

# Antinociceptive activity of *Mentha piperita* leaf aqueous extract in mice

Yousef A. Taher\*

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Tripoli University, Tripoli, Libya

**Background:** *Mentha piperita* L. (Labiatae) is an herbaceous plant, used in folk medicine for the treatment of several medical disorders.

**Methods and Results:** In the present study, the aqueous extract of *Mentha piperita* leaf, at the i.p doses 200 and 400 mg/kg, showed significant analgesic effects against both acetic acid-induced writhing and hot plate-induced thermal stimulation in mice, with protection values of 51.79% and 20.21% respectively. On the contrary, the *Mentha piperita* leaf aqueous extract did not exhibit anti-inflammatory activity against carrageenan induced paw oedema.

**Conclusion:** These findings indicate that *Mentha piperita* has a potential analgesic effect that may possibly have mediated centrally and peripherally, as well as providing a pharmacological evidence for its traditional use as a pain reliever.

Keywords: *Mentha piperita*; traditional medicine; analgesic activity; mice

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*Mentha piperita* Linn (family: Labiatae) is an herbaceous plant of small size with greenish-feathery leaves. Worldwide *M. piperita* leaves are used in various forms of preparations (as a tea preparation, alcohol extracts, a tincture or as an oil (1). The plant contains volatile oils, resins and flavonoids (2, 3). In Mediterranean region, *M. piperita* has been reported to possess diverse medicinal properties. Traditionally, it is used in treatment of various conditions including flatulence, carminative and as a local antiseptic (4). In addition, *M. piperita* is reported to have analgesic activity and its leaves are used to reduce tooth and abdominal pains (5). Besides, this plant has been indicated to relieve inflammatory disorders. Topically *M. piperita* essential oil is employed as an analgesic compound for diseases of the pharynx and in the relief of tension headache and migraines (6). In mouthwashes, it is utilized for oral hygiene, possibly due to its antimicrobial activities (3). *In vitro*, several studies have shown that *M. piperita* has antibacterial effects, strong antioxidant and antitumor action, and antiallergenic potential (2, 7, 8). Additionally, in animal studies, it was clearly demonstrated in the Ames test that *M. piperita* has antimutagenic effects once it significantly inhibited the shamma—a tobacco product—induced oral carcinogenesis in the hamster cheek pouch (9).

The genus *Mentha* L. comprises more than 20 species, mainly perennial herbs growing wildly in damp places throughout temperate regions of Eurasia, Australia, and Africa. Hence, the pharmacological activities of *M. piperita* (2, 10) and other species of the family Labiatae (11–13) was the subjects of certain studies, antinociceptive and anti-inflammatory activities included (10, 13, 14). Animal model studies have demonstrated that *Mentha suaveolens* (13) and *Mentha microphylla* (10) could be able to protect mice from peripheral pains. Furthermore, Atta and Alkofahi have shown that the ethanol extracts of *M. piperita* induced in addition to a dose-dependent pain-reducing protective activity anti-inflammatory effects against both exudative and proliferative inflammation (14). Keeping this in view, the present study aimed to evaluate the analgesic and the anti-inflammatory activities of the aqueous extract of *M. piperita* leaf in mice.

## Materials and methods

### Plant material

Samples of peppermint fresh green leaves, *M. piperita* plant, were purchased in November 2009 from local Al-Karaymia public market located at the south-western part of the capital, Tripoli, Libya. After identification, by Dr F. Elrateeb (Department of Botany, faculty of

Science, Tripoli University, Libya), a voucher specimen was deposited at the Herbarium of the same University.

### Aqueous extracts

*M. piperita* leaves were cleaned and air-dried in the open away from direct sun light. The dried powdered leaves (5 g) were extracted with 150 ml boiling water (decoction) for 10 min. The liquid extracts were filtered, combined, and concentrated almost to dryness using a water bath at 60°C for 3 h (the yields; 6.4%). Thereafter, the concentrated aqueous extract was stored in a refrigerator for subsequent evaluation of its pharmacological activities.

### Animals

Inbred Albino mice of either sex (weight 15–40 g,  $n = 8$  each group) reared in the local animal house, faculty of Pharmacy, Tripoli University, Tripoli, Libya, were used. Mice were kept under normal environmental condition and fed with standard diet with free access to water. Animal care and use were in strict compliance with the standard guidelines (Canadian Council). The experimental protocol, dated October 04, 2009; (Ref. No. Pharm/04/10/2009/2010), was approved by the Ethics Committee for Research Programs, faculty of Pharmacy, Tripoli University.

### Drugs and chemicals

The following drugs were used: acetylsalicylic acid, Indomethacin, Lambda-carrageenan Type IV (Sigma Chemical Co., St. Louis, USA) and morphine (Lavoisier, France). A bent blunted 25G needle connected to a 1 ml syringe was used for the i.p. administration.

### Acetic acid-induced abdominal constrictions in mice

This method was used to evaluate the possible peripheral effects of the plant as analgesic substance. The writhing acetic acid test (abdominal constriction test) was performed as previously described (15, 16). Briefly, *M. piperita* leaf aqueous extracts (200 and 400 mg/kg), acetyl salicylic acid (100 mg/kg), and equivalent volume of saline (as a negative control) were injected i.p., 30 min prior to the injection of 10 ml/kg of 0.6% v/v acetic acid. Acetyl salicylic acid is a well-known peripheral analgesic drug, and it was used as a positive control. Mice ( $n = 8$  each group) were then placed in an observation box, and the number of writhing movements (constriction of the abdominal muscles together with stretching of the hind limbs) was counted for each mouse for a period of 20 min after acetic acid injection. Antinociceptive activity was expressed as the number of abdominal constrictions between saline treated control and animals pre-treated with *M. piperita* leaf aqueous extracts or acetyl salicylic acid. Percentage inhibition of writhing was calculated using the formula:

% inhibition

$$= [\text{control mean} - \text{treated mean}] \times 100 / \text{control mean}$$

### Thermally-induced pain in mice

The hot plate test was employed for preferential assessment of possible centrally mediated analgesic effects of the *M. piperita* leaf aqueous extract. This test was used to measure response latencies according to the method previously described (17, 18). Briefly, for two consecutive days preceding the experiments, mice ( $n = 8$  each group) were placed kindly on a plate (Model-DS37) maintained at room temperature for 10 min each day. On third day each mouse was placed gently on  $55 \pm 1^\circ\text{C}$  hot plate for a maximum time of 25 sec. Latency to exhibit nociceptive responses such as licking forepaws, shaking or jumping off the plate was measured twice before distraction (basal) in 10 min interval ( $T_b$  = average basal responses). The *M. piperita* leaf aqueous extracts was given by i.p. injection at doses 200 and 400 mg/kg. The central analgesic drug, morphine, as a positive control was administered s.c. at a dose of 4.6 mg/kg. Control mice received 10 ml/kg normal saline. After 30 min distraction latency time was measured ( $T_a$ ). For each mouse, the mean latency of nociceptive responses was calculated (a latency period of 25 sec was defined as complete analgesia), permitting us to express the percentage of analgesia using the following formula:

$$\% \text{ analgesia} = (T_a - T_b) \times 100 / T_b$$

### Inflammatory paw oedema in mice

The anti-inflammatory activity was evaluated by the use of carrageenan-induced paw oedema test as described in rats (19, 20) with modification in mice (21). Briefly, 0.02 ml of freshly prepared 1% carrageenan (phlogistic agent) suspension in isotonic saline was injected subplantarily into the right hind paw of each mouse (Eight animals per group were tested). *M. piperita* leaf aqueous extracts at 200 and 400 mg/kgs were i.p. administered to mice 30 min before carrageenan injection. Control mice received normal saline while a reference group received indomethacin (10 mg/kg, i.p.). Mouse paw thickness was measured immediately before and then at 1 h intervals for 5 h after carrageenan injection, using micrometer-apparatus (Mitutoyo, Japan) (22). The thickness of hind paws was taken as an index of the increase in paw's dimension, and as the measure of acute inflammations. The increase in paw thickness (measured in mm) 3 h after carrageenan injection was taken as thickness of oedema. The inhibition percentage of the inflammatory reaction was determined for each mouse by comparison with control and calculated by the following formula:

$$\% = 100 - [( \text{experimental paw thickness} / \text{control paw thickness} ) \times 100]$$

### Statistical analysis

Data are expressed as a mean response  $\pm$  SEM. In the study of analgesic properties, comparisons were made on the individual algic reactions obtained for treated and control animals, in the writhing test, and between the individual response times obtained before and after each treatment, in the hotplate test. For the anti-inflammatory test, comparisons were made on the individual differences calculated between the paw thickness obtained after 3 h and those obtained before treatment. The statistical comparisons between the groups were assessed by one-way analysis of variance (ANOVA). When F ratios were significant ( $p < 0.05$ ), Tukey's *post hoc* test between groups was done using GraphPad Prism (GraphPad Software Inc., version 3.0, San Diego, USA).

### Results

The i.p administration of *M. piperita* leaf aqueous extracts at 200 and 400 mg/kg showed significant inhibitions of acetic acid-induced abdominal constrictions in mice (46.67% and 51.79% protection, respectively;  $p < 0.01$ ,  $n = 8$  each, Table 1). Besides, there was no important difference in analgesic effects between the two doses. The reference drug, acetyl salicylic acid, at 100 mg/kg produced 94.87% significant protective effect towards the acetic acid induced pain in this nociception model ( $p < 0.001$ , Table 1).

Using the hot-plate test, it was also shown that administration of *M. piperita* leaf aqueous extract (200 and 400 mg/kg) produced a significant increase in the latency to a response to thermal stimulation compared to control mice (20.21% and 17.13% protection, respectively,  $p < 0.001$ ,  $n = 8$  each, Table 2). In this model, the

**Table 1.** Antinociceptive effect of *M. piperita* leaf aqueous extract in the acetic acid-induced writhing test

Treatment	Dose (mg/kg, i.p) <sup>a</sup>	Writhing <sup>b</sup>	Inhibition (%)
control	10 ml/kg	39 $\pm$ 5.6	–
<i>M. piperita</i>	200	20.80 $\pm$ 6.15**	46.67
<i>M. piperita</i>	400	18.80 $\pm$ 8.36**	51.79
Acetyl salicylic acid	100	2 $\pm$ 5.6***	94.87

<sup>a</sup>Administered 30 min before 0.6% v/v acetic acid administration (10 ml/kg, i.p.).

<sup>b</sup>Counted for 20 min, starting 5 min after acetic acid administration; values are mean (8 mice per group)  $\pm$  SEM.

\* $p < 0.05$ , \*\*\* $p < 0.001$  vs. control, ANOVA followed by Tukey's test.

**Table 2.** Antinociceptive effect of *M. piperita* leaf aqueous extract in the hot-plate test

Treatment	Dose (mg/kg, i.p) <sup>a</sup>	Response time <sup>b</sup> (sec)	Analgesia (%)
Control	10 ml/kg	0.65 $\pm$ 1.36	–
<i>M. piperita</i>	200	4.92 $\pm$ 1.29**	20.21
<i>M. piperita</i>	400	4.17 $\pm$ 1.08**	17.13
Morphine <sup>c</sup>	4.6	13.28 $\pm$ 2.53***	54.53

<sup>a</sup>Administered 30 min before thermal stimuli at 55  $\pm$  1°C.

<sup>b</sup>Values are mean (8 mice per group)  $\pm$  SEM.

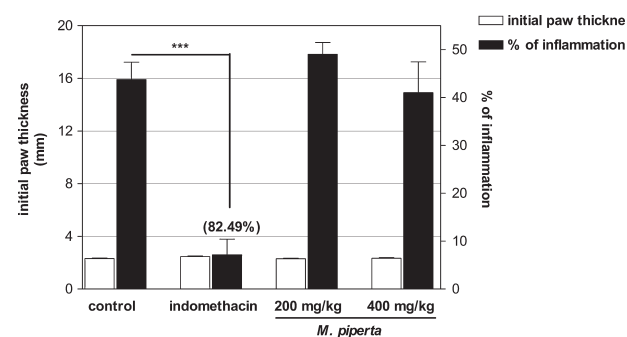
<sup>c</sup>Administered s.c.

\* $p < 0.05$ , \*\*\* $p < 0.001$  vs. control, ANOVA followed by Tukey's test.

reference drug morphine significantly increased the response time of mice to pain ( $p < 0.001$ , Table 2). In different experiment, the aqueous extract of *M. piperita* leaf at 200 and 400 mg/kg failed to protect mice from carrageenan induced acute inflammation assessed by increased paw oedema (Fig. 1). On the other hand, the reference drug indomethacin significantly inhibited the keen inflammation by 82.49% ( $p < 0.001$ , Fig. 1) compared to control mice.

### Discussion

In the present study, three animal models for investigation of the antinociceptive and anti-inflammatory effects of the *M. piperita* leaf aqueous extract were used. The hot plate thermal stimulation and the acetic acid induced writhes in mice were respectively selected to evaluate the central and peripheral antinociceptive activities. Carrageenan-induced paw oedema in mice was selected to represent a model of acute (exudative phase) inflammation.



**Fig. 1.** Effects of the i.p. administration of *M. piperita* leaf aqueous extract and indomethacin on the mouse paw oedema-induced by intraplantar injection of carrageenan at 3rd h. Results are expressed as mean  $\pm$  SEM. of paw oedema thickness. Eight mice were tested per group. Significant difference from control is shown as \*\*\* $p < 0.001$ .

The present study showed that the aqueous extract of *M. piperita* leaf has protective effects against the nociception to the intraperitoneally injected acetic acid-induced writhes, and to thermal pain stimuli. These findings are in agreement with the earlier observations by others (10, 13) who did evaluate the pain-reducing activities of other species of the family Labiatae. Studies by Moreno *et al.*, (13) have shown that the methanolic extract of *Mentha suaveolens* displayed analgesic effects against chemical and mechanical but not thermal stimulation, indicating the induction of only peripheral analgesic effects. Whereas, the studies by Atta and Abo (10) on nociception have demonstrated both central and peripheral analgesic effects for *Mentha microphylla* in mice treated systemically with merely large doses of methanol extract. Nonetheless, the discrepancies in mechanisms of actions seen in this study and others (10, 13) indicate that even though the genus *Mentha* exhibited antinociceptive activities, these effects are species and extract-form dependent.

In contrast, the present study showed that the aqueous extract of *M. piperita* leaf failed to exert anti-oedematogenic effect on the increase in paw thickness induced by carrageenan injection indicating a lack of peripheral anti-phlogestic activity. Hence, it is assumed that this extract has a non-inflammatory pain reliever activity. These data are not completely in line with the previous findings by Atta and Alkofahi, who demonstrated that *M. piperita* produced systemic anti-inflammatory activities (14). One likely explanation for this discrepancy may be that Atta and Alkofahi (14) used ethanolic extract whereas we used an aqueous extract.

On this basis, our present findings indicate that the extract evaluated has analgesic effect arising from both CNS and peripheral actions since it showed significant effects on both thermal and chemical pain stimuli. Such an efficacy on these two stimuli is characteristic of central analgesics, such as morphine, which inhibits inflammatory and non-inflammatory pains (23). Our data also indicate that the active constituents, which produced analgesic but not anti-inflammatory effects, in this extract are relatively polar compounds. In addition, since several studies have shown that the broad spectrum of bioactivity of this plant has usually been ascribed to the components of its essential oil, menthol and flavonoids (2, 10, 13) it seems likely that the *M. piperita* analgesic effect, observed in the present mice study, attributed to one of the/these active constituents.

Thus, as far as the analgesic effects are concerned our results strongly support the traditional indication of *M. piperita* as pain-reducing medicinal plant in folk medicine. Furthermore, these activities affirm the presence of biologically active compounds in the plant.

The pharmacological mechanisms that could contribute to this antinociceptive activity need further studies.

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## Conflict of interest and funding

The author has declared that there is no conflict of interest.

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**\*Yousef A. Taher**

Department of Pharmacology and Clinical Pharmacy  
 Faculty of Pharmacy  
 Tripoli University  
 P.O. Box: 83001  
 Tripoli, Libya  
 Tel: +218-92-3022980  
 Fax: +218-21-4625577  
 Email: ymadane@yahoo.co.uk