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Effect of *Boswellia serrata* on intestinal motility in rodents: inhibition of diarrhoea without constipation

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1 Clinical studies suggest that the Ayurvedic plant *Boswellia serrata* may be effective in reducing diarrhoea in patients with inflammatory bowel disease. In the present study, we evaluated the effect of a *Boswellia serrata* gum resin extract (BSE) on intestinal motility and diarrhoea in rodents.

2 BSE depressed electrically-, acetylcholine-, and barium chloride-induced contractions in the isolated guinea-pig ileum, being more potent in inhibiting the contractions induced by acetylcholine and barium chloride.

3 The inhibitory effect of BSE on acetylcholine-induced contractions was reduced by the L-type Ca^{2+} channel blockers verapamil and nifedipine, but not by the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid, by the phosphodiesterase type IV inhibitor rolipram or by the lipoxygenase inhibitor zileuton.

4 3-acetyl-11-keto-β-boswellic acid, one of the main active ingredients of *B. serrata*, inhibited acetylcholine-induced contractions.

5 BSE inhibited upper gastrointestinal transit in croton oil-treated mice as well as castor oil-induced diarrhoea. However, BSE did not affect intestinal motility in control mice, both in the small and in the large intestine.

6 It is concluded that BSE directly inhibits intestinal motility with a mechanism involving L-type Ca^{2+} channels. BSE prevents diarrhoea and normalizes intestinal motility in pathophysiological states without slowing the rate of transit in control animals. These results could explain, at least in part, the clinical efficacy of this Ayurvedic remedy in reducing diarrhoea in patients with inflammatory bowel disease.

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Abbreviations: BSE, *Boswellia serrata* gum resin extract; DMSO, dimethyl sulphoxide; PDE, phosphodiesterase

Introduction

Boswellia serrata Roxb (Fam *Burseraceae*), also called Indian olibanum, is a moderate to large branching tree native of India, North Africa and the Middle East. A gum resin, obtained tapping the tree trunk, is widely used in Ayurvedic medicine for the treatment of inflammatory diseases, including those affecting the gastrointestinal tract (e.g. diarrhoea, dysentery, and inflammatory bowel disease). The anti-inflammatory activity of *B. serrata* gum resin has been confirmed by experimental and clinical studies (Capasso *et al*., 2003). For example, extracts of *B. serrata* gum resin: (i) display a marked anti-inflammatory activity in carrageenan-, dextran-, and papaya-latex-induced models of inflammation in rodents (Singh & Atal, 1986; Gupta *et al*., 1992), (ii) reduce the infiltration of polymorphonuclear leucocytes in carrageenan-induced pleurisy (Sharma *et al*., 1988), and (iii) inhibit leukotriene synthesis from arachidonic acid in rat peritoneal polymorhonuclear leukocytes (Ammon *et al*., 1991). Boswellic acids (β-boswellic acid, 3-acetyl-β-boswellic acid, 11-keto-β-boswellic acid and 3-acetyl-11-keto-β-boswellic acid) have been suggested to be the active constituents of this herbal drug (Safayhi *et al*., 1992). These compounds are specific, nonredox inhibitors of 5-lipoxygenase without affecting 12-lipoxygenase and cyclooxygenase activities (Ammon *et al*., 1993; Wildfeuer *et al*., 1998; Safayhi *et al*., 2000).

A purified extract of the resin is actually used in modern herbal preparations to treat a number of inflammatory conditions including inflammatory bowel disease. Clinical studies have shown that *B. serrata* gum resin is effective in patients with ulcerative colitis (grades II and III). Treatment with this herbal drug is associated to improvement of a number of parameters of the pathology, including stool consistency and frequency (Gupta *et al*., 1997; 2001; Gerhardt *et al*., 2001) and it has been considered superior over mesalazine in terms of a benefit-risk evaluation (Gupta *et al*., 1997).

As a result of the clinically established symptomatic improvement of inflammatory bowel disease symptoms (including the reduction of the diarrhoea) seen under treatment with *B. serrata* and its traditional use in Ayurvedic medicine as

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antidiarrhoeal agent, we investigated the effect and the mode of action of this herbal drug on intestinal motility, both *in vitro* and *in vivo*. We also evaluated the effect of 3-acetyl-11-keto-β-boswellic acid, one of the main active ingredients of *B. serrata*.

Methods

Animals

Male ICR mice (20–22 g) and New Zealand guinea-pigs (300–450 g) were supplied by Harlan Nossan Italy, Corezzana, MI, U.S.A. All animals, used after 1 week of acclimation (temperature $23 \pm 2^{\circ}$C; humidity 60%), had free access to water and food. All experiments on animals complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European communities Council Directive of 24 November 1986 (86/609/ECC).

In vitro *experiments*

Guinea-pigs were killed by asphyxiation with CO_2 and segments (2–3 cm) of the terminal ileum were removed, flushed of luminal contents, and placed in Krebs' solution (composition in mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5, and glucose 11). The isolated organ was set up (in such a way to record contractions from the longitudinal axis) in an organ bath filled warm (37°C) aerated (95% O_2 : 5% CO_2) Krebs' solution. The tissues were connected to an isotonic transducer (load 0.5 g) connected to a PowerLab data-acquisition system (Ugo Basile, Comerio, Italy). At the beginning of each experiment, the ileum was stimulated with acetylcholine (10^{-3} M) in order to obtain a maximal contraction (100% contraction). After a minimal 1-h equilibration period, the tissues were subjected to electrical field stimulation (EFS, 10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration using a multiplexing pulse booster by Ugo Basile, Milan, Italy) *via* a pair of platinum electrodes (situated at a distance of 1.5 cm) placed around the intestine or stimulated with spasmogens, namely acetylcholine (10^{-6} M) or barium chloride (10^{-4} M). The concentrations of acetylcholine and barium chloride gave a contractile response which was similar in amplitude to that of EFS. Acetylcholine and barium chloride were added to the bath and left in contact with the tissue for 30 s and then washed out. The interval between each stimulation was 20 min. After at least three stable control contractions, the contractile responses were repeated in the presence of increasing (noncumulative) concentrations of *B. serrata* gum resin extract (BSE, 0.001–3 mg ml^{-1}) added 20 min before the contacting stimulus (i.e. after washing the tissue). Preliminary experiments showed that a 20 min contract time was sufficient for BSE to achieve the maximal inhibitory effect.

In some experiments, the effect of BSE on acetylcholine-induced contractions was evaluated in the presence of verapamil (10^{-6} M), nifedipine, (10^{-6} M), cyclopiazonic acid (10^{-5} M), rolipram (10^{-6} M), or zileuton (10^{-5} M) (contact time: 20 min for each drug). The concentrations of verapamil, nifedipine, cyclopiazonic acid, rolipram, and zileuton were selected on the basis of previous published work (Uyama *et al*., 1992; Izzo *et al*., 1999; Aronsson & Holmgren, 2000; Lis-Balchin & Hart, 2002). The presence of such inhibitors/antagonists did not affect the reproducibility and the stability of the contractions induced by acetylcholine. In another set of experiments, we evaluated the effect of 3-acetyl-11-keto-β-boswellic acid (2×10^{-7}–2×10^{-4} M, contact time: 20 min per concentration) on acetylcholine (10^{-6} M)-induced contractions.

Finally, some experiments were performed using the mouse terminal ileum (1–1.5 cm). This tissue was stimulated by acetylcholine (10^{-6} M) and the effect of BSE (1–1000 μg ml^{-1}) was evaluated. The experimental conditions and protocol of drugs administration were the same as described for the guinea-pig ileum.

In vivo *experiments*

Chronic intestinal inflammation Inflammation was induced as previously described (Pol & Puig, 1997; Izzo *et al*., 2000). Briefly, two doses of croton oil (20 μl mouse^{-1}) in two consecutive days were orally administered to mice and four days after the first administration of croton oil, upper gastrointestinal transit of mice was measured. This time was selected on the basis of a previous work (Pol & Puig, 1997), which reported that maximal inflammatory response occurred 4 days after the first treatment.

Upper gastrointestinal transit Upper gastrointestinal transit was measured in control and in croton oil-treated mice. A black marker (0.1 ml 10 g mouse^{-1}; 10% charcoal suspension in 5% gum Arabic) was administered orally to assess gastrointestinal transit as previously described (Pol & Puig, 1997; Izzo *et al*., 2001). After 20 min the mice were killed by asphyxiation with CO_2 and the gastrointestinal tract removed. The distance travelled by the marker was measured and expressed as a percentage of the total length of the small intestine from pylorus to caecum. BSE (100–400 mg kg^{-1}) or vehicle (carboxymethylcellulose 1%) was given orally 60 min before charcoal administration, both to control mice and to mice with chronic inflammation. In some experiments, we evaluated the effect of atropine (1 mg kg^{-1}), used as a reference drug, on motility in control mice.

Colonic propulsion Distal colonic propulsion was measured as previously described (Broccardo *et al*., 1998; Pinto *et al*., 2002). A single 3-mm glass bead was inserted 2 cm into the distal colon of each mouse with the aid of a catheter and the time to expulsion of the glass bead was determined for each animal. BSE (100–400 mg kg^{-1}), atropine (1 mg kg^{-1}, used as a reference drug), or vehicle (carboxymethylcellulose 1%) were given orally 60 min before glass bead insertion.

Castor oil-induced diarrhoea Diarrhoea was induced by oral administration of castor oil (0.2 ml mouse^{-1}) to mice fasted for a night. BSE (100–400 mg kg^{-1}) or vehicle (carboxymethylcellulose 1%) were given orally 60 min before cathartic administration. At 2 h after dosing with castor oil the individual mouse cages were inspected (by an observer unaware of the particular treatment) for the presence of unformed water fecal pellets; their absence was recorded as a positive result, indicating protection from diarrhoea at that time (Capasso *et al*., 1994). In some experiments, the effect of BSE (100–400 mg kg^{-1}) on castor oil-induced diarrhoea was evaluated in mice pretreated (i.p.) with zileuton (35 mg kg^{-1}, 30 min before BSE).

Drugs

Drugs used were: castor oil, acetylcholine chloride, barium chloride, verapamil, nifedipine, cyclopiazonic acid, rolipram, atropine, tetrodotoxin, polyethylene glycol, carboxymethylcellulose (all from Sigma, Milan, Italy), zileuton (Sequoia Research Products Ltd, U.K.), and 3-acetyl-11-keto-β-boswellic acid (LGC Promochem GmbH, Germany); *B. serrata* gum resin hydroalcoholic extract (BSE, standardized to contain 95% boswellic acids) was a gift from Carlo Sessa, Milan, Italy. Acetylcholine, barium chloride, atropine, and tetrodotoxin were dissolved in distilled water; zileuton, verapamil, nifedipine, cyclopiazonic acid, and 3-acetyl-11-keto-β-boswellic acid in dimethyl sulphoxide (DMSO). Rolipram was dissolved in DMSO to give 10^{-5} M stock solution and subsequent dilutions were made in distilled water. BSE was suspended in carboxymethylcellulose 1% (for *in vivo* experiments) or in polyethylene glycol (for *in vitro* experiments). Drugs were added in volumes $<0.01\%$ *in vitro* and given in the amount of 0.01 ml mouse^{-1} (DMSO) or 0.1 ml mouse^{-1} (carboxymethylcellulose) *in vivo*. The drug vehicles had no effect on the responses under study, both *in vitro* and *in vivo*.

Statistics

Data are mean $\pm$ s.e.m. Comparisons between two sets of data were made by Student's *t*-test for paired data. When multiple comparisons against a single control were made, one-way analysis of variance was used, followed by Turkey–Kramer multiple comparisons test. Analysis of variance (two way) was used to compare different cumulative concentration–effect curves.

Diarrhoea was expressed as total score and the χ^2 test was used to determine the significance between groups. A *P*-value <0.05 was considered significant.

Results

In vitro *experiments*

The contractile responses of guinea-pig ileum to EFS reached $55.3 \pm 4.56\%$ ($n=6$) of the contraction produced by acetylcholine 10^{-3} M. This concentration of acetylcholine produced a maximal contractile response in the ileum (100% contraction). EFS-induced contractions were abolished by tetrodotoxin (3×10^{-7} M) or atropine (10^{-6} M), thus suggesting that these contractions were mediated by the release of acetylcholine from enteric nerves. However, tetrodotoxin did not modify the contractions induced by either acetylcholine (10^{-6} M) or barium chloride (10^{-4} M) (data not shown).

BSE (1–1000 μg ml^{-1}) significantly and in a concentration-dependent manner, inhibited the contractions induced by acetylcholine, barium chloride or by EFS (Figure 1). BSE was significantly ($P<0.001$) more active in inhibiting the contractions induced by acetylcholine (or barium chloride) than the contractions induced by EFS (Figure 1). Figure 2 reports a typical trace showing the inhibitory effect of BSE on acetylcholine-induced contractions.

Verapamil (10^{-6} M) and nifedipine (10^{-6} M), but not cyclopiazonic acid (10^{-5} M), significantly reduced the inhibitory effect of BSE on acetylcholine-induced contractions

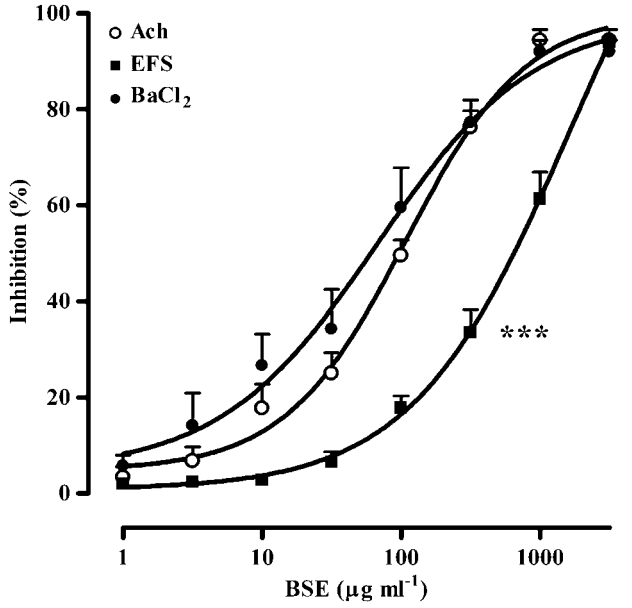

Figure 1 Inhibitory effect of *B. serrata* gum resin extract (BSE, 1–1000 μg ml^{-1}) on the contractile response induced by exogenous acetylcholine (ACh, 10^{-6} M), barium chloride ($BaCl_2$, 10^{-4} M), or electrical field stimulation (EFS, 10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration) in the isolated guinea-pig ileum. Each point represents mean $\pm$ s.e.m. of six to eight experiments. ***$P<0.001$ vs ACh (significance between the two dose–response curves).

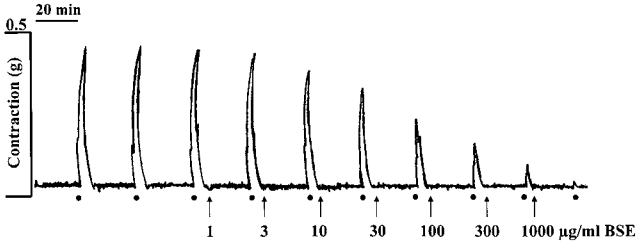

Figure 2 Typical trace showing inhibitory effect of *B. serrata* gum resin extract (BSE, 1–1000 μg ml^{-1}) on contractions produced by acetylcholine in isolated guinea-pig ileum. Dots indicate contractions induced by acetylcholine (10^{-6} M), arrows indicate the administrations of BSE in the organ bath.

(Figure 3). Rolipram (10^{-6} M) and zileuton (10^{-5} M) did not modify the effect of the extract on acetylcholine-induced contractions (rolipram % of inhibition: BSE 1 μg ml^{-1} 3.93 ± 2.53, BSE 3 μg ml^{-1} 5.13 ± 3.16, BSE 10 μg ml^{-1} 15.9 ± 4.23, BSE 30 μg ml^{-1} 29.2 ± 5.18, BSE 100 μg ml^{-1} 52.3 ± 6.34, BSE 300 μg ml^{-1} 76.6 ± 3.51, BSE 1000 μg ml^{-1} 96.0 ± 3.02; zileuton % of inhibition: BSE 1 μg ml^{-1} 3.01 ± 1.89, BSE 3 μg ml^{-1} 4.68 ± 2.58, BSE 10 μg ml^{-1} 18.9 ± 4.05, BSE 30 μg ml^{-1} 30.14 ± 5.01, BSE 100 μg ml^{-1} 55.9 ± 6.15, BSE 300 μg ml^{-1} 80.1 ± 3.92, BSE 1000 μg ml^{-1} 97.1 ± 3.11). When given alone (i.e. in absence of BSE) verapamil (10^{-6} M), nifedipine (10^{-6} M) and cyclopiazonic acid (10^{-5} M) reduced (verapamil: $45.51 \pm 5.41\%$ reduction, $P<0.001$; nifedipine: $43.26 \pm 3.45\%$ reduction, $P<0.001$; cyclopiazonic acid: 54.13 ± 6.22, $P<0.001$, $n=8$ for each drug) the contractions induced by acetylcholine. By contrast, rolipram (10^{-6} M) increased ($47.83 \pm 8.98\%$ increase, $P<0.01$) while zileuton (10^{-5} M) did not modify acetylcholine-induced contractions. Verapamil and nifedipine, at a concentration

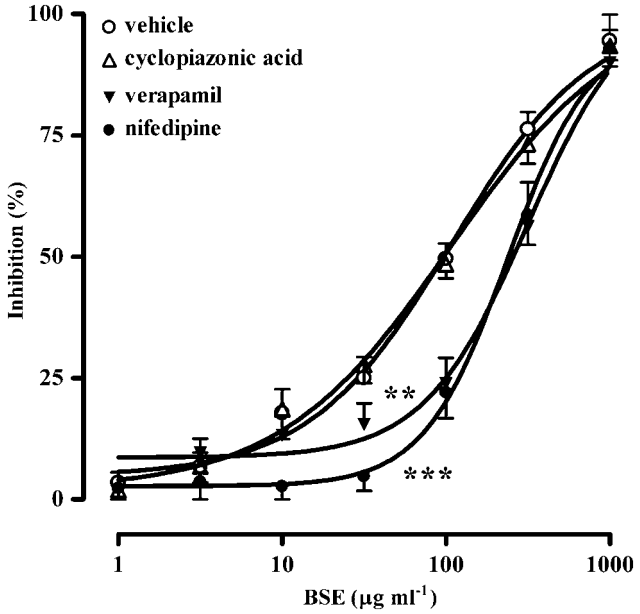

Figure 3 Acetylcholine-induced contractions in isolated guinea-pig ileum: effect of *B. serrata* gum resin extract (BSE, 1–1000 $\mu g\,ml^{-1}$) alone (vehicle) or in the presence of verapamil (10^{-6} M), nifedipine (10^{-6} M) or cyclopiazonic acid (10^{-5} M). Each point represents mean ± s.e.m. of six to eight experiments. $^{**}P<0.01$ and $^{***}P<0.001$ vs vehicle (significance between the two dose–response curves).

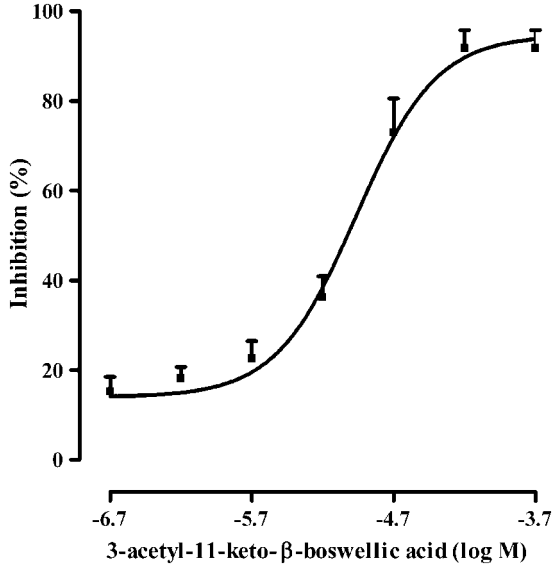

Figure 4 Inhibitory effect of 3-acetyl-11-keto-β-boswellic acid (2×10^{-7}–2×10^{-4} M) on the contractions induced by acetylcholine (10^{-6} M) in the isolated guinea-pig ileum. Each point represents mean ± s.e.m. of six to eight experiments.

higher than 10^{-6} M (i.e. 3×10^{-6} M) did not produce a further inhibitory effect on both acetylcholine-induced contractions and BSE antispasmodic effect (data not shown). 3-acetyl-11-keto-β-boswellic acid (2×10^{-7}–2×10^{-4} M), significantly and in a concentration dependent manner, inhibited the contractile response elicited by acetylcholine (Figure 4). BSE (1–1000 $\mu g\,ml^{-1}$) and 3-acetyl-11-keto-β-boswellic acid (2×10^{-7}–2×10^{-4} M) had no effect on the baseline mechanical activity of the intestine (data not shown).

Finally, BSE also inhibited the contractions induced by acetylcholine in the mouse ileum (percentage of inhibition: BSE 1 $\mu g\,ml^{-1}$ 3.54 ± 1.17, BSE 3 $\mu g\,ml^{-1}$ 7.02 ± 1.96, BSE 10 $\mu g\,ml^{-1}$ 20.9 ± 4.02, BSE 30 $\mu g\,ml^{-1}$ 32.8 ± 4.12, BSE 100 $\mu g\,ml^{-1}$ 51.3 ± 5.10, BSE 300 $\mu g\,ml^{-1}$ 79.8 ± 4.23, BSE 1000 $\mu g\,ml^{-1}$ 98.1 ± 3.44; $P<0.05$ for the 30 and 100 $\mu g\,ml^{-1}$ concentrations, $P<0.01$ for the 300 $\mu g\,ml^{-1}$ concentration and $P<0.001$ for the 1000 $\mu g\,ml^{-1}$ concentration, $n=6$).

In vivo *experiments*

Upper gastrointestinal transit and colonic propulsion in control mice Oral administration of BSE (100–400 $mg\,kg^{-1}$) had no effect on motility, both in the upper gastrointestinal tract (Figure 5a) and in the large intestine (Figure 5b). By contrast, atropine (1 $mg\,kg^{-1}$, used as a reference drug) inhibited motility both in the upper gastrointestinal tract and in the large intestine (Figure 5a and b).

Upper gastrointestinal transit in croton oil-treated mice According to previous studies (Pol & Puig, 1997; Capasso *et al*., 2001), administration of croton oil produced a significant increase ($P<0.01$) in upper gastrointestinal transit (Figure 6). BSE (100–400 $mg\,kg^{-1}$) counteracted the increase in motility induced by croton oil (Figure 6). The effect was significant starting from the 200 $mg\,kg^{-1}$ oral dose.

Castor oil-induced diarrhoea At 2 h after castor oil administration (0.2 $ml\,mouse^{-1}$) mice produced copious diarrhoea. BSE significantly ($P<0.01$) reduced castor oil-induced diarrhoea in a dose-related manner (Figure 7). Consistent with the experiments in mice treated with croton oil, a significant inhibitory effect was achieved for the 200–400 $mg\,kg^{-1}$ oral doses. Zileuton (35 $mg\,kg^{-1}$) did not modify castor oil-induced diarrhoea (% of mice with diarrhoea: castor oil 91.7; castor oil + zileuton 93.3; $n=12$–15 for each experimental group, $P>0.2$) or the antidiarrhoeal effect of BSE (% of mice with diarrhoea: castor oil 91.7; castor oil + BSE 200 $mg\,kg^{-1}$ 63.63; castor oil + zileuton + BSE 60.0; $n=12$–15 for each experimental group, $P>0.2$).

Discussion

B. serrata is an Ayurvedic remedy widely employed for the treatment of diarrhoea and inflammatory bowel disease. Suppression of leukotriene synthesis *via* inhibition of 5-lipoxygenase has been considered to be the main mechanism underlying its beneficial effect in intestinal inflammatory disease (Ammon, 1996). The present study provides evidence that BSE inhibits intestinal motility which may contribute to the clinical efficacy of this herbal drug in normalizing motility changes associated to inflammatory bowel disease.

We have shown that BSE produced a concentration-dependent inhibition of both acetylcholine- and electrical field stimulation-evoked contractions in the isolated guinea-pig ileum. Moreover, BSE preferentially inhibited the contractions induced by acetylcholine (which are due to a direct activation of muscarinic receptors located on smooth muscles) rather than the contractions elicited by EFS (which are mediated by

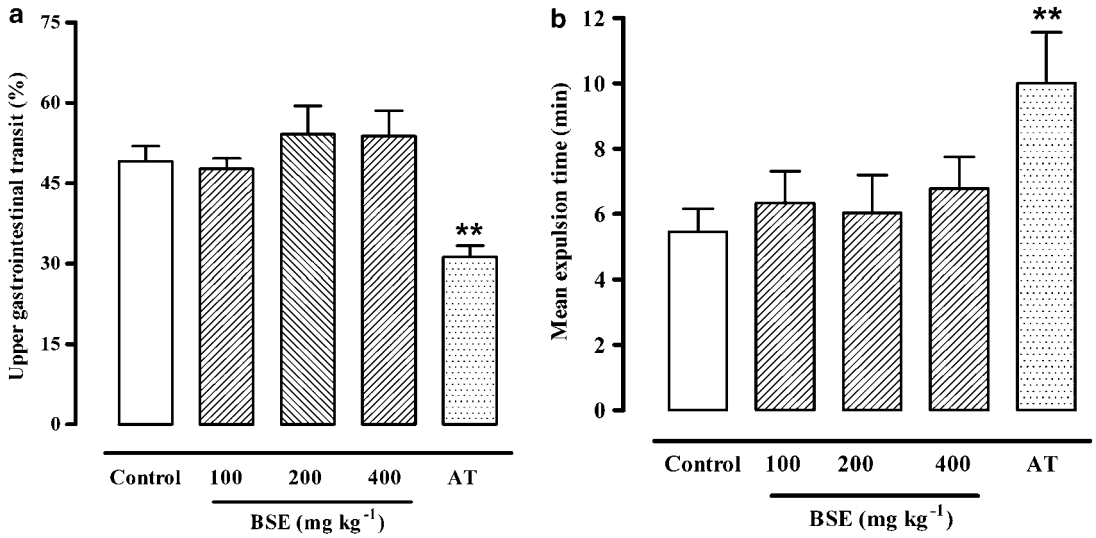

Figure 5 Effect of *B. serrata* gum resin extract (BSE, 100–400 mg kg^{-1}) and atropine (AT, 1 mg kg^{-1}) on upper gastrointestinal transit (a) and colonic propulsion in mice (b). Results are mean ± s.e.m. of 10–12 animals for each experimental group. $^{**}P<0.01$ vs control.

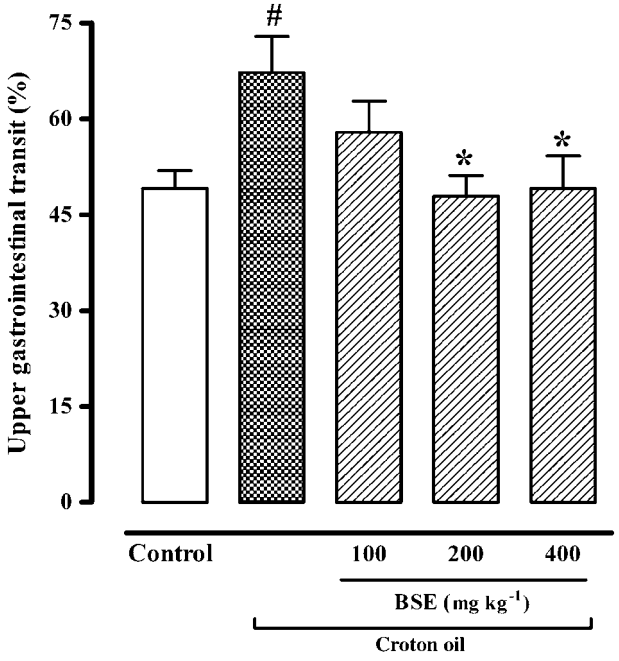

Figure 6 Effect of *B. serrata* gum resin extract (BSE, 100–400 mg kg^{-1}) on upper gastrointestinal transit in mice treated with croton oil (20 μl mouse^{-1}). Results are mean ± s.e.m. of 10–12 animals for each experimental group. $^{\#}P<0.01$ vs control; $^{*}P<0.05$ vs croton oil.

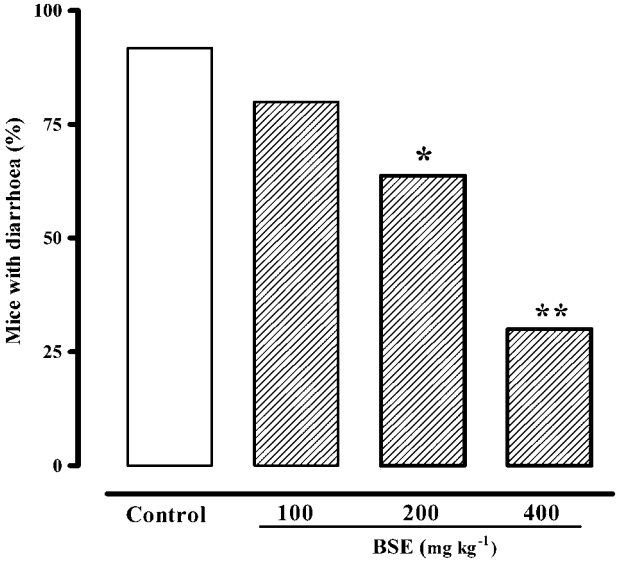

Figure 7 Effect of *B. serrata* gum resin extract (BSE, 100–400 mg kg^{-1}) on castor oil-induced diarrhoea. The effect of BSE was assessed 2 h after castor oil (0.2 ml mouse^{-1}) administration ($n=10$–12). $^{*}P<0.05$ and $^{**}P<0.01$ vs control.

the release of acetylcholine from myenteric nerves). These results indicate that BSE (i) could release from neural or non-neural sources endogenous substances which have an excitatory effect at prejunctional level and, more importantly, that (ii) exerts an antispasmodic effect by acting directly on intestinal smooth muscles. It is very unlikely that the antispasmodic effect of BES is due to antimuscarinic actions, since this herbal drug also inhibited the contractions induced by barium chloride which does not act through a receptor-mediated mechanism. As the inhibitory effect of BSE was exerted at postjunctional level, we investigated the mechanism of the antispasmodic activity of this herbal extract on the contractions induced by acetylcholine.

Contractions of all smooth muscles, including those of gastrointestinal tract, absolutely depend on the presence of Ca^{2+}. Agonists-induced contractions may be related to the release of intracellular Ca^{2+} from sarcoplasmic stores in addition to the influx, mainly through L-type Ca^{2+} channels of extracellular Ca^{2+} (Makhlouf, 1994). Consequently, smooth muscle contraction can be abolished by antispasmodic drugs through the inhibition of Ca^{2+} entry or release into the cells. In the present study, we have shown that verapamil and nifedipine, two blockers of L-type Ca^{2+} channels, reduced the

inhibitory effect of BSE on acetylcholine-induced contractions suggesting the involvement of such channels in the mode of BSE. The lack of effect of cyclopiazonic acid, a well known potent and specific inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase in smooth muscles (Grasa *et al*., 2004), excludes an action of BSE on the release of Ca^{2+} from sarcoplasmic stores.

Phosphodiesterase (PDE) enzymes are responsible for the breakdown of cyclic nucleotides (Beavo, 1995). At least seven PDE isoenzyme families exist including PDE IV, which is involved in the regulation of intestinal contractility (Bauer *et al*., 1991; Tomkinson & Raeburn, 1996). Blockade of PDE IV results in an increase of cAMP which in turn decreases calcium entry into the cell through L-type Ca^{2+} channels (Kaneda *et al*., 1997). In the present study, we have shown that the PDE IV inhibitor rolipram did not affect the inhibitory effect of BSE on intestinal motility, thus suggesting that BSE possibly affects L-type Ca^{2+} channels trough a PDE IV-independent mechanism. An unexpected result of the present study was the ability of the PDE IV inhibitor rolipram, given alone, to potentiate acetylcholine-induced contractions. Indeed, inhibition of phosphodiesterase IV is expected to reduce contractions by increasing the basic amount of the relaxing second messenger cAMP (Barbier & Lefebvre, 1995). The reason for this discrepancy remains to be examined.

Boswellic acids are considered to be the ingredients responsible of the plant anti-inflammatory activity, since these compounds inhibit leukotriene biosynthesis by impairing the lipoxygenase activity (Wildfeuer *et al*., 1998; Safayhi *et al*., 2000). In the present study, we evaluated the effect of 3-acetyl-11-keto-β-boswellic acid, one of the most effective compounds among the boswellic acids tested so far *in vitro* (Sailer *et al*., 1996; 1997). This compound has been found to attenuate experimental ileitis in rats (Krieglstein *et al*., 2001). Our findings show that 3-acetyl-11-keto-β-boswellic acid effectively reduced acetylcholine-induced contractions, thus suggesting that this compound may be responsible, at least in part, of the antispasmodic action of BSE. As compounds which modulate intestinal contractility *in vitro* may affect motility *in vivo*, and because BSE inhibited acetylcholine-induced contractions both in the guinea-pig ileum and in the mouse, we evaluated the effect of BSE on intestinal transit in the mouse *in vivo*. Moreover, because *B. serrata* has been clinically evaluated in patients with inflammatory bowel disease, we also studied the effect of these doses of herbal extract in a model of intestinal inflammation. Croton oil is a well known irritant used experimentally to induce inflammation in the mouse small intestine (Pol & Puig, 1997; Izzo *et al*., 2001). This inflammation is characterized by disruption of the mucosa and an infiltration of lymphocytes into the submucosa associated with an increase of intestinal transit (Pol & Puig, 1997). We found that BSE (in the dose range of 100–400 mg kg^{-1}) was without effect in control animals, but inhibited motility in animals treated with croton oil. Based on human pharmacokinetic data (Mack, 1990) and by assuming similar pharmacokinetics in mice, an oral dose of 100–400 mg kg^{-1} of BSE should result approximately in plasma concentrations of boswellic acids within the same range as the concentrations tested *in vitro*. Interestingly, Shi & Sarna (2000) found that Ca^{2+} influx through L-type Ca^{2+} channels, but not the release of intracellular Ca^{2+}, is involved in motility changes associated to intestinal inflammation (induced by exposure to ethanol and acetic acid), which is consistent with the ability of verapamil and nifedipine to reduce the inhibitory effect of BSE on motility *in vitro* (see above). Others have shown that *B. serrata* resin attenuated macroscopic and microscopic inflammatory features associated with indomethacin-induced ileitis in rats (Krieglstein *et al*., 2001). By contrast, it has been recently reported that *B. serrata* did not ameliorate symptoms of dextran sulfate- or trinitrobenzene sulfonic acid-induced colitis in mice (Kiela *et al*., 2005).

It is well known that drugs which inhibit intestinal transit in pathophysiological state may be effective in alleviating diarrhoea. In addition, because diarrhoea is a major pathophysiological feature in patients with inflammatory bowel disease, we evaluated the potential antidiarrhoeal effect of BSE. We used the castor oil test, which is extensively employed as a basic pharmacological test to screen antidiarrhoeal drugs. One of the assets of this model is the very reproducible evacuation of unformed stools 2-h after laxative administration. We found that BSE reduced castor oil-induced diarrhoea, a relevant finding in the light of the fact that BSE administration is not associated with constipating effects under physiological conditions (see results on intestinal transit described above). Indeed, one of the major side effects associated with oral administration of opiates (the most known antidiarrhoeal agents) is their constipating effect (Jafri & Pasricha, 2001). It is noteworthy that an involvement of L-type Ca^{2+} channels in the mode of action of BSE is consistent with the ability of verapamil to reduce diarrhoea during small intestinal inflammation (Lee *et al*., 1997). The effect of BSE could be either due to a direct effect on L-type channel activity or to an indirect effect on cellular membrane which reduce the activity of these channels (e.g. a change of the membrane potential). As a result of its action on L-type calcium channels, BSE could influence cardiovascular functions, including changes in blood pressure. However, BSE does not cause cardiovascular side effects when used in humans (Gupta *et al*., 1997).

Finally, we investigate the possible role of the lipoxygenase enzyme in the motility changes associated to BSE. Previous investigators have reported that boswellic acids are inhibitors of lipoxygenase enzyme. Moreover, leukotrienes may affect intestinal motility and have been involved in diarrhoeal diseases (Shahbazian *et al*., 2002). We have shown that the lipoxygenase inhibitor zileuton did not modify the antispasmodic and the antidiarrhoeal effect of BSE, thus suggesting a lack of involvement of leukotrienes in the mode of action of BSE.

In conclusion, the present results demonstrate that BSE can normalize the intestinal motility altered by an inflammatory stimulus and possesses antidiarrhoeal activity. *In vitro* studies have shown that the extract directly inhibits intestinal motility with a mechanism involving L-type Ca^{2+} channels. These results could explain, at least in part, the clinical efficacy of this Ayurvedic remedy in reducing diarrhoea in patients with inflammatory bowel disease. Moreover, BSE prevented experimental diarrhoea without slowing the rate of transit, which is of potential clinical interest since currently used antidiarrhoeal agents are often associated with constipating effects.

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