Effect of essential oil of *Anthemis mauritiana* Maire & Sennen flowers on intestinal smooth muscle contractility

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Abstract

The aim of the present study is to determine the chemical composition of the essential oil extracted from the flowers of *Anthemis mauritiana* Maire & Sennen (EOAM) and to investigate its antispasmodic effects on intestinal smooth muscle. The phytochemical composition was revealed by gas chromatography-mass spectrometry. Eighteen compounds were identified representing 90.56% of the oil. The major constituents were described as α-pinene (27.02%), sabinene (15.25%), cedrenol (14.53%) germacrene (9.61%) geraniol formate (6.82%), and caryophylene (5.38%). EOAM (10–100 µg/ml) elicited reversible relaxation of spontaneous contractions of isolated rabbit jejunal smooth muscle preparations, and similarly inhibited contractions induced by high-potassium solution ([K⁺]₀ = 76 mM) and carbachol (10⁻⁶ M) with IC₅₀ values of 14.98 and 27.29 µg/ml, respectively. Furthermore, EOAM exhibited an inhibitory effect on the dose-response curves induced by carbachol and CaCl₂ on rat jejunum preparations. These results clearly demonstrated the antispasmodic effect of EOAM which was strongly suggested to be mainly due to an inhibitory effect on Ca²⁺ influx through the membrane of jejunal smooth muscle cells.

Key words: essential oil, *Anthemis mauritiana* Maire & Sennen, jejunum, antispasmodic

Introduction

Gastrointestinal diseases consist of different combinations of chronic or recurrent symptoms, with physiological, biochemical, infectious, anatomical or structural causes that are not identifiable (Drossmann et al., 1990). Irritable bowel syndrome (IBS) is one such disease.
It is a bowel disorder characterized by a constellation of symptoms including abdominal pain associated with one or more symptoms of altered bowel function such as diarrhea, constipation or alternating diarrhea and constipation. It is a common functional disorder of the intestine, which affects many people. In developing countries, these diseases are a major health care problem. Medicinal herbs constitute a major component of the traditional medicine practiced worldwide due to their economic viability, accessibility and ancestral experience. In view of the wide traditional uses of medicinal plants in Morocco, the essential oil of *Anthemis mauritiana* Maire & Sennen flowers (EOAM) was studied for its possible spasmolytic activity.

The genus *Anthemis* (Asteraceae, syn. Compositae) is the second largest genus in this family, with more than 210 species that occur in the Mediterranean region, southwest Asia and eastern Africa (Oberprieler, 2001). *Anthemis mauritiana* Maire & Sennen is an endemic species that is distributed in Morocco. The species of this genus are widely used in the pharmaceutical, cosmetic and food industries. The active chemical components of these flowers and their applications have been well-documented. Their main components are natural flavonoids and essential oils (Williams et al., 2001; Saroglou et al., 2006). In the Mediterranean region, tinctures, tisanes, and salves of this genus are widely used as anti-inflammatory, antioxidant, antibacterial, and antispasmodic agents (Papaioannou et al., 2007, El Hanbali et al., 2007, Maschi et al., 2008).

**Materials and Methods**

**Solutions and drugs**

Normal Krebs-Henseleit buffer (KHB) solution composed of (in mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose 10. High K⁺ KHB ([K⁺]₀ = 76.2 mM); NaCl 48, KCl 75, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 10. Calcium-free high K⁺ KHB ([K⁺]₀ = 76.2 mM); NaCl 48, KCl 75, CaCl₂ 0, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 10. Calcium-free KHB; NaCl 121.7, KCl 4.7, CaCl₂ 0.0, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 10, made up in distilled water, and the pH of the solution was adjusted to 7.4.

The following drugs were used for the experiments: carbamylcholine chloride (carbachol, CCh) and dimethyl sulfoxide (DMSO) were purchased from Prolabo, papaverine hydrochloride from Fluka and verapamil hydrochloride from Sigma.

**Plant material**

Fresh plant material was collected locally during the flowering period (in May) from the north eastern area of Morocco, and botanically identified by Professor B. Haloui at the Department of Biology, Faculty of Science University Mohammed I Oujda, Morocco. A voucher specimen (No. 64666) had been previously deposited with the Scientific Institute of Rabat.

**Isolation of the essential oil**

Air-dried flowers (100 g) of *Anthemis mauritiana* were subjected to water distillation for 3 hours using a Dean stark apparatus (yield: 0.15%). The extracted oil was stored at +4°C.
Effect of essential oil extracted from flowers on intestinal contraction

**Gas chromatography-mass spectrometry (GC/MS) analysis**

The essential oil was analyzed by gas chromatography/mass spectrometry using the Trace GC Ultra/Polaris Q system. A VB5 column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness) was used with helium as the carrier gas (1 ml/min). The GC oven temperature was kept at 50°C for 5 min and programmed to increase to 250°C at a rate of 4°C/min, then to be kept constant at 250°C for 3 min and then to increase to 300°C at a rate of 25°C/min. Split flow was used, and the injector temperature was held at 250°C.

Mass spectrometry was performed at 70 eV. The mass range was from m/z 20 to 350, with the temperature of the interface held at 300°C. A library search was carried out using both the “Wiley GC/MS Library” Nist and PMW (Pfleger Maurer Weber).

**Spasmolytic study**

The spasmolytic activity of the essential oil was studied using isolated jejunum preparations from rabbits and Wistar rats. The animals were killed by cervical dislocation only. No anesthetic agent was administered that may have influenced the relaxation responses of the tissue. Segments of jejunum (2 cm) were removed and mounted in 10 ml organ baths containing Krebs-Henseleit buffer (KHB). The bath solution was maintained at 37°C, pH 7.4 and gassed continuously with air bubbling. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. EOAM was dissolved in DMSO (1%) and added to the organ bath. Each concentration of the essential oil was in contact with the tissue for at least 7–10 min before its effect was evaluated.

**Animals**

New Zealand rabbits (1.5–2 kg) and Wistar rats (200–250 g) were maintained under standard conditions in the animal house of the Department of Biology, Mohammed the First University, Oujda, Morocco, and fed with a standard diet with water ad libitum. Animals were fasted overnight prior to the experiments. All procedures concerning animals were carried out ethically following the guidelines set by the World Health Organization.

**Effect of EOAM on spontaneous contractions of jejunum segments**

After stabilization of the spontaneous smooth muscle contractions of segments of the rabbit jejunum, cumulative doses of EOAM were added (10–100 µg/ml).

**Relaxant effect on contractions produced by high-K+ solution and carbachol**

The segments of jejunum were contracted with high-K+ KHB ([K+]o = 76.2 mM) or carbachol (10⁻⁶ M) until the tone was maintained. At this point the essential oil was added to the bath.

**Inhibition of the dose-response to carbachol**

Cumulative dose-response curves for carbachol were obtained from rat jejunum preparations according to the method of Van Rossum (1963). After a stabilization period of 60 min, carbachol (10⁻⁸–10⁻⁵ M) was added to the organ bath, and different doses of the EOAM were added to the bath 5 min before commencing the dose-response curve of the agonist.
Inhibition of the dose-response to CaCl₂

After an initial incubation period of 60 min in normal KHB solution, the nutrient solution was replaced by calcium-free KHB for a period of 15 min, then replaced by calcium-free high-K⁺ KHB ([K⁺]₀ = 76.2 mM). Cumulative dose-response curves to CaCl₂ (0.1, 0.3, 1, 3, 10 mM) were obtained in rat jejunum preparations in the presence of different doses of essential oil (Farre et al., 1991).

Statistics

The results are expressed as the mean ± S.E.M. The statistical significance of data was analyzed using the Student’s t-test, with a P<0.05 considered to be significant. The 50% inhibitory concentration (IC₅₀) was determined using linear regression.

Results

Chemical composition of Anthemis mauritiana essential oil

GC-MS analysis of the essential oil resulted in the identification of eighteen compounds representing 90.56% of the oil. The major compounds were found as α-pinene (27.02%), sabinene (15.25%), cedrenol (14.53%) germacrene (9.61%) geraniol formate (6.82%), and caryophylene (5.38%), (Fig. 1, Table 1).

Antispasmodic effect of EOAM

When tested on smooth muscle preparations of spontaneously contracting rabbit jejunum, EOAM exhibited a spasmolytic effect in a dose-dependent manner (10–100 µg/ml), with the contraction at 100 µg/ml being totally reversible at wash-out. The median effective concentration (EC₅₀) was 32.26 ± 1.48 µg/ml (Fig. 2). Similarly, verapamil relaxed contractions with EC₅₀ values of 0.29 ± 0.03 µM and a total inhibition at 1 µM. With papaverine, even doses (30 µM) which were much higher than that of verapamil did not totally inhibit the contractions.

In rat jejunum preparations, carbachol yielded concentration-dependent contractions. The essential oil of this plant inhibited the jejunum contractions induced by carbachol (10⁻⁶ M) in a concentration-dependent manner with an IC₅₀ value of 27.29 µg/ml (Fig. 3). Moreover, to assess whether the spasmyotic activity of the tested EOAM involved the blockade of calcium channels, high-K⁺ KHB ([K⁺]₀ = 76.2 mM) was used to depolarize the preparations as described previously. High-K⁺ KHB was added to the tissue bath, in order to produce a sustained contraction, and addition of essential oil significantly reduced the maximal response to the high-K⁺ KBH in a concentration-dependent manner with an IC₅₀ value of 14.98 µg/ml (Fig. 4).

To confirm the calcium channel blocking activity of the test substances, dose-response curves of CaCl₂ were constructed in the absence and presence of EOAM in rat jejunum preparations. Pretreatment of the tissue with the plant essential oil (30, 50 and 100 µg/ml) shifted the CaCl₂ curves to the right (Fig. 5). Verapamil (10⁻⁶ M) produced a similar effect to that of 100 µg/ml of EOAM (Table 1).

In the rat jejunum, EOAM had also a significant inhibitory effect on the concentration-response curve produced by carbachol by reducing the maximum induced contraction (Fig. 6).
Papaverine ($10^{-5}$ M) produced a more potent relaxation effect than 100 $\mu$g/ml of EOAM (Table 2).

### Discussion and Conclusion

The therapy for some gastrointestinal disorders is directed towards the inhibition of smooth muscle contraction. In addition, herbal medicines are traditionally used for their spasmolytic activity (Bellakhdar, 1997). The present data show that the EOAM exerts concentration dependent reversible inhibitory effects on the contractile responses of the smooth muscle of isolated rabbit jejunum preparations. It is known that contraction of smooth muscle begins with...
an increase in the cytosolic concentration of Ca$^{2+}$, with the extra Ca$^{2+}$ coming either from the extracellular medium or from the sarcoplasmic reticulum (Karaki and Weiss, 1988). The inhibitory effect of the EOAM on spontaneous movements of the jejunum may be due to interference either with release of calcium ions from the sarcoplasmic reticulum or with Ca$^{2+}$ influx through voltage dependant Ca$^{2+}$ channels (VDCs). When the potassium ion concentration in the extracellular medium was increased to 30 mM and above, a depolarization of the membrane occurred, and consequently the VDCs opened and allowed Ca$^{2+}$ ions to enter the cytoplasm (Bolton, 1979). Agents that inhibited the contraction induced by KCl either inhibited the entry of Ca$^{2+}$ in some way or otherwise inhibited the intercellular contraction mechanism (Godfraind et al., 1986). Therefore, inhibition of the contraction of the rat jejunum by the EOAM reflects a reduction in the entry of Ca$^{2+}$ through VDCs. This hypothesis is further strengthened by the observation that when preparations were pretreated with the plant essential
Fig. 3. Relaxant effects of different concentrations of EOAM (0–90 µg/ml) on carbachol (10⁻⁶ M)-induced contractions in rabbit jejunum preparations. Amplitude of contractions relative to that produce in the absence of EOAM is shown (mean ± S.E.M, n=6). *, P<0.05; **, P<0.01 and ***, P<0.001 were statistically significant difference from control.

Fig. 4. Relaxant effects of different concentrations of EOAM (0–50 µg/ml) on high-K⁺-induced contractions ([K⁺]₀ = 75 mM) in rabbit jejunum preparations. *, P<0.05; **, P<0.01 and ***, P<0.001 were statistically significant difference from control (mean ± S.E.M, n=6).
Fig. 5. In rat jejunum preparations, a series of concentration-response curves were obtained after cumulative application of CaCl₂ in the presence of different concentrations of EOAM (● 0 µg/ml; ○ 30 µg/ml; ▲ 50 µg/ml; ■ 100 µg/ml). Concentrations of CaCl₂ are shown in logarithmic scale (mean ± S.E.M, n=6). *, P<0.05; **, P<0.01 and ***, P<0.001 were statistically significant difference from control (mean ± S.E.M, n=6).

Fig. 6. In rat jejunum preparations, a series of concentration-response curves were obtained after cumulative application of carbachol (CCh) in the presence of different concentrations of EOAM (● 0 µg/ml; ○ 30 µg/ml; ▲ 50 µg/ml; ■ 100 µg/ml). The amplitude of contraction was measured as relative to that produced by 10⁻⁵ M carbachol in the absence of EOAM. Mean ± S.E.M. (n=6; *, P<0.05).
oil, there was a concentration-dependent rightward shift in the concentration-response curves of CaCl$_2$ (Rojas et al., 1996).

Carbachol, a cholinomemetic drug, interacts with muscarinic receptors on intestinal smooth muscle cell membranes (Goyal, 1988). This effect is mediated by phospholipase C and inositol triphosphate (IP3). The latter, interacts with receptors in the endoplasmic reticulum and releases the stored reticulum Ca$^{2+}$ into the cytoplasm. Competitive antagonists of muscarinic receptors antagonised the response to ACh by antagonising muscarinic receptors and, therefore, without altering the maximum response they shift the ACh concentration-response curve to the right (Hajhashemi et al., 2000). We conducted an experiment by measuring the change of contraction elicited by carbachol in Ca$^{2+}$-free solution. The results showed that EOAM had an effect on the release of Ca$^{2+}$ from internal stores (data not shown here). Papaverine ($10^{-5}$ M), used as a positive control, caused a relaxation in the smooth muscle of the jejunum to a similar extent as 100 µg/ml EOAM. It causes relaxation by multiple mechanisms, but mainly via the intracellular accumulation of cAMP and/or cGMP, by inhibiting phosphodiesterase, by having effects on Ca$^{2+}$ movement and inhibition of mitochondrial respiration, although in ileal smooth muscle it is largely due to the last mechanism (Kaneda et al., 1998, 2005). Thus the inhibitory effect of EOAM on the carbachol concentration-response was like a noncompetitive antagonism attenuating the maximum response (Hajhashemi et al., 2000; Gilani et al., 2005). It is worth noting that $\alpha$-pinene is a major constituent of EOAM and according to Sadraei et al. (2001) this compound exhibits an inhibitory effect on the tonic contraction induced by either KCl or ACh. Thus, it is possible that the in vitro inhibitory effect on the jejunum contractions may be due to $\alpha$-pinene. However, the presence of other spasmolytic compound(s) must not be excluded. Further studies are needed to understand the mechanism of action of these EOAH compounds.

In conclusion, this study showed that *Anthemis mauritiana* essential oil exerts a significant inhibitory effect on contractions of the rodent jejunum and may explain some of the traditional use of the plant in the treatment of various gastrointestinal disorders. However, further studies are required to determine its actions on different smooth muscle and to examine the possibility of any unwanted effects on other organs.

### Table 2. EC$_{50}$ and maximum effect values obtained from the cumulative dose-response curves to carbachol (CCh) and CaCl$_2$ in rat jejunum preparations

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>CCh</th>
<th>CaCl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$ (M)</td>
<td>Emax ± S.E.M.</td>
</tr>
<tr>
<td>Control</td>
<td>$6.58 \times 10^{-6}$</td>
<td>100</td>
</tr>
<tr>
<td>EOAM (µg/ml)</td>
<td>$2.36 \times 10^{-5}$</td>
<td>$85.4 \pm 2.65$</td>
</tr>
<tr>
<td>30</td>
<td>$2.74 \times 10^{-5}$</td>
<td>$65.2 \pm 0.92^*$</td>
</tr>
<tr>
<td>50</td>
<td>$2.81 \times 10^{-5}$</td>
<td>$49.1 \pm 4.83^*$</td>
</tr>
<tr>
<td>100</td>
<td>$2.63 \times 10^{-5}$</td>
<td>$55.9 \pm 2.06^*$</td>
</tr>
<tr>
<td>Papaverine ($10^{-5}$ M)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Verapamil ($10^{-6}$ M)</td>
<td>–</td>
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</tbody>
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*, $P<0.05$ and **, $P<0.01$ were statistically significant difference from control.
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