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Antidiarrheal and antispasmodic activities of *Polygonum bistorta* rhizomes are mediated predominantly through K^+ channels activation

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Abstract

Polygonum bistorta is a popular medicinal herb used to treat diarrhea. This study provides pharmacological basis to its folk use in diarrhea using *in vivo* and *in vitro* assays. Administration of *P. bistorta* rhizomes extract to mice offered protection against castor oil-induced diarrhea at 300-1,000 mg/kg and was found safe up to the dose of 5 g/kg. In isolated rabbit jejunum, the extract caused a dose-dependent relaxation of spontaneous and low K^+ (25 mM)-induced contractions with weak effect against high K^+ (80 mM). In tissues pretreated with glibenclamide or tetraethylammonium chloride (TEA), the relaxant effect of the extract was markedly inhibited by TEA only. While verapamil showed complete relaxation of spontaneous, low K^+, low K^+ with TEA and high K^+-induced contractions. In guinea-pig ileum, mild atropine-sensitive effect was observed. This study indicates that *P. bistorta* possesses anti-diarrheal and antispasmodic activities mediated predominantly through K^+-channels activation along with weak Ca^{++} antagonist effect.

Introduction

Polygonum bistorta (Synonym; *P. vivparum*) belongs to family *Polygonaceae*, is locally known as Anjabar. It is commonly found in marshy places, in alpine areas as Chitral, Gilgit, Swat and Ladakh. *P. bistorta* is a small perennial shrub with woody root stock and 10-30 cm stem where leaves are 3.5-5 cm, linear, short pointed minutely round with sharp base give the shape of heart. Flowers are pink in color and solitary erect (Baquer, 1989). This shrub is known to be effective in adenopathy, amenorrhea, cancer, carbuncle, colitis, congestion, cramp, diarrhea, dysentery, dysmenorrhea, dyspepsia, epilepsy, fever, rhinitis sore throat and wound healing (Duke et al., 2002).

Phytochemical investigations revealed the presence of gamma-sitosterol, beta-sitosterol, beta-sitosterone, friedelin and cycloartane type triterpenoids like, 24(E)-ethylidenecycloartanone and 24(E)-ethylide-necycloartan-3alpha-ol (Manoharan et al., 2005), and some tannin-related compound, bistortaside A (Liu et al., 2006) in *P. bistorta* as plant constituents.

P. bistorta has also been studied for its anti-inflammatory (Duwiejua et al., 1999), CNS depressant (Datta et al., 2004) and interferon like activities (Smolarz and Skwarek, 1999). In addition, the plant has also been found effective in snake bites (Viegi et al., 2003). This plant has been popularly used to treat diarrhea; however, to the best of our knowledge, there is no known scientific evidence to support its medicinal use in diarrhea. This study provides scientific basis to its folkloric medicinal use in hyperactive gut disorders like diarrhea.

Materials and Methods

Collection of plant material and preparation of the crude extract

Rhizome of the plant was purchased from the local market, herbal medical store of Multan and was identified by the expert taxonomist Dr. Altaf A. Dasti at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, marked from the herbarium while voucher and specimens were deposited in the same Institute. The plant material was cleaned and coarsely powdered by the electrically driven machine. Powdered material was soaked in 80% aqueous ethanol for 7 days with occasional shaking (Williamson et al., 1998). It was filtered through muslin cloth to remove out the debris and further subjected for filtration using Whatman Qualitative Grade no. I filter paper. The filtrate was evaporated under reduce pressure in rotary evaporator to get thick paste like mass of dark brown color. It was transferred to Petri-dish to remove remaining solvent by keeping in desiccators. Percentage yield was around 12% (w/w) and the extract was solubilized in distilled water for further experimentation.

Preliminary phytochemical analysis

The crude extract was screened for the possible presence of anthraquinones, coumarins, flavonoids, saponins, tannins and terpenes by following methods described Tona et al. (1998).

Chemicals and animals

Acetylcholine, atropine sulfate, potassium chloride and loperamide hydrochloride were purchased from Sigma Chemicals Co, St Louis, MO, USA. Glibenclamide and tetraethylammonium chloride (TEA) were purchased from Tocris, Ellisville, MO and RBI Chemicals Co, Natick, MA, USA respectively. All chemicals used were of the analytical grade available and solubilized in distilled water/saline except glibenclamide, which was dissolved in DMSO (1%). The vehicle used for solubilization was found inert on isolated tissue preparations in control experiments. Stock solutions of all chemicals were made fresh in normal saline on the day of the experiment.

BALB/c mice (weighing 20–25 g, n=40) and locally bred rabbits (weighing 1–1.5 kg, n=8) and guinea-pigs (400-550 g) of both sexes, were housed at the Animal House of Aga Khan University under controlled environmental conditions (23–25°C). The animals were fasted for 16-18 hours before the experiment, whereas they were given tap water and standard diet routinely. Experiments were performed with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996). This study was part of the M. Phil thesis of the first author which was approved by the Board of Studies, Bahauddin Zakariya University, Multan.

In vivo and *in vitro* experiments

Castor oil-induced diarrhea

Mice of either sex were housed at the animal house of Aga Khan University fasted for 16-18 hours before the starting of experiments. They were housed in individual cages and divided into five groups (n=5/group). One group was treated with saline (10 mL/kg, orally) as negative control, while other was administered loperamide (10 mg/kg, orally) as positive control. Further, three groups were given increasing doses (300-1,000 mg/kg) of *P. bistorta* extract orally. One hour after the treatment, all groups were given castor oil (10 mL/kg) orally with feeding needle. At four hours after castor oil administration, the cages were inspected for typical diarrheal droppings, their absence was considered as protection against diarrhea (Janbaz et al., 2014).

Acute toxicity testing

The animals were divided into three groups with five animals each. First two groups were treated with increasing doses (3 and 5 g/kg) of *P. bistorta* extract and were kept in animal cages with free access to food and water. Third group was given saline (10 mL/kg) as negative control. The mice were kept under regular observations for any acute toxicity signs for 6 hours, while the lethality was monitored up to 24 hours.

Rabbit jejunum and guinea-pig ileum preparation

Rabbits and guinea-pigs were starved for 16-18 hours and were sacrificed by cervical dislocation. The gut modulatory effects of the test material were studied by using isolated rabbit jejunum and guinea-pig ileum preparations respectively. Individual segments of 2-3 cm were suspended in 10 mL tissue organ baths containing Tyrode's solution, maintained at 37°C and aerated by carbagen (a mixture of 95% oxygen and 5% of carbon dioxide). The Tyrode's solution used in these experiments contained KCI 2.68, NaCI 139.9, $MgCI_2$ 1.05, $NaHCO_3$ 11.90, $NaH2PO_4$ 0.42, $CaCl_2$ 1.8 and glucose 5.55 in mM (pH 7.4). The response of crude extracts on intestinal preparations was recorded using isotonic transducers coupled with oscillograph data acquisition setup. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with repeated administration of acetylcholine (Ach, 0.3 μM) at every 3-5 min interval, until similar responses were achieved. Under similar experimental conditions, guinea-pig ileum behaved as quiescent smooth muscle preparation and is considered more useful for the assessment of spasmogenic activity (Mehmood et al., 2011), while rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing the relaxant (spasmolytic) activity directly without the use of an agonist (Gilani et al., 2008; Khan et al., 2011; Rehman et al., 2013). The relaxant responses were quantified compared to the control spontaneous beating patent of isolated jejunal preparations. While the con-

tractile response of the test material were assessed as the percent of the maximum effect produced by ACh (0.3 or 1 µM).

Statistical analysis

All the data was expressed as mean ± standard error of mean (SEM, n=number of experiments) and the median inhibitory concentrations (IC_{50}) with 95% confidence intervals (CI). The statistical parameter applied is Student's t-test except in case of castor oil induced diarrhea where Chi-square-test was used. $P<0.05$ was considered as significant difference. Concentration-response curves were analyzed by non–linear regression using GraphPad program (San Diego, CA, USA).

Results and Discussion

Preliminary phytochemical analysis showed the presence of alkaloids, saponins, tannins and phenolic compounds as plant constituents. On account of medicinal use of *P. bistorta* in hyperactive gut disorders (Duke et al., 2002), it was first evaluated for its antidiarrheal activity against castor oil-induced diarrheal model in mice. Further to explore the scientific basis for its observed antidiarrheal activity, the test material was subjected studied for its spasmolytic effect on isolated tissues of rabbit jejunum and guinea pig ileum (Mehmood and Gilani, 2010; Shah et al., 2011; Janbaz et al., 2014; 2015a).

When administered to mice, *P. bistorta* offered protection against castor oil-induced diarrhea at a dose range of 300-100 mg/kg, similar to the effect of loperamide, a standard antidiarrheal agent (Reynolds et al., 1984) as shown in Table I. It was also found safe in mice at the highest tested doses of 3 and 5 g/kg both for acute toxic effects signs and mortality.

Castor oil administration in mice is known to cause a significant increase in intestinal fluid contents and promotes diarrhea through ricinoleic acid, its active constituent, which is formed by hydrolysis of oil (Iwao and Terada, 1962). It also alters transport of electrolytes and water through small bowel (Gaginella and Phillips, 1975) and generates massive contractions in transverse and distal colon (Croci et al., 1997). Any test material having inhibitory effect against castor oil-induced diarrhea is considered to possess marked antidiarrheal activity.

The plant extract when tested on spontaneously contracting rabbit jejunum for its possible gut inhibitory property, it caused inhibition of contractions. The spasmolytic effect was found dose-dependent with IC_{50} value of 7.3 mg/mL (4.5-11.8; 95% CI, n=3), similar to the effect of verapamil, a standard calcium channel blocker (Lee et al., 1997), which also inhibited spontaneous contractions with IC_{50} value of 0.3 µM (0.2-0.4, n=4) as shown in (Figure 1 and 2).

Against K^+-induced contractions, *P. bistorta* extract caused complete relaxation of low K^+ (20 mM)-induced contractions with respective IC_{50} value of 1.9 µM (0.9-3.1, n=5), while it had weak effect against high K^+ (80 mM) with resultant maximum relaxation of only 31.0 ± 1.2% (mean ± SEM) at the highest tested concentration of 10 mg/mL. Interestingly, when the relaxant effect of the extract was restudied in the presence of TEA, a non-selective K^+ channel blocker (Cook, 1989) or glibenclamide, an ATP-dependent K^+ channel blocker (Frank et al., 1994), it was markedly inhibited with remaining maximum relaxation of 63.0 ± 2.2% vs. 100% (its effect without TEA) at highest tested concentration of 10 mg/mL.

In smooth muscle contractions and gastrointestinal secretory system, the role of multiple types of physiological mediators, such as acetylcholine, histamine, substance P, cholecystokinins, prostaglandins and 5-hydroxytryptamine (Pasricha, 2006), and some ion channels like K^+ or Ca^{++} (Quast and Cook, 1989; Farre et al., 1991), is well established. It has been observed that most of the medicinal plant and plant-derived test compounds exhibit inhibitory effect through K^+ channel activation (Gilani et al., 2008; Mehmood et al, 2015; Quast, 1992) or Ca^{++} channel blockade like mechanisms (Shah et al., 2011; Janbaz et al., 2014). Challenge of isolated tissues with low K^+ (25 mM) and high K^+ (80 mM) induces depolarization which is usually practiced

Table I

Antidiarrheal effect of the crude extract of *P. bistorta* against castor oil-induced diarrhea in mice

Group	Treatment	Total No. of mice/group	No. of mice with diarrhea after 5 hours	% Protection
1	Saline (10 mL/kg) plus castor oil (10 mL/kg)	5	5	0
2	Loperamide (10 mg/kg) plus castor oil (10 mL/kg)	5	0[b]	100
3	Extract (300 mg/kg) plus castor oil (10 mL/kg)	5	4	80
4	Extract (600 mg/kg) plus castor oil (10 mL/kg)	**5**	3[a]	40
5	Extract (1000 mg/kg) plus castor oil (10 mL/kg)	5	2[a]	60

[a]$p<0.05$ and [b]$p<0.01$ versus Group No. 1 (Chi-square-test)

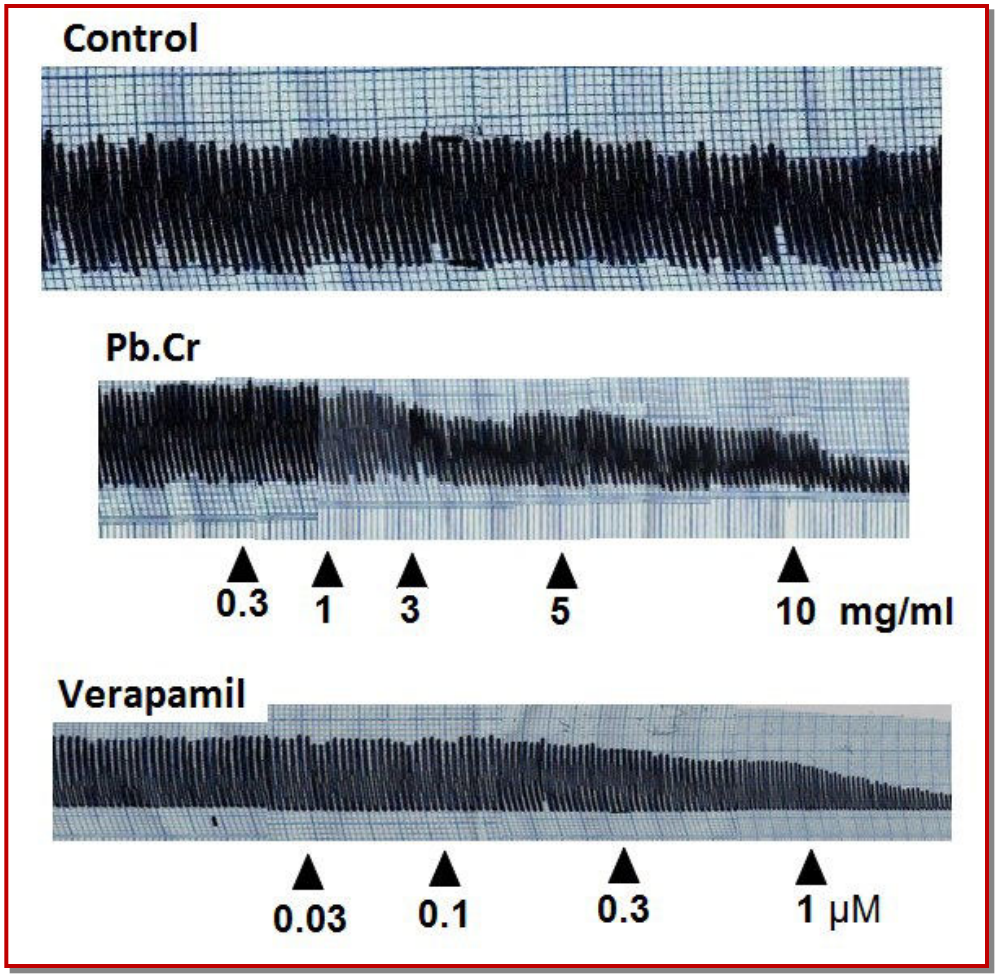

Figure 1: Tracing showing the concentration-dependent inhibitory effect of the crude extract of *P. bistorta* rhizomes (Pb.Cr) and verapamil on isolated spontaneously contracting rabbit jejunum preparations

to distinguish K^+ channel opening and Ca^{++} channel blocking like activities (Mehmood et al., 2015). Through the presence of K^+ channels and voltage dependent Ca^{++} channels in epithelial cells and smooth muscles of intestine, K^+ channel openers (increase in K^+ efflux) and Ca^{++} antagonists (inhibition of Ca^{++} entry) cause inhibitory effect on smooth muscle by decreasing intracellular free Ca^{++} through respective mechanisms of membrane hyperpolarization (Vogalis, 2002; Lee et al., 1997). The inhibitory effect of *P. bistorta* rhizomes extract was markedly inhibited in the presence TEA compared to its effect in the presence of glibenclamide, indicating the predominant involvement of non-specific K^+ channels in its exhibited spasmolytic action. As K^+ channels and abundantly present in intestinal smooth muscles and known for their inhibitory influence in hypermotile gut disorders (Vogalis, 2000; Poggioli et al., 1995).

The concentration of K^+ >30 mM, regarded as high K^+, is known to cause smooth muscle contractions through opening of voltage-dependent Ca^{++} channels (Karaki and Wiess, 1983). Thus, a substance that inhibits high K^+-provoked contractions is considered a blocker of Ca^{++} influx (Farre et al., 1991). The Ca^{++} antagonist effect on the part of plant extract was found week which was evident by its inhibitory effect against high K^+-induced contractions (Figure 1). However, the involvement of weak Ca^{++} antagonist-like effect cannot be ignored in the observed antidiarrheal efficacy of *P. bistorta* rhizomes extract in mice as the Ca^{++} antagonists are known for their antidiarrheal potential (Lee et al., 1997).

On the basis of presence of tannins and the observed maximum protection against diarrhea on relatively higher dose, the extract was screened on isolated guinea pig ileum for the presence of any gut stimulatory activity. In ileum, a quiescent gut preparation, the extract elicited mild stimulant response with maximum effect of 22.0 ± 5.3% (relative to 100% control response of acetylcholine at 0.3 µM) only at single concentration of 1 mg/mL, which was blocked when repeated in the presence of atropine (Figure 3).

Acetylcholine is released by the nerve endings of parasympathetic enteric nerve endings and plays an

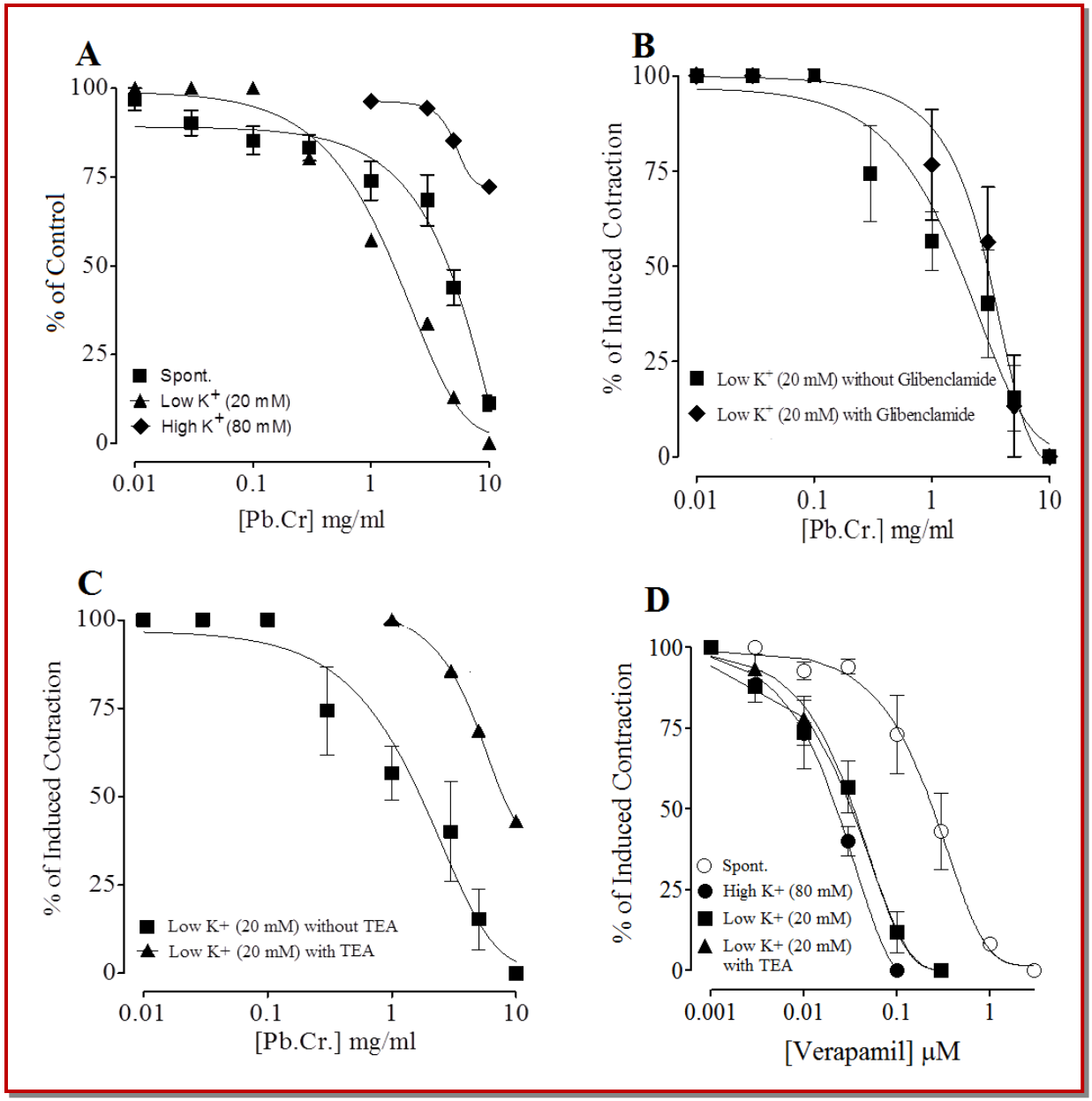

Figure 2: The concentration-dependent inhibitory effect of the crude extract of *P. bistorta* rhizomes (Pb.Cr) against (A) spontaneous, low K^+ (20 mM) and high K^+ (80 mM)-induced contractions, (B) low K^+-induced contractions without and with glibenclamide (10 µM) and (C) low K^+-induced contractions without and with tetraethylammonium chloride (TEA), while (D) shows concentration-dependent inhibitory effect of verapamil against low K^+, low K^+ with TEA and high K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values are expressed as mean ± S.E.M, n = 3-6

important role in regulation of gut motility, similarly the activation of muscarinic receptors in the gut are also known for their gut accelerating properties (Brown and Taylor, 2006). Since atropine is an antagonist at muscarinic receptors sites (Gilani et al., 1997), blocked the stimulant effect, which indicates the presence of some cholinergic-like constituents in *P. bistorta* rhizomes extract which might be presumably meant by the nature to offset an excessive gut relaxant effect, however, further investigation is required to prove this speculation.

Conclusion

These findings indicate that *P. bistorta* possesses antidiarrheal and antispasmodic activities mediated predominantly through TEA-sensitive K^+ channels activation along with weak Ca^{++} antagonist like mechanism. Thus, this study provided a rational to the folkloric use of *P. bistorta* in diarrhea.

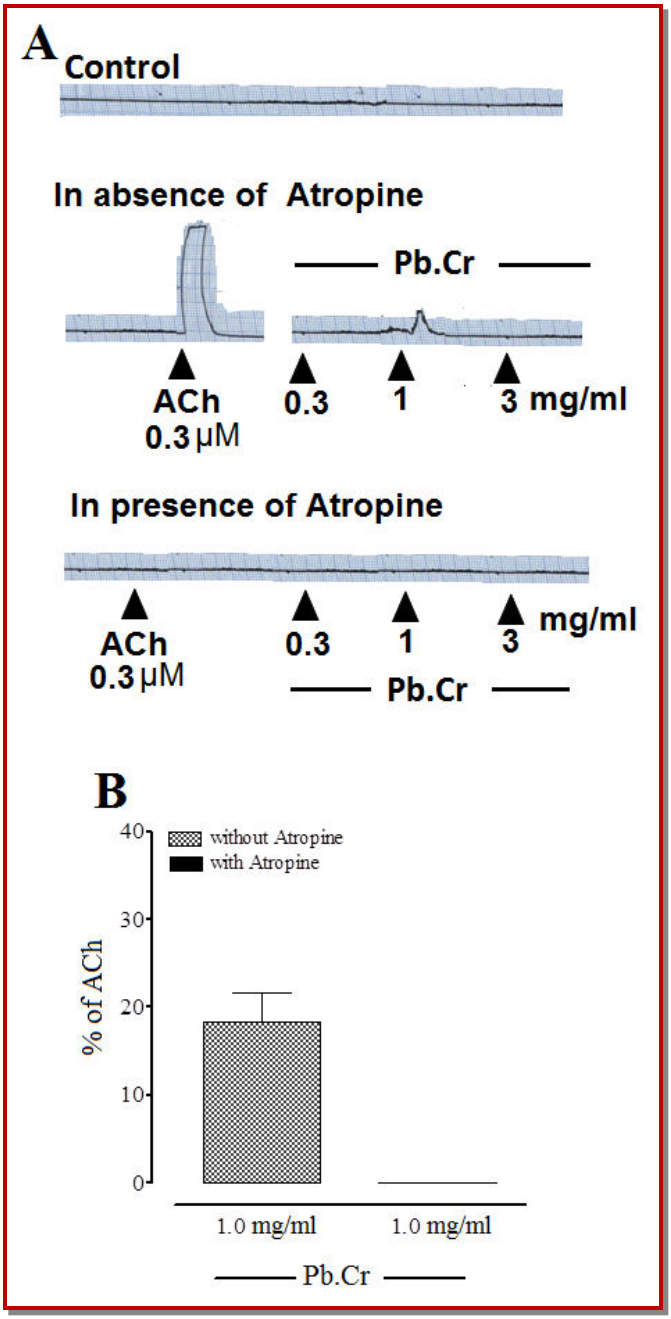

Figure 3. Typical tracing (A) and bar chart (B) represent the inhibitory effect of the crude extract of *P. bistorta* rhizomes (Pb.Cr) in the absence and presence of atropine (0.1 µM) on base line status of isolated guinea-pig ileum. Values are expressed as mean ± S.E.M, n = 3-5

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