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ORIGINAL PAPERS

Spasmolytic effect of *Jasonia glutinosa* on rodent intestine

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ABSTRACT

Introduction: *Jasonia glutinosa* is an endemic plant species of the Iberian Peninsula and Southern France traditionally used in infusions as a spasmolytic; this plant is also known as "té de roca" (rock tea) but there is no scientific evidence about the effects of this plant.

Aim: To evaluate the spasmolytic effect of rock tea.

Methods: We have studied the *in vitro* effect of a rock tea extract on rat duodenum spontaneous contractions and the *in vivo* effect on mice gastrointestinal transit.

Results: Rock tea extract reduced the spontaneous contractions of rat duodenal smooth muscle, inhibited KCl-induced contractions and blocked the contractions invoked by both extracellular Ca^{2+} and the agonist of L-type calcium channels Bay K8644. This inhibitory effect was similar to the one observed after the addition of the antagonist of L-type calcium channels verapamil. Rock tea did not modify gastrointestinal transit in healthy mice. However, after the treatment with dextran sulfate sodium, an inducer of colitis, rock tea extract reverted the increase in the gastrointestinal transit associated with this treatment.

Conclusion: Rock tea extract relaxed duodenal smooth muscle via L-type calcium channels and normalized gastrointestinal transit in a model of colitis. These results may validate the traditional use of *Jasonia glutinosa* in patients with gastrointestinal alterations. Thus, rock tea may be used as a spasmolytic agent to treat gastrointestinal disorders.

Key words: Rock tea. Duodenum. L-type calcium channels antagonist. Intestinal motility. Whole intestinal transit.

INTRODUCTION

The mammalian intestine presents a great variety of characteristic motor patterns, such as segmentation and propulsive movements and migratory motor complexes, which ensure an effective gastrointestinal motility. The alteration of these motor patterns is frequently associated with pain, abdominal distension, nausea, diarrhea and dyspepsia, among other symptoms, thus affecting patient's quality of life substantially. In addition, these alterations, of which the exact etiology remains unknown, have no effective treatments and their high prevalence generates large costs to health authorities (1).

Tea has well known stimulatory and inhibitory effects on intestinal, bronchial and cardiovascular functions as well as anti-inflammatory and antioxidant properties (2,3).

Among all tea types, Asian *Camellia sinensis* has been the best studied (4,5). In contrast, there are only a few studies about *Jasonia glutinosa (L.) CD,* an endemic species to the Iberian Peninsula (Spain) and the south of France. *Jasonia glutinosa* is a traditional medicinal plant belonging to the *Asteraceae* family commonly known in Spain as "té de roca" (rock tea). Infusions prepared with the flowering aerial parts of the plant have traditionally been used for the treatment of gastrointestinal problems such as diarrhea or abdominal pain (6,7). Other less common traditional uses include appendicitis, colds, respiratory diseases or hypertension (8,9).

These beneficial actions may be explained due to the presence of biologically active substances such as monoterpenes, sesquiterpenes and flavonoids (9-12).

The aim of this research was to examine if a rock tea extract had spasmolytic effects on the duodenal smooth muscle and intestinal transit as a pharmacological approach to validate its use in traditional medicine.

MATERIAL AND METHODS

Chemical compounds

Bay K8644, verapamil and Evans Blue were purchased from Sigma-Aldrich (Madrid, Spain). Dextran sodium sulphate was acquired from Panreac. Bay K8644 stock solution was prepared in dimethyl sulfoxide (DMSO). The final DMSO concentration did not exceed 0.5% and had no effect *per se* on intestinal tissue. The rest of products were dissolved in distilled water except for rock tea and DSS, which were re-suspended in drinking water.

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Plant material and extraction

Plant collection and preparation of the extract were carried out as previously described (9). This process was carried out following The United Nations Convention on Biological Diversity.

Animals

Male Wistar rats weighing 200-250 g and male C57BI/6 mice at age of 8-12 weeks were purchased from Janvier Lab (LeGenest St. Isle, France). Mice were fed with a standard diet and had free access to water. All procedures were carried out under Project Licenses PI45/11 and PI66/14 approved by the Ethic Committee for Animal Experiments from the University of Zaragoza. The care and use of animals were performed in accordance with the Spanish Policy for Animal Protection RD53/2013, which complies with the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Duodenal segments preparation

Rat duodenum segments were extracted, washed with Ringer Krebs and cleaned to remove adipose and connective tissues. Duodenal motility isometric measurements were made as previously described (13). After an adaptation period, duodenal spontaneous contractions were registered in Ringer Krebs, and this motility was considered as a control. In order to determine the Effective Concentration 50 (EC_{50}) on spontaneous contractions, accumulative contraction-response curves of rock tea extract (0.5-5 mg/ml) and verapamil (10^{-8} M-10^{-6} M) were produced.

Spasmolytic effect of rock tea

With the aim of evaluating the spasmolytic effect of rock tea extract, accumulative contraction-response curves of rock tea extract (0.5-5 mg/ml) and verapamil (10^{-8} M-10^{-6} M) were prepared on KCl (80 mM) precontracted intestine. To confirm the effect of calcium, after an initial period of incubation with Ringer Krebs, segments were incubated for 20 minutes with a calcium free buffer (that contained KCl [$(K^+)_o$ = 50 mM] instead of calcium). Calcium-free Ringer Krebs composition was (expressed as mM concentrations) as follows: NaCl 120, KCl 4.7, $CaCl_2$ 0, $MgSO_4$ 1.2, $NaHCO_3$ 24.5, KH_2PO_4 1, glucose 5.6 and ethylene glycol tetraacetic acid (EGTA) 1. Increasing concentrations of $CaCl_2$ (10^{-5} M-10^{-2} M) in the presence of rock tea extract (0.5 or 5 mg/ml), its vehicle (water) or verapamil (10^{-6} M) were added. In another series of experiments, rock tea extract (0.5 or 5 mg/ml) and verapamil (10^{-6} M) were added for 15 minutes before adding the agonist of L-type calcium channels Bay K8644 (10^{-5} M). The rock tea or verapamil responses were compared to vehicle (control). Each experimental protocol was systematically performed on two segments of duodenal longitudinal muscle of each rat and was repeated on 5-6 animals.

Effect of rock tea extract on gastrointestinal transit

Male C57BI/6 mice at age of 8-12 weeks were used. At the beginning of the study (day 0), their basal whole gastrointestinal transits were determined by the administration of 200 µl of a solution consisting of 5% Arabic gum and the non-absorbable color dye Evans Blue (5%). Transit was considered on each animal as the latency of the excretion of the colored pellet. Animals were then intragastrically gavaged with rock tea extract (50 mg/kg) or vehicle (water) for 17 days. At day 7, whole gastrointestinal transit was measured to evaluate the effect of rock tea on motility. From day 10 to day 17, animals were orally treated with DSS (2.5%), a well-known colitis inducer (18), dissolved in drinking water. At day 17, whole gastrointestinal transit was measured for a third time to study the effect of rock tea on DSS-associated motor disturbances.

Data analysis

Results are presented as the mean ± S.E.M. with respect to the control (%). The amplitude and frequency of spontaneous contractions were analyzed as previously described on duodenal segments (14). Data were analyzed by t-test or one-way analysis of variance (ANOVA) followed by *post hoc* Tukey test. p values < 0.05 were considered as statistically significant. The concentration of the agent that inhibits 50% of the maximal contraction (EC_{50}) was expressed as the geometric mean with 95% confidence intervals (IC); this value was calculated for the concentration-response curve experiments. *In vivo* experimental data were expressed as the percentage of transit time with respect to day 0 (basal transit, 100%) for each animal.

RESULTS

Effect of rock tea extract on spontaneous contractions

Figure 1 shows how rock tea extract (0.5-5 mg/ml) and verapamil (10^{-8} M-10^{-6} M), a L-type Ca^{2+} channel antagonist, induce a relaxant effect on spontaneous contractions in the longitudinal smooth muscle from rat duodenum in a concentration dependent manner, with an EC_{50} of 2.29 mg/ml (CI 2.5-2) and 0.16 µM (CI 0.22-0.19) respectively. Rock tea extract and verapamil significantly reduced the amplitude of spontaneous contractions in a concentration dependent manner, without modifying the frequency (Table I).

Effect of rock tea extract on duodenum precontracted with KCl

KCl (80 mM) induced a sustained contraction in the rat duodenum. Rock tea (0.5-5 mg/ml) and verapamil (10^{-8} M-10^{-6} M) relaxed the duodenal smooth muscle precontracted with KCl, in a concentration dependent manner, with an EC_{50} of 0.87 mg/ml (CI 0.9-0.8) and 0.03 µM (CI 0.04-0.02), respectively (Table II).

Effect of rock tea extract on the contraction induced by $CaCl_2$

Preincubation with rock tea extract, 0.5 or 5 mg/ml, inhibited the maximal contraction induced by $CaCl_2$ by

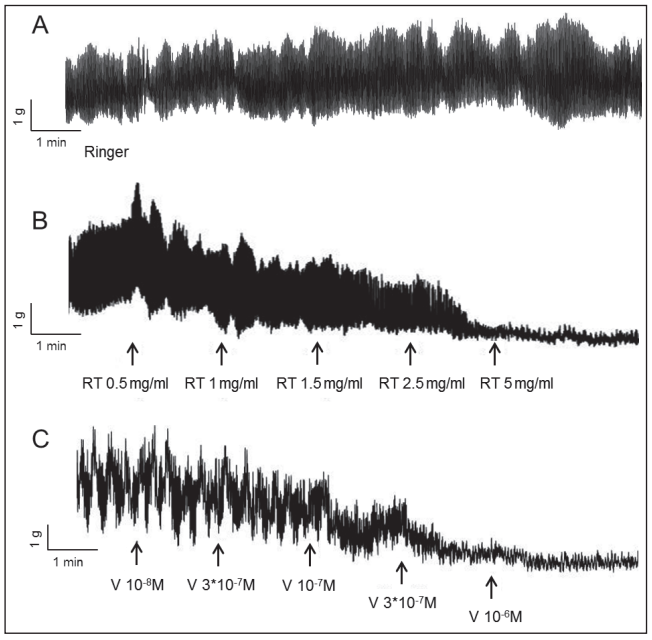

Fig. 1. Recording of spontaneous contractions from longitudinal smooth muscle of rat duodenum. A. In Ringer Krebs. B. In the presence of rock tea extract (RT). C. In the presence of verapamil (V) at different concentrations. Representative recordings from results obtained in 5-6 rats.

39.9% and 53.7%, respectively (Fig. 2). Verapamil 10^{-6} M induced an inhibition of 62.7% (Fig. 2).

Effect of rock tea extract on the contraction induced by Bay K8644

Bay K8644, an L-type Ca^{2+} channel agonist, induced a contractile response in the longitudinal muscle of the duodenum. Preincubation with rock tea extract, 0.5 or 5 mg/ml, diminished contractions evoked by Bay K8644 10^{-5} M by 85.7% and 94.4%, respectively (Fig. 3). Verapamil 10^{-6} M produced a similar inhibition to that induced by 5 mg/ml of rock tea extract (91.9%) (Fig. 3).

Table II. The relaxant effect of different concentrations of a) rock tea extract (RT) and b) verapamil (V) on high-K^+-induced contractions ($[K^+]_0$ = 80 mM) on segments isolated from rat duodenum

	% of contraction		*% of contraction*
Control	100.3 ± 0.2	Control	100.5 ± 0.3
RT 0.5 mg/ml	68.1 ± 3.7***	V 10^{-8} M	76.8 ± 6.9**
RT 1 mg/ml	44.4 ± 4.5***	V 3x10^{-7} M	45.6 ± 7.6***
RT 1.5 mg/ml	30.9 ± 3.0***	V 10^{-7} M	22.7 ± 3.5***
RT 2.5 mg/ml	18.0 ± 3.9***	V 3x10^{-6} M	11.7 ± 2.9***
RT 5 mg/ml	3.3 ± 1.7***	V 10^{-6} M	5.1 ± 2.2***

**p < 0.01; ***p < 0.001 *vs* control. Data are expressed as the mean of percentages with respect to the control ± S.E.M. Segments were obtained from 5-6 rats.

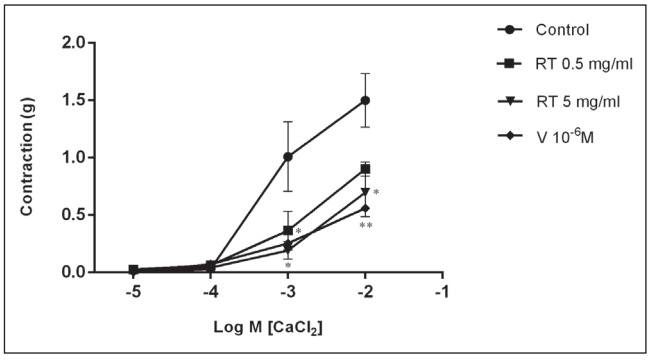

Fig. 2. Contraction (g of tension) produced by different concentrations of $CaCl_2$ in the absence or in presence of rock tea extract (RT, 0.5 or 5 mg/ml) and verapamil (V, 10^{-6} M) in rat duodenum. $CaCl_2$ contractions were induced after 20 minutes of incubation with different concentrations of rock tea extract and verapamil. *p < 0.05 and **p < 0.01 *vs* control. Data are expressed as the mean ± S.E.M. Segments were obtained from 5-6 rats.

Table I. Effect of rock tea extract (RT, 0.5-5 mg/ml) and verapamil (V, 10^{-8} M-10^{-6} M) after 15 minutes of incubation on the amplitude and frequency of spontaneous contractions in the longitudinal smooth muscle from rat duodenum

	Amplitude	*Frequency*		*Amplitude*	*Frequency*
Control	102.0 ± 0.2	100.4 ± 4.6	Control	101.0 ± 0.5	100.0 ± 0.7
RT 0.5 mg/ml	88.4 ± 5.3	104.2 ± 5.6	V 10^{-8} M	99.5 ± 1.9	97.9 ± 2.3
RT 1 mg/ml	81.9 ± 2.7*	105.6 ± 3.3	V 3x10^{-7} M	88.7 ± 3.7	95.7 ± 4.6
RT 1.5 mg/ml	74.8 ± 5.2**	107.2 ± 7.8	V 10^{-7} M	66.3 ± 7.5***	95.4 ± 4.8
RT 2.5 mg/ml	47.6 ± 6.6***	104.6 ± 6.5	V 3x10^{-6} M	39.6 ± 4.1***	94.1 ± 2.0
RT 5 mg/ml	25.1 ± 5.0***	105.1 ± 7.1	V 10^{-6} M	30.5 ± 2.5***	93.4 ± 2.3

*p < 0.05; **p < 0.01; ***p < 0.001 *vs* control. Values are expressed as mean ± S.E.M. Data are expressed as the percentage of the amplitude and frequency of spontaneous contractions compared to control conditions (Krebs). Segments were obtained from 5-6 rats.

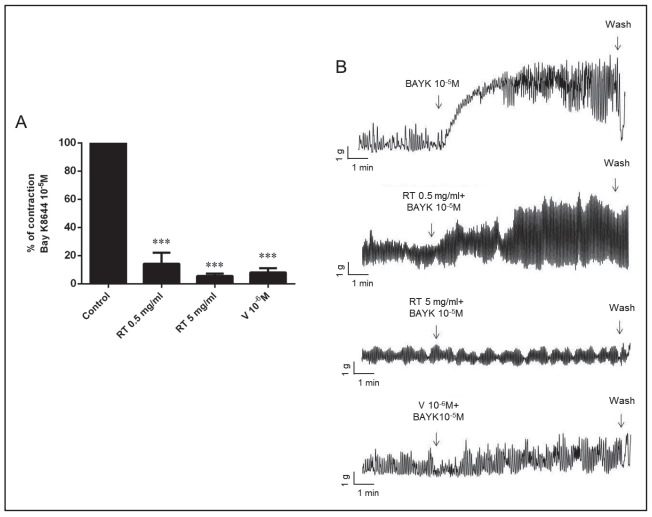

Fig. 3. A. Effect of rock tea extract (RT; 0.5 and 5 mg/ml) and verapamil (V; 10^{-6} M) on the contractile response induced by Bay K8644 in rat duodenum. ***p < 0.001 *vs* control. Data are expressed as the mean ± S.E.M. Segments obtained from 5 rats. B. Recording of contractions induced by Bay K8644 (10-5 M; control) and the decrease of contractile response induced by Bay K8644 in presence of rock tea extract or verapamil.

Effect of rock tea extract on the gastrointestinal transit

Seven days of treatment with the rock tea extract or vehicle did not alter the whole gastrointestinal transit time in physiological conditions (transit at day 7). Treatment with DSS accelerated the transit time ($p < 0.01$) effect that was reverted by treatment with rock tea extract (Table III).

DISCUSSION

Jasonia glutinosa is used in traditional medicine in the form of infusions as antispasmodic to treat several gastrointestinal alterations; however, there are no experimental studies that confirm this effect. The present study shows for the first time that rock tea extract has spasmolytic activity mediated by inhibition of L-type Ca^{2+} channels.

Table III. Whole gastrointestinal transit measured at day 0 (basal), day 7 (animals treated with rock tea at 50 mg/kg, or water) and day 17 (animals treated with rock tea at 50 mg/kg or water and DSS)

	Water group	*Rock tea group*
Basal (day 0)	100.0 ± 0.0	100.0 ± 0.0
Treatment (day 7)	88.4 ± 5.7	101.9 ± 4.3
Treatment + DSS (day 17)	66.0 ± 7.5*	83.5 ± 8.5

*p < 0.05 *vs* basal (control). Data are expressed as the percentage of the mean of whole gastrointestinal transit time of each animal with respect to whole gastrointestinal transit time at day 0 ± S.E.M. Data was obtained from 4-6 mice under each condition.

The spontaneous movements of the intestine are regulated by a periodic cycle of depolarization and repolarization. Depolarizations are evoked by the fast entry of Ca^{2+} into the cytoplasm via the voltage-dependent Ca^{2+} channels and the release of Ca^{2+} from intracellular stores, required for contractile responses and the maintenance of normal tone (15,16), whereas relaxation occurs due to the decrease of Ca^{2+} in the cytosol.

In this study, rock tea extract reduced, in a concentration dependent manner, spontaneous contractions in the longitudinal smooth muscle from rat duodenum. This inhibitory effect may be due to its effect on the entry or the release of Ca^{2+} into the cytosol. In fact, addition of verapamil, an L-type Ca^{2+} channels inhibitor, induces a similar response to our extract.

At high concentrations, K^+ causes contractions in the smooth muscle via the opening of L-type Ca^{2+} channels and the resulting entry of Ca^{2+} from the extracellular medium, as we have previously demonstrated in the rabbit duodenum (16). In a similar way, in the present study, K^+ induced contractions that were blocked by verapamil. Rock tea extract induced relaxation, in a concentration dependent manner, on the intestine precontracted with K^+, in a similar way to that obtained with verapamil, suggesting that in both cases the same mechanism of action was involved, *i.e.,* inhibition of L-type Ca^{2+} channels.

Rock tea extract produced a shift in the response curve to $CaCl_2$ towards the right and down, confirming a direct action on the intestinal smooth muscle by inhibiting the entry of Ca^{2+}. Furthermore, preincubation with the extract blocked in a concentration dependent manner the contraction induced by an activator of L-type Ca^{2+} channels, Bay K8644. Once again, these effects were similar to those produced by verapamil.

All these results suggest that rock tea decreases intestinal motility and contractility by inhibiting the entry of Ca^{2+} into the cell, probably by the presence of L-type Ca^{2+} channel antagonists in the extract.

On the other hand, rock tea did not modify whole gastrointestinal transit in healthy mice. However, in animals treated with DSS, a known inductor of colitis (17), rock tea extract reverted the increase in the intestinal transit observed in the animals treated with its solvent.

Several studies have demonstrated that the spasmolytic activity of different plants is usually due to the antagonistic effect that they exert on Ca^{2+}, and hence inhibitors of Ca^{2+} channels are used as antispasmodic and antidiarrheal agents (18,19).

Studies carried out by our group have demonstrated that rock tea is rich in flavonoids and terpenes (9). These results are in accordance with those previously obtained with flavonoids showing that they relax the precontracted intestinal smooth muscle and delay intestinal transit and peristalsis (20,21).

Our results suggest that rock tea extract relaxes the duodenal smooth muscle via the inhibition of L-type Ca^{2+}

channels and reverts the increase in the gastrointestinal transit produced by DSS. Therefore, *Jasonia glutinosa* could be useful in the prevention or treatment of disorders involving alterations in digestive motility.

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