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Gut modulator effects of *Conyza bonariensis* explain its traditional use in constipation and diarrhea

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Abstract. – BACKGROUND AND OBJECTIVES: The current investigation was carried out to explore the pharmacological basis of the crude extract of *Conyza bonariensis* (Cb.Cr) for its use in constipation and diarrhea.

MATERIALS AND METHODS: The plant extract of *Conyza bonariensis* (*C. bonariensis*) was prepared, isolated guinea-pig ileum and rabbit jejunum preparations were used to evaluate its gut modulator effects.

RESULTS: The Cb.Cr (0.3-10 mg/mL) exhibited spasmogenic effect in isolated guinea-pig ileum preparation, which was about 19-84% of the acetylcholine maximum. Pretreatment of the tissues with atropine (0.1 μM) abolished the contractile effect, similar to acetylcholine. Among the fractions, only the butanol fraction exhibited atropine sensitive contractile effect. In isolated rabbit jejunum preparations, Cb.Cr produced appreciable atropine-sensitive spasmogenic effect at lower concentrations (0.03-0.3 mg/mL) followed by spasmolytic effect at next higher concentration (1.0 and 3.0 mg/mL).

Cb.Cr caused an inhibition of the high K^+ induced contraction in isolated rabbit jejunum preparation with EC_{50} value of 0.62 mg/mL. Similarly, verapamil, a standard calcium blocker, inhibited high K^+ induced contraction in isolated rabbit jejunum preparations. Cb.Cr caused a right ward shift in the Ca^{++} concentration response curve, similar to verapamil. Among various fractions of *C. bonariensis*, only hexane and ethylacetate fractions showed spasmolytic effects.

CONCLUSIONS: The crude extract of *C. bonariensis* contains spasmogenic and spasmolytic constituents, which explains its medicinal use in constipation and diarrhea.

Key Words:
Conyza bonariensis, Spasmogenic, Constipation, Diarrhea, Spasmolytic, Calcium antagonism.

Introduction

Conyza bonariensis is a perennial native plant of the family Asteraceae. It plays an important role in supplying crude proteins and contributes a significant proportion of herbage fed to ruminant. This weed is widely available in local range lands of Indo-Pak[1] and Middle East[2]. Moreover, the plant is also commonly cultivated as an ornamental plant in gardens. In the South Asian traditional system of medicine, various parts of the plant are being used in a variety of ailments. Infusion of the petals is given as refrigerant and demulcent. Leaves are used as laxative, root is used in diarrhoea[3-4], cough[3] while flowers are considered as aphrodisiac, emollient[5], and in gastrointestional problems including diarrhea[6]. Phytochemical studies of *C. bonariensis* revealed the presence of several bioactive constituents including flavonoids, flavonoid glycosides, cyanin glycoside, taraxeryl acetate, β-sitosterol, campesterol, stigmasterol, ergosterol[7], cyclopropenoids[8] and anthocyanin pigments[5].

The plant has also been evaluated pharmacologically for the presence of anticonvulsant[4], antitumor[9], hypoglycaemic[10] and estrogenic[11] activities. This study was aimed at exploring the pharmacological rationale of *C. bonariensis* for its medicinal use in constipation and diarrhea.

Materials and Methods

Plant Materials

The whole plant of *Conyza bonariensis* (L.) Cronq. (Asteraceae) was collected from Oghi,

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Mansehra, Pakistan, in November 2002, and identified by Mr. Jan Alam (Taxanomist) at the Department of Botany, University of Karachi, Pakistan. A voucher specimen (KUH G. H. No. 68220) has been deposited at the herbarium of the above Department.

Extraction and Fractionation

The air dried whole plant of *Conyza bonariensis* (35 kg) was exhaustively extracted with MeOH (50 L×3) at room temperature. The extract was evaporated to yield the residue (815 g), which was partitioned between *n*-hexane (110 g), $CHCl_3$ (90 g), EtOAc (95 g), *n*-BuOH (495 g) and water (25 g).

Drugs and Animals

Acetylcholine perchlorate, atropine sulphate, potassium chloride, histamine diphosphate salt and verapamil hydrochloride, were purchased from Sigma Chemicals Co, St Louis, MO, USA and pyrilamine maleate USP grade was purchased from RB Industries Inc., Chicago, IL, USA. All other chemicals used were of the highest 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.

Animals used in this study were housed at the Animal House of The Aga Khan University, maintained at 23-25°C and were given a standard diet and tap water. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996). Rabbits (1.0-1.5 kg) and guinea pigs (500-600 g) of local breed and either sex were used for this study. Animals had free access to water but food was withdrawn 24 hours prior to the experiment. Rabbits were sacrificed by a blow on the back of the head and guinea pigs were sacrificed by cervical dislocation.

Isolated Tissue Preparations

The spasmogenic and spasmolytic activities of the plant material were studied using isolated guinea-pig ileum and rabbit jejunum preparations, as described previously[12]. Respective segments of 2 cm length were suspended in a 10 mL tissue baths containing Tyrode's solution, aerated with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37°C. The composition of the Tyrode's solution was: KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8 and glucose 5.55 mM. Intestinal responses were recorded isotonically using Bio-Science transducers and oscillographs. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug.

Under these experimental conditions, guinea pig ileum behaved as a quiescent preparation and is considered more useful for spasmogenic activity[13], whereas rabbit jejunum exhibits spontaneous rhythmic contractions, allowing to test the relaxant (spasmolytic) activity directly without the use of an agonist[14]. The contractile effect of the test material was assessed as the percent of the maximum effect produced by the control drug, acetylcholine (1.0 μM).

Determination of calcium antagonist activity: To assess whether the spasmolytic activity of the test substances, was through calcium channel blockade (CCB), K^+, as KCl, was used to depolarize the preparations according to method[15]. High K^+ (80 mM) 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[16]. The relaxation of intestinal preparations, precontracted with K^+ (80 mM) was expressed as percent of the control response mediated by K^+.

To confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{++} from the tissues. This solution was further replaced with a K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition: KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1 mM. Following an incubation period of 30 min, control concentration-response curves (CRCs) of Ca^{++} were obtained. When the control CRCs of Ca^{++} were found super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test a possible CCB effect. The CRCs of Ca^{++} were developed in the presence of different concentrations of the test material.

Statistical Analysis

The data is expressed in mean ± standard error of the mean (SEM). The median effective concentrations (EC_{50} values) were obtained using Graph Pad Prism version 4. Data was analyzed by one way ANOVA followed by the Student-Newman-Keuls multiple comparison test when significant difference were present. $p < 0.05$ was considered statistically significant.

Results

The plant extracts of *C. bonariensis* and its various fractions were evaluated for its effect in isolated rabbit jejunum and guinea pig ileum preparations. In isolated guinea-pig ileum preparation, Cb.Cr caused concentration-dependent contractile effect, which was about 19-84% of acetylcholine maximum effect (Figure 1A and B). Pretreatment of the tissues with atropine (0.1 μM), abolished the contractile effect of the crude extract, similar to that of acetylcholine. Among the fractions studied, only the butanol fraction of *C. bonariensis* (Cb.BUOH) exhibited atropine sensitive contractile effect (Figure 1C) in isolated guinea-pig ileum preparations, while other fractions, such as, ethylacetate (EtAc) and hexane (Hex) were found without any contractile effect at the maximum concentrations used.

The Cb.Cr and its fractions were further studied in isolated rabbit jejunum preparations. When tested on spontaneous contractions, Cb.Cr (0.3-3.0 mg/mL) caused relaxation of the spontaneous contraction of isolated rabbit jejunum preparation with an appreciable spasmogenic effect at lower concentrations of 0.03-0.3 mg/mL (Figure 2). Pre-treatment of tissue with atropine (0.1 μM) completely abolished the spasmogenic effect of

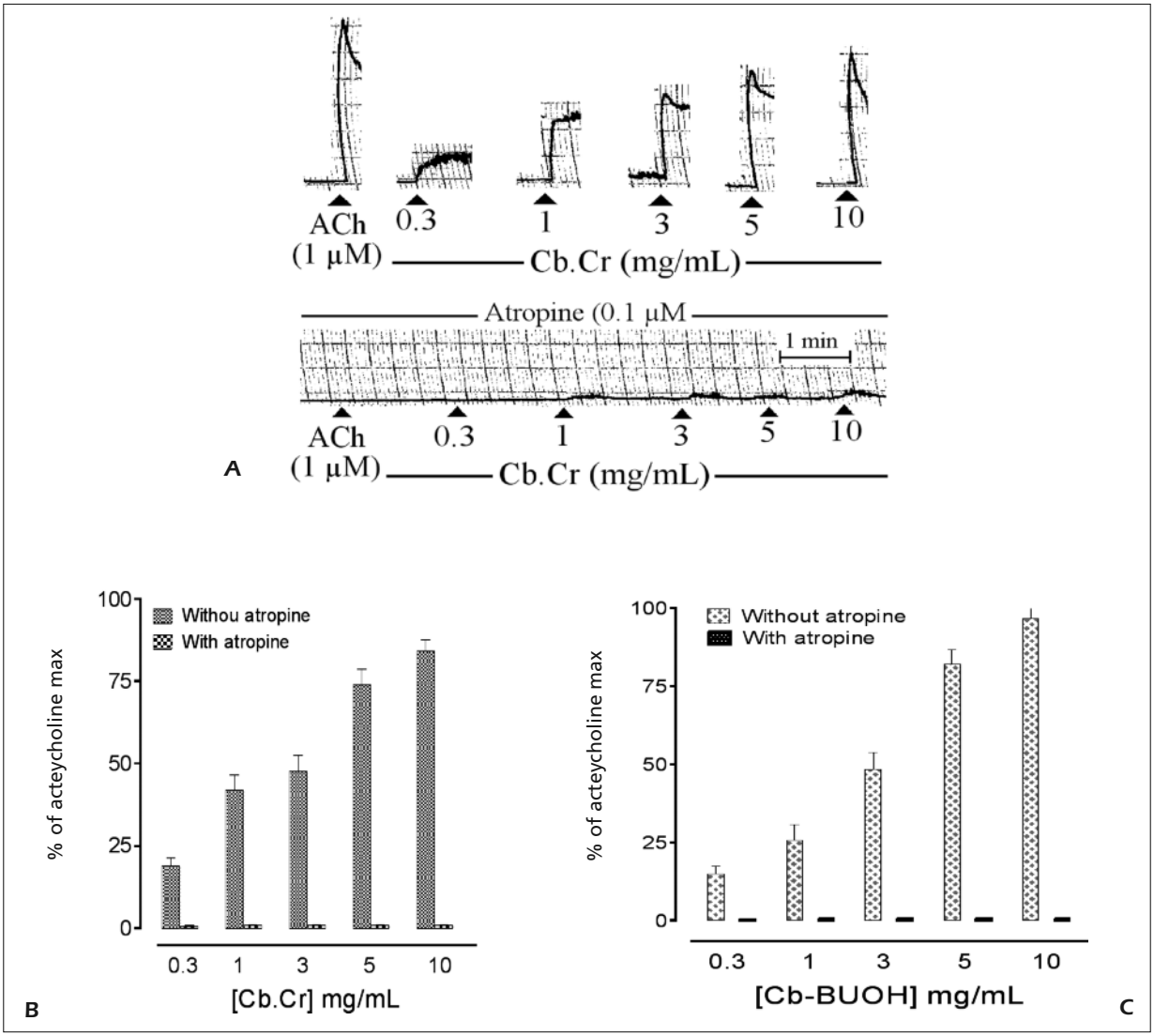

Figure 1. Tracings showing the dose dependent contractile effect of the crude extract of *Conyza bonariensis* (Cb.Cr) in the absence and presence of atropine in the isolated guinea-pig ileum preparations ***(A)***. Acteycholine (ACh) was used as positive control and concentration-response curve for the spasmogenic effects of Cb.Cr ***(B)*** and butanol fraction; BUOH ***(C)*** without and with atropine in isolated guinea-pig ileum preparations. Data are mean ± SEM of percentage of ACh maximum contractions (n=6).

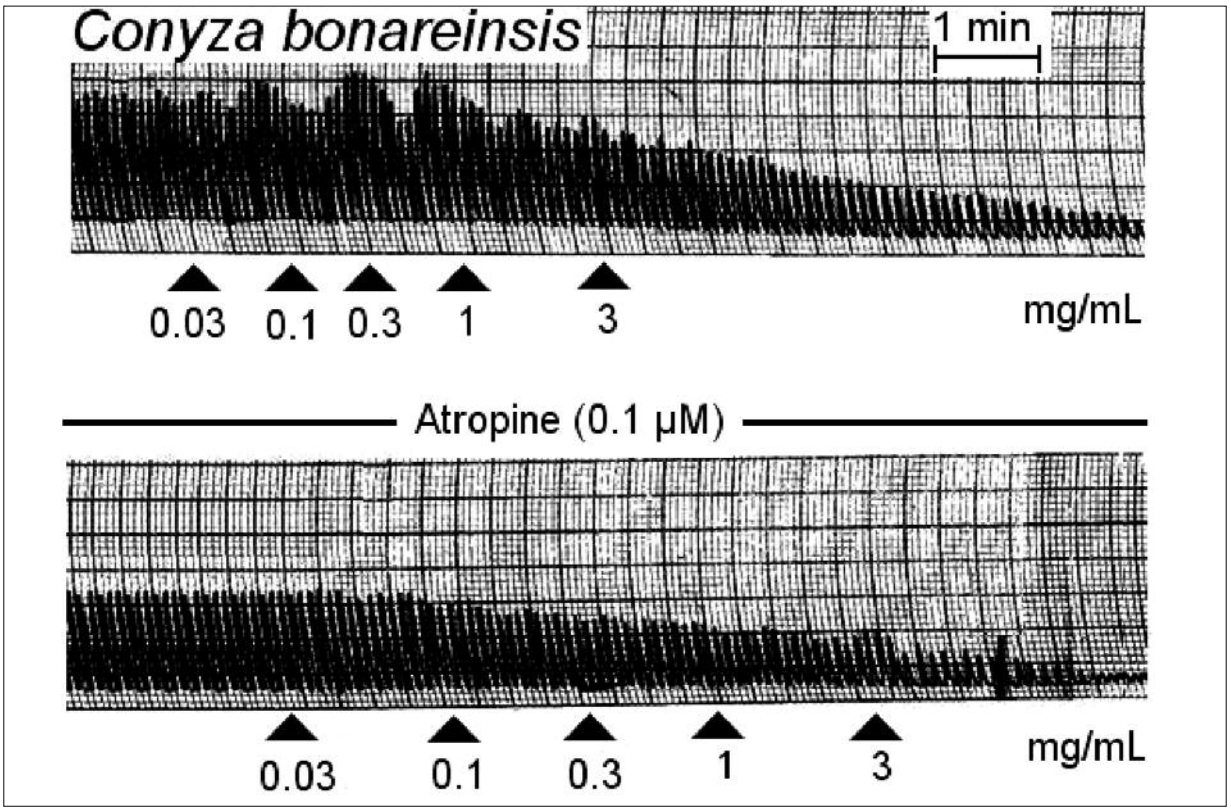

Figure 2. Tracing showing the spasmolytic effect of crude extract of *Conyza bonariensis* on spontaneously contracting isolated rabbit jejunum preparations in the absence and presence of atropine.

the plant extract (Figure 2) and relaxation was obtained at significantly ($p < 0.001$) lower concentration with respective EC_{50} values, in the absence and presence of atropine, were 0.44 and 0.05 mg/mL (Figure 3 A).

Cb.Cr caused a concentration-dependent relaxation of K^+-induced contractions with EC_{50} of 0.62 mg/mL (Figure 3A). Similar to the effects of Cb.Cr, Verapamil, used as a reference standard produced marked inhibition of spontaneous and high K^+-induced contractions in isolated rabbit jejunum preparation (Figure 3B).

Activity-directed fractionation revealed that the ethylacetate fraction of *C. bonariensis* (Cb.EtAc) antagonized spontaneous and high K^+-induced contractions in rabbit jejunum preparations with EC_{50} of 0.85 mg/mL (Figure 3 C). Similarly hexane fraction of *C. bonariensis* (Cb.Hex) caused relaxation of the spontaneous and high K^+-induced contractions in isolated rabbit jejunum preparations at comparatively lower concentrations than the parent crude extract with EC_{50} value of 0.25 mg/mL (Figure 3D). Cb.Cr caused a rightward shift in the Ca^{++} concentration-response curves, constructed in the Ca^{++}-free and K^+ rich medium (Figure 4A), similar to verapamil (Figure 4B).

Discussion

This study was aimed at exploring the pharmacological basis for the use of *Conyza bonariensis* in the management of constipation and diarrhea. Isolated guinea-pig preparation was used to test the possible contractile effect of the crude extract of *C. bonariensis*, which is considered more suitable preparation for the demonstration of spasmogenic effect[12]. Cb.Cr caused concentration-dependent contractile effect in isolated guinea-pig ileum preparation. Pretreatment of tissues with atropine (0.1 μM), abolished the contractile effect of the plant extract, similar to that of acetylcholine, indicating the involvement of cholinergic receptors in the observed contractile effect of the plant extracts. Acetylcholine is a neurotransmitter released by the parasympathetic nervous system, mediates its action in the gut by stimula-

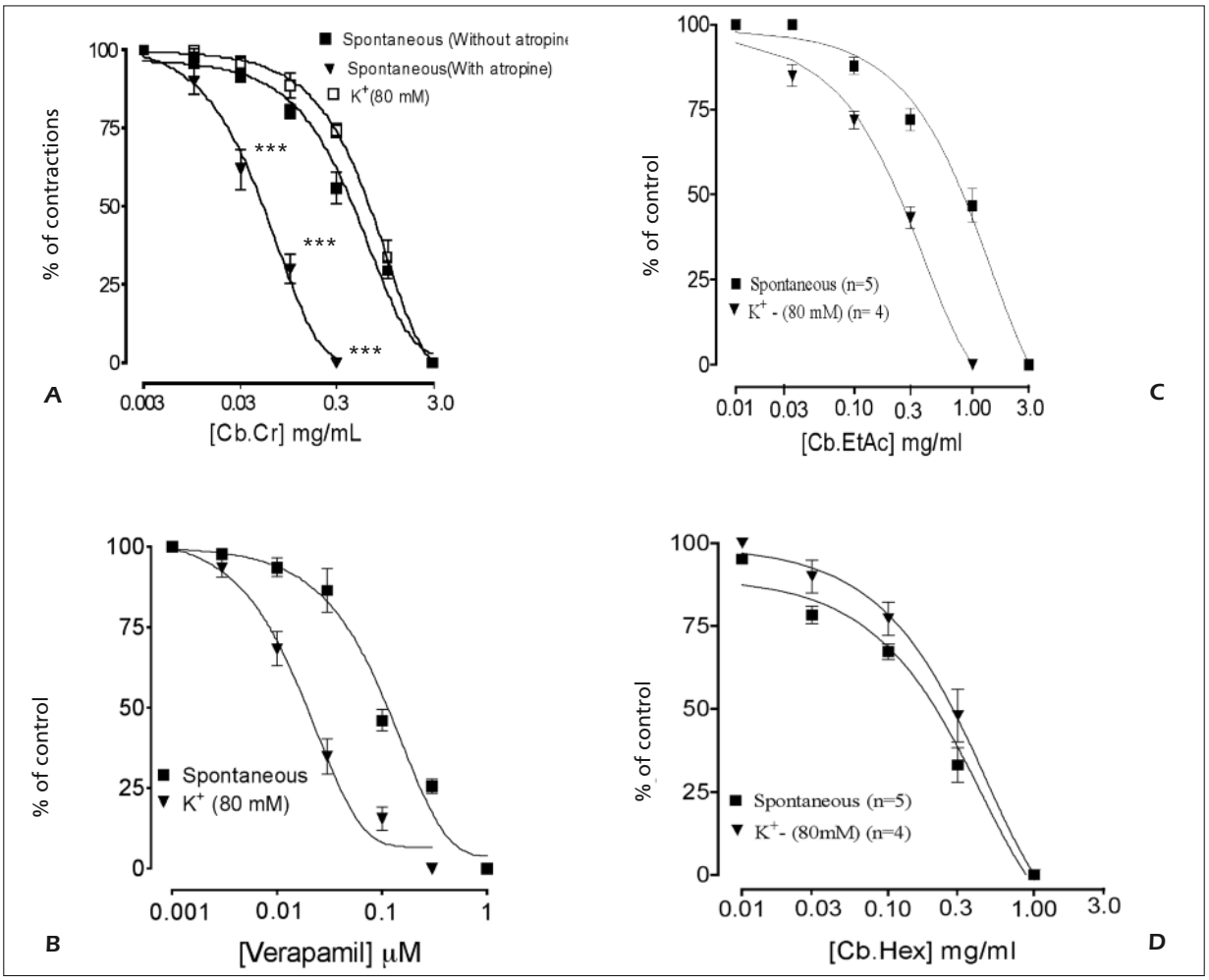

Figure 3. Inhibitory effect of the crude extract of *Conyza bonariensis*; Cb.Cr ***(A)***, verapamil ***(B)***, ethylacetate; Cb.EtAc ***(C)*** and hexane; Cb.Hex fraction ***(D)*** on spontaneous and high K^+-induced contractions in isolated rabbit jejunum preparations. Values are mean ± SEM (n=4-6). ***$p < 0.001$ compared to spontaneous contractions without atropine.

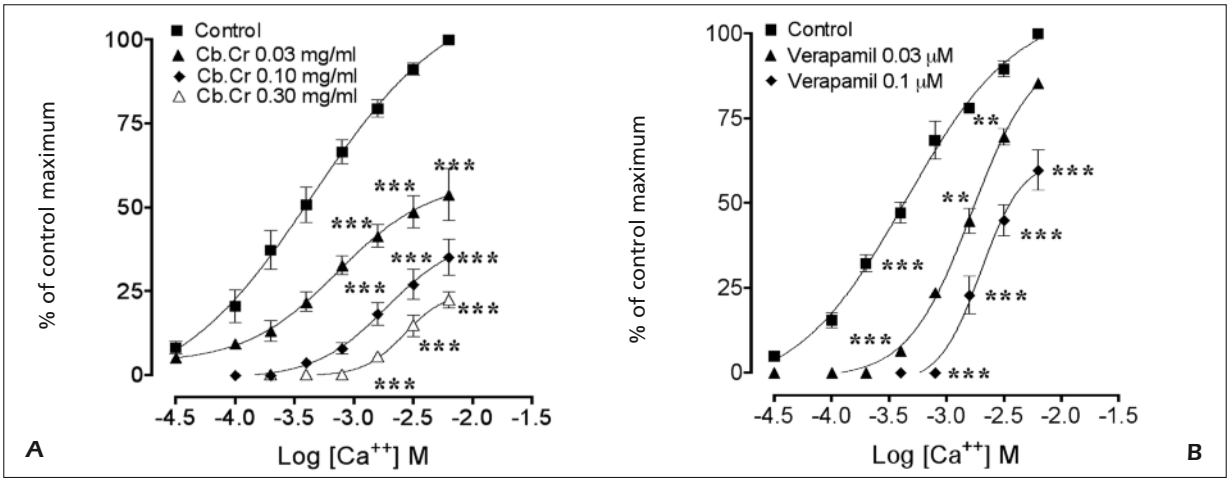

Figure 4. Effect of different concentration of ***(A)*** the crude extract of *Conyza bonariensis* (Cb.Cr) ad ***(B)*** verapamil on Ca^{++} concentration-response curves in isolated rabbit jejunum preparations. Values are mean ± SEM (n= 4-6). **$p < 0.01$, ***$p < 0.001$ compared to respective controls.

tion of muscarinic M_3 receptor subtypes and atropine blocks all muscarinic receptor sites. Acetylcholine plays an important physiological role to regulate the peristaltic movements of the gut[17]. Cholinergic agents are known to increase the secretory and motor activity of the gut[18]. The observed contractile effect of the crude extract, similar to that of acetylcholine, may explain the medicinal use of *C. bonariensis* in constipation[18]. However, the co-existence of other mechanisms cannot be excluded completely.

Activity guided fractionation study revealed that contractile constituents of *C. bonariensis* were concentrated in butanol fraction which can be used effectively in the treatment of constipation. The crude extract and its fractions were further studied in isolated rabbit jejunum preparations. *C. bonariensis* plant extract produced relaxation of the spontaneous contraction of isolated rabbit jejunum preparation with an appreciable spasmogenic effect at lower concentrations, suggesting that the plant extract has got spasmogenic effect which is accompanied by a spasmolytic effect. Similar to the effect of *C. bonariensis* in the guinea pig ileum preparation, the spasmogenic effect of the crude extract was abolished with pretreatment of the tissue with atropine (0.1 μM), an antimuscarinic agent[19]. In atropine pre-treated tissues the relaxation was obtained at lower concentration. The relaxant effect of *C. bonariensis* in the absence and presence of atropine were significantly different from each other, indicating the presence of spasmogenic and spasmolytic constituents in the plant extracts.

We have previously observed that the spasmolytic constituents present in medicinal plants usually mediate their effect through calcium channel blockade (CCB) properties[20-21]. To see whether the spasmolytic effect of *C. bonariensis* observed in this study is mediated through CCB, a high concentration of K^+ (80 mM) was used which caused sustained contraction. The crude extract caused a concentration-dependent relaxation of K^+-induced contractions, similar to verapamil. K^+ at high concentrations (>30 mM) is known to cause smooth muscle contractions through opening of voltage-dependent slow Ca^{++} channels, thus allowing influx of extracellular Ca^{++} causing a contractile effect[22]. Thus the inhibitory effect of the plant extract against K^+-induced contractions can be visualized as the CCB.

The presence of calcium antagonist(s) in the plant extract was confirmed when it caused a rightward shift in the Ca^{++} concentration-response curves, constructed in the Ca^{++}-free and K^+ rich medium, similar to that caused by verapamil, a standard Ca channel blocker[22]. Interestingly CCBs are known to be useful as antispasmodic and antidiarrhoeal[23]. Activity-directed fractionation studies revealed that the relaxant constituents of *C. bonariensis* plant extract were concentrated in the hexane and ethylacetate fraction. Both these fraction caused a significant relaxant effect in spontaneous and high K^+-induced contractions in isolated rabbit jejunum preparations. In contrast to their parent crude extract, the hexane and ethylacetate fractions of *C. bonariensis* did not produce any contractile effect in the isolated rabbit jejunum preparations.

Conclusions

The data from the present study indicate that the crude extract of *C. bonariensis* contains spasmogenic constituents, which are separated into the butanol fraction and spasmolytic constituents, concentrated in the ethylacetate and hexane fractions. The spasmogenic activity of the plant extract was similar to acetylcholine while the spasmolytic effect resembled calcium channel blockers. Moreover, theses finding validate the medicinal use of the *C. bonariensis* in constipation and diarrhea.

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