Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions

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Abstract

The relaxant effect of the essential oil of *Melissa officinalis* and its main component, citral, on rat isolated ileum contractions was evaluated. *M. officinalis* essential oil (MOEO) inhibited the response to KCl (80 mM), ACh (320 nM) and 5-HT (1.28 μM) in a concentration-dependent manner with an IC₅₀ of approximately 20 ng/ml. Citral also had a concentration-dependent inhibitory effect on contraction of rat ileum with IC₅₀ comparable to that of MOEO.

Keywords: *Melissa officinalis*; Citral; Essential oil; Spasmolytic; Ileum

1. Introduction

Irritable bowel syndrome (IBS) is a common functional disorder of the intestine, which affects many people. The cause of IBS is not known. Patients with IBS experience alternating bouts of constipation and diarrhoea. Abdominal pain and cramps are among the most common IBS symptoms. Many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders. One of the known natural medicines used for the treatment of IBS is peppermint oil [1] that has spasmyloytic effect. There are other herbal medicines that are traditionally...
being used for intestinal disorders and diarrhoea [2–5]. A common property of these medicinal plants is their spasmolytic activity. Therefore, selection of more effective herbal medicine in this area is of particular interest.

*Melissa officinalis* is a plant growing and cultivated in some parts of Iran. The leaves of lemon balm, *M. officinalis* L. (Lamiaceae) are used in Iranian folk medicine for their digestive, carminative, antispasmodic, sedative, analgesic, tonic and diuretic as well as for functional gastrointestinal disorders [6,7].

Several polyphenolic compounds such as caffeic acid derivatives and flavonoids have been identified in lemon balm [8–10]. The chemical composition of the essential oil of the plant leaf has also been studied. The major compounds were citronellal, citral and β-caryophyllene. Citral is a mixture of two monoterpenes, geranial and neral. Due to its intense lemon aroma and flavour, citral is used widely in foods and cosmetics [8,9,11]. Essential oil of *M. officinalis* was shown to have anti-bacterial, anti-fungal, anti-parasitic and anti-histaminic activities [8,12–14].

In this research we have studied the effect of essential oil of *M. officinalis* on contraction of rat isolated ileum in vitro in order to look for their possible anti-spasmodic activity. In addition, spasmolytic effect of citral, a reputed constituent of *M. officinalis* essential oil (MOEO), was also studied.

2. Experimental

2.1. Plant material

Aerial parts of *M. officinalis* were collected from cultivated plants in Kashan (Iran). The plant was identified by the staff at the Herbarium Department of Iranian Institute of Forest and Rangelands, Isfahan, Iran. A voucher specimen was deposited at Herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran).

2.2. Chemicals

Acetylcholine chloride (ACh), 5-hydroxytryptamine (5-HT), and citral were purchased from Sigma. Citral and the essential oil were made up as 100 µl/ml stock solution in 70% EtOH, further dilutions were made in distilled water. All chemicals, unless stated, were purchased from Merck.

2.3. Preparation and analysis of essential oil

Aerial parts of the plant were dried in shade and the volatile oil was obtained by hydrodistillation for 4 h according to *European Pharmacopoeia* (1975) [15].

The oil was analysed by GC and GC/MS. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (30 m×0.25 mm; film thickness, 0.25 µm). The operating conditions were as follows: carrier gas, helium with a flow rate of 2 ml/min; column temperature,
60–275 °C at 4 °C/min; injector temperature, 280 °C; volume injected, 0.1 μl of the oil; split ratio, 1:25.

GC/MS analysis was performed on a Hewlett-Packard 6890 MS selective detector coupled with Hewlett-Packard 6890 gas chromatograph equipped with a cross-linked 5% PHME siloxane HP-5MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm). The gas chromatography condition was as above. Mass spectrometer conditions were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200 °C; resolution, 1000; scan time, 1 s.

Identification of constituents was based on computer matching against the library spectra Wiley 275.L built up using pure substances and components of known constituents, MS literature data and evaluation of fragmentation patterns of compounds and confirmed by their gas chromatography retention time. The percentage composition of the essential oil computed in each case from gas chromatography peak areas without using correction factors and in comparison with n-decane as standard. A series of n-alkanes (C₅–C₃₀) were also used to calculate Kovats Indices from the gas chromatography analysis. Kovats Indices were calculated by the Kovates equations [16–18].

2.4. Animals

Male Wistar rats weighing 200–250 g were used. They were housed in standard environmental conditions and fed with standard rodent diet.

2.5. Antispasmodic study

Rats were killed and a portion of ileum was removed and placed in oxygenated Tyrode’s solution (NaCl, 139.9; KCl, 2.68; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.42 and glucose 5.55 mM) at room temperature. The tissue was then suspended in Tyrode’s solution at 37 °C and bubbled with O₂. Isotonic contractions were recorded using Harvard isotonic transducer and displayed on a Harvard Universal Oscillograph pen recorder device. Test compounds were added directly to the organ bath at the final concentrations ranging from 2.5 to 75 ng/ml. A concentration–response curve was obtained by cumulative addition of the drugs at 15-min intervals; after that a maximal contraction was reached by adding KCl (80 mM). Also the effects ACh (320 nM) and 5-HT (1.28 μM) induced contraction were evaluated. Each test compound’s concentration was in contact for 15 min with the tissue before their effects being evaluated against ACh and 5-HT. All experiments were conducted in parallel with time-matched controls using the tissue from the same animal and adding an equivalent volume of vehicle.

Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as area under the curve produced by tissue contraction and expressed as a percentage of control response for each tissue.

2.6. Statistical analysis

Mean and standard error of mean (S.E.M.) values were calculated for each group of results and significance of differences between the means was calculated by two-
Table 1
Chemical composition of *M. officinalis* essential oil

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Octen-3-ol</td>
<td>1.3</td>
</tr>
<tr>
<td>6-Methyl-5-hepten-2-one</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Trans</em>-β-ocimene</td>
<td>0.5</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.9</td>
</tr>
<tr>
<td><em>Para</em>-menth-3-en-8-ol</td>
<td>0.2</td>
</tr>
<tr>
<td>Citronellal</td>
<td>12.9</td>
</tr>
<tr>
<td>Citronellol</td>
<td>6.2</td>
</tr>
<tr>
<td>Neral</td>
<td>24.5</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0.7</td>
</tr>
<tr>
<td>Geranial</td>
<td>35.3</td>
</tr>
<tr>
<td>α-Cubebene</td>
<td>0.2</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.1</td>
</tr>
<tr>
<td>Geranyl acetate*</td>
<td>7.1</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>4.9</td>
</tr>
<tr>
<td>Germacrene</td>
<td>0.2</td>
</tr>
<tr>
<td>Eugenol acetate</td>
<td>0.1</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* May be an artefact.

Paired Student’s *t*-test or by one way analysis of variance (ANOVA). Differences were considered statistically significant when *P* < 0.05. Origin computer program was used for fitting non-linear curve and for the calculation of the IC<sub>50</sub> value (IC<sub>50</sub> = test compound concentration causing 50% of inhibition).

3. Results

3.1. Analysis of the essential oil

The aerial parts of *M. officinalis* yield 0.1% of a yellowish essential oil with a pleasant lemon odour. Seventeen constituents that were identified in the essential oil, account for 98.1% of the total oil components (Table 1). The oil was rich in monoterpenes and the major components were geranial (35.3%), neral (24.5%) and citronellal (12.9%).

3.2. Spasmolytic activity

Rat ileum suspended in Tyrode’s solution under 1 g of tension after 15 min had a stable tension. KCl (80 mM) produced a sustained tonic contraction, which was maintained during the course of experiments. ACh (320 nM) and 5-HT (1.28 μM) induced a phasic contraction in the tissue, reaching the peak within 30 s of contact. *M. officinalis* essential oil in a concentration-dependent manner (2.5–75 ng/ml) inhibited the ileum contraction induced by 80 mM KCl with an IC<sub>50</sub> value of 19 ± 2.1 ng/ml (Fig. 1). These inhibitory effects of essential oil could be seen
within 5-min contact with the tissue and were maintained as long as it was present in the bath and persisted 10–15 min after washing. As shown in Fig. 1, citral, a major constituent of the essential oil, also inhibited the tonic contraction induced by KCl in a concentration-dependent manner with an IC$_{50}$ value of $29\pm 3.7$ ng/ml ($n=6$). During the course of experiments there was a small reduction in the tonic responses of the tissues treated with equivalent volume of the vehicle (ethanol) but these changes were not statistically significant (ANOVA).

*M. officinalis* essential oil reduced significantly the effect of ACh response with an IC$_{50}$ of $20\pm 2.1$ ng/ml. Moreover, it reduced the response to 5-HT with an IC$_{50}$ value of $20\pm 4.1$ ng/ml (Fig. 3). Similar to the effect of essential oil, citral inhibited the contraction induced by ACh and 5-HT with an IC$_{50}$ of $32\pm 2.1$ ng/ml (Fig. 2) and $19\pm 3.5$ ng/ml (Fig. 3), respectively. Equivalent amount of the maximum volume of the vehicle (ethanol) used in this research caused no significant changes to the ACh and 5-HT response.

4. Discussion

Current therapy for some gastrointestinal disorder is directed towards inhibition of smooth muscle contractions. It is well known that herbal medicines are traditionally used for their spasmolytic activity [2–5]. The search of a new natural medicine with this property is still an important field of a research. Therefore, the objective of this work was to study the effect of *M. officinalis* essential oil and its main constituent, citral, on contractile activity induced by three different spasmogens on ileum to seek for scientific evidence for beneficial use of this herbal medicine.
Fig. 2. Effect of *M. officinalis* essential oil and citral on isolated rat ileum, on tension development to acetylcholine.

Fig. 3. Effect of *M. officinalis* essential oil and citral on isolated rat ileum, on tension development to 5-HT.
in gastrointestinal disorders. Both the essential oil and citral, had potent inhibitory effect on contraction of ileum induced by KCl, 5-HT and ACh. It seems that the inhibitory effect of the essential oil is closed to the effect of citral. As it can be seen from Fig. 1, at equal bath concentrations the inhibitory effect of essential oil is marginally better than that of citral. Adding that citral contributes for 60% of the constituents of the essential oil, it can be concluded that although citral has a major role in the inhibitory effect seen by MOEO but other constituents of MOEO also have some contribution.

The ileum contraction induced by KCl is a direct affect on ileum smooth muscle. High concentration of KCl added extracellularly results in cell depolarisation and consequently, activation of voltage-dependent calcium channels and Ca\(^{2+}\) influx into smooth muscle cell. Therefore, agents that inhibit contraction induced by KCl should somehow inhibit the entry of Ca\(^{2+}\) ions or otherwise inhibit the intercellular contraction mechanism. ACh, on the other hand, interact with muscarinic receptors on ileum smooth muscle cell membrane [19] and thereby increases the activity of membrane bound phospholipase-C enzymes and generation of inositol triphosphate (IP\(_3\)). IP\(_3\) being released in the cytoplasm will interact with the receptors on intracellular Ca\(^{2+}\) store sites and causes the release of intracellular Ca\(^{2+}\) stores. Competitive antagonists of muscarinic receptors, like atropine and dicyclomine, antagonised the response to ACh by antagonising the muscarinic receptors and, therefore, without altering the maximum response they shift ACh concentration–response curve to the right [2].

The 5-HT contraction is mediated by the release of ACh from the cholinergic neurone as well as activation of serotonergic receptors on the smooth muscles of ileum [20]. *M. officinalis* essential oil and citral relax the ileum contraction due to depolarisation (KCl) and inhibition of agonist effect on muscarinic or serotonergic receptors. This inhibitory effect may indicate a more general inhibitory mechanism of action, which includes a significant inhibition effect on Ca\(^{2+}\) ion channels [21]. In addition, by means of electrophysiological technique, it has been shown that peppermint oil can block L-type Ca\(^{2+}\) channels in the ileum [1].

In conclusion, this study showed that *M. officinalis* essential oil and its main component, citral, possess a significant inhibition effect on ileum contractions. Therefore, it seems to be a useful herbal medicine for the treatment of gastrointestinal spasms. However, further studies need to look for its possible action on other smooth muscle and any unwanted effect on other organs.

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**References**