wendelbo</td>
<td>Lamiaceae</td>
<td>- / Mohrkhosh</td>
<td>Aerial parts</td>
<td>KF1155</td>
<td>19.2</td>
</tr>
<tr>
<td>Achillea wilhelmsii C.Koch</td>
<td>Asteraceae</td>
<td>Santalin yarrow/ Boommadaran</td>
<td>Aerial parts</td>
<td>KF1387</td>
<td>21.4</td>
</tr>
<tr>
<td>Viola tricolor L.</td>
<td>Violaceae</td>
<td>Violet/ Banafsheh</td>
<td>flowers</td>
<td>KF1382</td>
<td>19.9</td>
</tr>
</tbody>
</table>
**Hot plate latency assay in mice**

In the hot-plate test, animals were habituated twice to the hot-plate in advance. The temperature of a metal surface was set at 55 ± 0.5 °C. The time that elapsed until the occurrence of either a hind paw licking or a jump off the surface was recorded as the hot-plate latency. After the determination of baseline response latencies, hot-plate latencies were determined at 30, 60 and 90 min after i.p administration of test drugs in comparison to control. A latency period of 30 s was defined as complete analgesia as cut off time to prevent damage to mice. Mice with baseline latencies of <5 s or >30 s were eliminated from the study (15, 16).

**Carrageenan-induced rat paw edema**

Edema was induced by injecting 0.1 ml of 0.5% carrageenan subcutaneously into the sub-plantar region of the left hind paw of Wistar rats. Equal volume of solvent was injected to right hind paw (16). Different doses of the extracts (100, 200 and 400 mg/kg) were administered i.p to left hind paw. The reference group received Ibuprofen (400 mg/kg, i.p) and the control group received an equal volume of solvent. All drugs were administrated just after injection of carrageenan (T=0). The volume of hind paws were measured with a plethysmometer at 0 h (just after carrageenan injection), 1, 2, 3 and 4 h. Difference of two paws volume was considered as carrageenan-induced edema.

**Statistical Analysis**

Results are expressed as mean ± S.E.M. Differences between the control and treated groups were tested for significance using a one-way analysis of variance (ANOVA), followed by Tukey’s t-test.

**RESULTS**

The yield of extraction and the other information of the plants are given in Table 1. The results of hot plate test showed that the extract of *T. foenum graecum* (dose of 100mg/kg) in all the test times showed significant antinociceptive effect compared to control (*p*<0.001). The extract of *Z. majdae* has shown the most antinociceptive effect at dose of 200mg/kg (30 and 90 min) and 400mg/kg (30, 60 and 90 min), in comparison to control (*p*<0.05) (Table 2). The results obtained from carrageenan-induced paw edema show the mean volume difference between right and left foot paws before and 4h. after i.p administration of normal saline and carrageenan respectively (Figure 1). As shown in this figure the index of carrageenan induced edema is completely different from the ones for normal saline. Methanol extract of *T. foenum graecum* seeds at the dose of 100 and 200mg/kg has inhibited paw edema at 1, 2 and 4h after carrageenan administration. As shown in Figure 2, these extracts notably inhibited paw edema in rat when given i.p at 4 h after carrageenan injection. At the dose of 400mg/kg, methanol extract of *V. tricolor* has shown the most inhibition of paw edema at 1, 2 and 4h after carrageenan administration. As shown in Figure 2, this extract notably inhibited paw edema in rat when given i.p at 4 h after carrageenan injection. None of these three active extracts showed significant activity in comparison to ibuprofen (*p*>0.05).

**DISCUSSION**

Pain and inflammation are induced in various clinical disorders like arthritis, cancer and vascular disease which disaster the patient. Some of medicinal plants have been traditionally used either for pain relief or as anti inflammatory agents. Our results of the antinociception in hot-plate test show that among the tested plants, the highest efficacy was exhibited by the methanol extracts of *T. foenum-graecum* (100mg/kg) and *Z. majdae* (200 and 400mg/kg) which significantly raised the pain threshold in comparison to control. The antinociception effect has occurred in different experimental times for each of the plants that would be due to the various analgesic metabolites of the plants which reached to maximum in different times [17]. *T. foenum-graecum* at the dose of 100mg/kg showed the
most latency in licking and jumping of mice paw (Table 2). The hot-plate method is known as a test for detecting of opioids as well as the other CNS depressants which can respond to thermal stimuli. It is suggested that the extracts which have exhibited anti nociception effect in this method, might act through central mechanisms [18]. A report shows that in the naloxone pre-treatment animals, the antinociception of T. foenum-graecum seeds has been partially antagonized, it seems that some of endogenous opioids are involved in antinociception effect of the plant seeds [19]. The seeds of T. foenum-graecum contain saponins, alkaloids, flavonoids and salicylate [18, 20, 21], which may be responsible of the antinociception effect of the plant. Our findings also show that the methanol extract of Z. majdae has increased significantly the tolerance to pain at the dose of 400mg/kg. This effect may derive from the contribution of active components composing the plant such as linalool. The essential oil of this plant contains linalool and camphor as main compounds (53 and 26% respectively)[9]. Linalool produces antinociception in two experimental models of pain [22]. As Hosseinzadeh et al., reports, the aqueous extract of the plant has shown antinociceptive and anti inflammatory activity at the doses of 1g and 800mg/k respectively [23]. Our findings show that the antinociceptive effect of methanol extract was more potent than that effect observed with aqueous extract of the plant which may be due to the presence of some monoterpenes like linalool in the methanol extract that can potentiate the antinociception of the extract. Linalool is a monoterpenic which is sparingly soluble in aqueous solvent. The methanol extract of A. wilhelmsii failed to prolong latency time compared with controls in mice hot plate test. Carrageenan-induced paw edema in rat has known as a sensitive method for studying of non steroidal anti-inflammatory agents and show a biphasic event which is attributed to the different mediators. At the first (about 2 h after carrageenan injection), hyperemia mainly induces because of the release of histamine and serotonin, whereas prostaglandins and bradykinin potentiates the second phase of edema by mobilization of leukocytes [24]. Our results showed that among the tested plants, the extract of T. foenum-graecum at the doses of 100 and 200mg/kg and V. tricolor at the dose of 400mg/kg significantly reduced the paw edema throughout the entire period of observation in comparison to control (p<0.05) without significant difference with Ibuprofen (Figure 2). Presence of saponins and flavonoids as the major compounds in T. foenum-graecum and V. tricolor [13, 20] can approximately explain the anti inflammatory activity of these plants. A number of flavonoids, including quercetin, are able to inhibit both the cyclooxygenase and lipoxygenase pathways at relatively high concentrations. Flavonoids can also inhibit the nitric oxide synthase [25]. Thakur et al, have reported no significant anti inflammatory activity of the aqueous extract of T. foenum-graecum seeds [26], whereas in another report the ethanol extract of the plant obtained in suxhelet method has exerted significant anti inflammatory effect. The latter report shows that the ethanol extract of T. foenum-graecum

**Table 2. Anti nociceptive effect of tested plant extract in hot-plate test at different doses (100, 200 and 400 mg/kg). Hot-plate latencies were determined at 0, 30, 60 and 90 min after i.p administration of test drugs to animal in comparison to control (n=6).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency time (sec) in definite intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td><strong>T. foenum-graecum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>9.3 ± 0.2</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>200</td>
<td>6.1 ± 0.2</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>400</td>
<td>5.9 ± 0.1</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td><strong>A. wilhelmsii</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5.5 ± 0.1</td>
<td>5.7 ± 0.07</td>
</tr>
<tr>
<td>400</td>
<td>3.7 ± 0.04</td>
<td>3.9 ± 0.02</td>
</tr>
<tr>
<td><strong>V. tricolor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5.8 ± 0.1</td>
<td>4.3 ± 0.05</td>
</tr>
<tr>
<td>200</td>
<td>6 ± 0.1</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>400</td>
<td>5 ± 0.1</td>
<td>3.6 ± 0.05</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5 ± 0.1</td>
<td>4.1 ± 0.08</td>
</tr>
</tbody>
</table>

*p < 0.001, vs. the control group.*
seeds increases the peritoneal exudates as well as macrophage cell counts which indicates that this plant probably acts via activation of macrophages (18). Gastroprotective effect reported for the seeds of this plant is a valuable factor regarding the gastrointestinal disturbance caused by non steroidal anti inflammatory (NSAIDs) [27].

The pharmacology of *V. tricolor* has been studies lesser than the other plants. The flowers of this plant are a rich source of many secondary metabolites with anti inflammatory effect such as saponins, flavonoids, salicylic acid, carboxylic phenolic acids (caffeic acid, coumaric acids) and mucilages and have been used in a wide variety of skin disease such as eczema, seborrhea, impetigo and acne [28]. Therefore, the results of this study not only provided partial experimental evidence for the therapeutic efficacy of *V. tricolor* in the treatment of skin inflammatory disease, but also would be beneficial to the future studies and exploitation of this plant. In conclusion, our results in this study not only provided partial experimental evidence (ANOVA). Significant differences with Ibuprofen group (p<0.05).

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