Journal of Ethnopharmacology 313 (2023) 116531

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Essential oil of Sicilian *Prangos ferulacea* (L.) Lindl. and its major component, β−ocimen, affect contractility in rat small and large intestine

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ARTICLE INFO

Keywords:
Apiaceae essential oil
Rat duodenum
Rat colon
Contractile activity
Calcium handling

ABSTRACT

Ethnopharmacological relevance: *Prangos ferulacea* (L.) Lindl is an Apiaceae plant, widely used in traditional medicine. Recently, chemical composition and biological activities of its essential oil (Prangroil) have been reported, but there are no studies on possible effects on intestinal contractility.
Aims of the study: We investigated the effects of essential oil Sicilian Prangoil on the contractility of rat small (duodenum) and large (colon) intestine and the related action mechanism.
Materials and methods: Responses to Prangoil and to its major component β−ocimen in intestinal segments were assessed *in vitro* as changes in isometric tension.
Results: Prangoil, induced in duodenum, depending upon doses, contraction and/or muscular relaxation. Instead, in colon Prangoil only reduced the phasic contractions and induced muscular relaxation. β−ocimen, in both segments, produced only reduction of the spontaneous contractions without affecting basal tone. Prangoil contractile effects were abolished by ω-conotoxin, neural N-type Ca^{2+} channels blocker, atropine, muscarinic receptor antagonist, neostigmine, acetylcholinesterase (AChE) inhibitor, suggesting that Prangoil-induced contraction would be the result of an increase in neuronal cholinergic activity. Prangoil and β−ocimen inhibitory effects were unaffected by ω−conotoxin, L-NAME, blocker of the NO synthase, ODQ, soluble guanylate cyclase inhibitor, excluding involvement of neurotransmitter release or NO synthesis in the inhibitory effects. Potassium channel blocker did not affect Prangoil or β-ocimen inhibitory responses. Prangoil or β-ocimen inhibited the Ca^{2+} and high-KCl solution -induced contractions and the Carbachol-induced contractions in calcium free solution.
Conclusion: Prangoil affects the contractility of small and large intestine in rat, with regional differences, via potentiation of neural cholinergic activity, blockade of L-type voltage-gated calcium channel and reduction of Ca^{2+} release from the intracellular store. The Prangroil main components, β−ocimen, contributes to the inhibitory effects.

1. Introduction

Visceral pain is a common symptom in gastrointestinal disorders, caused by smooth muscles spasms. So far, actual drug therapies present high incidence of systemic side effects (Chiou and Nurko, 2011; Grundy et al., 2019; Kim et al., 2020). As a result, there has been an increased interest towards the use of alternative therapeutic options for symptomatic treatment of functional dyspepsia, intestinal and colonic cramps, as botanic remedies, which are important source of antispasmodic compounds and perceived as “safe and natural” (Gwee et al., 2021; Pike et al., 2013).

Medicinal plants rich in essential oils are considered valuable and easily accessible natural resources for the development of new molecules, capable of becoming drug candidates. Essential oils are complex mixtures of volatile aromatic compounds; their composition varies among the plants by numerous factors including the seasonal variations, genetic, plant organ geographical origins or degree of maturity of the plant (Anwar et al., 2009; Fokou et al., 2020; Marotti et al., 2011).

Essential oils showed biological effects since they easily cross cellular membranes, influencing several molecular targets as ion channels or intracellular enzymes (Bakkali et al., 2008; Elshafie and Camele, 2017).

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https://doi.org/10.1016/j.jep.2023.116531
Received 7 March 2023; Received in revised form 12 April 2023; Accepted 18 April 2023
Available online 8 May 2023

Abbreviations

AChE	Acetylcholinesterase
CCh	Carbamylcholine chloride
DMSO	dimethysulphoxide;
EGTA	Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N'-tetraacetic acid tetrasodium salt
E_{MAX}	Maximal effect
GC-MS	Gas chromatography–mass spectrometry
L-NAME	Nω-nitro-l-arginine methyl ester
ODQ	1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one
Prangoil	*Prangos ferulacea* (L.) Lindl essential oil
TEA	tetraethylammonium

Both *in vitro* and *in vivo* studies report anti-oxidant, antimicrobial, antifungal, antiparasitary, anti-inflammatory, antinociceptive or anti-tumoral effects of essential oils (Spisni et al., 2020). Despite being extensively used in traditional medicine, antispasmodic effects of essential oils have been poor investigated. The major components of essential oils are aromatic terpenes, classified in monoterpenes and sesquiterpenes, which seem to be responsible for the pharmacological profiles of various medicinal plants (Bakkali et al., 2008; de Sousa Lima et al., 2018; Guimarães et al., 2013). Although some monoterpenes have direct effects upon smooth muscle contractility in different preparations (Boskabady and Jandaghi, 2003; Cardoso-Teixeira et al., 2018; Nascimento et al., 2009; Vasconcelos et al., 2016), for several of them there are no pharmacological studies yet and their possible effects should be investigated.

Apiaceae is one of the largest plant family (Burtt et al., 1993), characterized by pungent smell, rich in essential oils and popular for gastrointestinal disorders treatment, especially in the Middle East. It has been reported that several constituents of oils extracted from the Apiaceae family plants can exert beneficial effects on gut morphology, nutrient absorption, microbiota and oxidative status (Ali et al., 2022). Among Apiacae, different Prangos species are part of the traditional medicine. *Prangos ferulacea* (L.) *Lindl*, consisting of perennial herbaceous plants of eastern Mediterranean and western Asia, is one of the most commonly used species of this genus. Its leaves are traditionally recommended as antihypertensive and laxative agents, and widely used as carminative, emollient tonic for gastrointestinal and liver disorders, and as anti-flatulent, sedative, anti-inflammatory, anti-viral, anti-helminthic, antifungal and anti-bacterial agents (Badalamenti et al., 2022; Bruno et al., 2021; Ulubelen et al., 2018; Bagherifar et al., 2019; Bazdar et al., 2018; Çoruh et al., 2007; Mottaghipisheh et al., 2020). The antispasmotic effects of its extract have been already demonstrated in different smooth muscle preparations and could be related to the content of secondary metabolites like osthole (Sadraei et al., 2012, 2013). Recently chemical composition and biological activities of the essential oil from an unexplored accession of aerial parts of *Prangos ferulacea* (L.) Lindl (syn. *Cachrys ferulacea* (L.). Calest), an orophilous species growing wild in Sicily, have been reported, highlighting biological potential, in terms of radical scavenging activities (Badalamenti et al., 2022; Bruno et al., 2021). Studies showed also marked differences in the composition of *Prangos ferulacea* essential oil (Prangoil) compared to the extract. In detail, Prangoil lacks osthole and contains mainly aromatic monoterpenes, as β−ocimen (Badalamenti et al., 2022). In consideration of the absence of notion of possible effects on intestinal contractility, in this study we aimed to investigated, *in vitro*, whether Prangoil and its main component β−ocimen may affect the mechanical activity of rat small and large intestine and the related mechanisms of action.

2. Materials and Methods

2.1. Plant materials

Areal parts consisting in flowers (467g) and leaves (246 g) from twenty-five individuals of *Prangos ferulacea* (L.) Lindl., covering about 200 m^2, were collected at Piano Zucchi, Palermo, Sicily, Italy. The samples were stored in the Herbarium of the University of Palermo (Voucher No. PAL 109762). The essential oil was extracted by hydrodistillation (Department STEBICEF, University of Palermo, Italy), analyzed by GC\MS analysis, as reported by (Badalamenti et al., 2022). The major volatile constituents were identified as being β−ocimen (59%), α− pinene (5.6%), carvacrol (3,6%), sabinene (2,8%) and p-cymene (2.0%).The plant name has been verified by "The Plant List" (http://www.theplantlist.org).

2.2. Animals

The study was performed in accordance with national and European Community guidelines (EEC Directive of 1986; 86/609/EEC) for the handling and use of experimental animals and all efforts were made to minimize animal suffering and the number of animals used. Since the studies have been realized on tissues after animal sacrifice, there was no need for ethic committee approval.

Male albino Wistar rats (200–250 g, Envigo, S Pietro al Natisone-Italy) were euthanized using 2% isoflurane anesthesia followed by cervical dislocation. After laparotomy, intestine was removed and placed into a Petri dish containing physiological Krebs solution to dissect segment of duodenum and distal colon (20 mm length). Segments were then suspended in 10 mL four-channel organ bath filled with oxygenated (95% O_2 and 5% CO_2) and warmed (37 °C) Krebs solution. Mechanical activity of intestinal musculature was measured *in vitro* as previously described (Zizzo et al., 2011, 2016, 2017). Shortly, the distal end of the intestinal segments was tied up to an organ holder and the proximal end connected to a force transducer (FORT 10, Ugo Basile, Biological Research Apparatus, Comerio VA, Italy) with a silk thread and isometric muscular activity was recorded (PowerLab/400 system, Ugo Basile, Italy). Segments were allowed to equilibrate at 1 g tension for at least 40 min before starting experiments. Spontaneous mechanical activity developed in all segments.

2.3. Experimental protocol

At the end of the equilibration period, preparations were challenged with high-potassium chloride (KCl) solution (60 mM) and Carbachol (CCh) (1 μM) until stable responses were obtained. Preparations not responding to KCl or CCh were discarded.

Concentration-response curves for Prangoil 12.5–200 μg/mL were constructed; oil doses were applied for approximately 10 min. Since in duodenum Prangoil induced a dual response effect, contractile at lower doses and inhibitory at higher doses respectively, different protocols were performed to study these effects. Excitatory responses were tested in the presence of neostigmine (10 μM, Jarvie et al., 2008), acetylcholinesterase inhibitor (AChE), or atropine (1 μM, Zizzo et al., 2022), muscarinic receptor antagonist, or ω−conotoxin (100 nM, Zizzo et al., 2022), neural N-type Ca^{2+} channel inhibitor. Inhibitory responses to Prangoil, in both duodenum and colon were evaluated in the presence of ω−conotoxin (100 nM) or L-NAME (100 μM, Auteri et al., 2016), blocker of the NO synthase, or ODQ (10 μM, Pouokam et al., 2021), soluble guanylyl cyclase inhibitor, or TEA (20 mM, Zizzo et al., 2023), aspecific potassium channel blocker. Drugs were left in contact with the tissue for 30 min, before adding Prangoil.

In subsequent experiments, dose response curve for β−ocimen at the doses of 7–120 μg/mL, corresponding to the content of the monoterpene in the oil (59%), was constructed to evaluate its contribution in the Prangroil-induced effects.

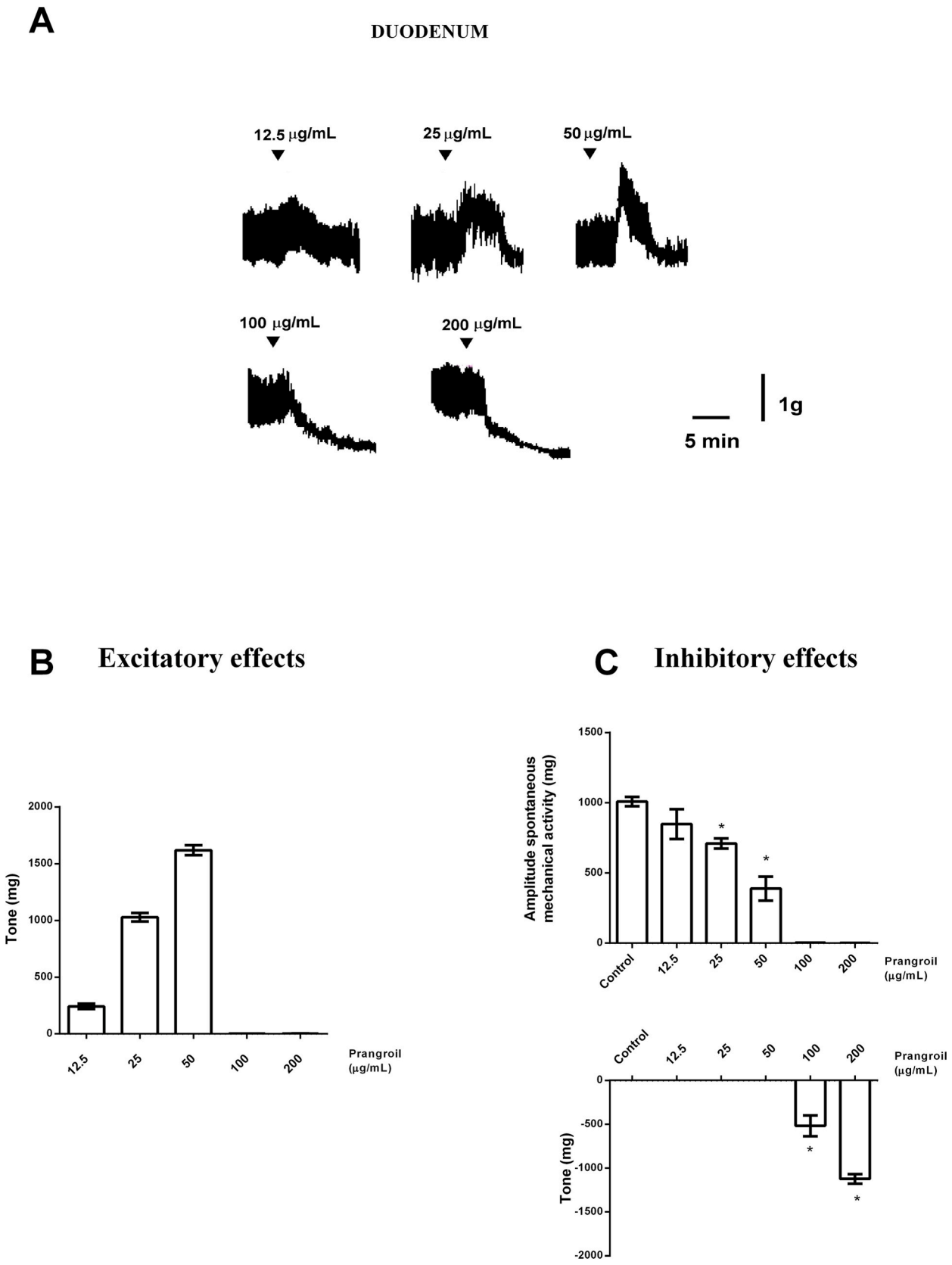


Fig. 1. A) Original tracings showing the effects induced by increasing concentrations of Prangoil on the spontaneous contractions of isolated rat duodenum. B) Histograms showing the increase in the basal tone induced by Prangoil (12.5–200 μg/mL) in isolated rat duodenum. C) Histograms showing the reduction in the amplitude of the spontaneous contraction and of the basal tone induced by Prangoil (12.5–200 μg/mL) in isolated rat duodenum. All data are means ± SEM (n = 11 each) and expressed in absolute value (mg). *$P < 0.05$ compared to the control condition.

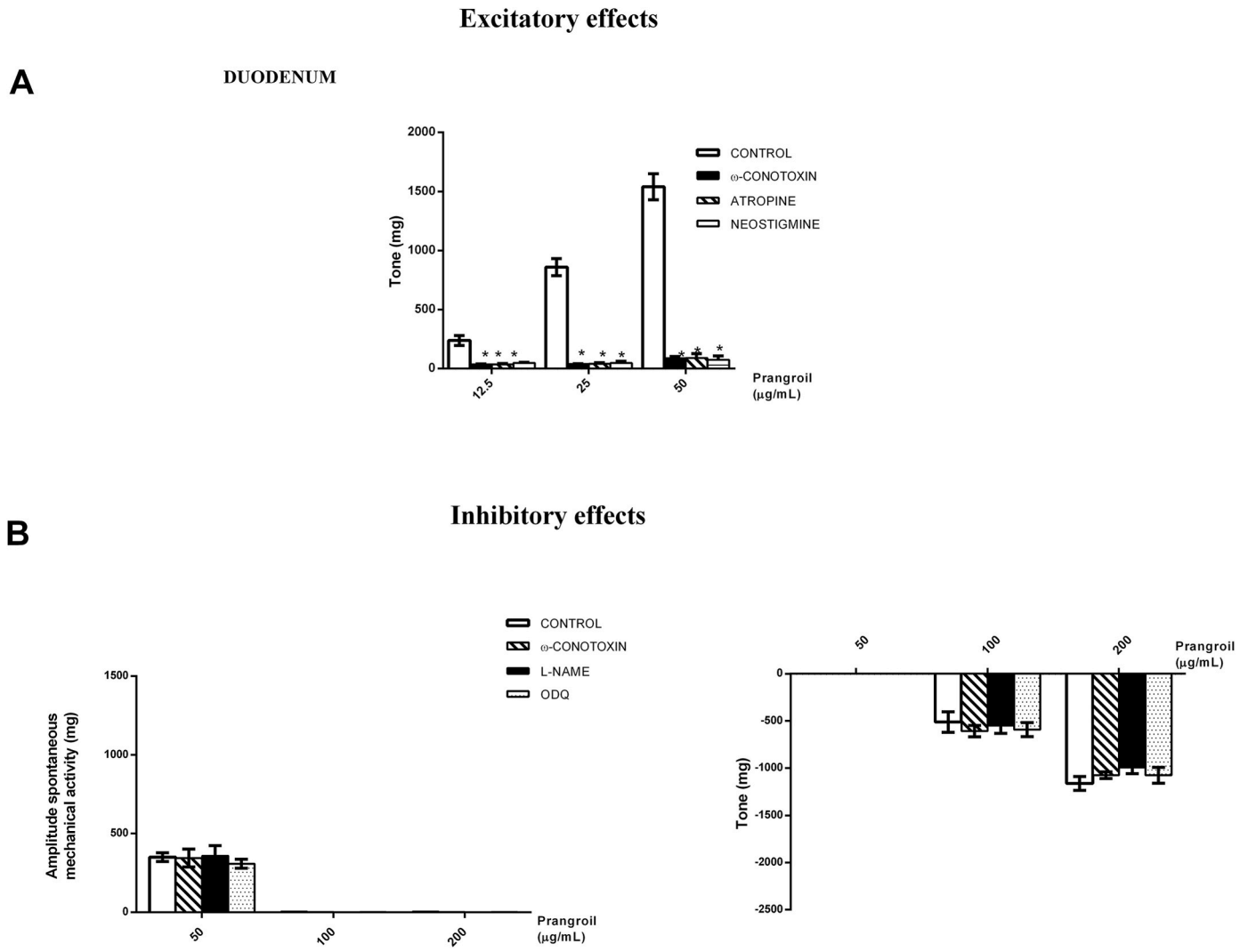


Fig. 2. A) Histograms showing the excitatory effects induced by Prangoil (12.5–50 μg/mL) before and after: ω−conotoxin (100 nM), blocker of the N-type Ca^{2+} channel, atropine (1 μM), muscarinic receptor antagonist, and neostigmine (10 μM), AChE inhibitor, on the basal tone in isolated rat duodenum. B) Histograms showing the amplitude of the spontaneous contraction and the reduction in the basal tone induced by Prangoil (50–200 μg/mL) before and after: ω−conotoxin (100 nM), blocker of the N-type Ca^{2+} channel, L-NAME (100 μM), a blocker of the NO synthase or ODQ (10 μM), soluble guanylyl cyclase in isolated rat duodenum. All data are means ± SEM (n = 5 each) and expressed in absolute value (mg). *$P < 0.05$ compared to the control condition.

To investigate whether the inhibitory responses of Prangoil or β−ocimen could be due to modulation of extracellular calcium influx, the effects of Prangoil or β−ocimen on the contraction evoked by high K^+ solution (60 mM) were evaluated. Segments of both duodenum and colon were pre-treated with the inhibitory doses of Prangoil or β−ocimen for 10 min and then KCl. In addition, the effects of increasing concentrations of Prangoil or β−ocimen on the contractile responses induced by extracellular calcium were investigated. After the equilibrium, the preparations were washed with calcium-free solution containing 0.1 mM EGTA until complete abolition of the spontaneous activity and cumulative concentration-response curve to $CaCl_2$ (0.1–10 mM) was performed. Then, Prangoil or β−ocimen were added to the organ bath 10 min before performing a second cumulative concentration-response curve to $CaCl_2$.

To evaluate the eventual contribution of intracellular calcium stores the effects of Prangoil or β−ocimen on the response to CCh (1 μM) in calcium free solution were analyzed.

2.4. Chemicals and reagents

The composition of Krebs solution was (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1 (Andrew BL, 1972). The following drugs were used: Atropine sulphate, β−ocimen, Carbamoylcholine chloride (CCh), $CaCl_2$, Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N’-tetraacetic acid tetrasodium salt (EGTA); Neostigmine, Nω-nitro-l-arginine methyl ester (L-NAME), 1H-[1,2,4] Oxadiazolo [4,3-a]quinoxalin-1-one (ODQ), Prangoil, Tetraethylammonium (TEA), ω−conotoxin (Sigma- Aldrich, Inc., St. Louis, USA). ODQ, β-ocimen and Prangoil were dissolved in dimethysulphoxide (DMSO) and further diluted in Krebs. The maximal final concentration of DMSO in the organ bath was 0.5%, which did not affect the contractility of the rat small or large intestine.

All the other drugs were dissolved in distilled water. The working solutions were prepared fresh on the day of the experiment by diluting the stock solutions in Krebs and were added to the organ bath.

2.5. Statistical analysis

Data are given as means ± SEM; *n* in the result section refers to the number of animals on which observations were made. The excitatory responses induced by Prangoil were measured 3 min after the application of the drugs, estimated as increase in tension above the basal tone

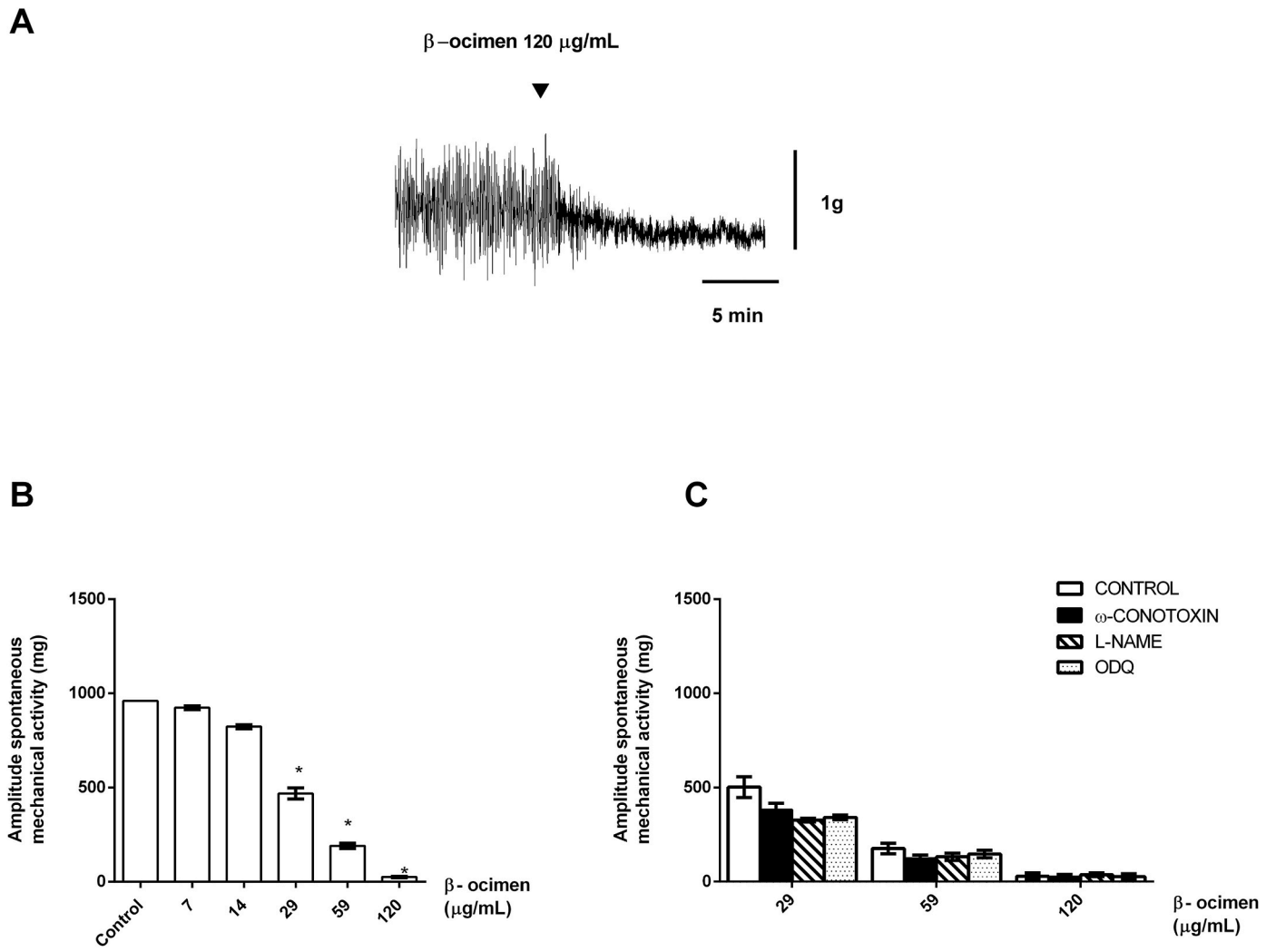


Fig. 3. A) Original tracing showing the maximal inhibitory effect of β−ocimen (120 μg/mL) on the spontaneous contractions of isolated rat duodenum. B) Histograms showing the amplitude of the spontaneous contraction in absence or in presence of β−ocimen (7–120 μg/mL) in isolated rat duodenum (n = 10). C) Histograms showing the effects on the amplitude of the spontaneous contraction induced by β−ocimen (29–120 μg/mL) alone and after: ω−conotoxin (100 nM), blocker of the N-type Ca^{2+} channel; L-NAME (100 μM), a blocker of the NO synthase or ODQ (10 μM), soluble guanylyl cyclase inhibitor, in isolated rat duodenum (n = 5 each). Data are means ± SEM and expressed in absolute value (mg). *$P < 0.05$ compared to the control condition.

set as baseline and expressed in absolute value (mg). The inhibitory effects induced by Prangoil and β−ocimen on the spontaneous contraction and basal tone were measured 10 min after the application of the drugs and expressed in absolute value (mg).

The amplitude of the contraction induced by high KCl solution or by CCh or $CaCl_2$ (both in Calcium free) was taken as 100% contraction to determine the effects of Prangoil and β−ocimen. Statistically significant differences were calculated by Student's t-test or by means of analysis of variance, followed by Dunnett's test, when appropriate. A probability value (*P*) less than 0.05 was regarded as significant.

3. Results

3.1. Prangoil and β−ocimen effects on rat small intestine contractility

Segments of rat duodenum, once mounted in the organ bath, developed a spontaneous mechanical activity characterized by rhythmic contractions with amplitude of 0.97 ± 0.28 g and frequency of 28.35 ± 6.11 contractions per minute (n = 11).

Prangoil (12.5–200 μg/mL) produced a dual response (Fig.1A and B). At the lower doses (12.5–50 μg/mL) Prangoil significantly increased the basal tone followed by a reduction of the spontaneous mechanical activity. At the higher doses (100–200 μg/mL), the initial contractile response was replaced by an increasing in amplitude muscular relaxation (Fig.1 A,C). Prangoil effects were fully reversible after washout. Maximal contractile response was observed in response to 50 μg/mL Prangoil, consisting in an increase in muscle tone of about 1.7 g, peaked on average after 3 min. Maximal inhibitory response was observed in response to 200 μg/mL Prangoil, consisting in the abolition of the spontaneous contractions and in a decrease in muscle tone of about 1.2 g, peaked on average after 10 min.

Parallel experiments using vehicle alone showed that it did not affect the mechanical activity and/or basal tone of duodenal segments.

The excitatory effects induced by Prangoil were abolished in the presence of ω-conotoxin (100 nM), neural N-type Ca^{2+} channels blocker, or atropine (1 μM), muscarinic receptor antagonist. Atropine *per se* reduced the amplitude of the spontaneous mechanical activity. Neostigmine (10 μM), AChE inhibitor, *per se* induced an increase in the amplitude of the spontaneous mechanical activity and a transitory increase of the basal tone of about 1.5 g. In the presence of neostigmine, Prangroil failed to induce any excitatory effect, suggesting that the excitatory effects of Prangoil would be likely the results of the potentiation of the effects of neural released ACh *via* inhibition of AChE activity (Fig. 2 A).

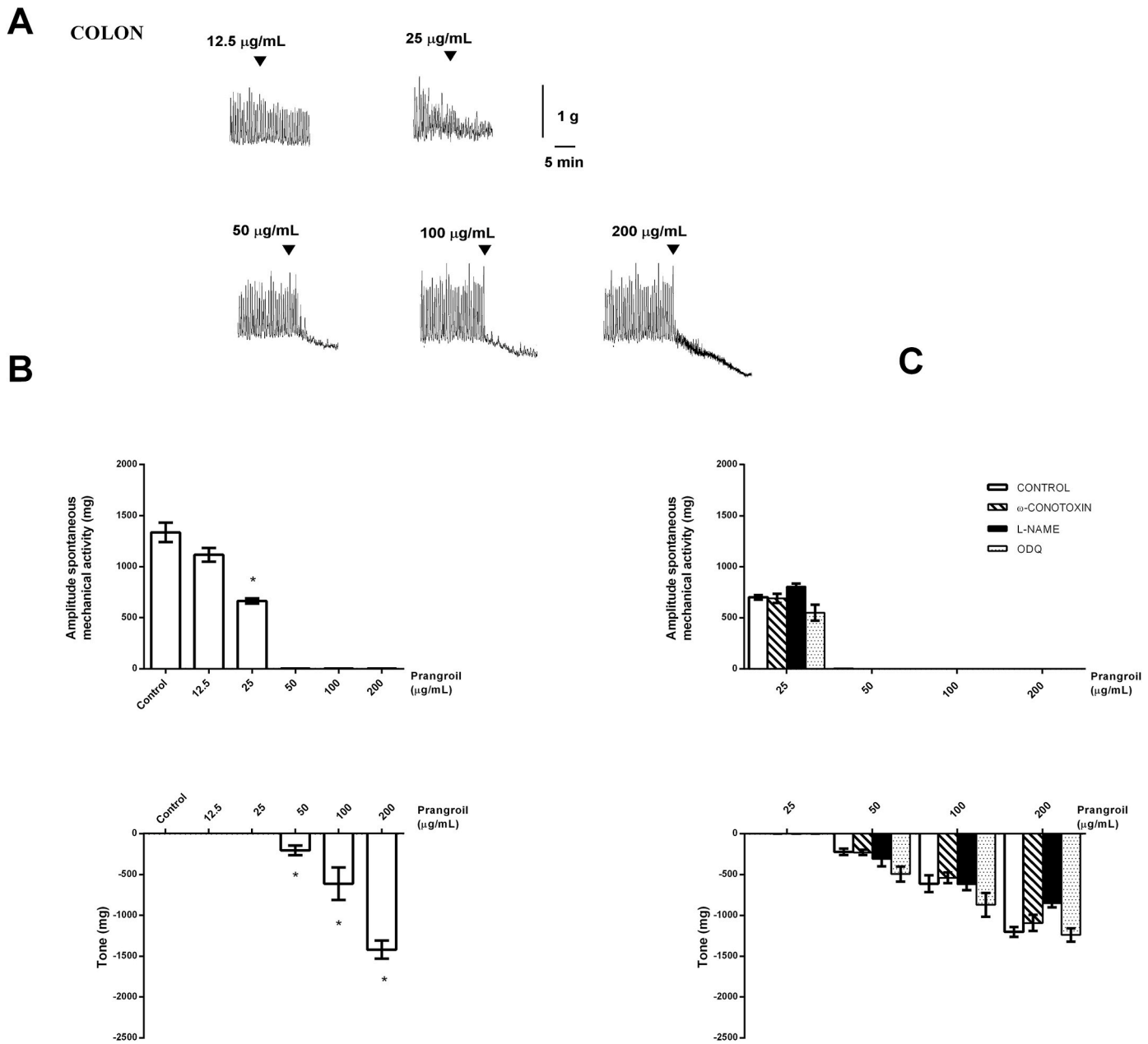


Fig. 4. A) Original tracings showing the effects induced by increasing concentrations of Prangoil on the spontaneous contractile activity of isolated rat colon. B) Histograms showing the reduction of the amplitude of the spontaneous contraction and of the basal tone induced by Prangoil (12.5–200 μg/mL) in isolated rat colon. C) Histograms showing the inhibitory effects induced by Prangoil (25–200 μg/mL) on the amplitude of the spontaneous contractions or on the muscular tone before and after: ω−conotoxin (100 nM), blocker of the N-type Ca^{2+} channel, L-NAME (100 μM) a blocker of the NO synthase or ODQ (10 μM), soluble guanylyl cyclase inhibitor, in isolated rat colon. Data are means ± SEM (n = 5 each) and expressed in absolute value (mg). *$P < 0.05$ compared to the control condition.

Instead, Prangoil inhibitory effects were not affected by ω−conotoxin (100 nM), L-NAME (100 μM), a blocker of the NO synthase, or ODQ (10 μM), soluble guanylate cyclase inhibitor, excluding an involvement of neurotransmitter release or nitrergic pathway in the inhibitory effects (Fig. 2B).

As above reported (Badalamenti et al., 2022), since the monoterpene β−ocimen is the main component of Prangoil we studied its own effects on duodenal contractility. β−ocimen (7–120 μg/mL) did not induce any contractile response, but produced only a concentration-dependent reduction of the spontaneous contractions. Maximal inhibitory response was observed in response to 120 μg/mL and consisted in the abolition of the spontaneous contractions without any reduction of basal tone (Fig. 3A and B). Neither ω−conotoxin (100 nM), L-NAME (100 μM) nor ODQ (10 μM) affected the inhibitory responses to β−ocimen, indicating that, as for Prangoil, neurotransmitter release or NO synthesis are not involved in the inhibitory effects (Fig. 3C).

3.2. Prangoil and β−ocimen effects on rat large intestine contractility

Segments of rat colon, once mounted in the organ bath, developed a spontaneous mechanical activity characterized by rhythmic contractions with amplitude of 1.31 ± 0.62 g and frequency of 4.5 ± 1.2 contractions per minute (n = 11).

Prangoil (12.5–200 μg/mL) induced a reproducible muscle inhibitory effect (Fig. 4A and B), consisting in a slowly developing reduction in the amplitude and frequency of phasic contractions and at the higher doses (50–200 μg/mL) in the abolition of the spontaneous contractions and in a decrease in basal muscle tone. The inhibitory effect was

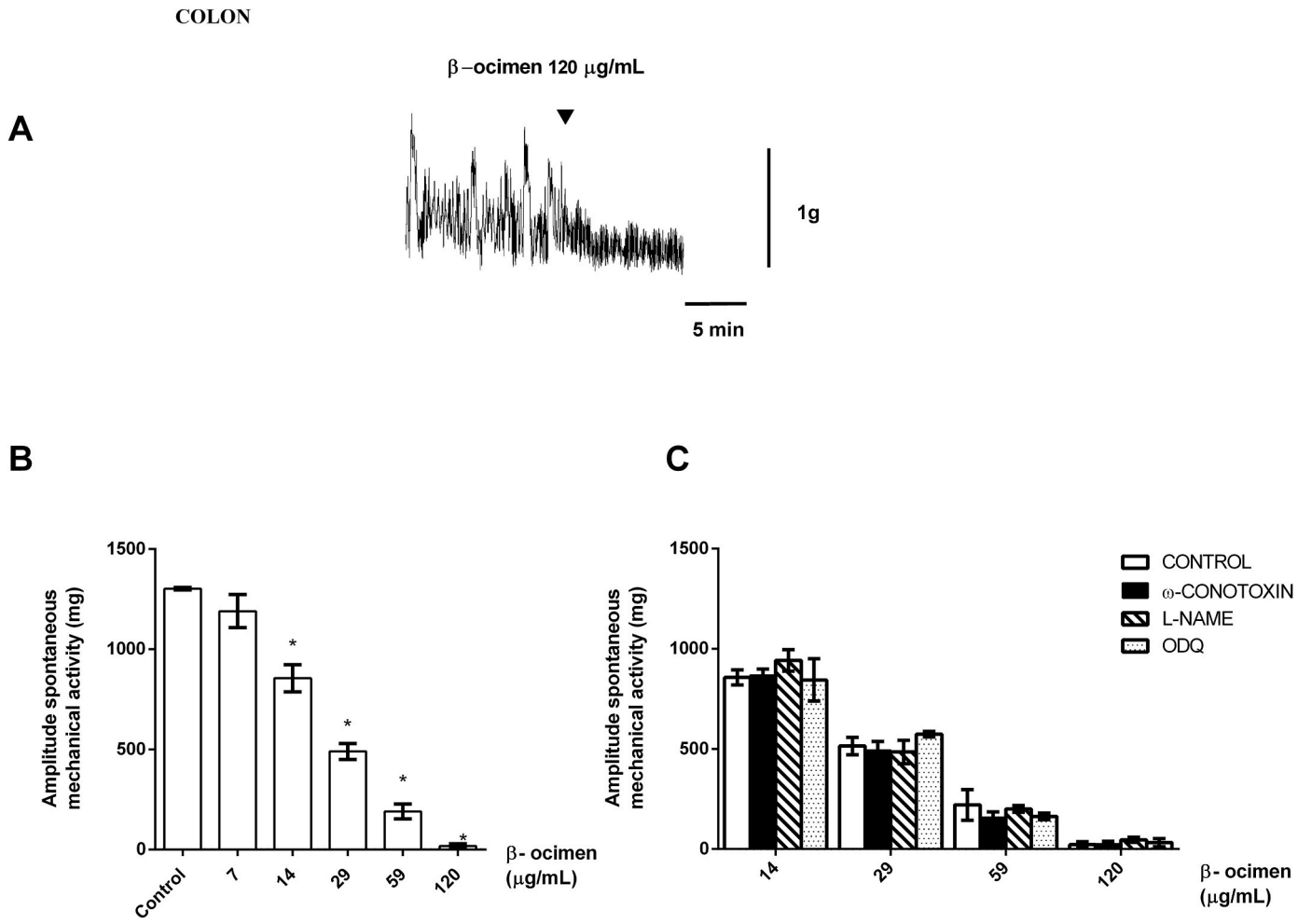


Fig. 5. Original tracing showing the maximal inhibitory effect of β−ocimen (120 μg/mL) on the spontaneous contractions of isolated rat colon. B) Histograms showing the amplitude of the spontaneous contraction in absence or in presence of β−ocimen (7–120 μg/mL) in isolated rat colon (n = 8). C) Histograms showing the effects on the amplitude of the spontaneous contraction induced by β−ocimen (14–120 μg/mL) alone and after: ω−conotoxin (100 nM), blocker of the N-type Ca^{2+} channel; L-NAME (100 μM), a blocker of the NO synthase or ODQ (10 μM), soluble guanylyl cyclase inhibitor, in isolated rat colon (n = 5 each). Data are means ± SEM and expressed in absolute value (mg). *$P < 0.05$ compared to the control condition.

reversible, being the spontaneous contractions and the basal tone restored after washout. Parallel experiments using vehicle alone showed no effect on the mechanical activity and/or basal tone. Maximal response was observed in response to 200 μg/mL Prangoil and consisted in the abolition of the spontaneous contractions and in a maximal decline in muscle tone of about 1.5 g that, peaked on average after 10 min (n = 14) (Fig. 4A and B).

β−ocimen (7–120 μg/mL) induced inhibitory effects consisting in a concentration-dependent reduction of spontaneous mechanical activity, without any change in the basal tone (Fig. 5A and B). As in duodenal segments, neither ω-conotoxin (100 nM) neural N-type Ca^{2+} channels blocker nor L-NAME (100 μM), a NO synthase blocker, nor ODQ (10 μM),inhibitor of soluble guanylate cyclase, affected inhibitory effects induced by Prangoil or by β−ocimen, implying that also in large intestine, neurotransmitter release or synthesis of nitric oxide are not responsible of the inhibitory effects (Fig. 4C; 5 C).

3.3. *Prangoil and β− ocimen inhibitory effects: action mechanism*

3.3.1. *K^+ channels*

In small and large intestinal preparations Prangoil or β-ocimen were tested in the presence of TEA (20 mM), non-selective K^+ channel inhibitor. As showed in Fig. 6 A,B the inhibitory effects induced by Prangoil (50–200 μg/mL) or β-ocimen (14–120 μg/mL) were not affected by TEA, which *per se*, induced an increase of about 40% of the amplitude of mechanical activity.

3.3.2. *Calcium modulation*

In both small and large intestine, application of high-KCl solution (60 mM) caused a contraction consisting in an initial increase in tone followed by a decline and plateau phase, maintained throughout the application time. Prangoil, at the concentrations able to induce inhibitory effects, caused a significant concentration-dependent reduction of the amplitude of high potassium-induced contraction. Also β−ocimen induced similar inhibitory effects on KCl (60 mM) -induced contraction. (Fig. 7A and B).

In both duodenum or colon segments, incubated in a Ca^{2+}-free solution containing 1 mM EDTA, $CaCl_2$ (0.1–10 mM) achieved a concentration-dependent contractile response, which was reduced by Prangoil or β-ocimen (14–120 μg/mL) (Fig. 7C and D).

Application of 1 μM CCh produced a contractile response, characterized by an increase in force followed by an oscillating force production, which is reduced in calcium free solution to 49% in duodenum and 47% in colon respectively. This residual response due to intracellular calcium mobilization was tested in the presence of Prangoil or its main component. Pretreatment with 50–200 μg/mL Prangoil or 14–120 μg/mL β−ocimen induced a significant concentration dependent reduction of the amplitude of CCh-induced contraction (Fig. 8).

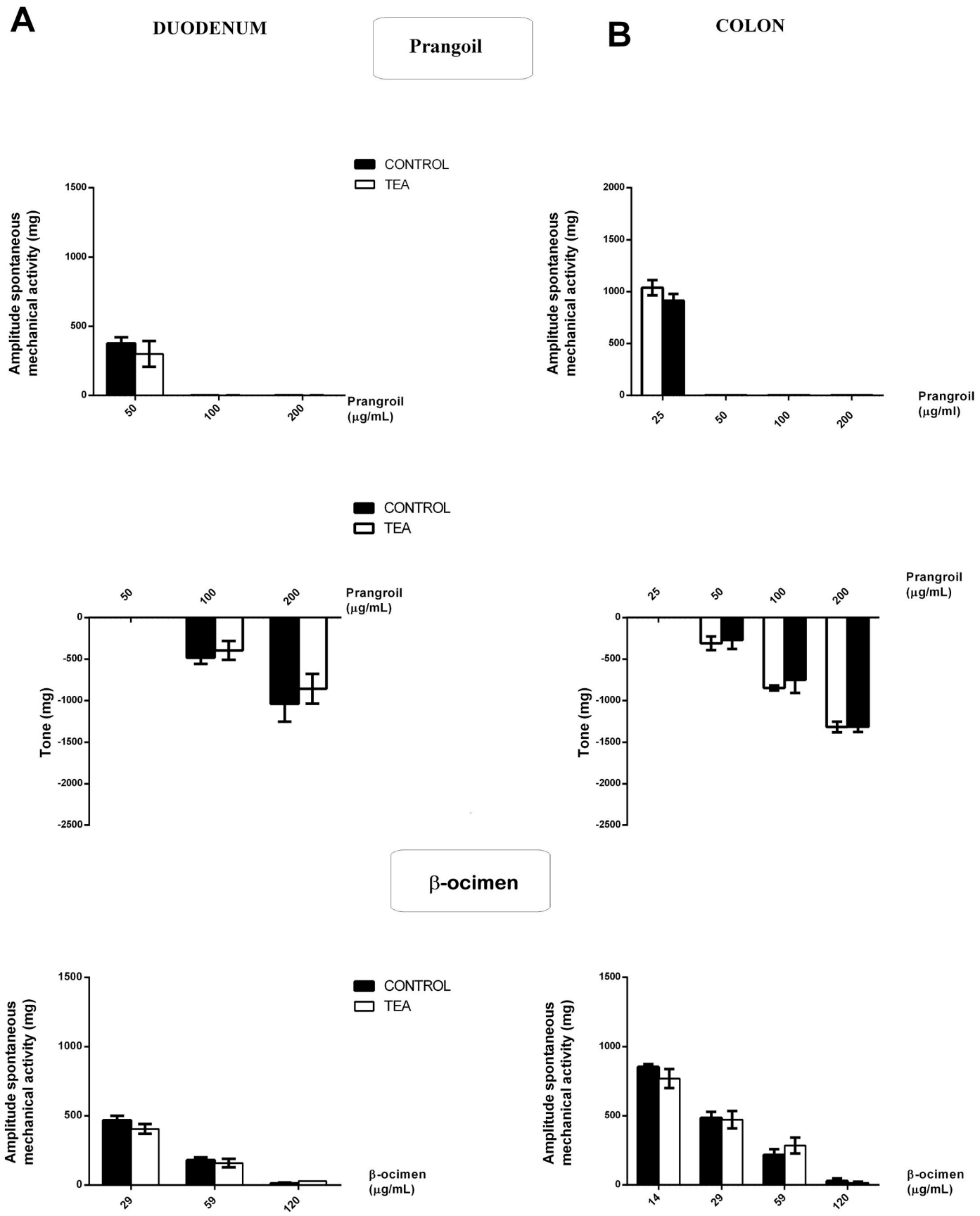


Fig. 6. Histograms showing the inhibitory effects induced by Prangoil or by β−ocimen on the amplitude of the spontaneous contractions or on the muscular tone before and after tetraethylammonium (TEA, 20 mM), non-selective K^+ channel blocker, in rat duodenum (A) or in rat colon (B). Data are means ± SEM and expressed in absolute value (mg).

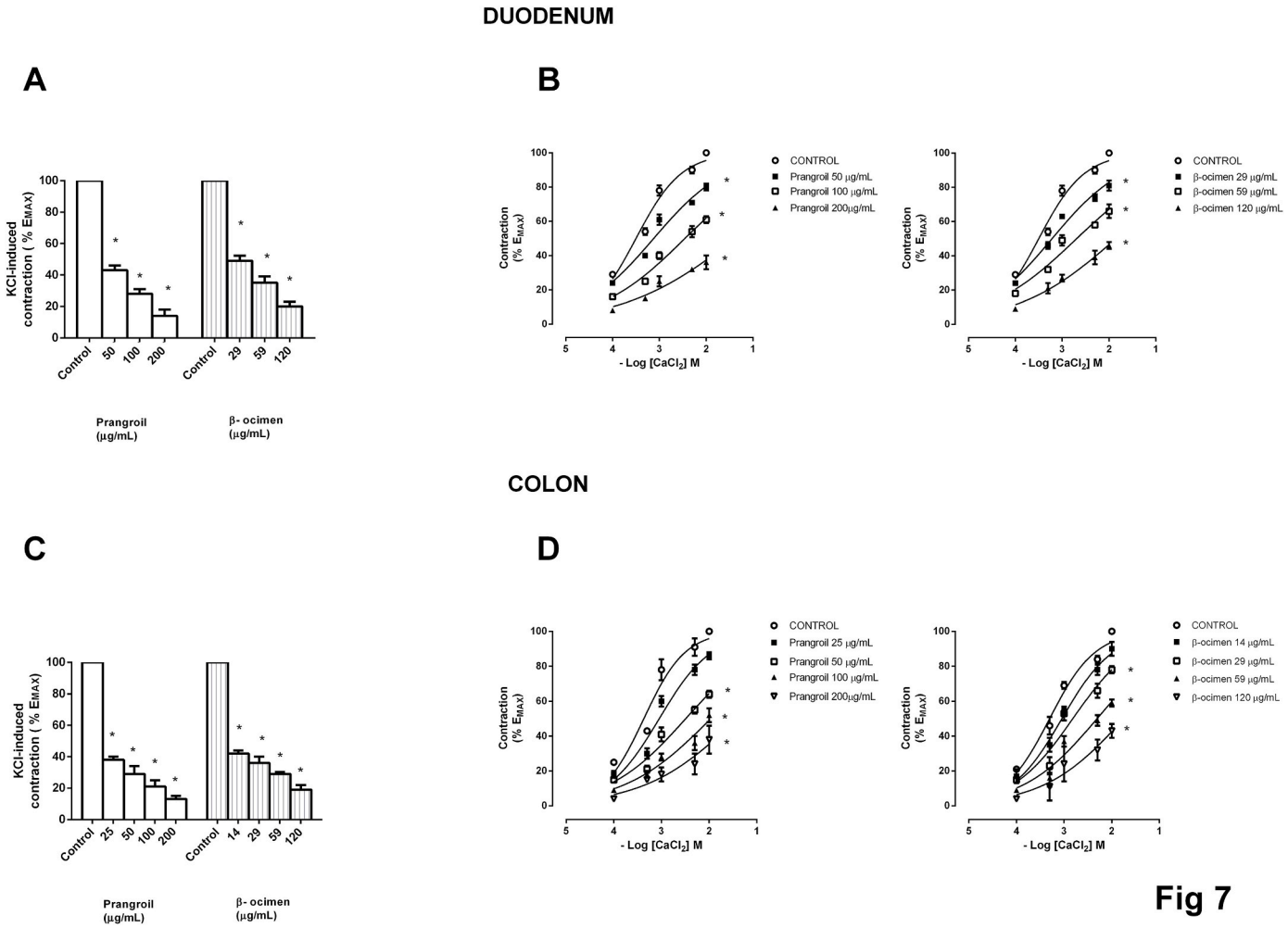


Fig. 7. A) Histograms showing the effects of pretreatment with Prangoil (50–200 μg/mL) or β−ocimen (29–120 μg/mL) on contraction induced by high-KCl solution (60 mM) in rat duodenum. Data are means ± SEM (n = 5) and expressed as the percentage of the excitatory effects (E_{MAX}) induced by 60 mM KCl, set as 100%. *$P <$ 0.05 compared to the control condition. B) Concentration-response curves of Calcium chloride (0.1–10 mM)-induced contraction in Ca^{2+} free solution before and after Prangoil (50–200 μg/mL) or β−ocimen (29–120 μg/mL) in rat duodenum. Data are means ± SEM (n = 5) and expressed as the percentage of the maximal excitatory effects (E_{MAX}) induced by 10 mM $CaCl_2$, set as 100%. *$P < 0.05$ compared to the control condition. C) Histograms showing the effects of pretreatment with Prangoil (25–200 μg/mL) or β−ocimen (14–120 μg/mL) on contraction induced by high-KCl solution (60 mM) in rat colon. Data are means ± SEM (n = 5) and expressed as the percentage of the excitatory effects (E_{MAX}) induced by 60 mM KCl, set as 100%.*$P < 0.05$ compared to the control condition. D) Concentration-response curves of Calcium chloride (0.1–10 mM)-induced contraction in Ca^{2+} free solution before and after Prangoil (50–200 μg/mL) or β−ocimen (29–120 μg/mL) in rat colon. Data are means ± SEM (n = 5) and expressed as the percentage of the maximal excitatory effects (E_{MAX}) induced by 10 mM $CaCl_2$, set as 100%. *$P <$ 0.05 compared to the control condition.

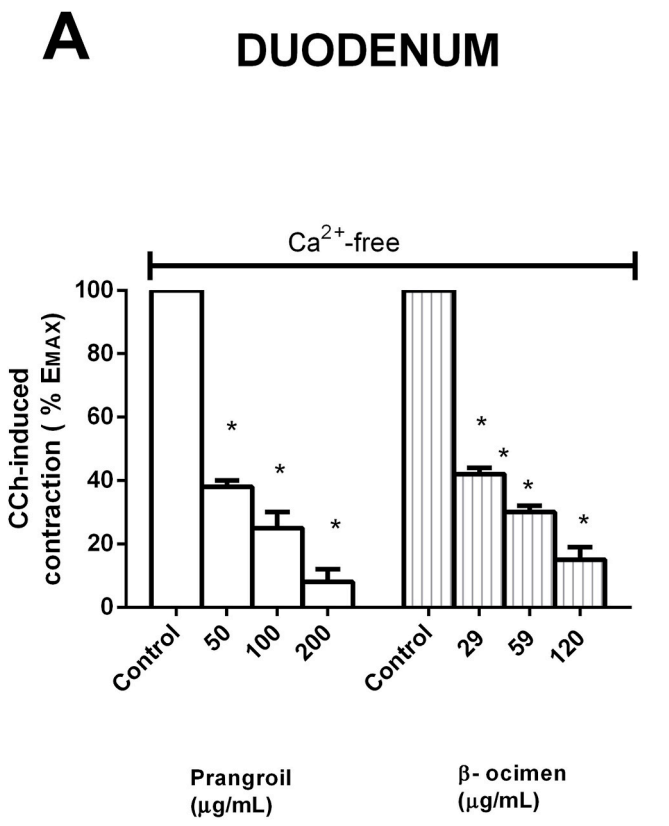
4. Discussion

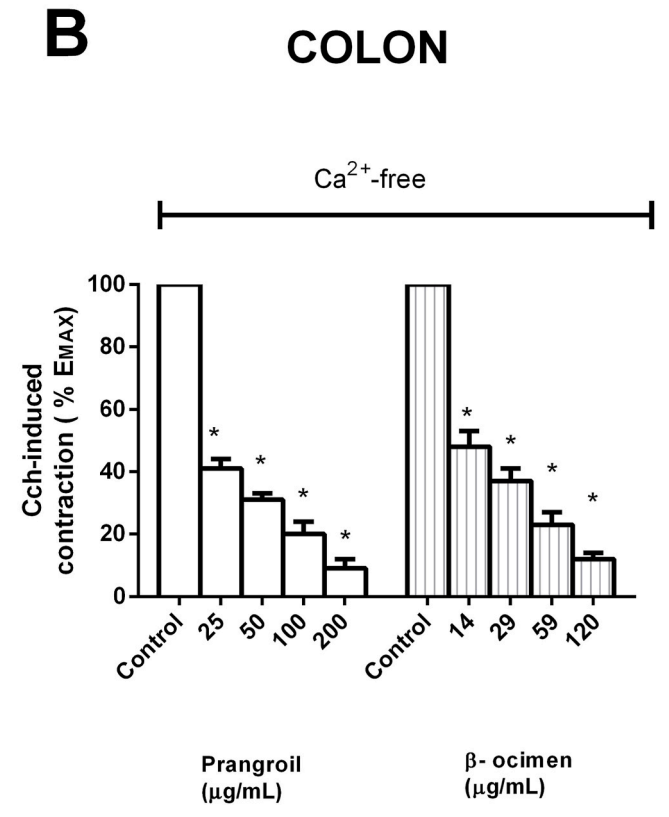
Apiaceae family, including the genus *Prangos Ferulacea,* is a rich source of essential oils, which make them valuable for a variety of purposes, as medicinal or industrial applications. In our study, Prangoil was tested on intestinal contractility and data indicate that the oil is active affecting the spontaneous intestinal activity in rat gut, with regional differences between small and large intestinal preparations.

In rat duodenal smooth muscle, Prangoil has been found to induce biphasic responses consisting in an initial concentration-dependent contraction, progressively followed by a reduction of the amplitude of the spontaneous contractions and, at the higher range of concentrations tested, by a significant muscular relaxation.

The observation that low doses of Prangoil were not able to induce excitatory effects after inhibition of neurotransmitter release or after muscarinic receptor block could suggest that in rat small intestine Prangoil would act likely modulating neurally released ACh effects. Indeed, as already demonstrated, rodent small intestine musculature is under a tonic control by ACh released from enteric nerves (Takeuchi et al., 2007; Tanahashi et al., 2013; Unno et al., 2006). Accordingly, in our preparations blockade of muscarinic receptor reduced spontaneous mechanical activity. Moreover, neostigmine, inhibitor of AChE, increased the amplitude of the spontaneous contractions. Interestingly, the contractile response induced by Prangoil was not observed when the AChE activity was inhibited by high dose of neostigmine, that in rat intestine has been demonstrated to induce the maximal increase of Ach released effect (Jarvie et al., 2008) Therefore, we can speculate that the mechanism underlying the Prangoil excitatory effect could be ascribable to the inhibition of ACh esterase activity. Indeed increasing evidence indicate that several plant essential oils are sources of potential AChE inhibitors (Dohi et al., 2009; Ulubelen et al., 2018) and in a recent study the ability of Prangoil to inhibit *in vitro* AChE was demonstrated (Bruno et al., 2021). However, future studies *in vitro, in silico or in vivo*, are needed to confirm such as hypothesis.

In rat colonic smooth muscle, Prangoil, in a dose dependent manner, inhibited the amplitude of the spontaneous contraction and at the higher doses decreased the basal tone. No excitatory effects have been observed. This is not surprising since colonic mechanical activity is not





(caption on next column)

Fig. 8. A) Histograms showing the effects of pretreatment with Prangoil (50–200 µg/mL) or β−ocimen (29–120 µg/mL) on contraction induced by 1 µM CCh in Ca^{2+}-free solution in rat duodenum. B) Histograms showing the effects of pretreatment with Prangoil (25–200 µg/mL) or β−ocimen (14–120 µg/mL) on contraction induced by 1 µM CCh in Ca^{2+}-free solution in rat colon. Data are means ± SEM (n = 5 each) and expressed as the percentage of the excitatory effects (E_{MAX}) induced by 1 µM CCh, set as 100%.*$P < 0.05$ compared to the control condition.

under a tonic control by cholinergic neurons(Gonzalez and Sarna, 2001), as duodenal mechanical activity. These differences are related to the different motor pattern of intestine regions, being mainly mixing movements in the small intestine, and prevalent propulsive activity in the large intestine.

In both intestinal preparations, the inhibitory effects resulted to be independent by neurotransmitter release since they remained unaltered in the presence of the neural N-type Ca^{2+} channel inhibitor. Moreover, since NO, the main inhibitory enteric mediator, can be synthesized not only by neural plexus, but also by intrinsic intestinal tissue as mast cells, epithelium, smooth muscle (Gantner et al., 2020; Salzman, 1995; Savidge, 2014), we tested if non neural NO may be involved in the inhibitory effects. However, we can exclude such a possibility since in our intestinal preparations, the response to Prangoil were unaffected by inhibition of NO synthesis, as well as by the block of soluble guanylate cyclase inhibitor.

The observation that in small and large intestinal preparations the inhibitory effects were fully reversible after washout indicate that these responses do not appear to be the result of non-specific properties of oils as the ability to modify the lipid bilayers of the cell membrane.

Prangos ferulacea (L.) Lindl extract has been reported to relax some smooth muscle preparations and the main role played by the prenylated coumarin, osthole has been suggested (Sadraei et al., 2012, 2013).

Prangoil composition analysis highlighted a high content of monoterpens (Badalamenti et al., 2022; Martínez-Pérez et al., 2018), which extensively reviewed natural antispasmodics, reported monoterpenoids as the chemical groups with the highest number of antispasmodic compounds, followed by flavonoids, triterpenes and alkaloids. Therefore, since the monoterpene β−ocimen is the main component of Prangoil, we evaluated its effects on our intestinal preparations.

Our results showed a marked reduction of spontaneous contractions in both small and large intestinal preparations in response to β-ocimen, suggesting its possible contribution in the inhibitory response to Prangoil. Oil resulted to be more potent compared to the monoterpene, inducing not only the reduction of the amplitude of spontaneous activity, but also muscular relaxation. Therefore, it is possible to suggest that other components in the oil may act in synergy with β−ocimen in inducing the inhibitory effects observed in rat intestine. Among these, we can speculate a role for other monoterpenes present in the oil as p-cymene and carvacrol, that have been demonstrated to relax other smooth muscle preparations as guinea pig trachea or rat aorta (Cardoso-Teixeira et al., 2018; Peixoto-Neves et al., 2010; Silva et al., 2015). Moreover, components other than β-ocimen should be responsible for excitatory effects in small intestine, since β-ocimen did not induced contractile responses at any concentration tested. However, further studies are needed to solve these issues.

It is well known that potassium channels are key player in the intestinal mechanical activity and different evidence indicate that natural compounds can interact with potassium channels (Rajabian et al., 2022). However, we can exclude that Prangoil and β-ocimen may act as potassium channel openers since the aspecific potassium channel blocker, TEA, did not modify the induced inhibitory effects.

Increase in intracellular calcium is necessary for triggering muscular contraction. Calcium entry by voltage-dependent channels and calcium mobilization from sarcoplasmic reticulum are the main events responsible for the increase in calcium concentration. Thereby, interfering with one or both of these mechanisms have the potential to relax intestinal

muscle. In this context, inhibition of voltage-dependent calcium channels has been reported to be the mechanism of action for many essential oils to induce antispasmodic effects (Heghes et al., 2019).

In our preparations, Prangoil and its main component reduced the contractions induced high K^+ solution and the calcium contractions induced by $CaCl_2$ in calcium free solution. In gut smooth muscle high K^+ induces membrane depolarization, opening of L-type voltage-gated Ca^{2+} channels, and thereby causes sustained contraction. Therefore, the inhibitory effects of Prangoil and its component are targeted at membrane Ca^{2+} channels, indeed we can suppose a modulation of Ca^{2+} influx due to their interaction with L-type voltage-gated calcium channel.

CCh-induced contraction *via* muscarinic cholinergic receptors consists in a component due to extracellular Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels and a second component due to intracellular Ca^{2+} release following the activation of phospholipase-C/inositol triphosphate (IP_3) pathway (Tanahashi et al., 2021). Therefore, we tested the sensitivity of CCh-induced contractions, in calcium-free solution, to Prangoil and β−ocimen in order to examine indirectly any effect on intracellular Ca^{2+} release from IP_3-sensitive stores. Our results indicate that both Prangoil and β-ocimen inhibited CCh-induced contraction in calcium-free solution, suggesting the ability of both compounds to interfere with the mobilization of Ca^{2+} from intracellular stores.

In conclusion, the net effect of Prangroil in the rat small intestine might be the sum of excitatory and inhibitory effects. At low concentration the excitatory effects, likely due to inhibition of ACh esterase activity dominate. Increasing the concentration, the ability to block Ca^{2+} influx through sarcolemma *via*, at least, L-type voltage-dependent Ca^{2+} channels and to reduce Ca^{2+} release from the intracellular store, would make inhibition the dominating response. In the rat large intestine, Prangoil leads only to the inhibition of the contractility *via* the same mechanisms observed in the small intestine. β-ocimen, its major component, seems to contribute to the inhibitory effects in both intestinal preparations.

Calcium handling plays crucial role in the normal functions of gastrointestinal smooth muscle cells. A calcium influx increase is related to disease conditions as diarrhea and intestinal spasms in gastrointestinal tract (De Ponti et al., 1993). Therefore, Prangoil showing calcium channel blocker activity could be used as antidiarrheal and antispasmodic agent, although it has to be taken into account the ability of essential oil of *Prangos ferulacea* (L.) Lindl to increase neuronal cholinergic activity in rat duodenum.

CRediT authorship contribution statement

Maria Grazia Zizzo: Conceptualization, Investigation, Formal analysis, and interpretation, Preparation figures, Writing – original draft, and, Writing – review & editing. **Adele Cicio:** Investigation, Formal analysis, Writing – review & editing. **Maurizio Bruno:** Writing – review & editing. **Rosa Serio:** Supervision, Conceptualization, Data interpretation, Writing – review & editing, All authors read and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by a grant from Ministero dell'Università e della Ricerca Scientifica (MIUR),(FFR).

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