

Calcium blocking activity as a mechanism of the spasmolytic effect of the essential oil of *Calamintha glandulosa* Silic on the isolated rat ileum

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Abstract. In this paper we present the results of studying the effects of the essential oil of *Calamintha glandulosa* Silic (EOCG) on the isolated rat ileum. *C. glandulosa* Silic has been used in folk medicine as an antispasmodic. EOCG (0.003–1 mg/ml) inhibited spontaneous contraction of the ileum (EC₅₀ of 210.48 ± 9.12 µg/ml). The calcium channel blocking activity was confirmed by inhibition of K⁺ (80 mmol/l) induced contractions with EOCG (EC₅₀ of 88.81 ± 6.01 µg/ml). EOCG shifted cumulative calcium curves in depolarizing medium downward (EC₅₀ of 18.18 ± 1.87 mmol/l), similar to the effects of verapamil. Our results confirm that the EOCG shows spasmolytic action in rat ileum. The spasmolytic effect of the EOCG was due to blockade of calcium influx. One of the main components of the EOCG is monoterpenoide pulegone. Namely, pulegone (0.15–50 µmol/l) inhibited the spontaneous (EC₅₀ of 9.02 ± 0.08 µg/ml), and K⁺ induced contractions of the ileum (EC₅₀ of 4.05 ± 0.14 µg/ml), and run rightward the dose response curve of calcium. Pulegone may have a main role in spasmolytic activities of the plant.

Key words: *Calamintha glandulosa* Silic — Spasmolytic — Calcium antagonism — Pulegone

Introduction

Family Labiatae (Lamiaceae), or Minth family, consists of perennial or annual aromatic herbs and shrubs (rarely trees) widely distributed around Mediterranean coast. The family comprises about 200 genera and 3500 species commonly utilized worldwide in folk remedies against a variety of complaints. The widespread use of infusions of dry peppermint leaves (*Mentha piperita* L., Lamiaceae) for their known antispasmodic, carminative and sedative effects is well established and documented (Della Logia et al. 1990). The species that have similar scent as peppermint are often used as folk remedies for the same purposes, even though they belong to a different genus. *Calamintha glandulosa* Silic is a perennial aromatic plant belonging to the family of Lamiaceae, with

the purple flowers and a strong, refreshing odor of the mint (name is derivate from Greek, “*cala*” meaning “good” and “*minth*” meaning “smell”). Some species of the *Calamintha* Miller genus have been traditionally used as a substitute for the mints, in infusion as a stomach tonic, an antiseptic and an expectorant (Mimica-Dukic et al. 2004). Due to its pleasant odor and a distinctive taste the aerial part of the plants can be added to meat stuffing as a seasoning. The leaves, flowers and stems of *Calamintha* species are used as herbal teas and in the production of traditional remedies, mainly as stimulants, antiseptics and antispasmodics (Kokkalou and Stefanou 1990; Baldovini et al. 2000). The extracts and essential oils exhibit hypoglycaemic (Lemhadri et al. 2004), sedating and antipyretic effects in rats (Ortiz et al. 1989), and antimicrobial activity (Kitic et al. 2002). In the last 15–20 years, there has been intensive phytochemical research of *Calamintha* species conducted on antimicrobial agent of herbs *in vitro*, which was mostly focused on their essential oils.

Generally, the essential (or volatile) oil composition is a mixture of various volatile secondary metabolites, mainly

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monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Other volatile compounds may include phenylpropenes and specific sulphur- or nitrogen-containing substances. In the last decades, the interest has been focused on the essential oils and various other extracts of plants, as they demonstrate a wide range of pharmacological effects, such as anti-inflammatory, antioxidant, cytotoxic. They have been screened for their potential uses as alternative remedies for the treatment of many diseases. The composition of the essential oil from the aerial part of plants of *C. glandulosa* has been recently described (Kitic et al. 2002). In the oils one constituent may prevail over all others. The analysis of the essential oil composition indicates that the main component in the oil was pulegone. The herbs with high pulegone content have been used as components of herbal teas for stomach disorders in Turkey (Özek 1990).

To the best of our knowledge, there has not yet been published any investigation of spasmolytic activity of the oil of *Calamintha* species. Guided by ethnobotanical literature and availability from natural sources, our main object was to assess the effects of the essential oil of *Calamintha glandulosa* Silic (EOCG) and pulegone, the principal component in the oil, on the contractility of isolated rat ileum.

Materials and Methods

Animals

In this study, Wistar albino rats ($n = 18$), were used age 12 weeks, body weight 200–250 g, obtained from the Animal Research Center of Medical Faculty, University of Niš, Serbia. The rats were housed in stainless steel cages under standard laboratory conditions. These animals were maintained at 20–24°C with a 12 h light-dark cycle (light on 08:00 to 20:00 h) at least 1 week before the experiment. All animals were fed with standard pellet and they had free access to food and water. All experimental procedures with animals were in compliance with the European Council Directive of November 24, 1986 (86/609/EEC).

The experiments in rat ileum were carried out as previously described (Radenkovic et al. 2006). The ileum portions were isolated out and cleaned off mesenteries. Preparations of 2 cm long parts of the ileum were mounted in 20 ml tissue baths containing Tyrode's solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. The composition of Tyrode's solution was (in mmol/l): NaCl 136.89, KCl 2.68, MgCl₂ 1.05, CaCl₂ 1.80, NaH₂PO₄ 0.42, NaHCO₃ 11.90 and glucose 5.5. The fragment were stretched to a sufficient tension and equilibrated for at least 30 min before starting experiments. The change of intestinal contractility was recorded using myograph transducer F-50 (Narco Bio-Systems,

Inc., Houston). After each assay, tissue was washed with fresh Tyrode and equilibrated for about 10 min.

Plant material

The aerial parts of flowering plant were collected in August 2006 from the population growing wild in Igalo-Sutorina River. A voucher specimen has been deposited in the Institute of Botany Herbarium (BEOU No. 16014), Botanical Garden, Faculty of Biology, University of Belgrade.

Drugs and chemicals

The following drugs were used: verapamil hydrochloride (Sigma Chemicals Company, St. Louis, MO, USA), pulegone (Sigma-Aldrich Chemie GmbH, Diesenhofen, Germany) and EOCG. Pulegone was dissolved in distilled water.

Preparation of EOCG

Dried and pulverized aerial parts of the plant (100 g) were hydrodistilled for 3 h using a Clevenger type apparatus (Pharmacopeia Jugoslavica). The oil was extracted from the distillate with Et₂O and then dried with anhydrous Na₂SO₄. After filtration, the solvent was removed by distillation under the atmospheric pressure and pure yellow oil, 0.6 ml, was kept at 4°C until analysis (Kitic 2003).

Experimental design

After stabilization period EOCG (0.003–1 mg/ml), verapamil (0.01–3 µmol/l) and pulegone (0.15–50 µmol/l) were added to the bath. Effects of different EOCG, verapamil and pulegone concentrations on ileal basal tonus are expressed as percent of changes in baseline values of basal tonus.

Ileal preparations were contracted with depolarizing solution KCl (80 mmol/l). A high concentration of K⁺ ions produced a tonic contraction. Then EOCG (0.003–1 mg/ml) was cumulatively added to the organ bath. The same protocol was carried out with verapamil (0.01–3 µmol/l) and pulegone (0.15–50 µmol/l). The relaxation of the intestinal preparations, precontracted with K⁺, was expressed as the percentage of the control response mediated by K⁺.

After the stabilization during 45 min in Tyrode, the external calcium was eliminated with Tyrode (Ca²⁺-free) and then muscle depolarized with Tyrode (Ca²⁺-free, 80 mmol/l K⁺ isoosmotic) (Karamenderes and Apaydin 2003). A cumulative concentration-response curves of Ca²⁺ were obtained by cumulatively adding CaCl₂ to reach concentrations from 0.01 to 10 mmol/l in the absence and presence of EOCG (0.1–0.3 mg/ml), verapamil (0.03–0.3 µmol/l) and pulegone (5–15 µmol/l), which were added to the bath 15 min before addition of Ca²⁺. This curve was compared with

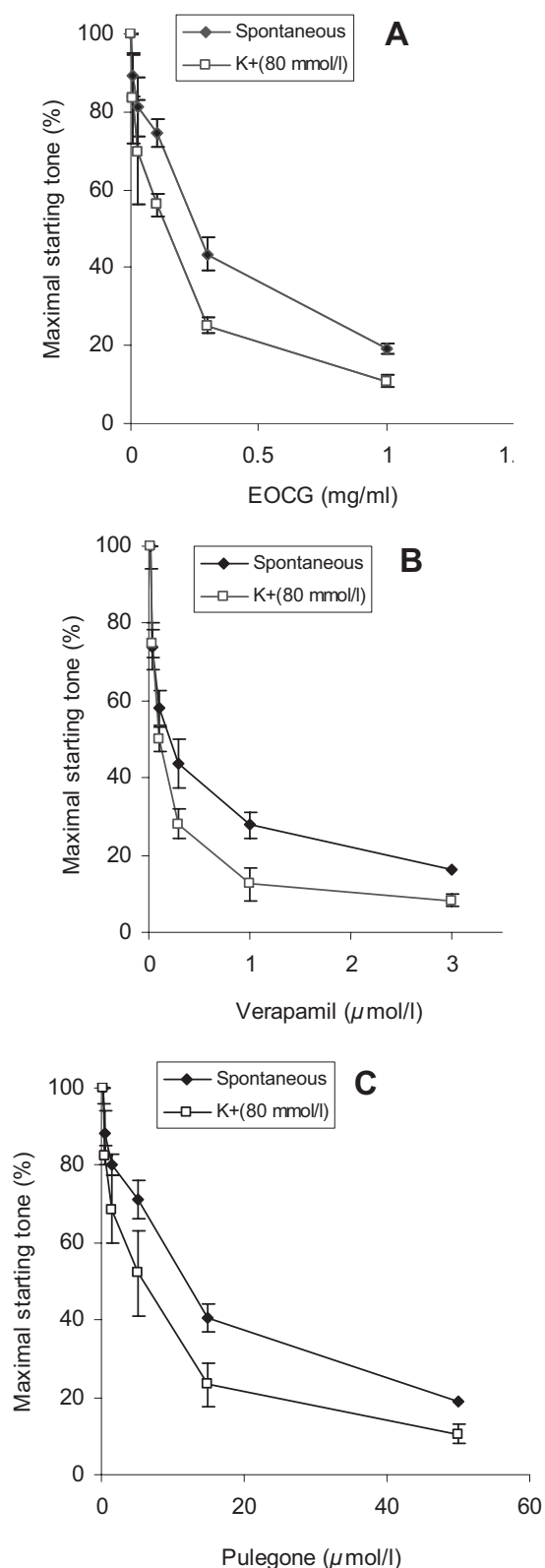


Figure 1. Relaxant effect of EOCG (A), verapamil (B) and pulegone (C) on spontaneous and K^+ (80 mmol/l)-induced contraction in isolated rat ileum ($n = 6$). The values are expressed as mean \pm SEM.

that obtained in the presence of CaCl_2 alone and expressed as percentages of the maximal response to CaCl_2 .

Statistical analysis

The results were expressed as mean \pm SD of six determinations. Statistical evaluation was performed using the Student's *t*-test or one-way analysis of variance (ANOVA). A probability value of $p < 0.05$ was considered to be significant. The mean effective concentration EC_{50} , that is the concentration which elicited 50% of maximal response, was established by regression analysis.

Results

Relaxant effects of EOCG, verapamil and pulegone on basal tonus of the ileum

A concentration dependent decreasing effect of the EOCG on the basal tonus of the rat ileum reaches its maximum effect at 1 mg/ml (Fig. 1A). The EC_{50} values for the EOCG induced relaxation was $210.48 \pm 9.12 \mu\text{g/ml}$. The relaxant effect of EOCG was reversible after washing the preparation. Verapamil (0.01–3 $\mu\text{mol/l}$, Fig. 1B) and pulegone (0.15–50 $\mu\text{mol/l}$, Fig. 1C) also relaxed the basal tonus of intestine in a concentration-dependent manner.

When concentrations of EOCG, verapamil and pulegone are expressed as $\mu\text{g/ml}$ (Table 1), pulegone was twenty three times as potent as EOCG in relaxing spontaneous contraction. Verapamil was ninety times as potent as pulegone in inhibiting spontaneous contraction of rat ileum. These results suggest that pulegone can be responsible for EOCG-induced relaxation.

Relaxant effects of EOCG, verapamil and pulegone on contraction induced by KCl

Solution of KCl (80 mmol/l) induced depolarization and tonic contraction of the ileum. EOCG (0.003–1 mg/ml) relaxed contraction with EC_{50} values of $88.81 \pm 6.01 \mu\text{g/ml}$. Verapamil (0.01–3 $\mu\text{mol/l}$) and pulegone (0.15–50 $\mu\text{mol/l}$) also relaxed contraction induced by K^+ (80 mmol/l), and they

Table 1. EC_{50} values ($\mu\text{g/ml}$) for the inhibition spontaneous and K^+ -induced contraction of isolated rat ileum

	EOCG	Verapamil	Pulegone
Spontaneous contraction	210.48 ± 9.12	0.10 ± 0.009	9.02 ± 0.08
K^+ -induced contraction	88.81 ± 6.01	0.059 ± 0.006	4.05 ± 0.14

were more potent than the oil with an EC₅₀ value of $0.059 \pm 0.006 \mu\text{g/ml}$ and $4.05 \pm 0.14 \mu\text{g/ml}$, respectively (Fig. 1).

Effect of EOCG, verapamil and pulegone on concentration response curves to CaCl₂ in Ca²⁺-free medium

Fig. 2 shows the effect of EOCG (A), verapamil (B) and pulegone (C) on cumulative CaCl₂-induced contractions in Ca²⁺-free and high K⁺ depolarizing medium. EOCG in concentration (0.1–0.3 mg/ml) inhibited the contractions induced by CaCl₂, in a concentration dependent manner. The concentration response curves of CaCl₂, in presence of EOCG were significantly shifted downward ($p < 0.01$). Verapamil (0.03–0.3 $\mu\text{mol/l}$) and pulegone (5–15 $\mu\text{mol/l}$) also significantly shifted the CaCl₂ response curves to the right and down ($p < 0.01$). The EC₅₀ values of calcium ions ($0.66 \pm 0.03 \text{ mmol/l}$) were affected by EOCG (EC₅₀ of $18.18 \pm 1.87 \text{ mmol/l}$), verapamil (EC₅₀ of $73.88 \pm 7.94 \text{ mmol/l}$) and pulegone (EC₅₀ of $22.79 \pm 1.02 \text{ mmol/l}$).

Discussion

EOCG exhibited relaxant activity in isolated rat ileum. The effect was reversible after washing, suggesting that the inhibition was not due to the damage of the intestine by the oil.

Depending on EOCG concentration, contraction induced by high concentration of potassium, were relaxed. Calcium is the regulator of tension in smooth muscles, and their contraction depends on Ca²⁺ influx from extracellular space through calcium channels. It is well known that the increase in external K⁺ concentration (KCl 80 mmol/l) induces smooth muscle contraction through the activation the voltage operated calcium channels and subsequent calcium release from the sarcoplasmic reticulum (Bolton 1979; Santos et al. 2007). Therefore, agents that inhibit contraction induced by KCl should somehow inhibit the entry of Ca²⁺ ions or otherwise inhibit the intercellular contraction mechanism (Sandraei et al. 2003).

Essential oil decreased the Ca²⁺ dose-response curves, constructed in the Ca²⁺-free medium, similar to that caused by verapamil. The common characteristic of verapamil is dose-dependent inhibition of the slow entry of calcium. The observed effect of the plant extract to inhibit Ca²⁺ contraction, similar to that of verapamil, suggests the presence of calcium antagonist in the extract (Godfrain et al. 1986; Gilani et al. 2005).

The antagonistic effect produced by extract on spontaneous activity of rat ileum or on contraction induced by KCl and CaCl₂ suggests spasmolytic action *via* the modulation of calcium entry through calcium channels. There is a report about antispasmodic effect of another species of *Satureja* L. (Cruz et al. 1990), and other plants of Lamiaceae family (Hajhashemi et al. 2000). Effect of this species is consistent with our results.

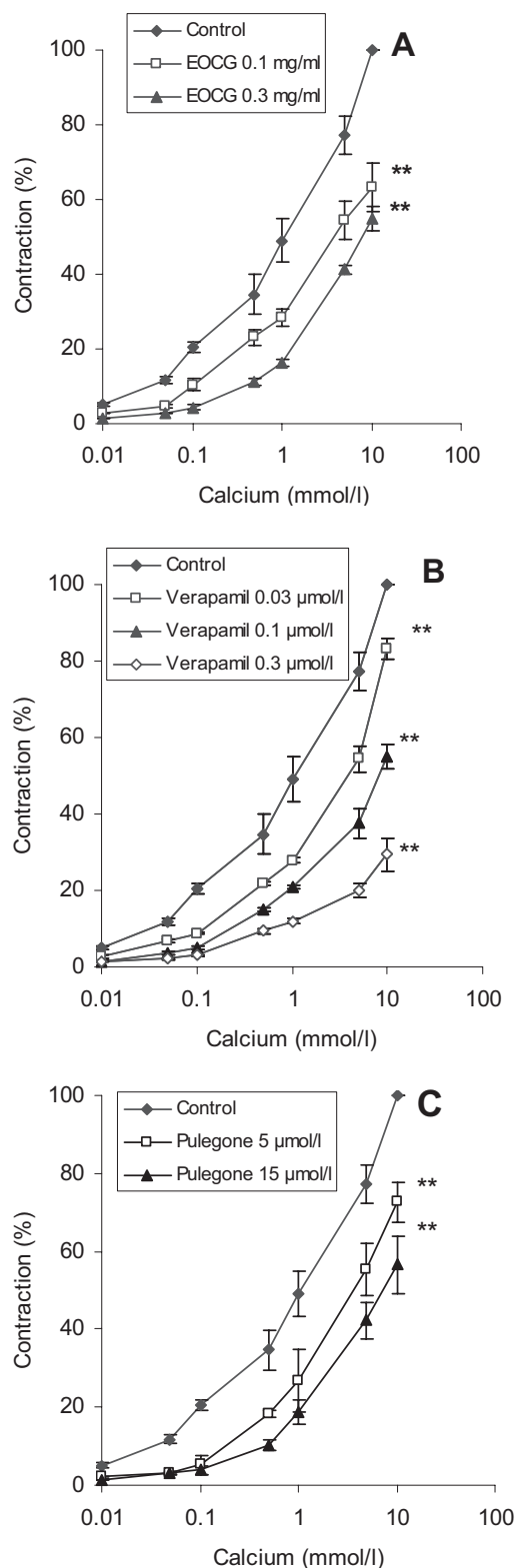


Figure 2. Relaxant effect of EOCG (A), verapamil (B) and pulegone (C) on Ca²⁺ induced contraction in isolated rat ileum ($n = 6$). Data are expressed as the mean \pm SEM. ** $p < 0.01$ vs. contractions induced in the presence of stimulator alone.

It is well known that some terpenoides can act as spasmolytic agents by involving calcium antagonism (Shabana et al. 2005). Terpenoid pulegone is the principal component of the essential oil of *Mentha pulegium* and induced an inhibitory effect on the contractile activity of the isolated rat myometrium (Soares et al. 2005).

The results of oil analysis of *C. glandulosa* shown that pulegone was the main component (37.5%) (Kitic et al. 2002). Pulegone exerts concentration dependent inhibition of spontaneous contraction of the ileum and effect was twenty three times as potent as EOCG in inhibiting contractions.

Conclusion

The results obtained in this work showed that EOCG exerts significant spasmolytic effect, which may underline the therapeutic action of the plant. Antispasmodic activity on rat ileum is probably caused by inhibition of calcium influx through voltage-operated Ca^{2+} channels. Pulegone, the principal constituent of EOCG, is most probably responsible for EOCG-induced relaxation.

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