

# The Effects of Methanolic Extract of *Melissa officinalis* on Experimental Gastric Ulcers in Rats

Arezoo Saberi,<sup>1</sup> Elham Abbasloo,<sup>1</sup> Gholamreza Sepehri,<sup>2,\*</sup> Mahnaz Yazdanpanah,<sup>2</sup> Ehsan Mirkamandari,<sup>3</sup> Vahid Sheibani,<sup>2</sup> and Zohreh Safi<sup>4</sup>

<sup>1</sup>Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, IR Iran

<sup>2</sup>Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, IR Iran

<sup>3</sup>Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, IR Iran

<sup>4</sup>Endocrinology and Metabolism Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, IR Iran

\*Corresponding author: Gholamreza Sepehri, Professor of Pharmacology, Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, IR Iran. Tel/Fax: +98-3433220081, E-mail: gsepehri@yahoo.com

Received 2014 October 21; Revised 2015 March 15; Accepted 2015 April 11.

## Abstract

**Background:** *Melissa officinalis* (MO) has potent antioxidant activity. Recent research has demonstrated the anti-ulcer properties of some medicinal plants through their antioxidant properties.

**Objectives:** The aim of this study was to evaluate the effects of methanolic extracts of MO on experimental gastric ulcers in rats.

**Materials and Methods:** Male Wistar rats (200 - 250 g) were starved for 24 hours prior to the induction of gastric ulceration by either indomethacin (48 mg/kg/oral) or water immersion restraint (WIR) stress. Experimental rats received either ranitidine (25 mg/kg) or MO extract (150, 300 and 450mg/kg) orally 2 hours prior to WIR stress or indomethacin treatment, for the evaluation of their gastroprotective effects. The control group received the same volume of saline. Gastric lesions were scored according to the surface of lesions on the ulcer index. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) were determined as measures of antioxidant defense, and malondialdehyde (MDA) was determined to measure tissue oxidation.

**Results:** MO extract (150 and 300 mg/kg) significantly decreased the ulcer index in both the indomethacin ( $1.3 \pm 0.09$  and  $1.5 \pm 0.19$ , respectively) and WIR stress groups ( $1.5 \pm 0.17$  and  $1.5 \pm 0.22$ , respectively), as compared to the control rats ( $2.5 \pm 0.28$ ) ( $P < 0.01$ ). MO extract (450 mg/kg) significantly reduced ulcer index readings in WIR stress rats ( $1.8 \pm 0.31$  vs.  $2.4 \pm 0.15$  in the WIR group), however, MO extract at a dose of 450 mg/kg did not prevent indomethacin-induced gastric ulceration ( $2.4 \pm 0.26$ ). There was no significant difference in the ulcer index for MO extract- (150 and 300 mg/kg) and ranitidine-treated rats ( $P > 0.05$ ). Also, MO extract (150 and 300 mg/kg) significantly reduced MDA serum levels ( $0.69 \pm 0.6 \mu\text{mol/L}$  and  $0.85 \pm 0.24 \mu\text{mol/L}$ , respectively, vs.  $4.5 \pm 1.9 \mu\text{mol/L}$  in the saline group) and significantly increased antioxidants' SOD activities ( $296.3 \pm 146.4 \text{ U/mL}$  and  $561.4 \pm 120 \text{ U/mL}$ , respectively, vs.  $190.2 \pm 63.8 \text{ U/mL}$  in the control group) and GPX levels ( $8273 \pm 3049 \text{ U/mL}$  and  $14574 \pm 5012 \text{ U/mL}$ , respectively), compared to the control ( $3236 \pm 1699 \text{ U/mL}$ ).

**Conclusions:** Our results showed that MO extract may have a gastroprotective effect against experimental gastric ulcers in rats. The exact mechanism has not yet been determined, but it may be due to enhancing enzymatic antioxidant defenses and inhibiting lipid peroxidation.

**Keywords:** Anti-ulcer, Ulcer index, Gastroprotective, Antioxidant, Water Immersion Restraint Stress, Indomethacin, *Melissa officinalis*

## 1. Background

Gastrointestinal tract (GIT) ulcers may be induced by the use of non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, alcohol consumption, continuous stress, long periods of starvation, and smoking. *Helicobacter pylori* is the most common cause of gastroduodenal ulcers, followed by the use of NSAIDs, such as aspirin (1,3). Peptic ulcer complications, including hemorrhage and perforation, remain a substantial healthcare problem that has a substantial economic impact on patients (1,4). Although H2-receptor blockers such as famotidine and proton pump

inhibitors (PPIs), including omeprazole, can effectively reduce the risk of peptic ulcer hemorrhage, the prevalence of adverse drug reactions may limit their use, so the search for and development of natural products with fewer adverse effects is of special interest (4).

The smooth muscle cells, macrophage, and metabolism of arachidonic acid generate reactive oxygen species or free radicals that may contribute to gastric mucosal damage. Therefore, natural products containing antioxidants such as flavonoids, tocopherol, and carotenoids may show protective effects against several human diseases related to the reactions of free radicals

(5-8). Antioxidants are potent free radical scavengers or have hydroxyl radical scavenging activity and inhibit the lipid peroxidation of cell membranes with decreases in malondialdehyde (MDA) production as an indicator of lipid peroxidation (9-11).

*Melissa officinalis* (MO) is a plant native to southern Europe and the eastern Mediterranean region. In traditional medicine, MO is used as an anti-seizure, mild sedative and/or calming agent, and for the attenuation of laboratory-induced stress in humans. It has also been shown to improve cognitive performance and mood (12, 13). Other studies have shown the beneficial effect of MO extract in the management of mild to moderate Alzheimer's disease (14).

MO possesses antispasmodic properties and is effective in the treatment of infantile colic (15). Other investigators have reported the radical scavenging properties and antiproliferative activities of extracts of *M. officinalis* (16).

*Melissa officinalis* possesses strong antioxidant activity, as reported by most investigators (17, 18); its major components include  $\alpha$ -tocopherol, rosmarinic acid, hydroxycinnamic acid derivatives, and flavonoids with caffeic acid, *m*-coumaric acid, eriodictyol-7-O-glucoside, naringin, hesperidin, rosmarinic acid, naringenin, and hesperetin (17, 19).

There are several reports indicating the anti-ulcer properties of some medicinal plants through their gastroprotective effects, antioxidant properties, and wound-healing effects (5, 6, 8, 20-22).

## 2. Objectives

Despite the strong antioxidant activity of MO and the contribution of antioxidant activity on gastric mucosal protection, there have been no documented reports on MO antioxidant activity and experimentally induced peptic ulcers, so it was considered important to investigate the possible gastroprotective/or anti-ulcer effects of MO's methanolic extracts on experimental gastric ulcers in rats.

## 3. Materials and Methods

### 3.1. Animals

This experimental study used male Wistar rats (200 - 250g). The rats had free access to food and water, and all rats were kept in 12 hours light/dark cycles. The temperature of the animals' room was kept constant, at  $23 \pm 1^\circ\text{C}$ . The humidity of animal room was the same as that of Kerman city's environment, which ranges between 35 and 42 percent in the spring season.

All of the experiments were designed to minimize the animals' suffering and to use the minimum number of rats ( $n = 6 - 8$ ). The animals were allowed to habituate to the laboratory surroundings for at least one hour before the start of the experiments. Animal care was approved by the local ethical committee of Kerman University of Medical Sciences, according to the guidelines for the Use of laboratory animals (K 92-25).

### 3.2. Preparation of Plant Extract

*M. officinalis* was collected in the spring of 2013 from local herbal medicines stores in Kerman, Iran and identified and confirmed by the department of botany, at Shahid Bahonar University in Kerman. MO leaves were air-dried and powdered, and then 100 g of powder was soaked in 1000 mL of 80% methanol (Merck Company, Germany) for 72 hours. The extract was shaken, filtered, and evaporated in a rotating evaporator under reduced pressure until dryness was achieved. A semi-solid mass of 7.6% W/W was prepared after evaporation and solvent removal of methanolic extract.

The extract was kept in a clean, dried bottle that was placed in a desiccator. A stock solution of the extract was prepared by dissolving 10 g of extract in 100 mL of distilled water to prepare a 100 mg/mL concentration. Stock solution was diluted with distilled water to prepare other concentrations. The extract was given orally by gavage at 0.5 mL/100g BW.

### 3.3. Indomethacin-Induced Gastric Ulceration

Rats from both the control and experimental groups were starved for 24 hours prior to the experiments, with free access to water. Indomethacin (48 mg/kg/oral) was used for induction of gastric ulceration (23). Experimental rats received either ranitidine (25 mg/kg) or MO extract (150, 300 and 450 mg/kg) orally 2 hours prior to indomethacin treatment for the evaluation of their gastroprotective effects. The control group received the same volume of saline.

### 3.4. Water Immersion Restraint (WIR) Stress

After 24 hours starvation, rats received MO methanolic extract (150, 300 and 450 mg/kg) orally, and after 2 hours the animals were restrained in a wire cage and immersed up to xyphoid process in water at  $22 - 23^\circ\text{C}$  for 5 hours to produce WIR stress-induced gastric mucosal lesions (24). Experimental groups received MO extract and ranitidine with the same protocol as the indomethacin-treated rats. The control group received the same volume of saline.

### 3.5. Biochemical Measurements and Histopathological Examination

Four hours after the last dose of the specific treatment, the WIR stress groups and indomethacin treated rats were sacrificed under thiopental (60 mg/kg/i.p.) anesthesia and serum samples were separated for the measurement of indices of lipid peroxidation activity (SOD activity, GPX, and MDA levels). Then, the stomachs were removed and fixed with 10% formaldehyde for 10 minutes and cut open along with the greater curvature. The gastric mucosa was carefully examined for gastric lesions under a stereoscopic microscope ( $\times 10$ ) by two pathologists, working independently, who were blind to the experiments. A kappa coefficient of greater than 80% was used to quantify actual levels of agreement indices between the two pathological results.

Gastric lesions were scored from 1 - 5, according to the surface of mucosal lesions as follows for the determination of the ulcer index: 0, no pathology; 1, a small pinhead ulcer spot; and 2 - 5 for mucosal lesions of 2 - 5 mm length (9). The mean ulcer index was determined as the sum of the total scores divided by the number of animals.

### 3.6. Lipid Peroxidation Measurement

Malondialdehyde (MDA) is the major product of lipid peroxidation, and its measurement has been used as an indicator of mucosal damage. Lipid peroxidation is analyzed by measuring thiobarbituric acid-reactive substances (TBARS) in plasma using the Botsoglou method adapted for our purposes (25). Briefly, the samples (250  $\mu$ L) were mixed with 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid. Then, samples were placed in a boiling water bath for 15 minutes and n-butyl-alcohol (2:1 v:v) was added to the solution. After centrifugation (4000 g, 15 minutes), TBARS were determined from the absorbance at 535 nm, using a spectrophotometer (Pharmacia Biotech; England). The results were expressed as  $\mu$ mol/L.

Superoxide dismutase's (SOD) role is to accelerate the dismutation of the toxic superoxide radical ( $O_2^*$ ), produced during oxidative energy processes, into hydrogen peroxide and molecular oxygen (26). Delmas-Beauvieux et al.'s method was used for superoxide dismutase (SOD) activity (26). SOD activity was measured at 505 nm by a spectrophotometer (Pharmacia Biotech; England). The generation of superoxide radicals by xanthine and xanthine oxidase is the basis of this method. Then, superoxide radicals react with 2[U+2011](4[U+2011]iodophenyl)[U+2011]3 (4[U+2011]nitrophenol)[U+2011]5[U+2011]phenyl tetrazolium chloride (INT) to form a red formazan dye, and

SOD could inhibit this reaction. The degree of inhibition of this reaction is considered the level of SOD activity.

One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. SOD activity value was expressed as U/mL serum.

Glutathione peroxidase (GPX) is an enzyme found in cytoplasmic and mitochondrial fractions of cells. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. The oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. In this reaction, the NADPH is oxidized to  $NADP^+$  simultaneously. A commercial kit was used to measure GPX levels in heparinized whole blood. The decrease in absorbance at 340 nm is then measured by a spectrophotometer (Pharmacia Biotech; England), according to the method used by Paglia and Valentine (27). GPX activity value was expressed as U/mL serum.

### 3.7. Statistical Analysis

The normality distribution of the data was evaluated by box-and-whisker plots and the Kolmogorov-Smirnov (K-S) of normality. The sample size was determined using the comparison of means in two different groups of rats with  $\alpha = 5\%$  and a power of 80%. All of the data were expressed as the means  $\pm$  SD of 6 - 8 rats in each group. The ulcer index values and SOD, GPX, and MDA levels were analyzed using one-way ANOVA, followed by Tukey's test. Differences with  $P < 0.05$  were considered significant.

## 4. Results

### 4.1. The Effects of MO Extract on WIR Stress-Induced Gastric Ulcers

The results of this study showed that gastric mucosal lesions developed in rats subjected to WIR stress over a 5 hours period, and the ulcer index in WIR stress rats ( $2.4 \pm 0.15$ ) was significantly different from the saline-treated rats ( $1.4 \pm 0.29$ ) (Table 1) ( $P < 0.01$ ). MO extract (150, 300 and 450 mg/kg) orally 2 hours prior to WIR stress significantly decreased the ulcer index in the WIR stress groups ( $1.5 \pm 0.17$ ;  $1.5 \pm 0.22$ ,  $1.8 \pm 0.31$ , respectively), as compared to the WIR group ( $2.4 \pm 0.15$ ) ( $P < 0.01$ ). There was no significant difference in the ulcer index in MO methanolic extract- (150 and 300 mg/kg) and ranitidine-treated rats ( $P > 0.05$ ). Also, the ulcer index following MO methanolic extract (450 mg/kg) in WIR stress rats ( $1.8 \pm 0.31$ ) was significantly different from the saline-treated rats ( $1.4 \pm 0.29$ ) ( $P < 0.05$ ) (Table 1).

**Table 1.** The Effects of MO Extract on WIR Stress Induced Gastric Ulcers in Rats<sup>a</sup>

Groups	Ulcer Index, Mean (SD)
Control	1.4 (0.29)
WIR + Saline	2.4 <sup>b</sup> (0.15)
WIR + RAN	1.6 <sup>c</sup> (0.14)
WIR + MO (150)	1.5 <sup>c</sup> (0.17)
WIR + MO (300)	1.5 <sup>c</sup> (0.22)
WIR + MO (450)	1.8 <sup>b,d</sup> (0.31)

Abbreviations: MO, *Melissa officinalis*; RAN, ranitidine; WIR, Water immersion restraint.

<sup>a</sup>The WIR stress method was used for the induction of gastric ulceration. Experimental rats received either ranitidine (25 mg/kg) or MO extract (150, 300 and 450 mg/kg) orally 2 hours prior to WIR stress for evaluation of their gastroprotective effects. The control group received the same volume of saline. N = 6 - 8 rats.

<sup>b</sup>P < 0.01 as compared to control.

<sup>c</sup>P < 0.01 as compared to WIR + saline.

<sup>d</sup>P < 0.05 as compared to WIR + MO extract (150, 300 mg/kg).

#### 4.2. The Effects of MO Extract on Indomethacin Induced Gastric Ulcers

In this study, indomethacin-treated rats showed gastric mucosal lesions over a 4 hours period, and the ulcer index in indomethacin-treated rats ( $2.5 \pm 0.28$ ) was significantly different from that of saline-treated rats ( $1.4 \pm 0.29$ ) ( $P < 0.01$ ). Pretreatment with MO methanolic extract (150 and 300 mg/kg) significantly decreased the development of gastric mucosal lesions ( $1.3 \pm 0.09$  and  $1.5 \pm 0.19$ , respectively), as compared to saline-treated rats ( $1.4 \pm 0.29$ ) ( $P < 0.01$ ). Also, ulcer index ratings in ranitidine-treated rats (25 mg/kg) ( $1.6 \pm 0.14$ ) were significantly lower than indomethacin-treated rats ( $2.5 \pm 0.28$ ) ( $P < 0.01$ ). Our results showed that ulcer index in MO methanolic extract at doses of 450 mg/kg ( $2.4 \pm 0.26$ ) prior to indomethacin was not significantly different from indomethacin-treated rats ( $2.5 \pm 0.28$ ) (Table 2). In other words, MO methanolic extract (450 mg/kg) did not prevent indomethacin-induced gastric ulcerations, as compared to the control rats (Table 2). Also, there was no significant difference in the ulcer index in the MO methanolic extract- (150 and 300 mg/kg) and ranitidine-treated groups ( $1.3 \pm 0.09$  and  $1.5 \pm 0.19$  in MO group, respectively, vs.  $1.6 \pm 0.14$  in the ranitidine group) ( $P > 0.05$ ) (Table 2).

#### 4.3. The Effects of MO Extract on Lipid Peroxidation

The administration of indomethacin caused a significant decrease in the levels of SOD ( $130 \pm 33.3$  U/mL) and GPX ( $2175 \pm 942$  U/mL) and an increase in the malondialdehyde MDA level ( $7.2 \pm 1.1$   $\mu\text{mol/L}$ ), as compared to the control ( $4.5 \pm 1.9$ ) ( $P < 0.01$ ). The administration of MO methanolic extract (150 and 300 mg/kg) reversed the trend. In this study, MO extract (150, 300 mg/kg) caused a significant decrease in MDA levels ( $0.69 \pm 0.6$   $\mu\text{mol/L}$ ,  $0.85 \pm 0.24$   $\mu\text{mol/L}$ , respectively) compared with that of the control group ( $4.5$

$\pm 1.9$   $\mu\text{mol/L}$ ) ( $P < 0.01$  and  $P < 0.001$ , respectively) (Table 3). Also, serum GPX activity was significantly increased in 150 mg/kg and 300mg/kg MO methanolic extract-treated groups ( $8273 \pm 3049$  U/mL,  $14574 \pm 5012$  U/mL vs.  $3236 \pm 1699$  U/mL in the control group) ( $P < 0.001$ ) (Table 3).

Furthermore, the level of SOD activity increased significantly in the MO methanolic extract-treated group (150 mg/kg and 300mg/kg), as compared with those of the saline group ( $296.3 \pm 146.4$  U/mL,  $561.4 \pm 120$  U/mL in MO-treated vs.  $190.2 \pm 63.8$  U/mL in control group;  $P < 0.01$  and  $P < 0.001$ ) (Table 3). However, the effect of high doses of MO methanolic extract (450 mg/kg) on indices of lipid peroxidation activity (SOD activity, GPX, and MDA levels) was not significantly different from the saline treated group (Table 3).

## 5. Discussion

In this study, we examined the effects of antioxidant properties and the gastroprotective effect of MO methanolic extract in experimental gastric ulcers with either WIR stress or indomethacin treatment in rats. The development of gastric mucosal lesions was found to occur with both WIR stress and indomethacin treatment. Our results showed that pretreatment with MO methanolic extract (150 mg/kg and 300 mg/kg) significantly reduced the ulcer index in both groups of rats exposed to either WIR stress/and or indomethacin treatment, the same as in ranitidine treated rats. The administration of indomethacin caused a significant decrease in the levels of SOD and GPX and an increase in the MDA level. This trend was reversed with MO methanolic extract pretreatment. MO methanolic extract significantly increased the SOD and GPX levels as measures of the antioxidative properties of cells and a

**Table 2.** The Effects of MO Extract on Indomethacin Induced Gastric Ulcers in Rats<sup>a</sup>

Groups	Ulcer Index, Mean (SD)
Control	1.4 (0.29)
Ind + saline	2.5 <sup>b</sup> (0.28)
Ind + RAN	1.6 <sup>c</sup> (0.14)
Ind + MO (150)	1.3 <sup>c</sup> (0.09)
Ind + MO (300)	1.5 <sup>c</sup> (0.19)
Ind + MO (450)	2.4 <sup>b,d</sup> (0.26)

Abbreviations: MO, *Melissa officinalis*; RAN, ranitidine; Ind, indomethacin.

<sup>a</sup>Indomethacin (48 mg/kg/oral) was used for the induction of gastric ulceration. Experimental rats received either ranitidine (25 mg/kg) or MO extract (150, 300, and 450 mg/kg) orally 2 hours prior to indomethacin treatment for the evaluation of their gastroprotective effects. The control group received the same volume of saline. N = 6 - 8 rats.

<sup>b</sup>P < 0.01 as compared to control.

<sup>c</sup>P < 0.01 as compared to Ind + saline.

<sup>d</sup>P < 0.05 as compared to Ind + MO extract (150, 300 mg/kg).

**Table 3.** Effect of *Melissa officinalis* (MO) on the Levels of Malondialdehyde (MDA), Superoxide Dismutase Activity (SOD), and Glutathione Peroxidase (GPX) Activity in Rat Serum<sup>a</sup>

Groups	MDA, $\mu\text{mol/L}$	SOD, U/mL	GPX, U/mL
Control	4.5 $\pm$ 1.9	190.2 $\pm$ 63.8	3236 $\pm$ 1699
Ranitidine	4.1 $\pm$ 2.0	225.4 $\pm$ 110.8	5474 $\pm$ 2112
Indomethacin	7.2 $\pm$ 1.1 <sup>b</sup>	130 $\pm$ 33.3 <sup>b</sup>	2175 $\pm$ 942 <sup>b</sup>
<i>Melissa officinalis</i> (150 mg/kg)	0.69 $\pm$ 0.6 <sup>c</sup>	296.3 $\pm$ 146.4 <sup>b</sup>	8273 $\pm$ 3049 <sup>c</sup>
<i>Melissa officinalis</i> (300 mg/kg)	0.85 $\pm$ 0.24 <sup>c</sup>	561.4 $\pm$ 120 <sup>c</sup>	14574 $\pm$ 5012 <sup>c</sup>
<i>Melissa officinalis</i> (450 mg/kg)	4.2 $\pm$ 1.9	165.2 $\pm$ 114	3420 $\pm$ 2483

<sup>a</sup>Indomethacin (48 mg/kg/oral) was used for the induction of gastric ulceration. Experimental rats received either ranitidine (25 mg/kg) or MO extract (150, 300, and 450 mg/kg) orally 2 hours prior to indomethacin treatment for the evaluation of their gastroprotective effects. The control group received the same volume of saline. N = 6 - 8 rats.

<sup>b</sup>P < 0.01 as compared to control group.

<sup>c</sup>P < 0.001 as compared to control group.

significant reduction in MDA levels, which is a biochemical indicator of oxidative damage and lipid peroxidation product.

The development of gastroduodenal ulcers may be modulated by aggressive factors (the rate of acid-pepsin secretion, mucosal blood flow, and acute inflammation) and endogenous protective agents, such as prostaglandins. Gastric ulcers may result in an imbalance between damaging factors, including oxidative stress and protective mechanisms such as antioxidants (1, 28, 29). Reactive oxygen species (ROS) are needed for normal functioning of the organism and usually are generated through numerous normal metabolic processes (29). Oxidative stress is believed to initiate and aggravate many diseases, including peptic ulcers. The mechanism of the development of gastroduodenal ulcers and experimental mucosal damage in rats' stomachs by either indomethacin and /or WIR stress may be through the impairment of the antioxidative enzyme activity of cells and the generation of ROS due to the gen-

eration of lipid peroxides (28).

The anti-ulcerogenic pharmacological effects of many plants is related to their flavonoid contents, which contain multiple OH substitutions with very strong antioxidant activity against peroxy radicals (10, 21). The plant extracts that possess potential antioxidant properties may improve oxidative stress conditions and DNA damage and inhibit the initiation of lipid peroxidation, or they may block the enzymes necessary for the process of lipid peroxidation (30, 31).

The major natural antioxidative components of MO include -tocopherol, rosmarinic acid, and flavonoids, naringin (17, 19). The antioxidant activity of MO methanolic extract has been reported by other investigators, and monoterpene aldehydes and ketones were found to be the most powerful scavenging compounds in MO extract (16, 30, 32, 33). Also, Neral and Beta-Caryophyllene were the most common components in the chemical analysis of essential oils of MO and the presence of phenolic com-

pounds (Naral) is an indicator of antioxidant activity (7). So, the gastroprotective effect of MO methanolic extract could be mediated via either an increase in the levels of the antioxidant enzymes SOD and GPX or a decrease in MDA as a biochemical indicator of cell damage and lipid peroxidation, or its potent natural antioxidant components, such as tocopherol (34), rosmarinic acid, and flavonoids (32). *M. officinalis* aqueous extract in Mn-treated mice significantly inhibited the antioxidant enzyme's activities and attenuated the oxidative damage, which shows its efficacy in attenuating Mn-induced oxidative stress in the mouse brain (35). Lin et al. (2012) also reported that the extract of lemon balm leaves (*Melissa officinalis*) showed Cyclooxygenase-2 (COX-2) inhibitory activities through 12-O-teradecanoylphorbol-13-acetate in KB cells (36).

GPX catalyses the reduction of hydrogen peroxide, and the hydroperoxides formed from fatty acids and superoxide dismutase (SOD) role is to accelerate the dismutation of the toxic superoxide radical ( $O_2^{\bullet}$ ), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen, thus antioxidants can effectively remove toxic peroxides from living cells.

MO methanolic extract's antioxidant activity and its gastroprotective effect may contribute to the reduction of the volume and total of acidity, or increase the gastric fluid's PH (37). Further investigation is needed to determine the effect of different antioxidative components of MO's methanolic extract on gastric ulcers. The gastroprotective effect of herbal plants may be mediated through other mechanism(s), such as wound healing effects, gastric mucosal protection, gastric mucous synthesis, and antibacterial effects against helicobacter pylori, so the anti-ulcer property of MO methanolic extract may be partly mediated through its antioxidant property. Further investigation is necessary to elucidate other possible mechanisms involved in its gastroprotective effects (5, 8, 36). However, MO extract at doses of 450 mg/kg did not prevent WIR stress and indomethacin-induced gastric ulceration. The underlying mechanism has not yet been determined, but MO extract may show toxic effects at doses of 450 mg/kg, which abolish its beneficial antioxidant effects. Further investigation is needed to elucidate whether the occurrence of potential adverse reactions is related to dosages.

Our study has some limitations. First, we did not investigate the effects of major natural antioxidative components of MO methanolic extract on the ulcer index and biochemical parameters. Second, we did not evaluate the underlying MO gastroprotective mechanism(s), which need further investigation and elucidation.

Our results showed that MO methanolic extract may have a gastroprotective effect against experimental gastric ulcers in rats. The exact mechanism has not yet been de-

termined, but it may be partly due to its enhancement of the enzymatic antioxidant defenses (SOD and GPX) and inhibiting the lipid peroxidation of cell membranes with decreases in MDA production as an indicator of lipid peroxidation. Also, the COX2 inhibitory activity of MO extract may play a role in its gastroprotective effects. Further research is needed to investigate the underlying mechanism(s).

## Acknowledgments

This study was carried out in the neuroscience research center of Kerman University of Medical Sciences. The funding for this study was provided by the neuroscience research center of Kerman University of Medical Sciences, Kerman, Iran (Grant No. 92/05/12/9225).

## Footnote

**Authors' Contribution:** This work was carried out in collaboration among all authors. Author Gholamreza Sepehri conducted the study and literature searches. Author Arezoo Saberi designed the study, performed the statistical analysis, and wrote the first draft of the manuscript. Authors Elham Abbasloo, Mahnaz Yazdanpanah, Ehsan Mirkamandari, Vahid Sheibani, and Zohreh Safi managed the analyses and experimental parts of study. All authors read and approved the final manuscript.

## References

1. Lau JY, Sung J, Hill C, Henderson C, Howden CW, Metz DC. Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. *Digestion*. 2011;**84**(2):102-13. doi: [10.1159/000323958](https://doi.org/10.1159/000323958). [PubMed: [21494041](https://pubmed.ncbi.nlm.nih.gov/21494041/)].
2. Abdollahi H, Shokoohi M, Savari M. The Prevalence of Helicobacter pylori babA2, iceA1 and iceA2 Genes and Their Association with Clinical Outcomes in Patients with Chronic Gastritis, Ulcerative Diseases and Non-Ulcer Dyspepsia in South East of Iran. *Jundishapur J Microbiol*. 2013;**6**(4):ee4739. doi: [10.5812/jjm.4739](https://doi.org/10.5812/jjm.4739).
3. Farjadi V, Abtahi H, Zolfaghari MR, Soufian S, Hasanzadeh L. Expression, Purification and Evaluation of Antigenicity of CagA Antigenic Fragment of Helicobacter pylori. *Jundishapur J Microbiol*. 2013;**6**(9):ee7367. doi: [10.5812/jjm.7367](https://doi.org/10.5812/jjm.7367).
4. Hernandez-Diaz S, Martin-Merino E, Garcia Rodriguez LA. Risk of complications after a peptic ulcer diagnosis: effectiveness of proton pump inhibitors. *Dig Dis Sci*. 2013;**58**(6):1653-62. doi: [10.1007/s10620-013-2561-9](https://doi.org/10.1007/s10620-013-2561-9). [PubMed: [23371011](https://pubmed.ncbi.nlm.nih.gov/23371011/)].
5. Barros MP, Lemos M, Maistro EL, Leite MF, Sousa JP, Bastos JK, et al. Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. *J Ethnopharmacol*. 2008;**120**(3):372-7. doi: [10.1016/j.jep.2008.09.015](https://doi.org/10.1016/j.jep.2008.09.015). [PubMed: [18930797](https://pubmed.ncbi.nlm.nih.gov/18930797/)].
6. Mota KS, Dias GE, Pinto ME, Luiz-Ferreira A, Souza-Brito AR, Hiruma-Lima CA, et al. Flavonoids with gastroprotective activity. *Molecules*. 2009;**14**(3):979-1012. doi: [10.3390/molecules14030979](https://doi.org/10.3390/molecules14030979). [PubMed: [19305355](https://pubmed.ncbi.nlm.nih.gov/19305355/)].

7. Lara MS, Gutierrez JI, Timon M, Andres AI. Evaluation of two natural extracts (*Rosmarinus officinalis* L. and *Melissa officinalis* L.) as antioxidants in cooked pork patties packed in MAP. *Meat Sci.* 2011;**88**(3):481-8. doi: [10.1016/j.meatsci.2011.01.030](https://doi.org/10.1016/j.meatsci.2011.01.030). [PubMed: [21345605](https://pubmed.ncbi.nlm.nih.gov/21345605/)].
8. Sumbul S, Ahmad MA, Mohd A, Mohd A. Role of phenolic compounds in peptic ulcer: An overview. *J Pharm Bioallied Sci.* 2011;**3**(3):361-7. doi: [10.4103/0975-7406.84437](https://doi.org/10.4103/0975-7406.84437). [PubMed: [21966156](https://pubmed.ncbi.nlm.nih.gov/21966156/)].
9. Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK. A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. *J Biol Chem.* 2003;**278**(13):10993-1001. doi: [10.1074/jbc.M210328200](https://doi.org/10.1074/jbc.M210328200). [PubMed: [12529378](https://pubmed.ncbi.nlm.nih.gov/12529378/)].
10. Kath RK, Gupta RK. Antioxidant activity of hydroalcoholic leaf extract of *ocimum sanctum* in animal models of peptic ulcer. *Indian J Physiol Pharmacol.* 2006;**50**(4):391-6. [PubMed: [17402269](https://pubmed.ncbi.nlm.nih.gov/17402269/)].
11. Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, et al. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *J Ethnopharmacol.* 2006;**103**(1):59-65. doi: [10.1016/j.jep.2005.06.043](https://doi.org/10.1016/j.jep.2005.06.043). [PubMed: [16169175](https://pubmed.ncbi.nlm.nih.gov/16169175/)].
12. Kennedy DO, Wake G, Savelev S, Tildesley NT, Perry EK, Wesnes KA, et al. Modulation of mood and cognitive performance following acute administration of single doses of *Melissa officinalis* (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology.* 2003;**28**(10):1871-81. doi: [10.1038/sj.npp.1300230](https://doi.org/10.1038/sj.npp.1300230). [PubMed: [12888775](https://pubmed.ncbi.nlm.nih.gov/12888775/)].
13. Kennedy DO, Little W, Scholey AB. Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (Lemon Balm). *Psychosom Med.* 2004;**66**(4):607-13. doi: [10.1097/01.psy.0000132877.72833.71](https://doi.org/10.1097/01.psy.0000132877.72833.71). [PubMed: [15272110](https://pubmed.ncbi.nlm.nih.gov/15272110/)].
14. Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Melissa officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. *J Neurol Neurosurg Psychiatry.* 2003;**74**(7):863-6. [PubMed: [12810768](https://pubmed.ncbi.nlm.nih.gov/12810768/)].
15. Savino F, Cresi F, Castagno E, Silvestro L, Oggero R. A randomized double-blind placebo-controlled trial of a standardized extract of *Matricaria recutita*, *Foeniculum vulgare* and *Melissa officinalis* (Col-Mil) in the treatment of breastfed colicky infants. *Phytother Res.* 2005;**19**(4):335-40. doi: [10.1002/ptr.1668](https://doi.org/10.1002/ptr.1668). [PubMed: [16041731](https://pubmed.ncbi.nlm.nih.gov/16041731/)].
16. Canadanovic-Brunet J, Cetkovic G, Djilas S, Tumbas V, Bogdanovic G, Mandic A, et al. Radical scavenging, antibacterial, and antiproliferative activities of *Melissa officinalis* L. extracts. *J Med Food.* 2008;**11**(1):133-43. doi: [10.1089/jmf.2007.580](https://doi.org/10.1089/jmf.2007.580). [PubMed: [18361749](https://pubmed.ncbi.nlm.nih.gov/18361749/)].
17. Koksal E, Bursal E, Dikici E, Tozoglu F, Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. *J Med Plant Res.* 2011;**5**(2):217-22.
18. de Sousa AC, Alviano DS, Blank AF, Alves PB, Alviano CS, Gattass CR. *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. *J Pharm Pharmacol.* 2004;**56**(5):677-81. doi: [10.1211/0022357023321](https://doi.org/10.1211/0022357023321). [PubMed: [15142347](https://pubmed.ncbi.nlm.nih.gov/15142347/)].
19. Mefthahzade H, Sargsyan E, Moradkhani H. Investigation of antioxidant capacity of *Melissa officinalis* L. essential oils. *J Med Plant Res.* 2010;**4**(14):1391-5.
20. Khayyal MT, el-Ghazaly MA, Kenawy SA, Seif-el-Nasr M, Mahran LG, Kafafi YA, et al. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. *Arzneimittelforschung.* 2001;**51**(7):545-53. doi: [10.1055/s-0031-300078](https://doi.org/10.1055/s-0031-300078). [PubMed: [11505785](https://pubmed.ncbi.nlm.nih.gov/11505785/)].
21. Sannomiya M, Fonseca VB, da Silva MA, Rocha LR, Dos Santos LC, Hiruma-Lima CA, et al. Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. *J Ethnopharmacol.* 2005;**97**(1):1-6. doi: [10.1016/j.jep.2004.09.053](https://doi.org/10.1016/j.jep.2004.09.053). [PubMed: [15652267](https://pubmed.ncbi.nlm.nih.gov/15652267/)].
22. Farzaei MH, Khazaei M, Abbasabadei Z, Feyzmahdavi M, Mohseni GR. Protective Effect of *Tragopogon Graminifolius* DC Against Ethanol Induced Gastric Ulcer. *Iran Red Crescent Med J.* 2013;**15**(9):813-6. doi: [10.5812/ircmj.7845](https://doi.org/10.5812/ircmj.7845). [PubMed: [24616792](https://pubmed.ncbi.nlm.nih.gov/24616792/)].
23. Sharma AV, Ganguly K, Paul S, Maulik N, Swarnakar S. Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibers via VEGF and MMP-2 mediated signaling. *Antioxid Redox Signal.* 2012;**16**(4):351-62. doi: [10.1089/ars.2011.4232](https://doi.org/10.1089/ars.2011.4232). [PubMed: [21942294](https://pubmed.ncbi.nlm.nih.gov/21942294/)].
24. Yasukawa K, Kasazaki K, Hyodo F, Utsumi H. Non-invasive analysis of reactive oxygen species generated in rats with water immersion restraint-induced gastric lesions using in vivo electron spin resonance spectroscopy. *Free Radic Res.* 2004;**38**(2):147-55. [PubMed: [15104208](https://pubmed.ncbi.nlm.nih.gov/15104208/)].
25. Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ, Trakatellis AG. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *J Agric Food Chem.* 1994;**42**(9):1931-17.
26. Delmas-Beauvieux MC, Peuchant E, Dumon MF, Receveur MC, Le Bras M, Clerc M. Relationship between red blood cell antioxidant enzymatic system status and lipoperoxidation during the acute phase of malaria. *Clin Biochem.* 1995;**28**(2):163-9. [PubMed: [7628075](https://pubmed.ncbi.nlm.nih.gov/7628075/)].
27. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 1967;**70**(1):158-69. [PubMed: [6066618](https://pubmed.ncbi.nlm.nih.gov/6066618/)].
28. Kwiecien S, Brzozowski T, Konturek SJ. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. *J Physiol Pharmacol.* 2002;**53**(1):39-50. [PubMed: [11939718](https://pubmed.ncbi.nlm.nih.gov/11939718/)].
29. Tandon R, Khanna HD, Dorababu M, Goel RK. Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. *Indian J Physiol Pharmacol.* 2004;**48**(1):115-8. [PubMed: [15270379](https://pubmed.ncbi.nlm.nih.gov/15270379/)].
30. Pereira RP, Fachineto R, de Souza Prestes A, Puntel RL, Santos da Silva GN, Heinzmann BM, et al. Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*. *Neurochem Res.* 2009;**34**(5):973-83. doi: [10.1007/s11064-008-9861-z](https://doi.org/10.1007/s11064-008-9861-z). [PubMed: [18853256](https://pubmed.ncbi.nlm.nih.gov/18853256/)].
31. Zeraatpishe A, Oryan S, Bagheri MH, Pilevarian AA, Malekiran AA, Baeeri M, et al. Effects of *Melissa officinalis* L. on oxidative status and DNA damage in subjects exposed to long-term low-dose ionizing radiation. *Toxicol Ind Health.* 2011;**27**(3):205-12. doi: [10.1177/0748233710383889](https://doi.org/10.1177/0748233710383889). [PubMed: [20858648](https://pubmed.ncbi.nlm.nih.gov/20858648/)].
32. Dastmalchi K, Damien Dorman HJ, Oinonen PP, Darwis Y, Laakso I, Hiltunen R. Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. *LWT Food Sci Tech.* 2008;**41**(3):391-400.
33. Marongiu B, Porcedda S, Piras A, Rosa A, Deiana M, Dessi MA. Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. *officinalis* and *Melissa officinalis* subsp. *inodora*. *Phytother Res.* 2004;**18**(10):789-92. doi: [10.1002/ptr.1549](https://doi.org/10.1002/ptr.1549). [PubMed: [15551397](https://pubmed.ncbi.nlm.nih.gov/15551397/)].
34. Munne-Bosch S, Alegre L. The significance of  $\beta$ -carotene,  $\alpha$ -tocopherol and the xanthophyll cycle in droughted *Melissa officinalis* plants. *Function Plant Biol.* 2000;**27**(2):139-46.
35. Martins EN, Pessano NT, Leal L, Roos DH, Folmer V, Puntel GO, et al. Protective effect of *Melissa officinalis* aqueous extract against Mn-induced oxidative stress in chronically exposed mice. *Brain Res Bull.* 2012;**87**(1):74-9. doi: [10.1016/j.brainresbull.2011.10.003](https://doi.org/10.1016/j.brainresbull.2011.10.003). [PubMed: [22020131](https://pubmed.ncbi.nlm.nih.gov/22020131/)].
36. Lin JT, Chen YC, Lee YC, Hou CWR, Chen FL, Yang DJ. Antioxidant, anti-proliferative and cyclooxygenase-2 inhibitory activities of ethanolic extracts from lemon balm (*Melissa officinalis* L.) leaves. *LWT Food Sci Tech.* 2012;**49**(1):1-7.
37. Bafna PA, Balaraman R. Anti-ulcer and antioxidant activity of DHC-1, a herbal formulation. *J Ethnopharmacol.* 2004;**90**(1):123-7. [PubMed: [14698519](https://pubmed.ncbi.nlm.nih.gov/14698519/)].