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# Studies on Spasmogenic and Spasmolytic Activities of *Calendula officinalis* Flowers

**Samra Bashir[1,2], Khalid H Janbaz[2], Qaiser Jabeen[1] and Anwar H. Gilani[1]***

[1]Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi-74800, Pakistan
[2]Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

**The aqueous-ethanol extract of *Calendula officinalis* flowers (Co.Cr) was studied for its possible spasmolytic and spasmogenic effects in isolated gut preparations. In rabbit jejunum, Co.Cr caused a dose-dependent (0.03–3.0 mg/mL) relaxation of spontaneous and $K^+$-induced contractions, suggestive of calcium channel blockade (CCB). In a few preparations, a mild non-reproducible spasmogenic effect was observed at lower doses, followed by relaxation. The CCB effect was confirmed when pretreatment of the jejunum preparations with Co.Cr produced a dose-dependent rightward shift in the $Ca^{++}$ dose-response curves, similar to that of verapamil. Activity-directed fractionation revealed that the spasmolytic activity of the plant was concentrated in its organic fractions. The aqueous fraction exhibited a marked atropine sensitive spasmogenic effect but was found to be devoid of any spasmolytic effect. These data indicate that the crude extract of *Calendula officinalis* flowers contains both spasmolytic and spasmogenic constituents, exhibiting these effects through calcium channel blocking and cholinergic activities and this study provides a scientific base for its traditional use in abdominal cramps and constipation. Copyright © 2006 John Wiley & Sons, Ltd.**

*Keywords: Calendula officinalis*; spasmogenic; spasmolytic; cholinergic; calcium antagonist; rabbit jejunum; guinea-pig ileum.

## INTRODUCTION

*Calendula officinalis* L. (Compositae), locally known as 'Zergul' is a medium sized erect annual herb and is commonly cultivated as an ornamental plant (Baquar, 1989). In the traditional system of medicine, the plant is considered as styptic and astringent. Various parts of *Calendula officinalis* have been used for a variety of ailments including abdominal cramps, constipation, fevers, cancer and as an emmenagogue ((Nadkarni, 1976; Tyler, 1993; Duke *et al.*, 2002).

Extensive phytochemical studies on *Calendula officinalis* revealed the presence of multiple chemicals including amino acids (Abasova *et al.*, 1995), alkaloids, carotenoids, flavonoids, glycosides, saponins, tannins (Duke, 1992), high molecular weight polysaccharides (Wagner *et al.*, 1984), triterpenoid monoesters (Neukirch *et al.*, 2004) and phenolic acids such as P-hydroxybenzoic, salicylic, vanillic and caffeic acids (Gora *et al.*, 1979). Alpha-cardinol (Chalchat *et al.*, 1991), delta-cadinol, delta-cadinine and gamma muurolene (Marczal *et al.*, 1987) have been identified in the essential oil.

*Calendula officinalis* has been shown through pharmacological investigations to possess activities including antiinflammatory (Della Loggia *et al.*, 1991), antioedematous, antihyperaemic (Peyroux *et al.*, 1981), anti-HIV (Kalvatchev *et al.*, 1997), antimicrobial (Tarle and Dvorzak, 1989; Iauk *et al.*, 2003), antimutagenic (Elias *et al.*, 1990), antioxidant (Cardova *et al.*, 2002), antiulcerous (Iatsyno *et al.*, 1978), counter irritant (Fuchs *et al.*, 2005), cytotoxic, antitumor (Boucaud-Maitre *et al.*, 1988) hypoglycemic, gastroprotective (Yoshikawa *et al.*, 2001) and wound healing (Klouchek-Popova *et al.*, 1982), providing a scientific base to several of its traditional uses.

This study reports the presence of spasmogenic and spasmolytic activities, mediated through cholinergic and calcium antagonistic constituents, which may explain the traditional use of the plant in constipation and abdominal cramps, respectively.

## MATERIALS AND METHODS

**Plant material.** Fresh flowers of *Calendula officinalis* were collected from a garden near Multan, Pakistan in February 2003, and identified by Professor Dr Altaf A. Dasti, a taxonomist at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

**Preparation of crude extract and fractions.** The plant material was cleaned, shade dried and ground to coarse powder. The powdered material (500 g) was soaked in 80% aqueous ethanol for 3 days with occasional shaking. The soaked material was rendered free of debris by passing through a muslin cloth and the fluid portion was filtered through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure (–760 mmHg) to a thick, paste like mass of light brown color, i.e. the crude extract (Co.Cr), yielding approximately 20%. The vehicle used for solubilization of the extract had no effect on tissue contractility in the control experiments.

* Correspondence to: Professor Anwarul Hassan Gilani, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi-74800, Pakistan.
E-mail: anwar.gilani@aku.edu (A.H. Gilani).
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For the purpose of fractionation, 50 g of the crude extract was dissolved in about 300 mL of distilled water and shaken vigorously with immiscible organic solvents in the order of increasing polarity (i.e. dichloromethane and ethyl acetate), followed by separation with a separating funnel. The procedure was repeated thrice with each organic solvent. Individually collected fractions and the aqueous solution left subsequent to the fractionation with organic solvents were evaporated on rotary evaporator to obtain the dichloromethane (Co.DCM), ethyl acetate (Co.EtAc) and aqueous fractions (Co.Aq) of *Calendula officinalis* with yields of 1%, 2% and 92%, respectively.

**Drugs and animals.** The reference drugs, acetylcholine perchlorate, atropine sulphate, histamine diphosphate, potassium chloride and verapamil hydrochloride were purchased from Sigma Chemicals Co, St Louis, MO, USA and all other 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.

Guinea-pigs (500–600 g) and rabbits (1.5–2.0 kg) of local breed, either sex were used for this study. Animals 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. Animals had free access to water but food was withdrawn 24 h prior to experiment. Guinea-pigs were killed through cervical dislocation and rabbits by a blow on back of the head. The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996).

**Preparation of rabbit jejunum and guinea-pig ileum.** The spasmolytic and spasmogenic activities of the plant materials were studied by using isolated rabbit jejunum and guinea-pig ileum as described previously (Gilani *et al.*, 1994a, 1994b). Respective segments of 2 cm length were suspended in a 10 mL tissue bath containing Tyrode's solution, bubbled 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 BioScience transducers and an oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these experimental conditions, the guinea-pig ileum behaves as a quiescent smooth muscle preparation and is considered more useful for studying the contractile responses of agonists like acetylcholine and histamine (Gilani and Aftab, 1992), whereas, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing the relaxant (spasmolytic) activity to be tested directly without the use of an agonist (Gilani *et al.*, 2005).

**Determination of calcium antagonist activity.** To assess whether the spasmolytic activity of the test substances was through calcium channel blockade, $K^+$ was used to depolarize the preparations as described by Farre *et al.* (1991). High $K^+$ (80 mM) was added to the tissue bath, which produced a sustained contraction. The test material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van Rossum, 1963). The relaxation of intestinal preparations, pre-contracted with $K^+$ (80 mM) was expressed as the percent of the control response mediated by $K^+$. Contraction of smooth muscle induced by $K^+$ is known to be mediated via the influx of $Ca^{++}$ from extracellular fluid and the substance, which inhibits this contraction, is considered to act through blockade of calcium channels (Bolton, 1979).

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 calcium from the tissues. This solution was further replaced with $K^+$-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 dose-response curves (DRCs) of $Ca^{++}$ were obtained. When the control DRCs of $Ca^{++}$ were found to be super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test the possible calcium channel blocking effect. The DRCs of $Ca^{++}$ were reconstructed in the presence of different concentrations of the test material.

## RESULTS AND DISCUSSION

The aqueous-ethanol crude extract of *Calendula officinalis* dried flowers (Co.Cr) caused inhibition when tested on spontaneously contracting rabbit jejunum preparations, thus showing spasmolytic (antispasmodic) action (Fig. 1). The spasmolytic effect was dose-dependent with a median effective concentration ($EC_{50}$

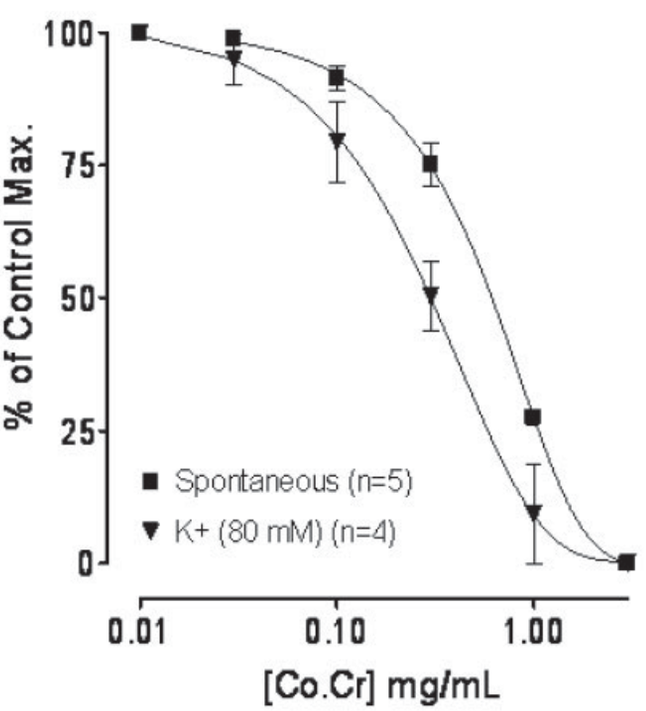


**Figure 1.** Dose-response curves of the crude extract of *Calendula officinalis* (Co.Cr) on spontaneous and $K^+$-induced contractions of isolated rabbit jejunum.



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value) of 0.78 mg/mL (0.61–1.01; 95% confidence intervals). The contraction of smooth muscle preparations including rabbit jejunum is dependent upon an increase in the cytoplasmic free [$Ca^{++}$], which activates the contractile elements (Karaki and Weiss, 1983). The increase in intracellular $Ca^{++}$ is due to either influx via voltage dependent $Ca^{++}$ 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 $Ca^{++}$ via VDCs (Brading, 1981). The inhibitory effect of the plant extract on spontaneous movements of rabbit jejunum may be due to interference either with the $Ca^{++}$ release or with the $Ca^{++}$ influx through VDCs. In a few preparations of rabbit jejunum, Co.Cr produced a mild spasmogenic effect at lower doses, followed by relaxation and this stimulant effect could not be characterized due to non-reproducibility of the contractile response. No spasmogenic effect could be seen in guinea-pig ileum, probably due to the presence of a dominant relaxant effect.

In guinea-pig ileum, a quiescent gut preparation (Gilani and Aftab, 1992), the inhibitory effect of the plant extract was studied against agonist-induced contractile responses. Both acetylcholine and histamine produced submaximal contractions at 1.0 μM. Pretreatment of the tissue with the plant extract (0.1 mg/mL) caused approximately 40% inhibition of both acetylcholine and histamine responses (Fig. 2). The next higher dose (0.3 mg/mL) further suppressed the agonist contractile responses to 80–90% inhibition, suggestive of a non-specific effect.

In order to elucidate the possible mode of the spasmolytic effect, a high dose of $K^+$ (80 mM) was used to obtain a sustained contraction, allowing dose dependent inhibitory response data to be obtained. The Co.Cr, when added to the tissue bath in a cumulative fashion, caused a dose dependent (0.03–3.0 mg/mL) relaxation of $K^+$-induced contraction (Fig. 1). $K^+$ at high doses (>30 mM) is known to cause smooth muscle contractions through the opening of VDCs, thus allowing an influx of extracellular $Ca^{++}$ causing a contractile effect and substances causing inhibition of high $K^+$-induced contraction are considered to be blockers of calcium influx (Bolton, 1979; Godfraind *et al.*, 1986). The presence of calcium antagonist constituent(s) was confirmed when the plant extract caused a dose-dependent rightward shift in the $Ca^{++}$ dose response curves, constructed in a $K^+$-rich and $Ca^{++}$-free medium, similar to that caused by verapamil, a standard calcium channel blocker (Fleckenstein, 1977) (Fig. 3). Calcium antagonists, such as verapamil form an important therapeutic group, which are particularly employed in the treatment of different cardiovascular diseases (Triggle, 1992). The common characteristic of these drugs is their dose-dependent inhibition of the slow entry of calcium and their capacity for reversal of this effect by $Ca^{++}$

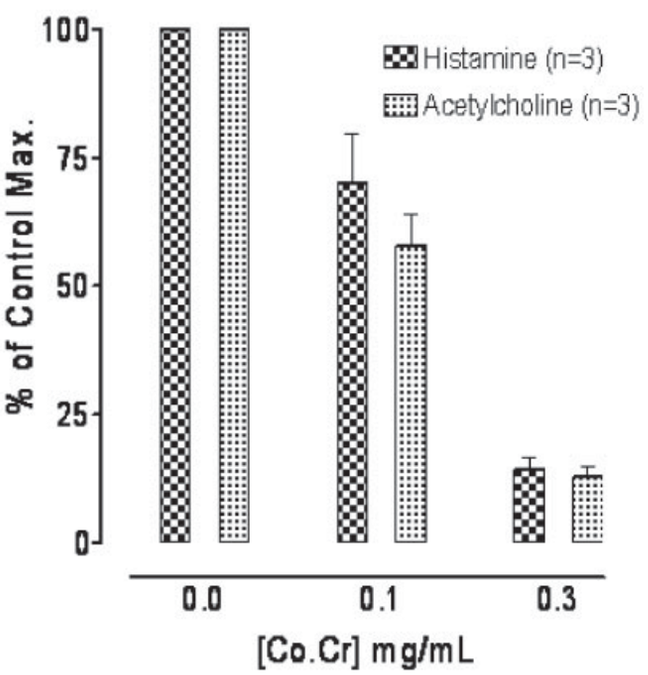


**Figure 2.** Bar diagrams showing the inhibitory effect of the crude extract of *Calendula officinalis* (Co.Cr) on acetylcholine and histamine-induced contractions in isolated guinea-pig ileum preparations.

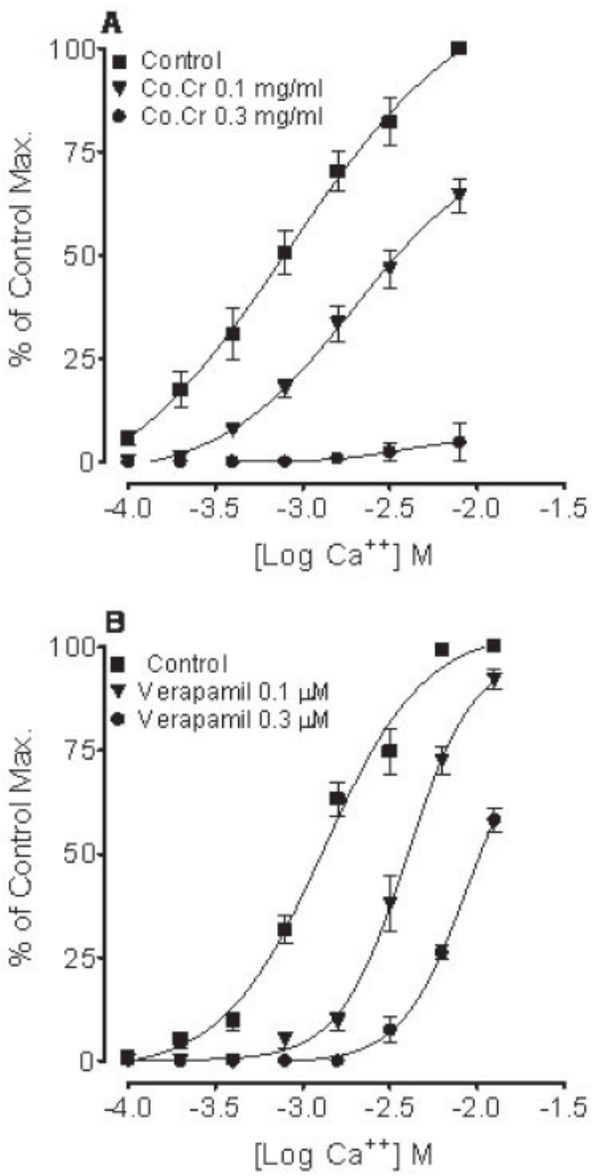


**Figure 3.** Dose-response curves of $Ca^{++}$ in the absence and presence of (A) crude extract of *Calendula officinalis* (Co.Cr) and (B) verapamil in isolated rabbit jejunum preparations.




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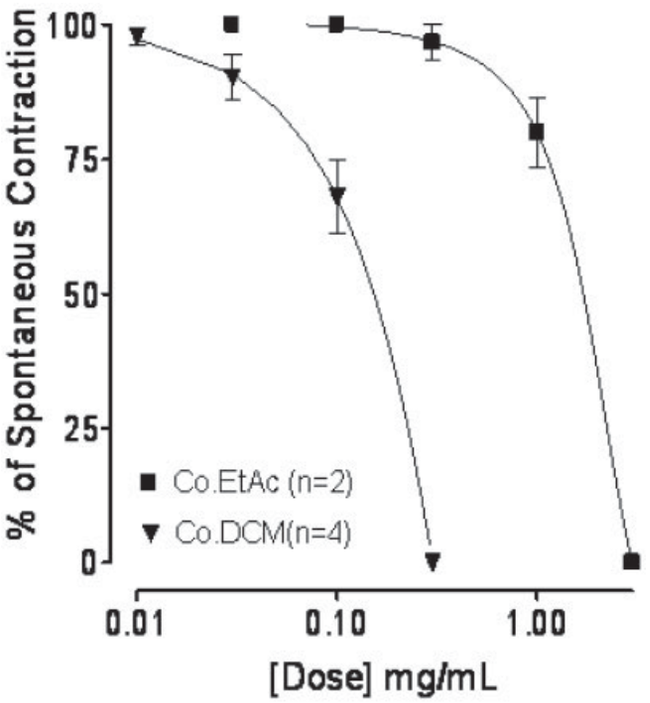


**Figure 4.** Inhibitory effect of organic fractions of the crude extract of *Calendula officinalis* namely dichloromethane (Co.DCM) and ethyl acetate (Co.EtAc) on spontaneous contractions in isolated rabbit jejunum preparations.

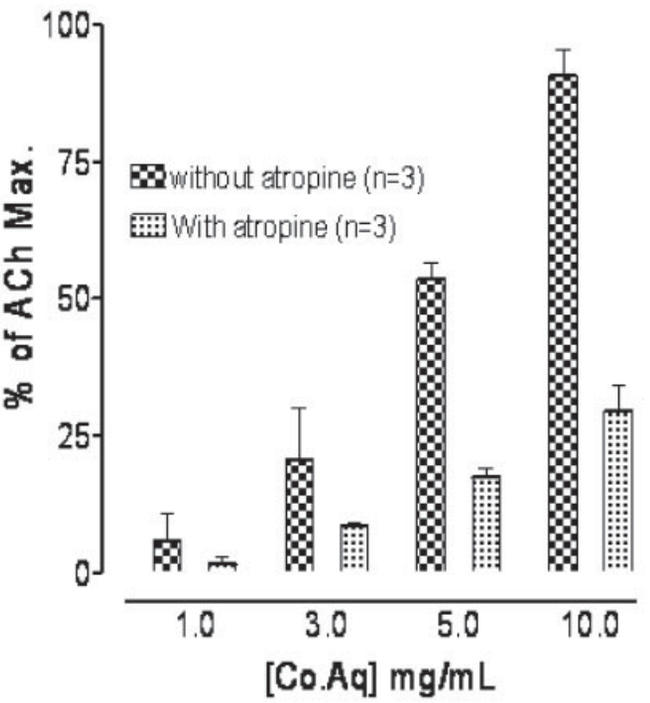


**Figure 5.** Bar diagrams showing the spasmogenic effect of the crude extract of *Calendula officinalis* (Co.Cr) on isolated guinea-pig ileum preparations.

(Fleckenstein, 1977). The observed effect of the plant extract to inhibit the induced contractions, followed by displacing the $Ca^{++}$ curves similar to that by verapamil strongly suggests the presence of a calcium antagonist(s) in the plant extract. The flowers of *Calendula officinalis* have been used traditionally in abdominal colic (spasm) and in irritation of the alimentary canal. The spasmolytic effect observed in this study, mediated through CCB may be a contributory factor for this traditional use, as calcium channel blockers are well known to be antispasmodic (Brunton, 1996).

Activity directed fractionation revealed that the spasmolytic component of the plant was separated in its organic fractions. The dichloromethane fraction (Co.DCM) inhibited spontaneous contractions at a dose range of 0.01–0.3 mg/mL, being ten times more potent than the parent crude extract, while the ethyl acetate fraction (Co.EtAc) exhibited this effect at doses similar to that of the parent crude extract (Fig. 4). The aqueous fraction (Co.Aq) was found to be devoid of any spasmolytic effect (data not shown).

When tested on isolated guinea-pig ileum, a stretched preparation considered suitable for the stimulant effect, Co.Aq exhibited a marked dose dependent spasmogenic effect mediated at a dose range of 1–10 mg/mL, with the maximum effect being 90.42 ± 4.73% (mean ± SEM; $n = 3$) of the acetylcholine maximum. Pretreatment of the tissue with atropine (1 μM), completely blocked the response of acetylcholine while the contractile effect of the crude extract was partially blocked (Fig. 5), indicating that the plant extract mediates its stimulatory action predominately through a mechanism similar to that of acetylcholine, though an additional mechanism cannot be ruled out. Acetylcholine is a neurotransmitter released by the parasympathetic nervous system, and its action in the gut is mediated by stimulation of $M_3$ muscarinic receptor subtype, the effect being blocked by atropine, a muscarinic receptor blocker (Brown and Taylor, 1996). Through this mechanism, acetylcholine plays an important physiological role in regulating the peristaltic movements of the gut.

These results clearly indicate the presence of both spasmolytic and spasmogenic constituents in *Calendula officinalis* flowers, mediating their effect through calcium channel blockade and cholinergic action, respectively, and this study provides a sound mechanistic basis for the use of the plant in gastrointestinal disorders, such as abdominal cramps and constipation.

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