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The Mechanism Underlying the Spasmolytic and Bronchodilatory Activities of the Flavonoid-rich Red Onion "*Allium cepa* L." Peel Extract

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Abstract: The flavonoid-rich hydro-acetone extract of red onion (*Allium cepa* peel, ACPE) was studied for its spasmolytic and bronchodilator activities using *ex-vitro* and *in-vivo assays*. In isolated rabbit jejunum preparations, ACPE produced a concentration-dependent (0.03-1 mg L^{-1}) relaxation of spontaneous and high K^+(80 mM)-induced contractions equipotently, nearly similar to that caused by papaverine, whereas, verapamil was relatively more potent against K^+-induced contractions. ACPE also caused the right ward shift in the Ca^{++} concentration-response curves (CRCs), similar to that of verapamil and papaverine. In normotensive anesthetized rats, ACPE dose-dependently (3-30 mg kg^{-1}) suppressed the carbachol (CCh, 1 mg kg^{-1}) induced bronchoconstriction similar to the effect observed with aminophylline. In guinea-pig tracheal preparation, ACPEexhibited concentration-dependent relaxation of both CCh (1 μM) and high K^+-induced contraction at similar concentrations (0.3-3 mg mL^{-1}) and also shifted the isoprenaline-induced inhibitory CRCs to the left, similar to that caused by papaverine. Theresults of this study indicated that the spasmolytic and bronchodilatory activities of ACPE are mediated through the dual inhibition of Ca^{++} channels and phosphodiesterase enzyme like-mechanisms, which might add an evidence-based medicinal value to the red onion peel in the treatment of gastrointestinal and respiratory disorders, e.g. diarrhea and asthma, respectively.

Key words: *Allium cepa* peel, *agrowaste,* spasmolytic, bronchodilator, Ca^{++} antagonist, phosphodiesterase inhibitor

INTRODUCTION

Allium cepa Linn (Fam. Liliaceae) is commonly known as onion. It is most widely used functional food worldwide. Onion bulb has been shown beneficial medicinal effects on various aspects including as antispasmodic, anti-asthmatic (Nadkarni, 1976) vasodilator (Naseri *et al*., 2008a), antihypertensive (Sakai *et al*., 2003; Naseri *et al*., 2008b), antidyslipidemic (Bang *et al*., 2009), antiplatelet (Makheja *et al*., 1979) antidiabetic (Taj Eldin *et al*., 2010), antioxidant (Campos *et al*., 2003) and antitumor (Challier *et al*., 1998). However, the non-edible part (an agrowaste), has not extensively been subjected to phytochemical and bioactivity evaluation compared to with the edible inner bulb.

The major flavonoids found in onion peels that have been considered usually as waste, contain large amounts of quercetin, quercetin glycoside and their oxidative product. HPLC-MS/MS analysis also showed the presence of ferulic, gallic, protocatechuic acids and kaempferol (Singh *et al*., 2009).

The flavonoids-rich extracts of onion peels were reported to exhibit anti-platelet (Furusawa *et al*., 2003), cell growth inhibitory (Furusawa *et al*., 2006), antioxidant enhancing (Park *et al*., 2007) and antimutagenic (Singh *et al*., 2009) activities. Moreover, a modulatory effect on the obesity-induced inflammation (Kim *et al*., 2012) and an insulin-sensitizing effect on streptozotocin-induced diabetes (Jung *et al*., 2009) in rats were also recorded for the quercetin-rich extract of onion peels. Nevertheless, there is no study to the best of knowledge for its effectiveness in gastrointestinal disorders except a preliminary report of an antispasmodic effect for the hydro-alcoholic extract of onion peels on rat ileum (Naseri *et al*., 2008a). The current study was designed to study the possible mechanism(s) underlying the antispasmodic and bronchodilatory activities of the crude

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extract of *Allium cepa* peels to rationalize the medicinal value of this agro-waste in treatment of diarrhea and asthma.

MATERIAL AND METHODS

Plant material: Red onion (*Allium cepa* L.) Ha'il cultivar, was purchased from local market at Riyadh, Saudi Arabia. A voucher specimen (ATA32-1) was deposited in the Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudia Arabia. The outer dry reddish-brown peels were separated from the bulbs, washed, air-dried and then coarsely powdered.

Extraction and isolation of quercetin: The peels powder (1 kg) was exhaustively extracted by maceration method using 70% aqueous acetone as a solvent. The organic extract was concentrated using a rotary evaporator at 45°C to yield a dark brown gummy extract (ACPE, 156.2 g, yield 15.62% w/w). The extract was suspended in water and partitioned with light petroleum ether, $CHCl_3$, EtOAc and n-BuOH saturated with water, successively to give the correspondent petroleum ether (1.2 g, 0.12%), $CHCl_3$ (3.0 g, 0.3%), EtOAc (80 g, 8.0%) and n-BuOH (41 g, 4.1%) solvent-free fractions. A part of EtOAc fraction (40 g) was subjected to two subsequent chromatographic separation using Si gel column and MeOH-$CHCl_3$ as a mobile solvent (0:100 to 80:20, gradient) to afford a fraction of pure qurecetin (1.4 g).

Quantitative determination of quercetin in ACPE: High performance thin layer chromatography (HPTLC) and TLC densitometry (Fischedick *et al*., 2009) were used for identification as well as quantification of quercetin in ACPE following. A standard solution was prepared by dissolving the isolated pure quercetin in methanol to obtain concentration of 1.0 mg mL^{-1}. A methanolic Solution of ACPE (0.1 g of ACPE was dissolved in 100 mL of MeOH by heating in water bath at 50°C for 5 min. This solution was used for HPTLC analysis and spectrophotometric determination of total quercetin in ACPE sample.

TLC densitometry: Chloroform- MeOH- AcOH in volume ratio 8.5:1.5:0.3 was used as mobile phase. Merck HPTLC plates (Silica Gel 60 F254, 10×10 cm) were used as stationary phase. Standards (1-8 µg) and samples (20 µg) were applied as 4 mm bands (in spray mode) using CAMAG automatic TLC sampler (ATS-4). After development in the CAMAG automatic developing chamber (ADC-2), the HPTLC plates were visualized and recorded at 254 and 366 nm. Spots identification was based on Rf value in comparison with the standard quercetin, which was confirmed by a UV scan from 200-400 nm (Fig. 1). Identification and quantification were performed by TLC densitometry using CAMAG TLC UV Scanner-3 and WinCATS software version1.3.4. The result of HPTLC analysis (via peak areas at Rf 0.38) corresponds to the total content of quercetin in ACPE was found to be 3.976% w/w.

Chemicals: The following reference chemicals were obtained from the source specified carbachol (CCh), isoprenaline, verapamil, papaverine, acetylcholine chloride (ACh) (Sigma Chemical Company, ST Louis, MO, USA), aminophylline and pentothal sodium (thiopental) (GlaxoSmithKline, Abbott Laboratories). The following chemicals were used to make the physiological salts solution: potassium chloride (Sigma Chemicals Co.), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride (BDH Laboratory Supplies, Poole, England). All chemicals used were of the analytical grade available and dissolved in distilled water.

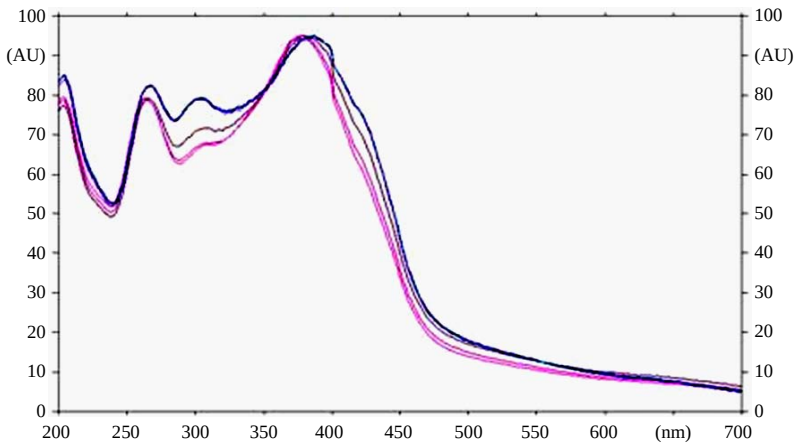

Fig. 1: Identification of quercetin-overlapping spectra of standards and ACPE samples

Animals: Rabbits (1000-1300 g), guinea-pigs (500-600 g) and Sprague-Dawley (SD) rats (180-200 g) of either sex were obtained from the animal house of the Aga Khan University, Karachi. The animals were housed in constant room temperature 23-25°C and given free access to food and water. Rabbits were starved for 24 h prior to experiment, sacrificed by a blow to the back of the head and guinea-pigs by cervical dislocation. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996).

Rabbit jejunum: The spasmolytic activity and possible mode of action of the plant materials were studied by using isolated rabbit jejunum preparations as described previously (Taqvi *et al*., 2006; Jabeen *et al*., 2007). Each segment (2-3 cm length) was suspended in a 10 ml tissue bath containing Tyrode's solution, maintained at 37°C and aerated with a carbogen gas (95% O_2 and 5% CO_2). The composition of the Tyrode's solution in mM 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. A tension of 1 g was applied to each of the tracheal strip and was kept constant throughout the experiment. The tissues were allowed to equilibrate for at least 30 min before the testing of any drug. The changes in the tissue were recorded via a force displacement transducer (model FT-03) coupled with Bridge Amplifier and Power Lab 4/25 data acquisition system connected to computer running chart 5.3 software (AD Instrument, Sydney Australia). The spasmolytic effect of the test materials was observed by addition of test drugs in a cumulative fashion.

To assess whether the spasmolytic activity of the test substance was through Calcium Channel Blockade (CCB), high K^+ (80 mM), as KCl, was used to depolarize the tissue (Farre *et al*., 1991). The high K^+ (80 mM) was added to the tissue bath, which produced a sustained contraction. The test materials were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of intestinal preparations, pre-contracted with K^+ (80 mM) was expressed as percent of the control response mediated by K^+.

To confirm the Ca^{++} antagonist property of the test substance, 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 15 min in order to remove Ca^{++} from the tissues. This solution was further replaced with K^+ rich and Ca^{++} free Tyrode's solution. Following an incubation period of 25 min, control concentration-response curves (CRCs) of Ca^{++} were obtained. When the control Ca^{++} CRCs were found super-imposable (usually after two cycles), the tissue was pre-treated with the test material for 45 min to test possible CCB activity. The CRCs of Ca^{++} were reconstructed in the presence of different concentrations of the plant extract (Khan *et al*., 2012).

Guinea-pig trachea: Trachea was dissected from guinea pigs and kept in normal Kreb's solution. The tracheal tube was cut into rings, 2-3 mm wide, each containing about two cartilages and then tracheal ring strips were mounted in a 20 mL tissue bath containing Kreb's solution, maintained at 37°C and aerated with carbogen gas to study the tracheal relaxant activity (Gilani *et al*., 2008). The composition of Kreb's solution was (mM): NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). A tension of 1 g was applied to each of the tracheal strip and was kept constant throughout the experiment. The tissues were allowed to equilibrate for at least 45 min before the addition of any drug. Carbachol (CCh) (1 μM) and K^+ (80 mM) were used to induce sustained contractions and the relaxant activity of crude extract was assessed by adding in cumulative fashion. The possible phosphodiesterase (PDE) inhibitory effect was studied indirectly through constructing isoprenaline-induced inhibitory CRCs against CCh-induced contractions in the absence and presence of the plant extract in tracheal preparations. The changes in the tissue responses were recorded on a force displacement transducer (model FT-03) coupled to a bridge amplifier and Power Lab 4/25 data acquisition system connected to computer running chart 5.3 software (AD Instrument, Sydney Australia).

Bronchodilatory activity: Rats were anaesthetized with sodium thiopental (Pentothal, 80-100 mg kg^{-1}), then incubated with a tracheal tube and ventilated with a volume ventilator (Miniature Ideal Pump, Bioscience, UK), adjusted at a rate of 70-80 strokes/min to deliver 7-10 mL kg^{-1} of room air. A polyethylene catheter was inserted into the jugular vein for drug administration. Changes in airways resistance (mmHg) were measured by a pressure transducer (MLT-1199) connected to the side arm of tracheal cannula and recorded by Power Lab 4/25 with running chart 5.3 software via a Quad bridge amplifier (ADInstruments, Bella Vista, NSW, Australia). Bronchoconstriction was induced with CCh (100 μg kg^{-1}), which was reversed within 10-15 min. The test drug was given to the animals 5-6 min prior to administration of CCh. The responses were expressed as the percent reduction of the CCh-induced bronchospasm (Khan and Gilani, 2009).

RESULTS

Effects on rabbit jejunum: In isolated rabbit jejunum preparation, the crud extract of *Allium cepa* (ACPE), inhibited the spontaneous contractions in a dose-dependent manner with EC_{50} value (95% CI) of

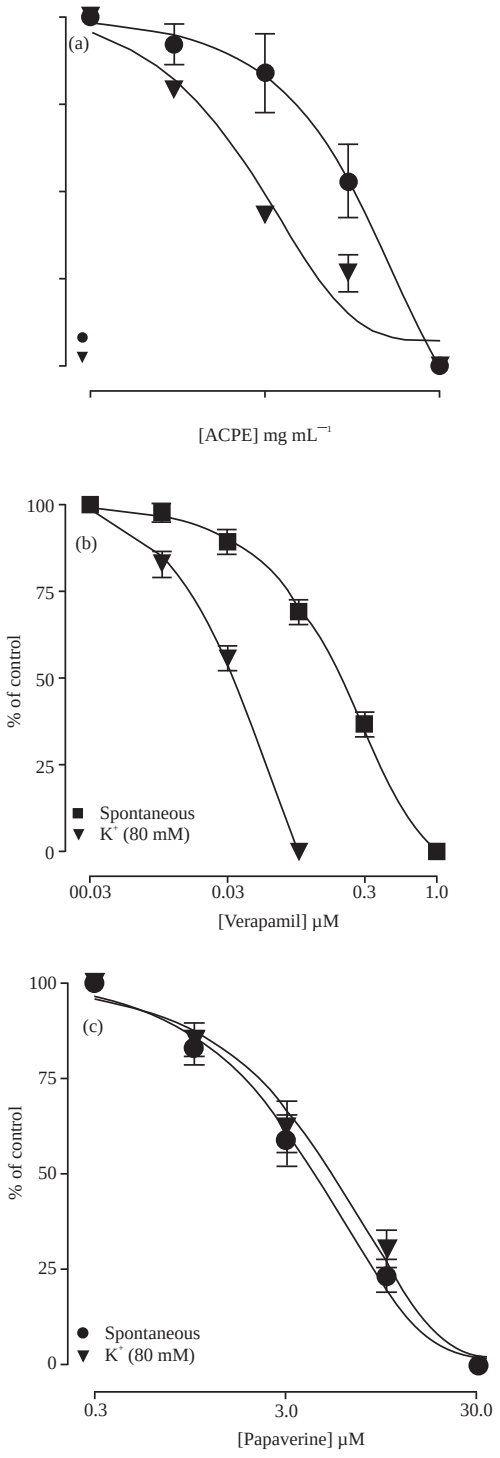

Fig. 2(a-c): Inhibitory effect of the crude extract of *A. cepa* peels (ACPE) (A) on spontaneous and high K^+ induced contractions in compared to papaverine and verapamil in isolated rabbit jejunum preparations

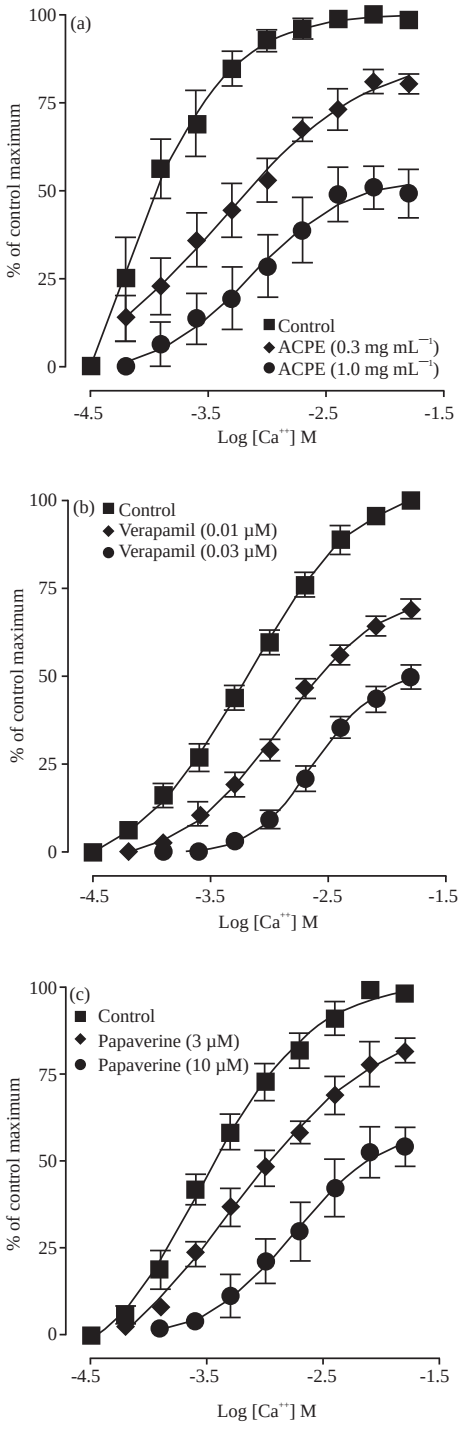

Fig. 3(a-c): Concentration-response curves of Ca^{++} in the absence and presence of the increasing concentrations of the crude extract of *A. cepa* peels (ACPE), papaverine and verapamil, constructed in Ca^{++} free and K^+ rich (80 mM) Tyrode's solution in isolated rabbit jejunum preparations (n = 3-4)

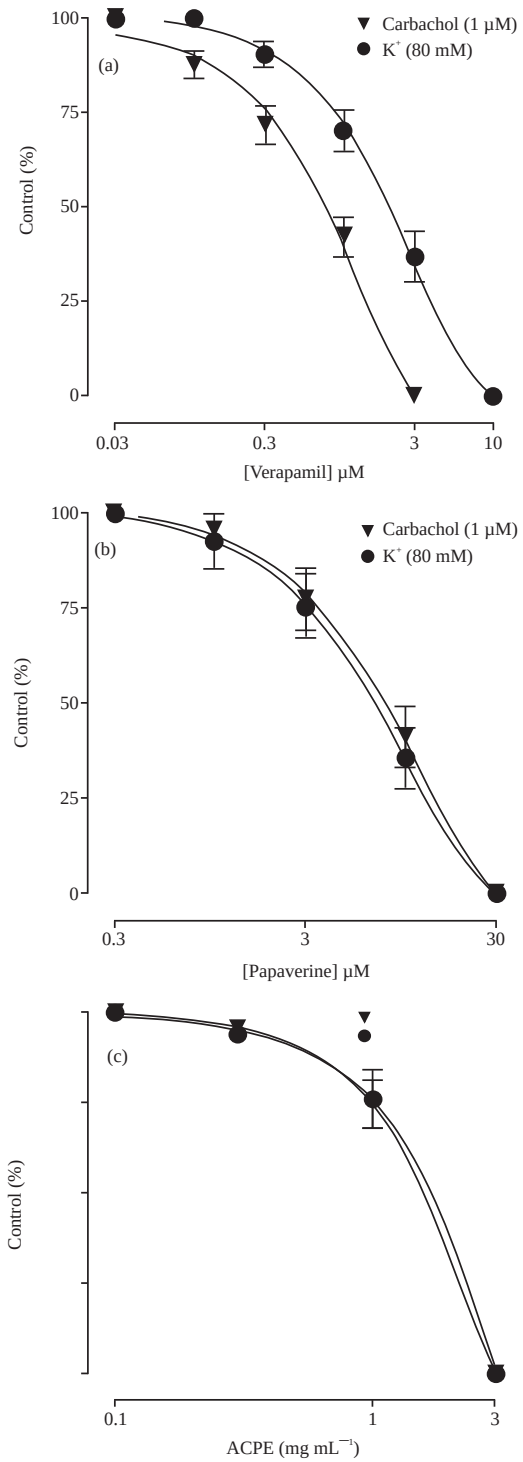

Fig. 4(a-c): Concentration-response curves showing comparisons of the crude extract of *Allium cepa* (ACPE), papaverine and verapamil on carbachol (CCh) and K^+-induced contractions in isolated guinea-pig tracheal preparations (n = 3-4)

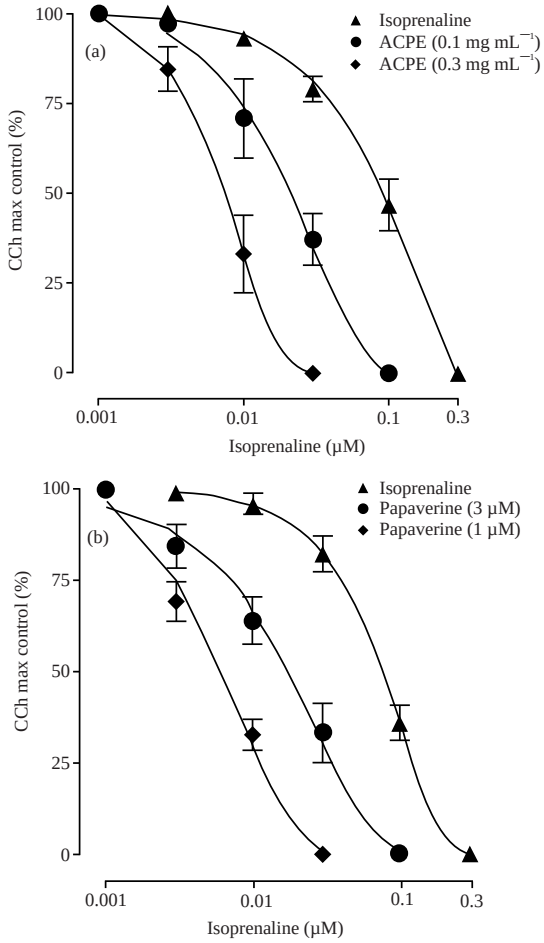

Fig. 5(a, b): Inhibitory concentration-response curves of isoprenaline against carbachol (CCh)-induced contractions in the presence of different concentrations of the crude extract of *A. cepa* peels (ACPE) and papaverine in isolated guinea-pig tracheal preparations (n = 3-4)

0.30 mg mL^{-1} (0.07-0.42; n = 4), similar to that of verapamil and papaverine as shown in Fig. 2. When tested against high K^+ (80 mM)-induced contractions, ACPE caused dose-dependent inhibiting effect with EC_{50} value of 0.11 mg mL^{-1} (0.05-0.12; n = 4) as shown in Fig. 2. ACPE caused inhibition of K^+ (80 mM)-induced contractions at same concentration compared to that of spontaneous contractions. Similar pattern was seen with papaverine, which produced inhibition of K^+ (80 mM)-induced contractions with EC_{50} value of 5.27 µM mL^{-1} (3.84-8.32; n = 4) similar EC_{50} value of 4.25 µM (3.00-5.97; n = 4) as shown in Fig. 2. Pre-treatment of the tissues with ACPE (0.3 or 1.0 mg mL^{-1}; n = 4) showed rightward shift in the Ca^{++} concentration response curves similar to that of verapamil and papaverine as shown in Fig. 3.

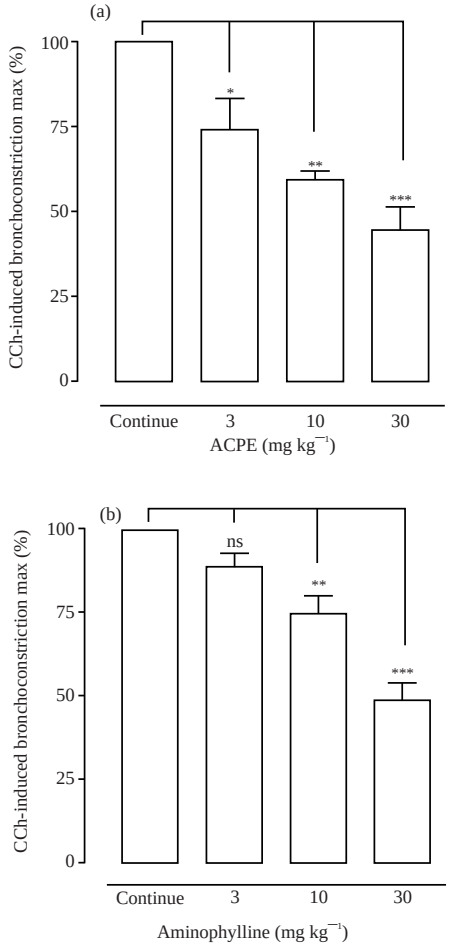

Fig. 6(a, b): Bar diagram showing the comparison of bronchodilatory effect of the crude extract of *Allium cepa* (ACPE) and aminophylline on carbachol-induced bronchoconstriction in anesthetized rat. Values shown are Mean±SEM, n = 3-4

Effect on guinea-pig trachea: In isolated guinea-pig tracheal preparations, ACPE inhibited carbachol and K^+ (80 mM) induced contraction dose-dependently (0.03-5.0 mg mL^{-1}) and equipotently, with EC_{50} value of 1.54 mg mL^{-1} (1.15-1.94; n = 4) and 1.45 mg mL^{-1} (1.19-1.58; n = 4) respectively. Similarly, papaverine caused the carbachol and K^+ (80 mM)-induced concretion at similar concentrations with respective EC_{50} values of 7.05 μM (4.10-8.88; n = 4) and 7.94 μM (4.53-9.80; n = 4), while, verapamil was more potent against high K^+ induced contractions with resultant EC_{50} values against carbachol and K^+ as 2.11 μM (1.26-2.88; n = 4) and 1.10 μM (0.54-1.10; n = 4) respectively. All the comparative results are shown in Fig. 4. Pre-treatment of the tissues with ACPE (0.1 or 0.3 mg mL^{-1}; n = 4) showed leftward shift in the isoprenaline concentration response curves similar to that of papaverine as shown in Fig. 5.

Effects on anesthetized rats: The ACPE was tested in anaesthetized SD rats for its possible bronchodilatory effect. The intravenous administrations of ACPE at different doses 3, 10 and 30 mg kg^{-1} significantly inhibit the CCh (100 μg kg^{-1})-evoked increased in respiratory pressure similarly, the same pattern of inhibition was observed with aminophylline. All the results are shown in Fig. 6.

DISCUSSION

The extract of *Allium cepa* peels (ACPE) was studied for its possible spasmolytic effect in the spontaneously contracting rabbit jejunum preparations, where it caused a concentration-dependent inhibition of both spontaneous and K^+ induced interactions with similar potency. Similarly, papaverine, a PDE and Ca^{++} influx inhibitor (Rang *et al*., 1999)also caused similar pattern of inhibitory effect with comparable potency against spontaneous and K^+ induced contractions, while verapamil, a standard Ca^{++} channel blocker (CCB) (Fleckenstein, 1977) was relatively selective in its inhibitory effect on high K^+ induced contraction, a typical characteristic of CCB (Godfraind *et al*., 1986). The presence of calcium antagonist constituent(s) was further confirmed when pretreatment of the tissue with the extract shifted the Ca^{++} CRCs to the right, similar to that of papaverine or verapamil. However, the similar inhibitory pattern of plant extract against spontaneous and K^+ induced contractions similar to that of papaverine, reflects that it may possess additional mechanism(s) involved in the spasmolytic effect, like PDE inhibition.

In view of the well-known medicinal use of onion in asthma, the extract was further studied for its possible bronchodilatory effect in anaesthetized rats, where it inhibited the carbachol-evoked bronchospasm, like that caused by aminophylline (soluble salt of theophylline), a well-known bronchodilator (Evans *et al*., 1980). The ACPE was then studied in isolated tracheal tissues, to elucidate the possible mode of bronchodilator action, where it caused relaxation of both CCh and K^+ induced contractions at similar concentrations, like papaverine. This suggests that the ACPE possesses papaverine-like PDE inhibitory constituent(s). The verapamil was found relatively more selective against high K^+ than carbachol-induced contractions, as expected from a CCB. The PDE inhibitory-like effect was further confirmed when the plant extract potentiated the isoprenaline-induced inhibitory effect, similar to that produced by papaverine, thus indicating the presence of additional PDE inhibitory-like bronchodilatory substance(s). The usefulness of PDE

inhibitors in asthma is well established (Teixeira *et al*., 1997). Interestingly, the CCBs have also been shown to be useful in such condition (Mathewson, 1985). Thus, the presence of combined inhibitory effect on PDE enzyme and calcium channels is likely to be responsible for the medicinal use of *Allium cepa* in gut and airways disorders, particularly in asthma. PDE inhibitors such as, theophylline is known to be very effective bronchodilator, but have limited clinical use primarily because of associate side-effect such as tachycardia as a result of increased levels of cAMP in the myocardium (Dobson, 1983). But co-existence of CCB with PDE inhibitory activity is likely to offer synergistic and side-effects neutralizing potential, which is common property in natural products (Gilani and Atta-ur-Rahman, 2005; Hasimun *et al*., 2011).

In summary, the findings of this study suggest that *Allium cepa* possesses spasmolytic and bronchodilator activities, which are mediated possibly, through dual inhibition of Ca^{++} channels and PDE enzyme. These finding may explain its medicinal use in diarrhea and asthma and the co-existence of Ca^{++} antagonist constituent(s) with that of PDE inhibitory activity is likely to offer not only the enhanced effect in airways and gut hyperactivity disorders but also likely to offset the cardiac stimulation usually seen as a major side effect, when PDE inhibitors like theophylline are used alone.

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