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## Antinociceptive effects of essential oil of *Melissa officinalis* L. in rats

Esam Qnais<sup>1\*</sup>, Yousra Bseiso<sup>1</sup>, Mohammad Wedyan<sup>1</sup> and Hakam Alkhateeb<sup>2</sup>

<sup>1</sup>Department of Biology and Biotechnology, Faculty of Science, Hashemite University

<sup>2</sup>Faculty of Medicine, Yarmouk University, Irbid, Jordan

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### ABSTRACT

Lemon balm (*Melissa officinalis*, Lamiaceae) is used by people in many regions for relieving toothache, fevers, colds, hyperthyroidism, depression, mild insomnia, epilepsy, and headaches. This study investigated the antinociceptive effect of the essential oil of *Melissa officinalis* (MOEO) in various experimental models. The median lethal dose (LD<sub>50</sub>) of MOEO was estimated using the method of Lorke. The antinociceptive effect was assessed using chemical (formalin and acetic acid) and thermal (hot-plate) nociceptive tests in rats. In all experiments, MOEO was administered intraperitoneally at the doses of 10, 31.6, 100 and 316, 1000 mg/kg. In the acute toxicity test, the value of estimated LD<sub>50</sub> for MOEO was 2250 mg/kg. MOEO at test doses (10, 31.6, 100, 316 and 1000 mg/kg, i.p.) significantly reduced in dose dependent manner ( $p < 0.05$ ) the pain response in all tests. Naloxone failed to antagonize the antinociceptive effect of the essential oil in all tests. It seems that mechanism(s) other than opioid receptors is (are) involved in the analgesic effect of MOEO. This study reported the peripheral and central antinociceptive activity of the MOEO and rationalized the traditional use of the plant in the treatment of different painful conditions.

**Keywords:** antinociceptive, opioid receptors, essential oil, *Melissa officinalis*, formalin test, hot plate.

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### INTRODUCTION

*Melissa officinalis* (Lemon balm) belongs to the Lamiaceae family, is a perennial herb, up to 1 m high, growing in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. Parts mostly used are dried leaves; which often present flowering tops [1-3]. Ancient Greeks and Romans used *Melissa officinalis* in surgical dressings for wounds and in preparations to treat venomous or infectious bites and stings such as caused by dogs and scorpions. Today, *Melissa officinalis* primary use involves the treatment of fevers and colds, indigestion associated with nervous tension, hyperthyroidism, depression, mild insomnia, epilepsy, headaches, and toothaches among others [1-4].

*Melissa officinalis* has been shown to possess several biological actions, such as antioxidant [1,5], sedative [6-8], anti-tumeral [9-10], anti-inflammatory, hepatoprotective [11-12], hypoglycaemic effects [13], antibacterial, antifungal, antiviral, antihistaminic [1,14-16], antilipidaemic [17], spasmolytic activities [14], anxiolytic [18] and controlling light to mild Alzheimer's cases [19-20]. The essential oil is mentioned also for migraine and rheumatism [21].

Phytochemical studies carried out with *M. officinalis* have demonstrated the occurrence of many classes of constituents, including polyphenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid), essential oils (geranial, neral, citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, germacrene D, and ocimene), monoterpenoid aldehydes, sesquiterpenes, flavonoids (luteolin) and tannins [1, 3, 6, 17, 22, 23, 24].

Taking into account the biological activities of *M. officinalis*, it is surprising that no pharmacological study has been carried out on the possible antinociceptive effects of the essential oils up to now. Here, we have therefore examined the possible antinociceptive action of the essential oils in chemical and thermal models of nociception in rats and, therefore, to determine the scientific basis for its use in traditional medicine in the treatment of pain. Attempts have been made to further investigate some of the possible mechanisms that underlie the antinociceptive action of the extract.

### MATERIALS AND METHODS

**Plant material:** Aerial parts of wild-growing *M. officinalis* were collected during April from Aum-Romanna (Jordan) by one of us (EYQ). The plant material was identified and authenticated taxonomically at the Hashemite University herbarium. A voucher specimen was deposited under the number HU-437 at the Hashemite University herbarium, Zarka, Jordan, for future reference.

**Determination of essential oil composition:** Samples of dried aerial parts (300g each sample) of *M. officinalis* were hydrodistilled for 4 h in a modified Clevenger-type apparatus to obtain the volatile constituents. The essential oil of *M. officinalis* was analysed by GC on a Trace GC ULTRA with FID detector gas chromatograph equipped with a column (30 m x 0.25 mm x 0.25  $\mu$ m) type VB-5 (methylpolysiloxane with 5% of phenyl) and split injection. Mass spectrometry (MS) analysis were performed on a Polaris Q MS mass spectrometer (with an ion-trap at 70 eV). The temperature program was 40 °C for 2 min, then raised to 180 °C at 4°C/min. The carrier gas was helium (1.4 mL/min). The volatile constituents of the essential oil (EO) were identified by automated comparison of their mass spectra with that of the NIST (National Institute of Standards and Technology) library.

**Drugs and chemicals:** All chemicals, unless otherwise stated, were purchased from Sigma Chemical Co., St. Louis, MO. All drugs were dissolved in saline. The essential oil was prepared in 1% v/v Tween 80 in sterile saline.

**Experimental animals:** Non-fasting male Wistar rats (150–250 g) or Swiss albino mice (25-35 g, only for acute toxicity experiment), housed at 22°–25°C under a 12 h light/dark cycle and with access to food and water *ad libitum*, were used throughout the experiments. All test solutions were administered in a volume of 10 ml/kg body weight [25]. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals at the Hashemite University.

**Hot-plate test:** The hot-plate test was assessed using groups of male rats, six animals per group. The temperature of the hotplate was maintained at 50°  $\pm$  1°C. Latency to a discomfort reaction (licking paws) was determined in seconds before and 60 min after intraperitoneal administration of vehicle, MOEO (10,31.6,100,316,1000 mg/kg) or morphine (5 mg/kg; positive control). The largest doses were determined on the basis of LD50 experiments. The doses were calculated to be located at approximately 0.5 log units from each other on a log scale. The cut-off time was 60 s. The prolongation of the latency times was compared to the values of the control and used for statistical comparison. Baseline was considered as the mean of three readings of the reaction time obtained before administration of vehicle, MOEO or morphine and was defined as the normal reaction time of animals to this temperature. The increase over baseline (in %) was calculated by the formula:  $(A-B/B) \times 100$ , where A is the mean of three readings of reaction time after treatment taken within 5-7 min; B is the mean of three readings of reaction time obtained before treatment. In this, and the following experiments, ID<sub>50</sub> was determined from the plot of individual experiments by the best visual fit.

**Acetic acid-induced abdominal writhing test:** Abdominal writhing was assessed in rats according to Matheus et al. (2005) [26]. Briefly, after an intraperitoneal administration of a 2% (v/v) acetic acid solution in a volume of 0.1 ml/10 g body weight. The number of writhes, a response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension, was counted during continuous observation for 20 min beginning from 5 min after the acetic acid injection. Rats were pretreated with MOEO (10,31.6,100,316,1000 mg/kg, i.p.), or vehicle (1% Tween 80 in normal saline, i.p.) 60 min before the administration of acetic acid. A positive control group was composed of animals pre-treated with morphine (5 mg/kg, i.p.). The percentage inhibitions of writhing was calculated as follows: percentage inhibitions of writhing =  $(N - N_t / N) \times 100$  where N is the average number of stretching of control group and N<sub>t</sub> is the average number of stretching of test group.

**Formalin test:** Rats were divided into groups (six rats each) and were injected intraperitoneally with either vehicle (control), MOEO (10, 31.6, 100, 316, and 1000 mg/kg), or 5 mg/kg morphine (positive control). Sixty min later, each rat received 50  $\mu$ L of formalin (5%) subcutaneously into the dorsal surface of the right hind paw using a microsyringe with a 27 gauge needle. Immediately after formalin injection, animals were placed individually in acrylic observation chambers (320 cm<sup>2</sup>  $\times$  40 cm). Mirrors were arranged at angles to allow clear observation of the paws of the animals. Licking of the injected paw was defined as the nociceptive response. The total time of the

response was measured during the periods of 0–5 min (early phase) and 15–40 min (late phase). The inhibition of licking (in %) was calculated by the formula:  $(A-B/A) \times 100$ , where A is the time of licking before treatment; B is the time of licking after treatment.

**Involvement of opioid system on antinociceptive action of *M. officinalis*:** To evaluate the mechanism of action of MOEO animals were pre-treated with the opioid antagonist naloxone (5 mg/kg). Naloxone was administered i.p. 15 min before administration of vehicle, the ED<sub>50</sub> of MOEO, or morphine (5 mg/kg). Using the acetic acid-induced abdominal writhing test, hot-plate test, and the formalin test as described above, inhibition of the writhing response, latency times and licking was calculated after 60 min of MOEO or morphine administration.

**Acute toxicity:** The intraperitoneal (i.p.) acute toxicity (lethal dose; LD<sub>50</sub>) of the MOEO was evaluated in mice [27]. The experiment was carried out in two phases; in the first phase, geometric doses of the MOEO (10, 100 and 1000 mg/kg) were administered i.p. to three groups of mice, and the control group received normal saline (10 ml/kg, i.p.). In the second phase, other doses of the MOEO (1600, 2900 and 5000 mg/kg) were administered. Signs of toxicity and mortality within 24 h were noted. The LD<sub>50</sub> was then calculated based on the pattern of death observed in the second phase using the Probit-log analysis from the graph of percent mortality against log dose of the MOEO.

**Statistical analysis:** The values were expressed as the mean  $\pm$  SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. Differences were considered significant when  $P < 0.05$ .

## RESULTS

**Essential oil extraction and analysis:** The chemical analysis of the MOEO sample used in the present investigations identified eighteen compounds, representing 94.8% of the total oil content (Table 1). Neral (13.7%),  $\beta$ -caryophyllene (12.7%), linalool (11.1%),  $\alpha$ -pinene (9.1%), citronellol (6.7%), camphene (6.1%) were found to be major constituents (Table 1).

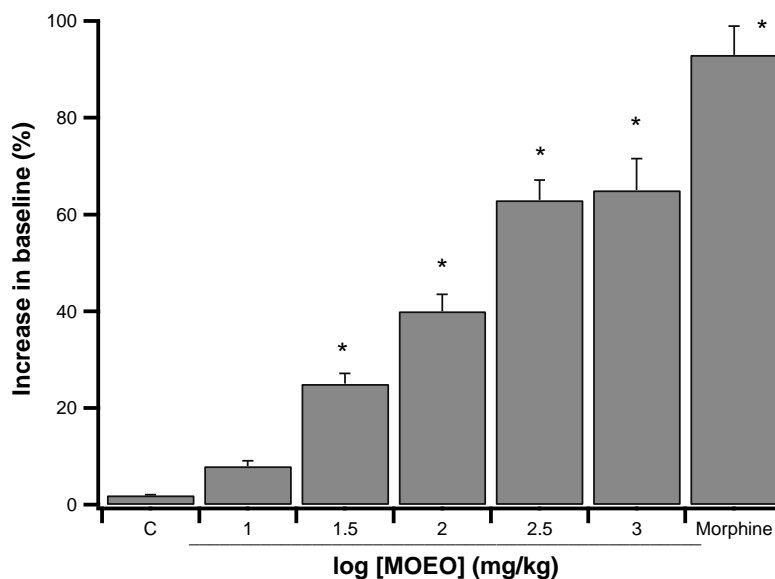
**Table 1: Chemical composition of the essential oil of aerial parts of *M. officinalis***

Constituents	Percentage (%)
$\beta$ -Caryophyllene	12.7
Citronella	1.1
Thymol	5.2
Caryophyllene oxide	3.7
$\alpha$ -pinene	9.1
Spathulenol	2.3
Camphene	6.1
$\beta$ -elemene	5.1
Myrtenal	3.6
Verbenone	2.1
Pinocarvone	3.7
Carvacrol	2.3
Linalool	11.1
Citronellol	6.7
Geranial	3.1
Neral	13.7
Estragole	2.1
Limonene	1.1
Total identified	94.8

**Acute toxicity:** The i.p. LD<sub>50</sub> of the MOEO in mice was calculated to be 2250 mg/kg indicating a low toxicity profile of the MOEO. No signs of toxicity, such as diarrhoea, motor impairment, ataxia, hyperexcitability or alterations on respiratory frequency or piloerection, were noted in the control or experimental animals at lower doses. Also, no gastric ulcerogenic effect was observed in controls or treated animals. Severe depression, abnormal gait, ataxia, increased respiration and decreased activity were observed at dose higher than 3000 mg/kg

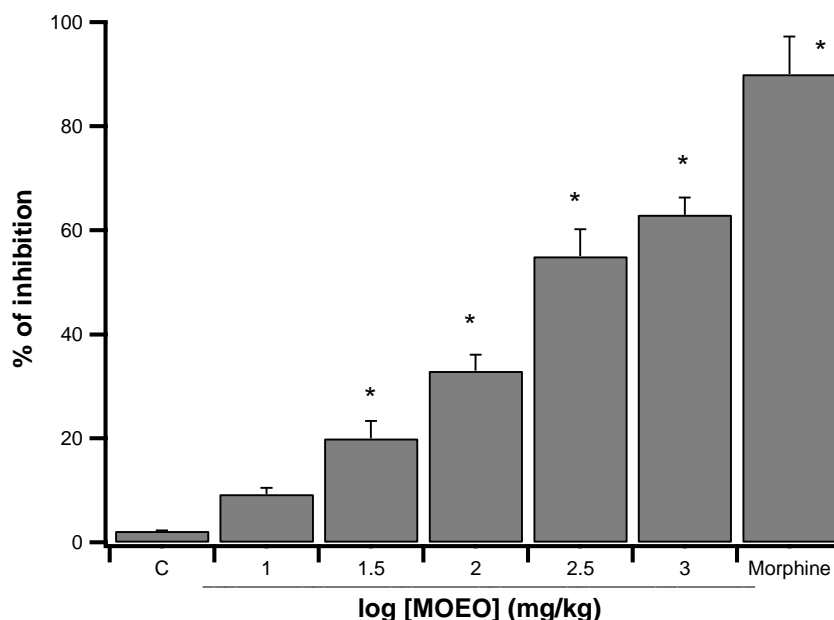
**Effect of MOEO on the latency time in the hot-plate test:** When rats were treated with MOEO (10, 31.6, 100, 361 and 1000 mg/kg, i.p.), there was a significant dose-dependent increase in time of response (latency) to thermal stimulation compared with control rats with ID<sub>50</sub> value of  $85.4 \pm 3.2$  mg/kg (Fig. 1). The percentage of increase in baseline produced by morphine (5 mg/kg, i.p.) was  $94.1 \pm 2.8\%$ .

**Fig. 1:** Effect of MOEO and morphine on the latency of rats submitted to hotplate test. Values were expressed as mean  $\pm$ SEM (n=6). Control values (C) indicate the animals injected with vehicle and the asterisks denote the significance levels when compared with control groups. ( $p > 0.05$ ).



**Effect of MOEO on acetic acid-induced abdominal writhing test:** The results in Figure 2 demonstrate that the MOEO, when administered intraperitoneally at different doses (10, 31.6, 100, 361 and 1000 mg/kg) caused an inhibition of the writhing response induced by acetic acid (i.p.) ( $p < 0.05$ ). This activity was dose-dependent. The calculated mean  $ID_{50}$  value was  $93.9 \pm 6.1$  mg/kg. The percentage inhibitions of writhing produced by morphine (5 mg/kg, i.p.) was  $91.5 \pm 5.4\%$ .

**Fig. 2:** Effects of MOEO and morphine administered intraperitoneally against acetic acid-induced writhing response in rats. Each column represents the mean of 6 animals and the error bars indicate the SEM. Control values (C) indicate the animals injected with vehicle and the asterisks denote the significance levels when compared with control groups. ( $p > 0.05$ )

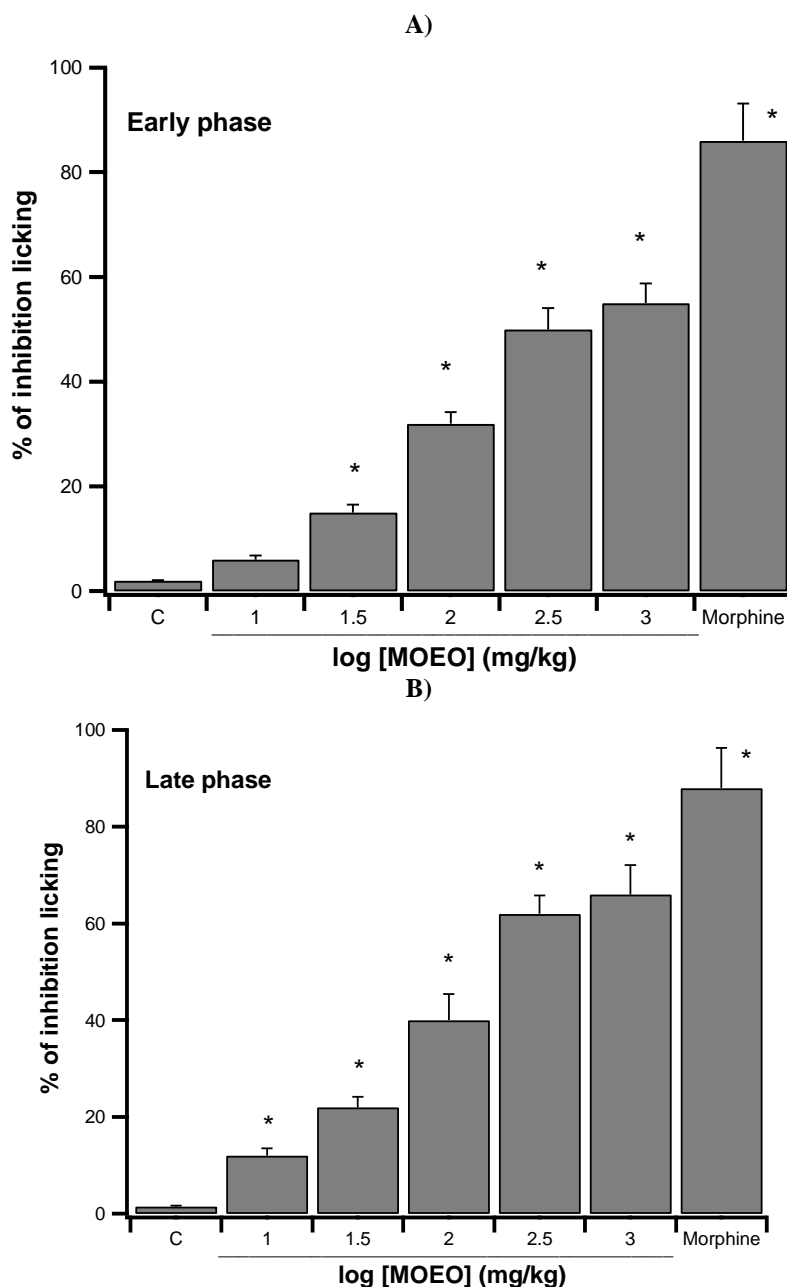


**Effect of MOEO on formalin-induced nociception:** The results depicted in Fig. 3A and B show that MOEO (10, 31.6, 100, 361 and 1000 mg/kg i.p.) significantly inhibited both the early (neurogenic, 0–5 min) and late (inflammatory, 15–30min) phases of formalin-induced licking. However, its antinociceptive effects were significantly more pronounced against the second (late) phase of this pain model. The calculated mean  $ID_{50}$  value for these effects were:  $97.1 \pm 3.7$  and  $78.8 \pm 3.6$  mg/kg, and the inhibitions observed were  $57.3 \pm 4.1\%$  and  $64.2 \pm 4.6\%$  at a dose of 1000mg/kg, for the first and second phases, respectively. (Fig. 3A and B). The percentage inhibitions of

licking produced by morphine (5 mg/kg, i.p.) were  $86.1 \pm 6.5$  and  $88.2 \pm 9.1\%$ , for the first and second phases, respectively.

**Opioid system involvement:** Pre-treatment with the non-selective opioid receptor antagonist naloxone (5mg/kg, i.p.) 30min before hand, did not reverse the antinociception caused by MOEO, but it completely reversed the antinociception caused by morphine (5 mg/kg, i.p.) during acetic acid-induced abdominal writhing test, hot-plate test, and formalin-induced pain (Table 2).

**Fig. 3:** Effects of MOEO (10, 31.6, 100, 361 and 1000 mg/kg) or morphine (5mg/kg) on the first (panel A) and second phase (panel B) of formalin-induced licking in rats. Control values (C) indicate vehicle administration, and the asterisks denote significance levels when compared with the control group ( $P > 0.01$ ). Values were expressed as mean  $\pm$  SEM (n=6).



**Table 2: Effect of naloxone (10 mg/kg) on MOEO antinociceptive activity in rats assessed using the hot-plate test, abdominal writhing test and formalin-induced pain test. Control indicates vehicle administration, and the asterisks denote significance levels when compared with the control group ( $P > 0.01$ ). Values were expressed as mean  $\pm$  SEM (n=6)**

Treatment	Increase in baseline (%) (Hotplate test)	Inhibition (%) of licking (PhaseI)	Inhibition (%) of licking (PhaseII)	Inhibition (%) of abdominal writhing
Control	2.1 $\pm$ 0.5	1.4 $\pm$ 0.4	1.9 $\pm$ 0.3	3.2 $\pm$ 0.2
MOEO	50.2 $\pm$	51.1 $\pm$	50.1 $\pm$	50.5 $\pm$
Morphine	79 $\pm$ 2.1	82 $\pm$ 1.8	85 $\pm$ 4.1	87 $\pm$ 3.4
Naloxone	3.1 $\pm$ 0.6	1.7 $\pm$ 0.9	2.7 $\pm$ 1.1	4.2 $\pm$ 0.4
Naloxone + MOEO	49.7 $\pm$ 3.7	50.1 $\pm$ 2.6	48.7 $\pm$ 4.5	51.3 $\pm$ 2.1
Morphine +Naloxone	4.1 $\pm$ 1.2	5.4 $\pm$ 1.4	3.7 $\pm$ 0.6	4.7 $\pm$ 1.3

*The doses of MOEO used were those used to give ID<sub>50</sub>.*

## DISCUSSION

The present study demonstrates for the first time that the MOEO induces antinociceptive effects in several model of nociception (acetic acid-induced abdominal writhing, hot plate and formalin). The experimental models used in this study employed chemical and thermal-induced nociception. They were selected such that both centrally and peripherally mediated effects were measurable. The acetic acid abdominal constriction method elucidates peripheral effects; hot-plate test method reveal central activity, while the formalin-induced nociception test investigate both central and peripheral effects [28]. Experiments on naloxone antagonism were conducted to further determine involvement of opioid receptors. Administrations of graded doses of MOEO (i.p.) in mice gave a LD<sub>50</sub> value of 2250 mg/kg in mice. This finding probably suggests that MOEO may have a reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lack of any reported side effects with the traditional use of this plant.

The formalin test is believed to represent a more valid model for clinical pain [29]. In this test, the early phase is thought to result from direct chemical activation of myelinated and unmyelinated nociceptive afferent fibers and the late phase as a consequence of noxious stimulus-evoked long-term changes in the properties of dorsal horn neurons [30]. Centrally acting drugs such as opioids exert an inhibition in both phases [31], as it is clearly consistent with our morphine results in the formalin test, whereas peripherally acting drugs such as indomethacin, aspirin and hydrocortisone only inhibit the late phase, which seems to be a result of an anti-inflammatory response [32-33]. Our data show that the plant essential oil is capable of exerting antinociception by acting at both phases suggesting that it may exert a central action. Notably, the activity of the MOEO was more pronounced in the late phase, which is commonly associated with inflammatory pain.

The hot-plate test was selected to investigate central analgesic activity, because it has several advantages, particularly the sensitivity to strong analgesics and limited tissue damage [34]. A significant antinociceptive effect with the hot-plate test was shown after MOEO (10–1000 mg/kg, i.p.) administration indicating central analgesic effect [35-37].

MOEO significantly inhibited the abdominal constriction induced by acetic acid in rats. Acetic acid causes an increase in peritoneal fluids of PGE<sub>2</sub> and PGF<sub>2a</sub>, serotonin and histamine as well as lipoxygenase products [38]. This model is commonly used for screening peripheral analgesics [38-39]. Although the writhing test has poor specificity [29], it is a very sensitive method of screening antinociceptive effects of compounds and with a good correlation between ID<sub>50</sub> values obtained in animals using this test and analgesic doses in humans [39]. MOEO exhibited marked dose dependent inhibitory effect on the writhing response induced by acetic acid. These results strongly suggest that MOEO possesses peripheral analgesic activity and its mechanism(s) of action may be mediated through inhibition of local peritoneal receptors or arachidonic acid pathways, involving cyclo-oxygenases and/or lipoxygenases. The present study shows that the opioid system is unlikely to be involved in the antinociceptive action of MOEO. This is inferred by the fact that the pretreatment of animals with naloxone, a nonselective opioid receptor antagonist, completely inhibited the antinociceptive effect of morphine but not the action of MOEO in the acetic acid-induced writhing test, hot plate test or formalin model.

Chemical composition of the MOEO was analyzed by gas GC/MS and identified the presence of eighteen compounds, representing 94.8% of total oil. The main components were the neral (13.7%),  $\beta$ -caryophyllene (12.7%), linalool (11.1%),  $\alpha$ -pinene (9.1%), citronellol (6.7%) and camphene (6.1%). The antinociceptive MOEO could be attributed to the single or synergic action of these main components or even other minor constituents present in the oil. It is more likely that the antinociceptive effect of the MOEO is caused by the presence of  $\beta$ -caryophyllene. This naturally occurring monoterpenoid was able to reduce the edema formation induced by

carrageenan, histamine, bradikinin, PGE2 and platelet activating factor [40]. Furthermore, caryophyllene is a known CB2-agonist. CB-2 is a cannabinoid receptor, and CB-2-selective agonists are promising candidates for pain treatment [41]. In addition, it is interesting to highlight that  $\alpha$ -pinene, citronellal, limonene and caryophyllene oxide of the MOEO, could also enhance the antinociceptive activity of MOEO, since they have shown antinociceptive effects in several animal models [42-47]. Furthermore, there is probably an involvement of carvacrol, which has been shown to reduce the number of writhings induced by acetic acid and decreases the linking time induced by formalin test with non-participation of the opioid system. Finally, estragole is also reported as responsible for the blocking of nerve excitability [48] and relax isolated smooth muscle [49]. Further studies are needed to clarify the mechanism of action and the components responsible for these pharmacological effects.

### CONCLUSION

Results suggest that MOEO possesses a potent antinociceptive effect which may be both peripherally and centrally mediated. It seems that mechanism(s) other than opioid receptors is (are) involved in the analgesic effect of MOEO. This finding supports the use of *M. officinalis* in traditional medicine for the treatment of painful disorders.

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