

# Pharmacological basis for the use of *Borago officinalis* in gastrointestinal, respiratory and cardiovascular disorders

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Received 5 December 2006; received in revised form 16 July 2007; accepted 14 August 2007

Available online 24 August 2007

## Abstract

**Aim of the study:** In this study, we investigated the crude extract of *Borago officinalis* leaves (Bo.Cr) for its antispasmodic, bronchodilator, vasodilator and cardio-depressant activities to rationalize some of the traditional uses.

**Materials and methods:** Bo.Cr was studied using different isolated tissue preparations including rabbit jejunum, trachea, aorta, and guinea-pig atria.

**Results:** Bo.Cr which was tested positive for flavonoids, coumarins, sterols and tannins produced a concentration-dependent relaxation of spontaneous and K<sup>+</sup> (80 mM)-induced contractions in isolated rabbit jejunum preparations, suggestive of Ca<sup>++</sup> antagonist effect, which was confirmed when pretreatment of the tissue with Bo.Cr produced a rightward shift in the Ca<sup>++</sup> concentration-response curves like that caused by verapamil. In rabbit tracheal preparations, Bo.Cr relaxed the carbachol (1 μM) and K<sup>+</sup>-induced contractions. Verapamil also produced non-specific inhibitory effect. In rabbit aorta preparations, Bo.Cr exhibited vasodilator effect against phenylephrine and K<sup>+</sup>-induced contractions similar to verapamil. When tested in guinea-pig atria, Bo.Cr caused inhibition of both atrial force and rate of contractions.

**Conclusions:** These results suggest that the spasmolytic effects of Bo.Cr are mediated possibly through Ca<sup>++</sup> antagonist mechanism, which might explain the traditional use of *Borago officinalis* in hyperactive gastrointestinal, respiratory and cardiovascular disorders.

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**Keywords:** *Borago officinalis*; Antispasmodic; Bronchodilator; Vasodilator; Cardio-depressant; Ca<sup>++</sup> antagonist

## 1. Introduction

*Borago officinalis* Linn. (family; Boraginaceae) is a hairy annual herb commonly known as ‘Borage’ and ‘Gaozaban’ or ‘Lisan al-Thawr’ locally in Pakistan. It has been known for its mood elevating properties as early as the first century A.D. (Tyler, 1993). The plant is reputed as antispasmodic, antihypertensive, antipyretic, aphrodisiac, demulcent, diuretic and is also considered useful to treat asthma, bronchitis, cramps, diarrhea, palpitations and kidney ailments (Usmanghani et al., 1997; Duke et al., 2002). Decoction of the plant is used as nerve and cardiac tonic and a home remedy for blood purification (Kybal, 1980).

Phytochemical studies reveal the presence of tannins, resins, ascorbic acid, beta-carotene, niacin, riboflavin, thiamine, silicic acid, choline arabinose, unsaturated pyrrolizidines alkaloids including amabiline (Duke, 1992), lycopsamine and supinidine (Larson et al., 1984), polyphenolics including phenolic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxy benzoic acid, gentisic acid, caffeic acid, rosmarinic acid and chlorogenic acid, scopoletin and flavonoids (Gudej and Tomczyk, 1996).

Borage oil has been reported to lower serum cholesterol, phospholipids and triglyceride levels (Gu et al., 1998) and increases the levels of Ω-6 polyunsaturated fatty acids in the plasma, liver, aorta and renal artery tissues. Dietary use of borage oil exhibited immuno-modulatory (Harbig et al., 2000) and blood pressure lowering effects in normal and spontaneously hypertensive rats through unknown mechanism (Engler and Engler, 1998). Borage oil is considered to improve task performance through attenuation of blood pressure, heart rate and

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temperature elevation in responses to stress (Mills et al., 1989). It exhibited kidney protective potential through angiotensin II receptor blockade (Engler et al., 1998), cytotoxic and free radical scavenging activities (Bandoniene and Murkovic, 2002; Lin et al., 2002).

Despite the fact that *Borago officinalis* has been used traditionally, it has not been widely studied to justify its use in abdominal colic, diarrhea, asthma and hypertension. In this investigation, we report the presence of  $\text{Ca}^{+2}$  antagonist-like constituents, which provide the pharmacological basis for the use of *Borago officinalis* in hyperactive gastrointestinal, respiratory and cardiovascular disorders.

## 2. Materials and methods

### 2.1. Plant material and preparation of crude extract

*Borago officinalis* leaves were purchased in dried condition from a local herbal store in Multan. The sample was identified with the help of Dr. Altaf A. Dasti, a taxonomist at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. The plant material was cleaned and ground into coarse powder by electrically driven device. The powdered material was soaked into aqueous ethanol (80% v/v) for 1 week with occasional shaking (Williamson et al., 1998). It was filtered through a muslin cloth and then through a Whatman Qualitative Grade-I filter paper. The filtrate was subjected to evaporation under reduced pressure on a rotary evaporator to a thick paste like mass of dark brown colour, i.e., the crude extract (Bo.Cr), yielding approximately 10.9%. Bo.Cr was completely dissolved in distilled water for *in vitro* experiments.

### 2.2. Phytochemical screening

Preliminary phytochemical analysis was carried out for the presence of flavonoids, coumarins, sterols, tannins, alkaloids, anthraquinones and saponins (Evans, 1996; Edeoga et al., 2005). Briefly, plant material was tested as positive for flavonoids when it gave yellow color on treatment with  $\text{AlCl}_3$  reagent and for tannins, when green or black color was produced with aqueous  $\text{FeCl}_3$ . For the detection of sterols, plant material was treated with petroleum ether and subsequently extracted with  $\text{CHCl}_3$ . The gradual appearance of green to pink was then noted after treatment of  $\text{CHCl}_3$  layer with acetic anhydride and concentrated HCl in succession. For coumarins, a piece of filter paper was moistened in NaOH and then kept over a test tube with boiling plant extract solution. The observation of yellow fluorescence on the examination of filter paper under UV light indicates the presence of coumarins. Alkaloids were screened by mixing with Dragendorff's reagent and saponins by the appearance of froth upon vigorous shaking of diluted samples. Lastly, for detecting anthraquinones, the extract was dissolved in 1% HCl, then in benzene and later if extract showed pink, violet or red color with  $\text{NH}_4\text{OH}$ , that indicate the presence of anthraquinones.

### 2.3. Drugs and animals

Acetylcholine perchlorate (ACh), atropine sulphate, carbachol (CCh), phenylephrine hydrochloride (PE), and verapamil hydrochloride were purchased from Sigma Chemicals Co, St. Louis, MO, USA. All chemicals used were of the analytical grade available. All drugs were dissolved in distilled water and dilutions were made fresh in normal saline (0.9% sodium chloride) on the day of experiment.

Rabbits (1–1.2 kg) and guinea-pigs (500–550 g) of local breed and either sex were used for this study, housed at the Animal House of the Aga Khan University, maintained at 23–25 °C and were given a standard diet and tap water. Rabbits starved for 24 h were sacrificed by blow on the back of head and guinea-pigs were sacrificed by cervical dislocation. Experiments performed complied with the rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996), approved by the Ethical Committee of the Aga Khan University.

### 2.4. Isolated tissue experiments

Isolated tissue experiments were carried out following the methods previously employed in our laboratory (Gilani et al., 2005a).

#### 2.4.1. Rabbit jejunum

The jejunum was dissected out, kept in Tyrode's solution and cleaned off mesenteries. Each segment of about 2 cm length was suspended in a 10 ml tissue bath containing Tyrode's solution, maintained at 37 °C and aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen). The composition of the Tyrode's solution in mM was: KCl 2.68, NaCl 136.9,  $\text{MgCl}_2$  1.05,  $\text{NaHCO}_3$  11.90,  $\text{NaH}_2\text{PO}_4$  0.42,  $\text{CaCl}_2$  1.8 and glucose 5.55. Intestinal responses were recorded isotonicly using Bioscience transducers and oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with a sub-maximal concentration of ACh (0.3  $\mu\text{M}$ ) with a 3 min interval until constant responses were recorded. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing the testing of relaxant (spasmolytic) activity directly without the use of an agonist (Gilani et al., 1994).

For the determination of  $\text{Ca}^{++}$  channel blocking (CCB) activity, high  $\text{K}^+$  (80 mM) was used to depolarize the preparations as described by Farre et al. (1991).  $\text{K}^+$  was added to the tissue bath, which produced a sustained contraction. Test materials were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van-Rosum, 1963).

To confirm the  $\text{Ca}^{++}$  antagonist effect of the test substance, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with  $\text{Ca}^{++}$ -free Tyrode's solution containing EGTA (0.1 mM) for 30 min in order to remove  $\text{Ca}^{++}$  from the tissues. This solution was further replaced with  $\text{K}^+$ -rich and  $\text{Ca}^{++}$ -free Tyrode's solution, having the following composition (mM): KCl 50, NaCl 91.04,  $\text{MgCl}_2$  1.05,  $\text{NaHCO}_3$  11.90,  $\text{NaH}_2\text{PO}_4$  0.42, glucose 5.55 and EGTA 0.1. Following an incubation period of 30 min, control concentration-response curves

(CRCs) of  $\text{Ca}^{++}$  were obtained. When the control  $\text{Ca}^{++}$  CRCs were found super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test the possible CCB effect. The CRCs of  $\text{Ca}^{++}$  were reconstructed in the presence of different concentrations of the test material.

#### 2.4.2. Rabbit trachea

The trachea was dissected out and kept in Krebs's solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about two cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite to the smooth muscle layer, forming a tracheal strip with a central part of smooth muscle in between the cartilaginous portions on the edges. The preparation was then mounted in a 20 ml tissue bath containing Krebs's solution, maintained at 37 °C and aerated with carbogen. The composition of Krebs's solution was (mM): NaCl 118.2,  $\text{NaHCO}_3$  25.0,  $\text{CaCl}_2$  2.5, KCl 4.7,  $\text{KH}_2\text{PO}_4$  1.3,  $\text{MgSO}_4$  1.2 and glucose 11.7 (pH 7.4). A tension of 1 g was applied to each of the tracheal strip and was kept constant throughout the experiment. The tissue was equilibrated for 1 h before the addition of any drug. CCh (1  $\mu\text{M}$ ) and  $\text{K}^+$  (80 mM) were used to stabilize the respective preparations until constant responses of each agonist were achieved (usually 3–4 concentrations). Then their sustained contractions were obtained and relaxant effect of the test substance was assessed by adding in a cumulative fashion. Isometric responses were recorded on a Grass model 7 Polygraph (Grass instrument company, Quincy, MA, USA).

#### 2.4.3. Rabbit aorta

To study the effect on vascular resistance, the thoracic aorta ring preparations from rabbit were used. Aortic rings 2–3 mm wide were individually mounted in 20 ml tissue baths containing Krebs's solution, at 37 °C and aerated with carbogen gas. A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 h was allowed before studying the effect of test materials. The changes in isometric tensions of the rings were measured via a force-displacement transducer (FT-03) using a Grass model 7 Polygraph. PE (1  $\mu\text{M}$ ) and  $\text{K}^+$  (80 mM) were used to induce sustained contractions and the vasodilator effect of the extract was assessed by adding in a cumulative fashion.

#### 2.4.4. Guinea-pig atria

Right atria from guinea-pigs were dissected out, cleaned off fatty tissue and mounted in 20 ml tissue baths containing Krebs's solution, at 32 °C and aerated with carbogen gas. The tissues were allowed to beat spontaneously (due to the presence of pacemaker cells) under the resting tension of 1 g. This preparation allows studying effect on both force and rate of atrial contractions. The effect on heart rate was measured by increasing the speed of chart recorder. An equilibrium period of 30 min was allowed before the application of any drug. Control responses of acetylcholine (1  $\mu\text{M}$ ) and isoprenaline (1  $\mu\text{M}$ ) were obtained at least in duplicate. Tension changes in the tissue were recorded via a Grass force-displacement transducer (model FT-03) using a Grass Model 7 Polygraph.

### 2.5. Statistical analysis

All the data expressed are mean  $\pm$  standard error of mean (S.E.M.,  $n$  = number of experiments) and the median effective concentrations ( $\text{EC}_{50}$  values) with 95% confidence intervals (CI). Concentration–response curves (CRCs) were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

## 3. Results

### 3.1. Phytochemical analysis

Bo.Cr was found to contain flavonoids, coumarins, sterols and tannins, while tested negative for rest of the classes.

### 3.2. Effect on jejunum

Bo.Cr caused a concentration-dependent inhibition of spontaneous and  $\text{K}^+$ -induced contractions of isolated jejunum preparations with respective  $\text{EC}_{50}$  values of 2.30 (1.40–3.80, 95% CI) and 2.50 mg/ml (1.40–3.76). Similarly, verapamil relaxed both types contractions with  $\text{EC}_{50}$  values of 0.27 (0.19–0.37) and 0.10  $\mu\text{M}$  (0.06–0.15), respectively (Figs. 1 and 2). Bo.Cr (0.3–1.0 mg/ml) shifted the  $\text{Ca}^{++}$  CRCs to the right (Fig. 3A) similar to that caused by verapamil (Fig. 3B). The respective  $\text{EC}_{50}$  values of  $\text{Ca}^{++}$  curves in the presence of lower concentrations of Bo.Cr (0.3 mg/ml) and verapamil (0.1  $\mu\text{M}$ ) were 0.004 (0.0001–0.008) and 0.003 M (0.00005–0.007), which were significantly greater ( $p < 0.05$ ) than those of the respective control  $\text{Ca}^{++}$  curves, constructed in the absence of any intervention. The  $\text{EC}_{50}$  values in the respective control  $\text{Ca}^{++}$  curves were 0.0007 (0.0003–0.001) and 0.00054 M (0.0002–0.0008). Some known constituents of borage namely: caffeic acid, chlorogenic acid and vanillic acid were also tested against spontaneous and  $\text{K}^+$  (80 mM)-induced con-

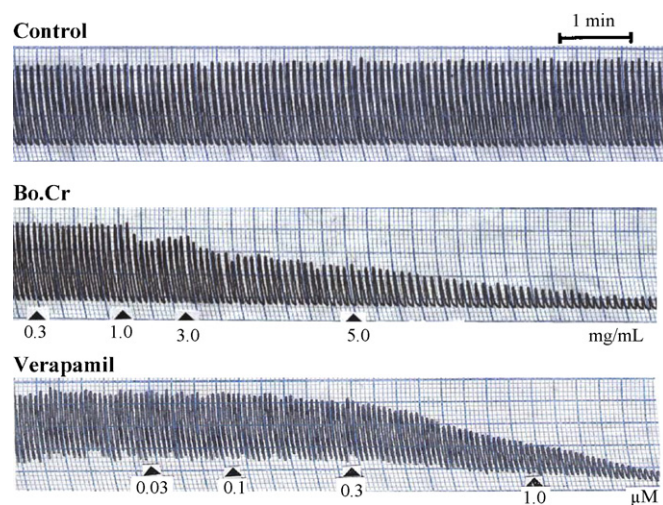


Fig. 1. Representative tracing showing concentration-dependent inhibitory effects of the crude extract of *Borago officinalis* (Bo.Cr) and verapamil on spontaneously contracting isolated rabbit jejunum preparations.

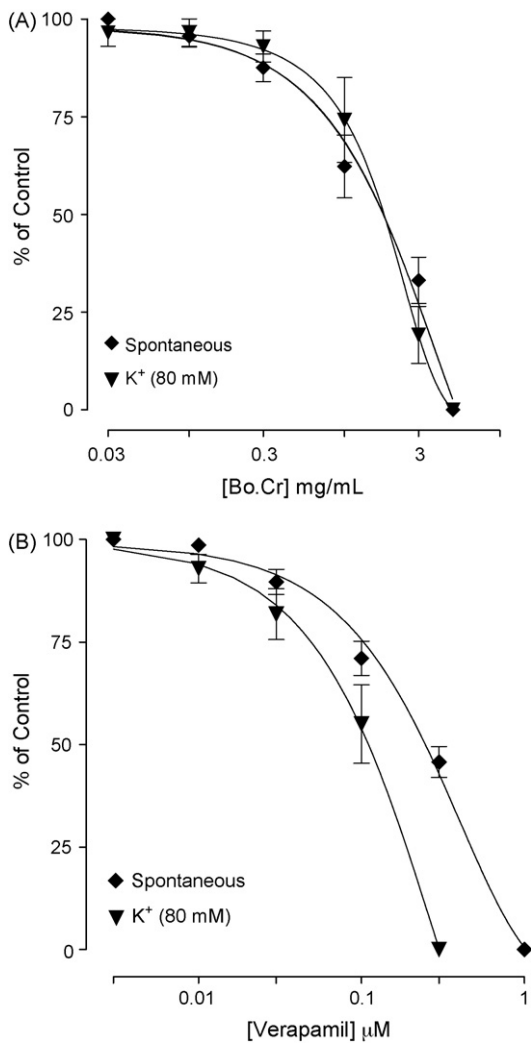


Fig. 2. Concentration-dependent inhibitory effects of the crude extract of (A) *Borago officinalis* (Bo.Cr) and (B) verapamil on spontaneous and K<sup>+</sup>-induced contractions of isolated rabbit jejunum preparations. Symbols represent mean  $\pm$  S.E.M.,  $n = 3-5$ .

tractions, and were found inactive up to the concentration of 300  $\mu$ g/ml.

### 3.3. Effect on trachea

In tracheal preparations, pre-contracted with CCh (1  $\mu$ M) and K<sup>+</sup> (80 mM), Bo.Cr caused concentration-dependent relaxant effect with respective EC<sub>50</sub> values of 3.17 (2.72–3.69) and 2.47 mg/ml (1.85–3.29) and verapamil with 0.21 (0.15–0.30) and 0.05  $\mu$ M (0.03–0.07), respectively, as shown in Fig. 4.

### 3.4. Effect on aorta

When tested against PE (1  $\mu$ M) and K<sup>+</sup> (80 mM)-induced contractions, Bo.Cr produced concentration-dependent vasodilator effect with respective EC<sub>50</sub> values of 2.5 (1.27–5.01) and 2.7 mg/ml (1.3–6.0) and verapamil with 1.53 (0.87–2.69) and 0.89  $\mu$ M (0.53–1.48), respectively (Fig. 5).

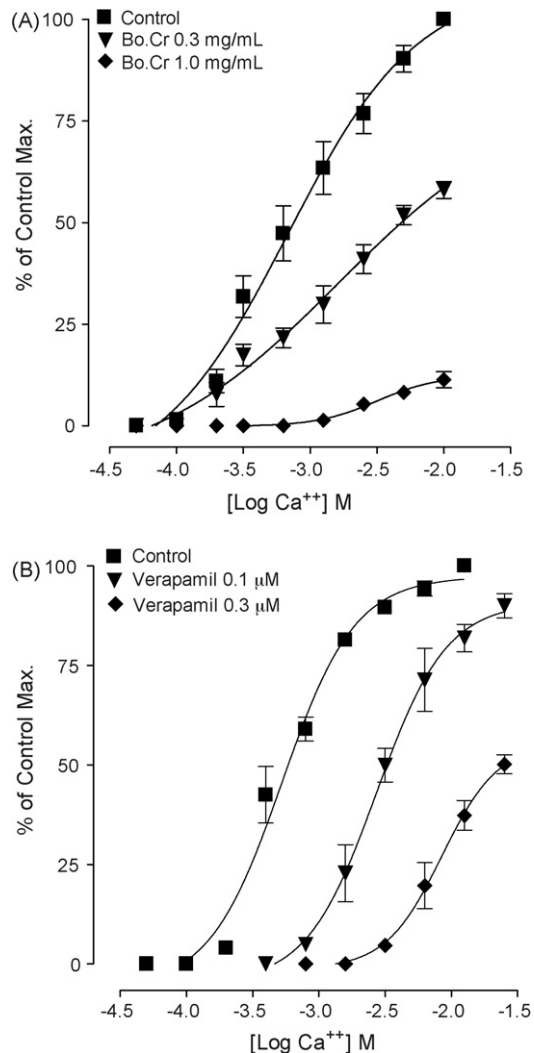


Fig. 3. Concentration–response curves of Ca<sup>2+</sup> in the absence and presence of increasing concentrations of (A) the crude extract of *Borago officinalis* (Bo.Cr) and (B) verapamil in isolated rabbit jejunum preparations. Symbols represent mean  $\pm$  S.E.M.,  $n = 4$ .

### 3.5. Effect on atria

Bo.Cr caused concentration-dependent inhibitory effect on spontaneously beating atrial force and rate of contractions with respective EC<sub>50</sub> values of 5.01 (4.51–5.52) and 5.90 mg/ml (5.53–6.30). Similarly, verapamil caused concentration-dependent inhibitory effect with respective EC<sub>50</sub> values of 0.99 (0.69–1.41) and 0.78  $\mu$ M (0.55–1.11), respectively (Fig. 6).

## 4. Discussion

Due to its folkloric use as an antispasmodic remedy, the extract of *Borago officinalis* was tested for its possible spasmolytic effect in isolated rabbit jejunum preparations, where it inhibited the spontaneous contractions in a concentration-dependent manner, thus showing antispasmodic effect. The contraction of smooth muscle preparations including rabbit

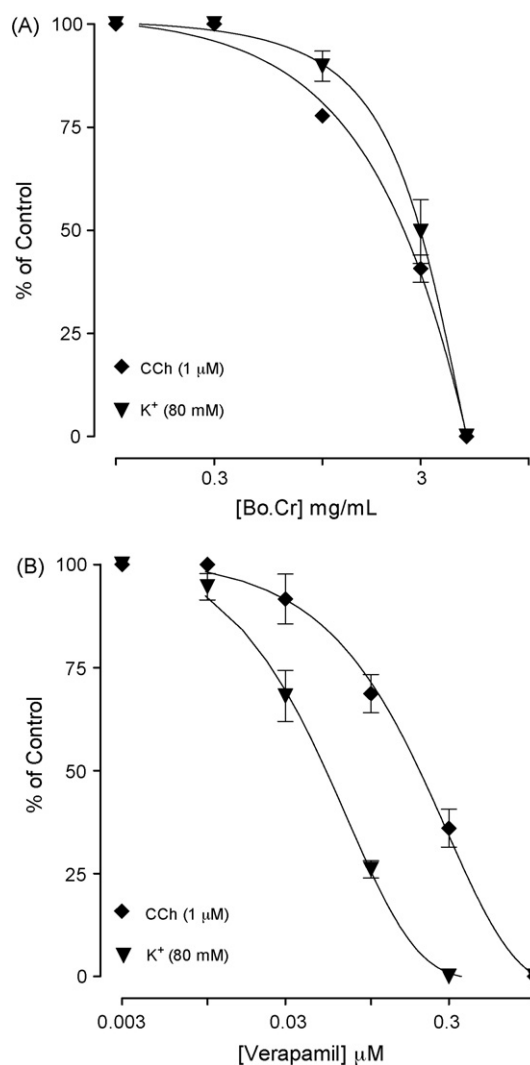


Fig. 4. Concentration-dependent inhibitory effects of (A) the crude extract of *Borago officinalis* (Bo.Cr) and (B) verapamil on carbachol (CCh) and K<sup>+</sup>-induced contractions of isolated rabbit tracheal preparations. Symbols represent mean  $\pm$  S.E.M.,  $n = 3-4$ .

jejunum is dependent upon an increase in the cytoplasmic free [Ca<sup>++</sup>], which activates the contractile elements (Karaki and Weiss, 1988; Grasa et al., 2004). The increase in intracellular Ca<sup>++</sup> is due to either influx via voltage dependant L-type Ca<sup>++</sup> channels (VDCs) or to release from intracellular stores in the sarcoplasmic reticulum. Periodic depolarization regulates the spontaneous movements of intestine and at the height of depolarization the action potential appears as a rapid influx of Ca<sup>++</sup> via VDCs (Brading, 1981). The inhibitory effect of the plant extract on spontaneous movements of jejunum may be due to interference either with the Ca<sup>++</sup> release or with the Ca<sup>++</sup> influx through VDCs.

In our earlier studies, we have observed that the spasmolytic effect of the medicinal plants is usually mediated through Ca<sup>++</sup> channel blockade (Gilani et al., 1994, 2005b,c). To see whether the spasmolytic effect of this plant is also mediated via the same mechanism, the extract was tested on high K<sup>+</sup> (80 mM)-induced contraction, which was completely relaxed by the plant

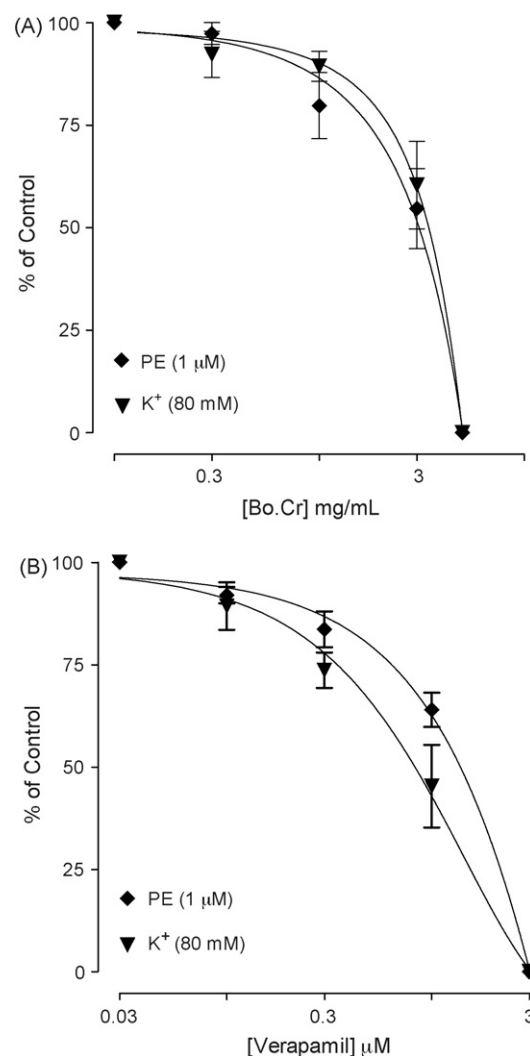


Fig. 5. Concentration-dependent inhibitory effects of (A) the crude extract of *Borago officinalis* (Bo.Cr) and (B) verapamil on phenylephrine (PE) and K<sup>+</sup>-induced contractions of isolated rabbit aortic preparations. Symbols represent mean  $\pm$  S.E.M.,  $n = 4$ .

extract. At high concentration (>30 mM), K<sup>+</sup> is known to cause smooth muscle contractions through opening of VDCs, thus allowing influx of extracellular Ca<sup>++</sup> causing a contractile effect (Bolton, 1979) and a substance causing inhibition of high K<sup>+</sup>-induced contraction is considered an inhibitor of Ca<sup>++</sup> influx (Godfraind et al., 1986). Verapamil, a standard Ca<sup>++</sup> channel blocker (Fleckenstein, 1977), also produced non-specific inhibition of spontaneous and K<sup>+</sup>-induced contractions. The presence of Ca<sup>++</sup> antagonist constituent(s) was further strengthened when the plant extract caused a rightward shift in the Ca<sup>++</sup> CRCs (constructed in the applied K<sup>+</sup>-rich and Ca<sup>++</sup>-free medium) and suppressed the maximal response similar to that caused by verapamil. Calcium antagonists constitute an important therapeutic group and the common characteristic of these drugs is their concentration-dependent inhibition of the slow entry of calcium and their capacity for reversal of this effect by Ca<sup>++</sup> (Triggle, 1992). *Borago officinalis* has been used in different hyperactive gastrointestinal disorders such as colic and diarrhea. Ca<sup>++</sup> antagonists have been known to be effective in these conditions

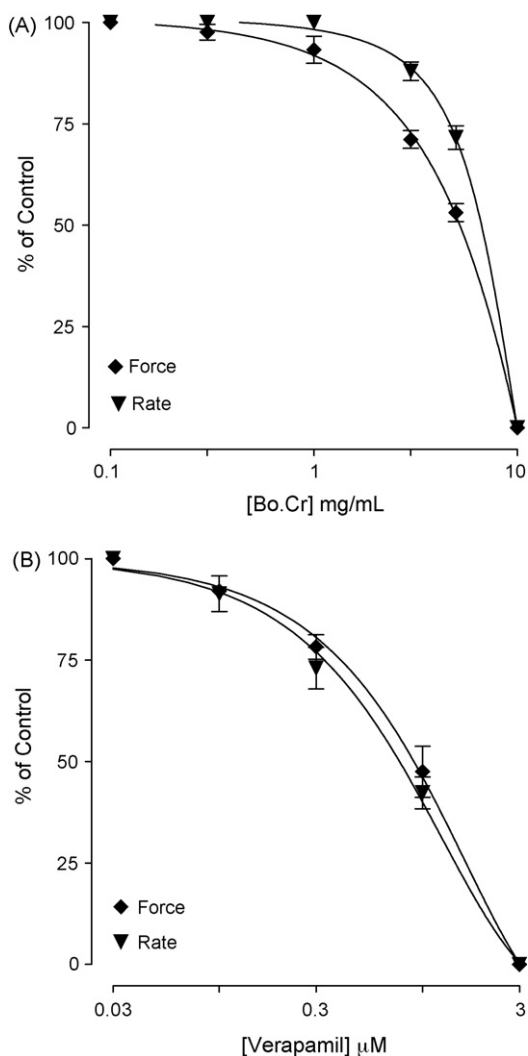


Fig. 6. Inhibitory effects of (A) the crude extract of *Borago officinalis* (Bo.Cr) and (B) verapamil on force and rate of contractions of isolated guinea-pig atrial preparations. The symbols represent mean  $\pm$  S.E.M.,  $n = 4-5$ .

(Brunton, 1996) and the observed calcium antagonist effect of the plant extract in this study may explain the use of this plant in such disorders.

Based on the traditional use of *Borago officinalis* in asthma, the plant was studied further for a possible bronchodilator effect. In trachea, the extract caused relaxation of both CCh and  $K^+$ -induced contractions like verapamil, suggestive of non-specific relaxation possibly mediated through a CCB like mechanism.  $Ca^{++}$  antagonists are known to be effective in hyperactive respiratory disorders (Mathewson, 1985) and the presence of this activity, as observed in this study may explain the use of *Borago officinalis* in asthma.

In view of the well establish therapeutic use of CCBs (Epstein, 1992) and that of *Borago officinalis* in cardiovascular disorders such as hypertension, the plant extract was studied further for its possible vasodilator effect. When tested in isolated aorta preparations, it caused inhibition of both phenylephrine and  $K^+$ -induced contractions, similar to that observed with verapamil. Phenylephrine produces vascular contraction

through increase in cytosolic  $Ca^{++}$ , partly due to  $Ca^{++}$  influx via receptor operated channels and partly via  $Ca^{++}$  release from intracellular stores (Graham et al., 1996). Inhibition of both phenylephrine and  $K^+$ -induced contractions by *Borago officinalis* suggests a non-specific vasodilator action, mediated through  $Ca^{++}$  antagonism. Blood pressure is a product of vascular resistance and cardiac output (Johansen, 1992), hence the extract was studied further for its possible inhibitory effect on heart. In spontaneously beating guinea-pig atria, the plant extract suppressed both the atrial force and rate of contractions. The cardiac inhibitory effect of the extract was similar to verapamil and was atropine resistant, a muscarinic receptor antagonist (Arunlakshana and Schild, 1959), indicating that the cardio-depressant effect of the plant is not mediated through muscarinic receptor stimulation, but possibly via CCB, as  $Ca^{++}$  antagonists are well known for their cardiac inhibitory effect (Billman, 1992). Borage oil is previously reported to reduce blood pressure in spontaneously hypertensive rats (Engler and Engler, 1998); however, this was a preliminary study with the precise mode of action remained to be elucidated. Hence, this study by virtue of exploring the possible mechanism of vasodilator and cardiac inhibitory effects provide pharmacological base for blood pressure lowering effect of *Borago officinalis*, though the presence of angiotensin-II receptor blocking activity previously reported in this plant associated with its renal protective mechanism (Engler et al., 1998) can be of added value.

When some of the commercially available known compounds of borage such as caffeic acid, chlorogenic acid and vanillic acid were screened, none of them was found active. Phytochemical analysis of the crude extract revealed the presence of flavonoids, coumarins, sterols and tannins. The observed  $Ca^{++}$  antagonist effect of the *Borago officinalis* extract may be due to the presence of flavonoids and coumarins, as such phytochemicals are known to possess  $Ca^{++}$  channel blocking action (Revelta et al., 1997; Gilani et al., 2000).

These results clearly indicate that *Borago officinalis*, possesses antispasmodic, bronchodilator and cardiovascular inhibitory effects mediated possibly through  $Ca^{++}$  channel blockade and this study may explain the traditional use of the plant in hyperactive gastrointestinal, respiratory and cardiovascular disorders.

## Acknowledgement

This study was supported by funds made available by the Higher Education Commission of Pakistan under the Scheme of Distinguished National Professor Research Allowance.

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