

Spasmolytic Activity of the Ethanol Extract of *Sideritis raeseri* spp. *raeseri* Boiss. & Heldr. on the Isolated Rat Ileum Contractions

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ABSTRACT *Sideritis raeseri* spp. *raeseri* Boiss. & Heldr., known as “mountain tea,” has been widely used in the Mediterranean region as a spice and in folk medicine as a very popular decoction because of its anti-inflammatory, carminative, analgesic, antitussive, stomachic, and antimicrobial properties. The study was aimed to investigate the effects of an ethanol extract of *S. raeseri* on intestinal activity. Air-dried and powdered aerial parts were extracted with 96% ethanol. The rat ileum preparations were incubated in Tyrode’s solution gassed (95% O₂/5% CO₂) at 37°C. The ethanol extract of *S. raeseri* (0.03–0.3 mg/mL) relaxed spontaneous contractions in isolated rat ileum, similar to that produced by papaverine. The plant extract in a concentration-dependent manner (0.015–0.15 mg/mL) significantly inhibited the contractile response to acetylcholine ($P < .01$). Atropine inhibited the response to acetylcholine. A similar relaxation-inducing effect of the *S. raeseri* extract was observed on the precontracted ileum by histamine and barium chloride. Plant extract (0.03–0.3 mg/mL) significantly shifted the histamine concentration–response curve to the right and down ($P < .01$). The *S. raeseri* extract (0.03–0.3 mg/mL) significantly inhibited the contractions induced by barium chloride ($P < .01$). The results show that the ethanol extract of *S. raeseri* can produce inhibition of the spontaneous rat ileum contractions and contractions induced by different spasmogens. These data indicate that *S. raeseri* acts as a spasmolytic on intestinal smooth muscle, which justifies its use in gastrointestinal disorders.

KEY WORDS: • ethanol extract • ileum • rat • *Sideritis raeseri*

INTRODUCTION

SIDERITIS RAESERI BOISS. & HELDR. spp. *raeseri* belongs to the genus *Sideritis* L. (Family Lamiaceae), which consists mostly of perennial herbs with a slightly woody base, often densely hairy. *Sideritis* L. comprises approximately 150 species of annuals and perennials distributed throughout North Africa, the Iberian Peninsula, the Mediterranean countries, and the Middle East region.¹ Aerial flowering parts of plants from this genus are known as “mountain tea,” which is widely used in Mediterranean folk medicine as a very popular tea because of its anti-inflammatory,^{2–4} carminative, analgesic, antitussive, stomachic, and antimicrobial properties.^{5,6} Anticataract,⁷ immunomodulating,⁸ anti-human immunodeficiency virus replication, antifeedant, anti-ulcerogenic, analgesic, antihypoglycemic,⁹ and antioxidative¹⁰ activities were also reported. In Serbian folk medicine, *S. raeseri* is used as a herbal tea in the treatment of inflammations, gastrointestinal disorders, and coughs and as a tonic, while extracts are used

as a component of dietary supplements for anemia. It is mostly imported from the former Yugoslav Republic of Macedonia and Albania.

The essential oils and extracts of *Sideritis* species have been the subject of many recent studies, but their physiological effects on the motility of intestinal smooth muscles have not been established yet. The present study was aimed to investigate the effects of an ethanol extract of *S. raeseri* spp. *raeseri* Boiss. & Heldr. on the contractile responses of isolated rat ileum.

MATERIALS AND METHODS

Animals

In this study the male Wistar albino rats (weighing 200–250 g) that were used were obtained from the Animal Research Center of the Medical Faculty, University of Niš, Niš, Serbia. The animals were housed in stainless steel cages under standard laboratory conditions. These animals were maintained at 20–24°C with a 12-hour light–dark cycle at least 1 week before the experiment. All animals 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).

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Plant material

The aerial parts of cultivated *S. raeseri* spp. *raeseri* were collected in the phase of full flowering, from the experimental field at the Institute for Medicinal Plants Research in Pančevo, Serbia. The upper 20 cm of the plants was harvested and open-air-dried in the shade. Air-dried and powdered aerial parts were extracted with 96% ethanol in a Soxhlet apparatus. The extracts were filtered and evaporated in a vacuum to dryness.

Drugs

Acetylcholine chloride, histamine dihydrochloride, and atropine sulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and papaverine hydrochloride was obtained from Merck (Darmstadt, Germany). All drugs were dissolved in distilled water.

Isolated tissue experiments

The ileum portions were isolated out, and mesenteries were cleaned off. Preparations 2 cm long were mounted in 10-mL tissue baths containing Tyrode's solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. The Tyrode's solution was composed of 136.89 mM NaCl, 2.68 mM KCl, 1.05 mM MgCl₂, 1.80 mM CaCl₂, 0.42 mM NaH₂PO₄, 11.90 mM NaHCO₃, and 5.5 mM glucose. The fragments were stretched to a sufficient tension and equilibrated for at least 30 minutes before experiments were started. The change of intestinal contractility was recorded using a TSZ-04-E Spell Iso system (Experimetria Ltd., Budapest, Hungary).

After each assay, tissues were washed with fresh Tyrode's solution and equilibrated for around 10 minutes. Rat ileum exhibits spontaneous rhythmic contractions. The isolated ileum had been treated with extract in cumulative concentrations. Papaverine was used as a positive control. Agonists such as acetylcholine, histamine hydrochloride, and barium chloride were cumulatively added to the bath in the absence and presence of *S. raeseri* extract (0.015–0.15 mg/mL). The relaxation of intestinal preparations precontracted with acetylcholine, histamine, and barium ions was expressed as a percentage of the control response mediated by agonist.

Statistical analysis

The results were expressed as mean ± SD values of six determinations. Statistical evaluation was performed using Student's *t* test. A probability value of $P < .05$ was considered to be significant. The mean effective concentration (EC₅₀), that is, the concentration that elicited 50% of maximal response, was established by regression analysis.

RESULTS

Effects of the extract on spontaneous contractions of isolated rat ileum

Figure 1 shows the effect of the *S. raeseri* extract on spontaneous contractions of rat ileum. The extract in a

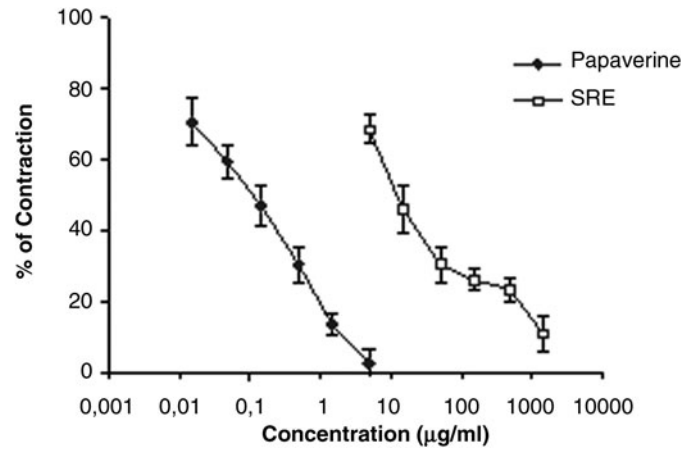


FIG. 1. Inhibitory effects of the ethanol extract of *S. raeseri* (SRE) and papaverine on spontaneous contractions in isolated rat ileum. Data are mean ± SEM values.

concentration-dependent manner inhibited contractility of the intestine. The EC₅₀ value for the *S. raeseri* extract-induced relaxation was 15.05 ± 0.97 mg/mL. Papaverine (0.015–5 µg/mL) also relaxed rat ileum in a concentration-dependent manner.

Effects of the extract on acetylcholine-evoked contraction of isolated rat ileum

The extract of *S. raeseri* (0.03–0.3 mg/mL) concentration-dependently inhibited the contraction induced with acetylcholine ($P < .01$), with an EC₅₀ value of 764.01 ± 64.23 nM (the EC₅₀ value of acetylcholine was 17.95 ± 0.97 nM) (Fig. 2). Atropine (140 nM) inhibited the response to acetylcholine.

Effects of the extract on histamine-evoked contraction of isolated rat ileum

Figure 3 shows the histamine-dependent contraction curve of rat ileum in the absence and presence of the extract

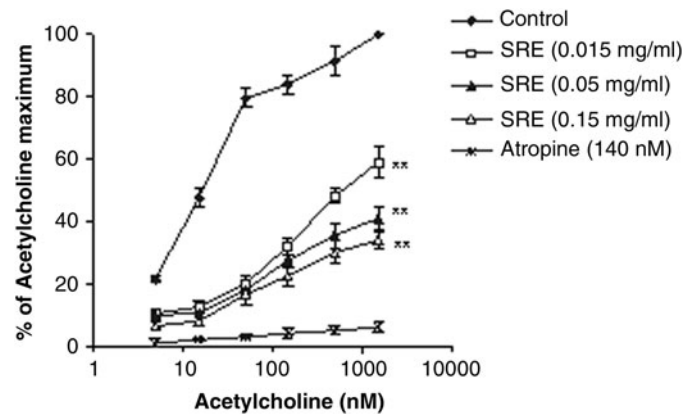


FIG. 2. Comparison of dose–response curves of acetylcholine in the absence and presence of SRE in isolated rat ileum. Data are mean ± SEM values. ** $P < .01$ versus contractions induced in the presence of stimulator alone.

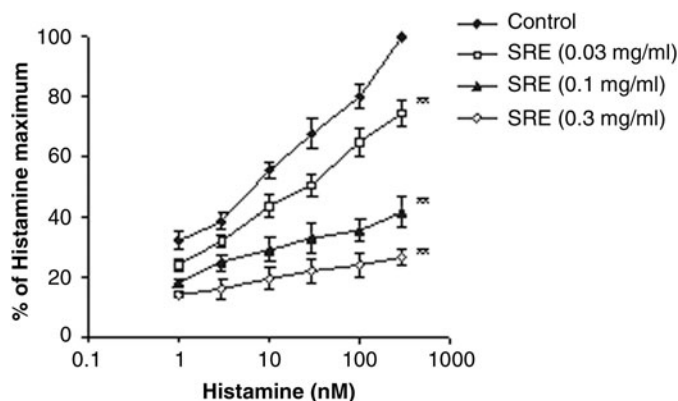


FIG. 3. Comparison of dose–response curves of histamine in the absence and presence of SRE in isolated rat ileum. Data are mean \pm SEM values. ** $P < .01$ versus contractions induced in the presence of stimulator alone.

of *S. raeseri*. Plant extract (0.03–0.3 mg/mL) significantly shifted the histamine concentration–response curve to the right and down ($P < .01$). The EC_{50} value of histamine (6.14 ± 0.58 nM) was affected by the extract of *S. raeseri* ($EC_{50} = 383.01 \pm 18.25$ nM).

Effects of the extract on barium chloride-evoked contraction of isolated rat ileum

The *S. raeseri* extract at a concentration of 0.03–0.3 mg/mL inhibited the contractions induced by barium chloride, in a concentration-dependent manner (Fig. 4). The concentration–response curves of barium chloride in the presence of *S. raeseri* extract were significantly shifted downward ($P < .01$). The EC_{50} value of barium ion (23.11 ± 1.47 μ M) was changed by the extract of *S. raeseri* ($EC_{50} = 488.30 \pm 28.36$ μ M).

DISCUSSION

The spasmolytic effects of the ethanol extract of *S. raeseri* were studied for scientific evaluation of its potential me-

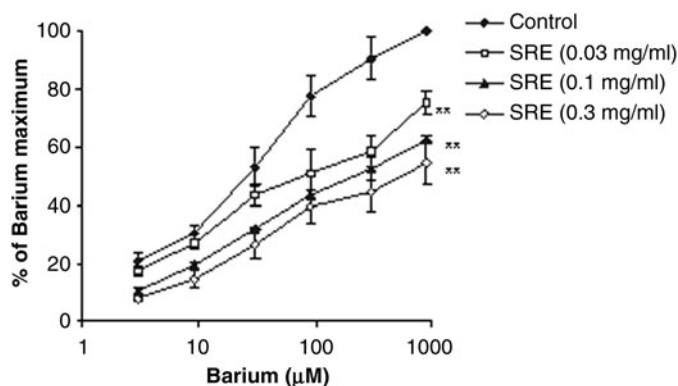


FIG. 4. Comparison of dose–response curves of barium ions in the absence and presence of SRE in isolated rat ileum. Data are mean \pm SEM values. ** $P < .01$ versus contractions induced in the presence of stimulator alone.

dicinal uses. The present data showed that the extract caused inhibition of spontaneous contraction, similar to the spasmolytic agent papaverine. The spasmolytic effect of the extract of *S. raeseri* was concentration-dependent and reversible after washing, suggesting that this inhibition was not due to damage of the intestine by the extract. These results are in good agreement with the traditional uses of this genus.

The rhythmic contractions of smooth muscle cells depend on an endogenous pacemaker driven by the cytosolic calcium (Ca^{2+}) oscillator that is responsible for the periodic release of Ca^{2+} from the endoplasmic reticulum. The periodic pulses of Ca^{2+} often cause membrane depolarization.¹¹ Action potential is generated at the peak of depolarization and constitutes influx of calcium ions through calcium channels.^{12–14}

We observed that the extract of *S. raeseri* decreased contractions induced by acetylcholine, histamine, and barium chloride. The interactions of acetylcholine and histamine with muscarinic and histamine receptors, respectively, cause depolarization and contraction of intestinal smooth muscle. Also, Walsh and Singer¹² showed that action potentials could be elicited when barium ions were present in high concentrations in extracellular fluid. Barium ions depolarize the cell membrane and open the voltage-dependent calcium channels, resulting in a calcium influx.¹⁵ It is possible that the extract of *S. raeseri* contains some compounds that interfere with calcium channel activity or with release of calcium ions from intracellular stores.

Phytochemical studies on samples of the genus *Sideritis* have shown that these plants are rich in flavones and diterpenoids.¹⁶ Actually, flavone glycosides, in particular, 8-hydroxyflavone glycosides, are a chemotaxonomic characteristic for some sections of the genus *Sideritis*. Several flavones, such as the 7-*O*-glycosides of 8-hydroxyflavones (isoscuteallarein, chryseriol) and apigenin, have been identified from *S. raeseri*.^{17,18} These compounds, isolated from *Sideritis* spp., have been shown to possess biological properties.¹⁹ The aglycone apigenin exhibited antispasmodic activities in isolated ileum, mainly caused by blockade of the calcium influx.²⁰

The spasmolytic effects of other plants of the Lamiaceae family have also been reported. The essential oil of *Melissa officinalis* and its constituent citral inhibited intestinal contractions induced by acetylcholine, histamine, and KCl.²¹ It is well known that some terpenoids can act as spasmolytic agents. Terpenoid pulegone and essential oil of *Calamintha glandulosa* (Family Lamiaceae) also relaxed spontaneous and K^+ -induced contraction of rat ileum.²²

In conclusion, the present results show that the ethanol extract of *S. raeseri* spp. *raeseri* Boiss. & Heldr. can inhibit spontaneous ileum contractions and contractions induced by acetylcholine, histamine, and barium ions. All the above findings are in agreement with their usage in traditional medicine. Based on our results, *S. raeseri* may be phytotherapeutically used, after full pharmacological and toxicological evaluation, as an alternative drug to synthetic spasmolytic agents.

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AUTHOR DISCLOSURE STATEMENT

No competing interests exist.

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