Antinociceptive effect of the aqueous extract obtained from *Foeniculum vulgare* in mice: the role of histamine H_1 and H_2 receptors

Zendehdel, M.[1*]; Taati, M.[2]; Amoozad, M.[1] and Hamidi, F.[1]

[1]Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Pathobiology, School of Veterinary Medicine, Lorestan University, Khorramabad, Iran

[*]**Correspondence:** M. Zendehdel, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: zendedel@ut.ac.ir

(Received 27 Dec 2010; revised version 24 Oct 2011; accepted 26 Nov 2011)

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 ED_{50} 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 H_1 and H_2 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-

spasmodic effects (Savino *et al*., 2005). Based on anti-spasmodic effects of FV, we examined the antinociceptive effect of the aqueous extract of FV using writhing test and investigated some possible mechanisms underlying this effect.

Materials and Methods

Preparation of crude extract

FV aerials parts were collected during the flowering period from Qazvin (Qazvin province, Iran). Voucher specimen was deposited and identified at the Herbarium (No. 84715) of the Division of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran. The crude aqueous extract of FV was prepared according to the standard method. One hundred grams of the powdered plant material was mixed with 500 ml of distilled water in a Soxhlet apparatus for 20-24 h. Then, 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±2ºC. 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:

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

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 ED_{50} for antinociceptive effects of FV was 87.6 mg/kg.

Effects of cyproheptadine, phentolamine 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

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

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

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

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

Treatment	Dose (mg/kg, IP)	Latency (sec)	Writhing count (Mean±SEM)	Inhibition (%)	P-value
Vehicle	10	155 ± 21	86.5 ± 7.8	—	—
FV	200	244 ± 35	27.5 ± 3.9	68.2	<0.001 vs. control
Phentolamine	20	163 ± 29	94 ± 11	—	>0.05 vs. control
Phentolamine + FV	20 + 200	236 ± 38	29 ± 4	66.4	<0.001 vs. control

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

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

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

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

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

Treatment	Dose (mg/kg, IP)	Latency (sec)	Writhing count (Mean±SEM)	Inhibition (%)	P-value
Vehicle	10	163 ± 20	94.5 ± 8.75	—	—
FV	200	244 ± 24	27 ± 3.68	71.4	<0.001 vs. control
Cimetidine	10	152 ± 82	90.17 ± 2.27	—	>0.05 vs. control
Cimetidine + FV	10 + 200	181 ± 23	60.5 ± 7.08	35.9	<0.001 vs. control P = 0.003 vs. FV

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 cycloxygenase 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 phentolamine had no effects. These results are consistent with our previous findings that reported H_1 and H_2 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 H_1-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 anti-nociception, which suggest an important role in the regulation of antinociception (Chung *et al*., 1984). Furthermore, it has been reported that the blockade of H_1 and H_2 receptors attenuate the antinociception induced by nefopam, decursinol and restraint (Girard *et al*., 2004). Both H_1 and H_2 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 H_2 receptors in the periaqueductal gray in histamine mediated antinoception (Thoburn *et al*., 1994). However, H_1 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 antinoceceptive effects of FV are partially mediated by histamine H_1 and H_2 receptors; however, it is also possible that other mechanisms influence its antinociceptive effects.

Acknowledgement

This research was supported by a grant from the research council of the Faculty of Veterinary Medicine, University of Tehran.

References

Albert-Puleo, M (1980). Fennel and anise as estrogenic agents. J. Ethnopharmacol., 2: 337-344.

Blumenthal, M (2000). *Herbal medicines*. Integrative Medicine Communications. 2nd Edn., Austin. PP: 419-423.

Chanh, PH; Ifansyah, N; Chahine, R; Mounayar-Chalfoun, A; Gleye, J and Moulis, C (1986). Comparative effects of total flavonoids extracted from *Ribes nigrum* leaves, rutin and isoquercitrin on biosynthesis and release of prostaglandins in the *ex vivo* rabbit heart. Prostaglandins Leukot. Med., 22: 295-300.

Choi, EM and Hwang, JK (2004). Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia. 75: 557-565.

Chung, YH; Miyake, H; Kamei, C and Tasaka, K (1984). Analgesic effect of histamine induced by intracerebral injection into mice. Agents Actions. 15: 137-142.

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, AW; Filho, VC; Olinger, L and Souza, MM (2008). Quercetin: further investigation of its antinociceptive properties and mechanisms of action. Arch. Pharm. Res., 31: 713-721.

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.

Giamberardino, MA (1999). Recent and forgotten aspects of visceral pain. Eur. J. Pain., 3: 77-92.

Girard, P; Pansart, Y; Coppe, MC; Verniers, D and Gillardin, JM (2004). Role of the histamine system in nefopam-induced antinociception in mice. Eur. J. Pharmacol., 503: 63-69.

Ikoma, A; Steinhoff, M; Stander, S; Yosipovitch, G and Schmelz, M (2006). The neurobiology of itch. Nat. Rev. Neurosci., 7: 535-547.

Kozak, W; Archuleta, I; Mayfield, KP; Kozak, A; Rudolph, K and Kluger, MJ (1998). Inhibitors of alternative pathways of arachidonate metabolism differentially affect fever in mice. Am. J. Physiol., 275: 1031-1040.

Kuiken, SD; Tytgat, GN and Boeckxstaens, GE (2005). Review article: drugs interfering with visceral sensitivity for the treatment of functional gastrointestinal disorders – the clinical evidence. Aliment. Pharmacol. Ther., 21: 633-651.

Levine, J and Taiwo, Y (1994). Inflammatory pain. In: Wall, PD and Melzack, R (Eds.), *Textbook of pain*. (3rd Edn.), New York, Churchill Livingstone. PP: 45-56.

Leza, JC; Lizasoain, I and Lorenzo, P (1990). H_1 and H_2 histamine receptor blockers and opiate analgesia in mice. Methods Find. Exp. Clin. Pharmacol., 12: 671-678.

Lorke, DA (1983). A new approach to acute toxicity testing. Arch. Toxicol., 54: 275-287.

Malmberg, AP; Lamberti, C; Ghelardini, C; Giotti, A and Bartolini, A (1994). Role of histamine in rodent antinociception. B. J. Pharmacol., 111: 1269-1279.

Miranda, HF; Puig, MM; Prieto, JC and Pinardi, G (2006). Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain. Pain. 121: 22-28.

Olsen, UB; Eltorp, CT; Ingvardsen, BK; Jørgensen, TK; Lundbæk, JA; Thomsen, C and Hansen, AJ (2002). ReN 1869, a novel tricyclic antihistamine, is active against neurogenic pain and inflammation. Eur. J. Pharmacol., 435: 43-57.

Parada, CA; Tambeli, CH; Cunha, FQ and Ferreira, SH (2001). The major role of peripheral release of histamine and 5-hydroxytryptamine in formalin-induced nociception. Neuroscience. 102: 937-944.

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

ethanolic stem extract of *Tinospora cordifolia*. Int. J. Drug Dev. Res., 3: 70-83.

Rang, HP; Dale, MM and Ritter, JM (1998). *Pharmacology*. 4th Edn., New York, Churchill Livingston. PP: 614-616.

Ren, SC; Suo, QF; Du, WT; Pan, H; Yang, MM; Wang, RH and Liu, J (2010). Quercetin permeability across blood-brain barrier and its effect on the viability of U251 cells. Sichuan. Da. Xue. Xue. Bao. Yi. Xue. Ban., 41: 751-754.

Robertson, JA; Hough, LB and Bodnar, RJ (1988). Potentiation of opioid and nonopioid forms of swim analgesia by cimetidine. Pharmacol. Biochem. Behav., 31: 107-112.

Satyanarayana, PSV; Jain, NK; Singh, A and Kulkarni, SK (2004). Isobolographic analysis of interaction between cyclooxygenase inhibitors and tramadol in acetic acid-induced writhing in mice. Prog. Neuropsycho-pharmacol. Biol. Psychiatry, 28: 641-649.

Savino, F; Cresi, F; Castagno, E; Silvestro, L and Oggero, R (2005). A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants. Phytother. Res., 19: 335-340.

Schmelz, M; Schmidt, R; Bickel, A; Handwerker, HO and Torebjork, HE (1997). Specific C receptors for itch in human skin. J. Neurosci., 17: 8003-8008.

Suh, HW; Song, DK; Choi, YS and Kim, YH (1996). Effects of intrathecally injected histamine receptor antagonists on the antinociception induced by morphine, beta-endorphin, and U50, 488H administered intrathecally in the mouse. Neuropeptides. 30: 485-490.

Thoburn, KK; Hough, LB; Nalwalk, JW and Mischler, SA (1994). Histamine-induced modulation of nociceptive responses. Pain. 58: 29-37.

Zendehdel, M and Babapour, V (2010). Study of antinociceptive effects of *Ziziphora tenuior* and its interference on opioidergic and serotoninergic systems. J. Vet. Res., 65: 57-60.

Zendehdel, M; Taati, M; Jadidoleslami, M and Bashiri, A (2011). Evaluation of pharmacological mechanisms of anti-nociceptive effect of *Teucrium polium* on visceral pain in mice. Iranian J. Vet. Res., 12: 292-297.

Zimmermann, M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 16: 109-110.