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# Antispasmodic and bronchodilator activities of *Artemisia vulgaris* are mediated through dual blockade of muscarinic receptors and calcium influx

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## ABSTRACT

**Aim of the study:** The present study describes antispasmodic, antidiarrheal, bronchodilatory and tracheo-relaxant activities of *Artemisia vulgaris* to rationalize some of its traditional uses.

**Materials and methods:** Crude extract of *Artemisia vulgaris* (Av.Cr) was studied in the isolated tissue preparations of rabbit jejunum and guinea-pig trachea, as well as in the *in vivo* castor oil-induced diarrhea and bronchodilatory techniques.

**Results:** Av.Cr which tested positive for alkaloids, coumarins, flavonoids, saponins, sterols, tannins and terpenes caused concentration-dependent (0.03–10 mg/mL) relaxation of jejunum spontaneous contractions. Av.Cr inhibited the carbachol (CCh, 1 μM) and K<sup>+</sup> (80 mM)-induced contractions in a pattern, similar to that of dicyclomine. Av.Cr shifted the Ca<sup>2+</sup> concentration–response curves to right, like that caused by verapamil and dicyclomine. Av.Cr produced rightward parallel shift in CCh-curves, followed by non-parallel shift at higher concentration with the suppression of the maximum response, similar to that caused by dicyclomine. It exhibited protective effect against castor oil-induced diarrhea and CCh-mediated bronchoconstriction in rodents. In trachea, Av.Cr relaxed the CCh (1 μM) and K<sup>+</sup> (80 mM)-induced contractions and shifted the CCh-curves to right.

**Conclusion:** These results indicate that *Artemisia vulgaris* exhibits combination of anticholinergic and Ca<sup>2+</sup> antagonist mechanisms, which provides pharmacological basis for its folkloric use in the hyperactive gut and airways disorders, such as abdominal colic, diarrhea and asthma.

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## 1. Introduction

*Artemisia vulgaris* Linn. belongs to family Compositae, commonly known as “Mugwort” and locally as “Afsantin”. It is an aromatic perennial shrub of 2–8 feet height and mostly found in Himalayas, India and Pakistan (Kapoor, 1990). The plant has folklore reputation as analgesic, antihelminthic, antipyretic, antispasmodic, antiseptic, diaphoretic, diuretic, emmenagogue, expectorant, hypoglycemic, larvicide, tonic and is considered useful in the treatment of asthma, cancer, colic, cough, diarrhea, dyspepsia, depression, epilepsy, headache, hemorrhage, hepatitis, inflammation and rheumatism (Baquar, 1989; Kapoor, 1990; Duke et al., 2002). It is known to contain adenine, amyryl, artemisiketone, borneol, cadinenol, coumarin, fernenol, esculin, esculetin, inulin, linalool, muurolool, myrcene, nerol, molybdenum, pinene, quercetin, scopoletin, β-sitosterol, spathulenol, stigmasterol, tau-

remisin, tetracosanol, thujone, vulgarin, vulgarol, vulgarole and umbelliferone (Nadkarni, 1976; Ikhsanova et al., 1986; Duke, 1992). *Artemisia vulgaris* is reported to possess antiallergic (Rodrigues-Alves et al., 2008), antibacterial (Chen et al., 1989), antihypertensive (Tigno et al., 2000), antinociceptive (Pires et al., 2009), antiplasmodial (Tran et al., 2003) and hepatoprotective (Gilani et al., 2005a) properties. It improves blood flow in the ischemic rat mesentery (Gumila and Tigno, 2000). We have observed, that plants with medicinal use in the overactive gut and respiratory disorders, usually exhibit spasmolytic effect through the combination of mechanisms (Gilani et al., 2005b,c, 2008a,b). In this investigation, we evaluated that the antispasmodic effects of *Artemisia vulgaris* are mediated through dual inhibition of muscarinic receptors and Ca<sup>2+</sup> influx, which justify its traditional use in the gastrointestinal and airways hyperactivity.

## 2. Materials and methods

### 2.1. Plant material and extraction

*Artemisia vulgaris* aerial parts (stem + leaves) were bought from local herbalist in Karachi and authenticated by Mr. Afzal Rizvi,

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a taxonomist at the Hamdard University and voucher specimen (AV-PL-03-02-44) was submitted to the herbarium at Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. The plant material was cleaned off adulterants, powdered and soaked into 70% aqueous-methanol at room temperature with occasional shaking. The soaked material was filtered through a muslin cloth and then through Whatman qualitative grade 1 filter paper (Williamson et al., 1998). This procedure was repeated thrice and the combined filtrate was evaporated with a rotary evaporator under reduced pressure to obtain a thick semi-solid paste, i.e., *Artemisia vulgaris* crude extract (Av.Cr), yielding approximately 5.5%. Av.Cr was solubilized both in saline and distilled water.

## 2.2. Chemicals

Acetylcholine chloride (ACh), carbachol (CCh), dicyclomine, loperamide and verapamil were purchased from Sigma Chemicals Co., St. Louis, MO, USA. Salbutamol, pentothal sodium (thiopental) and castor oil were respectively obtained from Glaxo-Wellcome, Abbot Laboratories and KCL Pharma, Karachi, Pakistan. Chemicals used for making physiological salt solutions were potassium chloride (Sigma Chemicals Co., St. Louis, MO, USA), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride from BDH Laboratory Supplies, Poole, England. The chemicals used in phytochemical analysis include acetic anhydride, aluminum chloride, ammonium hydroxide, dragendorff's reagent, ferric chloride (Sigma Chemical Co., St. Louis, MO, USA), benzene, chloroform, hydrochloric acid and petroleum ether (BDH Laboratory Supplies, Poole, England). All the chemicals used were of analytical grade available. All chemicals used were of analytical grade and solubilized in distilled H<sub>2</sub>O/saline. Vehicles used had no effect in control experiments.

## 2.3. Animals

Animals used in this study include, adult rabbits (1–1.2 kg), guinea-pigs (500–550 g), Sprague-Dawley rats (200–250 g) and Balb-C mice (20–25 g) of either sex and local breed were housed at Animal House of the Aga Khan University, maintained at 23–25 °C. Animals were given tap water *ad libitum* and a standard diet. Rabbits and mice had free access to water, but food was withdrawn 24 h prior to experiment. Rabbits and guinea-pigs were sacrificed by blow on the back of the head and cervical dislocation respectively. Experiments performed complied with rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by Ethical Review Committee of the Aga Khan University.

## 2.4. Phytochemical screening

Preliminary phytochemical analysis of Av.Cr was done for the presence of alkaloids, saponins, coumarins, sterols, terpenes, flavonoids, tannins and anthraquinones according to the reported procedures (Edeoga et al., 2005) with some modifications. Alkaloids were detected by dragendorff's reagent. Presence of saponins was detected based on the appearance of froth upon vigorous shaking of diluted samples. The observation of yellow fluorescence under ultraviolet light on examination of filter paper previously exposed to the vapors from boiling plant material indicated the presence of coumarins. For the detection of sterols and terpenes, the plant material was treated with petroleum ether and subsequently extracted with chloroform. The appearance of green to pink (for sterols) and pink to purple colors (for terpenes) was noted after the treatment of chloroform layer with acetic anhydride and concen-

trated hydrochloric acid in succession. Plant material was noted as positive for flavonoids, when it gave yellow color with aluminum chloride and for tannins when green or black color was produced on treatment with aqueous ferric chloride. For anthraquinones, extract was dissolved in 1% hydrochloric acid, then in benzene and appearance of pink, violet or red color with ammonium hydroxide indicated the presence of anthraquinones.

## 2.5. Isolated tissue preparations

### 2.5.1. Rabbit jejunum

Rabbit jejunum was dissected out and kept in Tyrode's solution (Ghayur and Gilani, 2005). Segments around 2 cm long were suspended individually in a 10 mL tissue bath containing Tyrode's solution, at 37 °C and aerated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (carbogen). The composition of Tyrode's solution in mM was KCl 2.68, NaCl 136.9, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 11.90, NaH<sub>2</sub>PO<sub>4</sub> 0.42, CaCl<sub>2</sub> 1.8 and glucose 5.55. Intestinal responses were recorded isotonically using Bioscience transducers and Harvard oscillograph. Tissues were allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with sub-maximal concentration of ACh (0.3 μM) at 3 min interval until constant responses were recorded. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing of relaxant (spasmolytic) activity of test extract and the control drug, dicyclomine, directly without the use of an agonist. For the elucidation of Ca<sup>2+</sup> channel blocking (CCB) effect, high K<sup>+</sup> (80 mM) was used to depolarize the preparations as described by Farre et al. (1991). K<sup>+</sup> was added to the tissue bath, which produced sustained contraction. High K<sup>+</sup> (>30 mM) is known to cause smooth muscle contractions through the opening of voltage-dependent L-type Ca<sup>2+</sup> channels, thus allowing influx of extracellular Ca<sup>2+</sup> causing a contractile effect (Bolton, 1979) and a substance causing inhibition of high K<sup>+</sup>-induced contraction is considered as an inhibitor of Ca<sup>2+</sup> influx (Godfraind et al., 1986). To elucidate the presence of any additional spasmolytic effect, the plant extract and dicyclomine were tested on the CCh-induced contractions. CCh is a cholinergic agonist, which causes smooth muscle contraction through the activation of muscarinic receptors (Jenkinson, 2002). Once plateau of the induced contractions was achieved (usually within 5–10 min), the test material was added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van-Rossum, 1963). To confirm the Ca<sup>2+</sup> antagonist action of test substance, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca<sup>2+</sup>-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca<sup>2+</sup> from the tissues. This solution was replaced with K<sup>+</sup>-rich and Ca<sup>2+</sup>-free Tyrode's solution with composition (mM): KCl 50, NaCl 91.04, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 11.90, NaH<sub>2</sub>PO<sub>4</sub> 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min control concentration-response curves (CRCs) of Ca<sup>2+</sup> (CaCl<sub>2</sub>) were constructed. When control Ca<sup>2+</sup>-CRCs were found super-imposable (usually after two cycles), the tissue was pretreated with the crude extract for 60 min to test for a possible CCB effect. The CRCs of Ca<sup>2+</sup> were reconstructed in the presence of different concentrations of the test material. For an investigation of competitive and non-competitive antagonism, the CCh-CRCs were constructed in the absence and presence of different concentrations of the plant extract and dicyclomine (Mitchelson and Ziegler, 1984; Jenkinson, 2002).

### 2.5.2. Guinea-pig trachea

Trachea was dissected and kept in Krebs' 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 ventral side, forming a tracheal chain with smooth muscle in the center and cartilaginous portions on the edges (Gilani et

al., 2005c). Each preparation was mounted in 20 mL tissue bath containing Krebs's solution at 37°C, aerated with carbogen. The composition of Krebs's solution was (mM): NaCl 118.2, NaHCO<sub>3</sub> 25.0, CaCl<sub>2</sub> 2.5, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.3, MgSO<sub>4</sub> 1.2 and glucose 11.7 (pH 7.4). A tension of 1 g was applied to the tracheal strips continuously throughout the experiment. The tissue was equilibrated for 1 h before the addition of any drug. In some preparations, CCh (1 μM) or K<sup>+</sup> (80 mM) was used to stabilize the respective preparations until constant responses of each agonist were achieved (usually 3–4 concentrations). When sustained contractions were obtained, tracheo-relaxant effect of test extract and dicyclomine was assessed by adding in a cumulative fashion. Cumulative curves to CCh were constructed using increasing concentration of an agonist. When a 3-fold increase in concentration produced no further increment in response, the tissue was washed to re-establish the base-line tension (usually 30–35 min). The CCh-curves were then repeated in presence of increasing concentrations of plant extract and dicyclomine. Isometric responses were recorded on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

## 2.6. In vivo experiments

### 2.6.1. Castor oil-induced diarrhea

The mice were fasted for 24 h before the experiment. Animals were housed in individual cages and divided in four groups, each containing five mice. The first group received saline (10 mL/kg, p.o.) and served as a negative control. Extract doses were selected on trial basis and then its two increasing doses were given orally. A group of mice were treated with loperamide (10 mg/kg, p.o.), as positive control. One hour after treatment, each animal received 10 mL/kg of castor oil orally through a feeding needle. Afterward, the cages were inspected for the presence of diarrhea droppings; their absence was noted as a positive result, indicating protection from diarrhea (Das et al., 1999).

### 2.6.2. Bronchodilatory activity

Sodium thiopental (Pentothal®, 80–100 mg/kg, i.p.) was administered to anaesthetize the rats, then incubated with tracheal tube and ventilated with volume ventilator (miniature ideal pump, Bioscience, UK) adjusted at rate of 70–80 strokes/min to deliver 7–10 mL/kg of room air (Dar and Channa, 1997). A polyethylene catheter was inserted into jugular vein for drug administration. Changes in airways resistance (mmHg) were measured by pressure transducer (MLT-1199) connected to side arm of tracheal cannula and recorded by PowerLab 4/25 with running chart software via Quad bridge amplifier (ADInstruments, Bella Vista, NSW, Australia). Bronchoconstriction was induced with CCh (1 μmol/kg), which was reversed within 7–10 min. The test drug was given to the animal 5–8 min prior to administration of CCh. The responses were expressed as percent reduction of CCh-induced bronchospasm (Gilani et al., 2008a).

### 2.6.3. Acute toxicity test

Animals were divided in groups of five mice each. The test was performed using increasing doses of plant extract, given orally in 10 mL/kg volume to different groups, serving as test groups (Sanmugapriya and Venkataraman, 2006). Another group of mice was administered saline (10 mL/kg, p.o.) as negative control. The mice were allowed food *ad libitum* and kept under regular observation for 6 h while the lethality was recorded after 24 h.

## 2.7. Statistical analysis

Data expressed are mean ± standard error of mean (SEM,  $n$  = number of experiment) and the median effective concentrations

(EC<sub>50</sub>) with 95% confidence intervals (CI). The statistical parameter applied in case of antidiarrheal study was Chi-square-test. Difference of  $P < 0.05$  was considered statistically significant. CRCs were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

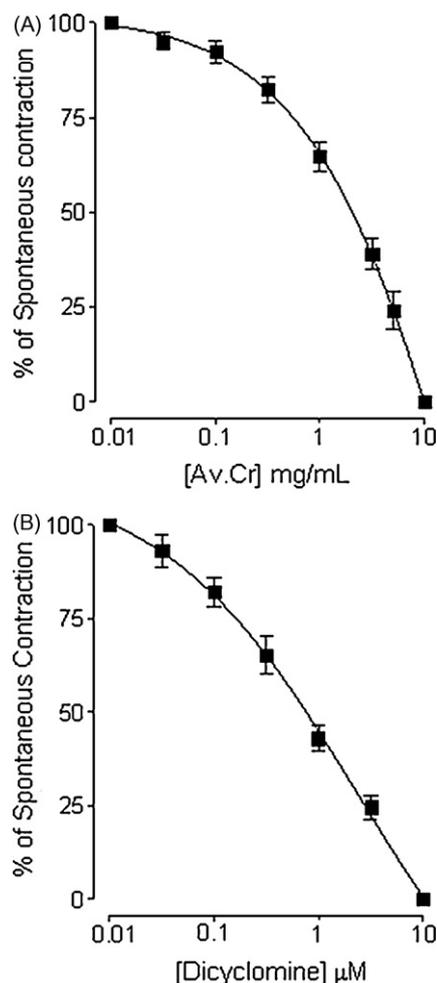
## 3. Results

### 3.1. Phytochemical analysis

Av.Cr was found to contain alkaloids, coumarins, flavonoids, saponins, sterols, tannins and terpenes, while tested negative for the presence of anthraquinones.

### 3.2. Effect on jejunum

Av.Cr caused relaxation of spontaneously contracting rabbit jejunum with EC<sub>50</sub> value of 2.3 mg/mL (1.7–3.1, 95% CI,  $n = 4$ ) as shown in Fig. 1A. Dicyclomine, used as a positive control, inhibited the jejunum spontaneous contractions (Fig. 1B) with EC<sub>50</sub> value of 0.80 μM (0.6–1.1,  $n = 3$ ). When tested against CCh (1 μM) and K<sup>+</sup> (80 mM)-induced contractions, Av.Cr relaxed the CCh (1 μM)-induced contractions at lower concentrations (Fig. 2A) with EC<sub>50</sub> value of 0.31 mg/mL (0.22–0.60,  $n = 5$ ), as compared to its effect

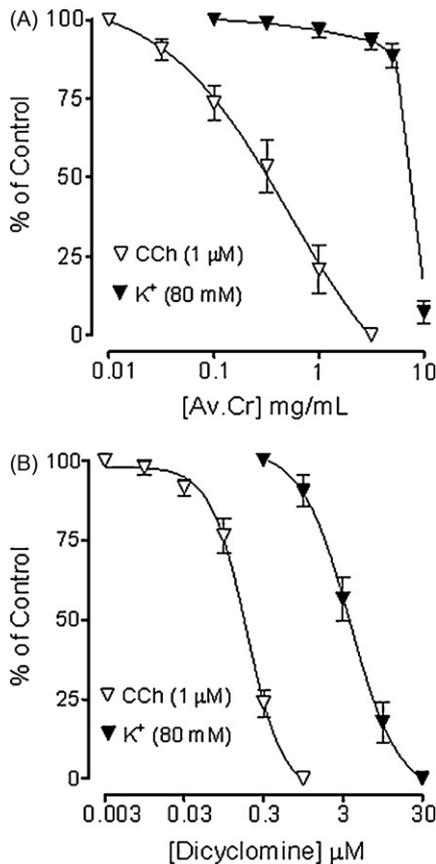


**Fig. 1.** Concentration–response curves representing spasmolytic effect of: (A) crude extract of *Artemisia vulgaris* (Av.Cr) and (B) dicyclomine on spontaneous contraction of isolated rabbit jejunum preparations. Test material was added into the tissue bath in a cumulative fashion and the concentrations shown are the final bath concentrations. Values shown are mean ± SEM,  $n = 3–5$ .

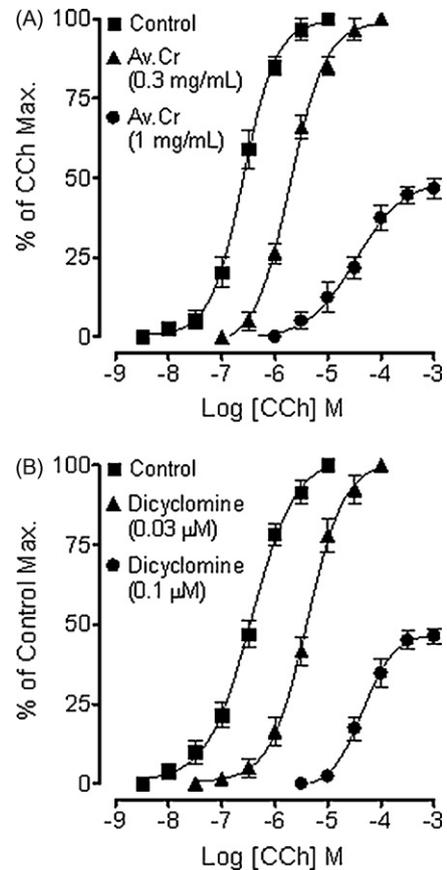
against  $K^+$  (80 mM) with  $EC_{50}$  value of 6.9 mg/mL (6.4–7.6,  $n=8$ ). Dicyclomine, also exhibited similar pattern of inhibitory effect against CCh (1  $\mu$ M) and  $K^+$  (80 mM)-induced contractions (Fig. 2B), with respective  $EC_{50}$  values of 0.24 (0.13–0.30,  $n=4$ ) and 3.4  $\mu$ M (2.2–5.2,  $n=4$ ). Pretreatment of the tissue with Av.Cr at 0.3 mg/mL caused rightward parallel shift in CCh-curves without the suppression of maximum contractile response, followed by a non-parallel shift with the suppression of maximum response at 1.0 mg/mL (Fig. 3A). Dicyclomine, at the concentration range of 0.03–0.1  $\mu$ M also shifted the CCh-curves in a similar pattern. It caused parallel displacement in CCh-curves at 0.03  $\mu$ M, followed by non-parallel shift with the suppression of the maximum contractile effect at 0.1  $\mu$ M, as shown in Fig. 3B. When tested for interaction with  $Ca^{2+}$  channels, Av.Cr caused rightward shift in  $Ca^{2+}$ -CRCs (Fig. 4A), but at relatively high concentrations (5–10 mg/mL), when compared against CCh, a pattern similar to that caused by dicyclomine (Fig. 4B). Verapamil, also shifted the  $Ca^{2+}$  CRCs to right, as expected (Fig. 4C).

### 3.3. Effect on castor oil-induced diarrhea

Av.Cr exhibited dose-dependent (300–1000 mg/kg) inhibitory effect against the castor oil-induced diarrhea in mice. The negative control treatment (saline) did not protect the animals from diarrhea. Pretreatment with Av.Cr, produced 20% protection from diarrhea at 300 mg/kg and 60% protection at 1000 mg/kg ( $P < 0.05$  vs. saline group). Loperamide at 10 mg/kg, showed complete protection (100%) from diarrhea in the positive control group (Table 1).



**Fig. 2.** Concentration–response curves showing inhibitory effect of: (A) crude extract of *Artemisia vulgaris* (Av.Cr) and (B) dicyclomine against carbachol (CCh) and  $K^+$ -induced contractions in isolated rabbit jejunum preparations. Values shown are mean  $\pm$  SEM,  $n=4$ –8.



**Fig. 3.** Concentration–response curves of carbachol (CCh) in the absence and presence of the increasing concentrations of: (A) crude extract of *Artemisia vulgaris* (Av.Cr) and (B) dicyclomine in isolated rabbit jejunum preparations. Values shown are mean  $\pm$  SEM,  $n=3$ –4.

### 3.4. Effect on carbachol-induced bronchoconstriction

Av.Cr at doses of 10, 30 and 100 mg/kg respectively caused  $18.5 \pm 3.3$ ,  $51 \pm 5.3$  and  $77.25 \pm 5.6\%$  ( $n=4$ ) inhibition of CCh (1  $\mu$ mol/kg)-induced increase in the inspiratory pressure of rats, under anaesthesia. Salbutamol (0.1 mg/kg), a standard bronchodilator, produced  $80 \pm 3.2\%$  ( $n=4$ ) suppression of the CCh (1  $\mu$ mol/kg)-induced bronchoconstriction (Fig. 5).

### 3.5. Effect on trachea

Like in gut preparation, Av.Cr caused inhibition of CCh (1  $\mu$ M)-induced contractions in tracheal preparations, at low concentrations with  $EC_{50}$  value of 0.20 mg/mL (0.12–0.30,  $n=4$ ) compared to that against  $K^+$  (80 mM)-induced contractions, with  $EC_{50}$  value of 3.4 mg/mL (2.1–5.3,  $n=4$ ) as shown in Fig. 6A. Dicyclomine, also showed similar pattern of inhibitory effect (Fig. 6B).

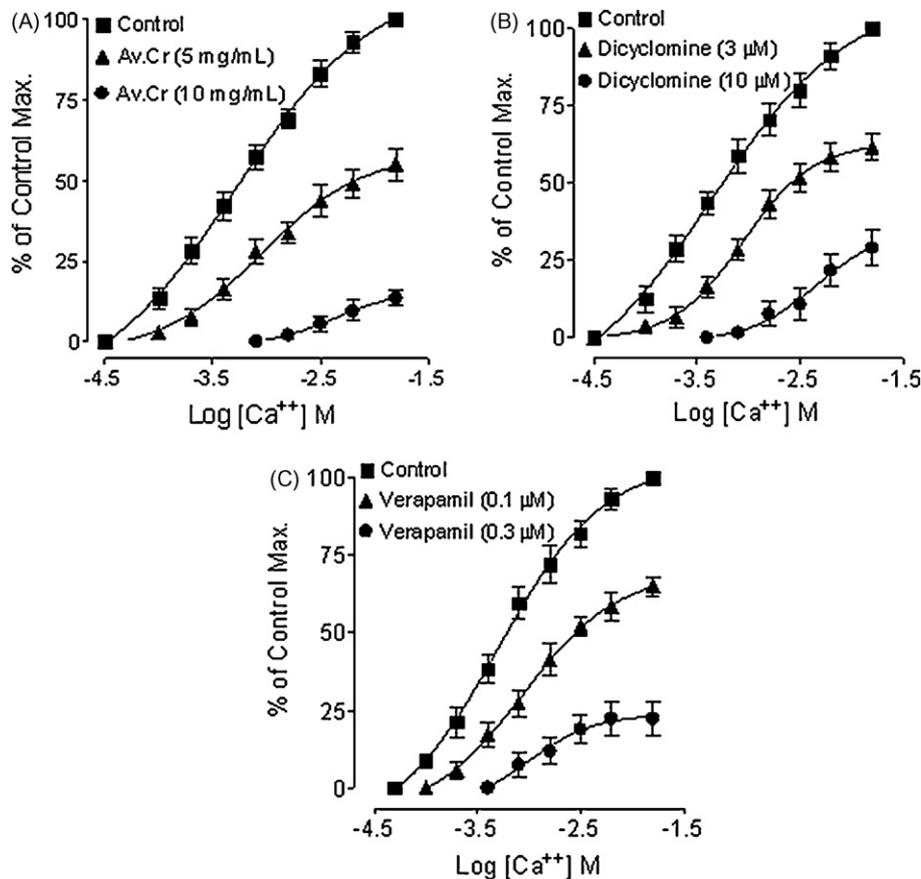
**Table 1**

Effect of the crude extract of *Artemisia vulgaris* (Av.Cr) on castor oil (C.oil, 10 mL/kg)-induced diarrhea in mice.

Treatment (p.o.)	No. of mice/five with diarrhea	% Protection
Saline (10 mL/kg) + (C.oil)	5	0
Av.Cr (300 mg/kg) + (C.oil)	4	20
Av.Cr (1000 mg/kg) + (C.oil)	2*	60
Loperamide (10 mg/kg) + (C.oil)	0**	100

\*  $P < 0.05$  compared to saline group, Chi-square test.

\*\*  $P < 0.01$  compared to saline group, Chi-square test.



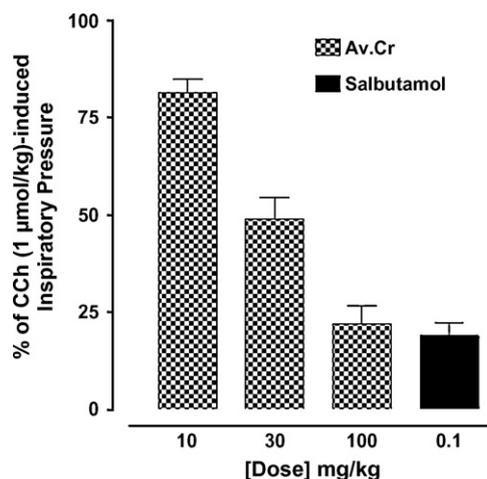
**Fig. 4.** Concentration–response curves of  $\text{Ca}^{2+}$  in absence and presence of the increasing concentrations of: (A) crude extract of *Artemisia vulgaris* (Av.Cr), (B) dicyclomine and (C) verapamil in isolated rabbit jejunum preparations. Values shown are mean  $\pm$  SEM,  $n=3-4$ .

with respective  $\text{EC}_{50}$  value of  $0.34 \mu\text{M}$  ( $0.2-0.7$ ,  $n=3$ ) against CCh ( $1 \mu\text{M}$ ), compared to that against  $\text{K}^+$  ( $80 \text{mM}$ )-induced contractions with  $\text{EC}_{50}$  value of  $6.1 \mu\text{M}$  ( $4.5-8.5$ ,  $n=3$ ). Similar to that in jejunum, Av.Cr produced rightward parallel displacement of CCh-curves without the suppression of the maximum contractile response at  $0.3 \text{mg/mL}$ , followed by non-parallel shift with suppression of the maximum effect at  $1.0 \text{mg/mL}$  (Fig. 7A). Dicyclomine ( $0.03-0.1 \mu\text{M}$ ) also shifted the CCh-curves in a similar pattern. It caused parallel displacement in CCh-curves at

$0.03 \mu\text{M}$ , followed by non-parallel shift with suppression of the maximum contractile effect at the concentration of  $0.1 \mu\text{M}$  (Fig. 7B).

### 3.6. Acute toxicity

The two different groups of mice were given Av.Cr in the graded doses of 1 and 3 g/kg, respectively. After 24 h, animals were observed for mortality. Av.Cr did not cause any mortality up to 3 g/kg.



**Fig. 5.** Inhibitory effect of the crude extract of *Artemisia vulgaris* (Av.Cr) and salbutamol on the carbachol (CCh)-mediated bronchoconstriction in anaesthetized rats. Values shown are mean  $\pm$  SEM,  $n=4$ .

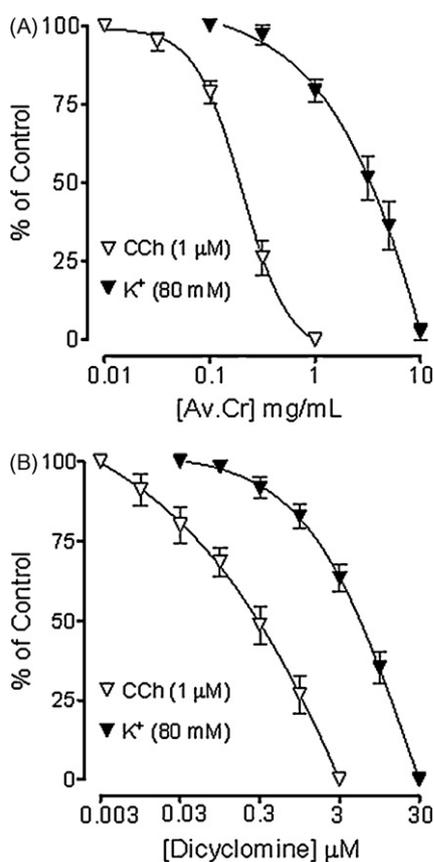
## 4. Discussion

In view of the medicinal use of *Artemisia vulgaris*, as an antispasmodic agent, its extract was tested for the possible inhibitory effect in spontaneously contracting rabbit jejunum preparations, where it relaxed the spontaneous contractions, thus showing an antispasmodic action. To assess the possible mechanisms of spasmolytic effect, the extract was studied against CCh and high  $\text{K}^+$ -induced contractions, where it reversed the induced contractions, being more potent against CCh, than  $\text{K}^+$ . Dicyclomine, a standard inhibitor of muscarinic receptors and  $\text{Ca}^{2+}$  influx (Downie et al., 1977) showed similar pattern of relaxant effect. It is thereby suggested that the inhibitory effect of the plant extract is mediated possibly through blockade of both muscarinic receptors and  $\text{Ca}^{2+}$  influx. The presence of dual inhibitory effects was further confirmed through constructing CCh and  $\text{Ca}^{2+}$  CRCs in the presence of different concentrations of test drugs. The plant extract caused a parallel displacement of CCh-curves without suppression of the maximum effect at the lower

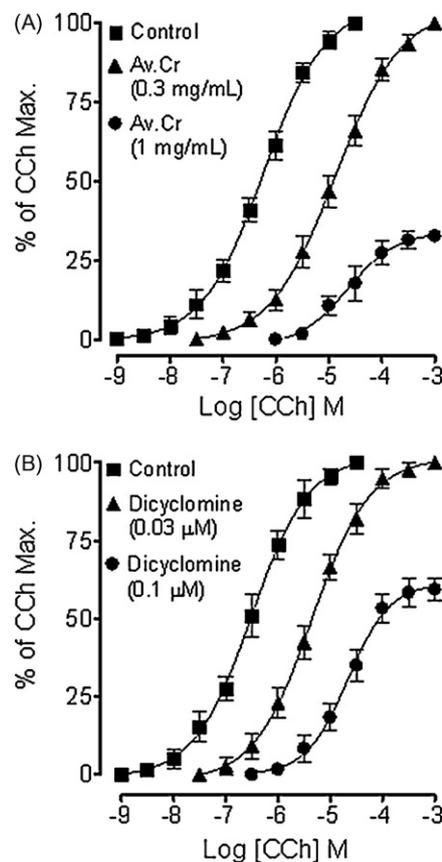
concentration, a characteristic of a competitive or specific antagonist (Arunlakshana and Schild, 1959), followed by non-parallel shift with suppression of the maximum effect at next higher concentration, pointing towards an additional non-competitive inhibition (VanDen Brink, 1973), known with  $\text{Ca}^{2+}$  antagonists (Irie et al., 2000; Gilani et al., 2008b), similar to that caused by dicyclomine. Av.Cr shifted the  $\text{Ca}^{2+}$  curves to the right, accompanied by the suppression of maximum response, like that caused by dicyclomine and verapamil, a standard  $\text{Ca}^{2+}$  antagonist (Fleckenstein, 1977).

Based on medicinal use in diarrhea, the *Artemisia vulgaris* extract was tested for possible protective effect against castor oil-induced diarrhea in mice. Diarrhea with castor oil results from the action of ricinoleic acid formed during hydrolysis (Iwao and Terada, 1962), which produces changes in the transport of electrolytes and water resulting in generation of giant contractions of the transverse and distal colon (Izzo et al., 1994; Croci et al., 1997). The observed antidiarrheal effect of the plant extract is possibly due to the dual blockade of muscarinic receptors and  $\text{Ca}^{2+}$  channels, as both these effects are known to mediate antidiarrheal action (Reynolds et al., 1984; Rang et al., 1999).

Since *Artemisia vulgaris* has medicinal use in asthma, the plant extract was evaluated for its possible bronchodilatory effect in anesthetized rats. The extract dose-dependently inhibited the CCh-mediated bronchoconstriction. Av.Cr was further studied in guinea-pig trachea to investigate the possible mode of bronchodilatory action. Similar to gut, the extract caused relaxation of CCh-induced contractions and displaced the CCh-curves to right in a parallel fashion without the suppression of the maximum response at low concentration, followed by non-parallel shift with



**Fig. 6.** Concentration–response curves showing inhibitory effect of: (A) crude extract of *Artemisia vulgaris* (Av.Cr) and (B) dicyclomine against carbachol (CCh) and  $\text{K}^+$ -induced contractions in isolated guinea-pig tracheal preparations. Values shown are mean  $\pm$  SEM,  $n=3-4$ .



**Fig. 7.** Concentration–response curves of carbachol (CCh) in the absence and presence of the increasing concentrations of: (A) crude extract of *Artemisia vulgaris* (Av.Cr) and (B) dicyclomine in isolated guinea-pig tracheal preparations. Values shown are mean  $\pm$  SEM,  $n=3-4$ .

suppression of the maximum effect at next higher concentration. Av.Cr also relaxed the  $\text{K}^+$ -induced contractions at higher concentration, suggesting the co-existence of anticholinergic and  $\text{Ca}^{2+}$  antagonist effects, similar to dicyclomine. The plant extract was found slightly more effective in trachea than gut. This could possibly be due to difference in the physiological modulators among various tissues and/or the extent of their regulatory influences (Gayton and Hall, 1996) or may as a result of better synergistic interaction of the different spasmolytic mechanisms in the airways compared to gut, though species differences cannot be ruled out (Ghayur et al., 2005). The observed anticholinergic and  $\text{Ca}^{2+}$  antagonist effects of *Artemisia vulgaris* might be due to the presence of alkaloids and flavonoids, evident in phytochemical screening, as the compounds of these classes have been reported to possess antimuscarinic (Broadley, 1996) and  $\text{Ca}^{2+}$  channel blocking (Di Carlo et al., 1993; Revuelta et al., 1997) activities, respectively. However, contribution of other constituents cannot be ignored. In acute toxicity testing, the extract was found safe up to maximum dose (3 g/kg) tested, which is in accordance with wide therapeutic use of the plant.

In conclusion, *Artemisia vulgaris* possesses smooth muscle relaxing effect, mediated possibly through the combination of anticholinergic and  $\text{Ca}^{2+}$  antagonist mechanisms, which provides sound mechanistic background for its application in traditional medical system for the hyperactive gut and airways disorders, such as abdominal colic, diarrhea and asthma. Moreover, the *in vivo* antidiarrheal and bronchodilatory activities prove effectiveness of the plant in such conditions and is a step forward towards the exploration of ethnomedicine.

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