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RESEARCH ARTICLE

Antinociceptive effect of methanol extract of *Capparis ovata* in mice

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Abstract

Context: Capparis ovata Desf. (Capparaceae) grows widely in Turkey. Flower buds and fruits of the plant are used in folk medicine for their analgesic, antirheumatismal, and diuretic effects.

Objective: This study evaluated the possible antinociceptive effect of the methanol extract of *C. ovata* (CME) in mice.

Materials: The antinociceptive effect of methanol extract, prepared with the *C. ovata* flower buds, was studied at the doses of 50, 100, and 200 mg/kg (i.p.) using tail-immersion, hot-plate, and writhing tests in mice. Morphine sulfate (5 mg/kg; i.p.) and dipyrone (100 mg/kg; i.p.) were used as reference analgesic agents. Naloxone (5 mg/kg; i.p.) was also tested.

Results: It was observed that the *C. ovata* extract had a significant antinociceptive effect in these tests. In the hot-plate and tail-immersion test results, the doses of 50, 100, and 200 mg/kg increased the percentage of the maximum possible effect (MPE%) value for nociception significantly according to the control value ($P < 0.001$). All doses of the extract decreased the number of acetic acid-induced abdominal constrictions in mice when compared with control group ($P < 0.001$). These effects were inhibited by pretreatment with naloxone.

Discussion and conclusion: Based on the results obtained, it can be concluded that CME is a potentially antinociceptive agent which acts as both at the peripheral and central levels.

Keywords: *Antinociception;* Capparis ovata*; hot-plate; tail-immersion; writhing test*

Introduction

Capparis species (Capparaceae), also called caper, grow naturally in various regions of the world (Özcan et al., 2004). Caper plants can be used for landscaping, animal feed, and to prevent erosion (Akgül, 1993; Baytop, 1999). Caper has a very rich content of aminoacids, vitamins, minerals, and glucosinolates and has been used traditionally for several therapeutic effects since ancient times (Özcan, 2005). The bitter bark of roots, flowers, seeds, and fruits of the plant are used in spleen diseases or as diuretic, laxative, stimulant, and particularly for antirheumatismal effects (Baytop, 1999; Jiang et al., 2007). Although caper is also used traditionally as an analgesic and anti-inflammatory agent, there are limited experimental studies about these effects. The ethanol and water extracts of *C. zeylanica* L. leaves, which contain alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, and carbohydrates, showed a dose-dependent analgesic activity in the tail-immersion and writhing tests (Ghule et al., 2007). *C. spinosa* L. and *C. decidua* Edgew. have also been studied for their analgesic and anti-inflammatory activities, but it has been found that they both possess anti-inflammatory effect (Ageel et al., 1986; Al-Said et al., 1988; Panico et al., 2005; Goyal et al., 2009).

Caper, which is known as kapari or gebere in Turkey, is one of the most popular herbal nutrients used as a component of a healthy diet. *C. ovata* Desf. and *C. spinosa* are the only species grown in Turkey and they are consumed

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in pickled form (Baytop, 1999; Bağcı et al., 1999). *C. ovata* grows widely in Mediterranean and Aegean regions of Turkey. Flower buds, root bark, and fruits of the plant are used in folk medicine due to their analgesic, wound healing, cell regeneration, tonic, and diuretic effects (Baytop, 1984; Bağcı et al., 1999). However, there is no experimental study which has reported its analgesic activity. The aim of this study is to evaluate the possible antinociceptive effect of CME in models of pain in mice.

Methods

Drugs and chemicals

The following drugs and chemicals were used in this study: methanol (Merck, Germany), DMSO (Merck), morphine sulfate (Sigma, St. Louis, MO), dipyrone (Sigma), naloxone (Sigma), and acetic acid (Merck, Darmstadt, Germany).

Plant materials

Plant material was provided by Aşçı Murat, Burdur, Turkey. The company collected the plant material around Burdur in 2008 August and it was dried under shade. The plant sample was confirmed by Sevim Alan and a voucher sample (ESSE-14487) is kept in the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey. Powdered dried buds of *C. ovata* (20 g) were extracted with 250 mL methanol using a Soxhlet apparatus. The solvent was removed from resulting solution under vacuum in a rotary evaporator. The dried extract yield was calculated as 8.5 g/20 g (42.5%).

Phytochemical screening

CME was tested for the presence of alkaloids, tannins, reducing sugar, and flavonoids by using standard phytochemical procedures. In each test we used 10% (w/v) methanol solution of extract (Trease & Evans, 2002; Ahmed et al., 2007).

Animals

Experimental groups consisted of six inbred Male Swiss Albino mice (25–30 g) per group. All animals were housed in cages with standard pellet diet and water *ad libitum*. They were placed in a quiet and temperature/humidity controlled room (22° ± 2°C/60 ± 5%, respectively) in which a 12 h light-dark cycle was maintained. The mice were allowed 1–2 days for adaptation to the laboratory conditions. In the research internationally accepted principles for animal use and care were followed. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised in 1985) and approved by the Local Ethics Committe of Osmangazi University, Medical School, Eskisehir (No: 100/2009).

Drugs and treatment

All drugs, administered intraperitoneally (i.p.) 30 min before the procedure, were dissolved in the vehicle (ratio DMSO saline 1:4). CME was given to the animals at the doses of 50, 100, and 200 mg/kg. The vehicle at the same volume (0.1 mL) was injected into the control group. Morphine sulfate (5 mg/kg) and dipyrone (100 mg/kg) were used as the reference analgesic agents. As an attempt to investigate the involvement of opioid system in the antinociceptive effect of CME, mice were pretreated with the non-selective opioid receptor antagonist, naloxone (5 mg/kg; i.p.) and injected 15 min before the administration of the extracts (50, 100, and 200 mg/kg).

Acute toxicity

Swiss albino mice were divided into six groups which contain six animals each. The first group served as normal control. CME was administered i.p. to different groups at the doses of 200, 400, 500, 1000, and 2000 mg/kg. The mice were allowed food and water *ad libitum* and all animals were observed for toxic symptoms and mortality for 72 h (Hosseinzadeh et al., 2002; Walker et al., 2008).

Tail-immersion

The tail-immersion test which used in evaluation of thermal pain was carried out as described by Schmauss and Yaksh (1984). An area of the tail was marked and immersed in the water bath thermostatically maintained at 52.5° ± 0.2°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time. The maximum cut-off time for immersion was 15 s to avoid injury of the tissues of the tail.

Hot-plate test

The hot-plate test, a thermal pain model, was based on the procedure described by Eddy and Leimbach (1953). A transparent glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface of the plate. The temperature of the hot-plate was set to 55° ± 0.5°C by using a thermo-regulated water-circulating pump (Heto, Allerod, Denmark). The time of latency was defined as the time period between the zero point, when the animal was placed on the hot-plate surface, and the time when animal licked its back paw or jumped off to avoid thermal pain. In order to minimize the damage on the animal paws, the cut-off time was taken as 20 s.

Acetic acid-induced writhing test

The antinociceptive activity was assessed by the acetic acid abdominal constriction test (writhing test), a chemical visceral pain model (Koster et al., 1959; Vogel & Vogel, 1997), was used. Mice were injected with 10 mL/kg of 0.6% acetic acid solution (i.p.) after 30 min of the i.p. administration of the drugs or extracts. The number of writhings was counted for each animal, starting 5 min after acetic acid injection over the period of 10 min. The number of stretching movements (arching of back, development of tension in the abdominal muscles, elongation of the body, and extension of the forelimbs) was observed.

Statistical analysis

The results of statistical analysis were expressed as the mean ± SEM. The results of the tail-immersion and hot-plate tests were given as a percentage of the maximal possible effect (MPE% ± SEM) which was defined by the following equation:

$$\text{MPE\%} = [(\text{postdrug latency}) - (\text{predrug latency}) / (\text{cutoff time}) - (\text{predrug latency})] \times 100$$

The statistical analyses were carried out using GraphPad Prism version 5.0 and performed by one-way ANOVA, followed by Tukey's multiple comparison tests. $P < 0.05$ was considered significant.

Results

Phytochemical screening

Phytochemical investigations of CME showed the presence of flavonoids and tannins. Total phenols were calculated as 51.10 ± 2.43 $mg_{GAE}/g_{extract}$.

Acute toxicity

Administration of CME at the doses of 200–2000 mg/kg did not produce mortality or significant changes in general behavior. Therefore, it was thought that LD_{50} of CME is more than 2000 mg/kg. This result indicates CME has a low toxicity profile.

Tail-immersion test

The tail-immersion test results are given in Figure 1. The MPE% values were calculated as 5.5% ± 1.12% (control), 28.72% ± 3.81% (dipyrone), 38.73% ± 2.84% (morphine), 31.12% ± 3.79% (CME 50 mg/kg), 37.9% ± 6.42% (CME 100 mg/kg), 44.23% ± 3.38% (CME 200 mg/kg) respectively. CME showed an antinociceptive activity ($P < 0.001$, compared with control). There were no significant differences in the antinociceptive effect of 50, 100, and 200 mg/kg of CME when compared with reference drugs. The antinociceptive activity of all drugs used in this test antagonized by naloxone. However, naloxone just significantly decreased the antinociceptive effect produced by morphine ($P < 0.001$) and at the dose of 200 mg/kg of CME ($P < 0.01$).

Hot-plate test

In the hot-plate test (Figure 2) MPE% values were observed as 12.59% ± 1.43% (control), 22.22% ± 2.29% (dipyrone), 46.75% ± 4.06% (morphine), 32.83% ± 1.28% (CME 50 mg/kg), 41.87% ± 3.05% (CME 100 mg/kg), 51.68% ± 5.39% (CME 200 mg/kg) respectively. According to these results, the doses of 50, 100, and 200 mg/kg increased the MPE% value for nociception significantly above the control value ($P < 0.001$). The antinociceptive effect of CME is dose-dependent. Naloxone influenced the antinociceptive activity of CME at both doses ($P < 0.001$). Administration of CME at the doses of 100 and 200 mg/kg demonstrated a significant antinociceptive effect when compared with dipyrone ($P < 0.001$).

Acetic acid-induced writhing test

The method was chosen to evaluate the peripheral antinociceptive effect of CME. The results are shown in

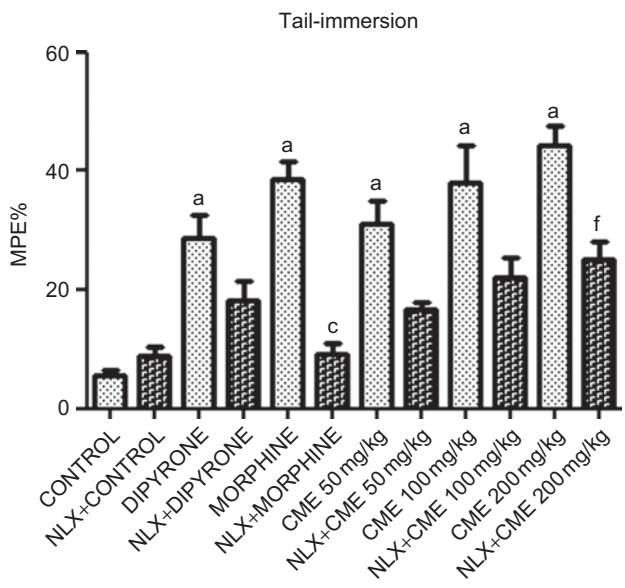

Figure 1. The antinociceptive effect of *C. ovata* extracts, morphine, dipyrone, and reversal effect of naloxone on tail-immersion test. Values are presented as the mean ± SEM (n = 6), (NLX; naloxone); [a] $P < 0.001$, significant difference from control; [c] $P < 0.001$, significant difference from morphine alone; [f] $P < 0.01$, significant difference from CME 200 mg/kg alone.

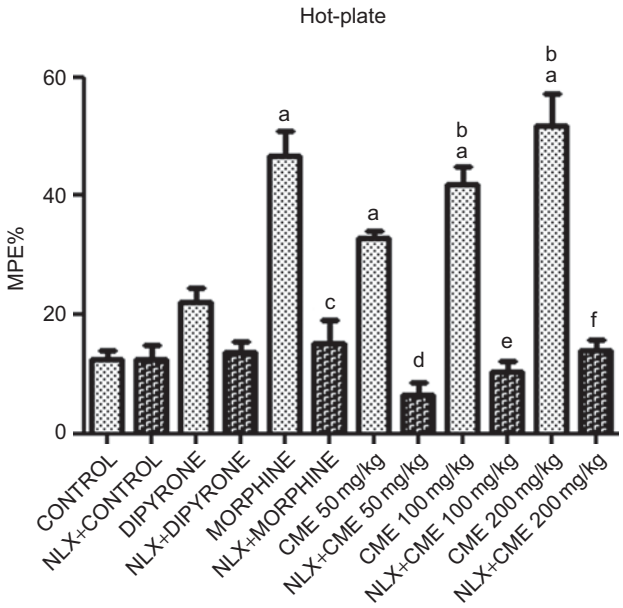

Figure 2. The antinociceptive effect of *C. ovata* extracts, morphine, dipyrone, and reversal effect of naloxone on the hot-plate test. Values are presented as the mean ± SEM (n = 6), (NLX; naloxone); [a]$P < 0.001$, significant difference from control; [b]$P < 0.001$, significant difference from dipyrone; [c]$P < 0.001$, significant difference from morphine alone; [d]$P < 0.001$, significant difference from CME 50 mg/kg alone; [e]$P < 0.001$, significant difference from CME 100 mg/kg alone, [f]$P < 0.001$, significant difference from CME 200 mg/kg alone.

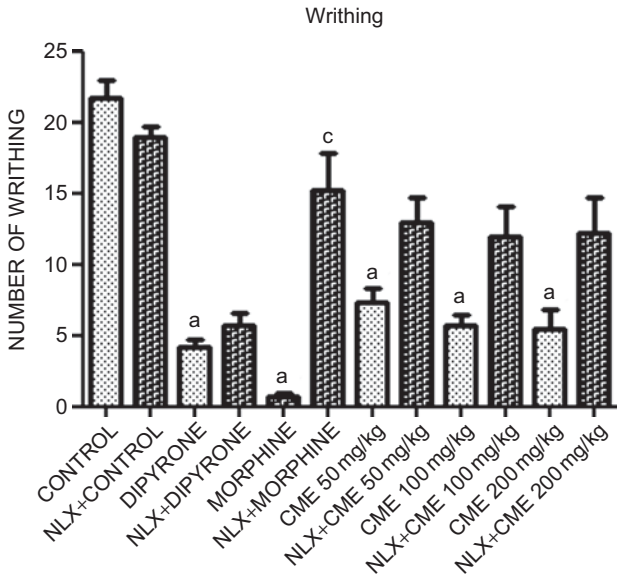

Figure 3. The antinociceptive effect of *C. ovata* extracts, morphine, dipyrone, and reversal effect of naloxone on acetic acid-induced writhing test. Values are presented as the mean ± SEM (n = 6), (NLX; naloxone); [a]$P < 0.001$, significant difference from control, [c]$P < 0.001$, significant difference from morphine alone.

Figure 3. The extract decreased the number of acetic acid-induced abdominal constrictions in mice and the values were found to be statistically significant at tested dose levels ($P < 0.001$), relative to controls only. The percentage inhibition of constrictions was calculated as 76.15% (dipyrone), 96.92% (morphine), 66.15% (CME 50 mg/kg), 73.85% (CME 100 mg/kg), and 74.62% (CME 200 mg/kg). The extract was statistically similar to the reference drug dipyrone. The antinociceptive effect of CME was antagonized by pretreatment with naloxone. The reversal effect of naloxone on the antinociceptive effect of CME (50, 100, and 200 mg/kg) was lower than its effect on morphine and there was no reversal effect on the activity of dipyrone.

Discussion

The present study demonstrated the central and peripheral antinociceptive effects of CME when assessed in chemical and thermal models of nociception as measured by writhing test and tail-immersion and hot-plate tests, respectively. Previous studies stated that the central activities are assessed using the hot-plate and tail-immersion tests to activate supraspinal nociceptive and spinal nociceptive pathways, respectively (Morales et al., 2001; Kılıç et al., 2006). The acetic acid-induced writhing test has been used for the evaluation of peripheral antinociceptive activity (Trongsakul et al., 2003). In addition, writhing response is widely accepted as a model visceral pain due to the release of endogen mediators of pain, such as prostaglandins, kinins, etc. (Derardt et al., 1980; Ahmed et al., 2007; Sulaiman et al., 2008; Zheng et al., 2009). Based on findings obtained in the present study, it was concluded that CME possesses a significant antinociceptive activity both at spinal and supraspinal levels. To approach possible antinociceptive mechanism(s) for CME, we examined the effect of naloxone, a non-selective opioid receptor antagonist, against the antinociceptive effect of CME. The results of the tail-immersion and hot-plate tests indicated that antinociceptive effect of CME was reversed by naloxone. The reversal effect was not as effective as that against morphine in the tail-immersion test, but similar in the hot-plate test. These data strongly suggested that the antinociceptive effect of CME may occur through opioid receptors not only at the spinal level, but also at the supraspinal level. Both tail-immersion and hot-plate tests are based on measuring the response of the animal to thermal stimuli. We mentioned above that tail-immersion monitors a spinal reflex, while the hot-plate is used for supraspinal reflex. In agreement with this suggestion, it has been demonstrated that μ2- and δ-opioid receptors are involved in spinal mechanism, while μ1/μ2-opioid receptors may mediate mainly supraspinal analgesia (Jinsmaa et al., 2004, 2005). Therefore, there is a high possibility that the central antinociceptive effect of CME may be prominent on μ-opioid receptors. It has

been proposed that the writhing test is non-selective for peripheral antinocieptive agents and is responsive to centrally acting antinociceptive compounds. However, the abdominal writhing induced by acetic acid still represents a model of peripheral nociception and is widely used for analgesic screening (Sanchez-Mateo et al., 2006). The inhibition of writhing response clearly indicated the peripheral antinociceptive effect of CME in addition to its central effect. This peripheral effect was as potent as dipyrone-induced antinociception and was inhibited by naloxone. Previous studies have shown that acetic acid acts indirectly by inducing the release of endogenous mediators of pain that stimulate the nociceptive neurons which are sensitive to non-steroidal anti-inflammatory drugs and opioids (Sanchez-Mateo et al., 2006). Hence, the peripheral component of CME-induced antinociception may be related to inhibition of the release of endogenous nociceptive mediators.

Our phytochemical screening results indicated that CME contains phenolic compounds such as flavonoids and tannins. Some phytochemical screening studies have reported that species belonging to the Cappariceae family contain these active compounds such as flavonoids, glucosinolates, alkaloids, etc. (Satyanarayana et al., 2008). Other studies suggested that plant materials which contain tannins, alkaloids, flavonoids, and phenolic acids possess analgesic and anti-inflammatory effects on experimental animals and these pharmacological effects are resulted from these contents (Mills & Bone, 2000; Morteza-Semnani et al., 2006). It was suggested that flavonoids demonstrate antinociceptive effect through opioid mechanisms (Anjaneyulu & Chopra, 2003; Katavic et al., 2007). There are few reports on the role of tannins in antinociceptive and anti-inflammatory activities (Starec et al., 1988). Hence, it was suggested that the antinociceptive action of CME can be related to its phenolic content.

Conclusions

In the present study, antinociceptive effects of CME in mice were observed. These effects were statistically significant at the doses of 50, 100, and 200 mg/kg (i.p.) of the methanol extract prepared with the *C. ovata* flower buds, in tail-immersion, hot-plate, and writhing tests comparing with morphine sulfate (5 mg/kg; i.p.) and dipyrone (100 mg/kg; i.p.), reference analgesic agents. Pretreatment with specific opioid antagonist naloxone (5 mg/kg; i.p.) inhibited CME-induced antinociception, suggesting an analgesia mediated by endogenous opioid mechanisms. The mechanisms of these activities need to be further studied with different antagonists (such as adrenergic, serotonergic, etc.). Taking these findings into account, it seems quite possible that *C. ovata* contains constituents with antinociceptive activity which may lead to the development of new natural products having analgesic effect. In further investigations, the different fractions of *C. ovata* will be evaluated and the structural characterization of responsible components will be clarified.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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