Anti-inflammatory and Antinociceptive Effects of *Melissa Officinalis* L. in Rodents

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**SUMMARY**

*Melissa officinalis* L. (lemon balm) is a traditional herbal medicine widely used as a mild sedative, spasmylytic and antibacterial agent. This paper aimed to examine possible anti-inflammatory, antinociceptive and antioxidant effects of *Melissa officinalis* L. in rats and mice. Anti-inflammatory effect of *Melissa officinalis* L. was investigated with the histamine- and carrageenan-induced paw edema tests. *Melissa officinalis* L. aqueous extract (MEL) was administered by gavage in doses of 50, 100, 200 and 400 mg/kg BW, and compared with indomethacin (10 mg/kg BW), and with a control group (the rats received distilled water). Antinociceptive effect of MEL were evaluated in the same conditions (same doses and compared with indomethacin) using acetic acid-induced writhing and formalin-induced paw licking tests. It was found that pretreatment with MEL significantly reduced inflammatory paw edema in rats and diminished the nociceptive response in mice. At the same time, MEL produced antioxidant effects in carrageenan-induced paw edema and formalin-induced paw licking tests. This study revealed that *Melissa officinalis* L. has an anti-inflammatory and an antinociceptive activity. These effects, at least in part, depend upon the antioxidative properties.

**Keywords** : *Melissa officinalis* L. anti-inflammatory, antinociceptive, antioxidant, rat, mouse

**RÉSUMÉ**

Effets anti-inflammatoires et antinociceptifs de *Melissa officinalis* L. chez les rongeurs

La mélisse officinale (*Melissa officinalis* L.) est une plante médicinale traditionnelle qui est largement utilisée pour ses propriétés calmantes, spasmylytiques et antibactériennes. Cette étude se propose d’examiner les possibles effets anti-inflammatoire, anti-douleur et antioxydant de *Melissa officinalis* L. chez les rats et les souris. L’effet anti-inflammatoire de *Melissa officinalis* L. a été exploré à l’aide de tests basés sur un œdème des pattes induit par du carrageenan ou de l’histamine. L’extrait aqueux de mélisse (MEL) a été administré par gavage à des doses de 50, 100, 200 et 400 mg/kg PV, et comparé à de l’indomethacin (10 mg/kg PV), et à un groupe témoin composé de rats recevant de l’eau distillée. L’effet antinociceptif de MEL a été évalué dans les mêmes conditions en utilisant les tests de contorsion induit à l’acide acétique et de léchage de la patte induit au formol. Il a été trouvé que le traitement avec MEL a réduit significativement l’œdème de patte chimi-induit chez les rats et a diminué la réponse nociceptive chez les souris. Parallèlement, MEL a eu des effets antioxydants dans l’œdème de patte chimi-induit et dans la plai de léchage de la patte induite au formol. Cette étude a montré que *Melissa officinalis* L. a une activité anti-inflammatoire et antinocicepti- ve. Ces effets, au moins en partie, dépendent de ses propriétés antioxydantes.

**Mots-clés** : *Melissa Officinalis* L., anti-inflammatoire, anti-douleur, antioxydant, rat, souris

**Introduction**

*Melissa officinalis* L., also called lemon balm, bee balm, melissa, sweet balm, a member of the mint (Labiatae) family, has been cultivated in the Mediterranean area for about 2000 years. Melissa is the Greek word for bee, and the plant is a favorite of honeybees. It is native of the southern Europe, western Asia, and northern Africa [31]. *Melissa officinalis* is a perennial plant, it growing wild in fields and gardens and along road sides. It is known for it’s lemony flavor and fragrance. Ancient Greeks and Romans used Lemon balm in surgical dressings for wounds and in preparations to treat venomous or infectious bites and stings such as caused by dogs and scorpions. Today, Lemon balm’s primary use involves the treatment of cold sores and teething. It is also commonly used as an antibacterial and antifungal agent. Lemon balm combined with other calming herbs, such as valerian, helps to reduce anxiety and insomnia [5,18]. Lemon balm is recommended to induce sweating and relieve fever due to cold and flu, and to ease menstrual cramps, insomnia, headaches and nervousness [3,15,31,34]. The balm also relieves cramps, dyspepsia, flatulence and colic [4,12,15,34]. Due to lemony smell and pretty white flowers, Lemon balm are extensively cultivated in gardens throughout the world and are commonly used today in perfumery, cosmetics and food industries.

Lemon balm (in Turkish “Ogulotu” or “Kovanotu”) is natively found in coastal part of Mediterranean, Aegean and Marmara regions of Turkey [3]. It is cultivated as a spice (flavouring salads), as a medicinal and as an ornamental plant in Turkey. Its infusion form (2-5.5% m/v) or tincture are traditionally used as sedative, anxiolytic, anti spasmodic, carminative, diaphoretic, digestive and antiseptic in Turkish folk medicine [3,31,34].

Reactive oxygen species (ROS) including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical are important mediators of cellular injury; they participate in the process of acute or chronic inflammation and pain in...
several tissues [6,14,22]. ROS can react with all macromolecules, such as lipids, proteins, nucleic acids, and carbohydrates, particularly polyunsaturated fatty acids on cell membrane. After the beginning of an initial reaction with ROS, a continuing chain reaction is started and cell injury and, ultimately, cell death occurs [17].

Inflammatory diseases and pain are still the most important health problem in the world. Although several agents are known to treat inflammatory disease and pain, their prolonged use often leads to serious side-effects such as gastric intolerance and water and salt retention. That’s why, there is a need to find and develop new anti-inflammatory and analgesic agents with low side-effect. Naturally originated agents with very little side-effects can substitute chemical therapeutics. The aim of this study was to investigate the possible anti-inflammatory and antinociceptive effects of *Melissa officinalis* L. by comparing its anti-inflammatory and antinociceptive potencies with those of conventional anti-inflammatory and analgesic drug. Additionally, antioxidant activity was investigated by measuring whole blood malondialdehyde (MDA) and reduced glutathione (GSH) levels in rats and mice.

**Materials and methods**

**PLANT MATERIAL**

The aerial parts of *Melissa officinalis* L. were collected in July 2003 from Bursa, western Anatolia, in the vicinity of Inegöl. The plant was identified by the department of Botany of Science and Arts Faculty, Atatürk University, Erzurum, Turkey, where a voucher specimen is kept (M03-1).

**EXTRACTION AND PREPARATION OF TEST SAMPLE**

Air-dried *Melissa officinalis* L. was pulverized with a blender. Obtained plant material (200 g) was mixed with 800 mL boiling distilled water and stirred on the hot plate for 15 min. Subsequently, it was filtered over Whatman No.1 paper. Finally, the filtrate was frozen and lyophilized in a lyophilizator (Labconco, Freezone 1L, USA) at 5 μHg pressure at 50 C (13.7 g). Then the extract were dissolved in distilled water. This *Melissa officinalis* L. aqueous extract was called MEL.

**ANIMALS**

Male albino Sprague-Dawley rats weighing 175-220 g and adult male albino mice weighing 25-32g were used for the experiments. All animals were fed with standard laboratory chow and tap water before the experiments. Food and water were ad libitum. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and approval has been received from our institutional Animal Ethics Committee.

**DRUGS**

Carrageenan (Sigma, USA), histamine (Sigma, USA), formaldehyde (Sigma, USA), indomethacin (Deva, Turkey), ketamine (Pfizer, Turkey) and naloxone (Abbott, USA) were used for the experiments. All drugs were dissolved in distilled water.

**ANTI-INFLAMMATORY STUDIES**

Histamine-induced paw edema in rats: MEL was administered by gavage in doses of 50, 100, 200 and 400 mg/kg BW, and compared with indomethacin at a dose of 10 mg/kg, and with a control group, in which the rats received the distilled water (n = 6, for each group). One hour after final drug administration, histamine 0.05 ml (1%, w/v) was subcutaneously injected into the plantar surface of the right hind paw. The paw volume was measured with a plethysmometer before injection, and four times at 1-h intervals after injection. The anti-inflammatory effects in the MEL administered groups were compared with the control and the indomethacin-administered group.

Carrageenan-induced paw edema in rats: MEL was administered by gavage in doses of 50, 100, 200 and 400 mg/kg BW, and compared with indomethacin at a dose of 10 mg/kg BW, and with a control group, in which the rats received the distilled water (n = 6, for each group). One hour after final drug administration, carrageenan 0.1 ml (1%, w/v) solution was subcutaneously injected into the plantar surface of right the hind paw. The paw volume was measured with a plethysmometer before injection, five times at 1-h intervals. The anti-inflammatory activity in animals receiving MEL were compared with that of the indomethacin and of the control group.

**ANALGESIC STUDIES**

Acetic acid-induced writhing test in mice: Distilled water, MEL at a doses of 50, 100, 200 and 400 mg/kg BW, or indomethacin at a dose of 10 mg/kg BW were administered by gavage (n = 6, for each group). One hour after treatment, the mice were injected intraperitoneally with 0.6% (v/v) acetic acid solution (0.1 ml.10g⁻¹ body weight) to induce characteristic writhings. The number of writhings occurring between 10 and 20 min after acetic acid injection was counted. The values from treated group were compared with those of control (distilled water) and indomethacin groups. Additionally, for evaluation of the inhibitor effect of naloxone, 2 mg/kg BW dose of naloxone was administered (subcutaneous injection) after the gavage with 400 mg MEL /kg BW.

Formalin-induced paw licking test in mice: Distilled water, MEL at doses of 50, 100, 200 and 400 mg/kg BW, or indomethacin at a dose of 10 mg/kg BW were administered by gavage (n = 6, for each group). One hour after treatment, 50 microliters of 4% formalin were injected into the plantar surface of the animals’ right hind paw. Mice were placed in chambers with a mirror mounted, and time spent licking the
injected paw (licking time) was recorded. Animals were observed for the first 5 min immediately after formalin injection (early phase) or for 10 min starting at the 20th min postformalin injection (late phase).

**BIOCHEMICAL ANALYSIS**

At the end of experiment, all groups were sacrificed with ketamine (100 mg/kg BW) anesthesia. Blood samples were collected by cardiac puncture using heparinised syringe. Whole blood was collected into heparinized tubes and whole blood MDA and GSH levels were studied on the same day of admission. Whool blood MDA (indicator of lipid peroxidation) levels were measured according to a method of JAIN [16]. The principle of the method was based on the spectrophotometric measurement of the color that occurred during the reaction of thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde–thiobarbituric acid complex and expressed in nmol/mL. Whole blood GSH concentration also was measured by a spectrophotometric method [13].

**STATISTICAL ANALYSIS**

Values were presented as mean ± S.E.M. and were analysed by one-way analysis of variance (ANOVA), following by a pair wise comparison with a Tukey test. All differences showing a p<0.05 were accepted as statistically significant.

**Results**

**ANTI-INFLAMMATORY STUDIES**

In histamin-induced edema, MEL at doses of 50, 100, 200 and 400 mg/kg BW, inhibited respectively for 64.2%, 45.7%, 57.6% and 88.0% the extent of edema, at 3 hours after histamine injection compared to the control group. However, a 76.85% inhibition occurred in the indomethacin group at the same time. Inflamed paw volume changes are presented in Table I.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg BW)</th>
<th>Edema rate percentage (mean+S.E.M, n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>34.2 ± 3.9a</td>
</tr>
<tr>
<td>MEL 50</td>
<td>36.8 ± 5.8a</td>
<td>22.2 ± 6.5a</td>
</tr>
<tr>
<td>MEL 100</td>
<td>36.6 ± 9.5a</td>
<td>20.2 ± 5.5a</td>
</tr>
<tr>
<td>MEL 200</td>
<td>32.4 ± 4.9a</td>
<td>15.8 ± 2.8a</td>
</tr>
<tr>
<td>MEL 400</td>
<td>18.8 ± 1.2b</td>
<td>9.0 ± 3.4b</td>
</tr>
<tr>
<td>Indomethacin 10</td>
<td>23.0 ± 4.9a</td>
<td>19.4 ± 3.8a</td>
</tr>
</tbody>
</table>

a,b,c, Means in the same column with unlike superscripts significantly differ (P<0.05).

**ANALGESIC STUDIES**

MEL at doses of 50, 100, 200 and 400 mg/kg BW, showed significant antinociceptive activity in the writhing test, as
also did 10 mg/kg BW dose of indomethacin (Table III). Antinociceptive activity of MEL at dose of 400 mg/kg BW was partly inhibited by pretreatment of naloxone. In Table IV, it is shown that the doses of 50, 100, 200 and 400 mg/kg BW of MEL caused significant inhibition of the both the early and the late phases of pain stimulus induced by formalin. Furthermore, MEL effects on second phase were more potent than first phase. In early phase, analgesic effect of indomethacin was lower than that of some MEL doses; but in the late phase, it was similar. All doses of MEL significantly but indomethacin insignificantly diminished the formalin-induced paw pain related lipid peroxidation, in mice (Figure 3). Significant increase in the GSH level was seen only for 50, 100 and 400 mg/kg BW MEL administered groups (Figure 4).

Discussion

*Melissa officinalis* L. is a traditional herbal medicine used widely as a mild sedative, spasmylytic, carminative, diaphoretic, digestive, emmenagogue, febrifuge, tonic, antihistaminic and antimicrobial agent [19,30,31,34]. An infusion of the leaves is used in the treatment of hyperthyroidism, fevers, colds, and headaches [2,3,31]. It is also externally used to treat sores, gout, insect bites and as an insect repellent. Phytochemical analyses of the *Melissa officinalis* L. have demonstrated the presence of volatile oil, flavonoids, polyphenols, tannins and triterpenoids [3,4,12,30,31]. These compounds appear to be responsible for the pharmacological actions of *Melissa officinalis* L.. Rosmarinic acid is a naturally occurring hydroxylated compound, present in many plants (e.g. *Rosmarinus officinalis, Melissa officinalis, Salvia officinalis*). It also shows a number of biological activities, such as antiviral, antibacterial, anti-inflammatory (inhibitions of several complement-dependent inflammatory processes) and antioxidant [26-28]. Luteolin and other some flavonoids are present in the constituent of *Melissa officinalis* (24), and luteolin is reported to inhibit NO production, active oxygen species, tumor necrosis factor- (TNF-) production, and metallopeptidases [33]. Those activities of Rosmarinic acid and luteolin may account for the anti-inflammatory action.

The inflammation response is usually initiated by cell injury or antigens, but its symptoms are related to the synthesis and the releasing of chemical mediators such as histamine, serotonin, kinins, reactive oxygen species, prostaglandins and leukotrienes [25]. In evaluation of the non-steroidal anti-inflammatory drug activity, inhibitory effects on cyclooxygenase and lipoxygenase products synthesis, releasing of inflammation mediators and generation of free radicals are considered [20]. Histamine, one of the important inflammation mediators, is a potent vasodilator substance and increases the vascular permeability [7,21]. This study showed that all doses of MEL significantly suppressed the edema produced by histamine at 3 h, so it may be suggested that its anti-inflammatory activity is possibly backed by its antihistaminic activity (Table I). Carrageenan-induced inflammation model is useful for the screening of the anti-inflammatory agents. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation and inflammation characterized by an increase of tissue water content.
and of plasma protein exudation associated with a neutrophil extravasation and increase in metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways [11,32]. MEL showed an anti-edematogenic effect on paw edema induced by carrageenan at 5 h. But this effect was less than that resulted from indomethacin. The maximal effect of the MEL occurred at 5 h (Table II). Indomethacin significantly inhibited the carrageenan-induced paw edema at 4 and 5 h. Depends on these results, it is suggested that anti-edematogenic effects of the MEL on histamine- and carrageenan-induced paw edema may be related to inhibition of inflammation mediators formation.

Acetic acid-induced abdominal writhing is a visceral pain model. The process or release of arachidonic acid metabolites via cyclooxygenase and prostaglandin biosynthesis play a role in the nociceptive mechanism of abdominal writhing induced by acetic acid [10]. Results of this study showed that all doses of the MEL produced a significant antinociceptive effect, and this effect may be due to an inhibition of the synthesis of arachidonic acid metabolites. Only the 400 mg/kg BW dose of MEL was more potent than the indomethacin. On the other hand, blockade of opioid receptors by naloxone diminished the antinociceptive effect of 400 mg/kg BW of MEL (Table III). This result showed that antinociceptive effect of MEL is partly related to opioidergic mechanism. A subcutaneous injection of formalin produces a distinct biphasic nociception. The first (early) phase starts immediately after the formalin injection and continues for 5 min. This phase reflects a direct effect of formalin on nociceptors (non-inflammatory pain). The second (late) phase is made by return to high levels of nociception beginning 15-20 min after the formalin injection and continued until 60 min. This phase reflects inflammatory pain [23]. These phases have different properties and are very useful tools, not only for assessing the potency of analgesics, but also for elucidating the mechanisms of pain and analgesia. Some drugs, such as narcotics, inhibit both phases equally, contrary to non-steroid anti-inflammatory or analgesic drugs that only inhibit the second

### Table II: Effects of Melissa officinalis L. extract (MEL) and indomethacin on carrageenan-induced rat paw edema (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg BW)</th>
<th>Edema rate percentage (mean+S.E.M)</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>38.7 ± 9.8</td>
<td>52.0 ± 10.7</td>
<td>51.3 ± 10.9</td>
<td>51.2 ± 6.1a</td>
<td>46.2 ± 5.6a</td>
</tr>
<tr>
<td>MEL 50</td>
<td>22.2 ± 4.1</td>
<td></td>
<td>28.0 ± 2.9</td>
<td>31.0 ± 3.8</td>
<td>38.4 ± 4.2</td>
<td>36.2 ± 4.5a</td>
<td>28.0 ± 3.0a</td>
</tr>
<tr>
<td>MEL 100</td>
<td>28.0 ± 2.9</td>
<td></td>
<td>31.0 ± 3.8</td>
<td>38.4 ± 4.2</td>
<td>36.2 ± 4.5a</td>
<td>28.0 ± 3.0a</td>
<td></td>
</tr>
<tr>
<td>MEL 200</td>
<td>19.8 ± 3.6</td>
<td></td>
<td>36.6 ± 6.2</td>
<td>34.0 ± 5.0</td>
<td>41.0 ± 5.4a</td>
<td>32.6 ± 2.7b</td>
<td></td>
</tr>
<tr>
<td>MEL 400</td>
<td>28.4 ± 4.6</td>
<td></td>
<td>28.0 ± 5.2</td>
<td>38.6 ± 6.5</td>
<td>37.2 ± 3.7a</td>
<td>27.6 ± 3.3b</td>
<td></td>
</tr>
<tr>
<td>Indomethacin 10</td>
<td>19.6 ± 3.9</td>
<td></td>
<td>24.0 ± 6.4</td>
<td>23.4 ± 4.7</td>
<td>19.0 ± 5.1b</td>
<td>18.8 ± 3.1b</td>
<td></td>
</tr>
</tbody>
</table>

### Table III: Effects of Melissa officinalis L. extract (MEL) and indomethacin on writhing induced by acetic acid in mice (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg BW)</th>
<th>Writhing number (mean ± SEM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>20.2 ± 3.7a</td>
<td>-</td>
</tr>
<tr>
<td>MEL 50</td>
<td>7.7 ± 1.3b</td>
<td>61.9</td>
<td></td>
</tr>
<tr>
<td>MEL 100</td>
<td>6.0 ± 1.9b</td>
<td>70.3</td>
<td></td>
</tr>
<tr>
<td>MEL 200</td>
<td>6.5 ± 1.6b</td>
<td>67.8</td>
<td></td>
</tr>
<tr>
<td>MEL 400</td>
<td>0.3 ± 0.3c</td>
<td>98.5</td>
<td></td>
</tr>
<tr>
<td>MEL + Naloxone</td>
<td>5.7 ± 1.4b</td>
<td>71.8</td>
<td></td>
</tr>
<tr>
<td>Indomethacin 10</td>
<td>2.7 ± 1.5b,c</td>
<td>86.6</td>
<td></td>
</tr>
</tbody>
</table>

a,b,c, Means in the same column with unlike superscripts significantly differ (P<0.05).
In formalin-induced paw licking test, all doses of MEL increased the GSH levels, with respect to control; but only 200 and 400 mg/kg BW doses of MEL lead to a statistically significant increase in the GSH levels, compared to the control, but indomethacin did not (Figure 2). In formalin-induced paw licking test, all doses of MEL increased the GSH levels, with respect to control; but only 50, 100 and 400 mg/kg BW doses of MEL effects were statistically significant (Figure 4). Lipid peroxidation is a well-established mechanism of cellular injury in humans, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds and radicals. These include reactive carbonyl compounds, in which the most abundant is malondialdehyde (MDA). Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems [9,29]. In carrageenan-induced paw edema test, all doses of MEL and indomethacin decreased the MDA levels with respect to control; but only 200 and 400 mg/kg BW doses of MEL and indomethacin effects were statistically significant (Figure 1). In formalin-induced paw licking test, all doses of MEL significantly decreased the MDA levels compared to the control (Figure 3) whereas decreasing effect of indomethacin was not significant.

In conclusion, results of our study showed that Melissa officinalis L. had anti-inflammatory and antinociceptive effects in rats and mice. Furthermore, Melissa officinalis L. decreased the lipid peroxidation in rodents and augmented their antioxidant capacity. On the basis of the results obtained, effects of Melissa officinalis L. on inflammagen-induced edema and chemical-induced nociception, at least in part, may be related to an inhibition of the formation of several inflammation mediators including histamine, and its antiperoxidative and antioxidant properties. Therefore, Melissa officinalis L. could be beneficial in the management of various inflammatory disease and pain therapy instead of chemical agents. However, the mechanism(s) of the anti-inflammatory and antinociceptive effects of Melissa officinalis L. in this are not yet clear. Further studies are necessary in order to establish its other mechanism(s) of anti-inflammatory and antinociceptive actions.

References
