The Journal of Animal & Plant Sciences, 23(1): 2013, Page: 330-336
ISSN: 1018-7081

INSIGHT INTO MECHANISM UNDERLYING THE MEDICINAL USE OF *CYDONIA OBLONGA* IN GUT AND AIRWAYS DISORDERS

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ABSTRACT

This study was intended to provide the pharmacological rationalization for the medicinal use of *Cydonia oblonga* in gut and airways diseases. Results showed that the crude extract of *Cydonia oblonga* seeds (Co.Cr) produced atropine-sensitive spasmodic effects in isolated ileum of guinea-pig and rabbit jejunum preparations. In rabbit jejunum, Co.Cr also showed relaxant activity at slightly higher concentrations (0.1-10 mg/mL). When analyzed on rabbit jejunum precontracted with K^+ (80 mM), the plant extract (0.003-10 mg/mL) produced relaxation. A rightward shifting of Ca^{++} dose-response curves along with decline in the maximum response was observed after pretreatment with Co.Cr (0.003-0.01 mg/mL), which was similar to the effect of verapamil. The plant extract (0.01-10 mg/mL) relaxed CCh (1 µM) and K^+ (80 mM)-induced contractions of isolated rabbit tracheal preparations, similar to the effect produced by verapamil. Data show that *Cydonia oblonga* possesses spasmogenic effect mediated through cholinergic pathway, while Ca^{++} antagonist like mechanism is found responsible for its spasmolytic activity, thus provides justification for the medicinal use of the plant in gut and airways disorders.

Key words: *Cydonia oblong;* Spasmodic; Cholinergic; Antispasmodic; Ca^{++} antagonist; Gut; Trachea.

INTRODUCTION

Cydonia oblonga Mill. (family; Rosaceae) found in central Asia and is commonly known as quince (Saeed, 1972). Major use of dried fruit is in making jelly and marmalade (Usmanghani *et al*., 1997). The name marmalade is said to be derived from "Marmelo" the Portuguese name for quince (Nadkarni, 1976).

The seeds of *Cydonia oblonga* have been used traditionally in diarrhea, dysentery, cough, sore throat and bronchitis (Nadkarni, 1976; Duke *et al*., 2002), intestinal colic and constipation (Prajapati *et al.,* 2006). In Europe, *Cydonia oblonga* extract along with lemon juice, namely Gencydo®, is one of the popular complementary therapies used for allergic rhinitis and asthma (De Bruin and Baars, 2001).

The plant has been reported to possess triterpenoic acids, sterols, carotenoids phytoene and phytofluene (Lorenz *et al.*, 2008), phenolic compounds (Oliveira *et al.*, 2007), organic acids like ascorbic, fumaric, shikimic, quinic, citric, and malic acids, while among free amino acids; the aspartic, glutamic and asparagines are the most abundant (Silva *et al*., 2005). Similarly, from fatty acids, the oleic, linoleic, capric and palmitic acids are the major constituents comprising around 85.06 % of its oil (Turkoz *et al*., 1998).

Despite the presence of many studies available on different medicinal aspects of *Cydonia oblonga*, this plant has not been analyzed for its use in diarrhea, constipation or airways disorders, like cough, bronchitis and asthma except preliminary reports on the usefulness of plant in cholera and asthma on account of its antibacterial (Guevara *et al*., 1994) and antiallergic (Baars and De Bruin, 2005; Gründemann, 2011) activities. These findings offer pharmacological rationalization for the medicinal use of *Cydonia oblonga* seeds in gut and airways diseases.

MATERIALS AND METHODS

Plant collection: *Cydonia oblonga* seeds were obtained from the local herbal market of Multan, Pakistan, in June 2008, and authenticated by Prof. Dr. Altaf A. Dasti, a taxonomist at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

Crude extract preparation: The seeds were coarsely -ground after removing the adulterants. A previously expressed method (Gilani *et al*., 1991) was followed for the extraction of plant material. The -ground seeds (1 Kg) were immersed in methanol-aqueous (80:20) for 72 h, as the methanol-aqueous, being commonly used solvent to extract maximum compounds with both polar and non-polar nature is known for the extraction of maximum plant constituents with appreciable yield (Sultana *et al*., 2009). Initially filtration was conducted by using muslin cloth and subsequently through filter paper (Whatman

No.1), and repeated the method three times. Rotary evaporator (Büchi R-200, Switzerland) coupled with vacuum pump (Büchi vac V-500), was used to evaporate the filtrate and to obtain a thick paste respectively, which was subsequently lyophilized to dry powder on a lyophilizer (Alpha 1-4 LD, Christ, Germany) with approximate yield of 6%. The methanol extract of *Cydonia oblonga* was found soluble in distilled water/saline for *ex-vivo* and *in vivo* studies, and dilutions were prepared a fresh on test day.

Chemicals and animals: Atropine sulphate, acetylcholine perchloride (ACh), verapamil hydrochloride, carbamylcholine (CCh) and some constituents of chemicals utilized for producing physiological salt solutions and the reagents used in phytochemical analysis; magnesium chloride ($MgCl_2$), potassium chloride (KCl), ethylenediamene-tetraacetic acid (EDTA), Dragedorff's reagent, ferric chloride ($FeCl_3$), aluminium chloride ($AlCl_3$), calcium chloride ($CaCl_2$), and aluminium hydroxide ($AlOH_3$) were acquired from Sigma-Aldrich Chemicals Company, St. Louis, MO, USA. While, glucose ($C_6H_{12}O_6$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), magnesium sulfate ($MgSO_4$), potassium dihydrogen phosphate (KH_2PO_4), and different solvents like petroleum ether, methanol, benzene, hydrochloric acid, and chloroform were purchased from E. Merck KGa, (Darmstadt, Germany). Chemicals used for the study were of analytical grade, and dissolved in distilled water/saline for the experiments.

Rabbits (1000-1500 g) and Guinea-pigs (500-600 g) of both sexes and local breed were kept at the Animal house of the Aga Khan University, and maintained at standard environmental conditions. The animals were given liberated access to tap water as per routine, but were kept on fasting for 01 day following the start of experiment. Experiments conducted under the guidelines of the Commission on Life Sciences, National Research Council (1996).

Phytochemical analysis: Phytochemical screening of the plant extract was carried out qualitatively for the possible occurrence of flavonoids, coumarins, anthraquinones, alkaloids, sterols, saponins, tannins, and terpenes by following previously expressed methods (Evans, 2006).

Isolated Rabbit jejunum tissue preparation: The antispasmodic/ prokinetic property of the Co.Cr was evaluated by using isolated jejunum of rabbit as explained earlier (Mehmood *et al*., 2010). Rabbits of either sex were sacrificed by using the method of cervical dislocation. Mesenteries were removed from jejunum and immersed in Tyrode's solution after separation. Around 2-3 cm lengthy jejunum surgical sections were hanged in Tyrode's solution filled tissue organ bath (10 mL), kept at 37°C, and ventilated with carbogen (5% CO_2 and 95% O_2). The composition of the Tyrode's solution in mM was: 5.55 glucose, 11.90 $NaHCO_3$, 1.05 $MgCI_2$, 136.9 NaCI, 1.8 $CaCl_2$, 2.68 KCI, and 0.42 NaH_2PO_4 (pH 7.4). Each tissue was applied a resting tension of 1g as preload. Intestinal responses were determined using isotonic transducer 50-6360 (Harvard Apparatus, Holliston, MA, USA) paired with student oscillograph (Harvard Apparatus) / Power Lab model ML-845, data acquisition system (AD Instruments; Sydney, Australia). Drugs were added after providing at least 30 min equilibration time to each tissue. Acetylcholine at a submaximal concentration of 0.3 μM was used to stabilize the tissues at 3 min periods until recorded responses became stable. These investigational conditions allowed jejunum to contract spontaneously, which is considered as suitable preparation for analyzing relaxant activity directly without applying an agonist (Janbaz *et al*., 2011).

To assess whether Ca^{++} antagonist mechanism is involved in the spasmolytic property of the test extract, isolated rabbit jejunum preparations were depolarized by using K^+, as KCl (Farre *et al*., 1991). L-type voltage dependent Ca^{++} channels are liable for the production of contractile effect via influx of extra cellular Ca^{++} and high K^+ (>30 mM) has been recognized for its property to open these channels (Bolton, 1979). A substance which blocks high K^+-stimulated contraction is known as Ca^{++} antagonist (Godfraind *et al*., 1986). The inhibition of K^+ (80 mM)-induced intestinal contraction was described as percent of the control response mediated by K^+. To further confirm (Ca^{++} channel blocking) CCB-like activity of the extract, the tissues were immersed in normal Tyrode's solution for stabilization, and substituted with Ca^{++} free Tyrode's solution for 10-15 min. EDTA (0.1 mM) present in Ca^{++} free Tyrode's solution is useful for Ca^{++} removal from tissues and the environment following by its substitution with Ca^{++} free and K^+ rich Tyrode's solution possessing following composition (mM): 0.1 EDTA, 50 KCL, 0.42 NaH_2PO_4, 91.04 NaCl, 11.90 $NaHCO_3$, 5.55 glucose, and 1.05 $MgCl_2$. The Ca^{++} concentration-response curves (CRCs) were developed after providing 30 min incubation period to Co.Cr with tissues. After getting the super imposable control Ca^{++} CRCs (generally after two cycles), the tissues were pre-incubated with Co.Cr for 01 hr to measure the presence of possible Ca^{++} antagonist activity.

Isolated guinea- pig ileum preparation: Guinea-pigs of local breed and both sexes, fasted for a day, were sacrificed by using the method of cervical dislocation, mesenteries were removed and the ileum was separated out. Previously described method for isolated rabbit jejunum tissue preparation was used for the preparation, equilibration, and stabilization of isolated guinea-pig ileum. Similarly, same isotonic transducers were used as adopted for rabbit jejunum preparation. Submaximal

doses of Ach were applied and constant responses were recorded, then extract was added in graded concentrations. The achieved stimulatory responses were calculated by the control effect ACh (0.3 μM), taking as 100%. Stimulant property of the plant extract was characterized by repeating the experiments after pretreatment of the tissue with antagonist (atropine) (Janbaz *et al*., 2011).

Isolated rabbit trachea preparation; Rabbits were sacrificed by cervical dislocation and trachea was separated out, adulterants were removed and isolated trachea was kept in Kreb's solution. 2-3 mm wide tracheal rings from main tracheal tube were prepared. Each ring consists of three to four cartilages. A tracheal chain was created by keeping cartilages on the edges and smooth muscles in the centre by cutting the ring longitudinally on the ventral side. The tissue was hanged in a Kreb's solution filled tissue organ bath, kept at 37°C, and ventilated continuously with carbogen. The composition of Kreb's solution was (mM): KH_2PO_4 1.3, KCI 4.7, glucose 11.7, NaCl 118.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, and $CaCl_2$ 2.5 (pH 7.4). Tracheal strips were provided 1 g resting tension as preload continuously during the experiment. Test substance was added after completion of 45 min equilibration period of the tissue. Isolated tracheal preparations were stabilized using carbachol (CCh, 1 μM) and K^+ (80 mM) until the identical responses were found, and achieved contractions became sustainable. The relaxant property of the Co. Cr was measured by dosing in the tissue bath in a cumulative manner, while the responses were recorded using isometric transducers attached with Power Lab (Shah and Gilani, 2010).

Statistical analysis: $P < 0.05$ was considered as statistically significant difference using unpaired *t* test. Values of median effective concentrations (EC_{50} values), mean ± standard error of mean (s.e.m; *n*=number of experiments), and 95% confidence intervals (CI) were calculated for the expression of data. Non-linear regression was used for the analysis of CRC through GraphPAD program (GraphPAD, San Diego, California, USA).

RESULTS AND DISCUSSION

When analyzed on isolated rabbit jejunum, which is considered a suitable preparation for the assessment of spasmodic/spasmolytic responses (Mehmood and Gilani, 2010), the plant extract caused a gentle prokinetic effect at lesser concentrations (0.003 and 0.03 mg/mL) with a maximum of 21.69 ± 8.47%, n=5 ($p < 0.01$ vs. basal spontaneous tone) of ACh (0.3 μM) maximum response. At the next higher concentrations (0.1-10 mg/mL), Co.Cr showed relaxant effect with an EC_{50} value of 0.73 mg/mL (95% CI, 0.36-1.15, n=5) as shown in Figure 1 and 2A. In order to describe the stimulatory activity, tissues were pretreated with an anti-muscarinic substance (Atropine, 0.1 μM) (Gilani *et al*., 1997) before redetermining the activity of the plant, which leads to the elimination of the contractile activity of Co. Cr. This shows the presence of ACh-like gut stimulatory effect. However, its relaxant activity was potentiated by pretreatment with atropine with a resultant EC_{50} value of 0.91 mg/mL (0.50-1.81, n=4), which could be an outcome of the masking of contractile elements. The gut stimulant effect was mild, which is possibly due to the presence of antispasmodic component(s) seen at slightly high concentrations, is possibly intended by nature to counterbalance the excessive stimulant activity. This excessive prokinetic activity could otherwise produce harmful effects by causing abdominal cramps, usually seen when synthetic chemical drugs are utilized in the treatment of constipation (Pasricha, 2006).

To know about the possible mechanism of spasmolytic activity, the plant extract was analyzed on sustained contractions induced by K^+ (80 mM). The extract inhibited K^+ (80 mM)-induced contraction with EC_{50} value of 0.86 mg/mL (0.66-1.98, n=4) similar to verapamil, a standard Ca^{++} antagonist (Fleckenstein, 1977), which inhibited the spontaneous and K^+ (80 mM)-induced contractions with EC_{50} values of 0.17 μM (0.11-0.28, n=3) and 0.05 μM (0.03-0.07, n=4), respectively (Figure 1 and 2B). Role of high K^+ (>30 mM) for producing contraction on smooth muscles is mediated through opening of L-type voltage-dependent calcium channels which causes influx of extracellular Ca^{++} (Reynolds *et al*., 1984). A substance producing relaxation of high K^+-mediated contraction is believed as Ca^{++} antagonist (Bolton, 1979), and thus the spasmolytic effect of Co.Cr against high K^+ similar to verapamil may demonstrate the occurrence of calcium channel blocker like constituents in the plant extract. A rightward shifting of Ca^{++} dose-response curves along with decline in maximum effect confirmed the Ca^{++} antagonist activity of Co.Cr (0.003 and 0.01 mg/mL). This effect of Co.Cr was comparable to verapamil, as shown in Figure 3. Thus, the Ca^{++} antagonist activity of Co.Cr may elucidate its medicinal use in diarrhea and intestinal colic, as Ca^{++} antagonists are well recognized for their effectiveness in gut motility disorders, such as diarrhea and intestinal colic (Reynolds *et al*., 1984).

The gut stimulatory effect of Co. Cr was further confirmed on isolated ileum of guinea-pig, a useful quiescent preparation for the assessment of the gut stimulant effect (Janbaz *et al*., 2011). The plant extract caused an atropine-sensitive spasmodic effect at 1-10 mg/mL with a resultant maximum of 31.22 ± 3.7% (n=4) of ACh (0.3 μM) contraction, which was evident by its inhibition after pretreatment with atropine (0.1 μM) as seen in Figure 4. This confirms the involvement and

activation of muscarinic receptors in gut stimulatory effect of the plant extract. ACh is discharged by the parasympathetic nervous system as a neurotransmitter and activates the M_3 muscarinic receptor subtypes responsible for the regulation of peristaltic movement in the gut (Gilani *et al*., 1997). Thus, the presences of ACh-like spasmodic constituent(s) in *Cydonia oblonga* extract is an evidence for its medicinal use in constipation (Duke *et al*., 2002). Such combinations of activities have been reported earlier (Gilani *et al*., 2000; 2005; Mehmood and Gilani, 2010).

Based on folkric use of *Cydonia oblonga* in airways diseases, such as bronchitis, cough and asthma (De Bruin and Baars, 2001), Co.Cr was tested for possible bronchodilator activity by using isolated tracheal preparations. The extract caused inhibition of high K^+ and CCh-induced contractions with EC_{50} values of 0.41 mg/mL (0.23-0.74, n=4) and 0.94 mg/mL (0.67-1.32, n=6) respectively, similar to verapamil as seen in Figure 5. The Ca^{++} antagonists are documented to possess bronchodilatory activities (Mathewson, 1985), and the bronchodilator property examined in this study may be due to the existence of Ca^{++} antagonist components in the plant extract, though presence of additional mechanism(s) is also possible and cannot be ruled out. A question arises, whether the presence of cholinergic component(s) in the plant extract is likely to cause bronchoconstriction due to involvement of muscarinic receptors activation (Brown and Taylor, 2006), which could be harmful for asthmatic patients. However, our experience working on such plant remedies with medicinal use both in airways and gut disorders reflects that the cholinergic component is usually water soluble (Gilani *et al*., 2005a; Jabeen *et al*., 2007; 2009) and being polar in nature is unlikely to get absorbed into systemic circulation, hence, its cholinergic side effect is unlikely to occur as systemic effect, like in airways, though the poor absorption of such moiety could be useful in exhibiting local action as gut stimulatory effect, a desired effect in constipation. On the other hand, the spasmolytic constituents are usually non-polar (Gilani *et al*., 2005b; Bashir *et al*., 2006), and being lipophilic in nature, these get absorbed quickly and are accountable for systemic effects in airways.

Another question arises, what is the rationale for the presence of spasmodic and antispasmodic constituents, when the specified diseases, like constipation or diarrhea, do not occur simultaneously being two opposite disease states of gut. The chemical drugs used to relieve constipation tend to cause abdominal cramps and even diarrhea at high doses. Similarly, the antidiarrheal drugs would tend to cause constipation at high doses (Gilani and Rahman, 2005), it is the beauty of nature that both gut stimulatory and inhibitory constituents co-exist in plant remedies, such as ginger (Ghayur and Gilani, 2005), ispaghula (Mehmood *et al*., 2011), black pepper (Mehmood and Gilani, 2010), which are probably meant

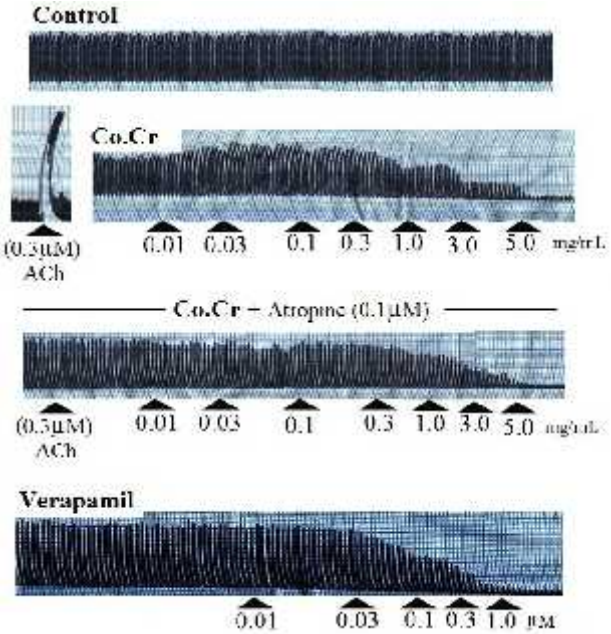

Figure 1. Typical tracing showing the stimulant and relaxant effects of the crude extract of *Cydonia oblonga* (Co.Cr) and verapamil on spontaneous contractions in isolated rabbit jejunum preparations.

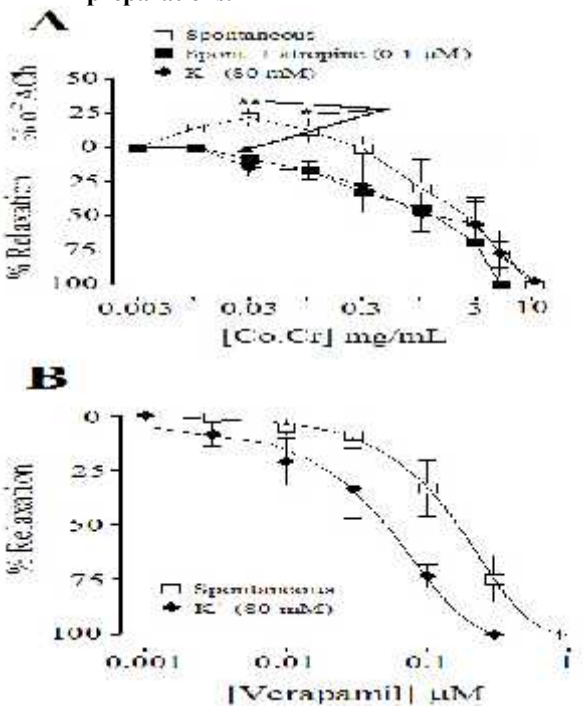

Figure 2. The mild spasmodic and antispasmodic effects of (A) the crude extract of *Cydonia oblonga* (Co.Cr) and (B) verapamil on spontaneous, in the absence and presence of atropine (0.1 μM) and K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values shown represent mean ± s.e.m of 3-5 determinations. *$p < 0.05$ and **$p < 0.01$ vs. control (Unpaired *t*- test)

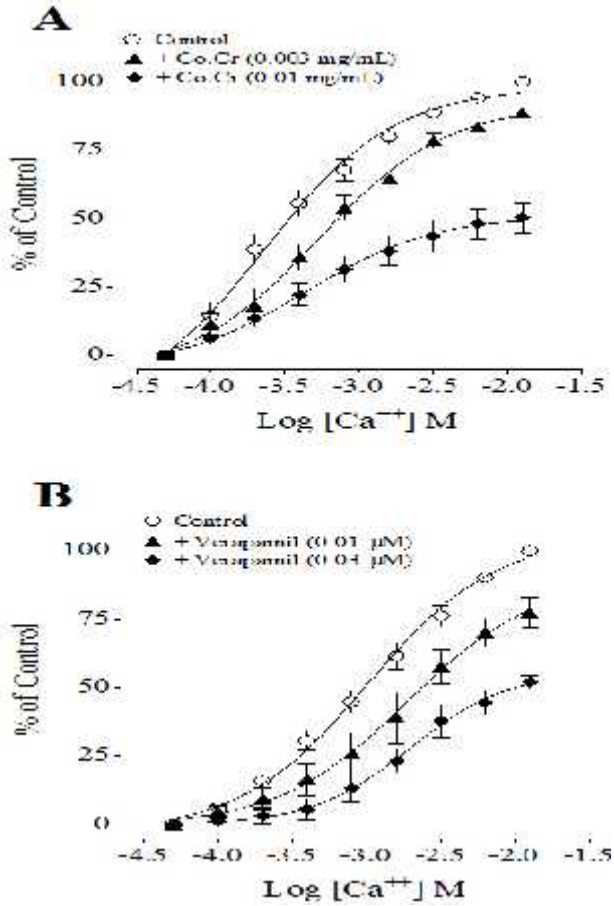

Figure 3. The concentration response curves of Ca^{++} in the absence and presence of (A) the crude extract of *Cydonia oblonga* (Co.Cr) and (B) verapamil in isolated rabbit jejunum preparations. Values shown represent mean ± s.e.m of 4-7 determinations.

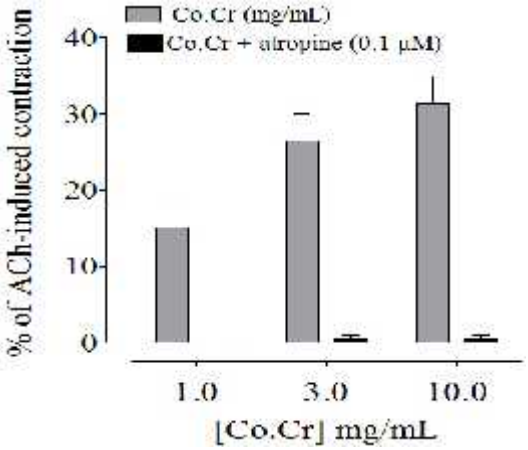

Figure 4. The spasmodic effect of the crude extract of *Cydonia oblonga* (Co.Cr) in the absence and presence of atropine (0.1 µM) on the base line of guinea-pig ileum. Values shown represent mean ± s.e.m of 3-4 determinations.

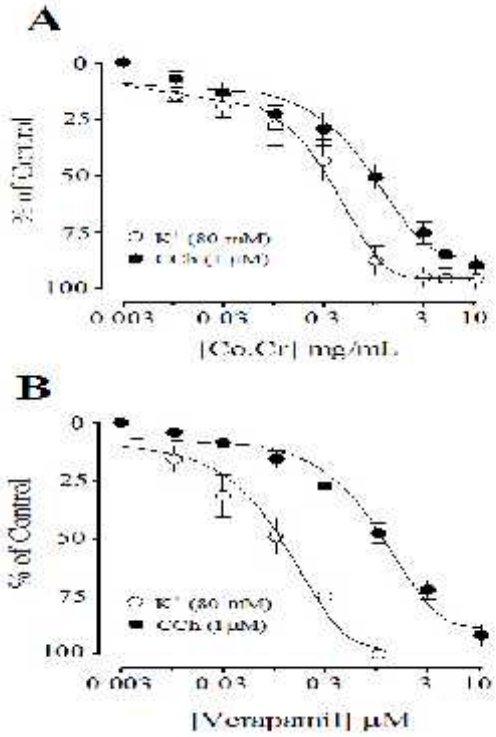

Figure 5. The antispasmodic effect of (A) the crude of *Cydonia oblonga* (Co.Cr) and (B) verapamil on K^+ (80 mM) and CCh (1 µM)-induced contractions in isolated rabbit tracheal preparations. Values shown represent mean ± s.e.m of 4-6 determinations.

by nature to offset the side effects due to extreme effect that occurs with chemical drug (Gilani and Rahman, 2005). It is possible that when using natural remedies with dual action, the gut stimulatory component becomes more active in constipation (the relaxed state of gut), while in diarrhea (hyperactive state of gut), the relaxant component dominates.

Preliminary analysis for the occurrence of phytochemical constituents showed the existence of tannins, triterpenes and sterols as plant components, which are known to possess spasmolytic activities (Budriesi *et al*., 2010; Kirimer *et al*., 1997; Ammar *et al*., 2009), thus, contributing towards the antispasmodic action of *Cydonia oblonga*. The data also showed the presence of alkaloids which may be responsible for its spasmodic effect, as some alkaloids have also been reported to possess the gut stimulant property (Fox *et al*., 1985). In addition, the plant is known to have antibacterial (Guevara *et al*., 1994) and antiallergic activities (Baars and De Bruin, 2005; Gründemann, 2011), which could offer an added benefit to its folk use in the management of gut and respiratory diseases.

Conclusion: This study clearly demonstrates the mild spasmodic property of the *Cydonia oblonga* seed extract is caused by the activation of muscarinic receptors, while Ca^{++} antagonist mechanism is possibly responsible for its antispasmodic actions seen in gut and tracheal tissues. Thus, these data provide a validation for the medicinal use of *Cydonia oblonga* in gut (diarrhea and constipation) and airways (bronchitis and asthma) disorders.

Acknowledgement: This study was conducted with partial support from the Higher Education Commission, Government of Pakistan.

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